SUPPLEMENTARY MATERIAL

Aspergivones A and B, Two New Flavones Isolated from a Gorgonian-Derived *Aspergillus candidus* Fungus

Jie Ma,^a Xiu-Li Zhang,^a Yu Wang,^a Ji-Yong Zheng,^{b,*} Chang-Yun Wang,^{a,c} and Chang-Lun Shao^{a,c,*}

^a *Key Laboratory of Marine Drugs, The Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China.*

^b *State Key Laboratory for Marine Corrosion and Protection, Luoyang Ship Material Research, Qingdao 266061, People's Republic of China.*

^c *Laboratory for Marine Drugs and Bioproducts, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266200, People's Republic of China*

* Correspondence

Prof. Dr. Chang-Lun Shao and Senior Engineer Ji-Yong Zheng

Key Laboratory of Marine Drugs, the Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, 5 Yushan Road, Qingdao, 266003, Shandong, P. R. China

State Key Laboratory for Marine Corrosion and Protection, Luoyang Ship Material Research, 149-1 Zhuzhou Road, Qingdao, 266101, Shandong, P. R. China

Tel/Fax: 86-532-82031381 (C.-L. Shao.); 86-532-68725022 (J.-Y. Zheng.)

E-mails: shaochenglun@163.com (C.-L. Shao.); zhengjy@sunrui.net (J.-Y. Zheng).

* Corresponding authors. E-mails: shaochenglun@163.com (C.-L. Shao.); zhengjy@sunrui.net (J.-Y. Zheng).

Aspergivones A and B, Two New Flavones from a Gorgonian-Derived *Aspergillus candidus* Fungus

Abstract

Two new flavones, aspergivones A (**1**) and B (**2**), were isolated from the fungus *Aspergillus candidus* cultured from the gorgonian coral *Anthogorgia ochracea* collected from the South China Sea. The structures of **1** and **2** were elucidated by NMR and MS methods and comparison with related known compounds. Only **2** showed slight inhibitory activity against alpha-glucosidase with an IC₅₀ value of 244 μ g/mL. Compounds **1** and **2** were also evaluated for their cytotoxic and antibacterial activities.

Keywords: *Aspergillus candidus*, gorgonian coral, flavone, alpha-glucosidase

List of supporting information

Experimental section

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

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

Figure S2. ^1H NMR (500 MHz, CDCl_3) spectrum of compound **1**

Figure S3. Partial ^1H NMR (500 MHz, CDCl_3) spectrum of compound **1**

Figure S4. ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **1**

Figure S5. Partial ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **1**

Figure S6. Partial ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **1**

Figure S7. ^1H - ^1H COSY (CDCl_3) spectrum of compound **1**

Figure S8. HMQC (CDCl_3) spectrum of compound **1**

Figure S9. HMBC (CDCl_3) spectrum of compound **1**

Figure S10. DEPT-90 (CDCl_3) spectrum of compound **1**

Figure S11. DEPT-135 (CDCl_3) spectrum of compound **1**

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

Figure S13. ESIMS spectrum of compound **1**

Table S2. ^1H and ^{13}C NMR Data for **2**

Figure S14. ^1H NMR (500 MHz, CDCl_3) spectrum of compound **2**

Figure S15. Partial ^1H NMR (500 MHz, CDCl_3) spectrum of compound **2**

Figure S16. ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **2**

Figure S17. Partial ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **2**

Figure S18. Partial ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **2**

Figure S19. DEPT-90 (CDCl_3) spectrum of compound **2**

Figure S20. DEPT-135 (CDCl_3) spectrum of compound **2**

Figure S21. ESIMS spectrum of compound **2**

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 was obtained from a Thermo MAT95XP High Resolution mass spectrometer. The HRESIMS was tested by UPLC-MS which was performed on a Waters ACQUITY UPLC H-Class system using a C_{18} (ACQUITY UPLC, $1.7\mu\text{m}$, $2.1\times 50\text{ mm}$, 0.5 mL/min) column coupled with a Waters ACQUITY UPLC PDA and λ PDA detector. Semi-preparative HPLC was performed on an Hitachi L-2000 system using a C_{18} column [HPLC (Kromasil $250\times 10\text{ mm}$, $5\mu\text{m}$, 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 *Aspergillus candidus* RA16-10 with the Genbank (NCBI) accession number KU877713 was cultured from a gorgonian *Anthogorgia ochracea* (GX-BH-WZ-16) collected from the Weizhou coral reefs in the South China Sea in 2011. 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. 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 2 months.

1.3. Extraction and isolation

After 2 months of cultivation, the fermented rice substrate was extracted for three times with EtOAc to give an organic extract (12 g). The organic extract was eluted by a gradient of petroleum ether/EtOAc to EtOAc, and then MeOH to afford five fractions (Fr.1–Fr.5). Fr.3 was subjected to Sephadex LH-20 gel column chromatography (petroleum ether: chloroform: methanol = 2: 1: 1) and reverse phase silica gel column chromatography to get three components (Fr. 31, Fr. 32, Fr. 33). Then Fr. 33 was purified by semi-preparation HPLC (60:40 MeOH– H_2O , 2 mL/min)

to give compound **1** (6.5 mg) and compound **2** (5.8 mg).

Aspergivones A (1):

C₁₉H₁₇O₇Cl, Yellowish powder. ¹H NMR (500 MHz, CDCl₃): 8.35 (1H, s, OH-2'), 7.69 (1H, d, *J* = 7.0 Hz, H-6'), 7.56 (1H, d, *J* = 6.8 Hz, H-4'), 7.04 (1H, t, *J* = 6.7 Hz, H-5'), 6.44 (1H, s, H-6), 4.00 (3H, s, MeO-5 and MeO-7), 3.91 (3H, s, MeO-3), 3.86 (3H, s, MeO-8). ¹³C NMR (125 MHz, CDCl₃): 172.9 (C-4), 156.8 (C-7), 156.5 (C-5), 151.6 (C-2), 151.4 (C-8a), 151.0 (C-2'), 139.6 (C-3), 132.8 (C-4'), 130.6 (C-8), 128.3 (C-6'), 124.1 (C-3'), 121.0 (C-5'), 119.9 (C-1'), 109.1 (C-4a), 92.6 (C-6), 62.0 (CH₃, MeO-3), 61.6 (CH₃, MeO-8), 56.5 (CH₃, MeO-7), 56.3 (CH₃, MeO-5). ESIMS *m/z* 393.1 [M + H]⁺. HRESIMS *m/z* 393.0736 [M + H]⁺ (calcd for C₁₉H₁₈ClO₇, 393.0736).

Aspergivones B (2):

C₁₉H₁₈O₇, Yellowish powder. ¹H NMR (500 MHz, CDCl₃): 8.04 (1H, s, OH-2'), 7.70 (1H, d, *J* = 8.1 Hz, H-6'), 7.35 (1H, t, *J* = 7.7 Hz, H-3'), 7.04 (2H, m, H-4' and H-5'), 6.34 (1H, s, H-6), 3.90 (3H, s, MeO-5 and MeO-7), 3.78 (3H, s, MeO-8 and MeO-3). ¹³C NMR (125 MHz, CDCl₃): 173.3 (C-4), 157.1 (C-7), 156.9 (C-5), 155.6 (C-2), 153.0 (C-2'), 152.0 (C-8a), 139.6 (C-3), 133.3 (C-4'), 130.8 (C-8), 129.8 (C-6'), 121.1 (C-3'), 119.9 (C-5'), 118.5 (C-1'), 109.5 (C-4a), 92.7 (C-6), 62.3 (CH₃, MeO-3), 62.0 (CH₃, MeO-8), 57.0 (CH₃, MeO-7), 56.0 (CH₃, MeO-5). ESIMS *m/z* 359.02 [M + H]⁺, 739.16 [2M + Na]⁺.

1.4. Bioactivity assays

1.4.1. Alpha-glucosidase Assays

Compounds **1** and **2** were assayed in vitro for alpha-glucosidase inhibiting activity. With deoxynojirimycin, acarbose, migltol as three positive controls. Alpha-glucosidase inhibition was determined spectrophotometrically using α-D pyran glycosidase (PNPG) as substrate by classical enzyme inhibitors in vitro screening method (Yeh et al. 2003; Van et al. 2005). PNPG is maltose analogues, after alpha-glucosidase added, which will show yellow on nitrophenol and can be directly used in the detection of spectrophotometer. The result showed that **2** has a slight activity with IC₅₀ values of 244 μg/mL (deoxynojirimycin, IC₅₀ = 42.40 μg/mL; acarbose, IC₅₀ = 457 μg/mL; migltol, IC₅₀ > 500 μg/mL). **1** only showed 30% inhibitory rate at the concentration of 100 μg/mL.

1.4.2. Cytotoxic Assays

The cytotoxic activities were evaluated against the leukemia (K562), colon cancer

(HCT-116), cervical cancer (HeLa), non-small cell lung cancer (A549), human breast cancer (MCF-7), human pancreatic cancer (BXPC-3) cell lines with adriamycin as a positive control. 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.

1.4.3. Antibacterial Assays

The antibacterial activity was evaluated by the conventional broth dilution assay (Pierce et al. 2008). Eight pathogenic bacterial strains, *Bacillus subtilis*, *Bacillus cereus*, *Candida albicans*, *Escherchia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus hemolyticus* and *Micrococcus luteus* were used, and ciprofloxacin was used as a positive control.

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

position	δ_{C} , type	δ_{H} , (J in Hz)
2	151.6, C	
3	139.6, C	
4	172.8, C	
4a	109.1, C	
5	156.5, C	
6	92.6, CH	6.44, s
7	156.8, C	
8	130.6, C	
8a	151.5, C	
1'	119.9, C	
2'	151.0, C	
3'	124.1, C	
4'	132.8, CH	7.56, d (7.0)
5'	121.0, CH	7.04, t (7.0)
6'	128.2, CH	7.69, d (7.0)
2'-OH		8.35, s
3-OMe	62.0, C	3.91, s
7-OMe	56.5, C	4.00, s
8-OMe	61.6, C	3.86, s
5-OMe	56.3, C	4.00, s

^a 500 MHz for ¹H NMR and 125 MHz for ¹³C NMR in CDCl₃.

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

Figure S2. ^1H NMR (500 MHz, CDCl_3) spectrum of compound **1**

Figure S3. Partial ^1H NMR (500 MHz, CDCl_3) spectrum of compound **1**

Figure S4. ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **1**

Figure S5. Partial ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **1**

Figure S6. Partial ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **1**

Figure S7. ^1H – ^1H COSY (CDCl_3) spectrum of compound **1**

Figure S8. HMQC (CDCl_3) spectrum of compound **1**

Figure S9. HMBC (CDCl₃) spectrum of compound **1**

Figure S10. DEPT-90 (CDCl₃) spectrum of compound **1**

Figure S11. DEPT-135 (CDCl₃) spectrum of compound **1**

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

Figure S13. ESIMS spectrum of compound **1**

Table S2. ^1H and ^{13}C NMR Data for **2**^a

position	δ_{C} , type	δ_{H} , (<i>J</i> in Hz)
2	155.6, C	
3	139.6, C	
4	173.3, C	
4a	109.5, C	
5	156.9, C	
6	92.7, CH	6.34, s
7	157.1, C	
8	130.8, C	
8a	152.0, C	
1'	118.5, C	
2'	153.0, C	
3'	121.1, C	7.35, d (7.7)
4'	133.3, CH	6.99, m
5'	120.0, CH	6.99, m
6'	129.8, CH	7.69, d (7.7)
2'-OH		8.00, s

3-OMe	62.3,C	3.78, s
7-OMe	57.0,C	3.90, s
8-OMe	62.0,C	3.78, s
5-OMe	56.8,C	3.90, s

^a 500 MHz for ¹H NMR and 125 MHz for ¹³C NMR in CDCl₃.

Figure S14. ¹H NMR (500 MHz, CDCl₃) spectrum of compound **2**

Figure S15. Partial ^1H NMR (500 MHz, CDCl_3) spectrum of compound **2**

Figure S16. ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **2**

Figure S17. Partial ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **2**

Figure S18. Partial ^{13}C NMR (125 MHz, CDCl_3) spectrum of compound **2**

Figure S19. DEPT-90 (CDCl₃) spectrum of compound **2**

Figure S20. DEPT-135 (CDCl₃) spectrum of compound **2**

Figure S21. ESIMS spectrum of compound **2**

References:

- Pierce C G, Uppuluri P, Teistan A R, Wormley F L J, Mowat E, Ramage G, Lopez-Ribot J L. 2008. A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. *Nat. Protoc.*, 3: 1494–1500.
- Yeh G Y, Eisenberg D M, Kaptchuk T J, Phillips R S. 2003. Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes care*, 26: 1277-1294.
- Van de Laar F A, Lucassen P L B J, Akkermans R P, Van de Lisdonk E H, Rutten G E, Weel C V. 2005. Alpha-glucosidase inhibitors for type 2 diabetes mellitus. *The Cochrane Library*.