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Cytotoxic Phyllactone Analogs from the Marine Sponge *Phyllospongia papyracea*

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Abstract

Background—A growing evidence indicates that marine sponge *Phyllospongia* sp. is one of rich sources of 20, 24-bishomoscalarane sesterterpenes with potent biological activities. In order to search more bioactive 20, 24-bishomoscalarane sesterterpenes for new drug discovery, chemical investigation was carried out on an Indonesian marine sponge *P. papyracea*.

Methods—Bioassay-guided fractionation was carried out on its dichloromethane extract. And nine compounds were purified and isolated using HPLC. Their chemical structures were determined by a combination of spectroscopic and spectrometric data, including 1D-, 2D-NMR and HRESI-MS. Their cytotoxic activities were performed on three human tumor cell lines A549, MCF-7 and HeLa using the CCK-8 method.

Results—One new 20, 24-bishomoscalarane sesterterpene, phyllactone H (**9**), was isolated and elucidated together with phyllactones A-B (**1–2**) and D-G (**3–6**), 12 α , 24-dihydroxy-20, 24-dimethyl-15, 17-scalaradien-25, 24-olides (**7–8**). Compounds **1** and **2**, **3** and **4**, **5** and **6**, **7** and **8** were C-24 anomers and inseparable mixtures, respectively. The ¹H and ¹³C-NMR data for **7/8** were firstly reported in this paper.

Conclusion—Compounds **1–9** possessed in vitro moderate cytotoxicities against A549, MCF-7 and HeLa cells with IC₅₀ values of less than 25 μ M.

Keywords

Marine sponge; *Phyllospongia papyracea*; Sesterterpene; Phyllactone; Cytotoxic activity

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

SUPPLEMENTARY MATERIAL

The isolation scheme, ¹H-NMR, ¹³C-NMR and ESI-MS spectra for compounds **3/4**, **7/8** and **9** are available online.

1. INTRODUCTION

Numerous studies have proven that marine sponges are a rich source of natural products with unique structures and remarkable bioactivities, many of which have potential value for new drug discovery [1–3]. Several carbolactone-based bishomoscalarane sesterterpenes have been isolated from marine sponges, which only limited to three different sponge genera, namely *Carteriospongia* [4–6], *Phyllospongia* [7–16], and *Strepsichordaia* [17]. And some *Phyllospongia* sponges were found to metabolite 20, 24-bishomoscalarane sesterterpenes with moderate in vitro cytotoxic activities against KB, BS-C-1, P388, NIH 3T3 cell lines and HIV-1 [11, 15, 16]. Therefore, this type sesterterpene has attracted worldwide attention in the field of marine natural product chemistry.

In our continuous investigation of bioactive small molecules from marine sponges [18–20], an Indonesian sponge *P. papyracea* (coll. no. 94561) was shown to produce one new 20, 24-bishomoscalarane sesterterpene, phyllactone H (**9**), together with eight known derivatives, phyllactones A–B (**1–2**), D–G (**3–6**) and 12 α , 24-dihydroxy-20, 24-dimethyl-15, 17-scalaradien-25, 24-olides (**7–8**) (Fig. 1). We hereby wish to report the isolation and identification of these 20, 24-bishomoscalarane sesterterpenes (**1–9**) from the marine sponge *P. papyracea* through bioassay-guided method and their *in vitro* antineoplastic activities.

2. RESULTS AND DISCUSSIONS

Bioassay-guided fractionation of the dichloromethane extract of the marine sponge afforded nine 20, 24-bishomoscalarane sesterterpenes (**1–9**) after repeated chromatography on semipreparative and analytical HPLC. Among these secondary metabolites, however, compounds **1** and **2**, **3** and **4**, **5** and **6**, **7** and **8**, were C-24 anomers and could not be separated using HPLC equipped with a cellulose chiral column. By a combination of ^1H -, ^{13}C -NMR, and ESI-MS techniques, the structures of these compounds were respectively identified as phyllactones A–B (**1–2**) and D–G (**3–6**) [11, 12], and 12 α , 24-dihydroxy-20, 24-dimethyl-15, 17-scalaradien-25, 24-olides (**7–8**) (CRC Numbers: PKL04-F, PKL05-G). However, the ^1H - and ^{13}C -NMR data for **7/8** were not available except their CRC numbers.

Compound **9** was isolated as amorphous colorless powder. Its molecular formula was deduced to be $\text{C}_{33}\text{H}_{48}\text{O}_7$ by HR-ESI-TOF-MS (m/z 579.3156, $[\text{M} + \text{Na}]^+$), which was 28 mu higher than that of **3/4** ($\text{C}_{32}\text{H}_{48}\text{O}_6$). To facilitate its structure elucidation, the ^1H - and ^{13}C -NMR data for compounds **3** and **4** were also presented in Table 1. The ^1H -NMR spectrum of **9** was very similar to that of **3/4** except the presence of a methoxyl group at δ_{H} 3.16 in **9** and the absence of an olefinic proton at δ_{H} 6.33 or 6.37 (C-16) in **3/4**. The correlation in HMBC spectrum of **9** confirmed that the methoxyl group at δ_{H} 3.16 was attached to C-24, suggesting the hydroxy group at C-24 of **3/4** was replaced by a methoxyl group.

By careful inspection of the ^{13}C -NMR spectrum of **9**, the chemical shift values (δ_{C}) at C-14 and C-16 were respectively 174.4 and 181.4, respectively, which greatly moved to downfield from δ_{C} 58.2/58.1 (C-14) and 102.3 (C-16) in **3/4**. This indicated that the sp^3 methine at

C-14 of **3/4** was dehydrogenated and changed to a sp^2 carbon in **9**. While the sp^2 carbon at C-16 in **3/4** was also oxidized and became a ketone in **9**. A new α,β -unsaturated carbonyl system was formed in the D ring of **9**. This assumption was subsequently confirmed by the HMBC correlations between H-15 (δ 6.41) and C-8 (δ 42.3), C-13 (δ 47.3) and C-17 (δ 149.8), between H-22 (δ 1.32) and C-8 (δ 42.3), C-9 (δ 54.3) and C-14 (δ 174.4), and between H-23 (δ 1.75) and C-12 (δ 75.1), C-13 (δ 47.3), C-14 (δ 174.4) and C-18 (δ 150.6) (Fig. 2). The ^1H - and ^{13}C -NMR data of 3-hydroxypentanoyl residue in compound **9** were consistent with those of **3/4** (Table 1), which resulted in assigning the location of 3-hydroxypentanoyl moiety at C-12.

The stereochemistry of ethyl group at C-4 of compound **9** was determined as β because of its NMR chemical shift value (δ 24.8) in good agreement with that of **3/4** (δ 24.4). The H-12 of **9** was assigned as β according to the NOESY correlation between H-12 and H-23 (Fig. 2). The NOE difference spectroscopy of **9** revealed cross-peaks between H-20 and H-21, between H-21 and H-22, between H-22 and H-23, suggesting the β methyl configurations at C-8, C-10 and C-13, which were identical to the configurations in **3/4**. However, the relative configuration at C-24 could not be determined since there was no NOE correlation between H-26 or OMe and other proton signals. Furthermore, the ^{13}C -NMR data of compound **9** were very close to those of the known compound 12 α -acetoxy-20, 24-dimethyl-16, 24-dioxoscalara-14, 17-dien-24-ol-25, 24-olide except the substituted group at C-12 [21].

Therefore, the chemical structure of compound **9** was unambiguously finalized as 12 α -[(3-hydroxypentanoyl)oxy]-20, 24-dimethyl-16, 24-dioxoscalara-14, 17-dien-24-ol-25, 24-olide and named as phyllactone H. According to the scalarane classification of sesterpenoid, phyllactone H (**9**) was denoted as type IIIa, C_{27} bishomoscalarane pentacyclic sesterterpene with one oxo ring.

Bioassay results showed that compounds **1–9** had *in vitro* moderate cytotoxicities against cell lines A549, MCF-7 and HeLa with IC_{50} values of less than 25 μM (Fig. 3). Among these 20, 24-bishomoscalarane sesterterpenes, phyllactones F/G (**5/6**) exhibited the strongest inhibitory effects on A549, MCF-7 and HeLa with IC_{50} values of 7.8, 5.2, and 9.6 μM , respectively. Considering the side effect of these marine sponge-derived sesterterpenes, the human hepatic normal cell line HL-7702 was respectively treated with **1–9**. As shown in Fig. 3, only higher concentration of each compound could inhibit cell viability of HL-7702. It indicated that **1–9** possessed more cytotoxic activities against tumor cell lines A549, MCF-7 and HeLa than on the normal cell HL-7702.

CONCLUSION

Bioassay-guided fractionation of the dichloromethane extract from an Indonesia sponge *P. papyracea* led to the isolation of nine 20, 24-bishomoscalarane sesterterpenes (**1–9**), including one new compound, phyllactone H (**9**), and eight inseparable anomers, phyllactones A-B (**1–2**), phyllactones D and E (**3–4**), phyllactones F and G (**5–6**), 12 α , 24-dihydroxy-20, 24-dimethyl-15, 17-scalaradien-25,24-olides (**7–8**). Furthermore, the ^1H - and ^{13}C -NMR data for **7/8** were firstly reported in this paper. Bioassay results suggested that

compounds **1–9** possessed *in vitro* moderate cytotoxicities against three cell lines A549, MCF-7 and HeLa with IC₅₀ values of no more than 25 μ M.

3. EXPERIMENTAL SECTION

3.1. General Experimental Procedures

All NMR experiments were run on a Varian Unity INOVA spectrometer (600 and 150 MHz for ¹H and ¹³C, respectively) equipped with a 5 mm triple resonance (HCN) cold probe. Optical rotation was obtained on a JASCO P-2000 polarimeter. UV spectrum was recorded on Hitachi-UV-3000 spectrometer. FT-IR spectrum was measured using a Perkin-Elmer 337 spectrophotometer. LR- and HR-ESI-TOF-MS spectra were recorded on an Applied Biosystems Mariner instrument. The analytical LC-UV-ELSD-MS system was controlled by the Empower software and comprised Waters HPLC components equipped with a reversed-phase column (Phenomenex, Luna C₁₈, 150 $\times$ 4.6 mm, 5 μ m) and ran at 1.0 mL/min. The operating method was the same with our previous report [22]. The semi-preparative HPLC system comprised Waters HPLC components (a gradient controller, two 515 pumps and one 2487 UV-Visible detector) and was equipped with a reversed-phase semi-preparative column (Phenomenex, Synergi Hydro-RP, 250 $\times$ 10.0 mm, 4 μ m). MeCN and H₂O used in HPLC system were of chromatographic grade and other chemicals were analytical.

3.2. Biological Material

The marine sponge *P. papyracea* (coll. no. 94561) was collected from Sangihe Island in the Indonesian Sea in July 1994 at 3°00.619 N 125°41.135 E. Taxonomic identification was performed by Karen Tenney at University of California, Santa Cruz (US), based on comparison of the biological characteristics to other voucher sample in our repository. And the voucher specimen and underwater photos are available at Crews lab.

3.3. Extraction and Isolation

The samples of *P. papyracea* were preserved in the field according to our previous validated procedures [23], shipped back to lab at ambient temperature, and stored at 4 °C until extraction and isolation. Accelerated solvent extraction was performed on 15.5 g of dry and lightly chopped specimen at high pressure and temperature (1500 psi N₂, 70 °C) using the solvent series, water, hexane, dichloromethane and methanol. The dichloromethane extract (DCM, 1.8 g) was biologically screened in a soft agar based disk diffusion assay employing a panel of murine and human cancer cell lines and exhibited moderate cytotoxic activity followed by prefractionation. Isolation scheme for compounds **1–9** was shown in Scheme S1 (see Supplementary Material).

Six fractions F1–F6 were afforded and found to have moderate cytotoxicity against the murine colon carcinoma cell line C38 at 10 μ g/mL. Fraction F4 (399.6 mg) was further fractionated into seven subfractions (F4.1–F4.7) using a HPLC system, utilizing a flow rate of 5 mL/min, 280 nm detection, and the following elution conditions: 30 min isocratic 80% CH₃CN in H₂O, 39 min gradient from 80% to 90% CH₃CN in H₂O, and 40 min at 80% CH₃CN. F4.1 was purified on the same HPLC system equipped with a reversed-phase column (Phenomenex, Luna C₁₈, 250 $\times$ 4.6 mm, 5 μ m) under an isocratic condition of 75%

CH₃CN in H₂O with a flow rate of 1.0 mL/min and 280 nm detection, to yield a mixture (1.1 mg) of **1** and **2**. Unfortunately, the anomers **1** and **2** could not be separated on the same HPLC system using a chiral column (Phenomenex, Lux Cellulose-1, 250 × 4.6 mm, 5 μm) under an isocratic condition of 60% CH₃CN in H₂O with a flow rate of 1.0 mL/min and 280 nm detection. Subfraction F4.2 was separated on the same HPLC system to afford two inseparable mixtures of **3/4** (0.7 mg) and **7/8** (0.5 mg) under an isocratic condition of 78% CH₃CN in H₂O. The inseparable mixture **5/6** (0.8 mg) was purified from F4.4 using the same HPLC system under an isocratic condition of 80% CH₃CN in H₂O. Subfraction F4.7 was further separated on the same HPLC system to yield a pure compound **9** (0.6 mg) under an isocratic condition of 82% CH₃CN in H₂O.

12α, 24-Dihydroxy-20, 24-dimethyl-15, 17-scalaradien-25,24-olides (7/8): amorphous colorless powder; ¹H- and ¹³C-NMR data see Table 1.

12α-[(3-Hydroxypentanoyl)oxy]-20, 24-dimethyl-16, 24-dioxoscalara-14, 17-dien-24-ol-25, 24-olide (9): amorphous colorless powder; +29.6 (c 1.0, CHCl₃); UV (MeOH) λ_{max} (log ε) 265 (3.72) nm; FT-IR (KBr) λ_{max} 3020, 2401, 1423, 1215, 929, 759, 669 cm⁻¹; ¹H- and ¹³C-NMR data see Table 1; HR-ESI-TOF-MS *m/z* [M + Na]⁺ 579.3156 (calc. for C₃₃H₄₈O₇Na, 579.3192).

3.4. Cytotoxicity Assay

Three human tumor cell lines A549, MCF-7, HeLa and one hepatic normal cell line HL-7702 were purchased from Shanghai Bioteleaf Technology Co. Ltd., China. Each cell line was grown in RPMI medium with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (50 μg/mL) and cultured in a 96-well plate at a density of 5 × 10⁵ cells per well. Each isolated compound was added to each well respectively with increasing concentrations. Cell lines without treatment by compound were used as the control. The incubation was performed in a humidified, 37 °C, 5% CO₂-containing incubator for 24 h. Then 10 μL CCK-8 dye (Be-yotime Institute of Biotechnology, China) was added to each well, cell lines were incubated at 37 °C for 2 h and plates were read in a Victor-V multilabel counter (Perkin-Elmer) using the default europium detection protocol. IC₅₀ values of each bishomoscalarane sesterterpene and the positive control gefitinib were calculated by comparison with DMSO-treated control wells and determined by the logit method from at least three independent tests [24].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Chemical structures of compounds **1–9**.

Fig. 2.
Selected HMBC and NOESY correlations of **9**.

Fig. 3.
Cytotoxic effects of **1–9** and the positive control gefitinib against three tumor cell lines (A549, MCF-7, HeLa) and one normal cell line (HL-7702).

Table 1

¹H- and ¹³C-NMR data (600 MHz and 150 MHz, in CDCl₃) of compounds 3/4, 7/8 and 9.

Position	δ_{H} (δ in Hz)			δ_{C}		
	3/4 ^a	7/8 ^a	9	3/4 ^a	7/8 ^a	9
1	ax: 0.76, <i>ddd</i> (14.4, 14.4, 3.6)	ax: 0.77, <i>ddd</i> (14.4, 14.4, 3.6)	ax: 0.77, <i>ddd</i> (15.0, 15.0, 4.2)	40.4	40.6	40.4
2	ax: 1.33–1.38, <i>m</i>	ax: 1.32–1.37, <i>m</i>	ax: 1.32–1.36, <i>m</i>	18.4	18.3/18.1	18.3
3	ax: 0.79–0.85, <i>m</i>	ax: 0.80–0.86, <i>m</i>	ax: 0.80–0.86, <i>m</i>	34.8/34.7	35.9	36.3
4				36.7	36.3	36.5
5	0.88–0.91, <i>m</i>	0.86–0.90, <i>m</i>	0.87–0.91, <i>m</i>	59.3	58.7/58.6	58.4
6	ax: 1.33–1.38, <i>m</i> eq: 1.45–1.50, <i>m</i>	ax: 1.32–1.37, <i>m</i> eq: 1.44–1.49, <i>m</i>	ax: 1.30–1.36, <i>m</i> eq: 1.44–1.50, <i>m</i>	20.8	19.7	18.7
7	ax: 0.85–0.91, <i>m</i> eq: 1.63–1.65, <i>m</i>	ax: 0.85–0.90, <i>m</i> eq: 1.62–1.65, <i>m</i>	ax: 0.86–0.91, <i>m</i> eq: 1.63–1.66, <i>m</i>	40.5	40.3	41.9
8				35.5/35.4	35.3	42.3
9	1.05–1.09, <i>m</i>	1.04–1.09, <i>m</i>	1.02–1.07, <i>m</i>	58.5	58.4	54.3
10				37.2	37.4	38.8
11	ax: 1.66–1.71, <i>m</i> eq: 1.94, <i>ddd</i> (15.0, 3.6, 3.0)	ax: 1.64–1.70, <i>m</i> eq: 1.92, <i>ddd</i> (15.0, 3.6, 3.0)	ax: 1.65–1.70, <i>m</i> eq: 1.95, <i>ddd</i> (15.0, 3.6, 3.0)	24.0	24.1/24.0	24.7
12	5.02/5.03, <i>dd</i> (13.8, 5.4)	5.37, <i>s</i>	4.88, <i>dd</i> (11.4, 6.0)	76.0/75.9	75.7/75.4	75.1
13				41.7/41.6	41.1	47.3
14	2.18, <i>d</i> (4.2)	2.20, <i>d</i> (4.2)		58.2/58.1	57.2/56.9	174.4
15	6.44/6.46, <i>t</i> (3.0)	6.50, <i>dd</i> (11.4, 3.0)	6.41, <i>s</i>	139.9/139.7	139.7/139.4	125.8
16	6.33/6.37, <i>dd</i> (11.4, 3.6)	6.31, <i>d</i> (10.8)		117.8/119.6	119.5/119.3	181.4
17				158.2/158.0	158.5/158.2	149.8
18				132.2/132.1	132.2/132.1	150.6
19	0.85, <i>s</i>	0.80, <i>s</i>	0.83, <i>s</i>	27.3/27.1	28.9/28.7	28.7
20	1.17, <i>dq</i> (16.8, 8.4)	1.18, <i>dq</i> (16.8, 8.4)	1.18, <i>dq</i> (16.8, 8.4)	24.4	24.6/24.4	24.8
21	1.10, <i>s</i>	0.99, <i>s</i>	1.32, <i>s</i>	19.0/18.9	19.0/18.9	22.7
22	0.87, <i>s</i>	0.87, <i>s</i>	0.97, <i>s</i>	17.5	17.9/17.7	17.2
23	1.17/1.16, <i>s</i>	1.02/1.01, <i>s</i>	1.75, <i>s</i>	12.7	12.7/12.6	20.7
24				102.3	102.9/102.8	106.6

Position	δ_{H} (<i>J</i> in Hz)			δ_{C}		
	3/4 ^a	7/8 ^a	9	3/4 ^a	7/8 ^a	9
25				168.0/167.5	168.6/167.9	167.4
26	1.65/1.55, <i>s</i>	1.57, <i>s</i>	1.78, <i>s</i>	22.3	24.0	22.9
27	0.84, <i>t</i> (6.0)	0.75, <i>t</i> (9.0)	0.77, <i>t</i> (7.2)	10.1/10.0	8.8	8.8
1'				173.3/173.1		173.1
2'	2.49–2.51, <i>m</i>		2.58–2.60, <i>m</i>	42.2/42.1		41.9
3'	4.03–4.05, <i>m</i>		3.98–4.01, <i>m</i>	69.6/69.7		69.4
4'	1.25–1.28, <i>m</i>		1.26–1.29, <i>m</i>	29.6		29.8
5'	0.95, <i>t</i> (6.0)		0.98, <i>t</i> (7.2)	10.1		10.0
24-OMe			3.16, <i>s</i>			51.5
3'-OH	5.30, <i>s</i>		5.30, <i>s</i>			

^aEach value may be interchanged.

ax and eq refer to axial and equatorial positions, respectively.