

Synthesis and Antibacterial Activities of Pleuromutilin Derivatives with Modified 7-Aminocephalosporin Acid and Thioether Moiety

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In order to study the effect of heterocyclic carboxamide group on pleuromutilin's antibacterial activity, seven novel pleuromutilin derivatives with modified 7-aminocephalosporin acid and thioether moiety were designed and synthesized. The antibacterial activities of the new compounds were tested *via* agar-well diffusion method *in vitro* under different concentrations. The results showed that three target compounds (**5a**, **5d**, **5f**) still had antibacterial activity against *Staphylococcus aureus* SC and *Staphylococcus aureus* ATCC26112 at the concentration 2.0 µg/mL.

Key Words: Pleuromutilin derivatives, 7-Aminocephalosporin acid, Synthesis, Antibacterial activity, Agar-well diffusion.

INTRODUCTION

The antibiotic pleuromutilin (Fig. 1) was first isolated in 1951 by Kavanagh and co-workers¹. Early research showed that this kind of antibiotic has modest activity *in vitro* against Gram-positive pathogens and mycoplasmas^{2,3}. Pleuromutilin selectively inhibits bacterial protein synthesis through interaction with prokaryotic ribosome⁴. A Sandoz group⁵ indicated that the modification at C14 side chain could be the most promising approach to obtain optimum activity and the combinations of both the thioether group and the basic group in the side chain would give excellent bioactivity. Based on these structure and activity relationships, tiamulin⁶, valunemulin^{7,8} and retapamulin^{9,10} were successfully developed as therapeutic pharmaceuticals. In 2008, pleuromutilin analogs with purine ring possessing excellent antibacterial activity had been reported by Hirokawa and coworkers¹¹.

In our previous works, some pleuromutilin derivatives containing substituent pyrazole carboxamide¹² and substituent thiazole carboxamide¹³ (Fig. 1) were reported to exist excellent antibacterial activity. Herein, for the propose of further exploring the effect of heterocyclic carboxamide groups on the antibacterial activity of pleuromutilin, some modified 7-aminocephalosporin acids (7-ACA) was introduced into pleuromutilin by amido bond. The synthetic route was showed in **Scheme-I**, the antibacterial activities of the target compounds were tested *via* agar-well diffusion method.

EXPERIMENTAL

Compounds **5a-g** were prepared as shown in **Scheme-I**. Intermediates **2a-g** were prepared according to the literature with little modification¹⁴. Intermediates **2** was suspended in dried dichloromethane and Et₃N at 0 °C where 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and

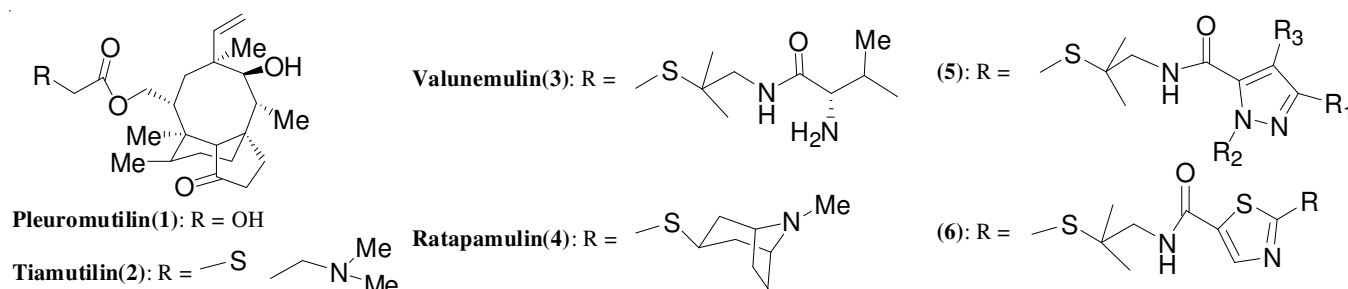
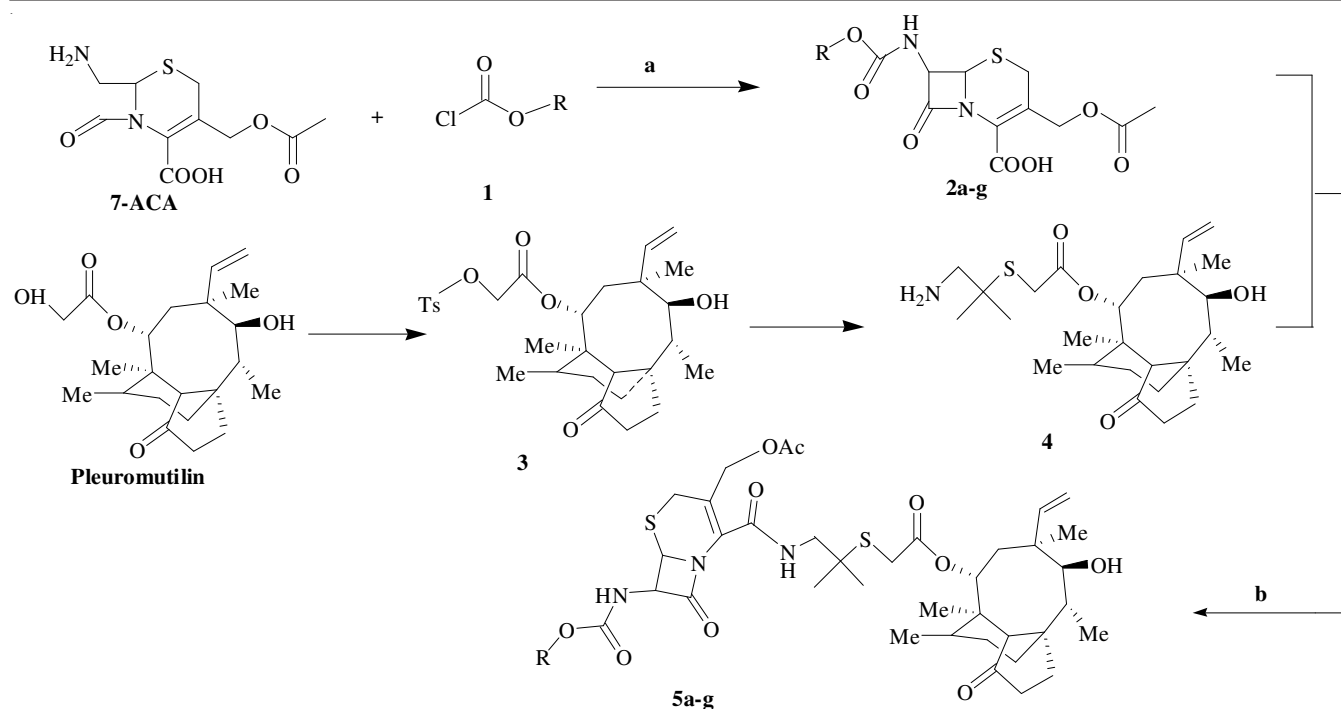


Fig. 1. Pleuromutilin and pleuromutilin derivatives



2a,5a: R=CH₃; 2b,5b: R=CH₃CH₂; 2c,5c: R=(CH₃)₂CH; 2d,5d: R=(CH₃)₂CHCH₂; 2e,5e: R=(CH₃)₃C; 2f,5f: R=C₆H₅CH₂; 2g,5g: R=CCl₃CH₂

Scheme-I: Synthetic route of compound 5a-g

1-hydroxybenzotriazole (HOBt) were rapidly added and the reaction mixture was maintained at room temperature for 2 h. Then compound 4 (which was synthesized according to the literature¹²) was added into the solution at 0 °C and the resulting solution was allowed to be stirred at room temperature for another 24 h. The solution was then washed with H₂O and saturated sodium chloride solution. The organic layer was dried, filtered and evaporated under reduced pressure. The crude products were purified on silica-gel column (petroleum ether/ethyl acetate as the eluent).

Mutilin-14-O-[1-(7-methoxycarbamidocephalosporanic carboxamide)-2-methylpropane-2-yl] thioacetate (5a): Orange solid; yield: 76.34 %; m.p.: 106–108 °C; IR (KBr, $\nu_{\max}$, cm⁻¹): 3402, 3084, 2931, 2870, 1729, 1664, 1537, 1459, 1375, 1280, 1117, 1022, 915, 587; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) 0.73 (d, 3H, J = 6.8 Hz), 0.90 (d, 3H, J = 6.8 Hz), 1.10–1.15 (m, 1H), 1.18 (s, 3H), 1.26–1.28 (m, 3H), 1.31 (s, 3H), 1.33–1.45 (m, 3H), 1.47 (s, 3H), 1.60 (s, 3H), 1.65–1.71 (m, 3H), 1.77–1.81 (m, 1H), 2.06 (s, 3H), 2.07–2.14 (m, 3H), 2.18–2.35 (m, 3H), 3.12–3.30 (m, 6H), 3.32–3.38 (m, 2H), 3.73 (s, 3H), 5.19–5.23 (m, 2H), 5.32 (dd, 1H, J_1 = 10.8 Hz, J_2 = 1.2 Hz), 5.74–5.78 (m, 2H), 6.48 (dd, 1H, J_1 = 17.6 Hz, J_2 = 11.2 Hz); HR-MS (-ESI): Calcd. for C₃₈H₅₄N₃O₁₀S₂ [M-H]⁻: 776.3251, Found: 776.3265.

Mutilin-14-O-[1-(7-ethoxycarbamidocephalosporanic carboxamide)-2-methylpropane-2-yl] thioacetate (5b): Yellow solid; yield: 75.83 %; m.p.: 94–96 °C; IR (KBr, $\nu_{\max}$, cm⁻¹): 3393, 3075, 2960, 2929, 2857, 1775, 1726, 1677, 1537, 1457, 1377, 1262, 1115, 1022, 914, 802, 731; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) 0.67 (d, 3H, J = 6.8 Hz), 0.85 (d, 3H, J = 6.8 Hz), 1.02–1.08 (m, 1H), 1.12 (m, 3H), 1.18 (s, 3H), 1.19 (s, 3H), 1.26–1.37 (m, 3H), 1.40 (s, 3H), 1.43–1.57 (m, 3H), 1.65–1.77 (m, 1H), 1.99 (s, 3H), 2.02–

2.09 (m, 3H), 2.12–2.33 (m, 3H), 3.06–3.18 (m, 3H), 3.20–3.29 (m, 3H), 3.31–3.34 (m, 2H), 3.65 (m, 2H), 4.61 (d, 1H, J = 12.8 Hz), 4.84 (d, 1H, J = 12.8 Hz), 4.90 (s, 1H), 5.14 (dd, 1H, J_1 = 17.2 Hz, J_2 = 9.2 Hz), 5.23 (dd, 1H, J_1 = 10.4 Hz, J_2 = 8.4 Hz), 5.40 (m, 1H), 5.48–5.51 (m, 1H), 6.56 (s, 1H), 7.60 (s, 1H); HR-MS (-ESI): Calcd. for C₃₉H₅₆N₃O₁₀S₂ [M-H]⁻: 790.3406, Found: 790.3412.

Mutilin-14-O-[1-(7-isopropoxycarbamidocephalosporanic carboxamide)-2-methylpropane-2-yl] thioacetate (5c): Yellow solid; yield: 61.28 %; m.p.: 98–102 °C; IR (KBr, $\nu_{\max}$, cm⁻¹): 3441, 2961, 2927, 2857, 1778, 1726, 1532, 1459, 1379, 1260, 1111, 1024, 914, 803, 616; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) 0.73 (d, 3H, J = 6.8 Hz), 0.90 (d, 3H, J = 7.2 Hz), 1.11–1.14 (m, 1H), 1.17 (s, 3H), 1.22–1.28 (m, 6H), 1.30 (s, 3H), 1.32–1.42 (m, 3H), 1.46 (s, 3H), 1.56 (s, 3H), 1.62–1.70 (m, 3H), 1.80–1.83 (m, 1H), 2.05 (s, 3H), 2.10–2.16 (m, 3H), 2.17–2.44 (m, 3H), 3.14–3.30 (m, 6H), 3.34–3.56 (m, 2H), 4.69 (d, 1H, J = 12.8 Hz), 4.90–5.03 (m, 2H), 5.20 (dd, 1H, J_1 = 17.6 Hz, J_2 = 9.6 Hz), 5.31 (d, 1H, J = 7.6 Hz), 5.36–5.49 (m, 1H), 5.80 (d, 1H, J = 8.8 Hz), 6.44 (dd, 1H, J_1 = 17.2 Hz, J_2 = 11.2 Hz), 7.52 (s, 1H), 7.56 (s, 1H); HR-MS (-ESI): Calcd. for C₄₀H₅₈N₃O₁₀S₂ [M-H]⁻: 804.3563, Found: 804.3567.

Mutilin-14-O-[1-(7-isobutoxycarbamidocephalosporanic carboxamide)-2-methylpropane-2-yl] thioacetate (5d): Yellow solid; yield: 63.57 %; m.p.: 71–73 °C; IR (KBr, $\nu_{\max}$, cm⁻¹): 3403, 3079, 2921, 2928, 2865, 1729, 1663, 1540, 1461, 1375, 1280, 1117, 1023, 915; ¹H NMR spectrum (400 MHz; d₁-CDCl₃; TMS): δ (ppm) 0.73 (d, 3H, J = 7.2 Hz), 0.90 (d, 3H, J = 6.8 Hz), 0.99 (d, 6H, J = 6.8 Hz), 1.10–1.15 (m, 1H), 1.18 (s, 3H), 1.21–1.28 (m, 3H), 1.31 (s, 3H), 1.35–1.43 (m, 3H), 1.46 (s, 3H), 1.48–1.62 (m, 3H), 1.65–1.70 (m, 3H), 1.76–1.80 (m, 1H), 1.99 (s, 3H), 2.05–2.18 (m, 3H), 2.20–

2.35 (m, 4H), 3.11-3.31 (m, 6H), 3.36-3.40 (m, 2H), 4.09 (d, 2H, $J = 6.8$ Hz), 5.27 (dd, 1H, $J_1 = 17.6$ Hz, $J_2 = 1.6$ Hz), 5.32 (dd, 1H, $J_1 = 11.2$ Hz, $J_2 = 1.6$ Hz), 5.74 (d, 1H, $J = 8.4$ Hz), 6.43 (dd, 1H, $J_1 = 17.2$ Hz, $J_2 = 10.8$ Hz), 7.52 (m, 1H); HR-MS (-ESI): Calcd. for $C_{41}H_{60}N_3O_{10}S_2$ $[M-H]^-$: 818.3719, Found: 818.3725.

Mutilin-14-O-[1-(7-*t*-butoxycarbamidocephalosporanic carboxamide)-2-methylpropane-2-yl] thioacetate (5e): Yellow solid; yield: 56.78 %; m.p.: 86-88 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3419, 2959, 2924, 2855, 1726, 1526, 1459, 1372, 1261, 1110, 1023, 914, 803, 618, 588; ^1H NMR spectrum (400 MHz; $\text{d}_1\text{-CDCl}_3$; TMS): δ (ppm) 0.73 (d, 3H, $J = 6.8$ Hz), 0.88 (d, 3H, $J = 6.8$ Hz), 1.10-1.14 (m, 1H), 1.17 (s, 3H), 1.21-1.24 (m, 3H), 1.25 (s, 9H), 1.28 (s, 3H), 1.36-1.39 (m, 3H), 1.45 (s, 3H), 1.48-1.69 (m, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.17-2.33 (m, 3H), 3.12-3.31 (m, 6H), 3.33-3.80 (m, 2H), 4.67-4.79 (m, 2H), 4.96 (d, 1H, $J = 11.6$ Hz), 5.19 (dd, 1H, $J_1 = 17.6$ Hz, $J_2 = 8.0$ Hz), 5.47 (d, 1H, $J = 8.4$ Hz), 5.55 (m, 1H), 5.76 (d, 1H, $J = 8.4$ Hz), 6.45 (dd, 1H, $J_1 = 16.4$ Hz, $J_2 = 6.0$ Hz); HR-MS (-ESI): Calcd. for $C_{41}H_{60}N_3O_{10}S_2$ $[M-H]^-$: 818.3719, Found: 818.3728.

Mutilin-14-O-[1-(7-benzoxycarbamidocephalosporanic carboxamide)-2-methylpropane-2-yl] thioacetate (5f): Yellow solid; yield: 64.66 %; m.p.: 58-60 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3388, 2962, 2924, 2855, 1729, 1661, 1524, 1458, 1377, 1261, 1097, 1022, 912, 800, 698, 586; ^1H NMR spectrum (400 MHz; $\text{d}_1\text{-CDCl}_3$; TMS): δ (ppm) 0.73 (d, 3H, $J = 6.8$ Hz), 0.90 (d, 3H, $J = 6.8$ Hz), 1.10-1.15 (m, 1H), 1.18 (s, 3H), 1.26-1.28 (m, 3H), 1.31 (s, 3H), 1.36-1.43 (m, 3H), 1.46 (s, 3H), 1.49-1.62 (m, 3H), 1.65-1.70 (m, 3H), 1.76-1.80 (m, 1H), 2.02 (s, 3H), 2.05-2.13 (m, 3H), 2.20-2.35 (m, 3H), 3.11-3.27 (m, 6H), 3.29-3.37 (m, 2H), 5.21 (dd, 2H, $J_1 = 17.2$ Hz, $J_2 = 1.2$ Hz), 5.33 (d, 2H, $J = 12.0$ Hz), 5.75 (d, 1H, $J = 8.8$ Hz), 6.48 (dd, 1H, $J_1 = 17.6$ Hz, $J_2 = 11.2$ Hz), 7.34 (m, 5H); HR-MS (-ESI): Calcd. for $C_{44}H_{58}N_3O_{10}S_2$ $[M-H]^-$: 852.3563, Found: 852.3581.

Mutilin-14-O-[1-(7-N-carbamate-2,2,2-trichloroethyl)-cephalosporanic carboxamide)-2-methylpropane-2-yl] thioacetate (5g): Yellow solid; yield: 57.89 %; m.p.: 68-70 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3347, 2962, 2926, 2858, 1733, 1679, 1533, 1457, 1380, 1261, 1101, 1020, 866, 799, 702, 570; ^1H NMR spectrum (400 MHz; $\text{d}_1\text{-CDCl}_3$; TMS): δ (ppm) 0.74 (d, 3H, $J = 6.8$ Hz), 0.88 (d, 3H, $J = 6.8$ Hz), 1.10-1.15 (m, 1H), 1.17 (s, 3H), 1.31 (s, 3H), 1.33 (s, 3H), 1.37-1.43 (m, 3H), 1.47 (s, 3H), 1.49-1.58 (m, 3H), 1.61-1.70 (m, 3H), 1.76-1.80 (m, 1H), 2.02 (s, 3H), 2.05-2.20 (m, 3H), 2.22-2.35 (m, 3H), 3.16-3.30 (m, 6H), 3.35 (d, 1H, $J = 6.4$ Hz), 3.48 (dd, 1H, $J_1 = 14.1$ Hz, $J_2 = 6.8$ Hz), 5.17 (d, 1H, $J = 17.5$ Hz), 5.26 (d, 1H, $J = 11.12$ Hz), 5.77 (d, 1H, $J = 8.5$ Hz), 6.47 (dd, 1H, $J_1 = 17.6$ Hz, $J_2 = 11.2$ Hz), 7.67 (s, 1H), 7.89 (d, 1H, $J = 8.0$ Hz); HR-MS (-ESI): Calcd. for $C_{39}H_{53}N_3O_{10}S_2Cl_3$ $[M-H]^-$: 892.2237, 894.2208. Found: 892.2247, 894.2225 [isotope peaks].

The antibacterial activities of the target compounds were tested *via* agar-well diffusion method *in vitro*. Target compound (1000 μg) was dissolved with ethanol (1 mL) and diluted to 20 $\mu\text{g}/\text{mL}$ with ethanol, later, **5a**, **5d** and **5f** were further diluted to 2 $\mu\text{g}/\text{mL}$. A 50 μL solution of each compound (target compounds or pleuromutilin) was injected into the corresponding well in the Luria Bertani (LB) culture medium, which was

covered with bacteria suspension in advance and the plates were incubated at 37 °C for 24 h. The results of average diameters of the bacteriostatic circle were listed in Tables 1 and 2.

TABLE-1
In vitro ANTIBACTERIAL ACTIVITY OF **5a-g** AT
THE CONCENTRATION 20 $\mu\text{g}/\text{mL}$

Compound	Diameter of inhibition zone (mm)	
	<i>Staphylococcus aureus</i> SC	<i>Staphylococcus aureus</i> ATCC26112
5a	21	17
5b	20	15
5c	14	12
5d	20	16
5e	15	12
5f	24	19
5g	15	13
Pleuromutilin ^a	22	13
Ethanol ^b	-	-

^aConcentration of pleuromutilin: 20.0 $\mu\text{g}/\text{mL}$; as the positive control;

^bNegative control: ethanol; Diameter of the well in each plate: 6 mm

TABLE-2
In vitro ANTIBACTERIAL ACTIVITY OF **5a**, **5d** and **5f**
AT THE CONCENTRATION 2 $\mu\text{g}/\text{mL}$

Compound	Diameter of inhibition zone (mm)	
	<i>Staphylococcus aureus</i> SC	<i>Staphylococcus aureus</i> ATCC26112
5a	10	7
5d	11	7
5f	14	11
Pleuromutilin ^a	7	-
Ethanol ^b	-	-

^aConcentration of pleuromutilin: 2.0 $\mu\text{g}/\text{mL}$; as the positive control;

^bNegative control: ethanol; Diameter of the well in each plate: 6 mm

RESULTS AND DISCUSSION

The results of antibacterial activities showed that all the target compounds exhibited antibacterial activity against two bacterial strains at the concentration 20 $\mu\text{g}/\text{mL}$. Three compounds (**5a**, **5d**, **5f**) were chosen to further test the antibacterial activity at the concentration 2 $\mu\text{g}/\text{mL}$, they still displayed antibacterial activity against *Staphylococcus aureus* SC and *Staphylococcus aureus* ATCC26112.

Conclusion

In conclusion, seven novel pleuromutilin derivatives with modified 7-aminocephalosporin acid and thioether moiety in the C14 side chain were successfully synthesized. The results of antibacterial activities indicated that three target compounds (**5a**, **5d**, **5f**) still exist antibacterial activity against *Staphylococcus aureus* SC and *Staphylococcus aureus* ATCC26112 at the concentration 2.0 $\mu\text{g}/\text{mL}$. Our exploration has further enriched the content of SAR of pleuromutilin and it is helpful for us to design more pleuromutilin derivatives.

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