SUPPLEMENTARY MATERIAL

Penimethavone A, a Flavone from a Gorgonian-Derived Fungus

Penicillium chrysogenum

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Abstract

A novel flavone, penimethavone A (**1**), possessing a rare unique methyl group at ring-B, was isolated from the fungus *Penicillium chrysogenum* cultured from a gorgonian *Carijoa* sp. collected from the South China Sea. The structure was elucidated by extensive spectroscopic analysis and by comparison with related known compound. Compound **1** showed selective and moderate cytotoxicity against cervical cancer (HeLa) and rhabdomyosarcoma (RD) cell lines with IC₅₀ values of 8.41 and 8.18 μ M, respectively

Keywords: *Penicillium chrysogenum*, gorgonian *Carijoa* sp., flavone, cytotoxicity

List of supporting information

Experimental section

Table S1. ^1H and ^{13}C NMR Data for **1**

Table S2. Cytotoxic activity of compound **1**

Figure S1. The key HMBC correlations for **1**

Figure S2. ^1H NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of compound **1**

Figure S3. Partial ^1H NMR (500 MHz, $\text{DMSO-}d_6$) spectrum of compound **1**

Figure S4. ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) spectrum of compound **1**

Figure S5. Partial ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) spectrum of compound **1**

Figure S6. Partial ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) spectrum of compound **1**

Figure S7. ^1H – ^1H COSY ($\text{DMSO-}d_6$) spectrum of compound **1**

Figure S8. HMQC ($\text{DMSO-}d_6$) spectrum of compound **1**

Figure S9. HMBC ($\text{DMSO-}d_6$) spectrum of compound **1**

Figure S10. DEPT-90 ($\text{DMSO-}d_6$) spectrum of compound **1**

Figure S11. DEPT-135 ($\text{DMSO-}d_6$) spectrum of compound **1**

Figure S12. HRESIMS spectrum of compound **1**

Experimental section

1.1. General experimental procedures

NMR spectra were recorded on a JEOL JEM-ECP NMR spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C), using TMS as internal standard. The ESIMS and HRESIMS were obtained from a Thermo MAT95XP High Resolution mass spectrometer. UV spectra were obtained on a Beckman DU 640 spectrophotometer. IR spectra were recorded on a Nicolet-Nexus-470 spectrometer using KBr pellets. Melting points were determined on an X-6 micromelting point apparatus. Semipreparative HPLC was performed on an Hitachi L-2000 system using a semipreparative C_{18} column [HPLC (Kromasil 250×10 mm, $5 \mu\text{m}$, 2.0 mL/min)]. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh), octadecylsilyl silica gel (Unicorn; $45\text{--}60 \mu\text{m}$), and Sephadex LH-20 (GE Healthcare) were used for

column chromatography (CC). Precoated silica gel plates (Yan Tai Zhi Fu Chemical Group Co.; G60, F-254) were used for thin layer chromatography.

1.2. Fungus material and culture conditions

The fungus *Penicillium chrysogenum* TA01-16 was cultured from a gorgonian *Carijoa* sp. collected from the Weizhou coral reefs in the South China Sea in April, 2010. The strain was deposited at the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, PR China, with the Genbank (NCBI) accession number KP759287. The fungus was grown stationary on sea water-added rice solid medium (fifty 1000 mL Erlenmeyer flasks, each containing 50 g of rice and 50 mL of sea water) at room temperature for 45 days.

1.3. Extraction and isolation

After 45 days of cultivation, the fermented rice substrate was extracted three times with an equal volume of EtOAc to give an organic extract (10 g). The extract was subjected to silica gel column chromatography (CC) and eluted by a gradient of petroleum ether/EtOAc to EtOAc, and then MeOH to afford four fractions (Fr. 1–Fr. 4) on the basis of TLC analysis. Fr. 3 was rechromatographed on CC with petroleum ether–EtOAc to provide three subfractions (Fr. 31–Fr. 33). Fr. 33 was subjected to Sephadex LH-20 column chromatography eluting with MeOH and then purified by semipreparative HPLC (75:25 MeOH–H₂O, 2 mL/min) to give compound **1** (2.5 mg).

Penimethavone A (1):

C₁₆H₁₂O₆, Yellowish powder. M.p. 214–215 °C. IR (KBr) $\nu_{\max}$ 3169, 2925, 1650, 1506, 1458, 1346, 1164 cm⁻¹. ¹H NMR (500 MHz, DMSO-*d*₆): 12.91 (1H, s, OH-5), 10.84 (1H, s, OH-7), 9.83 (1H, s, OH-2'), 9.73 (1H, s, OH-4'), 6.35 (1H, d, *J* = 1.8 Hz, H-8), 6.28 (1H, d, *J* = 1.5 Hz, H-3'), 6.20 (1H, d, *J* = 1.8 Hz, H-6), 6.21 (1H, d, *J* = 1.5 Hz, H-5'), 6.21 (1H, s, H-3), 2.13 (3H, s, H-7'). ¹³C NMR (125 MHz, DMSO-*d*₆): 181.8 (C-4), 164.2 (C-7), 164.1 (C-2), 161.6 (C-5), 159.9 (C-4'), 158.2 (C-8a), 157.0 (C-2'), 139.0 (C-6'), 111.6 (C-3), 111.2 (C-1'), 108.8 (C-5'), 103.8 (C-4a), 100.3 (C-3'), 98.7 (C-6), 93.8 (C-8), 19.9 (C-7'). ESIMS *m/z* 301.1 [M + H]⁺. HRESIMS *m/z* 301.0709 [M + H]⁺ (calcd for C₁₆H₁₃O₆, 301.0707).

1.4. Bioactivity assay

1.4.1. Cytotoxic Assay

The cytotoxic activities were evaluated against the cervical cancer (HeLa), human laryngeal epithelial (Hep-2), rhabdomyosarcoma (RD), and non-small cell lung cancer (A549) cell lines. Cells in the logarithmic phase (approximately 5×10^4 cells ml^{-1}) were seeded into 96-well plates and maintained at 37 °C overnight in an atmosphere of 10% CO_2 . Compounds and adriamycin were diluted in DMSO at the indicated concentrations. After 72 h of incubation, 100 μL of neutral red (3-amino-7-dimethylamino-2-methyl-phenazine hydrochloride) medium was added into the 96-well plates and incubated for 2 h, then the OD absorbance was measured in a microplate reader at 540 nm and the IC_{50} values were further calculated.

1.4.2. Acetyl cholinesterase (AChE) Inhibition Assays

AChE inhibition was determined spectrophotometrically using acetylthiocholine iodide (ATCI) as substrate by modifying the method of Ellman (Ellman et al. 1961; ur Rahman et al. 2001; Orhan et al. 2004). The reaction was carried in 0.1 M sodium phosphate buffer (pH 8.0) at room temperature. In a typical assay 140 μL buffer, 15 μL enzyme preparation and 20 μL test compound solution were mixed and incubated at 4 °C for 20 min. 10 μL of 0.002 M 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was added and the reaction was then started by adding 10 μL of 0.015 M ATCI. The optical density was measured in a 96-well plate reader at 405 nm immediately after incubating the reaction solution at 37 °C for 20 min. A blank positive control was set up by adding 20 μL huperzine A (0.100 mg/mL in phosphate buffered saline) or/and 20 μL galantamine hydrobromide (250 μM in deionized water) instead of 20 μL sample solution. Blank was set up by adding 20 μL PBS buffer solution instead of 20 μL sample solution. Experiment control was set up by adding 15 μL PBS buffer solution instead of 15 μL enzyme solution in order to deduct sample background.

Table S1. ^1H and ^{13}C NMR Data for **1**^a

position	δ_{C} , type	δ_{H} , (J in Hz)
2	164.1, C	
3	111.6, CH	6.21, s
4	181.8, C	
4a	103.8, C	
5	161.6, C	
6	98.7, CH	6.20, d (1.8)
7	164.2, C	
8	93.8, CH	6.35, d (1.8)
8a	158.2, C	
1'	111.2, C	
2'	157.0, C	
3'	100.3, CH	6.28, d (1.5)
4'	159.9, C	
5'	108.8, CH	6.21, d (1.5)
6'	139.0, C	
7'	19.9, CH ₃	2.13, s
5-OH		12.91, s
7-OH		10.84, s
2'-OH		9.83, s
4'-OH		9.74, s

^a 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR in DMSO- d_6 .

Table S2. Cytotoxic activity of compound **1**^a

compound	IC ₅₀ (μM)			
	HeLa	RD	Hep-2	A-549
1	8.41	8.18	52.08	>100
adriamycin ^b	0.43	0.36	0.09	7.32

^a Data are expressed in IC₅₀ values (μM). ^b Adriamycin was used as a positive control.

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