The Journal of Antibiotics (2018) 71:672–676
https://doi.org/10.1038/s41429-018-0051-1

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

BRIEF COMMUNICATION

Glycosylated piericidins from an endophytic streptomyces with cytotoxicity and antimicrobial activity

Ning-Ning Shang[1,2] · Zhouxin Zhang[1] · Jian-Ping Huang[1] · Li Wang[1] · Jianying Luo[1] · Jing Yang[1] · Teng Peng[2] · Yijun Yan[1] · Ya-Tuan Ma[1] · Sheng-Xiong Huang[1]

Received: 15 December 2017 / Revised: 19 February 2018 / Accepted: 7 March 2018 / Published online: 12 April 2018

Abstract

Two new glycosylated piericidins, glucopiericidinol A_3 (**1**) and 7-demethyl-glucopiericidin A (**2**), along with four known analogs were isolated from the culture broth of *Streptomyces* sp. KIB-H1083. The chemical structures of new compounds were elucidated by spectroscopic analyses. Their cytotoxicity on HL-60, SMMC-772, A-549, MCF-7, and SW480 cell lines, as well as antimicrobial activities was evaluated. The results showed that glucopiericidin A (**4**) has potent cytotoxicity against HL-60, SMMC-772, A-549, and MCF-7 cell lines with IC_{50} values of 0.34, 0.65, 0.60, and 0.50 μM, respectively. For the antimicrobial activity, piericidin A (**6**) showed most powerful inhibitory activities against *Xanthomonas oryzae* pv. *oryzicola*, and *Penicillium decumbens*.

Microbes have played a very important role in the production of natural antimicrobial drugs [1]. So far, more than 23,000 biologically active compounds produced by microbes have been reported [2]. Furthermore, 45% of the reported microbial metabolites is produced by actinomycetes, with ~75% of metabolites obtained from the genus *Streptomyces* [1, 3]. However, as the increasing problem of drug resistance and the human's growing desire of health, the development of new antibiotics and anticancer agents has become a major problem to be solved [4]. In the continuation of our chemical and biological screenings of extracts libraries from endophytes (mainly actinomycetes) [5-7], the extract of *Streptomyces* sp. KIB-H1083, which is an endophyte isolated from traditional Chinese medicinal plant *Diaphasiastrum veitchii*, indicated antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. In this context, we describe the isolation, structure elucidation, cytotoxicity, and antimicrobial evaluation of two new glycosylated piericidins, glucopiericidinol A_3 (**1**) and 7-demethyl-glucopiericidin A (**2**), as well as four known analogs from the fermentation broth of strain KIB-H1083.

The fermentation broth of strain KIB-H1083 was centrifuged to obtain supernatant and mycelia cake, which were extracted with EtOAc and acetone, respectively. Both extracts were combined and then purified using various chromatographic techniques. Two new glycosylated piericidin derivatives, glucopiericidinol A_3 (**1**) and 7-demethyl-glucopiericidin A (**2**), as well as four known congeners BE-14324-113 (**3**) [8], glucopiericidin A (**4**) [9], glucopiericidinol A (**5**) [10], and piericidin A (**6**) [11] were obtained.

Compound **1** was obtained as faint yellow oil, with the molecular formula, $C_{37}H_{57}NO_{15}$ (10 double-bond equivalents), as derived from high-resolution ESI mass spectrometry ($[M + Na]^+$ at *m/z* 778.3633, calcd 778.3620). The IR spectrum exhibited absorption bands for hydroxyl (3420 cm^{-1}) and aromatic ring (1606, 1575, and 1471 cm^{-1})

These authors contributed equally: Ning-Ning Shang and Zhouxin Zhang.

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41429-018-0051-1) contains supplementary material, which is available to authorized users.

✉ Sheng-Xiong Huang
sxhuang@mail.kib.ac.cn

[1] State Key Laboratory of Phytochemistry and Plant Resources in West China, and Yunnan Key Laboratory of Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences, 650201 Kunming, China

[2] Key Laboratory of Standardization of Chinese Herbal Medicine, State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicine Resources, Ministry of Education, College of Pharmacy, Chengdu University of Traditional Chinese Medicine, 611137 Chengdu, China

Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for compounds **1** and **2** in CD_3OD

No.	1		2	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	6.76 (d, $J = 15.3$)	124.1	3.34 (m)	35.5
2	6.82 (d, $J = 15.3$)	141.8	5.28 (t, $J = 7.0$)	123.6
3		74.3		135.9
4	2.42 (dd, $J = 13.8$, 6.8)	47.6	2.73 (d, $J = 7.1$)	44.1
	2.38 (dd, $J = 13.8$, 8.1)			
5	5.65 (dt, $J = 15.5$, 7.4)	123.8	5.53 (dt, $J = 14.2$, 7.1)	131.4
6	6.16 (d, $J = 15.5$)	139.6	6.05 (m)	131.2
7		134.8	6.05 (m)	133.5
8	5.43 (d, J = 9.2)	136.4	5.79 (m)	137.1
9	2.79 (m)	37	2.44 (m)	40.6
10	3.74 (m)	94	3.67 (m)	93.8
11		136.6		136.8
12	5.47 (q, $J = 6.8$)	125	5.45 (q, $J = 6.8$)	124.9
13	1.63 (d, $J = 6.8$)	13.3	1.62 (d, $J = 6.8$)	13.5
14	1.61 (s)	12	1.59 (s)	11.9
15	0.82 (d, $J = 6.9$)	17.9	0.84 (d, $J = 7.0$)	17.4
16	1.75 (s)	13.5		
17	1.34 (s)	27.8	1.73 (s)	16.8
1′		145.8		151.8
2′		114.9		114.5
3′		156.8[a]		157.4
4′		130.7		130.3
5′		156.1		156.2
6′	2.14 (s)	10.5	2.03 (s)	11
7′	3.76 (s)	61	3.73 (s)	61
8′	3.92 (s)	53.4	3.90 (s)	53.9
1″	4.25 (d, $J = 7.9$)	104.2	4.24 (d, $J = 7.8$)	104.2
2″	3.16 (t, $J = 8.5$)	76	3.19 (t, $J = 8.6$)	75.9
3″	3.30 (m)	78.3	3.30 (m)	78.4
4″	3.37 (m)	71.4	3.32 (m)	71.4
5″	3.32 (m)	76.6	3.31 (m)	76.6
6″	3.95 (overlap)	67.3	3.93 (m)	67.4
	3.55 (brd, $J = 10.6$)		3.55 (dd, $J = 10.8$, 2.3)	
1‴	4.84 (d, $J = 3.0$)	100.4	4.83 (d, $J = 3.3$)	100.5
2‴	3.74 (m)	70.7	3.74 (m)	70.7
3‴	3.75 (m)	71.8	3.75 (m)	71.8
4‴	3.88 (m)	71.1	3.88 (m)	71.2
5‴	3.85 (m)	72.3	3.84 (m)	72.4
6‴	3.69 (d, $J = 6.0$)	62.8	3.38 (m)	62.7

Assignments were done by HSQC, ^{1}H-^{1}H COSY, and HMBC experiments

[a] Weak signal, shift taken from HMBC spectrum

functional groups. The ^{1}H and ^{13}C NMR (Table 1) spectra of **1** revealed the presence of 37 carbon atoms. The substructures of C-1 to C-2, C-4 to C-6, C-8 to C-10, C-12 to C-13, C-1″ to C-6″, and C-1‴ to C-6‴ was identified by the ^{1}H-^{1}H COSY spectrum (Fig. 1). In the HMBC experiment, correlations were observed from H_3-17 to C-2, C-3, C-4, from H_3-16 to C-6, C-7, C-8, and from H_3-14 to C-10, C-11, C-12, thereby revealed the connection of these partial structures. Further HMBC correlations from H_3-6′ to C-1′, C-2′, and C-3′, from H_3-7′ to C-4′, and from H_3-8′ to C-5′ defined a fully substituted pyridine ring. HMBC correlations from H_2-1 to C-1′ and C-2′ revealed that C-1 to C-13 chain was connected to the pyridine ring at C-1′ (Fig. 1). The ^{13}C-chemical shifts of C-3 and C-10 (74.3 and 94.0 ppm, respectively) suggested that both carbons were oxygenated. This was supported by the similarities of ^{1}H and ^{13}C NMR signals between **1** and the known compounds, glucopiericidinol A (**5**). [10] However, a set of signals at δ_C 100.4 (C-1‴), 70.7 (C-2‴), 71.8 (C-3‴), 71.1 (C-4‴), 72.3 (C-5‴), and 62.8 (C-6‴), which did not existed in the ^{13}C NMR spectrum of **5**, was exhibited in that of **1**. On the other hand, the molecular weight variation (Δ 162) between **1** and **5** suggested that **1** possessed one more glycosyl group than **5**. The ^{1}H and ^{13}C NMR data of **1** exhibited specific signals for the α-galactopyranose of **1** [δ_H 4.84 (d, $J = 3.0$ Hz, H-1‴), 3.74 (m, H-2‴), 3.75 (m, H-3‴), 3.88 (m, H-4‴), 3.85 (m, H-5‴), and 3.69 (d, $J = 6.0$ Hz, H-6‴). [12, 13] The galactopyranose in **1** is bonded to C-6″ according to the correlations in the HMBC spectrum from H-1‴ to C-6″ (δ_C 67.3). The geometry of the disubstituted olefins Δ^1 and Δ^5 was

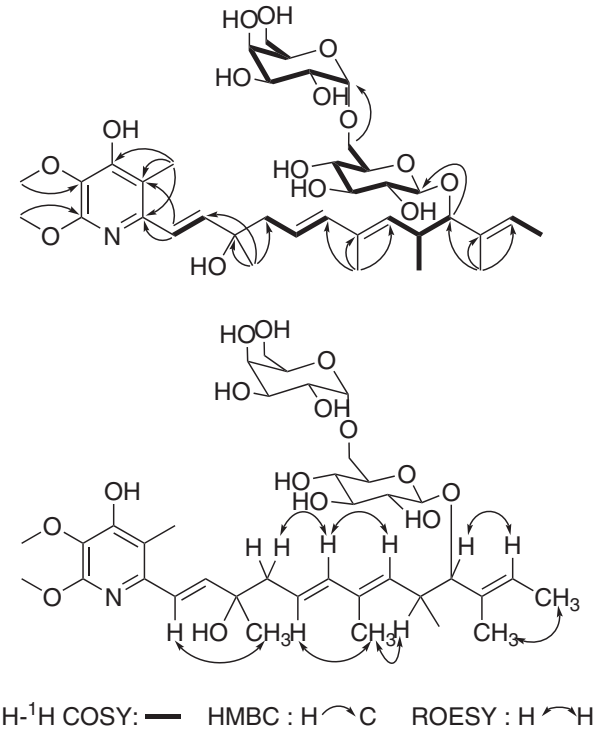

Fig. 1 Key 2D NMR correlations of compound **1**

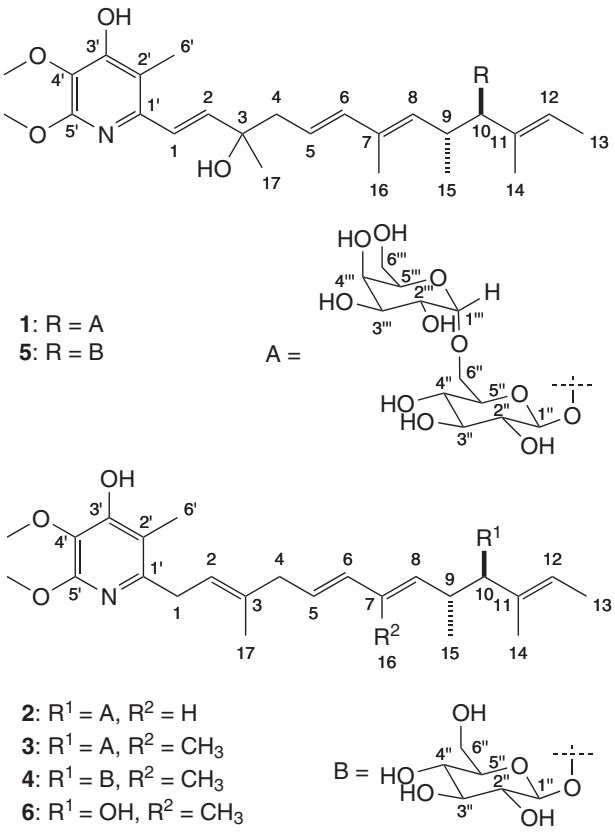

Fig. 2 The chemical structures of compounds **1**–**6**

determined to be *E* type, respectively, from the large vicinal proton coupling constant ($J_{1,2} = 15.3$ Hz and $J_{5,6} = 15.5$ Hz). The geometry of the trisubstituted olefins Δ^7 and Δ^{11} was determined by ROESY experiments (Fig. 1). ROE correlations H-6/H-8 and H-10/H-12 indicated the geometry of Δ^7 and Δ^{11} were all *E* type. Thus, the structure of **1** (6′′-*O*-*α*-galactopyranosyl glucopiericidinol A) was identified as shown in Fig. 2.

7-demethyl-glucopiericidin A (**2**) was also obtained as faint yellow oil. Compound **2** was found to possess the molecular formula $C_{36}H_{55}NO_{14}$ from the HRESIMS data (*m/z* 726.3698 $[M+H]^+$, calcd for 726.3695), indicating an unsaturation index of ten. The ^1H and ^{13}C NMR spectra of **2** (Table 1) were quite similar to those of **1**. Through the comparison of ^{13}C NMR data at pyridine ring and sugar moiety, the structure of pyridine ring and sugar moiety was concluded to be the same with that of **1**. However, a signal at δ_H 1.75 (s, H-16) observed in the ^1H NMR spectrum of glucopiericidinol A3 (**1**) disappeared in that of 7-demethyl-glucopiericidin A (**2**). On the other hand, a signal at δ_H 6.05 (m, H-7), which was not existed in the ^1H NMR spectrum of glucopiericidinol A3 (**1**), was exhibited in that of 7-demethyl-glucopiericidin A (**2**). Further HMBC correlation from H_2-1 to C-2, C-3, from H-2 to C-1, C-4, C-17, and from H_3-17 to C-2, C-3, C-4 revealed the side chain as shown in Fig. 2. This was supported by the NMR data similarities between **2** and 7-demethylpiericidin A_1 indicated that both compound **2** and 7-demethylpiericidin A_1 contained the same polyketide chain [14], and the HMBC from δ_H 4.24 to δ_C 93.8 showed the position (C-10) of the sugar moiety. Thus, the structure of **2** was identified as shown in Fig. 2.

Table 2 Cytotoxic activities (IC_{50} [μM]) of compounds **1**–**6**

Compound	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	>40	>40	>40	>40	>40
2	>40	21.66	22.78	10.88	>40
3	>40	>40	>40	>40	>40
4	0.34	0.65	0.60	0.50	>40
5	2.08	5.32	9.92	2.69	32.59
6	>40	>40	>40	>40	>40
Cisplatin[a]	1.81	9.62	8.58	13.93	13.73
Paclitaxel[a]	<0.008	<0.008	<0.008	<0.008	<0.008

[a] Positive control.

Cytotoxic activities of the six piericidins (**1**–**6**) were evaluated against HL-60, SMMC-7721, A-549, MCF-7, and SW480 tumor cell lines by MTS method [15]. Compounds **1**, **3**, and **6** were inactive against all six tumor cell lines (IC_{50} values >40 μM). Compound **2** exhibited moderate cytotoxicity against SMMC-7721, A-549, and MCF-7 cell lines with the IC_{50} values of 21.66, 2.78, and 10.88 μM, respectively. Compounds **4** and **5** showed significant cytotoxicity to HL-60, SMMC-7721, A-549, and MCF-7 cell lines with the IC_{50} values ranging from 0.34 to 0.65 μM and from 2.08 to 9.92 μM, respectively (Table 2). It's the first report for the cytotoxicity of compounds **4** and **5** to HL-60, SMMC-7721, A-549, and MCF-7 cell lines.

Compounds **1**–**6** were also assessed for their in vitro antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Xanthomonas oryzae* pv. *oryzicola*, and *Penicillium decumbens* by the filter paper method [16]. The result demonstrated that compounds **1**, **4**, and **6** exhibited potent inhibitory activities against *Staphylococcus aureus* and *Bacillus subtilis* (Table 3). At the same time, compound **6** showed powerful inhibitory activities against *Xanthomonas oryzae* pv. *oryzicola* and *Penicillium decumbens*.

In this study, we have isolated and characterized two new glycosylated piericidins, glucopiericidinol A_3 (**1**) and 7-demethyl-glucopiericidin A (**2**), together with four known piericidins from a plant-derived endophytic *Streptomyces* sp. KIB-H1083. All the isolates were evaluated for their cytotoxic and antimicrobial activities. Piericidins are some of the most common compounds exiting in the metabolite of actinomycetes. More than 40 piericidins, including nine piericidin glycosides, have been isolated to date [17].

Table 3 Antimicrobial activities (zone [mm]) of compounds **1–6**

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Xanthomonas oryzae* pv. *oryzicola*	*Penicillium decumbens*
1	9.8 ± 0.3	7.3 ± 0.3	0	0
2	0	0	0	0
3	0	0	0	0
4	8.3 ± 0.3	8.1 ± 0.2	0	0
5	6.0	0	0	0
6	7.2 ± 0.3	8.0 ± 0.5	30.5 ± 0.5	10.2 ± 0.3
Carbendazim[b]				37.7 ± 0.3
Kanamycin[b]	13.3 ± 0.3	25.0 ± 0.5	29.8 ± 0.3	
DMSO[c]	0	0	0	0

[a] The antimicrobial activities were evaluated at 20 μg/disk

[b] Positive control

[c] Blank control

Previous studies showed that piericidins are potent inhibitors of both mitochondrial and bacterial NADH-ubiquinone oxidoreductase (complex I), therefore leading to their strong antimicrobial activity. In addition, structure activity relationships studies indicate that the sugar component of the piericidin glycosides is important in modulating their physiological activities [18]. In this study, piericidin A (**6**) with no sugar moiety showed the most powerful antimicrobial activity without cytotoxicity, however, glucopiericidin A (**4**) has powerful antitumor activity. Above observation may indicate that the antimicrobial activity and cytotoxicity of different piericidins have distinct modes of action.

Glucopiericidinol A_3 (**1**). Faint yellow oil. $[\alpha]_{24.2\ D}$ + 35.6 (*c* 0.19, MeOH). UV (MeOH): 295 (3.89), and 220 (4.46) nm. IR (KBr): 3420, 2927, 1709, 1626, 1606, 1575, 1471, 1411, 1385, 1307, 1259, 1229, 1194, 1150, 1077, 1038, and 974 cm^{-1}.^{1}H- and ^{13}C-NMR data, see Table 1. HRESIMS: *m/z* 778.3633 $[M + Na]^+$ (calcd for $C_{37}H_{57}NO_{15}Na^+$, 778.3620).

7-demethyl-glucopiericidin A (**2**). Faint yellow oil. $[\alpha]_{18.7\ D}$ + 61.9 (*c* 0.05, MeOH). UV (MeOH): 268 (1.49), 228 (2.05), and 204 (2.19) nm. IR (KBr): 3417, 3407, 2927, 1630, 1610, 1587, 1473, 1412, 1355, 1251, 1191, 1151, 1126, 1076, 1044, and 973 cm^{-1}. ^{1}H- and ^{13}C-NMR data, see Table 1. HRESIMS: *m/z* 726.3698 $[M + Na]^+$ (calcd for $C_{36}H_{55}NO_{14}Na^+$, 726.3695).

Acknowledgements This work was financially supported by the National Natural Science Foundation of China to S-XH. (Nos. 81522044 and U1702285), Applied Basic Research Foundation of Yunnan Province to Y-TM and S-XH (Nos. 2016FB021 and 2013HA022), and foundation from Chinese Academy of Sciences to S-XH (QYZDB-SSW-SMC051).

Author Contribution S-XH designed the research, and ZZ and J-PH wrote the paper. NNS and ZZ performed experiments. JYL, JY, YY, and TP analyzed the NMR data and performed the structures determination of isolated compounds. LW and S-XH corrected the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Solecka J, Zajko J, Postek M, Rajnisz A. Biologically active secondary metabolites from Actinomycetes. Open Life Sci. 2012;7:373–90.
2. Demain AL, Sanchez S. Microbial drug discovery: 80 years of progress. J Antibiot. 2009;62:5–16.
3. Demain AL. Importance of microbial natural products and the need to revitalize their discovery. J Ind Microbiol Biot. 2014;41:185–201.
4. D'Costa VanessaM, et al. Antibiotic resistance is ancient. Nature. 2011;477:457–61.
5. Cao P, et al. New duclauxamide from *Penicillium manginii* YIM PH30375 and structure revision of the duclauxin family. Org Lett. 2015;17:1146–9.
6. Yan Y, et al. Tropolone ring construction in the biosynthesis of rubrolone B, a cationic tropolone alkaloid from endophytic Streptomyces. Org Lett. 2016;18:1254–7.
7. Yang R, et al. Lorneic acid analogues from an endophytic actinomycete. J Nat Prod. 2017;80:2615–9.
8. Oka, H et al. Antitumor glycosides BE-14324 and their manufacture with Streptomyces. JP 03081283 (1991).
9. Matsumoto M, et al. New piericidin glucosides, glucopiericidins A and B. J Antibiot. 1987;40:149–56.
10. Funayama S, et al. Novel cytocidal antibiotics, glucopiericidinols A1 and A2. J Antibiot. 1989;42:1734–40.
11. Yoshida S, Yoneyama K, Shiraishi S, Watanabe A, Takahashi N. Chemical structures of new piericidins produced by Streptomyces pactum. Agric Biol Chem. 1977;41:855–62.
12. Wu J, Zhang S, Li Q, Huang J, Xiao Z, Long L. Two new cyclolignan glycosides from *Acanthus ilicifolius*. *Z.* Naturforsch B. 2004;59:341–4.
13. Luo JG, Ma L, Kong LY. New triterpenoid saponins with strong α-glucosidase inhibitory activity from the roots of Gypsophila oldhamiana. Bioorg Med Chem. 2008;16:2912–20.

14. Kimura KI, Takahashi H, Miyata N, Yoshihama M, Uramoto M. New piericidin antibiotics, 7-demethylpiericidin A1 and 7-demethyl-3'-rhamnopiericidin A1. J Antibiot. 1996;49:697–9.
15. Monks A, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer I. 1991;83:757–66.
16. Hamid AA, et al. Chemical composition, antimicrobial and free radical scavenging activities of *Grewia pubescens*. Chem Int. 2016;2:254–61.
17. Zhou X, Fenical W. The unique chemistry and biology of the piericidins. J Antibiot. 2016;69:582–93.
18. Helmke E, Kelter G, Laatsch H, Fiebig HH, Shaaban KA. Glucopiericidin C: a cytotoxic piericidin glucoside antibiotic produced by a marine *Streptomyces* isolate. J Antibiot. 2011;64:205–9.