SUPPLEMENTARY MATERIAL

Penimethavone A, a Flavone from a Gorgonian-Derived Fungus

*Penicillium chrysogenum*

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Penimethavone A, a Flavone from a Gorgonian-Derived Fungus *Penicillium chrysogenum*

**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 IC$_{50}$ values of 8.41 and 8.18 µM, respectively.

**Keywords:** *Penicillium chrysogenum*, gorgonian *Carijoa* sp., flavone, cytotoxicity
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1.1. General experimental procedures

NMR spectra were recorded on a JEOL JEM-ECP NMR spectrometer (500 MHz for $^1$H 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 μm, 2.0 mL/min)]. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh), octadecysilyl silica gel (Unicorn; 45–60 μm), 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) νmax 3169, 2925, 1650, 1506, 1458, 1346, 1164 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆): 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-d₆): 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 $5 \times 10^4$ cells ml$^{-1}$) were seeded into 96-well plates and maintained at $37 \, ^\circ\text{C}$ 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 $\mu$L 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 er al. 2004). The reaction was carried in 0.1 M sodium phosphate buffer (pH 8.0) at room temperature. In a typical assay 140 $\mu$L buffer, 15 $\mu$L enzyme preparation and 20 $\mu$L test compound solution were mixed and incubated at 4 $^\circ\text{C}$ for 20 min. 10 $\mu$L of 0.002 M 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was added and the reaction was then started by adding 10 $\mu$L 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 $^\circ\text{C}$ for 20 min. A blank positive control was set up by adding 20 $\mu$L huperzine A (0.100 mg/mL in phosphate buffered saline) or/and 20 $\mu$L galantamine hydrobromide (250 $\mu$M in deionized water) instead of 20 $\mu$L sample solution. Blank was set up by adding 20 $\mu$L PBS buffer solution instead of 20 $\mu$L sample solution. Experiment control was set up by adding 15 $\mu$L PBS buffer solution instead of 15 $\mu$L enzyme solution in order to deduct sample background.
| position | $\delta_C$, type | $\delta_H$ (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 $^1$H NMR and 125 MHz for $^{13}$C NMR in DMSO-$d_6$. 
### Table S2. Cytotoxic activity of compound 1

| compound          | HeLa  | RD   | Hep-2 | A-549 |
|-------------------|-------|------|-------|-------|
| 1                 | 8.41  | 8.18 | 52.08 | >100  |
| adriamycin<sup>b</sup> | 0.43  | 0.36 | 0.09  | 7.32  |

<sup>a</sup> Data are expressed in IC<sub>50</sub> values (μM). <sup>b</sup> Adriamycin was used as a positive control.

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