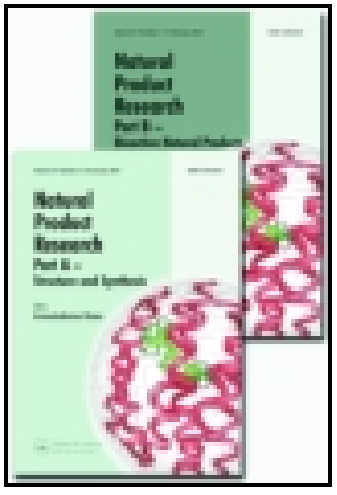

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A new curvularin glycoside and its cytotoxic and antibacterial analogues from marine actinomycete *Pseudonocardia* sp. HS7

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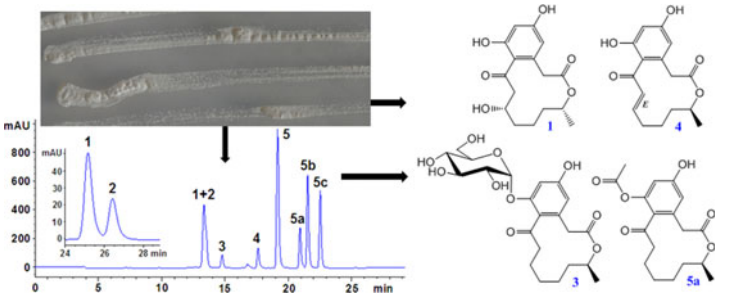

Five curvularin macrolides (**1**–**5**) were isolated from the cultured broth of marine actinomycete *Pseudonocardia* sp. HS7 that was obtained from the cloacal aperture of sea cucumber *Holothuria moebii*. The structures of these isolates were characterized as (11*S*,15*R*)-11-hydroxycurvularin (**1**), (11*R*,15*R*)-11-hydroxycurvularin (**2**), curvularin-7-*O*-α-D-glucopyranoside (**3**), *trans*-dehydrocurvularin (**4**) and curvularin (**5**) based on their NMR and HRESIMS data as well as chemical degradation. Compound **3** is a new macrolide with a rare α-D-glucopyranose substituent. Compounds **1**–**4**, **5a** and **5c** (the acyl products of **5**), suppressed the proliferation of all six tested cancer cell lines and **4** is the most active compound with IC_{50} values ranging from 0.59 to 3.39 μM. The 11-hydroxycurvularins **1** and **2** also showed antibacterial activity inhibiting the growth of *Escherichia coli*.

Keywords: *Pseudonocardia* sp. HS7; curvularin glycoside; cytotoxic; antibacterial activity

1. Introduction

Gliomas are one of the most challenging cancers to treat and account for 80% of all malignant brain tumours. Chemotherapy is an important adjunctive therapy for treating gliomas and temozolomide (TMZ) is the only drug that has been independently used for the treatment of gliomas. The efficacy of the currently used anticancer drugs including TMZ is limited (Chamberlain 2010; Patil et al. 2013). There is therefore a need to discover lead compounds for the development of novel anti-glioma drugs. Natural products are highly significant sources of new drug leads (Newman & Cragg 2012).

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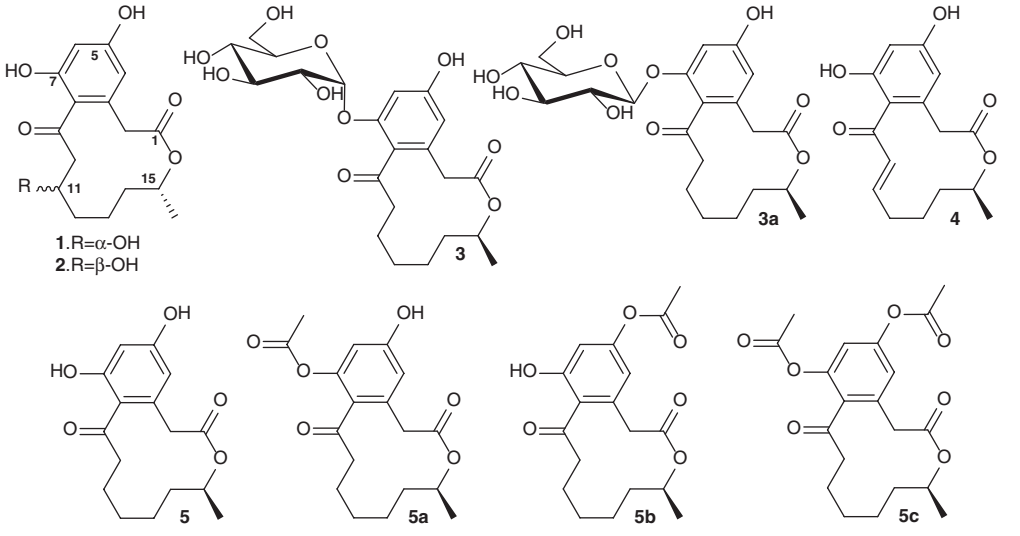

Figure 1. Structures of compounds **1**–**5**, **3a** and **5a**–**5c**.

During the course of our ongoing programme for the discovery of novel anti-glioma agents from natural sources (Xin et al. 2012; Ye et al. 2014; Yu, Ye, Chen et al. 2014; Yu et al. 2015; Yu, Ye, Xin et al. 2014), the cultured broth of marine actinomycete strain HS7 isolated from the cloacal aperture of sea cucumber *Holothuria moebii* was found to be active against the proliferation of glioma cells. The strain HS7 was identified by 16S rDNA sequence analysis and its top16S rDNA sequence was 100% sequence match to *Pseudonocardia antitumoralis* strain SCSIO 01299. This actinomycete strain SCSIO 01299 was recently isolated as a new species from deep-sea sediment collected from the northern South China (Tian et al. 2013). Previously chemical investigation indicated that strain SCSIO 01299 produced deoxynyboquinone and three new diazaanthraquinone derivatives of pseudonocardians A, B and C. These diazaanthraquinones were shown to have potent cytotoxic and antibacterial activities (Li et al. 2011).

In this study, chromatographic separation of an active EtOAc extract prepared from the cultured actinomycete strain HS7 afforded five curvularin analogues (**1**–**5**, Figure 1). Three acyl products (**5a**–**5c**) of curvularin (**5**) were also prepared for bioactivity assay. Compound **3** is a new curvularin macrolide glycoside with a rare α-D-glucopyranose substituent and **5a** is a new synthetic compound. We herein report the isolation and structural elucidation of these compounds and their activities inhibiting the proliferation of cancer cells and the growth of *Staphylococcus aureus* and *Escherichia coli*.

2. Results and discussion

Marine actinomycete strain HS7 was isolated from the cloacal aperture of sea cucumber *H. moebii*. The taxonomic identity of this isolated actinomycete was determined by 16S rDNA sequence analysis. The top sequence of HS7 was 100.0% sequence similarity to *P. antitumoralis* strain SCSIO 01299 (accession number: NR_109460.1) and 99% sequence similarity to other nine *Pseudonocardia* sp. strains Table S1) in the GenBank database. Therefore, the taxonomy of actinomycete HS7 was proposed to be *Pseudonocardia* sp. HS-7.

An EtOAc extract obtained from the cultured broth of strain HS7 showed inhibitory activity against glioma cells. This active EtOAc crude was separated by HPLC to afford compounds **1**–**5**.

Based on NMR and HRESIMS spectral analyses and comparison with published NMR data, compounds **1**, **2**, **4** and **5** were identified as (11*S*,15*R*)-11-hydroxycurvularin (**1**), (11*R*,15*R*)-11-hydroxycurvularin (**2**), *trans*-dehydrocurvularin (**4**) and curvularin (**5**) (Lai et al. 1989; Greve et al. 2008; Dai et al. 2010).

Compound **3** had a molecular formula of $C_{22}H_{30}O_{10}$ deduced from its HRESIMS at *m*/*z* $[M + Na]^+$ 477.1730 (calcd for $C_{22}H_{30}NaO_{10}$, 477.1737). The ^{13}C NMR spectrum of **3** exhibited 22 carbon signals, of which 16 were assigned to the aglycone and the remaining six to a sugar moiety. The 16 carbons (in acetone-d_6) of aglycone included two carbonyls (δ 207.0, C-9; δ 171.1, C-1), six aromatic carbons (δ 160.0, C-5; δ 157.5, C-7; δ 136.1, C-3; δ 124.3, C-8; δ 113.7, C-4; δ 102.6, C-6), one oxymethine (δ 73.0, C-15), six methylenes (δ 44.7, C-10; δ 39.2, C-2; δ 33.2, C-14; δ 27.9, C-12; δ 25.2, C-13; δ 23.6, C-11) and one methyl (δ 20.8, C-16). These carbon signals were almost the same as those (Table S2) of the aglycone of compound **3a** (curvularin-7-*O*-β-D-glucopyranoside) (Zhan & Gunatilaka 2005), implying that **3** and **3a** shared a same aglycone and had a same glycosylated position at C-7, which was further supported by big different chemical shifts ($\Delta\delta$ 1.5 ppm for C-7 and $\Delta\delta$ 3.0 ppm for C-8) between **3** and **5**. Acid hydrolysis of **3** produced its aglycone. The aglycone and curvularin (**5**) had a same HPLC retention time and very close negative optical rotation values, suggesting that both compounds had a same configuration at C-15 (Dai et al. 2010; Lai et al. 1989). Therefore, the aglycone of **3** was proved to be curvularin (**5**). Further comparison of the NMR data of **3** with those of **3a** included that the structural difference between **3** and **3a** was their sugar part. The anomeric proton signal of sugar in **3** resonated at δ_H 5.49 with a small $^3J_{H1,H2}$ coupling constant (3.1 Hz), which was quite different from its counterpart at δ_H 4.94 with a larger coupling constant (7.6 Hz). The ^{13}C NMR data of the sugar in **3** resonated at δ 99.5 (C-1′), 73.1 (C-2′), 74.8 (C-3′) and 75.0 (C-5′), which were also quite different from their counterparts of **3a** at δ 102.3 (C-1′), 74.6 (C-2′), 77.8 (C-3′) and 78.2 (C-5) (Table S2). The foregoing evidence suggested the present of α-glucopyranose in **3** (Bock & Pederson 1983; Yamamoto et al. 2002). Acid hydrolysis of **3** furnished D-glucose as detected by GC analysis. The structure of **3** was thus assigned as curvularin-7-*O*-α-D-glucopyranoside, a new macrolide glycoside.

Three synthetic derivatives of 7-acetyl-curvularin (**5a**), 5-acetyl-curvularin (**5b**) and 5,7-diacetyl-curvularin (**5c**) (Elzner et al. 2008) were made for bioactive assay. The activity of compounds **1**–**5** and **5a**–**5c** inhibiting the proliferation of six cell lines of glioma C6, U87-MG, SHG-44, U251 and colorectal cancer HCT-15 and SW620 was determined by Sulforhodamine B (SRB) assay (Xin et al. 2012; Yu, Ye, Chen et al. 2014; Yu et al. 2015; Yu, Ye, Xin et al. 2014). Doxorubicin (DOX, one of the most potent of the chemotherapeutic drugs) (Tacara et al. 2013) was used as positive control. The data (Table 1) indicated that compounds **1**, **2** and**4** had good activity with $IC_{50} < 11.0$ μM for most tested cell lines with **4** being the most active compound

Table 1. Activity of compounds **1**–**5** and **5a**–**5b** inhibiting the proliferation of cancer cells (IC_{50}: μM).

Compounds	C6	U87-MG	SHG-44	U251	HCT-15	SW620
1	5.95 ± 0.96	3.04 ± 0.14	20.30 ± 0.48	5.25 ± 0.19	2.66 ± 0.14	2.20 ± 0.13
2	3.16 ± 0.10	3.27 ± 0.03	31.90 ± 1.92	10.86 ± 0.28	2.02 ± 0.09	2.39 ± 0.20
3	44.47 ± 4.36	81.01 ± 4.54	43.66 ± 0.10	32.15 ± 2.41	23.29 ± 0.96	20.84 ± 0.28
4	0.59 ± 0.12	2.40 ± 0.19	1.99 ± 0.20	3.39 ± 0.73	2.47 ± 0.34	0.85 ± 0.12
5	>100	>100	>100	>100	>100	71.30 ± 2.89
5a	4.21 ± 0.47	9.98 ± 0.60	73.74 ± 7.05	13.47 ± 0.48	17.67 ± 0.28	2.58 ± 0.09
5b	2.44 ± 0.25	47.92 ± 2.10	>100	>100	20.86 ± 0.33	6.97 ± 0.51
5c	49.61 ± 1.00	24.18 ± 1.79	16.32 ± 1.48	25.15 ± 2.31	21.99 ± 0.95	8.35 ± 0.52
DOX	0.96 ± 0.07	1.35 ± 0.01	4.64 ± 0.16	2.26 ± 0.07	1.13 ± 0.08	1.33 ± 0.06

(IC_{50}: 0.59–3.39 μM). The new compound **3** and the synthetic compounds **5a** and **5c** also displayed activity against all the six cancer cell lines with IC_{50} values ranging from 2.58 to 81.01 μM. Compound **5b** only showed activity against C6, U87-MG, HCT-15 and SW620, while curvularin (**5**) had no activity against the tested cell lines. The positive control DOX had activity against all six ed tumor cells wtestith IC_{50} values of 0.96–4.64 μM. It was noted that, compared to curvularin (**5**), the activity of compounds **1**–**4** and **5a**–**5c** was significantly enhanced because of the presence of a hydroxyl group at C-11 (**1** and **2**), a sugar group at C-7 (**3**) and the acetyl groups at C-5 and C-7 (**5a**–**5c**). The result from this study supports the previous reports that acyl groups are for the activity of some cytotoxic compounds (Chan 2007; Zhang & Li 2007; Wang et al. 2010; Ye et al. 2014).

The compounds (**1**–**5**, **5a**–**5c**) were also assayed for their activity against *S. aureus* and *E. coli*. The antibacterial activity of compounds was initially tested at 100 μg/mL by spot method (Supplementary material). Only compounds **1** and **2** showed activity inhibiting the growth of *E. coli*. None of tested compounds was active against *S. aureus*. The MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of the active compounds **1** and **2** were further determined by micro broth dilution method (Supplementary material) and norfloxacin was used as positive control. The results indicated that both **1** and **2** had activity against *E. coli* with an MIC value of 20 μg/mL and an MBC value of 30 μg/mL. The positive control drug norfloxacin had activity against *E. coli* with MIC 1.2 μg/mL.

Curvularin macrolides were previously found in fungi mainly from genus *Curvularia* (Greve et al. 2008) and *Penicillium* (Meng et al. 2013). This type of macrolides was reported to be cytotoxic towards tumour cells (He et al. 2004; Greve et al. 2008; Meng et al. 2013) and also inhibit the growth of fungal and bacterial organisms (Dai et al. 2010). In the current study, several curvularin macrolides including a new curvularin macrolide glycoside (**3**) were isolated from marine actinomycete strain HS7. This new macrolide and other isolates were shown to be active against the proliferation of different cancer cell lines, further confirming the antitumour property of this type of macrolides. (11*S*,15*R*)-11-hydroxycurvularin (**1**) and (11*R*,15*R*)-11-hydroxycurvularin (**2**) were found to have antibacterial activity inhibiting the growth *E. coli*. To the best of our knowledge, this type of curvularin macrolides is isolated from a bacterial source for the first time.

3. Experimental

Experimental section was supplied as online Supplementary material.

4. Conclusions

A marine actinomycete strain HS7 was isolated from the cloacal aperture of sea cucumber *H. moebii*. This strain was identified by 16S rDNA sequence analysis as *Pseudonocardia* sp. HS7. Five curvularin macrolides (**1**–**5**) and three curvularin acyl products (**5a**–**5c**) of compound **5** were obtained in this study. The structures of these compounds were assigned as (11*S*,15*R*)-11-hydroxycurvularin (**1**), (11*R*,15*R*)-11-hydroxycurvularin (**2**), curvularin-7-*O*-α-D-glucopyranoside (**3**), *trans*-dehydrocurvularin (**4**), curvularin (**5**), 7-acetyl-curvularin (**5a**), 5-acetyl-curvularin (**5b**), 5,7-diacetyl-curvularin (**5c**) mainly based on their NMR and HRESIMS spectral analyses as well as the published NMR data comparison. Compound **3** is a new macrolide glycoside with a rare α-D-glucopyranose substituent and **5a** is a new synthetic acetyl-curvularin. New compounds (**3** and **5a**) and others (**1**, **2**, **4**, and **5c**) showed to inhibit the proliferation of all tested cancer cell lines, confirming the antitumour property of this type of macrolides. (11*S*,15*R*)-11-hydroxycurvularin (**1**) and (11*R*,15*R*)-11-hydroxycurvularin (**2**) were found to have activity against the growth of *E. coli* for the first time.

Supplementary material

Supplementary material relating to this paper is available online.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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