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ScienceDirect
Chinese Journal of Natural Medicines 2018, **16**(3): 0219–0224
doi: 10.3724/SP.J.1009.2018.00219
Chinese Journal of Natural Medicines

Antioxidant xanthones and anthraquinones isolated from a marine-derived fungus *Aspergillus versicolor*

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Available online 20 Mar., 2018

[ABSTRACT] Chemical examination of an EtOAc extract of cultured *Aspergillus versicolor* fungus from deep-sea sediments resulted in the isolation of four xanthones, eight anthraquinones and five alkaloids, including a new xanthone, oxisterigmatocystin D (**1**) and a new alkaloid, aspergillusine A (**13**). High resolution electron impact mass spectrometry (HR-EI-MS), FT-IR spectroscopy, and NMR techniques were used to elucidate the structures of these compounds, and the absolute configuration of compound **1** was established by its NMR features and coupling constant. Furthermore, the biosynthesis pathway of these xanthones and anthraquinones were deduced, and their antioxidant activity and cytotoxicity in human cancer cell lines (HTC-8, Bel-7420, BGC-823, A549, and A2780) were evaluated. The trolox equivalent antioxidant capacity (TEAC) assay indicated most of the xanthones and anthraquinones possessing moderate antioxidant activities. The Nrf2-dependent luciferase reporter gene assay revealed that compounds **6**, **7**, **9**, and **12** potentially activated the expression of Nrf2-regulated gene. In addition, compounds **5** and **11** showed weak cytotoxicity on A_{549} with the IC_{50} values of 25.97 and 25.60 μmol·L^{-1}, respectively.

[KEY WORDS] *Aspergillus versicolor*; Xanthones; Anthraquinones; Antioxidant activity; Cytotoxicity

[CLC Number] R284 **[Document code]** A **[Article ID]** 2095-6975(2018)03-0219-06

Introduction

Over the past years, more than 180 *Aspergillus* strains have been isolated from a host of terrestrial ecological niches, and they provide a steady stream of diverse small molecules [1]. Aflatoxin pathway, as a main biosynthesis pathway in the genus of *Aspergillus* [2-3], can produce abundant of xanthones and anthraquinones, which always show antioxidant activity and cytotoxicity [4-5]. The fungal strain *Aspergillus versicolor*, a species of this genus, also has been proven to be a rich source of diverse secondary metabolites with novel structures and interesting bioactivities [6-8].

[Received on] 30-May-2017
[Research funding] This work was supported by the Knowledge Innovation Program of Shenzhen (JCYJ20160428181415376 and-JCYJ20150529153646078).
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These authors have no conflict of interest to declare.

As part of our ongoing research on structurally novel and bioactive compounds, the EtOAc extract of a fungus strain *A. versicolor* A-21-2-7, which was obtained from the marine sediment samples, has been studied. Chemical investigation resulted in the isolation of a new xanthone, oxisterigmatocystin D (**1**) and a new alkaloid, aspergillusine A (**13**), along with another three known xanthones (**2**−**4**), eight known anthraquinones (**5**−**12**), and four known alkaloids (**14**−**17**) (Fig. **1**). Their structure elucidation and biological activities are described here in detail.

Results and Discussion

Compound identification and structure elucidation

Chromatographic separation of the EtOAc extract of *A. versicolor* cultured in solid rice medium, including semipreparative HPLC purification, resulted in the isolation of compounds **1**−**17**, two of which were new.

Compound **1** was obtained as a pale yellow needle-like crystal, with the molecular formula of $C_{19}H_{16}O_7$ based on HRESIMS, ^{1}H NMR and ^{13}C NMR data (12 degrees of unsaturation). The IR absorption bands showed the presence of

hydroxyl (2 917 cm^{-1}) and carbonyl (1 649 cm^{-1}) groups. The NMR data exhibited 18 carbon signals, including two benzene rings, two methoxy groups, one methylene, two methines, and a keto carbonyl group (δ_C 180.9). Careful analysis of 1D NMR data (Table **1**) revealed that compound **1** had a similar sterigmatocystin skeleton as that of oxisterigmatocystin C (**2**) [9], which also could be confirmed by the COSY correlations of H-5/H-6/H-7 and H-1′/H-2′/H-3′/H-4′, and the HMBC correlations from H-2 to C-1/C-3/C-4/C-9a, H-6 to C-8a/C-10a, and OH-8 to C-7/C-8/C-8a, as shown in Fig. **2**. Furthermore, the methoxyl groups could be assigned at C-1 and C-4′, respectively, based on the HMBC correlations from 1-OMe (δ_H 3.90, s) to C-1 (δ_C 163.5) and from 4′-OMe (δ_H 3.37, s) to C-4′ (δ_C 107.0) (Fig. **2**). The ^{1}H and ^{13}C NMR data of compound **1** were almost identical to those of compound **2** except that they had different coupling patterns of H-2′, H-3′, and H-4′ (Table **1**). Thus, compound **1** was proposed as a diastereomer of compound **2**, with the absolute configurations of 1′*R*, 2′*S* and 4′*S* [9]. Thus, the structure of **1** was elucidated, and named as oxisterigmatocystin D.

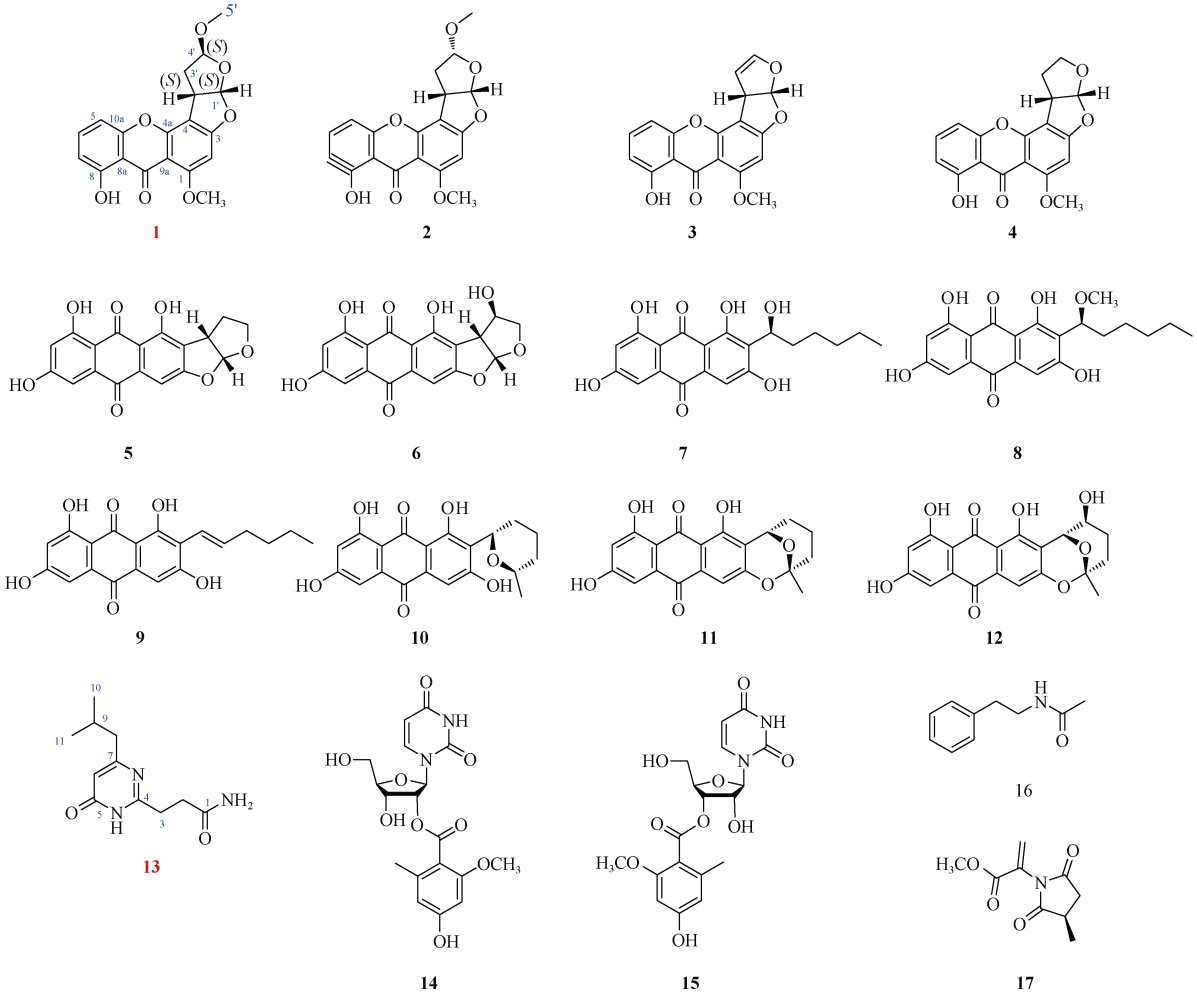

Fig. 1 Structures of compounds 1–17

Aspergillusine A (**13**), isolated as a colorless needle-like crystal, was assigned the molecular formula of $C_{11}H_{17}N_3O_2$ by the HRESIMS data (*m/z* 224.138 5 $[M + H]^+$) with 5 degrees of unsaturation. The IR absorptions at 3 404 cm^{-1} and 1 652 cm^{-1} suggested the presence of amino group (-NH) and carbonyl group. The 1D NMR and HSQC (Table 2) spectra provided the resonances for 11 carbon signals, including two methyl (δ_C, 22.3; δ_H 0.84, d), three methylene (δ_H 2.79, t; 2.41, t; 2.24, d), one methine (δ_H 1.90, m), one tri-substituted double bond (δ_H 7.00, s), two quaternary carbons, and an amide carbonyl group (δ_C 174.2). The ^{1}H-^{1}H COSY correlations between H_2-2 and H_2-3, H-9 and H_2-8/H_3-10/H_3-11, together with the HMBC correlations from H-6 to C-5/C-7, from H_2-8 to C-6/C-7, from H_2-3 to C-4, from H_2-2 to C-1, and from NH_2 to C-1 indicated the structure units and the basic skeleton of compound **13** (Fig. 2). The lower field location of NH group (δ_H 12.05, s) in ^{1}H NMR indicated the NH was nearby with the carbonyl. All the information mentioned above showed that the structure of **13** probably contained a pyrimidine ring, which could be further certified by the chemical shifts of C-4, C-5 and C-7 in ^{13}C-APT spectrum (Table 2), and the linkage of the two residues was also assigned (Fig. 2). Thus, the structure of **13** was determined (Fig. 1).

Table 1 ^{1}H and ^{13}C NMR data for compounds 1–2 in DMSO-d_6

Position	1[a]		2[b]	
	δ_H	δ_C	δ_H	δ_C
1		163.5, C		163.4, C
2	6.66, s	91.3, C	6.59, s	91.1, C
3		165.2, C		165.4, C
4		107.2, C		108.2, C
4a		154.2, C		153.6, C
5	6.97, d (8.2)	106.8, CH	6.92, d (8.1)	106.6, CH
6	7.62, t (8.2)	136.6, CH	7.59, t (8.1)	136.5, CH
7	6.74, d (8.2)	111.1, CH	6.72, t (8.1)	111.0, CH
8		161.8, C		161.9, C
8a		108.7, C		108.6, C
9		180.9, C		180.9, C
9a		105.4, C		105.0, C
10a		154.9, C		154.9, C
1-OCH_3	3.90, s	57.1, CH	3.88, s	57.1, CH
1'	6.54, d (5.8)	112.1, CH	6.60, d (6.4)	114.2, CH
2'	4.27, ddd (9.0, 5.8, 4.2)	42.3, CH	4.20, dd (8.2, 6.4)	42.3, CH
3'	2.26, ddd (13.4, 9.0, 5.0) 2.38, dt (13.4, 4.2)	36.4, CH_2	2.23, d (13.2) 2.40, ddd (13.2, 8.2, 4.8)	37.1, CH_2
4'	5.19, dd (5.0, 5.0)	107.0, CH	5.27, d (4.8)	106.9, CH
4'-OCH_3	3.37, s	56.4, CH_3	3.10, s	55.0, CH_3
8-OH	13.32, s		13.37, s	

a. ^{1}H NMR 500 MHz, ^{13}C NMR 125 MHz; b. ^{1}H NMR 400 MHz, ^{13}C NMR 100 MHz

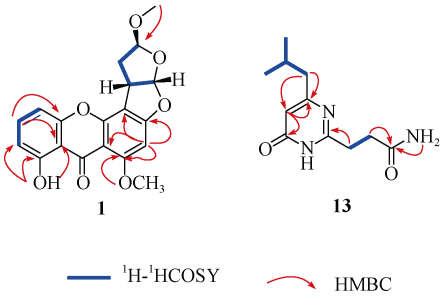

Fig. 2 Key ^{1}H–^{1}H COSY and HMBC correlations of compounds 1 and 13

On the basis of the NMR and MS spectroscopic comparison with those reported in the literatures, in addition to the specific rotation, the other 15 compounds were identified as oxisterigmatocystin C (**2**) [9], sterigmatocystine (**3**) [4], dihydrosterigmatocystine (**4**) [10], versicolorin B (**5**) [11], UCT1072M1 (**6**) [12], averantin (**7**) [4], methyl-averantin (**8**) [4], averythrin (**9**) [13], averufanin (**10**) [14], averufine (**11**) [4], nidurufin (**12**) [4], kipukasin H (**14**) [15], kipukasin I (**15**) [15], N-Phenethylacetaminde (**16**) [16], and versimide (**17**) [17]. The biosynthesis pathways of xanthones and anthraquinones from anthrone were also deduced (Fig. 3) [18-19].

Table 2 ^{1}H and ^{13}C NMR data for compound 13 in DMSO-d_6

Position	13 (^{1}H NMR 400 MHz, ^{13}C NMR 100 MHz)	
	δ_H	δ_C
1		174.2, C
2	2.41, t (7.4)	31.5, CH_2
3	2.79, t (7.4)	28.0, CH_2
4		155.6, C
5		155.8, C
6	7.00, s	121.4, CH
7		138.5, C
8	2.24, d (7.1)	38.8, CH_2
9	1.90, m	27.9, CH
10	0.85, d (6.2)	22.3, CH_3
11	0.85, d (6.2)	22.3, CH_3
-NH/NH_2	-NH_2 (7.28, s; 6.69, s) -NH 12.05, s	

The antioxidant capability of xanthones and anthraquinones was firstly studied by TEAC assay [20]. The activity of the tested compounds was expressed as TEAC (trolox equivalent antioxidant capacity) values. TEAC value is defined as the concentration of standard trolox (1 mmol·L^{-1}) to be set as 1. As shown in Table 3, the potent free-radical scavenging activity.

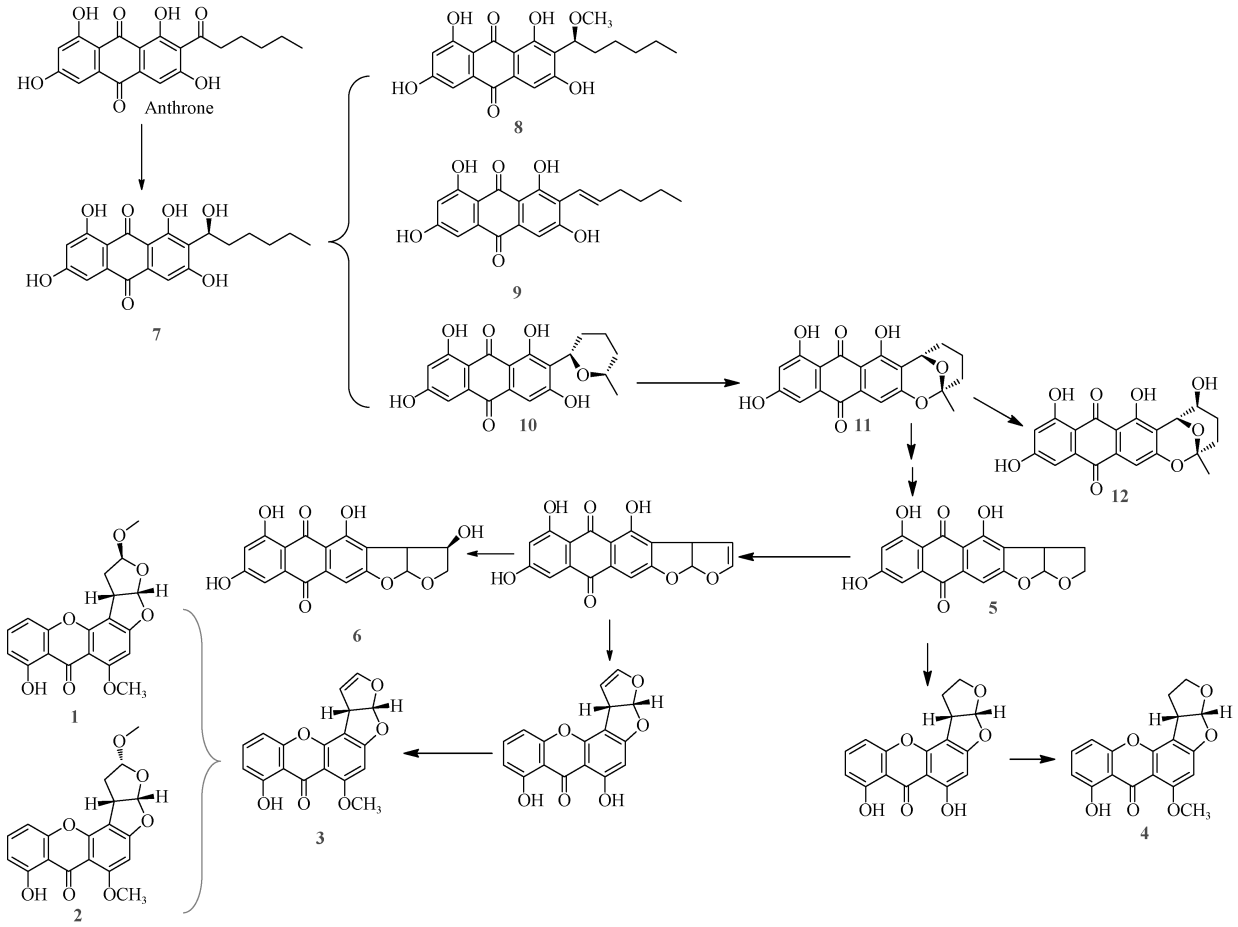

Fig. 3 The biosynthesis pathways of xanthones and anthraquinones (1−12)

Table 3 Antioxidant effects of compounds 1−3 and 5−12

Compound	Antioxidant capacity (Trolox equivalents)	ARE luciferase activity (folds to control)
tBHQ	N/A	3.0 ± 0.3
Trolox	1	N/A
1	0.55 ± 0.13	
2	1.16 ± 0.18	
3	0.65 ± 0.13	
5	1.03 ± 0.11	1.27 ± 0.12
6	0.97 ± 0.01	1.49 ± 0.28
7	0.89 ± 0.10	1.58 ± 0.11
8	0.86 ± 0.08	1.05 ± 0.09
9	0.22 ± 0.10	1.46 ± 0.08
10	0.82 ± 0.01	0.86 ± 0.04
11	0.94 ± 0.19	1.10 ± 0.06
12	0.62 ± 0.14	1.41 ± 0.05

TEAC values were expressed as Trolox equivalents needed for neutralizing the ABTS$^{\bullet+}$ radical (mmol of Trolox/g of compound). Results are expressed as average SD (n = 4).

ARE luciferase activity of compounds (10 μmol·L^{-1}) in HepG2C8 cell which was stably transfected with an ARE-driven luciferase reporter plasmid; Blankcontrol: DMSO; Positivecontrol: tBHQ (a Nrf2 activator), 50 μmol·L^{-1}

of compounds **2**, **5**, **6**, and **11** was approximately equivalent to that of trolox (1.0 mmol·L^{-1}). Comparison of the TEAC value of oxisterigmatocystin D (**1**) (TEAC 0.55) with that of oxisterigmatocystin C (**2**) (TEAC 1.16) revealed that the configuration of 4′-OMe in oxisterigmatocystins had a great influence on their antioxidant capability.

All of these anthraquinones were also evaluated to determine whether they were able to regulate the nuclear factor E2-related factor 2 (Nrf2), a transcription factor that responds to oxidative stress by binding to the antioxidant response element (ARE) in the promoter of genes coding for antioxidant enzymes and proteins for glutathione synthesis, and its activity can be measured by ARE-driven luciferase reporters [21-22]. As shown in Table **3**, significant induction of luciferase was observed at 10 μmol·L^{-1} for compounds **6**, **7**, **9**, and **12** with 1.41−1.58 folds more than that of blank control (DMSO), and with approximately half of the positive control tBHQ (tertiary butylhydroquinone) at 50 μmol·L^{-1}.

Compounds **1**−**12** were also evaluated for their cytotoxicity on the HCT-8, Bel-7402, BGC-823, A549, and A2780 cancer cell lines using the MTT method. Compounds **5** and **12** showed selective weak inhibition against A549, with IC_{50} values of 25.60, and 25.97 μmol·L^{-1} respectively, and compound **5** also showed weak cytotoxicity on A2780 with IC_{50}

value of 38.76 μmol·L^{-1}.

In summary, this work provided series of antioxidant xanthones and anthraquinones derived from marine-derived fungus, indicating that the deep-sea afforded a new source for the antioxidant discovery. The structural skeleton of aspergillusine A (**13**) was found for the first time from *A. versicolor*.

Experimental

General procedures

IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer. Optical rotations were measured using an Autopol III automatic polarimeter (Rudolph Research Co.). NMR spectra were measured on a Bruker Avance-500 FT NMR spectrometer (500 MHz) and a Bruker Avance-400 FT NMR spectrometer (400 MHz) using TMS as the internal standard. HRESIMS spectra were obtained on an FT-MS-Bruker APEX IV (7.0 T). Column chromatography was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Plant, Qingdao, PR China). Sephadex LH-20 (18 mm × 110 mm) was obtained from Pharmacia Co., and ODS (50 mm) was provided by YMC Co. TLC analyses were carried out using pre-coated silica gel GF_{254} plates (Yantai Chemical Industry Research Institute, China). High-performance liquid chromatography (HPLC) was performed on a Waters e2695 Separation Module coupled with a Waters 2998 photodiode array detector. A Kromasil C_{18} preparative HPLC column (250 mm × 10 mm, 5 μm) was used. All the solvents used in the present study were of analytical grade.

Fungal Material and Fermentation

The fungal strain *A. versicolor* A-21-2-7 was isolated from the deep-sea sediment (3 002 m) in South China Sea. The fungus was identified by morphological observation and analysis of the ITS region of the 16S rDNA, whose sequence data have been deposited to GenBank with the accession number KC899773. The strain A-21-2-7 was preserved at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, China. Fermentation of the strain was initiated in 28 500-mL sized Erlenmeyer flasks, each preloaded with 80 g of rice and 100 mL of sterilized artificial seawater. The seed was prepared by inoculating activated fungal cakes from an agar Petri dish into 200 mL of potato dextrose broth medium. Approximately 20 mL of the inoculum were then transferred to fermentation medium and further incubated for 35 days at 25 °C statically.

Extraction and Isolation

The fermented materials were extracted with ethyl acetate (EtOAc) (3 × 10 L), *n*-butanol (BuOH) (2 × 10 L), and H_2O (10 L), successively, while the organic and H_2O solutions were evaporated under vacuum to afford EtOAc, BuOH, and H_2O extracts. The EtOAc extract (6.4 g) was fractionated by silica gel vacuum liquid chromatography (VLC) using CH_2Cl_2–MeOH gradient elution to provide six fractions (Fr. 1−Fr. 6). The Fr. 2 (1.5 g) was subjected to ODS eluting with MeOH–H_2O gradient (20%−100%) to obtain Fr. 2A−Fr. 2E; Fr. 2B (400 mg) and Fr. 2C (90 mg) were further purified by the semi-preparative RP HPLC (Kromasil C_{18} preparative HPLC column, 250 mm ×10 mm, 5 μm, 2 mL·min^{-1}) eluted with 80% MeOH–H_2O for **3** (109.3 mg), and **4** (0.6 mg) and 83% MeOH–H_2O for **7** (6.5 mg), respectively; Fr. 2E (215 mg) was subjected to Sephadex LH-20 CC eluting with MeOH to give Compounds **8** (4.0 mg), **10** (1.0 mg), and **11** (7.6 mg). Fr. 3 (1.3 g) was subjected to ODS eluted with MeOH–H_2O gradient (20%–100%) to provide Fr. 3A−Fr. 3E. Both Fr. 3B (270 mg) and Fr. 3C (185 mg) were first isolated by Sephadex LH-20 (CH_2Cl_2–MeOH, *V*/*V*, 1: 1), and then purified by semi-pHPLC eluted with 45% MeOH-H_2O for Compounds **16** (3.3 mg), and **17** (19.7 mg), and 75% MeOH–H_2O for Compounds **1** (1.2 mg), **2** (5.0 mg), **5** (4.0 mg), **6** (1.5 mg), and **12** (5.8 mg). Fr.3D (110 mg) was separated by Sephadex LH-20 CC (MeOH) to afford compound **9** (6.6 mg). Fr. 4 (600 mg) was subjected to ODS eluted with MeOH–H_2O gradient (20%–100%), and then purified by semi-pHPLC eluted with 30% ACN–H_2O to give compound **4** (2.7mg), **14** (24.0 mg), and **15** (10.2 mg).

Oxisterigmatocystin D (**1**): pale yellow needle-like crystal, $[\alpha]_D^{25}$ −89.7 (*c* 0.21, CH_2Cl_2); UV (MeOH) λ_{max} 196.5, 248.4, 325.6; IR (KBr): 2 917, 2 849, 1 649, 1 586, 1 460, 1 275, 1 234, 1 131, 750 cm^{-1}; ^{1}H and ^{13}C NMR, see Table **1**; HRESIMS *m*/*z* 357.096 2 $[M + H]^+$ (Calcd. for $C_{19}H_{17}O_7$, 357.096 8).

Aspergillusine A (**13**): colorless crystal, $[\alpha]_D^{25}$ −1.8 (*c* 0.50, MeOH); UV (MeOH) λ_{max} 225.9, 323.4; IR (KBr): 3 404, 2 957, 2 868, 1 652, 1 613, 1 433, 1 054, 1 032, 1 014 cm^{-1}; ^{1}H and ^{13}C NMR, see Table **2**; HR-ESI-MS *m*/*z* 224.138 5 $[M + H]^+$ (Calcd. for $C_{11}H_{18}N_3O_2$, 224.139 3).

Biological activity testing in vitro

Antioxidant assay: The direct antioxidant capacity of the isolated compounds was evaluated by the modified 2, 2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) radical cation decolorization assay using a total antioxidant capability assay kit following the manufacturer's instruction (Beyotime Institute of Biotechnology, Jiangsu, China) [23]. The capability to induce ARE-driven antioxidant gene expression was tested in HepG2C8 cells which was stably transfected with ARE-luciferase reporter plasmids. The HepG2C8 cells were treated with 10 mmol·L^{-1} of indicated compounds for 6 h and then lysed in reporter lysis buffer; 50 mL of lysate were used to measure luciferase activity using Firefly Luciferase Reporter Gene assay kit (Beyotime Institute of Biotechnology, Jiangsu, China) and a Centro LB960 microplate luminometer (Berthold, Germany). The luciferase activity was normalized by protein concentration and expressed as folds of control. TBHQ was used as a positive control [24-25].

Cytotoxicity assay: The cytotoxic properties of the isolated compounds were tested *in vitro* using human cancer cell lines including HCT-8 (colon cancer); BeL-7402, BGC-823, and A-549 (lung adenocarcinoma); and A2780 (ovarian cancer). The bioassays used the MTT method as described in the

literature [26].

References

[1] Zhou YM, Mandi A, Debbab A, *et al*. New austalides from the sponge-associated fungus *Aspergillus* sp. [J]. *Eur J Org Chem*, 2011, **2011**(30): 6009-6019.

[2] Roze LV, Laivenieks M, Hong SY, *et al*. Aflatoxin biosynthesis is a novel source of reactive oxygen species−a potential redox signal to initiate resistance to oxidative stress? [J]. *Toxins*, 2015, **7**(5): 1411-1430.

[3] Yu J, Bhatnagar D, Ehrlich KC. Aflatoxin biosynthesis [J]. *Rev Iberoam Micol*, 2002, **19**(4): 191-200.

[4] Lee YM, Li H, Hong J, *et al*. Bioactive metabolites from the sponge-derived fungus *Aspergillus versicolor* [J]. *Arch Pharm Research*, 2010, **33**(2): 231-235.

[5] Wu ZH, Wang YR, Liu D, *et al*. Antioxidative phenolic compounds from a marine-derived fungus *Aspergillus versicolor* [J]. *Tetrahedron,* 2016, **72**(1)**:** 50-57.

[6] Wu G, Yu G, Kurtán T, *et al*. Versixanthones A-F, cytotoxic xanthone-chromanone dimers from the marine-derived fungus *Aspergillus versicolor* HDN1009 [J]. *J Nat Prod*, 2015, **78**: 2691-2698.

[7] Wang MZ, Sun MW, Hao HL, *et al*. Avertoxins A-D, prenyl asteltoxin derivatives from *Aspergillus versicolor* Y10, an endophytic fungus of *Huperzia serrata* [J]. *J Nat Prod*, 2015, **78:** 3067-3070.

[8] Wang JF, He WJ, Huang XL, *et al*. Antifungal new oxepine-containing alkaloids and xanthones from the deep-sea-derived fungus *Aspergillus versicolor* SCSIO 05879 [J]. *J Agr Food Chem,* 2016, **64:** 2910-2916.

[9] Cai SX, Zhu TJ, Du L, *et al*. Sterigmatocystins from the deep-sea-derived fungus *Aspergillus versicolor* [J]. *J Antibiot*, 2011, **64**(2): 193-196.

[10] Zhu F, Lin YC. Three xanthones from a marine-derived mangrove endophytic fungus [J]. *Chem Nat Compd*, 2007, **43**(2): 132-135.

[11] Shao CL, Wang CY, Wei MY, *et al*. Structural and spectral assignments of six anthraquinone derivatives from the mangrove fungus (ZSUH-36) [J]. *Magn Reson Chem*, 2008, **46**(9): 886-889.

[12] Asai A, Yamashita Y, Ando K, *et al*. UCT1072s, new antitumor antibiotics with topoisomerase II mediated DNA cleavage activity, from *Aspergillus sp.* [J]. *J Antibiot*, 1999, **52**(11): 1046-1049.

[13] Roberts JC, Roffey P. Studies in mycological chemistry. 17. averythrin an anthraquinonoid pigment from *Aspergillus Versicolor* (Vuillemin) Tiraboschi [J]. *J Chem Soc*, 1965, **33**: 3666-3672.

[14] Sakai K, Ohte S, Ohshiro T, *et al*. Selective inhibition of acyl-CoA: cholesterol acyltransferase 2 isozyme by flavasperone and sterigmatocystin from *Aspergillus* species [J]. *J Antibiot*, 2008, **61**(9): 568-572.

[15] Chen M, Fu XM, Kong CJ, *et al*. Nucleoside derivatives from the marine-derived fungus *Aspergillus versicolor* [J]. *Nat Prod Res*, 2014, **28**(12): 895-900.

[16] Choi JH, Ozawa N, Yamakawa Y, *et al*. Leccinine A, an endoplasmic reticulum stress-suppressive compound from the edible mushroom *Leccinum extremiorientale* [J]. *Tetrahedron*, 2011, **67**(35): 6649-6653.

[17] Brown AG. Versimide, a metabolite of *Aspergillus versicolor* [J]. *J Chem Soc C-Org*, 1970, **18**(18): 2572-2573.

[18] Dutton MF. Enzymes and aflatoxin biosynthesis [J]. *Microbiol Rev*, 1988, **52**(2): 274-295.

[19] Yu JJ, Chang PK, Ehrlich KC, *et al*. Clustered pathway genes in aflatoxin biosynthesis [J]. *Appl Environ Microbiol*, 2004, **70**(3): 1253-1262.

[20] Re R, Pellegrini N, Proteggente A, *et al*. Antioxidant activity applying an improved ABTS radical cation decolorization assay [J]. *Free Radic Biol Med*, 1999, **26**(10): 1231-1237.

[21] Johnson JA, Johnson DA, Kraft AD, *et al*. The Nrf2-ARE pathway an indicator and modulator of oxidative stress in neurodegeneration [J]. *Ann N Y Acad Sci*, 2008, **1147**(1147): 61-69.

[22] Gan L, Johnson JA. Oxidative damage and the Nrf2-ARE pathway in neurodegenerative diseases [J]. *BBA Mol Basis Dis*, 2014, **1842**(8): 1208-1218.

[23] Ozgen M, Reese RN, Tulio AZ, *et al*. Modified 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) methods [J]. *J Agr Food Chem*, 2006, **54**(4): 1151-1157.

[24] Keum YS, Owuor ED, Kim BR, *et al*. Involvement of Nrf2 and JNK1 in the activation of antioxidant responsive element (ARE) by chemopreventive agent phenethyl isothiocyanate (PEITC) [J]. *Pharm Res*, 2003, **20**(9): 1351-1356.

[25] Nioi P, McMahon M, Itoh K, *et al*. Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD(P)H: quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence [J]. *Biochem J*, 2003, **374**(2): 337-348.

[26] Mosmann T. Rapid colorimetric assay for cellular growth and survival application to proliferation and cyto-toxicity assays [J]. *J Immunol Method*, 1983, **65**(1-2): 55-63.

Cite this article as: WU Ze-Hong, LIU Dong, XU Ying, CHEN Jian-Liang, LIN Wen-Han. Antioxidant xanthones and anthraquinones isolated from a marine-derived fungus *Aspergillus versicolor* [J]. *Chin J Nat Med*, 2018, **16**(3): 219-224.