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Synthesis and antibacterial activity of pleuromutilin derivatives with novel C(14) side chain

Li Qiang Fu, Chen Yu Ling, Xing Sheng Guo, Hui Li He, Yu She Yang *

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

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Abstract

In order to find novel antibacterial agents with superior antibacterial activity and overcoming multidrug resistance, a series of pleuromutilin derivatives with novel C(14) side chain were synthesized and evaluated for their *in vitro* antibacterial activities. The results of antibacterial acticities indicated that most of the derivatives showed potent activities against Gram-positive organisms. In particular, compound **10d** exhibited the most potent inhibitory activity compared with pleuromutilin and linezoid, emerged as potential molecule for further investigation.

Keywords: Synthesis; Pleuromutilin; Novel side chains; Antibacterial activity

Pleuromutilin (Fig. 1) was first isolated in 1951 from basidiomycetes *Pleurotus* and *P. passeckerianus* and displays modest activity against Gram-positive organisms *in vitro* [1,2]. Further studies have shown that this class of antibiotics interfered with bacterial protein synthesis via a specific interaction with the 23S rRNA of the 50S bacterial ribosome subunit [3]. These compounds offered a distinct profile and showed no cross-resistance with any other class of antibiotics. Tiamulin and valnemulin as semisynthetic pleuromutilin 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. Other pleuromutilin derivatives from Nabriva Therapeutics such as BC-3205, BC3781 and BC-7013 have entered clinical trials in 2009 [7].

Recently, several structure–activity relationship (SAR) studies demonstrated the importance of the substituent at C(14) in pleuromutilin analogues [8–12]. Poul Nielsen's group synthesized a series of pleuromutilin derivatives conjugated with different nucleoside fragments as side chain extensions by a click chemistry protocol, for example, derivative **1** (Fig. 1) [8]. In the publications reported by Hirokawa *et al.*, a series of structurally novel thioether pleuromutilin analogue bearing a purine were prepared and evaluated. From SAR studies, they identified the compound **2** (Fig. 1) showed not only excellent *in vitro* antibacterial activity against MRSA, PRSP and VRE, but also potent *in vivo* efficacy [9–12]. Inspired by these good results, we hypothesize that the hetero-aromatic substituent side chains may have unique interaction with domain II of the 50S bacterial ribosome subunit [3,8,13]. The increased interaction with

* Corresponding author.
E-mail address: ysyang@mail.shcnc.ac.cn (Y.S. Yang).

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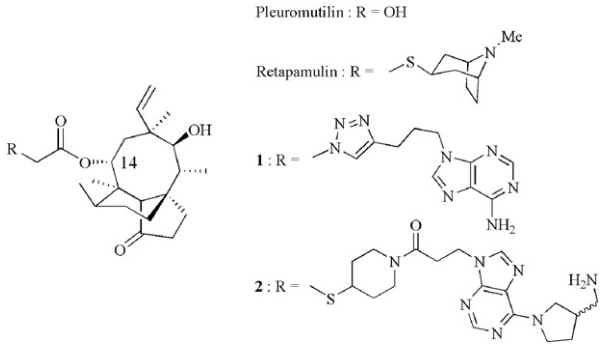
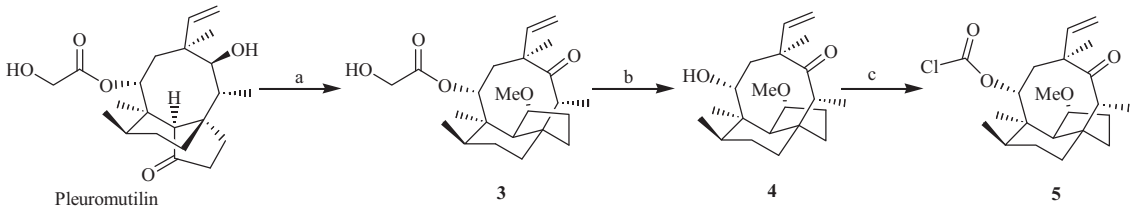


Fig. 1. Structures of pleuromutilin derivatives.

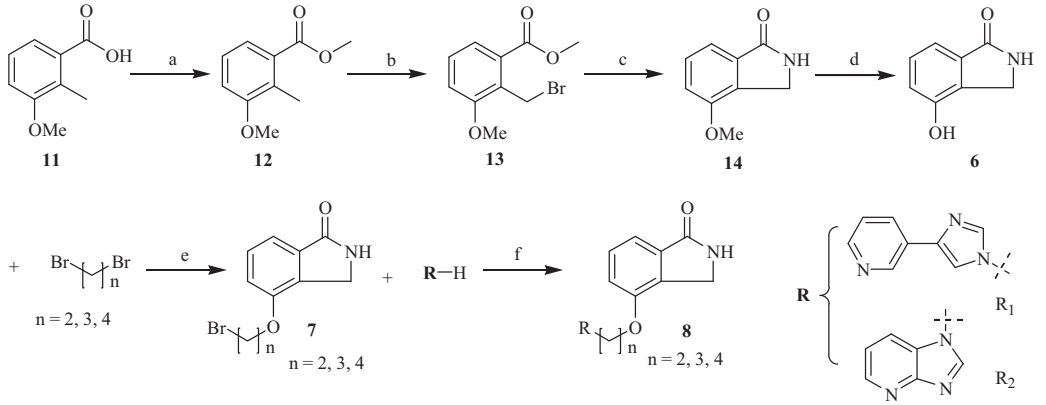
domain II was presumed to impart potent antibacterial activity. In this letter, we report the synthesis and antibacterial activities of pleuromutilin derivatives with substituted 4-hydroxyisoindolin-1(1*H*)-one as a novel C(14) side chain scaffold and heterocyclic fragments as side chain extensions.

The 4-*epi*-mutilin 14-chloroformate **5** was synthesized from commercially available pleuromutilin in three steps as shown in Scheme 1 [14]. The skeletally rearranged intermediate **4**, colloquially known as 4-*epi*-mutilin, was synthesized in two steps form pleuromutilin via an acid-catalysed 1,5-hydride shift and hydrolysis [15]. Then 14-chloroformate **5** was formed by treatment of 4-*epi*-mutilin with phosgene [16].

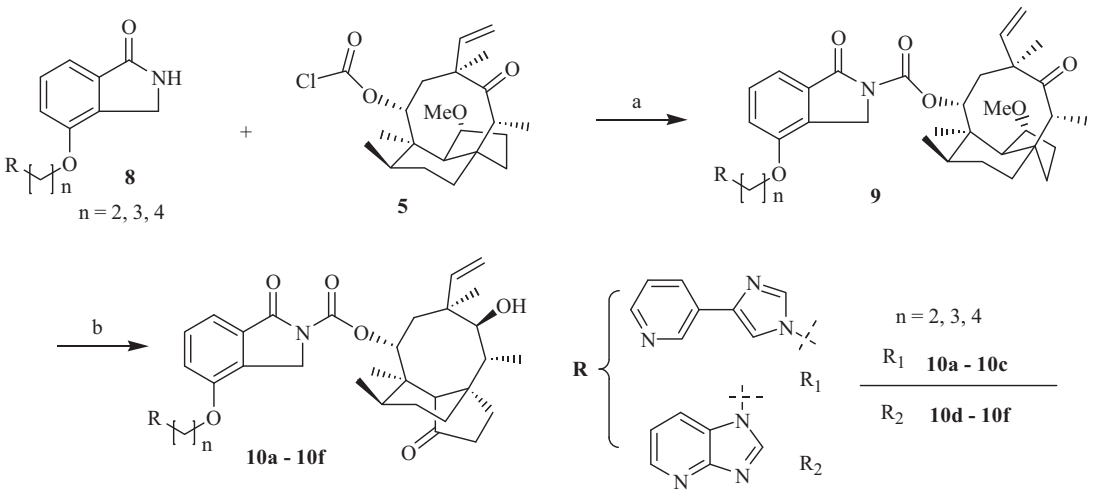
The scaffold 4-hydroxyisoindolin-1(1*H*)-one (**6**) was prepared from 2-methyl-3-methoxybenzoic acid (**11**) according to reported method as shown in Scheme 2 [17]. Thus, **11** was treated with thionyl chloride and methanol to obtain the corresponding methyl ester **12** in quantitative yield. Treatment of **12** with *N*-bromosuccinimide under radical conditions afforded the corresponding brominated derivative **13**, which was then cyclized in the presence of methanolic ammonia to obtain the isoindolinone derivative **14** in quantitative yield. The latter was finally



Scheme 1. *Reagents and conditions*: (a) $(CH_3O)_3CH$, MeOH, H_2SO_4, rt, 18 h, 90%; (b) NaOH, MeOH, H_2O, 65 °C, 2 h, 95%; (c) Py, phosgene, THF, 0 °C, 2 h, 76%.



Scheme 2. *Reagents and conditions*: (a) MeOH, $SOCl_2$, reflux, 6 h, 98%; (b) NBS, BPO, CCl_4, 80 °C, 85%; (c) NH_3–MeOH, reflux, 2 h, quantitative; (d) BBr_3, reflux, 6 h, 70%; (e) acetone, K_2CO_3, reflux, 3 h, 71–85%; (f) DMF, NaH, rt, 2 h, 36–52%.



Scheme 3. *Reagents and conditions*: (a) NaH, THF, rt, 6 h, 61–85%; (b) dioxane, $ZnCl_2$–con. HCl, 25 °C, 3 h, 51–70%.

demethylated by treatment with boron tribromide to obtain the desired intermediate (**6**) in 70% yield. Then alkylation of compound **6** to install the 2 to 4-carbon linker followed by coupling to selected heterocyclic fragments [18,19] afforded two regioisomers. The ratio of the *N*-alkylation product distribution was dependent on the nature of the alkyl group and reaction time. The objective compounds **8** was easily separated by column chromatography.

Finally, the pleuromutilin derivatives **10a**–**10f** were prepared as shown in Scheme 3. Completion of the synthesis required only the coupling of **5** and **8** to produce **9** followed by treatment with saturated solution of zinc chloride in hydruochloride acid to afford compounds **10a**–**10f** in satisfying yield [20].

The *in vitro* antibacterial activities of the compounds were tested against a spectrum of resistant and susceptible Gram-positive bacteria with pleuromutilin and linezolid as positive controls. Minimum inhibitory concentration (MIC) values were determined using agar dilution method according to NCCLS. The results were summarized in Table 1. It showed that most of the compounds displayed potent antibacterial activities and compounds **10a** and **10d** had more potent activities compared with linezolid. However, the antibacterial activity of compounds **10e** and **10f** which owned longer linker than **10d** greatly decreased. Moreover, compounds **10b** and **10c** were also less potent than references. A general trend is that compounds with a 2-carbon linker have better antibacterial potency than those with 3 or 4-carbon linker. As the result suggested, the length of the linker plays an important role in the activity of the compounds.

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)
10a	0.5	0.5	0.5	0.5	1
10b	2	2	2	2	2
10c	2	1	2	2	4
10d	0.25	0.25	0.25	0.25	0.25
10e	32	32	32	32	32
10f	16	8	16	16	32
Pleuromutilin	2	2	2	2	2
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*.

In conclusion, a series of pleuromutilin derivatives with novel C(14) side chain were synthesized and evaluated. The results of antibacterial acticities indicated that most of the pleuromutilin derivatives retained appreciable antibacterial activities and the length of the linker played an important role. In particular, the compound **10d** exhibited the most potent inhibitory compared with linezoid, which emerged as potential molecule for further investigation.

Acknowledgments

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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] R. Novak, D.M. Shlaes, Curr. Opin. Invest. Drugs 11 (2010) 182.
[8] L. Lolk, J. Pohlsgaard, A.S. Jepsen, et al. J. Med. Chem. 51 (2008) 4957.
[9] Y. Hirokawa, H. Kinoshita, T. Tanaka, et al. J. Med. Chem. 51 (2008) 1991.
[10] Y. Hirokawa, H. Kinoshita, T. Tanaka, et al. Bioorg. Med. Chem. Lett. 18 (2008) 3556.
[11] Y. Hirokawa, H. Kinoshita, T. Tanaka, et al. Bioorg. Med. Chem. Lett. 19 (2009) 170.
[12] Y. Hirokawa, H. Kinoshita, T. Tanaka, et al. Bioorg. Med. Chem. Lett. 19 (2009) 175.
[13] R. Berisio, N. Corti, P. Pfister, et al. Antimicrob. Agents Chemother. 50 (2006) 3816.
[14] Y. Andemichael, J. Chen, J.S. Clawson, et al. Org. Process Res. Dev. 13 (2009) 729.
[15] H. Berner, G. Schulz, H. Schneider, Tetrahedron 36 (1980) 1807.
[16] G. Brooks, W. Burgess, D. Colthurst, et al. Bioorg. Med. Chem. 9 (2001) 1221.
[17] R. Pellicciari, E. Camaioni, G. Costantino, et al. Chem. Med. Chem. 3 (2008) 914.
[18] S. Kumar, D. Jaller, B. Patel, et al. J. Med. Chem. 51 (2008) 4968.
[19] H. Yoshizawa, H. Itani, K. Ishikura, et al. J. Antibio. 55 (2002) 975.
[20] Selected data of title compounds: **10a**: White solid, yield 59%, mp: 145–146 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.02 (d, 1H, J = 2.0 Hz), 8.50 (dd, 1H, J = 3.1 Hz, 1.5 Hz), 8.31 (d, 1H, J = 8.0 Hz), 7.85 (s, 1H), 7.40–7.55 (3m, H), 7.06 (d, 1H, J = 8.1 Hz), 6.87 (m, 1H), 6.58 (dd, 1H, J = 17.3, 11.1 Hz), 5.90 (d, 1H, J = 8.8 Hz), 5.39 (d, 1H, J = 11.0 Hz), 5.22 (d, 1H, J = 17.3 Hz), 4.62 (s, 2H), 4.50 (m, 2H), 4.41 (m, 2H), 3.40 (br, 1H), 2.38 (t, 1H, J = 6.6 Hz), 2.10–2.30 (m, 4H), 1.10–1.80 (m, 8H), 1.28 (s, 3H), 1.19 (s, 3H), 0.92 (d, 3H, J = 6.7 Hz), 0.75 (d, 3H, J = 6.6 Hz); MS (ESI) m/z (689.1 $[M+Na]^+$); Anal. Calcd. for $C_{39}H_{46}N_4O_6$: C, 70.25; H, 6.95; N, 8.40. Found: C, 70.22; H, 7.00; N, 8.37. **10d**: White solid, yield 63%, mp: 166 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (s, 1H), 8.46 (d, 1H, J = 4.5 Hz), 8.20 (d, 1H, J = 8.1 Hz), 7.46 (d, 1H, J = 6.7 Hz), 7.30–7.42 (m, 2H), 6.95 (d, 1H, J = 7.6 Hz), 6.60 (dd, 1H, J = 17.3, 11.1 Hz), 5.92 (d, 1H, J = 8.8 Hz), 5.40 (d, 1H, J = 11.3 Hz), 5.21 (d, 1H, J = 17.5 Hz), 4.82 (m, 2H), 4.60 (s, 2H), 4.50 (m, 2H), 3.40 (br, 1H), 2.38 (t, 1H, J = 6.6 Hz), 2.10–2.30 (m, 4H), 1.10–1.80 (m, 8H), 1.28 (s, 3H), 1.20 (s, 3H), 0.91 (d, 3H, J = 7.1 Hz), 0.76 (d, 3H, J = 6.2 Hz); MS (ESI) m/z (663.1 $[M+Na]^+$); Anal. Calcd. for $C_{37}H_{44}N_4O_6$: C, 69.35; H, 6.92; N, 8.74. Found: C, 69.28; H, 7.11; N, 8.78.