Study on the structure-activity relations of pleuromutilin derivatives with an aromatic amide and a thioether group in the C_{14} side chain

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Nine novel pleuromutilin derivatives having benzamide substituents have been synthesised permitting structure-activity relations of pleuromutilin derivatives with aromatic amide and thioether groups in the C_{14} side chain to be studied. The results showed that the heterocyclic carboxamide group was necessary to enhance bio-activities.

Keywords: benzamide, pleuromutilin, structure–activity, antibacterial activities

The antibiotic Pleuromutilin **1** was first isolated in 1951[1] with moderate bioactivity against drug-resistant Gram-positive bacteria and mycoplasmas *in vitro*.[2,3] Earlier research reported[4–6] that modifications of the side chain at C_{14} including both a thioether group and a basic group offered the most possibilities for achieving the best bioactivities. On this basis, Tiamulin **2**[6,7] was synthesised with excellent bioactivity against Gram-positive bacteria and mycoplasmas. Further researches led to the development of Valnemulin **3**[8] with much better bio-activity than Tiamulin, which was the best pleuromutilin antibiotic for use in animals.[9] In the 1980s, Azamulin **4**[10] was discovered with good bio-activity, but it has limited solubility in water.[11] Later, Retapamulin **5**[12] with excellent bioactivity was produced as the first pleuromutilin antibiotic for potential use in humans.[13,14] Recently, **6** and related compounds having excellent bioactivity and good solubility in water were synthesised (Fig. 1).[11,15,16]

Some potent antibacterial derivatives with heterocyclic carboxamide groups in the C_{14} side chain were designed and synthesised in our previous work[17]. In this series, **7** and related compounds displayed excellent bioactivities against Gram-positive bacteria *in vitro*. This is a very interesting result. So we focused our attention on a functional group, the heterocyclic carboxamide group. In order to find out whether the heterocyclic carboxamide group is necessary to enhance bio-activities, nine novel pleuromutilin derivatives **11a–f** and **12c–e** with benzamide substituents in place of the heterocyclic carboxamide have been synthesised and their structure-activity relations were studied. The synthetic route is shown in Scheme 1.

Experimental

Melting points were determined with XRC-1 melting point apparatus (Sichuan University Instrument Inc., Chengdu, China) without being corrected. ^{1}H NMR spectra were run on a Varian INOVA-400 spectrometer (Varian Inc., Palo Alto, CA, USA) with $CDCl_3$ as the solvent and TMS as the internal standard. Mass spectra were recorded with Agilent 6210 (DOF-MAS) spectrometer (Agilent Inc., Santa Clara, CA, USA) using the EI method. IR spectra were recorded with Perkin-Elmer 16PC-FT instrument (Perkin-Elmer Inc., Norwalk Conn, CA, USA). Elemental analyses were carried out by the Euro EA 3000 instrument (Euro Vector S.P.A., Italy). X-ray diffraction were recorded with Philips X-ray Diffraction apparatus (X'Pert Pro MPD Dy1291).

8: *p*-Methylbenzenesulfonyl chloride (1.91 g, 0.010 mol) in 1,2-dichloroethane (10 mL) was slowly added to a mixture of pleuromutilin (3.54 g, 0.0094 mol), triethylamine (1.20 g, 0.012 mol) and pyridine (three drops) in 1,2-dichloroethane (10 mL). The mixture was stirred at 10–15 °C for 20h, washed with water (3 × 10 mL), then concentrated almost to dryness. Purification was achieved by

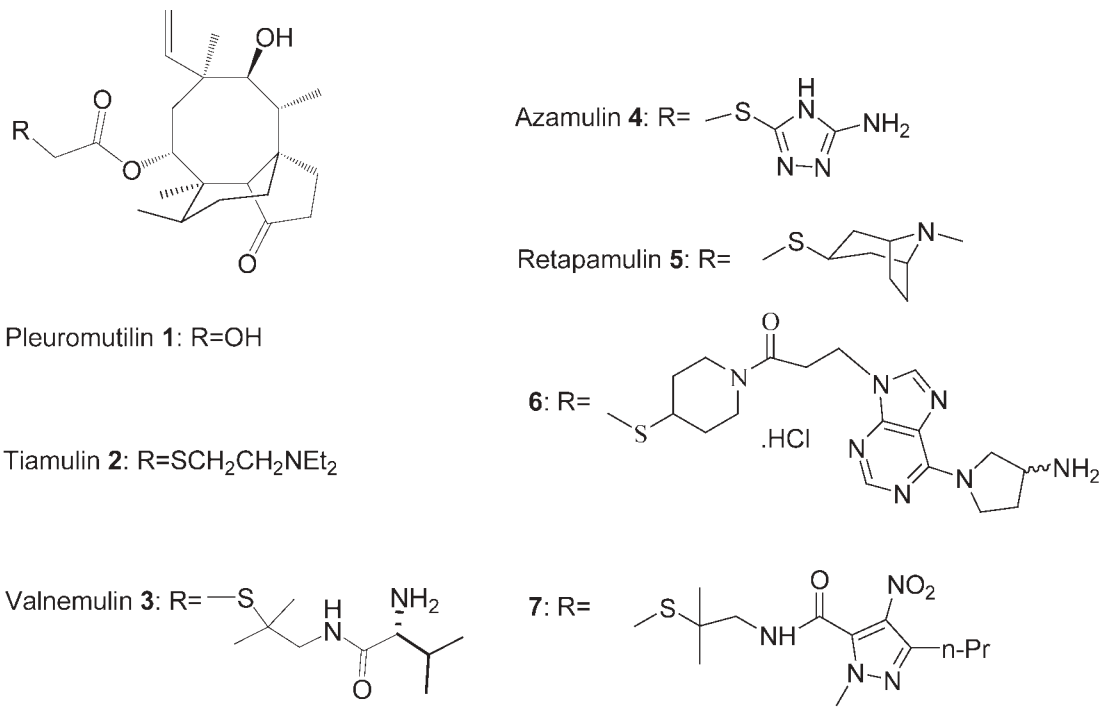

Fig. 1 Structure of pleuromutilin derivatives.

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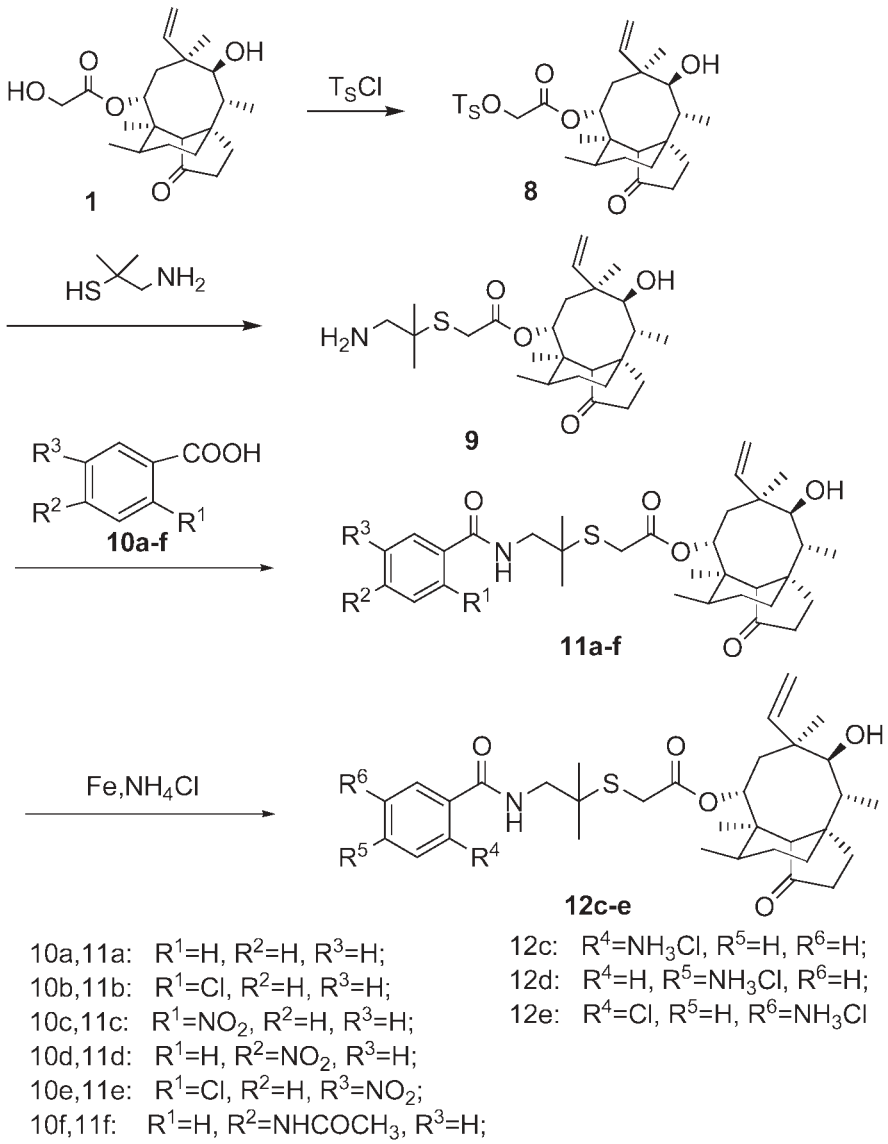

10a,11a: R^1=H, R^2=H, R^3=H;
10b,11b: R^1=Cl, R^2=H, R^3=H;
10c,11c: R^1=NO_2, R^2=H, R^3=H;
10d,11d: R^1=H, R^2=NO_2, R^3=H;
10e,11e: R^1=Cl, R^2=H, R^3=NO_2;
10f,11f: R^1=H, R^2=$NHCOCH_3$, R^3=H;

12c: R^4=NH_3Cl, R^5=H, R^6=H;
12d: R^4=H, R^5=NH_3Cl, R^6=H;
12e: R^4=Cl, R^5=H, R^6=NH_3Cl

Scheme 1 The synthetic route of compounds **11a–f** and **12c–e**.

recrystallisation from 85% ethyl alcohol to afford a white solid 3.90g (yield 78%).

9: Sodium (1.71 g, 0.074 mol) was added portionwise to absolute ethanol (150 mL). The mixture was stirred at room temperature until the sodium was dissolved completely. 1-Amino-2-methyl-2-propanethiol hydrochloride (5.00 g, 0.035 mol) and compound **8** (17.10 g, 0.032 mol) were added to the mixture. The mixture was stirred for 1h at room temperature, poured into ice water (200 mL) and extracted with ethyl acetate (3 × 100 mL). The ethyl acetate extract was washed with water, extracted with dilute hydrochloric acid (12 × 20 mL, 0.0001 mol L^{-1}). The aqueous layer was made alkaline and extracted with ethyl acetate which had only one product as checked by TLC. Then the combined organic phases were washed with water (3 × 10 mL), dried over sodium sulfate and evaporated in vacuum to afford a white solid 13.39 g (yield 90%).

10e: Synthesised by a literature[18] procedure.

10f: 4-Aminobenzoic acid (0.28 g, 0.002 mol) was added to acetic anhydride. The mixture was heated to 130 °C and kept at this temperature for 3h, cooled to room temperature and neutralised with 30% sodium hydroxide to pH 7. The mixture was filtered, and the subsequent wet cake was washed with water and dried to afford a white solid 0.28g (yield 78%).

11a: Benzoic acid (0.12g, 1.0 mmol) in thionyl chloride (2.5 mL) was refluxed for 3h. The excess thionyl chloride was evaporated *in vacuo* and the residue was cooled to 0 °C. Dichloromethane (8 mL), triethylamine (0.40 g, 4.0 mmol) and 14-O-[(1-amino-2-methylpropane-2-yl)thioacetyl-]mutilin (0.42 g, 0.9 mmol) were added in turn. The mixture was stirred at room temperature for 2h, concentrated and then ethyl acetate (20 mL) was added. The organic phase was washed with water (3 × 10 mL) and then 2N HCl (3 × 10 mL), dried with sodium sulfate and evaporated *in vacuo* to give the crude product. The crude product was chromatographed on silica gel using petroleum ether/ethyl acetate (1:1) as eluent to afford a white solid 0.26 g (yield 50%).

Preparation of **11b–e**; *general procedure*

10b–e (1.5 mmol) in thionyl chloride (3 mL) was refluxed for 3h. The excess thionyl chloride was evaporated *in vacuo* and the residue was cooled to 0 °C. Dichloromethane (10 mL), triethylamine (3.0 mmol) and 14-O-[(1-amino-2-methylpropane-2-yl)thioacetyl-]mutilin (1.2 mmol) in dichloromethane (5 mL) were added in turn. The mixture was stirred at room temperature for 1–2h, evaporated and ethyl acetate (20 mL) was added. The organic phase was washed with water (3 × 10 mL), 2N HCl (3 × 10 mL) and saturated brine (10 mL), dried with sodium sulfate and evaporated *in vacuo* to afford solid (yield 80–95%).

11f: 10f (0.27 g, 1.5 mmol) in thionyl chloride (5 mL) was heated at 45 °C for 1h. The excess thionyl chloride was evaporated *in vacuo* and the residue was cooled to 0 °C. Dichloromethane (10 mL), triethylamine (0.61 g, 6.0 mmol) and 14-O-[(1-amino-2-methyl-propane-2-yl)thioacetyl-]mutilin (0.46 g, 1.0 mmol) in dichloromethane (5 mL) were added in turn. The mixture was stirred at room temperature for 2.5h, concentrated and ethyl acetate (20 mL) was added. The organic phase was washed with water (3 × 10 mL), 2N HCl (3 × 10 mL) and

saturated salt water (10 mL), dried with sodium sulfate and evaporated *in vacuo* to give the crude product. The crude product was chromatographed on silica gel using petroleum ether/ethyl acetate (2:1) as eluent to afford a yellow solid 0.36 g (yield 58%).

Preparation of **12c-e***; general procedure*

A mixture of compounds **11c–e** (0.67 mmol), iron (1.96 mmol) and ammonium chloride (5.89 mmol) in 80% ethanol (7.5 mL) was heated at 45 °C for 2h, filtered and the cake was washed with ethanol. The filtrate was extracted with ethyl acetate (3 × 15 mL) and the extract was washed with water (3 × 10 mL) and 2N HCl (3×5 mL). Then the ethyl acetate layer was extracted with 6N HCl (6 × 4 mL), and the water layer was evaporated *in vacuo* to afford a solid (yield 51–58%).

Mutilin 14-O-[1-benzamide-2-methylpropane-2-yl]thioacetate (**11a**): White solid (50%); melting point: 62–63 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 7.92 (1H, d, *J* = 7.6 Hz), 7.46–7.55 (4H, m), 6.42 (1H, dd, J_1 = 14.0 Hz, J_2 = 10.4 Hz), 5.74 (1H, d, *J* = 7.6 Hz), 5.13 (1H, d, *J* = 13.6 Hz), 5.12 (1H, d, *J* = 10.4 Hz), 3.29–3.51 (2H, m), 3.34 (1H, d, *J* = 6.4 Hz), 3.27 (1H, d, *J* = 16.8 Hz), 3.19 (1H, d, *J* = 16.4 Hz), 2.28–2.32 (1H, m), 2.24–2.26 (1H, m), 2.16–2.22 (1H, m), 2.10 (1H, s), 2.05–2.12 (1H, m), 1.74–1.80 (1H, m), 1.57–1.71 (3H, m), 1.46 (3H, s), 1.42–1.53 (2H, m), 1.33–1.34 (1H, m), 1.34 (3H, s), 1.31 (3H, s), 1.14 (3H, s), 1.09–1.17 (1H, m), 0.87 (3H, d, *J* = 6.8 Hz), 0.73 (3H, d, *J* = 7.2 Hz); IR (KBr, cm^{-1}) 3414, 3080, 2928, 2860, 1728, 1652, 1600, 1537, 1458, 1381, 1286, 1115, 805, 713, 619;. Calcd for $C_{33}H_{47}NO_5S$ (%): C, 69.56; H, 8.31; N, 2.46; S, 5.63. Found (%):C, 69.54; H, 8.25; N, 2.50; S, 5.60%; X-ray: amorphism; HR-MS (ESI): Calcd for $C_{33}H_{47}NO_5S$ ($M\text{-}H^+$):568.3102, Found: 568.3110.

Mutilin 14-O-[1-(2-chloro)benzamide-2-methylpropane-2-yl]thioacetate (**11b**): White solid (81%); melting point: 56–58 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 7.67 (1H, d, *J* = 7.2 Hz), 7.32–7.44 (3H, m), 7.02–7.08 (1H, m), 6.40 (1H, dd, J_1 = 17.2 Hz, J_2 = 10.8 Hz), 5.70 (1H, d, *J* = 8.8 Hz), 5.18 (1H, d, *J* = 10.8 Hz), 5.12 (1H, d, *J* = 17.6 Hz), 3.51 (1H, dd, J_1 = 14.0 Hz, J_2 = 6.0 Hz), 3.42 (1H, dd, J_1 = 14.0 Hz, J_2 = 6.0 Hz), 3.33 (1H, d, *J* = 6.0 Hz), 3.23 (1H, d, *J* = 16.0 Hz), 3.17 (1H, d, *J* = 15.6 Hz), 2.28–2.32 (1H, m), 2.21–2.26 (1H, m), 2.16–2.21 (1H, m), 2.07 (1H, s), 2.01–2.05 (1H, m), 1.74–1.78 (1H, m), 1.50–1.70 (3H, m), 1.44 (3H, s), 1.40–1.50 (2H, m), 1.28–1.33 (1H, m), 1.37 (3H, s), 1.36 (3H, s), 1.13 (3H, s), 1.08–1.16 (1H, m), 0.87 (3H, d, *J* = 6.8 Hz), 0.73 (3H, d, *J* = 7.2 Hz); IR (KBr, cm^{-1}) 3424, 3080, 2927, 2855, 1732, 1652, 1515, 1456, 1379, 1281, 1115, 751, 619, 438; Calcd for $C_{33}H_{46}ClNO_5S$ (%): C, 65.70; H, 7.52; N, 2.32; S, 5.32. Found: C, 65.68; H, 7.66; N, 2.44; S, 5.01%; X-ray: amorphism; HR-MS (ESI): Calcd for $C_{33}H_{46}ClNO_5S$ ($M\text{-}H^+$): 602.2712; Found 602.2705.

Mutilin 14-O-[1-(2-nitro)benzamide-2-methylpropane-2-yl]thioacetate (**11c**): Buff solid (95%); melting point: 68–69 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 8.05 (1H, d, *J* = 7.6 Hz), 7.58–7.71 (3H, m), 7.19 (1H, t, *J* = 5.2 Hz), 6.36 (1H, dd, J_1 = 17.6 Hz, J_2 = 11.2 Hz), 5.67 (1H, d, *J* = 8.8 Hz), 5.14 (1H, d, *J* = 10.4 Hz), 5.13 (1H, d, *J* = 17.2 Hz), 3.33–3.48 (2H, m), 3.34 (1H, d, *J* = 6.4 Hz), 3.25 (1H, d, *J* = 16.4 Hz), 3.17 (1H, d, *J* = 16.4 Hz), 2.28–2.31 (1H, m), 2.21–2.26 (1H, m), 2.16–2.21 (1H, m), 2.08 (1H, s), 2.04–2.10 (1H, m), 1.75–1.78 (1H, m), 1.53–1.70 (3H, m), 1.43–1.51 (2H, m), 1.43 (3H, s), 1.29–1.31 (1H, m), 1.38 (3H, s), 1.37 (3H, s), 1.14 (3H, s), 1.08–1.17 (1H, m), 0.89 (3H, d, *J* = 6.4 Hz), 0.69 (3H, d, *J* = 7.2 Hz); IR (KBr, cm^{-1}) 3430, 3080, 2927, 2860, 1729, 1658, 1533, 1458, 1351, 1282, 1115, 699, 619, 438; Calcd for $C_{33}H_{46}N_2O_7S$ (%): C, 64.47; H, 7.54; N, 4.56; S, 5.22. Found: C, 64.26; H, 7.50; N, 4.57; S, 5.22%; X-ray: amorphism; HR-MS (ESI): Calcd for $C_{33}H_{46}N_2O_7S$ ($M\text{-}H^+$): 613.2943; Found 613.2940.

Mutilin 14-O-[1-(4-nitro)benzamide-2-methylpropane-2-yl]thioacetate (**11d**): Buff solid (80%); melting point:70–72 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 8.33 (2H, d, *J* = 8.4 Hz), 8.12 (2H, d, *J* = 8.8 Hz), 7.85 (1H, d, *J* = 5.6 Hz), 6.39 (1H, dd, J_1 = 17.2 Hz, J_2 = 10.8 Hz), 5.73 (1H, d, *J* = 8.4 Hz), 5.13 (1H, d, *J* = 17.2 Hz), 5.05 (1H, d, *J* = 10.8 Hz), 3.52 (1H, dd, J_1 = 14.0 Hz, J_2 = 6.4 Hz), 3.35 (1H, d, *J* = 8.8 Hz), 3.31 (1H, d, *J* = 16.8 Hz), 3.22–3.28 (1H, m), 3.20 (1H, d, *J* = 17.6 Hz), 2.08–2.31 (4H, m), 2.10 (1H, s), 1.76–1.80 (1H, m), 1.61–1.72 (2H, m), 1.50–1.54 (1H, m), 1.43–1.51 (2H, m), 1.47 (3H, s), 1.36–1.41 (1H, m), 1.34 (3H, s), 1.31 (3H, s), 1.16 (3H, s), 1.11–1.18 (1H, m), 0.88 (3H, d, *J* = 6.8 Hz), 0.72 (3H, d, *J* = 7.2 Hz); IR (KBr, cm^{-1}) 3430, 2925, 1725, 1644, 1610, 1528, 1456, 1420, 1400, 1348, 1288, 1115, 718, 619; Calcd for $C_{33}H_{46}N_2O_7S$ (%): C, 64.47; H, 7.54; N, 4.56; S, 5.22. Found: C, 64.30; H, 7.52; N, 4.59; S, 5.21%; X-ray: amorphism; HR-MS (ESI): Calcd for $C_{33}H_{46}N_2O_7S$ ($M\text{-}H^+$): 613.2943; Found 613.2955.

Mutilin 14-O-[1-(5-nitro-2-chloro)benzamide-2-methylpropane-2-yl]thioacetate (**11e**): Buff solid (93%); melting point: 66–68 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 8.54 (1H, d, *J* = 2.8 Hz), 8.24 (2H, dd, J_1 = 8.8 Hz, J_2 = 2.8 Hz), 7.63 (1H, d, *J* = 8.8 Hz), 7.46(1H, t, *J* = 5.6 Hz), 6.37 (1H, dd, J_1 = 18.0 Hz, J_2 = 10.8 Hz), 5.72 (1H, d, *J* = 8.4 Hz), 5.13 (1H, d, *J* = 12.8 Hz), 5.12 (1H, d, *J* = 16.0 Hz), 3.50 (1H, dd, J_1 = 14.4 Hz, J_2 = 6.4 Hz), 3.39 (1H, dd, J_1 = 14.0 Hz, J_2 = 6.0 Hz), 3.34 (1H, d, *J* = 6.4 Hz), 3.25 (1H, d, *J* = 16.8 Hz), 3.17 (1H, d, *J* = 16.8 Hz), 2.27–2.31 (1H, m), 2.24–2.26 (1H, m), 2.14–2.23 (1H, m), 2.08 (1H, s), 2.05–2.12 (1H, m), 1.75–1.79 (1H, m),1.49–1.68 (3 H, m), 1.45–1.49 (2H, m), 1.44 (3H, s), 1.37 (3H, s), 1.36 (3H, s), 1.15 (3H, s), 1.08-1.17 (1H, m), 0.89 (3H, d, *J* = 7.2 Hz), 0.69 (3H, d, *J* = 7.2 Hz); IR (KBr, cm^{-1}) 3437, 3090, 2928, 1730, 1662, 1611, 1528, 1460, 1349, 1282, 1116, 909, 741, 619, 523; Calcd for $C_{33}H_{45}Cl\ N_2O_7S$ (%): C, 61.05; H, 6.99; N, 4.31; S, 4.94. Found: C, 61.00; H, 6.80; N, 4.35; S, 4.85%; X-ray: amorphism; HR-MS (ESI): Calcd for $C_{33}H_{45}Cl\ N_2O_7S$ ($M\text{-}H^+$): 647.2563; Found 647.2567.

Mutilin 14-O-[1-(4-acetamino)benzamide-2-methylpropane-2-yl] thioacetate (**11f**): Yellow solid (58%); melting point: 108–110 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 7.91 (2H, d, *J* = 7.6 Hz), 7.63 (2H, d, *J* = 7.2 Hz), 7.46 (1H, m), 7.34 (1H, s), 6.42 (1H, dd, J_1 = 18.0 Hz, J_2 = 10.8 Hz), 5.75 (1H, d, *J* = 8.4 Hz), 5.14 (1H, d, *J* = 12.8 Hz), 5.12 (1H, d, *J* = 16.0 Hz), 3.51 (1H, dd, J_1 = 14.0 Hz, J_2 = 6.4 Hz), 3.34 (1H, d, *J* = 6.8 Hz), 3.20–3.25 (1H, m), 3.16–3.29 (2H, m), 2.29–2.33 (1H, m), 2.06–2.28 (3H, m), 2.22 (3H, s), 2.09 (1H, s), 1.77–1.80 (1H, m), 1.61–1.69 (2H, m), 1.51–1.58 (1H, m), 1.46 (3H, s), 1.43–1.48 (2H, m), 1.37–1.41 (1H, m), 1.33 (3H, s), 1.29 (3H, s), 1.15 (3H, s), 1.11–1.18 (1H, m), 0.88 (3H, d, *J* = 6.8 Hz), 0.73 (3H, d, *J* = 6.8 Hz); IR (KBr, cm^{-1}) 3395, 3107, 2931, 2855, 1728, 1682, 1645, 1600, 1530, 1505, 1460, 1406, 1372, 1290, 1186, 1117, 1018, 981, 916, 854, 766; Calcd for $C_{35}H_{50}\ N_2O_6S$ (%): C, 67.06; H, 8.04; N, 4.47; S, 5.12. Found: C, 69.95; H, 8.01; N, 4.50; S, 5.08%; X-ray: amorphism; HR-MS (ESI): Calcd for $C_{35}H_{50}\ N_2O_6S$ ($M\text{-}H^+$): 626.3390; Found 626.3383.

Mutilin 14-O-[1-(2-amino)benzamide-2-methylpropane-2-yl]thioacetate hydrochlorate (**12c**): White solid (51%); melting point: 120–123 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 8.35 (1H, s), 7.90–8.14 (2H, m), 7.61–7.79 (1H, m), 7.42–7.59 (1H, m), 6.42 (1H, s), 5.74 (1H, s), 5.06–5.30 (2H, m), 3.15–3.48 (5H, m), 2.10–2.35 (5H, m), 1.60–1.84 (4H, m), 1.38–1.58 (3H, m), 1.47 (3H, s), 1.33 (6H, s), 1.17 (3H, s), 1.07–1.22 (1H, m), 0.88 (3H, s), 0.74 (3H, s); IR (KBr, cm^{-1}) 3425, 3090, 2929, 2860, 1725, 1645, 1541, 1460, 1383, 1286, 1118, 1022, 748, 619, 442; Calcd for $C_{33}H_{49}ClN_2O_5S$ (%): C, 63.80; H, 7.95; N, 4.51; S, 5.16. Found: C, 63.78; H, 7.90; N, 4.62; S, 5.10%; X-ray: amorphism; HR-MS (ESI): Calcd for $C_{33}H_{49}ClN_2O_5S$ ($M\text{-}Cl^-$): 585.3357; Found 585.3356.

Mutilin 14-O-[1-(4-amino)benzamide-2-methylpropane-2-yl]thioacetate hydrochlorate (**12d**): White solid (50%); melting point: 126–128 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 7.55–8.06 (5H, m), 6.39 (1H, s), 5.39 (1H, s), 5.05 (2H, s), 3.16–3.54 (5H, m), 2.05–2.38 (5H, m), 1.58–1.80 (4H, m),1.35–1.50 (3H, m), 1.42 (3H, s), 1.32 (6H, s), 1.00–1.20 (4H, m), 0.86 (3H, s), 0.70 (3H, s); IR (KBr, cm^{-1}) 3410, 3090, 2930, 2860, 2596, 1724, 1644, 1620, 1545, 1503, 1460, 1383, 1288, 1118, 1018, 982, 848, 758, 618; Calcd for $C_{33}H_{49}ClN_2O_5S$ (%): C, 63.80; H, 7.95; N, 4.51; S, 5.16. Found: C, 63.75; H, 7.88; N, 4.58; S, 5.05%; X-ray: amorphism; HR-MS (ESI): Calcd for $C_{33}H_{49}ClN_2O_5S$ ($M\text{-}Cl^-$): 585.3357; Found 585.3355.

Mutilin 14-O-[1-(4-amino-2chloro)benzamide-2-methylpropane-2-yl]thioacetate hydrochlorate (**12e**): White solid (55%); melting point: 126–128 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 7.20–8.15 (4H, m), 6.37 (1H, s), 5.70 (1H, s), 5.10–5.18 (2H, m), 3.16–3.80 (5H, m), 2.04–2.30 (5H, m), 1.58–1.80 (4H, m), 1.30–1.50 (3H, m), 1.42 (3H, s), 1.25 (6H, s), 1.20–1.50 (4H, m), 0.82–0.92 (3H, m), 0.64–0.78 (3H, m); IR (KBr, cm^{-1}) 3419, 3085, 2929, 2855, 2597, 1727, 1648, 1532, 1467, 1383, 1282, 1119, 822, 619; Calcd for $C_{33}H_{48}Cl_2N_2O_5S$ (%): C, 60.45; H, 7.38; N, 4.27; S, 4.89. Found: C, 60.43; H, 7.30; N, 4.35; S, 4.80%; X-ray: amorphism; HR-MS (ESI): Calcd for $C_{33}H_{48}Cl_2N_2O_5S$ ($M\text{-}Cl^-$): 619.2967; Found 619.2960.

Table 1 The antibacterial activities of 0.1 g L^{-1} target compounds against three bacterial strains

Compound	Diameter of bacteriostatic circle/mm		
	E. coli	*S. lignieres*	*S. aureus*
11a	16.3	13.4	15.4
11b	15.4	12.6	16.9
11c	17.2	14.2	16.8
11d	15.4	14.1	15.7
11e	15.3	13.8	15.8
11f	17.1	14.2	18.2
12c	16.0	14.7	15.2
12d	16.2	14.4	17.6
12e	15.9	14.1	15.4
Pleuromutilin	18.9	13.6	21.9
Blank	14.9	13.3	17.4

Biological assay

The antibacterial activities of the target compounds were tested according to the method of bacteriostatic circles *in vitro*. Target compound (2.5mg) was dissolved in 0.3% Tween-water (25 mL), the resulting solution was added to the flat plate of an Oxford cup which was covered with culture and kept at 37 °C for 24h. The results of average diameters of the bacteriostatic circle are listed in Table 1.

Discussion

The bioactivities of compounds **11a–f** and **12c–e** against *E. coli, S. lignieres*, and *S. aureus* are shown in Table 1. The results show that all of the new derivatives in which the heterocyclic carboxamide group was replaced by a benzamide group were inactive. This proved that the heterocyclic carboxamide group was essential to enhance bioactivities. Based on this conclusion, we will continue further to study novel pleuromutilin derivatives with heterocyclic carboxamide and thioether groups in the C_{14} side chain.

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