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Synthesis and antibacterial activity of C-2(S)-substituted pleuromutilin derivatives

Li Qiang Fu [a,1], Xin Sheng Guo [b,1], Xin Liu [a], Hui Li He [a], Yu Lin Wang [b], Yu She Yang [a,*]

[a] *Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*
[b] *School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110015, China*

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Abstract

In order to probe the effect of C-2(S)-substituted groups in the antibacterial activity, a series of novel C-2(S)-substituted pleuromutilin analogues of SB-225586 were synthesized and evaluated for their *in vitro* antibacterial activity. The results of antibacterial activities indicated that C-2(S)-substituted pleuromutilin derivatives retained appreciable antibacterial activity, and the 2-fluorination compounds **6a** and **6b** are more potent than the corresponding 2-hydroxylation analogues **7a** and **7b**.

Keywords: Synthesis; Pleuromutilin; C-2(S)-substituted; Antimicrobial activity

Pleuromutilin (Fig. 1) was first isolated in 1951 from basidiomycetes *Pleurotus* and *P. passeckerianus* and display modest activity against Gram-positive organisms *in vitro* [1,2]. Further studies have shown that this class of antibiotics interfere with bacterial protein synthesis via a specific interaction with the 23S rRNA of the 50S bacterial ribosome subunit [3]. These compounds offer a distinct profile and show no cross-resistance with any other class of antibiotics. Tiamulin and valnemulin as semisynthetic derivatives are currently employed as veterinary medicine to treat serious infections in swine and poultry [4,5]. GlaxoSmithKline's novel pleuromutilin analogue retapamulin [6], with excellent activity *in vitro*, was first approved for human use as a topical antimicrobial agent to treat skin infections in 2007.

Although semisynthetic pleuromutilin analogues generally exhibit potent antibacterial activity, they displayed poor oral absorption and a short half-life due to rapid metabolism and subsequent excretion. Further studies have shown that hepatic metabolism mediated by cytochrome P450 represents the major obstacle to developing a semisynthetic pleuromutilin for oral treatment of bacterial infections in the community [7]. Our previous research [8] has revealed that a water-soluble phosphate prodrug of pleuromutilin analogue SB-225586 exhibited potent protective effects against MSSA and MRSA strains *in vivo*, but a short half-life was also found (less than 1 h). However, reports have also shown that metabolic stability can be increased by involvement of fluorination or hydroxylation at C-2 [9]. In order to probe the effect of C-2(S)-substituted groups in the antibacterial activity, pleuromutilin analogues SB-225586

* Corresponding author.
E-mail address: ysyang@mail.shcnc.ac.cn (Y.S. Yang).
[1] Both the authors contributed equally to this work.

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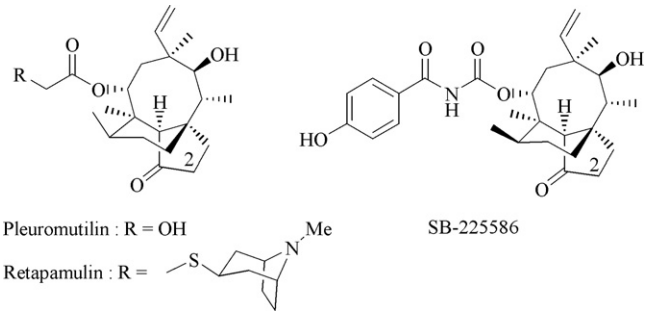


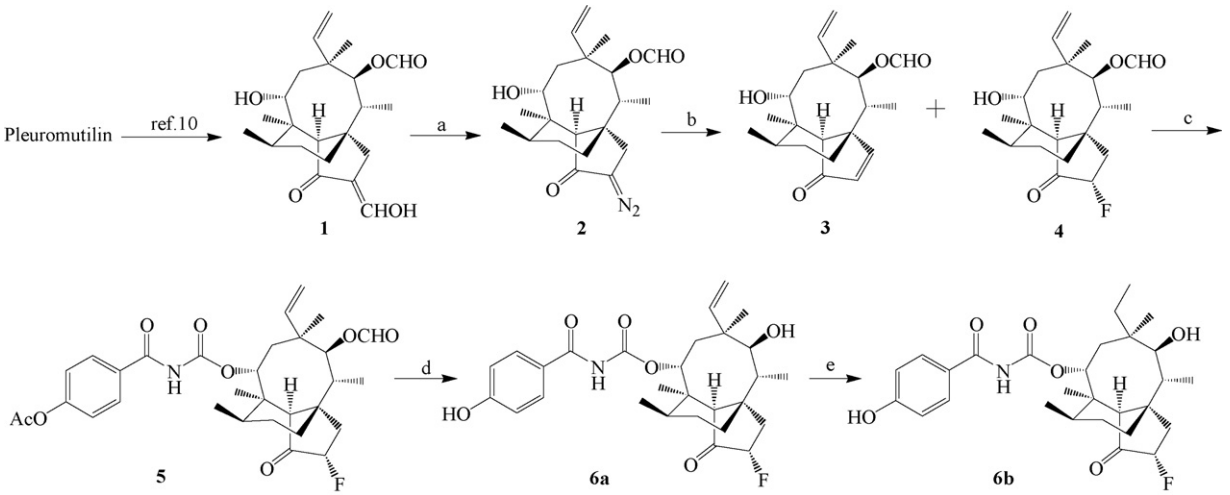
Fig. 1. Structures of pleuromutilin derivatives.

were selected as C-2 unsubstituted parent. In this letter, we report the synthesis and antibacterial activity of C-2(S)-substituted derivatives of SB-225586.

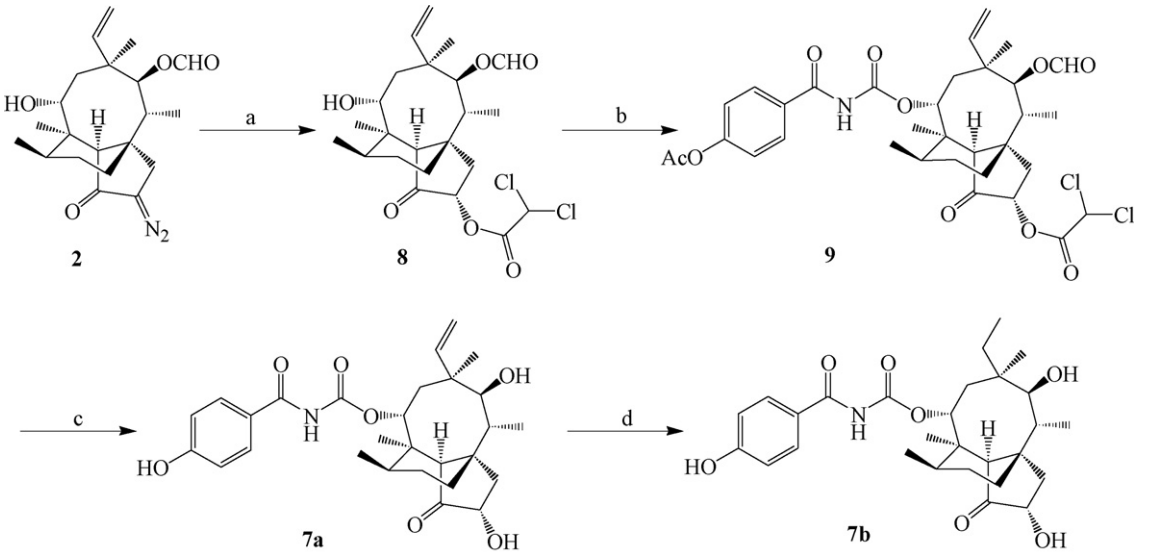
Compounds **6a** and **6b** were prepared as shown in Scheme 1. The 2-hydroxymethylene-11-formate **1** was synthesized form commercially available pleuromutlilin in two steps according to reported method [10]. A solution of **1** in dichloromethane was treated with tosylazide to generate α-diazo carbonyl intermediate **2** [10]. C-2(S) fluoro-substituted compound **4** was obtained by the reaction of **2** with hydrogen fluoride-pyridine in moderate yield [10]. The nucleophilic substitution was taken place from less hinder side and the main side product of this step was the enones **3** in 20–30% yield. Intermediate **5** was prepared in satisfying yield by treatment 4-acetoxybenzoyl chloride, silver cyanate with compound **4** in dichloromethane [11]. Then **5** was treated with 0.5 N potassium hydroxide in ethanol solution at ambient temperature to give the deprotected compound **6a** [12]. The reduced compounds **6b** was obtained by hydrogenation of the double bond in high yield (95.1%) without further purification.

Compounds **7a** and **7b** were prepared as shown in Scheme 2. A solution of **2** in dichloromethane was treated dropwise with dichloracetic acid. The mixture was stirred continued until the solution was colourless, compound **8** was obtained by silica gel chromatography [13], and the enones **3** was also observed as the main side product. Thus, **8** reacted with 4-acetoxybenzoyl chloride and silver cyanate successfully to produce compound **9**, which was subjected to deprotection and smoothly to afford the target compound **7a**. The reduced compounds **7b** was obtained by hydrogenation as compound **6b** [12].

The *in vitro* antibacterial activity of the compounds against a spectrum of resistant and susceptible Gram-positive bacteria was tested with SB-225586 and Linezolid as positive controls. Minimum inhibitory concentration (MIC) values were determined using agar dilution method according to NCCLS. The result is summarized in Table 1. It



Scheme 1. Regents and conditions: (a) TEA, TsN_3, CH_2Cl_2, −10 °C to r.t., 44.1%; (b) HF-Py, ether, −0 °C to r.t., 46.9%; (c) 4-acetoxybenzoyl chloride, CH_2Cl_2, AgOCN, 50 °C to r.t., 85.8%; (d) KOH, EtOH, r.t., 1 h, 88.3%; (e) H_2, 10% Pd-C, THF, 95.1%.



Scheme 2. Regents and conditions: (a) $Cl_2CHCOOH$, CH_2Cl_2, 0 °C to r.t., 58.1%; (b) 4-acetoxybenzoyl chloride, CH_2Cl_2, AgOCN, 50 °C to r.t., 72.9%; (c) KOH, EtOH, r.t., 1 h, 70.2%; (d) H_2, 10% Pd-C, THF, r.t., 96.0%.

Table 1
In vitro antibacterial activity of synthetic compounds.

Compounds	MIC(μg/mL) MSSA[a] $n = 5$	MRSA[b] $n = 6$	MSSE[c] $n = 5$	MRSE[d] $n = 5$	S.p.[e] $n = 3$
SB-225586	0.125	0.125	0.0625	0.125	0.25
6a	0.0625	0.0625	0.125	0.125	0.125
6b	0.0625	0.0625	0.0625	0.0625	0.125
7a	1	1	1	1	0.5
7b	0.5	0.5	0.5	0.5	0.25
Linezolid	0.5	1	0.5	1	1

[a] MSSA = Methicillin-susceptible *Staphylococcus aureus*.
[b] MRSA = Methicillin-resistant *Staphylococcus aureus*.
[c] MSSE = Methicillin-susceptible *Staphylococcus epidermidis*.
[d] MRSE = Methicillin-resistant *Staphylococcus epidermidis*.
[e] S.p. = *Streptococcus pneumoniae*.

clearly showed that all of the compounds displayed excellent antibacterial activities and most of the compounds had more potent activities compared with Linezolid. C-2(S) fluorination compounds **6a** and **6b** are were twofold more potent than 2-unsubstitued SB-225586, however, C-2(S) hydroxylation compounds **7a** and **7b** were less potent than references. Moreover, the double bond reduction compounds **6b** and **7b** are more potent than the corresponding vinyl analogues **6a** and **7b**.

In conclusion, a series of C-2(S)-substituted derivatives of pleuromutilin analogues SB-225586 were synthesized and evaluated. The results of antibacterial activities indicated that C-2(S)-substituted pleuromutilin derivatives retained appreciable antibacterial activity, especially the C-2(S)-fluoro derivatives **6a** and **6b** which antimicrobial activity has been obviously improved.

References

[1] F. Kavanagh, A. Hervey, W.J. Robbins, Proc. Natl. Acad. Sci. U.S.A. 37 (1951) 570.
[2] M. Anchel, J. Biol. Chem. 199 (1952) 133.
[3] C. Davidovich, A. Bashan, R. Yaggie, et al. Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 4291.
[4] K.M. Islam, U. Klein, G.S. Burch, Poult. Sci. 88 (2009) 2353.

[5] I.A. Aitken, J.H. Morgan, R. Dalziel, et al. Vet. Rec. 144 (1999) 128.
[6] S. Rittenhouse, S. Biswas, J. Broskey, et al. Antimicrob. Agents Chemother. 50 (2006) 3882.
[7] I. Schuster, C. Fleschurz, J. Hildebrandt, 13th Int. Cong. Chemother., Aug 28–Sept 2 Vienna, 1983, Abst PS 4.6/2-11.
[8] L.Q. Fu, Z.T. Jiang, Y.S. Yang, et al. Bioorg. Med. Chem. Lett. 19 (2009) 5407.
[9] E. Hunt, Drug Future 25 (2000) 1163.
[10] G. Brooks, E. Hunt, WO 00/07974 [P].
[11] G. Brooks, W. Burgess, D. Colthurst, et al. Bioorg. Med. Chem. 9 (2001) 1221.
[12] The data of **6a**, **6b**; **7a**, **7b**; **6a:** White solid, yield 88.3%, mp: 127–129 °C; $[\alpha]_D^{20}$ −4.7 (*c* 0.75, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (d, 3H, *J* = 7.0 Hz), 0.94 (d, 3H, *J* = 6.4 Hz), 1.07–1.38 (m, 4H), 1.08 (s, 3H), 1.39 (s, 3H), 1.80–1.95 (m, 4H), 2.05–2.10 (m, 2H), 2.36 (s, 1H), 3.36 (d, 1H), 4.79 (t, 1H, *J* = 8.1 Hz), 5.25 (d, 1H, *J* = 17.9 Hz), 5.36 (d, 1H, *J* = 11.2 Hz), 5.76 (d, 1H, *J* = 8.1 Hz), 6.41 (dd, 1H, *J* = 18.0 Hz, 11.2 Hz), 7.23 (d, 2H, *J* = 8.6 Hz), 7.80 (d, 2H, *J* = 8.5 Hz), 9.40 (s, 1H). MS (ESI) *m/z*: 500.2 ([M−H]$^-$); Anal. Calcd. for $C_{28}H_{36}FNO_6$:C, 67.23; H, 7.14; N, 2.95. Found: C, 67.05; H, 7.23; N, 2.79; **6b:** White solid, yield 95.1%, mp: 141–143 °C; $[\alpha]_D^{20}$ +5.6 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (d, 3H, *J* = 6.8 Hz), 0.91 (d, 3H, *J* = 6.7 Hz), 0.96 (t, 3H), 1.09–1.35 (m, 6H), 1.13 (s, 3H), 1.41 (s, 3H), 1.77–1.94 (m, 4H), 2.03–2.10 (m, 2H), 2.35 (s, 1H), 3.15 (d, 1H), 4.75 (t, 1H, *J* = 8.0 Hz), 5.74 (d, 1H, *J* = 8.2 Hz), 7.20 (d, 2H, *J* = 8.3 Hz), 7.79 (d, 2H, *J* = 8.4 Hz), 9.65 (s, 1H). MS (ESI) *m/z*: 502.2 ([M−H]$^-$); Anal. Calcd. for $C_{28}H_{38}FNO_6$:C, 66.61; H, 7.33; N, 2.76. Found: C, 66.78; H, 7.61; N, 2.78; **7a:** White solid, yield 70.2%, mp: 161–163 °C; $[\alpha]_D^{20}$ −1.6 (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (d, 3H, *J* = 7.1 Hz), 0.92 (d, 3H, *J* = 7.4 Hz), 1.05–1.39 (m, 4H), 1.10 (s, 3H), 1.36 (s, 3H), 1.60–1.86 (m, 4H), 2.06–2.10 (m, 2H), 2.39 (s, 1H), 3.32 (d, 1H), 4.04 (t, 1H, *J* = 8.1 Hz), 5.21 (d, 1H, *J* = 17.7 Hz), 5.39 (d, 1H, *J* = 11.0 Hz), 5.58 (d, 1H, *J* = 8.4 Hz), 6.20 (dd, 1H, *J* = 17.9 Hz, 11.4 Hz), 6.80 (d, 2H, *J* = 8.8 Hz), 7.66 (d, 2H, *J* = 8.4 Hz), 10.44 (s, 1H). MS (ESI) *m/z*: 498.2 ([M−H]$^-$); Anal. Calcd. for $C_{28}H_{38}NO_7$:C, 67.06; H, 7.27; N, 2.91. Found: C, 67.31; H, 7.43; N, 2.80; **7b:** White solid, yield 96.0%, mp: 192–194 °C; $[\alpha]_D^{20}$ +5.2 (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.81 (d, 3H, *J* = 7.2 Hz), 0.90 (d, 3H, *J* = 6.9 Hz), 0.96 (t, 3H), 1.08–1.40 (m, 6H), 1.14 (s, 3H), 1.34 (s, 3H), 1.65–1.91 (m, 4H), 2.09–2.13 (m, 2H), 2.43 (s, 1H), 3.19 (d, 1H), 4.09 (t, 1H, *J* = 7.9 Hz), 5.65 (d, 1H, *J* = 8.1 Hz), 6.87 (d, 2H, *J* = 8.3 Hz), 7.73 (d, 2H, *J* = 8.1 Hz), 10.87 (s, 1H). MS (ESI) *m/z*: 500.2 ([M−H]$^-$); Anal. Calcd. for $C_{28}H_{39}NO_7$:C, 66.87; H, 7.67; N, 2.92. Found: C, 67.04; H, 7.84; N, 2.79.
[13] G. Brooks, E. Hunt, WO 01/74788 [P].