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Article type : Research Article

Synthesis and antibacterial activities of novel pleuromutilin derivatives bearing an aminothiophenol moiety

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Abstract

A series of novel thioether pleuromutilin derivatives incorporating 2-aminothiophenol moieties into the C14 side chain were synthesized via acylation reactions under mild conditions. The *in vitro* antibacterial activities of the derivatives against methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC43300), *Staphylococcus aureus* (ATCC 29213) and

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cbdd.13328
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Escherichia coli (ATCC25922) were evaluated. The majority of the synthesized derivatives possessed moderate antibacterial activities. Compound **8** was found to be the most active antibacterial derivative against MRSA. Docking experiments were conducted to understand the possible mode of interactions between compounds **8**, **9b**, **11a** and 50S ribosomal subunit. The docking results proved that there is a reasonable correlation between the binding free energy and the antibacterial activity. Compound **8** was evaluated for its *in vivo* antibacterial activity and showed higher efficacy than tiamulin against MRSA in mouse infection model.

Introduction

Multidrug-resistant bacteria represent a severe threat to human communities worldwide[1]. However, there have not been any developments for antibacterial against antibiotic-resistant bacteria in recent decades [2]. Among those antibiotic-resistant bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been reported resistance to clinical antibiotics, including the class of β-lactam antibiotics[3], linezolid[4], vancomycin[5] and daptomycin[6]. Infections with MRSA have been the key cause of higher costs, longer hospitalization[7], immense morbidity and mortality, and so on [8]. Thus, there is an urgent need for antibacterial agents with novel mechanisms of action to treat infections caused by MRSA.

Pleuromutilin **1** (Figure 1) is a naturally tricyclic diterpene natural product, first isolated in 1951 from *Pleurotus mutiliz* and *Pleurotus passeckeranius*, that displays potent antibacterial activity against Gram-positive bacteria and mycoplasmas [9, 10]. The pleuromutilin class of antibiotics selectively interferes bacterial protein synthesis through binding to the bacterial 50S ribosome subunit at the peptidyl transferase center (PTC) [11-13]. This distinct mode of

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action of pleuromutilin also made it an attractive lead compound in the discovery and development of new antibacterial agents for the treatment of resistant bacterial infections [14, 15].

Numerous semisynthetic pleuromutilin derivatives have been prepared and evaluated [11, 15-18]. In order to improve the pharmacokinetic of pleuromutilin derivatives, much synthetic effort has also been focused on total synthesis of pleuromutilin [19, 20]. The most successful of pleuromutilin synthetic modications was the replacement of the hydroxyl group in C14 glycolic ester side chain of pleuromutilin with a substituent containing a sulfide linkage. This has led to the discovery of three pleuromutilin drugs tiamulin (**2**, Figure 1) [21], valnemulin (**3**, Figure 1) [22] and retapamulin (**4**, Figure 1) [14]. Tiamulin and valnemulin are approved in veterinary medicine for pigs and poultry. Retapamulin is the first pleuromutilin drug approved for human use to treat topical skin infections both in USA and Europe [23]. Although it was not licensed for use in MRSA infections, retapamulin displayed clinical successes in clinical trails for few cases (100%:8/8) [24]. Additionally, BC-3781 (**5**, Figure 1) and BC-7013 (**6**, Figure 1) have been reported in clinical trail for human use [25, 26]. All above pleuromutilin derivatives either licensed or in clinical trails vary only in the C14 side chain of pleuromutilin, furthermore all of them have a thioether moiety.

Previous work in our group has led to several semisynthetic pleuromutilin derivatives with powerful antibacterial activity against *S. aureus* including ATCC 29213 [27] and MRSA [28]. One of the synthesized pleuromutilin derivative in our group possessed superior *in vivo* efficacy to that of tiamulin in MRSA systemic infection model [28]. This research background motivated us to develop the pleuromutilin derivatives containing a thioether

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moiety, and thus, 13 pleuromutilin derivatives were designed, synthesized and evaluated for their antibacterial activities against three strains including MRSA. The antibacterial and docking results in this study may help us to design novel pleuromutilin derivatives.

Experimental

Materials

All reagents and solvents were purchased from commercial suppliers as reagent grade and were used as supplied unless noted otherwise. Column chromatography was carried out using silica gel (200-300 mesh). Melting points were measured on a Shenguang X-4 apparatus (China) and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at Bruker AV-400 or Bruker AV-600 spectrometer. The chemical shift values (δ) are reported in ppm relative to tetramethylsilane as internal standard. Fourier transform infrared(FT-IR) were obtained on a Nicolet 6700 Research FTIR spectrometer using KBr pellets, and the absorptions are reported in cm^{-1}. High-resolution mass spectra were conducted using a LTQ-Orbitrap mass spectrometer (Thermo Fisher) with an electro spray ionization (ESI) source.

Synthesis

A general synthesis strategy based on the usual 22-*O*-tosylpheuromutilin, 2-aminophenol and a variety of phenylacetyl chlorides were used (Scheme 1).

Scheme 1 here

22-*O*-tosylpleuromutilin (7)

A solution of pleuromutilin 1(5.4g, 14.27 mmol) in pyridine (30.0 mL) was stirred at 0 ℃ in a three-necked round bottom flask, and *p*-toluenesulfonyl chloride (8.6 g, 45.11 mmol) was added. The solution was stirred at 0 ℃ for 3h. $CHCl_3$ (50 mL) and Ice-cold water (50 mL)

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were added to the solution. The organic phase was washed with a 2 M aqueous solution of H_2SO_4, a saturated aqueous solution of $NaHCO_3$ and water, respectively. Then the organic phase was dried with anhydrous Na_2SO_4 for 2 h and evaporated in vacuum. The residue was precipitated from isopropanol to give a white solid (6.0 g, 79%) [29].

22-(2-Amino-phenylsulfanyl)-22-deoxypleuromutilin (8)

22-***O***-tosylpleuromutilin (1g, 1.87 mmol) was dissolved in ethyl acetate (35 mL) and sodium iodide (0.31g, 2.07 mmol) was added. The mixture was stirred for 0.5 h at 70 ℃. After 22-***O***-tosylpleuromutilin had been dissolved completely, a solution of 2-aminothiophenol (0.35g, 2.79mmol) in 20% aqueous NaOH (10mL) was added dropwise, and the resulting reaction mixture was stirred at 70 ℃ until complete consumption of 22-***O***-tosylpleuromutilin was obtained. The reaction mixture was concentrated in vacuo. Chloroform was added, and the mixture was washed with brine and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (ethyl acetate: petroleum ether = 3:4) using silica gel to afford the desired compounds (0.51g, 56%).

White powder; yield: 56%; m.p.: 184.5-186.8 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 (1H, dd, *J* = 7.7, 1.6 Hz), 7.12 (1H, m), 6.68 (2H, m), 6.48 (1H, *J* = 17.4, 11.0 Hz, H19), 5.72 (1H, d, *J* = 8.5 Hz, H14), 5.36 (1H, dd, *J* = 11.0, 1.5 Hz, H20), 5.21 (1H, dd, *J* = 17.4, 1.6 Hz, H20), 4.40 (2H, s), 3.44 (1H, d, *J* = 14.8Hz, H22), 3.38 (1H, d, *J* = 14.9 Hz, H22), 3.35 (1H, s, H11), 2.12 (5H, dd, *J* = 16.0, 8.6 Hz), 1.76 (1H, dd, *J* = 14.6, 3.3 Hz, H6), 1.56 (4H, m, H1, H7), 1.39 (3H, s, H15), 1.28 (2H, m, H18, H13), 1.16 (3H, s, H18), 1.10 (1H, d, *J* = 13.9, 4.4 Hz, H8), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.65 (3H, d, *J* = 7.0 Hz, H16) ; ^{13}C NMR (151 MHz,

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$CDCl_3$) δ 217.0 (C3), 168.9 (C21), 147.4, 139.1 (C19), 136.4, 130.6, 119.4, 117.1 (C20), 115.8, 74.6 (C11), 69.5 (C14), 58.1 (C4), 45.4 (C9), 44.6 (C13), 43.9, 41.7, 37.8, 36.7, 36.0 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.3 (C18), 24.8 (C1), 16.6 (C16), 14.8 (C15), 11.5 (C17); IR (KBr, cm^{-1}) 3497, 3346, 2923, 1726, 1614, 1482, 1449, 1407, 1312, 1278, 1122, 1020, 983, 964, 915, 751, 668; HR-MS (ESI): Calcd for $C_{28}H_{40}NO_4S$ ($M+H^+$): 486.2673; Found: 486.2674.

General procedure for the synthesis of compounds **9a-9c, 10a-10c, 11a-11c and 12a-12c**

Compound **8** (1g, 2.28mmol) was dissolved in ethyl acetate (35 mL), acyl chloride derivatives (2.28mmol) was added dropwise, and the resulting reaction mixture was stirred at 70 ℃ for 1 h. The mixture was washed with water, followed by separation of organic layer. The aqueous layer was extracted with chloroform and the combined organic layer extracts were washed with brine, water and then dried with anhydrous Na_2SO_4. After evaporation the organic solvent in vacuo, the residue was purified by silica gel column chromatography to give a pure product.

22-(2-(2-Fluoro-benzoylamino)- phenylsulfanyl)-22-deoxypleuromutilin (9a)

White powder; yield: 62%; m.p.: 129.5-130.7 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.89 (1H, d, *J* = 12.6 Hz), 8.59 (1H, d, *J* = 8.3 Hz), 8.19 (1H, m), 7.61 (1H, dd, *J* = 7.8, 1.6 Hz), 7.56 (1H, m), 7.41 (1H, m), 7.34 (1H, m), 7.23 (1H, dd, *J* = 11.9, 8.2 Hz), 7.09 (1H, dd, *J* = 7.5, 1.4 Hz), 6.36 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.66 (1H, d, *J* = 8.5 Hz, H14), 5.24 (1H, dd, *J* = 10.9, 1.6 Hz, H20), 5.12 (1H, dd, *J* = 17.3, 1.6 Hz, H20), 3.51 (1H, d, *J* = 15.3 Hz, H22), 3.46 (1H, d, *J* = 15.4 Hz, H22), 3.31 (1H, d, *J* = 6.5 Hz, H11), 2.20 (5H, m), 1.95 (1H, dd, *J* = 16.0, 8.5 Hz), 1.75 (1H, m, H6), 1.53 (5H, m, H1, H7), 1.35 (3H, s, H15), 1.23 (2H, m, H18, H13),

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1.10 (3H, s, H18), 1.06 (1H, d, *J* = 16.0 Hz, H8), 0.86 (3H, d, *J* = 7.0 Hz, H17), 0.58 (3H, d, *J* = 7.1 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.0 (C3), 168.1 (C21), 140.0, 138.8, 138.7 (C19), 135.7, 133.7, 129.9, 124.6, 121.4, 117.2 (C20), 74.5 (C11), 69.8 (C14), 65.1 (C22), 58.0 (C4), 45.4 (C9), 44.5 (C13), 43.9 (C12), 41.7 (C5), 38.9, 36.6 (C6), 35.9 (C10), 34.4 (C2), 30.4 (C8), 26.8 (C7), 26.4 (C18), 26.2, 24.8 (C1), 16.6 (C16), 14.7 (C15), 11.5 (C17); IR (KBr, cm^{-1}) 3529, 3334, 2941, 1728, 1674, 1581, 1529, 1454, 1435, 1375, 1311, 1192, 1149, 1115, 1020, 918, 764, 663; HR-MS (ESI): Calcd for $C_{35}H_{43}FNO_5S$ ($M+H^+$): 608.2840; Found: 608.2867.

22-(2-(3-Fluoro-benzoylamino)- phenylsulfanyl)-22-deoxypleuromutilin (9b)

Faint yellow powder; yield: 47%; m.p.: 130.9-132.1 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.90 (1H, s), 8.46 (1H, dd, *J* = 8.3, 1.4 Hz), 7.93 (1H, dd, *J* = 7.8, 1.2 Hz), 7.89 (1H, dd, *J* = 9.5, 2.2 Hz), 7.61 (1H, dd, *J* = 7.8, 1.6 Hz), 7.53 (1H, m), 7.42 (1H, m), 7.32 (1H, m), 7.11 (1H, m), 6.33 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.64 (1H, d, *J* = 8.6 Hz, H14), 5.14 (1H, dd, *J* = 11.0, 1.4 Hz, H20), 5.10 (1H, dd, *J* = 17.5, 1.5 Hz, H20), 3.57 (1H, d, *J* = 16.3 Hz, H22), 3.50 (1H, d, *J* = 16.0 Hz, H22), 3.30 (1H, d, *J* = 16.0 Hz, H11), 2.10 (5H, m), 1.74 (1H, dd, *J* = 14.7, 3.3 Hz, H6), 1.53 (4H, m, H1, H7), 1.36 (3H, s, H15), 1.22 (3H, m, H18, H13, 11-OH), 1.09 (3H, s, H18), 1.01 (1H, d, *J* = 16.0 Hz,, H8), 0.86 (3H, d, *J* = 7.0 Hz, H17), 0.46 (3H, d, *J* = 7.1 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 216.7 (C3), 169.3 (C21),163.9, 140.3, 138.6 (C19), 137.3, 136.3, 130.7, 130.3, 124.9, 123.1, 122.5, 121.5, 118.8, 117.4 (C20), 114.8, 74.5 (C11), 70.3 (C14), 65.0 (C22), 58.0 (C4), 45.4(C9), 44.5 (C13), 43.9 (C12), 41.7, 40.5, 36.6 (C6), 36.0 (C10), 34.4 (C2), 30.4 (C8), 26.8 (C7), 26.2 (C18), 24.8 (C1), 16.5 (C16), 14.8 (C15), 11.5 (C17) ; IR (KBr, cm^{-1}) 3567, 2937, 2864, 1732, 1675, 1598, 1579, 1510,

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1455, 1372, 1291, 1190, 1176, 1117, 1037, 978, 775, 663; HR-MS (ESI)): Calcd for $C_{35}H_{43}FNO_5S$ ($M+H^+$): 608.2840; Found: 608.2856.

22-(2-(4-Fluoro-benzoylamino)- phenylsulfanyl)-22-deoxypleuromutilin (9c)

Faint yellow powder; yield: 65%; m.p.: 129.5-130.7 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.82 (1H, s), 8.46 (1H, dd, *J* = 8.3, 1.3 Hz), 8.16 (2H, m), 7.60 (1H, dd, *J* = 7.7, 1.6 Hz), 7.41 (1H, m), 7.23 (1H, s), 7.09 (1H, t, *J* = 7.6, 1.4 Hz), 6.31 (1H, dd, *J* = 17.4, 11.1 Hz, H19), 5.60 (1H, d, *J* = 8.6 Hz, H14), 5.13 (1H, m, H20), 5.09 (1H, m, H20), 3.56 (1H, d, *J* = 16.2 Hz, H22), 3.48 (1H, d, *J* = 16.2 Hz, H22), 3.29 (1H, d, *J* = 6.5 Hz, H11), 2.10 (5H, m), 1.73 (1H, m, H6), 1.52 (4H, m, H1, H7), 1.35 (3H, s, H15), 1.20 (3H, m, H18, H13, 11-OH), 1.07 (3H, s, H18), 0.96 (1H, m, H8), 0.85 (3H, d, *J* = 7.0 Hz, H17), 0.47 (3H, d, *J* = 7.1 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 216.8 (C3), 169.3 (C21), 165.9, 164.1, 138.6 (C19), 140.4, 136.2, 130.7, 129.9, 124.7, 122.3, 121.3, 117.2 (C20), 115.8, 77.4 (C11), 70.5 (C14), 57.9 (C4), 45.3 (C9), 44.5 (C13), 43.9 (C12), 41.6, 40.4, 36.5 (C6), 35.9 (C10), 34.4 (C2), 30.3 (C8), 26.8 (C7), 26.1 (C18), 24.8 (C1), 16.4 (C16), 14.8 (C15), 11.5 (C17) ; IR (KBr, cm^{-1}) 3508, 3331, 2939, 1736, 1664, 1581, 1529, 1456, 1437, 1317, 1298, 1192, 1147, 1115, 1020, 918, 854, 766, 579; HR-MS (ESI)): Calcd for $C_{35}H_{43}FNO_5S$ ($M+H^+$): 608.2840; Found: 608.2836.

22-(2-(2-Chloro-benzoylamino)- phenylsulfanyl)-22-deoxypleuromutilin (10a)

Yellow powder; yield: 48%; m.p.: 157-160 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.91 (1H, s), 8.44 (1H, dd, *J* = 8.3, 1.4 Hz), 8.19 (1H, m), 8.02 (1H, dd, *J* = 7.7, 1.4 Hz), 7.60 (2H, m), 7.50 (1H, dd, J = 7.9, 1.3 Hz), 7.42 (1H, m), 7.10 (1H, t), 6.34 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.67 (1H, d, J = 8.5 Hz, H14), 5.32 (1H, s), 5.13 (1H, dd, *J* = 10.9, 1.5 Hz, H20), 5.09 (1H, dd, *J* = 17.4, 1.6 Hz, H20), 3.56 (1H, d, *J* = 15.9 Hz, H22), 3.50 (1H, d, *J* = 15.9 Hz,

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H22), 3.30 (1H, d, *J* = 6.6 Hz, H11), 2.21 (5H, m,H2, H4, H10, H13), 1.74 (1H, dd, *J* = 14.4, 3.2 Hz, H6), 1.53 (5H, m, H1, H7), 1.36 (3H, s, H15), 1.22 (3H, m, H18, H13), 1.09 (3H, s, H18), 1.03 (1H, m, H8), 0.86 (3H, d, *J* = 7.0 Hz, H17), 0.48 (3H, d, *J* = 7.1 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 216.8 (C3), 169.4 (C21), 164.0, 140.3, 138.7 (C19), 136.8, 136.4, 134.9, 131.8, 130.7, 130.0, 127.9, 125.7, 124.9, 122.7, 121.6, 117.3 (C20), 74.5 (C11), 70.5 (C22), 58.0 (C4), 45.4 (C9), 44.6 (C13), 43.9 (C12), 41.7, 40.6, 36.6 (C6), 36.0 (C10), 34.4 (C2), 30.4 (C8), 26.8 (C7), 26.2 (C18), 24.8 (C1), 16.4 (C16), 14.8 (C15), 11.5 (C17) ; IR (KBr, cm^{-1}) 3545, 3332, 2945, 1720, 1678, 1579, 1516, 1435, 1308, 1194, 1149, 1112, 1018, 917, 766, 747, 627; HR-MS (ESI): Calcd for $C_{35}H_{43}ClNO_5S$ ($M+H^+$): 624.2545; Found: 624.2575.

22-(2-(3-Chloro-benzoylamino)- phenylsulfanyl)-22-deoxypleuromutilin (10b)

Faint yellow powder; yield: 35%; m.p.: 63.7-64.5 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.92 (1H, s), 8.44 (1H, dd, *J* = 8.3, 1.3 Hz), 8.19 (1H, t, *J* = 1.9 Hz), 8.03 (1H, d, *J* = 7.8 Hz), 7.61 (1H, dd, *J* = 7.8, 1.5 Hz), 7.58 (1H, m), 7.50 (1H, t, *J* = 7.9 Hz), 7.42 (1H, m), 7.11 (1H, d, *J* = 7.6, 1.3 Hz), 6.34 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.67 (1H, d, *J* = 8.5 Hz, H14), 5.13 (1H, m, H20), 5.08 (1H, dd, *J* = 17.4, 1.5 Hz, H20), 3.56 (1H, d, *J* = 16.5 Hz, H22), 3.50 (1H, m, H22), 3.30 (1H, d, *J* = 6.6 Hz, H11), 2.10 (6H, m), 1.74 (1H, d, *J* = 14.5, 3.3 Hz, H6), 1.53 (5H, m, H1, H7), 1.36 (3H, s, H15), 1.22 (2H, m, H18, H13), 1.08 (3H, s, H18), 1.02 (1H, d, *J* = 16.0 Hz, H8), 0.86 (3H, d, *J* = 7.0 Hz, H17), 0.47 (3H, d, *J* = 7.1 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 216.8 (C3), 169.4 (C21), 164.0, 140.3, 138.6 (C19), 136.3, 134.9, 130.7, 130.0, 127.9, 125.7, 124.9, 122.7, 121.6, 117.2 (C20), 74.5 (C11), 70.5 (C14), 58.0 (C4), 45.4 (C9), 44.6 (C13), 43.9 (C12), 41.7 (C5), 40.6, 36.5 (C6), 36.0 (C10), 34.4 (C2), 30.3 (C8),

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26.8 (C7), 26.2 (C18), 24.8 (C1), 16.4 (C16), 14.8 (C15), 11.5 (C17) ; IR (KBr, cm^{-1}) 3458, 3348, 2931, 1728, 1678, 1579, 1435, 1375, 1303, 1246, 1190, 1151, 1115, 1016, 979, 914, 752; HR-MS (ESI): Calcd for $C_{35}H_{43}ClNO_5S$ ($M+H^+$): 624.2545; Found: 624.2562.

22-(2-(4-Chloro-benzoylamino)- phenylsulfanyl)-22-deoxypleuromutilin (10c)

Faint yellow powder; yield: 50%; m.p.: 155-157 ℃; ^1H NMR (600 MHz, $CDCl_3$) δ 9.85 (1H, s), 8.47 (1H, d, *J* = 8.3 Hz), 8.10 (2H, m), 7.61 (1H, dd, *J* = 7.8, 1.6 Hz), 7.53 (2H, m), 7.41 (1H, t, *J* = 8.0 Hz), 7.10 (1H, t, *J* = 7.6 Hz), 6.29 (1H, dd, *J* = 17.3, 11.1 Hz, H19), 5.59 (1H, d, *J* = 8.6 Hz, H14), 5.12 (1H, d, *J* = 3.8 Hz, H20), 5.09 (1H, d, *J* = 10.8 Hz, H20), 3.56 (1H, d, *J* = 16.2 Hz, H22), 3.48 (1H, d, *J* = 16.3 Hz, H22), 3.29 (1H, d, *J* = 6.5 Hz, H11), 2.09 (5H, m), 1.73 (1H, m, H6), 1.52 (4H, m, H1, H7), 1.35 (3H, s, H15), 1.21 (3H, s, H15), 1.07 (3H, s, H18), 0.96 (1H, d, *J* = 16.0 Hz, H8), 0.85 (3H, d, *J* = 7.0 Hz, H17), 0.46 (3H, d, *J* = 7.1 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 216.7 (C3), 169.3 (C21), 164.2, 140.3, 138.6 (C19), 138.2, 136.2, 133.4, 130.7, 129.0, 124.8, 122.3, 121.4, 117.1 (C20), 74.5 (C11), 70.5 (C14), 57.9 (C4), 45.3 (C9), 44.5 (C13), 43.9 (C12), 41.7 (C5), 40.4, 36.5 (C6), 35.9 (C10), 34.5 (C2), 30.3 (C8), 26.8 (C7), 26.2, 24.8 (C1), 16.5 (C16), 14.7 (C15), 11.5 (C17) ; IR (KBr, cm^{-1}) 3504, 3321, 2937, 1722, 1662, 1581, 1529, 1491, 1435, 1317, 1288, 1192, 1149, 1111, 1014, 935, 914, 849, 766, 752, 642; HR-MS (ESI): Calcd for $C_{35}H_{43}ClNO_5S$ ($M+H^+$): 624.2545; Found: 624.2540.

22-(2-(2-Methy-benzoylamino)- phenylsulfanyl)-22-deoxypleuromutilin (11a)

White powder; yield: 47%; m.p.: 76.6-77.4 ℃; ^1H NMR (600 MHz, $CDCl_3$) δ 7.19 (1H, dd, *J* = 7.6, 1.5 Hz), 6.93 (1H, m), 6.63 (1H, d, *J* = 8.0 Hz), 6.51 (1H, t, *J* = 7.5 Hz), 6.24 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.48 (1H, d, *J* = 8.5 Hz, H14), 5.14 (1H, m, H20), 4.97 (1H, dd, *J* =

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17.4, 1.6 Hz, H20), 3.23 (1H, d, *J* = 15.8 Hz, H22), 3.17 (1H, d, *J* = 15.9 Hz, H22), 3.11 (1H, d, *J* = 6.6 Hz, H11), 1.92 (5H, m), 1.53 (1H, m, H6), 1.33 (4H, m, H1, H7), 1.16 (3H, s, H15), 1.04 (2H, m, H18, H13), 0.92 (3H, s, H18), 0.87 (1H, d, *J* = 16.0 Hz, H8), 0.65 (3H, d, *J* = 7.0 Hz, H17), 0.38 (3H, d, *J* = 7.1 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.0 (C3), 169.0 (C21), 139.1 (C19), 136.5, 130.6, 119.9, 117.2 (C20), 116.3, 74.6 (C11), 69.6 (C14), 65.0 (C22), 58.1 (C4), 45.4 (C9), 44.6 (C13), 43.9 (C12), 41.7 (C5), 38.0, 36.7 (C6), 36.0 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.3 (C18), 24.8 (C1), 16.6 (C16), 14.8 (C15), 11.5 (C17) ; IR (KBr, cm^{-1}) 3479, 3339, 2931, 1732, 1678, 1579, 1510, 1433, 1373, 1304, 1190, 1176, 1117, 1016, 980, 914, 756, 661; HR-MS (ESI): Calcd for $C_{36}H_{46}NO_5S$ ($M+H^+$): 604.3091; Found: 604.3117.

22-(2-(3-Methy-benzoylamino)- phenylsulfanyl)-22-deoxypleuromutilin (11b)

White powder; yield: 56%; m.p.: 65.8-66.7 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (1H, dd, *J* = 7.7, 1.5 Hz), 7.14 (1H, m), 6.80 (1H, d, *J* = 8.0 Hz), 6.71 (1H, t, *J* = 7.5 Hz), 6.47 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.71 (1H, d, *J* = 8.5 Hz, H14) , 5.37 (1H, dd, *J* = 11.1, 1.5 Hz, H20), 5.20 (1H, dd, *J* = 17.5, 1.5 Hz, H20), 3.45 (1H, d, *J* = 16.0 Hz, H22), 3.39 (1H, d, *J* = 16.0 Hz, H22), 3.34 (1H, d, *J* = 6.5 Hz, H11), 2.17 (5H, m), 1.76 (1H, m, H6), 1.55 (4H, m, H1, H7), 1.39 (3H, s, H15), 1.24 (2H, m, H18, H13), 1.15 (3H, s, H18), 1.11 (1H, m, H8), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.63 (3H, d, *J* = 7.1 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.0 (C3), 169.0 (C21), 139.1 (C19), 136.4, 130.6, 119.5, 117.2 (C20), 115.9, 74.6 (C11), 69.7 (C14), 65.0 (C22), 58.1 (C4), 45.4 (C9), 44.6 (C13), 43.9 (C12), 41.8 (C5), 37.8 (C6), 36.7, 36.0 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.3 (C18), 24.8 (C1), 16.6 (C16), 14.8 (C15), 11.5 (C17) ; IR (KBr, cm^{-1}) 3487, 3349, 2929, 1732, 1674, 1579, 1518, 1435, 1373, 1304, 1190,

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1176, 1117, 1018, 980, 914, 812, 756, 663; HR-MS (ESI): Calcd for $C_{36}H_{46}NO_5S$ ($M+H^+$): 604.3091; Found: 604.3110.

22-(2-(4-Methy-benzoylamino)- phenylsulfanyl)-22-deoxypleuromutilin (11c)

White powder; yield: 53%; m.p.: 133.1-133.8 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (1H, d, *J* = 7.9 Hz), 7.15 (1H, dd, *J* = 7.7, 1.5 Hz), 7.03 (1H, m), 6.89 (1H, td, *J* = 7.7, 1.5 Hz), 6.47 (1H, d, *J* = 8.0 Hz), 6.42 (1H, m), 6.25 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.48 (1H, d, *J* = 8.6 Hz, H14), 5.13 (1H, dd, *J* = 11.1, 1.6 Hz, H20), 4.97 (1H, dd, *J* = 17.4, 1.6 Hz, H20), 3.20 (1H, d, *J* = 14.9 Hz, H22), 3.14 (1H, d, *J* = 14.9 Hz, H22), 3.12 (1H, d, *J* = 6.5 Hz, H11), 2.19 (1H, s), 1.93 (5H, m), 1.53 (1H, m, H6), 1.33 (4H, m, H1, H7), 1.16 (3H, s, H15), 1.04 (2H, m, H18, H13), 0.92 (3H, s, H18), 0.87 (1H, dd, *J* = 13.9, 4.2 Hz, H8), 0.64 (3H, d, *J* = 7.0 Hz, H17), 0.42 (3H, d, *J* = 7.1 Hz, H16); ^{13}C NMR (151 MHz, $CDCl_3$) δ 216.8 (C3), 168.9 (C21), 138.7 (C19), 136.0, 129.9, 129.4, 128.1, 127.5, 121.2, 117.3 (C20), 74.5 (C11), 70.3 (C14), 65.0 (C22), 58.0 (C4), 45.4 (C9), 44.5 (C13), 43.9 (C12), 41.9 (C5), 41.6, 40.1, 36.6 (C6), 35.9 (C10), 34.4 (C2), 30.3 (C8), 26.8 (C7), 26.4 (C18), 26.1, 24.8 (C1), 21.7, 16.6 (C16), 14.8 (C15), 11.5 (C17) ; IR (KBr, cm^{-1}) 3491, 3332, 2939, 1728, 1660, 1579, 1529, 1506, 1435, 1371, 1306, 1176, 1149, 1115, 1020, 914, 818, 764, 663; HR-MS (ESI): Calcd for $C_{36}H_{46}NO_5S$ ($M+H^+$): 604.3091; Found: 604.3110.

22- (2- (2-Methoxybenzamido) phenylsulfanyl) -22-deoxypleuromutilin(12a)

Yellow powder; yield: 42%; m.p.: 140.7-141.5 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.06 (1H, s), 8.69 (1H, dd, J = 8.3, 1.3 Hz), 8.33 (1H, dd, J = 7.8, 1.8 Hz), 7.58 (1H, dd, *J* = 7.8, 1.6 Hz), 7.53 (1H, m), 7.37 (1H, m), 7.16 (1H, d, J = 7.6, 1.0 Hz), 7.07 (2H, m), 6.40 (1H, m, H19), 5.67 (1H, d, J = 8.5 Hz, H14), 5.26 (1H, dd, J = 11.0, 1.5 Hz, H20), 5.11 (1H, dd, J = 17.4,

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1.6 Hz, H20), 4.12 (3H, s), 3.52 (1H, d, J = 15.0 Hz, H22), 3.47 (1H, d, J = 15.0 Hz, H22), 3.30 (1H, d, J = 6.5 Hz, H11), 2.11 (5H, m), 1.75 (1H, dd, J = 14.7, 3.1 Hz, H6), 1.49 (5H, m, H1, H7), 1.29 (3H, s, H15), 1.11 (2H, m, H18, H13), 1.05 (3H, d, J = 4.3 Hz, H18), 0.86 (3H, d, J = 7.0 Hz, H17), 0.60 (3H, d, J = 7.1 Hz, H16); ^{13}C NMR (151 MHz, $CDCl_3$) δ 216.9 (C3), 168.0, 163.5 (C21), 157.5, 140.7, 138.9 (C19), 135.1, 133.4, 132.7, 130.2, 124.0, 121.4, 117.0 (C20), 111.4, 74.5 (C11), 69.7 (C14), 65.0 (C22), 58.0 (C4), 56.2, 45.4 (C9), 44.5 (C13), 43.9 (C12), 41.7, 38.5, 36.6 (C6), 35.9 (C10), 34.4 (C2), 30.4 (C8), 26.8 (C7), 26.1 (C18), 24.8 (C1), 16.6 (C16), 14.7 (C15), 11.5 (C17) ; IR (KBr, cm^{-1}) 3502, 3284, 2937, 1732, 1666, 1579, 1529, 1485, 1439, 1373, 1308, 1176, 1117, 1018, 980, 914, 816, 756, 660; HR-MS (ESI): Calcd for $C_{36}H_{46}NO_6S$ ($M+H^+$): 620.3040; Found: 620.3037.

22- (2- (3-Methoxybenzamido) phenylsulfanyl) -22-deoxypleuromutilin (12b)

White powder; yield: 45%; m.p.: 99.2-100.7 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.78 (1H, s), 8.49 (1H, d, *J* = 8.3 Hz), 7.69 (2H, m), 7.60 (1H, d, *J* = 7.6 Hz), 7.46 (1H, t, *J* = 8.1 Hz), 7.41 (1H, t, *J* = 7.8 Hz), 7.14 (1H, dd, *J* = 8.1, 2.4 Hz), 7.09 (1H, t, *J* = 7.5 Hz), 6.34 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.63 (1H, d, J = 8.5 Hz, H14), 5.32 (1H, s), 5.15 (1H, d, *J* = 11.0 Hz, H20), 5.09 (1H, d, *J* = 17.4 Hz, H20), 3.93 (3H, s), 3.55 (1H, d, *J* = 16.0 Hz, H22), 3.48 (1H, d, *J* = 16.0 Hz, H22), 3.29 (1H, d, *J* = 6.5 Hz, H11), 2.09 (5H, m,H2, H4, H10, H13), 1.73 (1H, dd, *J* = 14.7, 3.3 Hz, H6), 1.52 (4H, m, H1, H7), 1.34 (3H, s, H15), 1.20 (3H, m, H18, H13, 11-OH), 1.06 (3H, s, H18), 0.97 (1H, d, *J* = 16.0 Hz, H8), 0.85 (3H, d, *J* = 7.0 Hz, H17), 0.49 (3H, d, *J* = 7.1 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 216.8 (C3), 168.9 (C21), 165.0, 160.0, 140.3, 138.6 (C19), 136.4, 136.0, 130.6 , 129.7, 124.6, 122.2, 121.3, 119.3, 118.3, 117.2 (C20), 112.6, 74.5 (C11), 70.3 (C14) , 58.0 (C4) , 55.5 (C22) , 45.4 (C9), 44.6

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(C13), 43.9 (C12), 41.7 (C5), 40.2, 36.5 (C6), 35.9 (C10), 34.4 (C2), 30.3 (C8), 26.8 (C7), 26.1 (C18), 24.8 (C1), 16.4 (C16), 14.7 (C15), 11.5 (C17); IR (KBr, cm^{-1}) 3494, 3344, 2937, 1720, 1655, 1579, 1522, 1489, 1435, 1385, 1306, 1192, 1151, 1115, 1036, 914, 796, 760, 683; HR-MS (ESI): Calcd for $C_{36}H_{46}NO_6S$ ($M+H^+$):620.3040; Found: 620.3062.

22- (2- (4-Methoxybenzamido) phenylsulfanyl) -22-deoxypleuromutilin (12c)

Maple powder; yield: 37%; m.p.: 204.5-205.6 ℃; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.69 (1H, s), 8.48 (1H, d, *J* = 8.3 Hz), 8.09 (2H, d, *J* = 8.5 Hz), 7.59 (1H, d, *J* = 7.8 Hz), 7.38 (1H, t, *J* = 7.9 Hz), 7.05 (3H, m), 6.34 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.62 (1H, d, *J* = 8.6 Hz, H14), 5.15 (1H, d, *J* = 11.0 Hz, H20), 5.10 (1H, d, *J* = 17.4 Hz, H20), 3.91 (3H, s), 3.55 (1H, d, *J* = 15.9 Hz, H22), 3.48 (1H, d, *J* = 15.9 Hz, H22), 3.29 (1H, d, *J* = 6.5 Hz, H11), 2.09 (5H, m,H2, H4, H10, H13), 1.73 (2H, m, H6), 1.51 (4H, m, H1, H7), 1.33 (3H, s, H15), 1.21 (3H, m, H18, H13, 11-OH), 1.07 (3H, s, H18), 0.98 (1H, d, *J* = 16.0 Hz, H8), 0.84 (3H, d, *J* = 6.9 Hz, H17), 0.49 (3H, d, *J* = 7.2 Hz, H16) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 216.8 (C3), 168.9, 164.8 (C21), 162.6, 138.7 (C19), 136.0, 130.6, 129.3, 127.2, 124.3, 122.0, 121.2, 117.2 (C20), 113.9, 74.5 (C11), 70.3 (C14), 58.0 (C4), 55.5 (C22), 45.4 (C9), 44.6 (C13), 43.9 (C12), 41.7 (C5), 40.1, 36.5 (C6), 35.9 (C10), 34.4 (C2), 30.3 (C8), 26.8 (C7), 26.1 (C18), 24.8 (C1), 16.5 (C16), 14.8 (C15), 11.5 (C17); IR (KBr, cm^{-1}) 3477, 3325, 2937, 1726, 1657, 1579, 1531, 1458, 1435, 1387, 1319, 1174, 1149, 1115, 1020, 916, 816, 768, 663; HR-MS (ESI): Calcd for $C_{36}H_{46}NO_6S$ ($M+H^+$): 620.3040; Found: 620.3067.

Minimal inhibitory concentration(MIC) testing

The MIC values of these novel pleuromutilin derivatives, and pleuromutilin were determined on MRSA, *E. coli* and *S. aureus* by broth dilution in accordance with the "*Clinical and*

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Laboratory Standards Institute" (CLSI, 2008). Stock solutions of these compounds were prepared in N,N-Dimethylformamide (DMF) at the concentrations of 5120 μg/mL. The working solutions (256 μg/mL) were obtained by diluting stock solutions in sterile Mueller Hinton broth.

The drug susceptibility testing was performed in 96-well plate. All dates were tested in duplicate in each plate. Medium was inoculated with single colonies and incubated overnight. 100 μL of diluted culture was mixed with 100 μL of drug solutions in a series with 2-fold concentration steps. The tested concentration ranges were 0.015-64 μg/mL. Valnemulin and tiamulin were used as positive controls against bacteria. The final concentration of DMF in the first well column was 1.25%. Initially, preliminary analyses were conducted with 1.25% (v/v) DMF/MHB and this affect neither the growth of the tested bacteria nor the determination of MIC. The MIC value was defined as the lowest concentration of the sample which inhibits the visible growth of test bacteria.

Molecular Modeling

The docking experiments were performed by use of the AutoDockTools and Pymol. The crystal structure of *Deinococcus radiodurans* in complex with tiamulin (PDB ID:1XBP) [13] was used to build the peptidyl transferase center (PTC) mode. The PTC model was constructed containing all residues within a spherical cut of 30 Å around the PTC binding site of 1XBP. The compounds were built with the CORINA online service and optimized by Avogadro 1.1.1 [30], with 5000 steps Steepest Descent and 1000 steps Conjugate Gradients geometry optimization using MMFF94 force field.

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MRSA infection model

All experimental procedures and animal care were in accordance with South China Agricultural University (SCAU) guidelines and were approved by the Animal Ethical and Experimental Committee (AEEC) of SCAU. All mice were acclimatized to the new environment for at least one week before treatment initiation. Institute of Cancer Research mice (the Hunan SLAC Jingda Laboratory Animal Company, Changsha, China), weighing 25±1 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. Intragastric administration of the test compounds at dose of 50 mg/kg were given to the mice 1 h after infection. Tiamulin was used as a control in the same manner at the same doses as **8**. Formulation of the test compounds was dissolved in vehicle (20% DMSO, 5% Tween-80, and 75% normal saline). The survival of the mice at seventh day after infection was selected as the end-point.

Results

Chemistry

As shown in Scheme 1, compound 7 was prepared by the reaction of pleuromutilin **1** with *p*-toluenesulfonyl chloride in pyridine. Compound **8** was then prepared by nucleophilic substitution of 22-*O*-tosylpleuromutilin (compound **7**) with 2-aminothiophenol under basic conditions. Compounds **9a-9c, 10a-10c, 11a-11c** and **12a-12c** were prepared by acylation of compound **8** with the corresponding acyl chloride derivatives.

All compounds were purified using silica gel column chromatography. The structure of all

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synthesized pleuromutilin derivatives were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR and HR-MS.

***In vitro* antibacterial activity**

The minimum inhibitory concentrations (MIC) of these synthesized pleuromutilin derivatives were tested against methicillin-resistant *S. aureus* ATCC 43300 (MRSA), *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 by the broth dilution methods using tiamulin and valnemulin as reference drugs. The results of these studies were summarized in Table 1. The MICs of these synthesized pleuromutilin derivatives against MRSA and *S. aureus* ATCC 29213 ranged from 0.0156~1 μg/mL. It could also be observed that all these derivatives possessed pretty weak antibacterial activities against the gram-negative bacteria *E. coli* ATCC 25922.

Table 1 here

The antibacterial activities of compound **8**, which was an intermediate of other pleuromutilin derivatives, were evaluated at first. To our surprise, compound **8** displayed higher activity against MRSA and ATCC 29213 than all the other pleuromutilin derivatives. The *in vitro* antibacterial activities of compound **8** (MIC = 0.0156μg/mL) were even superior to that of both tiamulin (MIC = 0.5μg/mL) and valnemulin (MIC = 0.0625μg/mL). Compounds **9b**, **10b** and **11a** showed lower potent activity against both *S. aureus* when compared to that of valnemulin while possessed higher activity when compared to that of tiamulin. All other compounds displayed comparable or slightly less antibacterial activities against MRSA and ATCC 29213 to that of tiamulin.

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Molecular Docking Study

Due to their superior *in vitro* antibacterial activity to that of other synthesized pleuromutilin derivatives, compounds **8**, **9b** and **11a** were selected for molecular docking studies. Compounds**8**, **9b** and **11a**possessed multiple binding modes into 1XBP with the binding free energies at -7.28, -6.93 and -6.94 kcal/mol, respectively. As shown in Figure 2, two strong hydrogen bonds were formed through the interaction of **8** with G2044 (O/NH distance: 1.79Å) and C2431 (OH/O distance: 1.99Å). It could also be observed that the amino group of **8** protruding into the area formed by resides of C2046 and U2564 with the distance at 2.55Å and 2.43Å, respectively. This might be one possible reason that why compound **8** possessed higher binding affinity as well as better *in vitro* antibacterial activities than that of **9b** and **11a**.

Figure 2, 3 and 4 here

***In vivo* efficacy of compound 8**

Compound **8** (0.0156 μg/mL) possessed higher *in vitro* antibacterial activity against MRSA 43300 than tiamulin (0.5 μg/mL) in our experiment. The *in vivo* efficacy of compound **8** was evaluated using a systemic MRSA infection mice model. Mice which were intragastric administrated with vehicle alone showed 100% mortality in this MRSA infection model. As shown in Figure 5, both tiamulin and compound **8** possessed *in vivo* antibacterial efficacy and led to the survival of the MRSA infection mice. Treatment with tiamulin and compound **8** displayed protection with 30% and 50% survival of the MRSA infection mice in this model, respectively. Thus, Compound **8** showed higher antibacterial activity than that of tiamulin against MRSA-43300 in the mouse infection model. The result of *in vivo* efficacy

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demonstrated that compound **8** might act as a potent antibacterial drug candidate against MRSA.

Figure 5 here

Discussion

As shown in Table 1, compounds **9b** and **10b**, bearing an electron withdrawing substituent in the benzene ring at the *meta*- position to the side chain, presented improved activity against MRSA and ATCC 29213 compared with the compounds **9a**, **9c**, **10a** and **10c**. The substitution position on the benzene ring of the phenyl amide side chain appeared to have influence on the antibacterial activity of these pleuromutilin derivatives. Electron donating substituents were then introduced to investigate the optimal substituents of this series of pleuromutilin derivatives. Compound **11a** possessed superior antibacterial activity to that of tiamulin. Compounds **11b**, **11c** and **12a-12c** displayed lower or comparable antibacterial activities to that of tiamulin.

Generally, all of the benzoylamino substituted pleuromutilin derivatives bearing phenylsulfanyl linker in Table 1 showed less *in vitro* antibacterial activity against MRSA and ATCC 29213 when compared to that of valnemulin. Some of them possessed slightly superior activities to that of tiamulin. Compound **8** possessed the highest antibacterial activity against MRSA and ATCC 29213 than all the other synthesized pleuromutilin derivatives. It displayed even better *in vitro* antibacterial activity than both tiamulin and valnemulin against MRSA and ATCC 29213. The results of MIC values from Table 1 indicated that the introduction of both electron withdrawing and electron donating groups in benzene ring of the phenyl amide could decrease antibacterial activity.

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Molecular docking studies revealed that compound **9b** formed just one hydrogen bond between its oxygen atom of OH (8-membered ring) with C2589 (O/NH distance: 1.91 Å). The molecular docking results of compound **11a** were shown in Figure 4. Two strong hydrogen bonds were observed through the interaction of its amide with G2484 (NH/O distance: 1.97 Å) and its oxygen atom of OH (8-membered ring) with C2565 (O/NH distance: 2.12 Å). Although adopted one hydrogen bond more than **9b**, compound **11a** displayed similar binding free energy as **9b** (-6.94 kcal/mol vs -6.93 kcal/mol). The similar binding affinity of **11a** compared to **9b** might be explained by the interaction between **9b** and A2482 with the distance at 2.48 Å. Additionally, both **11a** and **9a** displayed lower antibacterial activity when compared to **8**, perhaps because of the longer distance of the hydrogen bonds between both compounds and the residues around them (Figure 2, 3 and 4).

Conclusion

In conclusion, a series of novel pleuromutilin derivatives bearing 2-aminothiophenol moieties have been reported. These synthesized derivatives were evaluated for their *in vitro* antibacterial activities against MRSA, *S. aureus* and *E. coli*. The obtained MIC values revealed that all the synthesized derivatives possessed moderate antibacterial activity. Compound **8** was the most active pleuromutilin derivative, it possessed superior antibacterial activity to that of both tiamulin and valnemulin against MRSA. Docking studies were conducted on compounds **8**, **9b** and **11a**. The docking results showed that the binding free energies were -7.28, -6.93 and -6.94 kcal/mol, respectively. This demonstrates that there is a rational correlation between the binding free energy and the antibacterial activity of compounds **8**, **9b** and **11a**. Compound **8** exhibited higher in vivo antibacterial activity than

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that of tiamulin against MRSA at th dose of 50 mg/kg in the mouse infection model. The results indicated that compound **8** may serve as possible lead compound for the development of new potent antibacterial drugs based on pleuromutilin. A more comprehensive study of compound **8** will be reported in our future research.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 31672602 and 21405051) and the National Key Research and Development Program of China (No. 2016YFD0501300).

Conflict of interest

The authors declare that they have no conflict of interest.

Figure Captions

Scheme 1 Reagent and conditions: (i) *p*-toluenesulfonyl chloride, pyridine, 0℃, 3h; (ii) 2-aminothiophenol, 20% aqueous NaOH (10ml), chloroforme, reflux; (iii) acyl chloride derivatives, ethyl acetate, reflux.

Figure 1 Structure of pleuromutilin (1), tiamulin (2), valnemulin (3), retapamulin (4) BC-3004 (5) and BC-3080 (6).

Figure 2 Result of the docking of compound **8** into the PTC model binding site. Residue numbers shown are according to *E. coli* 23s RNA numbering.

Figure 3 Result of the docking of **9b** into the PTC model binding site. Residue numbers shown are according to *E. coli* 23s RNA numbering.

Figure 4 Result of the docking of **11a** into the PTC model binding site. Residue numbers

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shown are according to *E. coli* 23s RNA numbering.

Figure 5 Efficacy of compound **8** and tiamulin in mouse systemic infection model.

Table 1 MIC (μg/mL) for MRSA (ATCC 43300), *Staphylococcus aureus* (*S. aureus*) ATCC 29213 and *E. coli* ATCC 25922.

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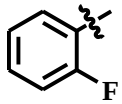
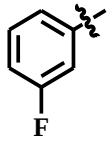
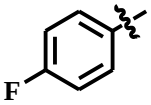
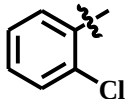
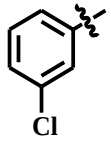
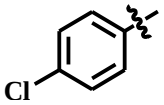
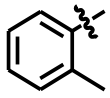
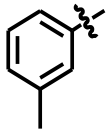
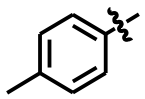
Compounds	R	MRSA ATCC 43300	*S.aureus* ATCC 29213	*E. coli* ATCC 25922
9a		0.5	0.5	>64
9b		0.125	0.125	>64
9c		1	1	>64
10a		0.5	0.5	>64
10b		0.25	0.25	>64
10c		0.5	0.5	>64
11a		0.25	0.25	>64
11b		0.5	1	>64
11c		0.5	0.5	>64

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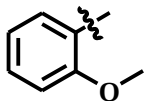
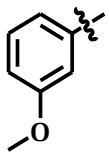
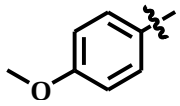
12a		0.5	1	>64
12b		1	1	>64
12c		1	1	>64
8		0.0156	0.0156	>64
pleuromutilin		0.5	1	>64
Tiamulin		0.5	0.5	>64
Valnemulin		0.0625	0.0625	32

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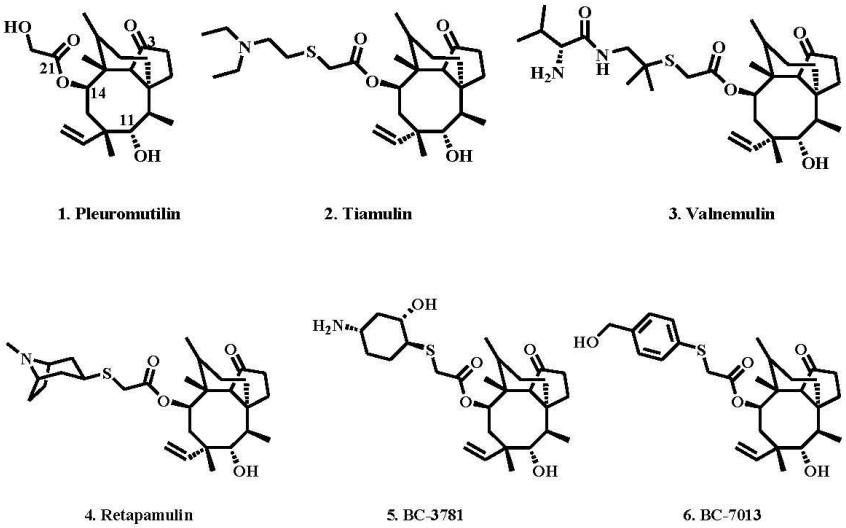

1. Pleuromutilin 2. Tiamulin 3. Valnemulin

4. Retapamulin 5. BC-3781 6. BC-7013

Figure 1

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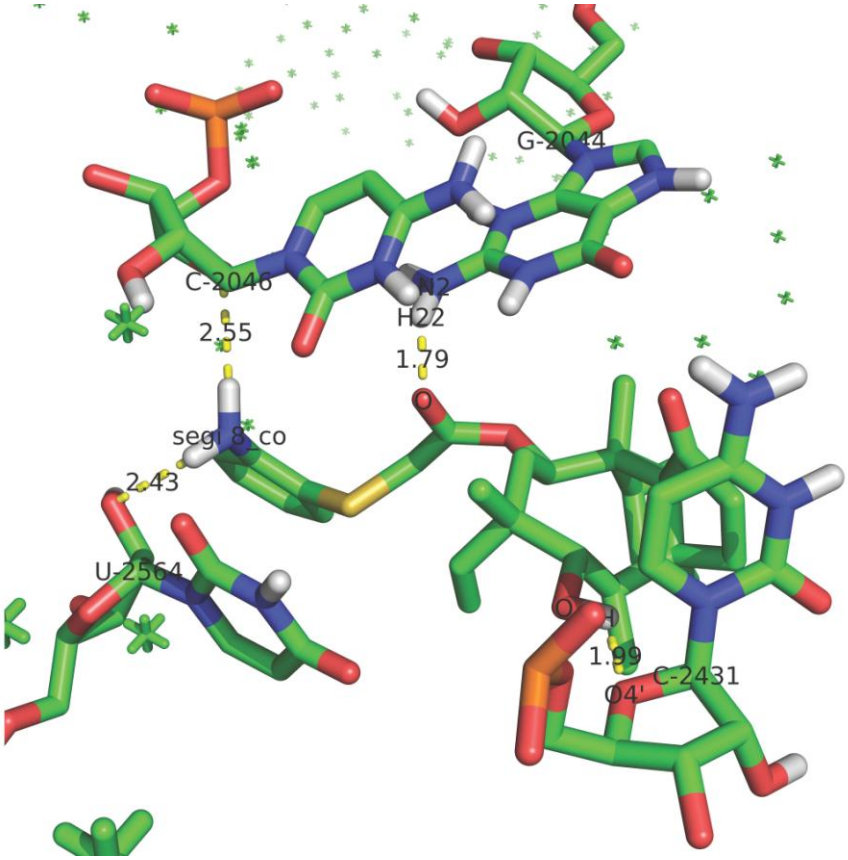

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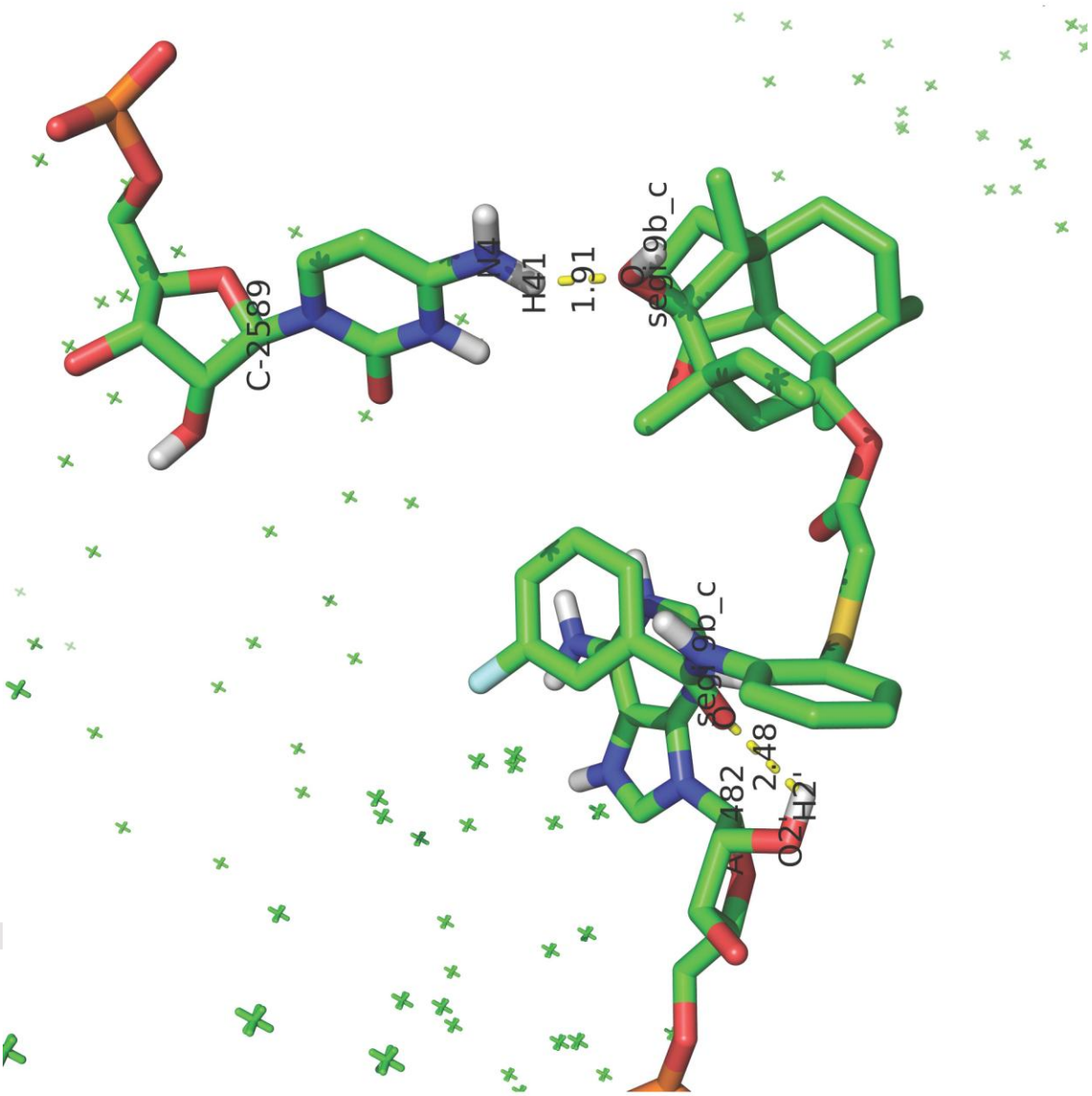

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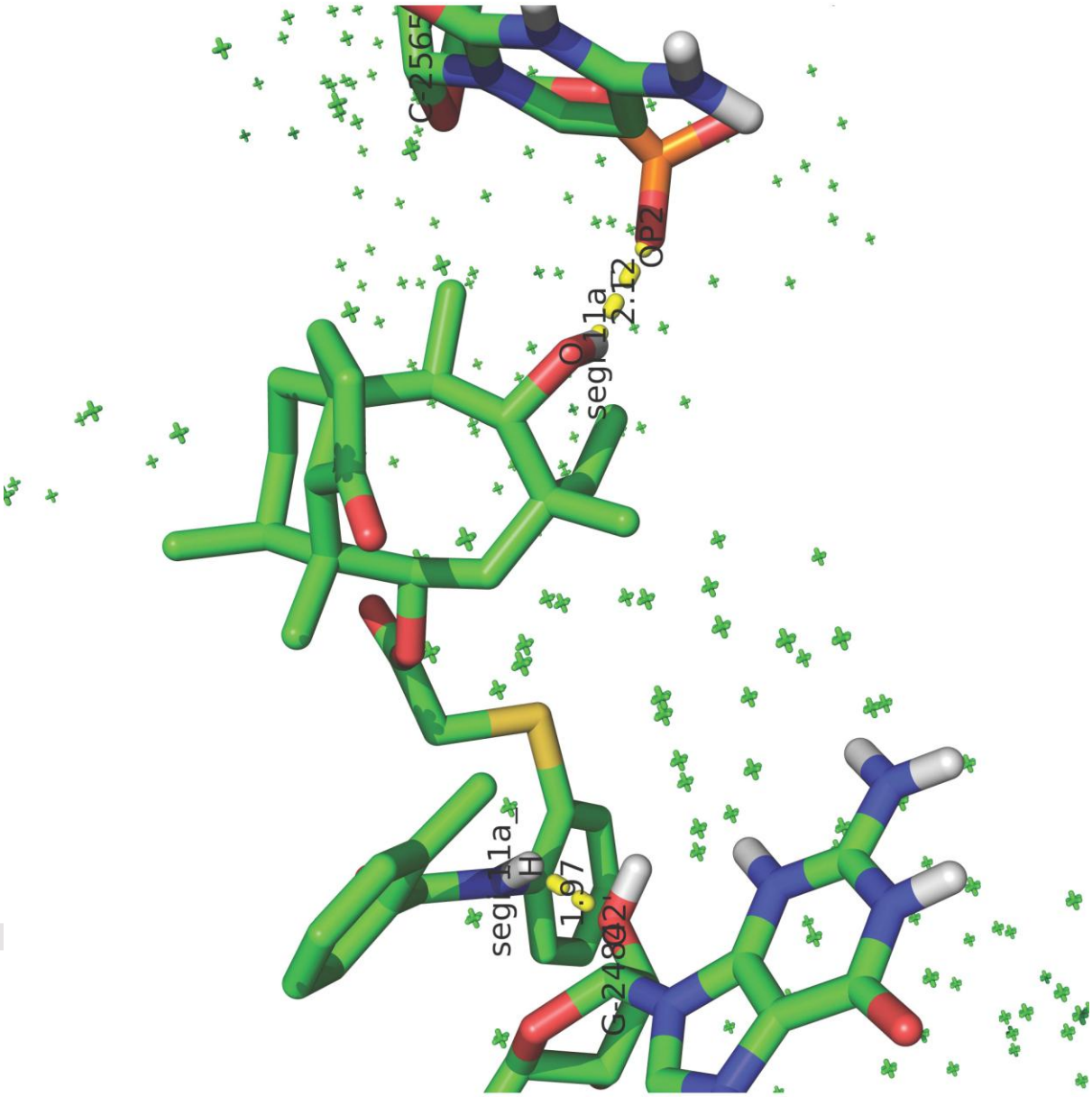

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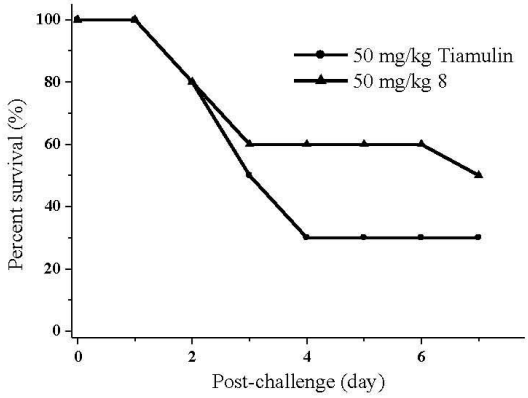

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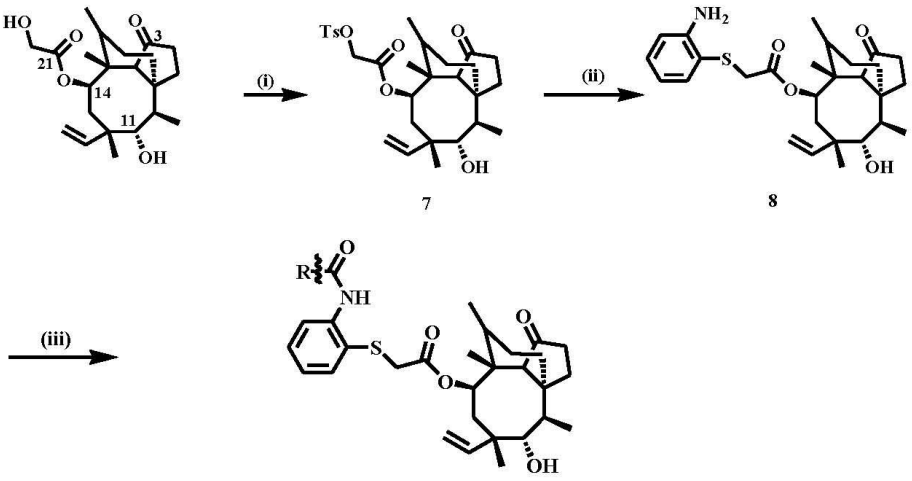

Scheme 1

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