Arch Pharm Res Vol 33, No 2, 231-235, 2010
DOI 10.1007/s12272-010-0207-4

Archives of Pharmacal Research
www.springer.com/12272

Bioactive Metabolites from the Sponge-Derived Fungus *Aspergillus versicolor*

Yoon Mi Lee[1], Huayue Li[1], Jongki Hong[2], Hee Young Cho[3], Kyung Sook Bae[4], Mi Ae Kim[1], Dong-Kyoo Kim[5], and Jee H. Jung[1]

[1]College of Pharmacy, Pusan National University, Busan 609-735, Korea, [2]College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea, [3]Korea Research Institute of Chemical Technology, Daejon 305-343, Korea, [4]Korea Research Institute of Bioscience and Biotechnology, Daejon 305-333, Korea, and [5]Department of Biomedicinal Chemistry and Institute of Functional Materials, Inje University, Gimhae 621-749, Korea

(Received July 25, 2009/Revised October 30, 2009/Accepted December 16, 2009)

As part of an ongoing search for bioactive metabolites from the fungus *Aspergillus versicolor* derived from a marine sponge *Petrosia* sp., an aromatic polyketide derivative (**1**), two xanthones (**2** and **3**), and five anthraquinones (**4**-**8**) were isolated by bioactivity-guided fractionation. The gross structures were determined based on the NMR and MS spectroscopic data, and the absolute configurations were defined by comparison of optical rotation data with those of reported. Compounds **2**, **4**, **5**, and **7** exhibited significant cytotoxicity against five human solid tumor cell lines (A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT-15) with IC_{50} values in the range of 0.41-4.61 μg/mL. Compounds **4** and **7** exhibited antibacterial activity against several clinically isolated Gram-positive strains with MIC values of 0.78-6.25 μg/mL.

Key words: *Aspergillus versicolor*, Cytotoxicity, Antibacterial activity, Polyketide, Xanthone, Anthraquinone

INTRODUCTION

Marine invertebrate-associated microorganisms have attracted recent attention as an important source of novel, biologically active secondary metabolites (Belofsky et al., 1998). The fungal genus *Aspergillus* has been reported to produce a considerable number of cytotoxic compounds as well as other bioactive compounds (Belofsky et al., 1998; Fremlin et al., 2009). A previous study searching for bioactive metabolites from the marine sponge-derived fungus, *Aspergillus versicolor*, yielded three polyketides (Lee et al., 2007). In a continuing study on the same fungus, an aromatic polyketide derivative (**1**), two xanthones (**2** and **3**), and five anthraquinones (**4**-**8**) were isolated by bioactivity-guided fractionation. This paper reports the isolation, structure elucidation and bioactivity evaluation of these compounds.

Correspondence to: Jee H. Jung, College of Pharmacy, Pusan National University, Busan 609-735, Korea
Tel. 82-51-510-2803, Fax. 82-51-513-6754
E-mail: jhjung@pusan.ac.kr

MATERIALS AND METHODS

General experimental procedures

The ^1H and ^{13}C NMR spectra were recorded on Varian INOVA 500 MHz and Varian UNITY 400 MHz spectrometers. The chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H = 3.30 and δ_C = 49.0 for CD_3OD, δ_H = 2.50 and δ_C = 39.5 for DMSO). The FABMS data were obtained on a JEOL JMS SX-102A spectrometer. The optical rotations were measured using a JASCO P-1020 digital polarimeter. HPLC was performed on Shodex C8-5E (250 × 10 mm, 5 μm, 100 Å), and Shodex C18-10E (250 × 10 mm, 5 μm, 100 Å) columns using a Shodex RI-71 detector.

Fungal isolation and culture conditions

The sponge was collected by hand by a SCUBA diver (20 m in depth) in 2004 off the coast of Jeju Island, Korea. This specimen was identified as *Petrosia* sp. and its morphology is described elsewhere (Lim et al., 2001). After rinsing with sterile sea water,

small pieces of the surface and inner tissue of the sponge were homogenized and then inoculated on malt extract agar (20 g/L of malt extract (Difco), 1 g/L of peptone (Difco), 20 g/L of glucose, 20 g/L of agar, and 5 mL/L of penicillin-streptomycin solution in 75% aged sea water) at 25°C for 10-14 days to allow colony development (Hyde et al., 1987). One of the pure fungal strains (designated as PF10M), of which the EtOAc extract showed strong brine shrimp lethality (LC_{50} = 32 μg/mL), was identified as *Aspergillus versicolor* by morphological and biochemical analyses.

Extraction and isolation

The pure *Aspergillus versicolor* colonies were subcultured in 500 mL Erlenmeyer flasks each containing 250 mL of malt media (20 g/L of malt extract, 1 g/L of peptone, and 20 g/L of glucose in 75% aged sea water). For a large scale culture, 250 mL of the subculture was transferred to a 2 L Erlenmeyer flask each containing 1 L of malt media, and incubated at 32°C for 21 days on a rotary shaker (150 rpm). The culture (8 L) was extracted with 16 L of EtOAc to produce the EtOAc extract (2.0 g of residue, brine shrimp lethality, LC_{50} = 32 μg/mL), which was partitioned between *n*-hexane (0.7 g, LC_{50} = 51 μg/mL) and 90% MeOH (1.27 g, LC_{50} = 0.4 μg/mL). The 90% MeOH layer was subjected to a stepped-gradient MPLC (ODS-A, 120 Å, S-30/50 mesh) eluting with 50% to 100% MeOH to afford 13 fractions. Fraction 3, one of the bioactive fractions (LC_{50} < 0.1 μg/mL), was subjected to reversed-phase HPLC (Shodex C8-5E, 250 × 10 mm, 5 μm, 100 Å) eluting with 55% MeOH to afford 9 subfractions. Compound **1** (1.5 mg) was obtained by separation of subfraction 3 on a reversed-phase HPLC eluting with 55% MeOH. Another bioactive fraction 6 (122.0 mg, LC_{50} < 0.1 μg/mL) was eluted with 65% MeOH to afford compounds **2** (3.2 mg) and **3** (0.8 mg). Compounds **4**-**8** were obtained from fractions 9 and 10 eluting with 90% MeOH.

2,4-Dihydroxy-6-((*R*)-4-hydroxy-2-oxopentyl)-3-methylbenzaldehyde (1)

Yellow, amorphous powder; $[\alpha]_D$: +29.0 (*c* 0.03, MeOH); IR (acetone) υ_{max} 3400, 1700, 1680 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 12.59 (1H, br s, 2-OH, DMSO-d_6), 10.68 (1H, br s, 4-OH, DMSO-d_6), 9.80 (1H, br s, H-7), 6.25 (1H, s, H-5), 4.24 (1H, m, H-12), 4.04 (2H, br s, H-9), 2.72 (1H, dd, *J* = 15.5, 10.5 Hz, H-11a), 2.63 (1H, dd, *J* = 15.5, 4.0 Hz, H-11b), 2.03 (3H, s, H-8), 1.23 (3H, d, *J* = 6.0 Hz, H-13); ^{13}C NMR (CD_3OD, 100 MHz) δ 208.7 (C-10), 194.9 (C-7), 165.2 (C-2), 164.8 (C-4), 140.0 (C-6), 113.8 (C-3), 111.7 (C-5), 110.6 (C-1), 64.0 (C-12), 50.7 (C-11), 46.0 (C-9), 22.6 (C-13), 6.4 (C-8); LRFABMS: *m/z* 253 $[M + H]^+$; HRFABMS: *m/z* 253.1108 $[M + H]^+$ (calcd. for $C_{13}H_{17}O_5$: 253.1076).

Sterigmatocystin (2)

White crystals; $[\alpha]_D^{25}$ -206.0 (*c* 0.18, MeOH); IR (acetone) υ_{max} 3450, 3000, 1660, 1550 cm^{-1} ; ^1H NMR (CD_3OD, 500 MHz) δ 7.52 (1H, t, *J* = 7.0 Hz, H-6), 6.85 (1H, d, *J* = 7.0 Hz, H-5), 6.85 (1H, d, *J* = 2.5 Hz, H-4'), 6.78 (1H, d, *J* = 7.0 Hz, H-7), 6.46 (1H, s, H-2), 6.25 (1H, t, *J* = 2.5 Hz, H-3'), 5.47 (1H, dt, *J* = 2.5 Hz, H-2'), 4.83 (1H, dt, *J* = 2.5, 7.0 Hz, H-1'), 4.02 (3H, s, OCH_3); ^{13}C NMR (CD_3OD, 75 MHz) δ 181.3 (C-9), 164.6 (C-3), 163.3 (C-1), 162.3 (C-8), 154.9 (C-10a), 154.0 (C-4a), 145.4 (C-3'), 135.7 (C-6), 113.5 (C-4'), 111.4 (C-7), 109.2 (C-8a), 106.7 (C-4), 106.1 (C-9a), 105.9 (C-5), 102.5 (C-2'), 90.5 (C-2), 56.8 (C-11), 48.2 (C-1'); LRFABMS *m/z* 325 $[M + H]^+$.

Dihydrosterigmatocystin (3)

Light yellow amorphous powder; ^1H NMR data (CD_3OD, 500 MHz) δ 7.52 (1H, t, *J* = 7.0 Hz, H-6), 6.85 (1H, d, *J* = 7.0 Hz, H-5), 6.75 (1H, d, *J* = 7.0 Hz, H-7), 6.52 (1H, d, *J* = 2.5 Hz, H-4'), 6.50 (1H, s, H-2), 4.22 (1H, m, H-1'), 4.10 (1H, m, H-3'a), 3.60 (1H, m, H-3'b), 4.01 (3H, s, OCH_3), 2.30 (1H, m, H-2'a), 2.05 (1H, m H-2'b).

Averantin (4)

Deep orange amorphous powder; $[\alpha]_D^{25}$ +120.8 (*c* 0.28, MeOH); ^1H NMR data (CD_3OD, 500 MHz) δ 7.11 (1H, d, *J* = 2.5 Hz, H-7), 7.07 (1H, s, H-4), 6.45 (1H, d, *J* = 2.5 Hz, H-5), 5.21 (1H, dd, *J* = 5.5 Hz, H-1'), 1.90/1.80 (2H, m, H-2'), 1.34 (2H, m, H-3'), 1.30 (2H, m, H-4'), 1.28 (2H, br s, H-5'), 0.89 (3H, t, *J* = 7.0 Hz H-6'); HRFABMS *m/z* 373.1312 $[M + H]^+$ (calcd for $C_{20}H_{21}O_7$, 373.1287).

Methyl-averantin (5)

Deep yellow amorphous powder; $[\alpha]_D^{25}$ -58.0 (*c* 0.12, MeOH); ^1H NMR data (CD_3OD, 500 MHz) δ 7.18 (1H, d, *J* = 2.0 Hz, H-7), 7.16 (1H, s, H-4), 6.53 (1H, d, *J* = 2.0 Hz, H-5), 4.96 (1H, dd, *J* = 5.5 Hz, H-1'), 3.50 (3H, s, OCH_3), 2.00/1.80 (2H, m, H-2'), 1.45/1.30 (2H, m, H-3'), 1.30 (2H, m, H-4'), 1.28 (2H, br s, H-5'), 0.89 (3H, t, *J* = 7.0 Hz, H-6'); ^{13}C NMR (CD_3OD, 75 MHz) δ 191.0 (C-9), 182.0 (C-10), 166.7 (C-8), 166.3 (C-6), 165.0 (C-3), 164.8 (C-1), 133.6 (C-10a), 133.4 (C-4a), 110.3 (C-2), 110.0 (C-8a), 108.9 (C-5), 108.7 (C-7), 108.5 (C-4), 108.0 (C-9a), 75.5 (C-1'), 58.0 (OCH_3), 33.6 (C-2'), 31.4 (C-3'), 25.2 (C-4'), 22.2 (C-5'), 14.0 (C-6'); LRFABMS *m/z* 409 $[M + Na]^+$; HRFABMS *m/z* 387.1444 $[M + H]^+$ (calcd for $C_{21}H_{23}O_7$, 387.1451).

Averufin (6)
Light yellow amorphous powder; $[\alpha]_D^{25}$ -201.0 (*c* 0.10, MeOH); ^{1}H NMR data (CD_3OD, 500 MHz) δ 7.15 (1H, d, *J* = 2.5 Hz, H-7), 7.12 (1H, s, H-4), 6.50 (1H, d, *J* = 2.5 Hz, H-5), 5.30 (1H, d, *J* = 3.0 Hz, H-1'), 2.00/2.15 (2H, m, H-4'), 2.00/2.07 (2H, m, H-2'), 1.55 (3H, s, CH_3), 1.30 (2H, m, H-3'); LRFABMS *m/z* 369 $[M + H]^+$.

Nidurufin (7)
Light yellow amorphous powder; $[\alpha]_D^{25}$ +165.0 (*c* 0.07, MeOH); ^{1}H NMR data (CD_3OD, 500 MHz) δ 7.16 (1H, br s, H-7), 7.15 (1H, s, H-4), 6.49 (1H, br s, H-5), 5.12 (1H, m, H-1'), 3.94 (1H, m, H-2'), 2.20/1.90 (2H, m, H-4'), 1.58 (3H, s, CH_3), 1.70 (2H, m, H-3').

Versiconol (8)
Light yellow crystal; $[\alpha]_D^{25}$ -101.5 (*c* 0.20, MeOH); ^{1}H NMR data (CD_3OD, 500 MHz) δ 7.21 (1H, s, H-4), 7.15 (1H, s, H-7), 6.53 (1H, d, *J* = 2.5 Hz, H-5), 3.97 (2H, m, H-2'), 3.73 (1H, m, H-1'), 3.53 (2H, m, H-4'), 2.17/2.07 (2H, m, H-3').

Cytotoxicity
The cytotoxicity was evaluated at the Korea Research Institute of Chemical Technology. The rapidly growing cells (A-549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF-498, human CNS cancer; HCT-15, human colon cancer) were harvested, counted and inoculated at the appropriate concentrations ((1-2) × 10^4 cells/well) into 96-well microtiter plates. After incubation for 24 h, the compounds dissolved in the culture medium (RPMI 1640, Gibco; 10% FBS, Gibco) were applied to the culture wells in triplicate, and incubated for 72 h at 37°C in an atmosphere containing 5% CO_2. The culture was fixed with cold TCA, and was stained with 0.4% SRB (sulforhodamine B, Sigma) dissolved in 1% acetic acid. The bound dye was dissolved in 10 mM of an unbuffered Tris base using a gyrotatory shaker, and the absorbance at 520 nm was measured with a microplate reader (Dynatech Model MR 700). The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance by 50% compared to the control level in the untreated wells.

Antibacterial activity
The antibacterial assay was performed at the Korea Research Institute of Chemical Technology. Mueller Hinton Agar plates were impregnated with 17 serial dilutions of the sample and positive control (meropenem: provided by Yuhan Corporation) to make final concentrations ranging from 12.5 to 0.002 μg/mL. The strains were inoculated into Fleisch extract broth (containing 10% horse serum depending on strains) and incubated for 18 h at 37°C. The cultured strains were inoculated onto the Mueller Hinton agar plates with 10^4 CFU per spot population using an automatic inoculater (Dynatech). The MIC values were measured after 18 h of incubation.

RESULTS AND DISCUSSION

Compound **1** was obtained as a light yellow, amorphous powder with a molecular formula of $C_{13}H_{17}O_5$ according to HRFABMS. The exact mass of the $[M + H]^+$ ion (*m/z* 253.1108) matched well with the expected formula $C_{13}H_{17}O_5$ (Δ + 3.2 mmu). Strong absorption at 3400 cm^{-1} in the IR spectrum indicated a hydroxyl group, and the two intense bands at 1700 cm^{-1} and 1680 cm^{-1} indicated two carbonyl groups. Compound **1** was identified as 2,4-dihydroxy-6-((*R*)-4-hydroxy-2-oxopentyl)-3-methylbenzaldehyde by a comparison of the NMR (^{1}H, ^{13}C, COSY, HSQC, and HMBC) and MS data with those of reported in the literature

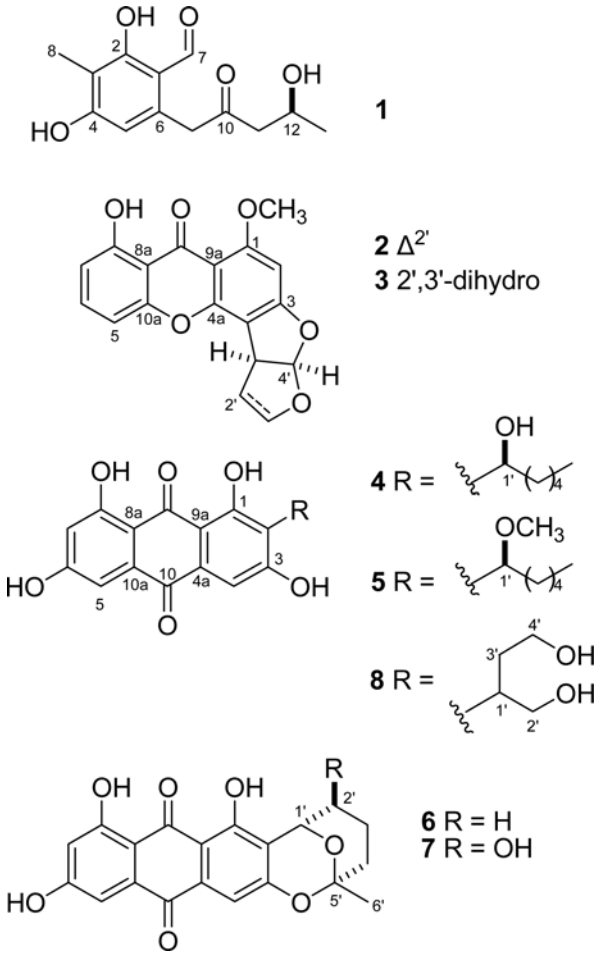

Fig. 1. Chemical structures of compounds **1**-**8**

(Yamaguchi et al., 2004). This compound was first isolated from *Aspergillus versicolor* as a new ultraviolet light-absorbing substance (Arai and Sano, 1994), and was also found to be produced as a major secondary metabolite by the fungus *Pseudobotrytis* sp. FKA-25 (Yamaguchi et al., 2004). However, the stereochemistry of the hydroxyl group at C-12 has not been defined. Interestingly, the optical rotations given in the two papers showed opposite directions, $[\alpha]_D^{26}$ +22 (*c* 0.1, MeOH) by Takayuki and Hiroshi (1994), and $[\alpha]_D^{23}$ -8.0 (*c* 0.1, MeOH) by Yamaguchi et al. (2004), which indicates that the two reported compounds isolated from *Aspergillus versicolor* and *Pseudobotrytis* sp. might be stereoisomers. Our optical rotation data ($[\alpha]_D$ +29.0 (*c* 0.03, MeOH)) was almost identical to the compound previously isolated from *Aspergillus versicolor*. In this study, the stereochemistry of the hydroxyl group at C-12 was assigned by comparing the optical rotation data with the reported compound globosumone B (Bashyal et al., 2005). Globosumone B has a similar structure with **1** except for the substitutions at benzene ring, and the absolute configuration of hydroxyl group was defined as *S* by Mosher's method. As the optical rotation data of **1** ($[\alpha]_D^{25}$ +29.0 (*c* 0.03, MeOH)) and globosumone B ($[\alpha]_D^{25}$ +4.95 (*c* 0.05, MeOH)) both showed positive values, it is speculated that the stereochemistry of the hydroxyl group at C-12 of **1** is 12*S* configuration.

Compounds **2**-**8** were identified as sterigmatocystin (**2**), dihydrosterigmatocystin (**3**), averantin (**4**), methylaverantin (**5**), averufin (**6**), nidurufin (**7**), and versiconol (**8**) by a comparison of the ^1H and ^{13}C NMR data with those of reported in the literature (Zhu and Lin, 2007; Bennett et al., 1980; Augamp and Holzapfel, 1970; Shier et al., 2005; Fredenhagen et al., 1995). These xanthones (**2** and **3**) and anthraquinones (**4**-**8**) are related to the aflatoxin biosynthesis in *Aspergillus* species.

As part of our ongoing investigation into the bioactivities of these aflatoxin related compounds, the cytotoxicity and antibacterial activity the compounds isolated in the present study were evaluated. The cytotoxicity was determined against five human tumor cell lines (A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT-15). Compounds **2**, **4**, **5**, and **7** showed strong activity with IC_{50} values ranging from 0.41 to 4.61 μg/mL, but compounds **6** and **8** exhibited weak activity (Table I). However, compound **3**, which had the same xanthone skeleton as **2**, showed no activity. Considering the only structural difference between compounds **2** and **3**, i.e. compound **2** has a double bond at C-2', it is believed that the additional (allylic ether) moiety (Radin et al., 2007) plays an important role in the cytotoxicity of compound **2**. Among the anthraqui-

Table I. Cytotoxicity of compounds **1**-**8**[a] against human solid tumor cell lines

Compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	>30.0	>30.0	>30.0	>30.0	>30.0
2	1.86	2.53	1.22	2.75	4.61
3	>30.0	>30.0	>30.0	>30.0	>30.0
4	3.15	3.88	3.57	3.04	3.13
5	0.64	1.17	1.10	0.41	0.49
6	14.92	14.07	14.56	12.04	11.97
7	1.83	3.39	3.16	1.78	2.20
8	20.45	15.29	15.86	23.73	19.02
Doxorubicin	0.004	0.019	0.002	0.01	0.034

[a]Data expressed in IC_{50} values (μg/mL). A-549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF-498, human CNS cancer; HCT-15, human colon cancer.

Table II. Antibacterial activity of compounds **4**-**7**

Strain[b]	Compound (μg/mL)[a]				
	4	**5**	**6**	**7**	meropenem
Streptococcus pyogenes 308A	0.78	6.25	6.25	3.13	0.01
Streptococcus pyogenes 77A	3.13	>12.50	>12.50	6.25	0.01
Staphylococcus aureus SG511	3.13	>12.50	12.50	6.25	0.10
Staphylococcus aureus 285	3.13	>12.50	12.50	3.13	0.10
Staphylococcus aureus 503	1.56	>12.50	6.25	3.13	0.05

[a]Data expressed in MIC (μg/mL)
[b]Gram-positive strains.

nones (**4**-**8**), compound **5** showed most potent cytotoxicity. The free hydroxyl derivatives of compounds **4**, **5** and **8** showed much attenuated cytotoxicity, suggesting that their activity is modulated by the polarity of these compounds.

The antibacterial activities of these anthraquinones (**4**-**8**) were evaluated against clinically isolated bacterial strains. In the agar dilution method, compounds **4** and **7** showed weak activity against all five Gram-positive bacterial strains with minimal inhibitory concentration (MIC) values ranging from 0.78-6.25 μg/mL (Table II). The *Staphylococcus pyogenes* 308A strain was most sensitive to these compounds.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Chung Ja Sim of Hannam University for identifying the sponge. This study was supported by a grant from the Marine Biotechnology Program, Ministry of Land, Transport, and Maritime Affairs, Korea.

REFERENCES

Arai, T. and Sano, H., Novel UV-absorbing compounds FK17-P2a, FK-17-P2b1, FK17-P2b2, and FK17-P3 and manufacture of the compounds with *Aspergillus* sp. Jpn. Kokai Tokkyo Koho, pp. 6, (1994)

Augamp, P. J. and Holzapfel, G. W., Polyhydroxyanthraquinones from *Aspergillus versicolor*, *Aspergillus nidulans* and *Bipolaris* sp. *J. S. Afr. Chem. Inst.*, 23, 40-56 (1970).

Bashyal, B. P., Wijeratne, E. M. K., Faeth, S. H., and Gunatilaka, A. A. L. Globosumones A-C, cytotoxic orsellinic acid esters from the sonoran desert endophytic fungus *Chaetomium globosum*. *J. Nat. Prod.*, 68, 724-728 (2005).

Belofsky, G. N., Jensen, P. R., Renner, M. K., and Fenical, W., New cytotoxic sesquiterpenoid nitrobenzoyl esters from a marine isolate of the fungus *Aspergillus versicolor*. *Tetrahedron*, 54, 1715-1724 (1998).

Bennett, J. W., Lee, L. S., Shoss, S. M., and Boudreaux, G. H., Identification of averantin as aflatoxin B_1 precursor: Placement in the biosynthetic pathway. *Appl. Environ. Microbiol.*, 39, 835-839 (1980).

Fredenhagen, A., Hug, P., Sauter, H., and Peter, H. H., Paeciloquinones A, B, C, D, E and F: New potent inhibitors of protein tyrosine kinase produced by *Paecilomyces carneus*. *J. Antibiot.*, 48, 199-204 (1995).

Fremlin, L. J., Piggott, A. M., Lacey, E., and Capon, R. J., Cottoquinazoline A and cotteslosins A and B, metabolites from an Australian marine-derived strain of *Aspergillus versicolor*. *J. Nat. Prod.*, 72, 666-670 (2009).

Hyde, K. D., Farrant, C. A., and Jones, E. B. G., Isolation and culture of marine fungi. *Botanica Marina*, 30, 291-303 (1987).

Lee, Y. M., Mansoor, T. A., Hong, J., Lee, C. O., Bae, K. S., and Jung, J. H., Polyketides from a sponge-derived fungus, *Aspergillus versicolor*. *Nat. Prod. Sci.*, 13, 90-96 (2007).

Lim, Y. J., Park, H. S., Im, K. S., Lee, C. O., Hong, J., Lee, M. Y., Kim, D. K., and Jung, J. H., Additional cytotoxic polyacetylenes from the marine sponge *Petrosia* species. *J. Nat. Prod.*, 64, 46-53 (2001).

Radin, N. S., Meta-analysis of anticancer drug structures-significance of their polar allylic moieties. *Anticancer Agents Med. Chem.*, 7, 209-222 (2007).

Shier, W. T., Lao, Y., Steele, T. W. J., and Abbas, H. K., Yellow pigments used in rapid identification of aflatoxin-producing *Aspergillus* strains are anthraquinones associated with the aflatoxin biosynthetic pathway. *Bioorg. Chem.*, 33, 426-438 (2005).

Yamaguchi, Y., Masuma, R., Kim, Y. P., Uchida, R., Tomoda, H., and Omura, S., Taxonomy and secondary metabolites of *Pseudobotrytis* sp. FKA-25. *Mycoscience*, 45, 9-16 (2004).

Zhu, F. and Lin, Y., Three xanthones from a marine-derived mangrove endophytic fungus. *Chem. Nat. Com.*, 43, 132-135 (2007).