Synthesis and antibacterial activities of pleuromutilin derivatives with quinazolinone and thioether groups

Yuan-peng Yao[a], Fu-ying Dai[b], Kui-kui Dong[a], Qiang Mao[b], Yu-liang Wang[a]* and Tian Chen[b]*

[a]*Faculty of Chemistry, Sichuan University, Chengdu 610065, P. R. China*

[b]*Department of Pathogenic Biology, Chengdu Medical College, Chengdu 610083, P. R. China*

Ten novel pleuromutilin derivatives with quinazolinone and thioether groups in the C_{14} side chain have been designed and synthesised. The antibacterial activities of the target compounds were tested via the agar-well diffusion method *in vitro* in the concentration of 5.0 μg mL^{-1} The results showed that all target compounds had displayed obvious antibacterial activity against *Staphylococcus aureus ATCC26112* and *Staphylococcus aureus SC*.

Keywords: pleuromutilin derivatives, quinazolinone, antibacterial activity

Pleuromutilin (Fig. 1, **1**), a naturally occurring antibiotic, was first isolated in 1951 from the *Basidiomycete Pleurotus mutilus*. [1,2] The tricyclic diterpenoid structure was elucidated by Arigoni and Birch in the 1960s and subsequently confirmed by X-ray crystallography.[3–5] The earlier research results showed that pleuromutilin displayed modest activity against Gram-positive pathogens *in vitro* and weak activity *in vivo*.[6,7] Pleuromutilin exerts its antibacterial activity by disturbing bacterial protein synthesis via interaction with prokaryotic ribosomes.[8] In order to exploit new pleuromutilin derivatives with potent activity, many efforts have been made worldwide. It has been reported that chemical modification of the side chain at C_{14} may lead to optimum activity, and decisive improvement in antibacterial activity could be produced when both a thioether moiety and a basic group were arranged in the same side chain.[9,10] As a result, tiamulin[11] (Figs 1 and 2) and valnemulin[12] (Figs 1 and 3) were successfully developed as therapeutic agents for veterinary use. Azamulin (Figs 1 and 4) was another derivative of pleuromutilin introduced in the 1980s, although it showed good antibacterial activity *in vitro*, it did not go into further clinical trials because of its atrocious solubility in water.[13,14] Retapamulin (Figs 1 and 5) which showed excellent

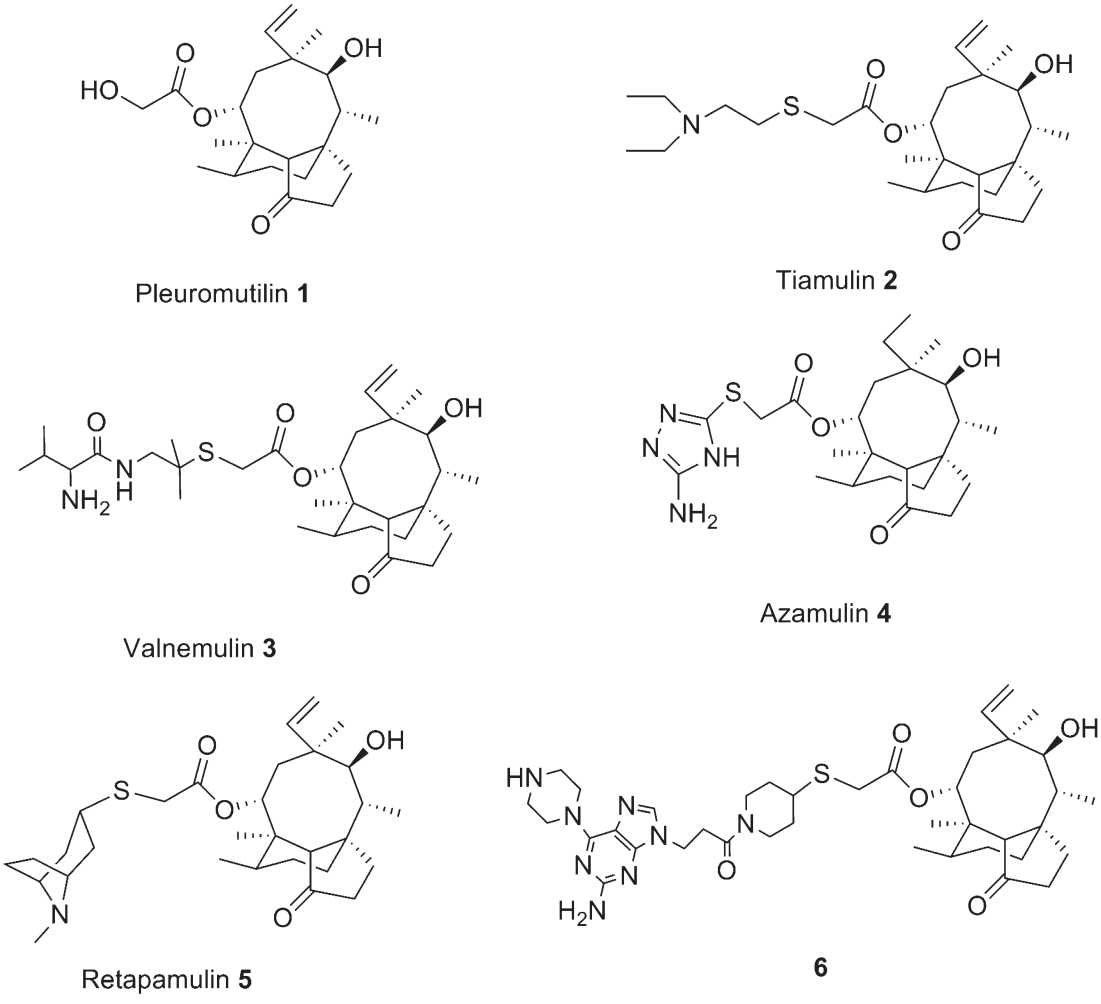


Fig. 1 Pleuromutilin and related derivatives.

* Correspondent. E-mail: Yu-liang Wang

antibacterial activity *in vitro*, was first approved for human use as a topical antimicrobial agent to treat skin infections.[15] Recently, **6** (Figs 1 and 6) and related compounds with excellent bioactivity and good solubility in water were synthesized.[16]

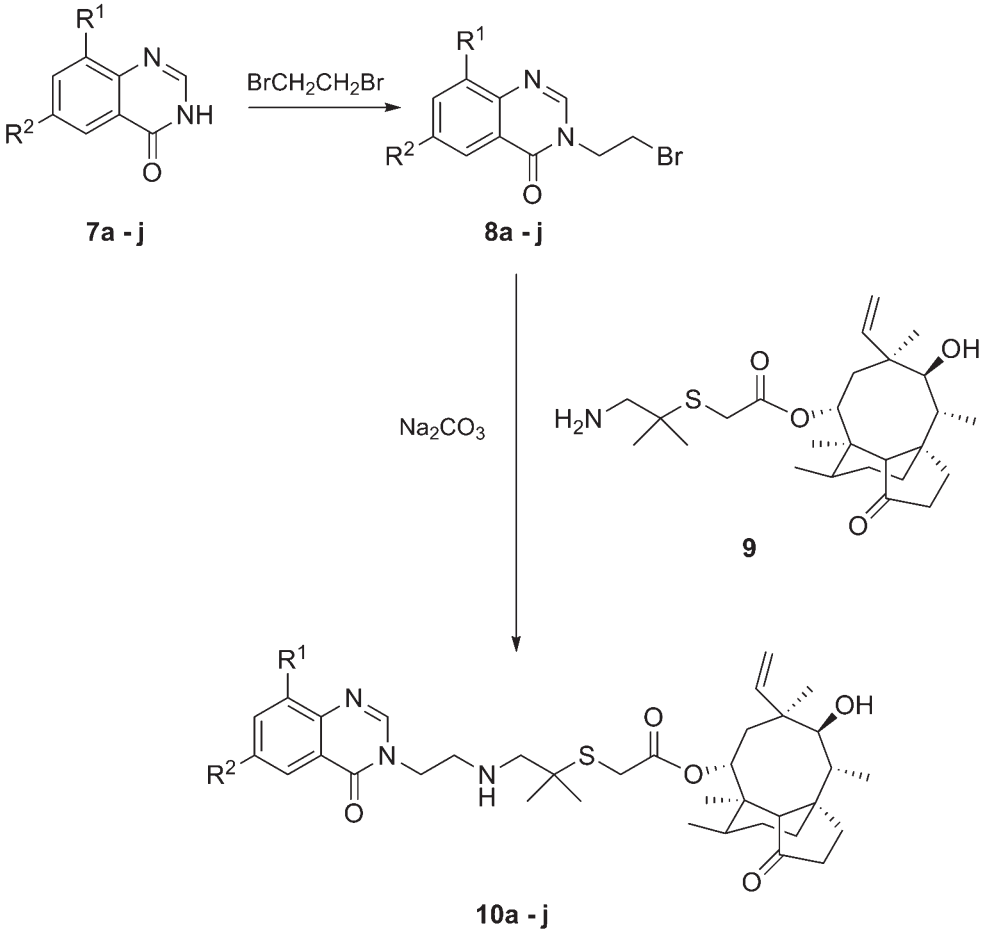
In our previous research, it was found that the pleuromutilin derivatives with substituted heterocyclic carboxamide at C_{14} showed excellent activity.[17,18] Here, the heterocyclic moiety is introduced at the C_{14} side chain in another way. A series of novel pleuromutilin derivatives containing quinazolinone which possess extensive biological potency including antimicrobial activity and a thioether group at the C_{14} side chain were designed and synthesised. The antibacterial activities of the target compounds were tested via the agar-well diffusion method *in vitro*. From the results it is evident that all target compounds exhibited distinct antibacterial activity against *Staphylococcus aureus ATCC26112* and *Staphylococcus aureus SC*. The synthetic route is shown in Scheme 1.

Experimental

Melting points were recorded on an 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 an Agilent 6210 (DOF-MAS) spectrometer (Agilent Inc., Santa Clara, CA, USA) using the electrospray ionisation (ESI) method. IR spectra were recorded with a Perkin-Elmer 16PC-FT instrument (Perkin-Elmer Inc., Norwalk Conn, CA, USA). Compounds **7a–j** were synthesised according to the literature[19]. Compound **9** was synthesised according to the literature.[18]

Preparation of **8a–j**; *general procedure*

A mixture of **7a–j** (5.5 mmol) and NaH (0.27 g, 11.3 mmol) in dry DMF (30 mL) was stirred at room temperature for 3 h. The resulting mixture was added dropwise to 1,2-dibromothane (10.4 g, 55 mmol), the reaction mixture was stirred at 60 °C for 6h, cooled, poured into water (100 mL), then the mixture was extracted with ethyl acetate and the organic layer was washed with water, dried with anhydrous



a: $R^1 = R^2 = H$; **f**: $R^1 = CH_3$, $R^2 = H$;

b: $R^1 = H$, $R^2 = F$; **g**: $R^1 = Br$, $R^2 = F$;

c: $R^1 = H$, $R^2 = CH_3$; **h**: $R^1 = H$, $R^2 = I$;

d: $R^1 = H$, $R^2 = Cl$; **i**: $R^1 = Cl$, $R^2 = Cl$;

e: $R^1 = Cl$, $R^2 = H$; **j**: $R^1 = H$, $R^2 = Br$

Scheme 1 The synthetic route of compounds **8a–j, 10a–j**.

Na_2SO_4, and evaporated *in vacuo* to give the crude product. The crude product was chromatographed on silica gel using petroleum ether/ethyl acetate (3:1) as eluent to afford a pure product (yield 68–79%).

Preparation of **10a–j**; *general procedure*

A mixture of **8a–j** (1.3 mmol), Na_2CO_3 (0.42 g, 3.9 mmol) and compound **9** (0.61 g, 1.3 mmol) in dry DMF (25 mL) was stirred at 60 °C for 24h, then the mixture was cooled slowly, poured into ice water (80 mL), the resulting precipitate was filtered to give the crude product. The crude product was chromatographed on silica gel using ethyl acetate/acetone (6:1) as eluent to afford a pure product (yield 35–46%).

Mutilin 14-O-[1,1-dimethyl-2-(quinazolin-4(3H)-one-3-yl)ethylamine]ethylthioacetate (**10a**): White solid; yield: 43%; m.p. 93–94 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 0.71 (3H, d, *J* = 7.2 Hz), 0.88 (3H, d, *J* = 7.2 Hz), 1.14 (3H, s), 1.31 (2H, m), 1.39 (3H, s), 1.40 (3H, s), 1.47 (3H, s), 1.48 (1H, m), 1.61–1.77 (4H, m), 2.03–2.09 (3H, m), 2.16–2.32 (3H, m), 3.22 (2H, s), 3.34 (1H, d, *J* = 6.2 Hz), 3.80 (2H, s), 4.01(2H, t, *J* = 4.8 Hz), 4.20 (2H, t, *J* = 4.8 Hz), 5.18 (1H, d, *J* = 17.6 Hz), 5.30 (1H, d, *J* = 11.2 Hz), 5.70 (1H, d, *J* = 8.4 Hz), 6.44 (1H, dd, J_1 = 17.2 Hz, J_2 = 11.2 Hz), 7.37 (1H, m), 7.62 (2H, m), 8.02 (1H,s), 8.11 (1H, d, *J* = 8.4 Hz) ; IR (KBr, cm^{-1}) 3438, 2926, 1727, 1637, 1605, 1560, 1460, 1380, 1119, 765; HR-MS (ESI): Calcd for $C_{36}H_{51}N_3O_5S$ $[M+H]^+$: 638.3628. Found: 638.3625.

Mutilin 14-O-[1,1-dimethyl-2-(6-fluoro-quinazolin-4(3H)-one-3-yl) ethylamine] ethylthioacetate (**10b**): White solid; yield: 38%; m.p. 93–95 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$;TMS): δ (ppm) = 0.72 (3H, d, *J* = 6.8 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 1.13 (3H, s), 1.30 (2H, m), 1.39 (3H, s), 1.42 (3H, s), 1.45 (3H, s), 1.48 (1H, m), 1.62–1.778 (4H, m), 2.06–2.09 (3H, m), 2.19–2.34 (3H, m), 3.23 (2H, s), 3.35 (1H, d, *J* = 6.2 Hz), 3.82 (2H, s), 4.01 (2H, t, *J* = 4.8 Hz), 4.16 (2H, t, *J* = 4.8 Hz), 5.18 (1H, d, *J* = 16.2 Hz), 5.30(1H, d, *J* = 12.1Hz), 5.73 (1H, d, *J* = 8.4 Hz), 6.47 (1H, dd, J_1 = 17.2 Hz, J_2 = 11.2 Hz), 7.04–7.44 (1H, m), 7.64 (1H, d, *J* = 7.2 Hz), 8.15–8.17 (2H, m); IR (KBr, cm^{-1}) 3438, 2926, 1727, 1635, 1609, 1485, 1383, 1119, 773; HR-MS (ESI): Calcd for $C_{36}H_{50}FN_3O_5S$ $[M+H]^+$: 656.3534. Found: 656.3529.

Mutilin 14-O-[1,1-dimethyl-2-(6-methyl-quinazolin-4(3H)-one-3-yl) ethylamine] ethylthioacetate (**10c**): White solid; yield: 35%; m.p. 94–96 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 0.71 (3H, d, *J* = 6.8 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 1.13 (3H, s), 1.32 (2H, m), 1.39 (3H, s), 1.40 (3H, s), 1.45 (3H, s), 1.48 (1H, m), 1.61–1.77 (4H, m), 2.01–2.09 (3H, m), 2.19–2.33 (3H, m), 2.52 (3H, s), 3.23 (2H, s), 3.35 (1H, d, *J* = 6.2 Hz), 3.91 (2H, s), 4.00 (2H, t, *J* = 4.8 Hz), 4.19 (2H, t, *J* = 4.8 Hz), 5.18 (1H, d, *J* = 17.6 Hz), 5.28 (1H, d, *J* = 11.2 Hz), 5.72 (1H, d, *J* = 8.4Hz), 6.44 (1H, dd, J_1 = 17.2 Hz, J_2 = 11.2 Hz), 7.43 (1H, d, *J* = 8.0 Hz), 7.52 (1H, d, *J* = 8.1 Hz), 7.89 (1H, s), 7.92 (1H, s); IR (KBr, cm^{-1}) 3431, 2954, 2868, 1727, 1638, 1608, 1489, 1383, 1118, 771; HR-MS (ESI): Calcd for $C_{37}H_{53}N_3O_5S$ $[M+H]^+$: 652.3785. Found: 652.3793.

Mutilin 14-O-[2-(6-chloro--quinazolin-4(3H)-one-3-yl)ethylamine-1,1-dimethyl] ethylthioacetate (**10d**): White solid; yield: 38%; m.p. 91–92 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 0.72 (3H, d, *J* = 6.8 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 1.14 (3H, s), 1.34 (2H, m), 1.39 (3H, s), 1.40 (3H, s), 1.45 (3H, s), 1.49 (1H, m), 1.61–1.77 (4H, m), 2.03–2.09 (3H, m), 2.19–2.34 (3H, m), 3.23 (2H, s), 3.35 (1H, d, *J* = 6.2 Hz), 3.83 (2H, s), 4.00 (2H, t, *J* = 4.8 Hz), 4.14 (2H, t, *J* = 4.8 Hz), 5.18 (1H, d, *J* = 17.6 Hz), 5.29 (1H, d, *J* = 11.2 Hz), 5.73 (1H, d, *J* = 8.4 Hz), 6.48 (1H, dd, J_1 = 17.2 Hz, J_2 = 11.2 Hz), 7.52 (2H, s), 7.89 (1H, s), 8.04 (1H, s); IR (KBr, cm^{-1}) 3438, 2955, 2868, 1727, 1637, 1606, 1471, 1385, 1119, 835, 646; HR-MS (ESI): Calcd for $C_{36}H_{50}ClN_3O_5S$ $[M+H]^+$: 672.3239, 674.3209. Found: 672.3233, 674.3218 (3:1).

Mutilin 14-O-[2-(8-chloro-quinazolin-4(3H)-one-3-yl)ethylamine-1,1-dimethyl] ethylthioacetate (**10e**): White solid; yield: 39%; m.p. 92–94 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$;TMS): δ (ppm) = 0.72 (3H, d, *J* = 6.8 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 1.14 (3H, s), 1.30 (2H, m), 1.37 (3H, s), 1.38 (3H, s), 1.47 (3H, s), 1.48 (1H, m), 1.61–1.79 (4H, m), 2.03–2.09 (3H, m), 2.19–2.33 (3H, m), 3.22 (2H, s), 3.35 (1H, d, *J* = 6.2 Hz), 3.83 (2H, s), 3.98 (2H, t, *J* = 4.8 Hz), 4.13 (2H, t, *J* = 4.8 Hz), 5.18 (1H, d, *J* = 17.6 Hz), 5.31 (1H, d, *J* = 11.2 Hz), 5.71 (1H, d, *J* = 8.0 Hz), 6.47 (1H, dd, J_1 = 17.2 Hz, J_2 = 11.2 Hz), 7.23 (2H, m), 7.66 (1H, d, *J* = 8.1 Hz), 7.93 (1H, s); IR (KBr, cm^{-1}) 3430, 2926, 2864, 1727, 1637, 1604, 1457, 1384, 1118, 756, 721; HR-MS (ESI): Calcd for $C_{36}H_{50}ClN_3O_5S$ $[M+H]^+$: 672.3239, 674.3209. Found: 672.3230, 674.3219 (3:1).

Mutilin 14-O-[1,1-dimethyl-2-(8-methyl-quinazolin-4(3H)-one-3-yl) ethylamine] ethylthioacetate (**10f**): White solid; yield: 42%; m.p. 94–96 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 0.71 (3H, d, *J* = 6.8 Hz), 0.87 (3H, d, *J* = 6.8 Hz), 1.14 (3H, s), 1.30 (2H, m), 1.37 (3H, s), 1.39 (3H, s), 1.45 (3H, s), 1.48 (1H, m), 1.61–1.77 (4H, m), 2.03–2.09 (3H, m), 2.17–2.34 (3H, m), 2.63 (3H, s), 3.23 (2H, s), 3.35 (1H, d, *J* = 6.2 Hz), 3.83 (2H, s), 4.01 (2H, t, *J* = 4.8 Hz), 4.21 (2H, t, *J* = 4.8 Hz), 5.16 (1H, d, *J* = 17.6 Hz), 5.31 (1H, d, *J* = 11.2 Hz), 5.71 (1H, d, *J* = 8.4 Hz), 6.44 (1H, dd, J_1 = 17.2 Hz, J_2 = 11.2 Hz), 7.42 (1H, m), 7.66 (1H, d, *J* = 8.0 Hz), 7.81 (1H, s), 8.15 (1H, m); IR (KBr, cm^{-1}) 3435, 2928, 2864, 1728, 1635, 1606, 1457, 1380, 1278, 1118, 768; HR-MS (ESI): Calcd for $C_{37}H_{53}N_3O_5S$ $[M+H]^+$: 652.3785; found: 652.3792.

Mutilin 14-O-[2-(8-bromo-6-fluoro-quinazolin-4(3H)-one-3-yl) ethylamine-1,1 -dimethyl] ethylthioacetate (**10g**): White solid; yield: 37%; m.p. 93–94 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 0.71 (3H, d, *J* = 6.8 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 1.14 (3H, s), 1.30 (2H, m), 1.37 (3H, s), 1.38 (3H, s), 1.45 (3H, s), 1.48 (1H, m), 1.61–1.77 (4H, m), 2.03–2.09 (3H, m), 2.14–2.34 (3H, m), 3.22 (2H, s), 3.35 (1H, d, *J* = 6.2 Hz), 3.83 (2H, s), 3.98 (2H, t, *J* = 4.8 Hz), 4.21 (2H, t, *J* = 4.8 Hz), 5.18 (1H, d, *J* = 17.6 Hz), 5.29 (1H, d, *J* = 11.2 Hz), 5.72 (1H, d, *J* = 8.4 Hz), 6.44 (1H, dd, J_1 = 17.4 Hz, J_2 = 11.2 Hz), 7.64 (1H, dd, J_1 = 7.6 Hz, J_2 = 2.4 Hz), 7.78 (1H, m), 7.89 (1H, s); IR (KBr, cm^{-1}) 3438, 2926, 2864, 1727, 1635, 1604, 1460, 1386, 1279, 1117, 867, 769; HR-MS (ESI): Calcd for $C_{36}H_{49}BrFN_3O_5S$ $[M+H]^+$: 734.2639, 736.2619. Found: 734.2649, 736.2632 (1:1).

Mutilin 14-O-[1,1-dimethyl-2-(6-iodo-quinazolin-4(3H)-one-3-yl) ethylamine] ethylthioacetate (**10h**): White solid; yield: 46%; m.p. 98–99 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 0.71 (3H, d, *J* = 6.8 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 1.14 (3H, s), 1.30 (2H, m), 1.38 (3H, s), 1.40 (3H, s), 1.46 (3H, s), 1.48 (1H, m), 1.61–1.77 (4H, m), 2.03–2.09 (3H, m), 2.18–2.34 (3H, m), 3.22 (2H, s), 3.34 (1H, d, *J* = 6.2 Hz), 3.83 (2H, s), 3.98 (2H, t, *J* = 4.8 Hz), 4.12 (2H, t, *J* = 4.8 Hz), 5.18 (1H, d, *J* = 17.6 Hz), 5.31(1H, d, *J* = 11.2 Hz), 5.72 (1H, d, *J* = 8.4 Hz), 6.48 (1H, dd, J_1 = 17.2 Hz, J_2 = 11 Hz), 7.29 (1H, s), 7.82 (1H, d, *J* = 1.6 Hz), 7.85 (1H, s), 8.31 (1H, d, *J* = 1.2 Hz); IR (KBr, cm^{-1}) 3439, 2954, 2868, 1727, 1636, 1606, 1468, 1385, 1280, 1119, 832, 760; HR-MS (ESI): Calcd for $C_{36}H_{50}IN_3O_5S$ $[M+H]^+$: 764.2595. Found: 764.2581.

Mutilin 14-O-[1,1-dimethyl-2-(6,8-dichloro-quinazolin-4(3H)-one-3-yl)ethylamine] ethylthioacetate (**10i**): White solid; yield: 40%; m.p. 93–95 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 0.71 (3H, d, *J* = 7.2 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 1.14 (3H, s), 1.31(2H, m), 1.38 (3H, s), 1.39 (3H, s), 1.45 (3H, s), 1.48 (1H, m), 1.61–1.77 (4H, m), 2.03–2.09 (3H, m), 2.14–2.33 (3H, m), 3.11 (2H, s), 3.34 (1H, d, *J* = 6.2 Hz), 3.83 (2H, s), 3.93 (2H, t, *J* = 4.8 Hz), 4.11 (2H, t, *J* = 4.8 Hz), 5.18 (1H, d, *J* = 17.6 Hz), 5.29 (1H, d, *J* = 11.2 Hz), 5.72 (1H, d, *J* = 8 Hz), 6.48 (1H, dd, J_1 = 17.2 Hz, J_2 = 11.1 Hz), 7.65 (1H, d, *J* = 2.0 Hz), 7.91 (1H, s), 7.94 (1H, d, *J* = 2.1 Hz); IR (KBr, cm^{-1}) 3440, 2955, 2868, 1727, 1638, 1603, 1456, 1386, 1280, 1118, 843, 777, 708; HR-MS (ESI): Calcd for $C_{36}H_{49}Cl_2N_3O_5S$ $[M+H]^+$: 706.2849, 708.2819, 710.2790. Found: 706.2840, 708.2823, 710.2815 (9:6:1).

Mutilin 14-O-[2-(6-bromo--quinazolin-4(3H)-one-3-yl)ethylamine-1,1-dimethyl] ethylthioacetate (**10j**): White solid; yield: 45%; m.p. 90–91 °C; ^{1}H NMR spectrum (400 MHz; d_1-$CDCl_3$; TMS): δ (ppm) = 0.71 (3H, d, *J* = 7.2 Hz), 0.88 (3H, d, *J* = 7.2 Hz), 1.14 (3H, s), 1.32 (2H, m), 1.38 (3H, s), 1.40 (3H, s), 1.45 (3H, s), 1.48 (1H, m), 1.61–1.77 (4H, m), 2.03–2.09 (3H, m), 2.18–2.33 (3H, m), 3.24 (2H, s), 3.34 (1H, d, *J* = 6.0 Hz), 3.82 (2H, s), 3.99 (2H, t, *J* = 4.8 Hz), 4.12 (2H, t, *J* = 4.8 Hz), 5.18 (1H, d, *J* = 17.6 Hz), 5.31 (1H, d, *J* = 11.2 Hz), 5.72 (1H, d, *J* = 8.4 Hz), 6.48 (1H, dd, J_1 = 17.2 Hz, J_2 = 11.2 Hz), 7.43 (1H, d, *J* = 8.8 Hz), 7.66 (1H, dd, J_1 = 8.8 Hz, J_2 = 1.5 Hz), 7.85 (1H, s), 8.17(1H, d, *J* = 2.1 Hz); IR (KBr, cm^{-1}) 3436, 2955, 2868, 1728, 1636, 1607, 1468, 1384, 1277, 1119, 834, 758, 670; HR-MS (ESI): Calcd for $C_{36}H_{50}BrN_3O_5S$ $[M+H]^+$: 716.2734, 718.2713. Found: 716.2731, 718.2717 (1:1).

Biological assay

The wild *Staphylcoccus aures* were isolated from Sichuan province in China. The standard Staphylcoccus aures were obtained from the Basic Medical and Forensic College Sichuan University. McF was used to evaluate the bacteria

count and 0.5 McF (amount of bacteria: about 1.5×10^8 colony-forming unit (CFU)/mL) was formulated according to the condition: 0.048 M $BaCl_2$ 0.5 mL, 0.36 N H_2SO_4 99.5 mL. The antibacterial activities of the target compounds *in vitro* were tested with the agar-well diffusion method.

The *Staphylococcus aureus ATCC26112* and *Staphylococcus aureus SC* were inoculated in LB (Luria Bertani) culture medium respectively and then kept at 37 °C for 18 h after both of them were recovered. Later, the bacteria solution was corrected to 0.5 McF (amount of bacteria: about 10^8 CFU/mL) with saline.

Every sample (1000 μg) was dissolved with ethanol (1 mL) and diluted to 5 μg mL^{-1} with ethanol on a super clean bench.

The LB culture medium (with 2.2% agar) was calmed down at about 55 °C after sterilisation by autoclave. 1 mL bacteria solution (0.5 McF) was added into the LB culture medium (100 mL) (the amount of bacteria: about 10^6 CFU/mL), and the resulting mixture was shaken up until it was well-distributed, then the mixture was poured into three plates (20 mL for each). Four wells (6 mm) were made in each plate using a sterile cork borer when the mixture became curdled. A 50 μL solution of each compound (target compounds or contrast compounds) was injected into the corresponding well and the plates were incubated at 37 °C for 24 h.

Results and discussion

All the target compounds exhibited better antibacterial activities than pleuromutilin at the concentration of 5.0 μg mL^{-1}.

As shown in Scheme 1, the intermediates (**8a–j**) were synthesised by the reaction of substituted quinazolinone (**7a-7j**) with 1,2-dibromoethane. Because the 1,2-dibromoethane molecule has two reaction sites, 10 equivalent of 1,2-dibromoethane was used to avoid the side reaction and **8a–j** were produced in good yield.

In the last step, when K_2CO_3 was used as the base, significant amounts of by-products formed and the purification process of the product became very difficult. When Na_2CO_3 was used in place of K_2CO_3, the by-products decreased and a pure product could be obtained easily as a white powder via silica gel column chromatography.

Table 1 The antibacterial activity of the target compounds in the concentration of 5.0 μg mL^{-1} towards *S. aureus ATCC26112* and *S. aureus SC.* (Isolated from Sichuan, China)

Compound	Diameter of inhibition zone /mm	
	S. aureus ATCC26112	*S. aureus SC*
10a	13	17
10b	14	17
10c	15	16
10d	13	17
10e	13	16
10f	14	16
10g	14	16
10h	13	15
10i	15	18
10j	13	16
Pleuromutilin	11	13
Ethanol	6	6

Concentration of pleuromutilin: 5.0 μg mL^{-1}; negative control: ethanol, positive control: pleuromutilin; diameter of the well in each plate: 6 mm.

Biological activity

Since pleuromutilin had modest activity against Gram-positive bacteria while it is not sensitive to Gram-negative bacteria, two Gram-positive pathogen *S. aureus ATCC26112* and *S. aureus SC* were chosen to test the antibacterial activities of the target compounds. The results showed that all the target compounds exhibited better antibacterial activity than pleuromutilin, especially toward *S. aureus SC*.

Conclusion

A series of novel pleuromutilin derivatives with quinazolinone and thioether groups in the C_{14} side chain have been designed and synthesised. The antibacterial activity results of the target compounds indicated that all the target compounds displayed better antibacterial activity against *S.aureus ATCC26112* and *S.aureus SC* than pleuromutilin. Our exploration has further enriched the content of SAR of pleuromutilin and it is helpful for us to design more pleuromutilin derivatives.

Electronic Supplementary Information

Pictures of the inhibition zones have been deposited in the ESI available through stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data.

We appreciate the financial support from the National Science Foundation of China (No. 21072135). We thank Mr Qiang Mao a student in 2008 grade in the School of Biomedical Science for experiments in the laboratory of Professor Tian Chen in the Department of Pathogenic Biology, Chengdu Medical College.

Received 19 September 2010; accepted 15 November 2010
Paper 1000364 doi: 10.3184/174751911X556675
Published online: 21 January 2011

References

1 F. Kavanagh, A. Hervey, W.J. Robbins, *Proc. Natl. Acad. Sci. USA*, 1951, **37**, 570.
2 M. Dane, Springer, T. Jason, Goodricha and Stella Huangb, *Tetrahedron Lett*., 2002, **43**, 4857.
3 D. Arigoni, *Gazz. Chim. Ital.*, 1962, **92**, 884.
4 A.J. Birch, C.W. Holzapfel, R.W. Rickards, *Tetrahedron*., 1966, **8**, 359.
5 M. Dobler, G.G. Durr, *Cryst.struct.Commun.*,1975, **4**, 259.
6 M. Anchel, *J. Biol. Chem*., 1952, **199**, 133.
7 J. Drews, G. Georgopoulos and G. Laber, *Antimicrob. Agents Chemother*., 1975, **7**, 507.
8 G. Hogenauer, *Eur.J. Biochem.*, 1975, **52**, 93.
9 Y.Y. Zhang, K.P. Xu, Y.L. Wang and Y.Z. Wang, *Chin. Chem. Lett.*, 2009, **20**, 29.
10 H. Egger, H. Reinshagen, *J. Antibiot*., 1976, **29**, 923.
11 G. Laber, E. Schultze, *Antimicrob. Agents Chemother*., 1975, **7**, 517.
12 P.C.T. Hannan, H.M. Windsor and P.H. Ripley, *Res. Vet. Sci*., 1997, **63**, 157.
13 Y. Hirokawa, H. Kinoshita and T. Tanaka, *Bioorganic. Med. Chem. Lett.*, 2008, **18**, 3556.
14 Y. Hirokawa, H. Kinoshita and T. Tanaka, *Bioorganic. Med. Chem. Lett.*, 2009, **19**, 175.
15 L. Fu, Z. Jiang, Z. Cai, Y. Yang, *Bioorganic. Med. Chem. Lett.*, 2009, **19**, 5407.
16 Y. Hirokawa, H. Kinoshita and T. Tanaka, *J. Med. Chem*., 2008, **51**, 1991.
17 P. Xu, Y.Y. Zhang, Y.X. Sun and J.H. Liu, *Chem. Biol. Drug*., 2009, **73**, 655.
18 K.P. Xu, Y.Y. Zhang and Y.L. Wang, *J. Chem. Res*., 2010, **34**, 354.
19 J.H. You, C.W. Ye, Y.L. Wang, *ARKIVOC*., 2008, **17**, 1.