SHORT REPORT

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Three New Chromone Derivatives from the Deep-Sea-Derived Fungus *Penicillium thomii*

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Abstract: Chemical investigation of the EtOAc extract of a deep-sea-derived fungus *Penicillium thomii Maire* YPGA3 cultured on rice solid medium led to the isolation of three new chromone analogs, named penithochromones U–W (**1**–**3**), along with six known compounds (**4**–**9**). The structures were determined by extensive analyses of spectroscopic data (NMR and HRESIMS data). Compounds **1**–**3** are 5,7-dioxygenated chromone analogs bearing an aliphatic acid side chain. All isolated compounds were inactive toward the α-glucosidase at the concentration of 200 μM

Keywords: *Penicillium thomii*; deep-sea fungus; chromone derivative; penithochromone. © 2022 ACG Publications. All rights reserved.

1. Plant Source

The fungal strain YPGA3 was isolated from the deep-sea sediments that collected in the Yap Trench (West Pacific Ocean) at a depth of –4500 m. The strain was identified as *Penicillium thomii* based on microscopic examination and by internal transcribed spacer (ITS) sequencing. The ITS sequence has been deposited in GenBank (http://www.ncbi.nlm.nih.gov) with the accession number MG835903. The strain YPGA3 (MCCC 3A01052) was deposited at the Marine Culture Collection of China

2. Previous Studies

In the past ten years, marine-derived fungi have gained increasing attention from natural product chemists, lots of small molecules bearing diverse structures and significant pharmacological effects have been reported. The strains belonging to the genera *Penicillium* from marine environment are continually studied and proved to be productive, leading to the discovery of DMOA-based

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meroterpenoids with unprecedented skeletons [1, 2], chlorinated diphenyl ethers possessing significant inhibitory effects against α-glucosidase [3], *p*-terphenyls with promising anti-HSV activity [4], antimicrobial alkaloids [5, 6], terpenoids [7, 8], and steroids [9, 10]. In our continuing research for new or bioactive metabolites from marine-derived fungi [7, 11, 12] , three new chromone analogs (**1**–**3**) and six known compounds (**4**–**9**) were identified from a deep-sea derived strain *P. thomii* (Figure 1). Herein, the isolation and structural elucidation of the metabolites were described.

3. Present Study

The fermentation was conducted in 30 Fernbach flasks (500 mL), each containing 70 g of rice. Artificial seawater (90 mL) was added to each flask, and the contents were soaked for 3 h before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 3.0 mL of the spore inoculum and incubated at room temperature for 30 days. The fermented material was extracted successively with EtOAc (4000 mL) for three times. After evaporation under vacuum, the EtOAc extract (3.1 g) was subjected to a middle chromatogram isolated gel (MCI) with MeOH/H_2O (10:90→100:0) as eluent to obtain 10 fractions (Fr. 1 to Fr. 10). Fr. 3 (280 mg) was chromatographed over RP-18 silica gel eluted with MeOH/H_2O (30:70 → 90:10) to afford 14 subfractions (Fr. 3a–Fr. 3n). Fr. 3b (32 mg) was separated by HPLC (MeCN/H_2O = 34:66, 3 mL/min) to yield **1** (4.6 mg, t_R = 9.5 min), **2** (11.3 mg, t_R = 11.2 min) and **3** (1.9 mg, t_R = 13.4 min). Fr. 3c (23 mg) was purified by HPLC (MeCN/H_2O = 44:56, 3 mL/min) to give **6** (5.3 mg, t_R = 32.2 min). Fr. 3g (27 mg) was subjected to HPLC (MeCN/H_2O = 51:49, 3 mL/min) to afford **5** (6.7 mg, t_R = 13.4 min) and **7** (2.3 mg, t_R = 9.8 min). Fr. 7 (150 mg) was chromatographed over ODS silica gel CC eluted with MeOH/H_2O (30:70→100:0) to give seven subfractions (Fr. 7a–Fr. 7g). Fr. 7a (19 mg) was further purified by HPLC (MeCN/H_2O=15:85, 3 mL/min) to yield **4** (2.3 mg, t_R = 31.1 min) and **8** (3.2 mg, t_R = 27.3 min). Fr. 7b (52 mg) was purified by HPLC (MeCN/H_2O=20:80, 3 mL/min) to give **9** (19.6 mg, t_R = 33.7 min).

*Penithochromone U (**1**)*: light yellow oil; UV (MeOH) λ_{max} 249 (3.93), 289 (3.64) nm; ^{1}H and ^{13}C NMR data, see Table 1; Negative HRESIMS *m/z* 291.0863 [M − H]$^-$ (calcd for $C_{15}H_{15}O_6^-$, 291.0874).

*Penithochromone V (**2**)*: light yellow oil; UV (MeOH) λ_{max} 247 (3.92), 290 (3.65) nm; ^{1}H and ^{13}C NMR data, see Table 1; Negative HRESIMS *m/z* 277.0704 [M − H]$^-$ (calcd for $C_{14}H_{13}O_6^-$, 277.0718).

*Penithochromone W (**3**)*: light yellow oil; UV (MeOH) λ_{max} 250 (4.08), 294 (3.67) nm; ^{1}H and ^{13}C NMR data, see Table 1; Negative HRESIMS *m/z* 249.0392 [M − H]$^-$ (calcd for $C_{12}H_9O_6^-$, 249.0405).

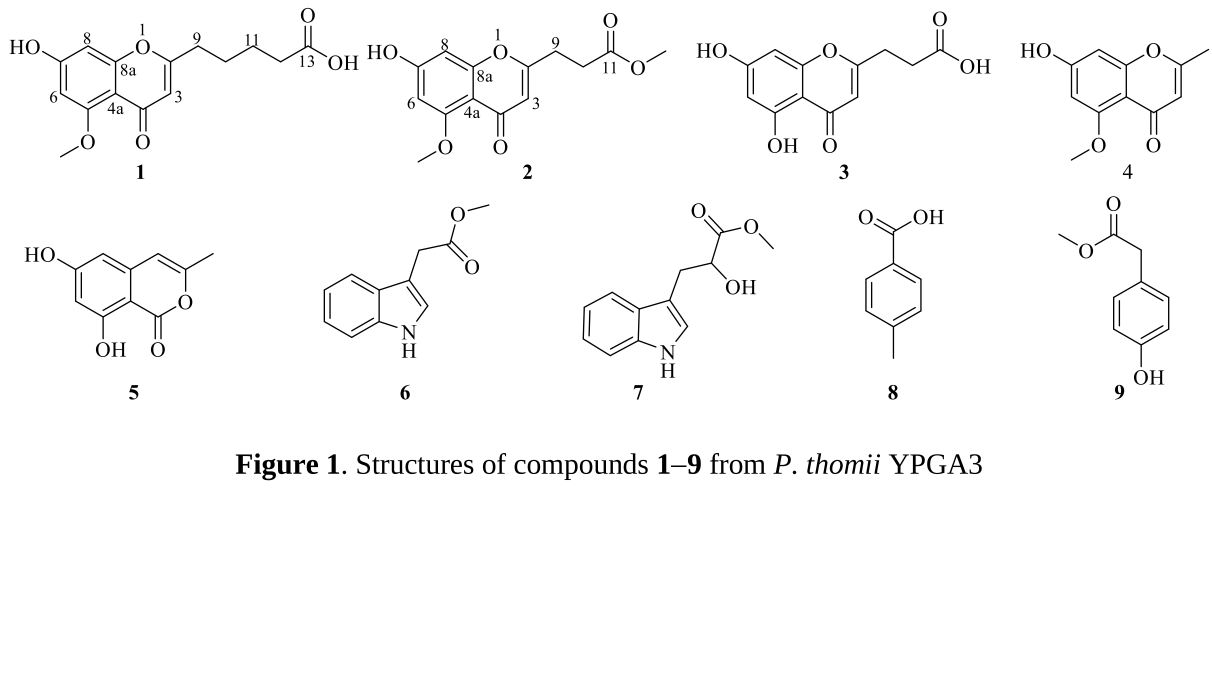

Figure 1. Structures of compounds **1**–**9** from *P. thomii* YPGA3

The molecular formula of compound **1** was defined as $C_{15}H_{16}O_6$, based on analysis of the HRESIMS (*m/z* 291.0863 [M − H]$^-$ (calcd 291.0874)) and the ^{13}C NMR data, requiring eight double bond equivalents. The ^{1}H NMR spectrum displayed the signals of two aromatic protons for a meta-coupling system [δ_H 6.34 (1H, d, *J* = 1.9 Hz), 6.35 (1H, d, *J* = 1.9 Hz)], an aromatic singlet (δ_H 5.87, s), an aromatic methoxyl group [δ_H 3.75 (3H, s)], as well as several aliphatic protons (Table 1). Analysis of the ^{13}C NMR (Table 1) and HSQC spectra revealed a total of 15 carbon resonances, which were attributed to a benzene ring, a double bond, two carbonyl carbons (δ_C 175.7, 174.5), a methoxyl carbon (δ_C 55.9), and four methylene carbons (δ_C 33.4, 32.3, 25.6, 23.9). The above-mentioned functional groups accounted for seven double bond equivalents, the remaining one revealed an additional ring in the structure. The presence of meta-coupled aromatic protons (δ_H 6.34 and 6.35) indicated a 1,2,3,5-tetra substituted benzene ring. The HMBC correlations (Figure 2) from H-3 (δ_H 5.87) to C-2 (δ_C 165.8), C-4 (δ_C 175.7), C-4a (δ_C 107.0), H-6 (δ_H 6.34) to C-4a, and H-8 (δ_H 6.35) to 4a, 8a (δ_C 159.5), in combination with the additional ring enabled the construction of a 5,7-dioxygenated chromone nucleus. The methoxy group was located at C-5 by the HMBC correlation from the methoxyl protons at δ_H 3.75 to the oxygenated aromatic carbon C-5 (δ_C 160.7). Analysis of the COSY spectrum for **1** revealed the presence of a spin system including four methylenes (CH_2-9/ CH_2-10/ CH_2-11/ CH_2-12) (Figure 2). The HMBC correlations from the protons H_2-11 (δ_H 1.53) and H_2-12 (δ_H 2.24) to the carbonyl carbon C-13 (δ_C 174.5) allowed a pentanoic acid side chain to be defined, which was anchored to the chromone nucleus at C-2 by HMBC correlations of H_2-9 to C-2 (δ_C 165.8) and C-3 (δ_C 110.4). Thus, the structure of **1** was determined as 7-hydroxy-5-methoxy-2-pentanoic acid-chromone and was named penithochromone U.

Table 1. ^{1}H (400 Hz) and ^{13}C NMR (100 Hz) Data of **1**–**3** (δ in ppm)

No	**1** ^{a}		**2** ^{b}		**3** ^{b}	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		165.8		167.6		170.9
3	5.87, s	110.4	6.00, s	111.1	6.09, s	108.5
4		175.7		179.9		183.9
4a		107.0		108.1		105.2
5		160.7		162.5		163.3
6	6.34, d (1.9)	95.1	6.39, d (1.9)	96.2	6.19, d (2.1)	100.1
7		162.6		165.0		166.0
8	6.35, d (1.9)	96.4	6.39, d (1.9)	97.4	6.33, d (2.1)	94.9
8a		159.5		161.6		159.8
9	2.53, t (7.0)	32.3	2.90, t (7.1)	29.6	2.93, t (7.2)	30.3
10	1.61, m	25.6	2.75, t (7.1)	31.4	2.73, t (7.2)	31.6
11	1.53, m	23.9		174.0		175.4
12	2.24, t (7.2)	33.4				
13		174.5				
5-OCH_3	3.75, s	55.9	3.86, s	56.4		
11-OCH_3			3.69, s	52.4		

^{a} in DMSO-d_6, ^{b} in methanol-d_4

The molecular formula of **2** was determined to be $C_{14}H_{14}O_6$ by the HRESIMS data. The NMR data showed similar structural features as those of **1**, presenting the signals for the same chromone moiety [δ_H 6.00 (s), 6.39 (d, *J* = 1.9 Hz, 1H), 6.51 (d, *J* = 1.9 Hz, 1H); δ_C 96.2, 97.4, 108.1, 111.1, 161.6, 162.5, 165.0, 167.6, 179.9] as that of **1**. The structure of **2** was established by 2D NMR analyses (Figure 2). Analysis of the COSY spectrum for **2** revealed the connectivity of the two methylenes [δ_H 2.90 (t, *J* = 7.1 Hz, 2H), 2.75 (t, *J* = 7.1 Hz, 2H)], both showed HMBC correlations to the ester carbonyl carbon C-11 (δ_C 174.0). The HMBC correlation from the remaining methoxyl protons (δ_H 3.69) to C-11 assigned a methyl propionate side chain, which was attached to the chromone unit at C-2 by HMBC correlations of H_2-9 to C-2 (δ_C 167.6) and C-3 (δ_C 111.1). Thus, the structure of **2** was determined as 7-hydroxy-5-methoxy-2-methyl propionate-chromone and was named penithochromone V.

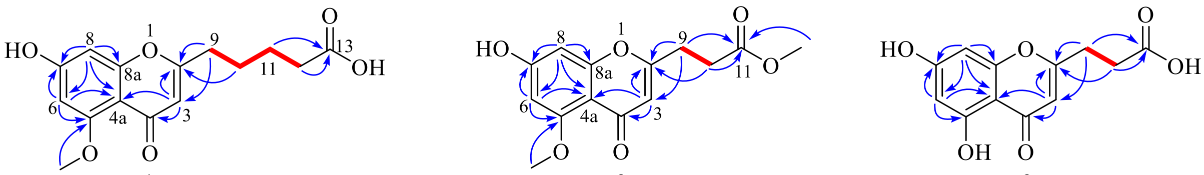

Figure 2. Key ^{1}H-^{1}H COSY (—) and HMBC (→) correlations of **1**–**3**

Compound **3** had a molecular formula of $C_{12}H_{10}O_6$, as determined by the HRESIMS along with ^{13}C NMR data. The ^{1}H NMR and ^{13}C NMR spectra of **3** were very similar to those of **2** with differences due to the absence of the two methoxy groups in **2**, indicating **3** was demethylated derivative of **2**. The structure of **3** was confirmed by the 2D NMR analyses (Figure 2). Compound **3** was established as 5,7-dihydroxy-2-propanoic acid-chromone and was named penithochromone W.

Additionally, the known compounds **4**–**9** were identified to be isoeugenin (**4**) [13], 6,8-dihydroxy-3-methylisocoumarin (**5**) [14], methyl indole-3-acetate (**6**) [15], methyl indole-3-lactate (**7**) [16], crithminic acid (**8**) [17], and methyl (4-hydroxyphenyl)acetate (**9**) [18] by comparing their NMR data with those in the literature.

The isolated compounds were screened for their inhibitory effect toward the α-glucosidase at the initial concentration of 200 μM, while all compounds were inactive with inhibition rate less than 30%.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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