Natural Product Sciences
22(2) : 82-86 (2016)
http://dx.doi.org/10.20307/nps.2016.22.2.82

Compounds from a jellyfish-derived fungus *Aspergillus fumigates*

Guan-yu Tao[1,2], Juan Liu[1], Jee H. Jung[3], Wei Guo[4], Xiao-qiong Wen[4], and Yonghong Liu[1,5,*]

[1]*Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 510301 Guangzhou, China*
[2]*School of Life Science, South China Normal University, 510361 Guangzhou, China*
[3]*College of Pharmacy, Pusan National University, Busan 609-735, Korea*
[4]*School of Traditional Chinese Medicine, Southern Medical University, 510515 Guangzhou, China*
[5]*South China Sea Bio-Resource Exploitation and Utilization Collaborative Innovation Center*

Abstract – Six compounds were isolated from the secondary metabolites of the jellyfish-derived fungus *Aspergillus fumigates*, whose structures were identified by chemical methods and spectroscopic analysis as pseurotin F1 (**1**), azaspirofurans B (**2**), (22*E*, 24*R*)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol (**3**), 5α,8α-epidioxyergosta-6,22-dien-3β-o1 (**4**), cyclo-(L-Pro-L-Tyr) (**5**), fumitremorgin C (**6**). The compounds **1 - 5** were isolated from the fungus *Aspergillus fumigates* for the first time. The isolated compounds (**1 - 6**) were evaluated for antibiotic activity and cytotoxicity against six bacterial strains and ten human tumor cell lines, respectively.
Keywords – *Nemopilema nomurai,* Jellyfish-derived fungus, *Aspergillus fumigates*, Isolation, Structural elucidation

Introduction

The symbiotic microbe inhabits in the issue of marine invertebrate, holding an ecological interactions with hosts either in a symbiotic or competitive way.[1] Endozoic microorganisms synthesize unique secondary metabolites delivering remarkable pharmacological properties.[2] Numerous researches have been done on the microbe derived from sponges, tunicates, and corals. However, fewer studies on the endozoic microorganisms in jellyfish have been reported, which are also recognized as a productive resource of bioactive molecules.[3] In our preliminary study, several fungus strains were obtained from giant jellyfish *Neopilema nomurai,* of which massive blooms have become increasingly frequent.[4] By the silica gel, Sephadex LH-20 column chromatograph and HPLC, six compounds were isolated from the crude extract of the jellyfish-derived fungus *Aspergillus fumigates*. On the basic of spectral analysis, the structures were identified as pseurotin F1 (**1**), azaspirofurans B (**2**), (22*E*, 24*R*)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol (**3**), 5α,8α-epidioxyergosta-6,22-dien-3β-o1 (**4**), cyclo-(L-Pro-L-Tyr) (**5**), fumitremorgin C (**6**). The compounds **1 - 5** were isolated from the fungus *Aspergillus fumigates* for the first time. (Fig. 1)

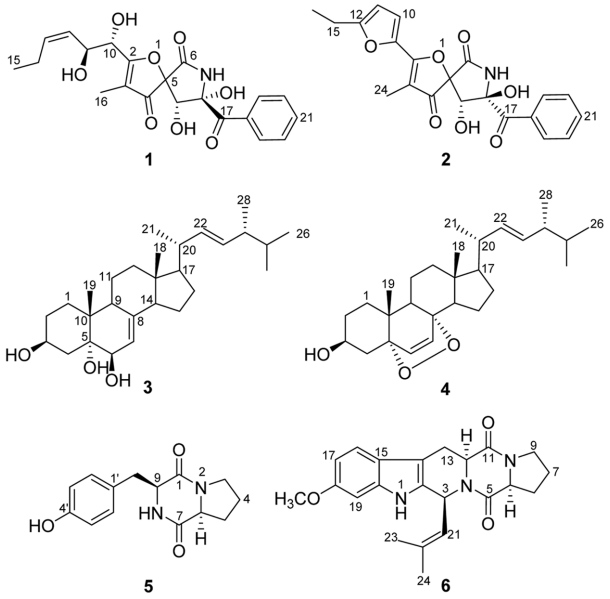

Fig. 1. The structures of the compounds from the jellyfish-derived fungus *Aspergillus fumigatus*.

Experimental

General experimental procedures – ^{1}H and ^{13}C NMR spectra were recorded using Bruker AM-500 MHz NMR spectrometer. Mass spectra were measured on a Bruker BioTOF Q mass spectrometer. The chromatographic system of preparative HPLC consisted a HITACHI L-213 pump

*Author for correspondence
Yong-hong Liu, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 510301 Guangzhou, China
Tel: +86-020-89023244; E-mail: yonghongliu@scsio.ac.cn

and a L-2400 refractive index detector. The adsorbent used for column chromatography was silica gel 200 - 300 mesh and 300 - 400 mesh. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). TLC was carried out using BUCHI Pump Manager C-615. Spots were detected under UV light or by heating after spraying with 2% vanillin sulfuric acid solution. CD spectra were measured with a Chirascan circular dichroism spectrometer (Applied Photophysics). Δε values were expressed in liter·mol^{-1} cm^{-1}.

Fungal Materials – *Aspergillus fumigates* was isolated from jellyfish *Nemopilema nomurai* collected off the southern coast of Korea in June 2007. The fungus strain was identified by K. S. Bae. The specimen was preserved at the Marine Natural Product Laboratory, PNU, marked as fungal strains J08NF-10. The fungus preserved under 80°C was inoculated on malt extract agar (MEA) and cultured at 25 ºC for 3 - 5 days. Fungi growing out of the MEA were separated to malt extract liquid medium until a pure culture was obtained. Afterwards the culture was inoculated on rice medium and cultivated under natural lighting at 25°C for a month, in total of 25 L.

Extraction and isolation – The culture medium were extracted with EtOAc. The EtOAc extract were concentrated to give residue. The chloroform-MeOH layer was subjected to multi-step gradient MPLC, eluting with 0% to 100% MeOH, to afford 27 fractions (J10-1 to J10-27).

Fractions J10-5, J10-7, J10-14 were separated by silica gel chromatography, respectively. Elution was carried out using stepwise gradient elutions of petroleum ether-ethyl acetate to provide subfractions (J10-5-1 to J10-5-9, J10-7-1 to J10-7-5, J10-14-1 to J10-14-3). Subfraction J10-5-4 yielded compounds **6** (3.1 mg) and **3** (7.8 mg) by thin-layer chromatography, as well as J10-7-4 yielded compound **2** (44.0 mg). Fraction J10-9 was subjected to Sephadex LH-20 column to provide 9 subfractions (J10-9-1 to J10-9-9). Compounds **1** (4.6 mg) and **5** (5.4 mg) were isolated from J10-9-6 by thin-layer chromatography; compound **4** (64.5 mg) was obtained from J10-9-2 by preparative scale chromatography.

Pseurotin F1 (1) – Colorless crystal. CD ($c = 4.8 \times 10^{-4}$ M, MeOH): Δε (nm) +7.60 (311.0), –16.61 (280.0), +0.43 (250.0), –0.96 (240.0), 12.23 (208.0); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.74 (s, NH-7), 8.40 (2H, d, J = 10.0 Hz, H-17, H-21), 7.62 (1H, t, J = 10.0 Hz, H-19), 7.49 (2H, t, J = 10.0 Hz, H-20), 6.86 (s, OH-8), 5.56 (1H, m, H-13), 5.14 (1H, dd, J = 15.0, 10.0 Hz, H-12), 4.86 (s, OH-9), (1H, s, H-11), 4.80 (1H, dd, J = 10.0, 5.0, H-10), 4.62(1H, d, J = 5.0 Hz, H-3), 2.06 (s, OH-10,11), 1.68 (3H, s, H-15), 1.02 (3H, t, J = 5.0 Hz, H-16); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 198.8 (C-4), 194.3 (C-17), 188.8 (C-6), 165.1 (C-2), 136.4 (C-13), 134.6 (C-21), 133.0 (C-18), 131.7 (C-19, C-23), 128.5 (C-20, C-22), 126.2 (C-12), 112.9 (C-3), 95.0 (C-5), 89.4 (C-8), 71.8 (C-11), 71.6 (C-9), 70.8 (C-10), 21.4 (C-14), 14.0 (C-15), 6.3 (C-16). ESI-MS *m/z*: 418 [M+H]$^+$.

Azaspirofuran B (2) – Pale yellow crystal. CD ($c = 4.7 \times 10^{-4}$ M, MeOH): Δε (nm) +5.41 (357.0), –15.92 (319.0), +3.95 (282.0), –2.16 (262.0) 1.91 (243.0) , 14.85 (205.0); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.39 (2H, d, J = 10.0 Hz, H-17, H-21), 7.66 (1H, t, J = 10.0 Hz, H-19), 7.51 (2H, t, J = 10.0 Hz, H-10, H-20), 7.20 (1H, d, J = 5.0 Hz, H-4), 6.39 (1H, d, J = 5.0 Hz, H-3), 4.59 (1H, s, H-14), 2.80 (2H, dd, J = 10.0, 5.0, H-15), 2.01 (3H, s, H-24), 1.31 (3H, t, J = 5.0 Hz, H-23); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 196.0 (C-4), 195.4 (C-17), 171.8 (C-2), 167.9 (C-6), 163.9 (C-12), 143.5 (C-14), 133.8 (C-21), 133.5 (C-18), 130.4 (C-19, C-23), 128.2 (C-20, C-22), 117.9 (C-10), 107.6 (C-3), 107.5 (C-11), 92.4 (C-5), 92.2 (C-8), 75.2 (C-9), 51.5 (C-25), 21.2 (C-15), 10.8 (C-16), 4.9 (C-24); ESI-MS *m/z*: 420 [M+Na]$^+$.

(22*E*, 24*R*)-24-methyl- cholesta-7, 22-diene-3β, 5α, 6β-triol (3) – Colorless crystal. ^{1}H NMR (CD_3OD, 500 Mz): δ 5.20 (2H, dd, J = 15.0, 10.0 Hz, H-22, H-23), 5.08 (1H, m, H-7), 4.22 (1H, m, H-6), 3.77 (1H, m, H-3), 0.99 (3H, d, J = 10.0 Hz, H-29), 0.90 (3H, s, H-19), 0.89 (3H, d, J = 5.0 Hz, H-21), 0.81 (3H, d, J = 10.0 Hz, H-26), 0.80 (3H, d, J = 10.0 Hz H-27), 0.54 (3H, s, H-18); ^{13}C NMR (CD_3OD, 125 MHz): δ 31.7 (C-1), 21.5 (C-2), 66.5 (C-3), 39.4 (C-4), 75.0 (C-5), 72.6 (C-6), 119.9 (C-7), 140.2 (C-8), 42.8 (C-9), 37.1 (C-10), 21.8 (C-11), 39.1 (C-12), 43.5 (C-13), 54.7 (C-14), 23.1 (C-15), 28.2 (C-16), 55.8 (C-17), 12.6 (C-18), 18.2 (C-19), 40.7 (C-20), 21.8 (C-21), 135.9 (C-22), 131.9 (C-23), 42.5 (C-24), 33.0 (C-25), 20.2 (C-26), 20.0 (C-27), 17.8 (C-28); ESI-MS *m/z*: 445 [M+H]$^+$.

5α,8α-epidioxyergosta-6,22-dien-3β-ol (4) – Colorless needles. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.52 (d, J = 6.0 Hz, H-7), 6.26 (d, J = 6.0 Hz, H-6), 5.24 (dd, J = 14.0 Hz, H-23), 5.16 (1H, dd, J = 14.0 Hz, H-22), 3.99 (m, H-3), 2.14 (m, H-25), 2.03 (m, H-4a), 1.97 (m, H-20), 1.95 (m, H-1a), 1.93 (m, H-12a), 1.87 (m, H-4b), 1.83 (m, H-2a), 1.73 (m, H-16a), 1.69 (m, H-1b), 1.60 (m, H-15a), 1.54 (m, H-2b), 1.51 (m, H-11a), 1.50 (m, H-9), 1.40 (m, H-15b), 1.26 (m, H-16b), 1.24 (m, H-11,12b), 1.20 (m, H-17), 1.01 (d, J = 5.0 Hz, H-26), 0.93 (d, J = 5.0 Hz, H-27), 0.90 (s, H-19), 0.83 (s, H-18); ^{13}C-NMR (CD_3COCD_3, 125 MHz) δ: 36.3 (C-1), 31.7 (C-2), 66.8 (C-3), 38.4 (C-4), 83.0 (C-5), 137.0 (C-6), 131.7 (C-7), 80.0 (C-8), 53.1 (C-9), 38.6 (C-10), 24.6 (C-11), 40.9 (C-12), 45.8 (C-13),

53.3 (C-14), 21.9 (C-15), 29.9 (C-16), 57.6 (C-17), 13.8 (C-18), 19.0 (C-19), 44.1 (C-20), 21.9 (C-21), 137.1 (C-22), 133.5 (C-23), 44.3 (C-24), 34.4 (C-25), 20.5 (C-26), 20.8 (C-27), 18.6 (C-28). ESI-MS *m/z*: 451 $[M+Na]^+$.

Cyclo-(L-Pro-L-Tyr) (5) – White powder. CD ($c = 1.2 \times 10^{-3}$ M, MeOH): Δε (nm) +1.34 (277.0), +17.56 (202.0); ^{1}H NMR (CD_3OD, 500 MHz): δ 7.08 (2H, d, $J = 10.0$ Hz, H-2' and H-6'), 6.81 (2H, d, $J = 5.0$ Hz, H-3' and H-5'), 5.89 (1H, s, NH), 4.24 (1H, dd, $J = 10.0$, 2.9 Hz, H-9), 4.11 (1H, $J = 10.0$ Hz, H-6), 3.63 (2H, m, H-3), 3.50 (1H, dd, $J = 15.0$, 15.0 Hz, H-10), 2.80 (1H, dd, $J = 15.0$, 15.0 Hz, H-10), 2.37 (1H, m, H-5a), 2.02 (1H, m, H-5b), 1.95 (2H, m, H-4); ^{13}C NMR (CD_3OD, 125 MHz): δ 169.7 (C-7), 165.2 (C-1), 155.6 (C-4'), 130.3 (C-2' and C-6'), 127.0 (C-1'), 116.2 (C-3' and C-5'), 59.2 (C-6), 56.3 (C-9), 45.5 (C-3), 35.9 (C-10), 28.4 (C-5), 22.5 (C-4); EI-MS *m/z*: 163 $[M-H]^-$.

Fumitremorgin C (6) – Yellow powder. CD ($c = 5.3 \times 10^{-4}$ M, MeOH): Δε (nm) +3.20 (299.0), +5.63 (272.0), −6.00 (226.0), −1.87 (215.0), −6.56 (205.0); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.72 (1H, s, H-1), 7.44 (1H, d, $J = 8.5$ Hz, H-16), 6.86 (1H, d, $J = 2.0$ Hz, H-19), 6.82 (1H, dd, $J = 8.5$, 2.5 Hz, H-17), 5.98 (1H, d, $J = 10.0$ Hz, H-3), 4.91 (1H, d, $J = 5.0$ Hz, H-21), 4.19 (1H, dd, $J = 10.0$, 5.0 Hz, H-12), 4.11 (1H, t, $J = 10.0$ Hz, H-6), 3.84 (3H, s, 18-OCH_3), 3.64 (2H, m, H-9), 3.52 (1H, dd, $J = 15.5$, 5.0 Hz, H-13b), 3.10 (1H, dd, $J = 16.0$, 11.0 Hz, H-13a), 2.41 (1H, m, H-7b), 2.24 (1H, m, H-7a), 2.00 (3H, s, H-24), 1.65 (3H, s, H-23); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.6 (C-5), 165.8 (C-11), 156.6 (C-18), 137.0 (C-20), 134.0 (C-22), 132.1 (C-2), 124.2 (C-21), 120.8 (C-15), 118.9 (C-16), 109.6 (C-17), 106.3 (C-14), 95.3 (C-19), 59.3 (C-6), 56.8 (C-12), 55.8 (18-OCH_3), 51.0 (C-3), 45.4 (C-9), 28.6 (C-7), 25.7 (C-23), 23.1 (C-8), 21.9 (C-13), 18.1 (C-24); ESI-MS *m/z*: 380 $[M+H]^+$.

Biological assay – Compounds **1 - 6** were evaluated for their antibacterial activity against a panel of strains including *Staphyloccocus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Aeromonas hydrophila* ATCC 7966, and *Enterococcus faecalis* ATCC 29212. In addition, the cytotoxicity evaluation of compounds **1 - 6** was also carried against a panel of ten human solid tumor cell lines (K562, A549, Huh-7, H1975, MCF-7, U937, BGC823, HL60, Hela, and MOLT-4).

Result and Discussion

Compound **1** was obtained as colorless crystal. The ^{1}H NMR ($CDCl_3$, 500 MHz) of **1** indicated that there existed

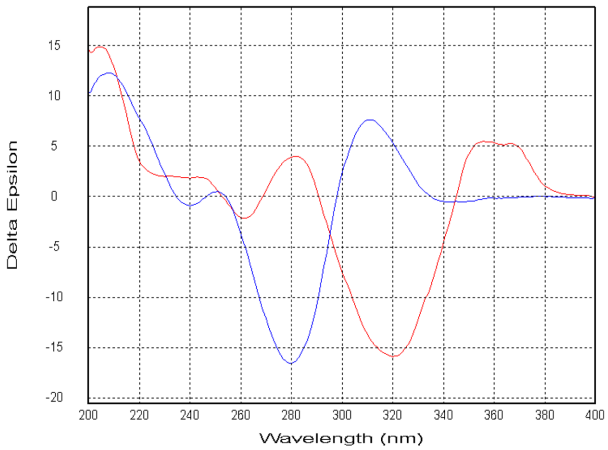

Fig. 2. CD spectra of compounds **1** and **2**.

two alkenyl protons at δ_H 5.56 (1H, m, H-13), 5.14 (1H, dd, $J = 15.0$, 10.0 Hz, H-12), two methine groups substituted hydroxyl at δ_H 4.86 (1H, s, H-11), 4.80 (1H, dd, $J = 10.0$, 5.0, H-10), and two methyls at δ_H 1.68 (3H, s, H-15), 1.02 (3H, t, $J = 5.0$ Hz, H-16). The compound **1** was confirmed as pseurotin F1, according to the reported ones.[5] The relative configuration was determined from a NOESY spectrum. Pseurotins were isolated from *Pseudeurotium ovalis* and different strains of *Aspergillus fumigatus*, which varied slightly in their chemical structure.[6] Although these compounds are chitin synthase inhibitors, only the epoxy-pseurotin and synerazol have antifungal activity.[7]

Compound **2** was isolated as yellow crystal. The ^{1}H NMR ($CDCl_3$, 500 MHz) indicated that the structure of compound **2** possesses two hydroxyls, two methyl groups, amide group and phenyl group. The spectroscopic data of **2** was similar with those of **1**, implying the existence of 1-oxa-7-azaspiro [4,4] non-2-ene-4,6-dione skeleton. Compared ^{1}H and ^{13}C NMR data with reported ones, the structure of compound **2** was confirmed as azaspirofurans B.[8] With respect to corresponding CD transitions, the pattern exhibited by compound **2** was opposite to that of compounds **1** (Fig. 2), thus the absolute configuration at C-8 was proposed as 8*S*.

The main difference between azaspirofurans B and pseurotin F1 is that the vicinal diol moiety was replaced by an ethyl furan ring, bearing to 1-oxa-7-azaspiro [4,4] non-2-ene-4,6-dione skeleton. Although azaspirofurans B has ever been isolated from *Aspergillus sydowi* derived from marine sediment, it's the first time to be obtained from jellyfish symbiotic fungus *Aspergillus fumigates*. Moreover, it is also the first time to isolate azaspirofurans B and pseurotin F1 from the same strain *Aspergillus fumigates*, both have an unusual spiro-ring structure.

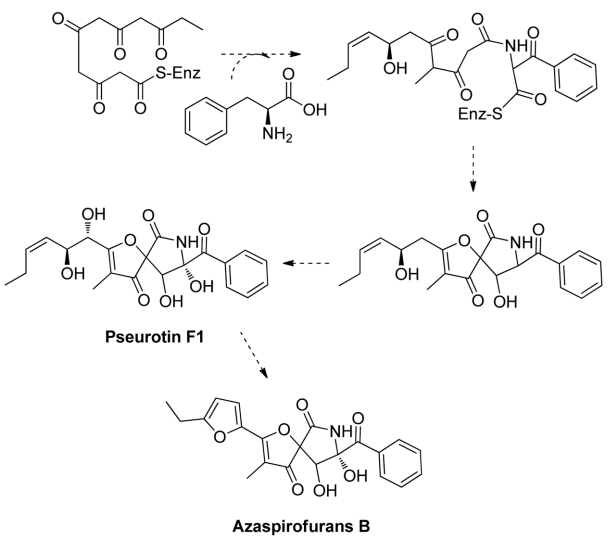

Fig. 3. Proposed biosynthetic pathway of compounds **1** and **2**.

According to previous researches, the building blocks of pseurotin included basically 1 unit of propionate (starter unit), 4 units of malonate and 1 unit of phenylalanine. Thus **1** and **2** were probably biosynthesized via a mixed amino acid - malonate pathway. The precursor skeleton of pseurotins was reported to be formed from the condensation of a polyketide chain with phenylalanine. However, the detailed mechanism of the spiro-ring core formation still remains undefined. It could be proposed that compound **1** was produced from the hydroxyl substitution of 1-oxa-7-azaspiro [4,4] non-2-ene-4,6-dione skeleton, while **2** may derive from **1** by cyclization.[9,10] (Fig. 3)

Compound **3** was isolated as a colorless crystalline solid. The molecular formula of material was determined to be $C_{29}H_{48}O_3$ based on its ^{1}H and ^{13}C NMR, and ESI-MS data. The ^{1}H NMR ($CDCl_3$, 500 MHz) spectrum suggested that compound **3** was a cholesterol derivative, containing six methyl groups and three alkenes including a pair of alkene at δ_H 5.20 (2H, dd, J = 15.0, 10.0 Hz, H-22, H-23), which indicated three hydroxyl groups and two double bonds. Compared with reported data, the compound **3** was determined as (22*E*, 24*R*)-24-methyl- cholesta-7, 22-diene-3β, 5α, 6β-triol, which was isolated from the sponge *Spongionella gracilis*[11] It's the first time to isolate (22*E*, 24*R*)-24-methyl-cholesta-7, 22-diene-3β, 5α, 6β-triol from the strain of fungus *Aspergillus fumigates*. Compound **3** exhibited neither antibacterial activity nor cytotoxicity, although it has only slight distinctions with stigmasta-7,22-diene-3β,5α,6α-triol which displayed significant cytotoxicity against a panel of human tumor cell lines and showed high levels of selective antimicrobial activity.[12]

Compound **4** was isolated as colorless needles, without having UV absorption at 254 nm. The molecular formula was determined to be $C_{28}H_{44}O_3$ from the molecular ion peak $[M+Na]^+$ *m/z* at 451 in the ESI-MS. The ^{1}H NMR data of **4** showed a methyne at δ_H 3.99 (m, H-3), and six methyl group signals, which implied **4** was sterene compound. Compound **4** was identified as 5α,8α-epidioxy-24-methyl-cholesta-6,22-dien-3β-ol, a member of ergosterol endoperoxide family, compared its spectroscopic data with the reported ones.[13] Although compound **4** was obtained from several species of sponge such as *Luffariella cf. variabilis* and *Homaxinella sp*., it is the first time to be isolated from the jellyfish-derived fungus *Aspergillus fumigates*.[14] Over seven derivatives of sterol 5α,8α-endoperoxides were isolated from the gorgonian *Eunicella cavolini* and the ascidian *Tridemnum inarmatum* which exhibited weak cytotoxicity against human solid tumor cell lines. Also no clear correlations between structure and cytotoxicity could be delineated due to diverse variations of the side chain. So far the natural origin of the 5α,8α-epidioxy-sterols in the investigated organisms through enzymatic processes in the living cells are ambiguously proposed.[15]

Compound **5** was isolated as white powder. The ^{13}C NMR spectrum showed two characteristic amide signals at δ_C 169.7 and 165.2, and the ^{1}H NMR data also showed two α-methine residues at δ_H 4.11 and 4.24. These spectral data indicated the presence of diketopiperazine ring system in compound **5**. From ^{1}H and ^{13}C NMR chemical shift, tyrosine was identified as the second amino acid residue. Its structure can be confirmed as cyclo-(L-Pro-L-Tyr), compared the spectroscopic data of **5** with the reported ones.[16] The compound cyclo-(L-Pro-L-Tyr) has been isolated from various terrestrial yeast, lichens, and fungi.[17] Nevertheless, it is rare that this kind of compounds was isolated from fungi derived from jellyfish. Recently, cyclo-(L-Pro-L-Tyr), one of compounds in 2,5-diketopiperazines family, is interested the scientists for its chemical structures, reactions, pharmacological properties, and potential therapeutic applications.[18]

Compound **6** was isolated as yellow powder. The ^{1}H NMR data of compound **6** indicated the presence of a 1,2,4-trisubstituted benzene at δ_H7.44 (1H, d, J = 8.5 Hz, H-16), 6.86 (1H, d, J = 2.0 Hz, H-19), 6.82 (1H, dd, J = 8.5, 2.5 Hz, H-17) and a methoxyl at δ_H 3.84 (3H, s, 18-OMe). The ^{13}C NMR ($CDCl_3$, 125 MHz) revealed the presence of two amide-carbonyls at δ_C 169.6 (C-5), 165.8 (C-11) and a carbon in benzene ring connected with methoxy group at δ_C 156.6 (C-18). The ^{1}H and ^{13}C NMR spectra suggested that compound **6** was also diketopiperazine, the same as compound **5**. The structure of com-

pound **6** was confirmed as fumitremorgin C, according to the reported sample.[19] Diketopiperazines are widespread microbial products, but it's the first time to obtained cyclo-(L-Pro-L-Tyr) and fumitremorgin C from fungus derived from jellyfish.

Although there is the same skeleton in compounds **5** and **6**, their bioactivities are different. In previous report, the culture of *Lysobacter capsici* AZ78, a strain producing compound **5**, showed anti-oomycete activity against sporangia of *Phytophthora infestans* and *Plasmopara viticola*; and the toxic activity of **5** was confirmed in a bioassay carried out on detached tomato leaves.[20] Compound **6** was reported to exhibit mild antibacterial activity against *Staphyloccocus aureus,* methicillin-resistant *S. aureus* and multidrug-resistant *S. aureus.*[18,19] Nevertheless neither compound **5** nor **6** showed remarkable antibacterial and anti-tumor effects, according to our cytotoxicity assay.

In our preliminary experiments, the ethyl acetate extracts of culture medium displayed various antibacterial and anti-tumor effects. None-the-less, in our following bioactivity assay compounds **1 - 6** showed no antibacterial activity against the tested strains including *S. aureus*, *E. coli*, *K. pneumonia*, *A. baumannii*, *A. hydrophila* and *E. faecalis.* Also the cytotoxicity evaluation of compounds **1-6** against human solid tumor cell lines offered negative results.

Acknowledgments

The research was supported by the Natural Science Foundation of China (21202177), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA11030403), Guangzhou Science and Technology Project (201604010081). We are grateful to the faculty of Key Laboratory of Tropical Marine Bio-resource and Ecology, Chinese Acadamy of Science for antimicrobial activities and ESI-MS experiments.

References

(1) Mille-Lindblom, C.; Fischer, H.; Tranvik, L. *J. OIKOS.* **2006**, *113*, 233-242.
(2) Zhang, H. W.; Song, Y. C.; Tan, R. X. *Nat. Prod. Rep.* **2006**, *23*, 753-771.
(3) Blunt, J. W.; Copp, B. R.; Keyzers, R. A. Munro, M. H.; Prinsep, M. R. *Nat. Prod. Rep.* **2014**, *31*, 160-258.
(4) Uye, S. *Plankton Benthos Res.* **2008**, *3*, 125-131.
(5) Wink, J.; Grabley, S.; Gareis, M.; Thiericke, R.; Kirsch, R. *European Patent Application* **1993**, EP0546474A1.
(6) Frisvad, J. C.; Rank, C.; Nielsen, K. F. *Med. Mycol.* **2009**, *47*, S53-S71.
(7) Wenke, J.; Anke, H.; Sterner, O. *Biosci. Biotech. Biochem.* **1993**, *57*, 961-964.
(8) Ren, H.; Liu, R.; Chen, L. Zhu, T.; Zhu, W. M.; Gu, Q. Q. *Arch. Pharm. Res.* **2010**, *33*, 499-502.
(9) Mohr, P.; Tamm, C. *Tetrahedron* **1981**, *37*, 201-212.
(9) Mohr, P.; Tamm, C. *Tetrahedron Lett.* **1987**, *28*, 391-394
(10) Tsunematsu, Y.; Fukutomi, M.; Saruwatari, T. Noguchi, H.; Hotta, K.; Tang, Y.; Watanabe, K. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 8475-8479.
(11) Vincenzo, P.; Donato, S. *J. Nat. Prod.* **1987**, *50*, 915-920.
(12) Wang, X.; Wang, H.; Liu, T.; Xin Z. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 4875-4885.
(13) Ioannou, E.; Abdel-Razik, A. F.; Zervou, M.; Christofidis, D.; Alexi, X.; Vagias, C.; Alexis, M. N.; Roussis, V. *Steroids* **2009**, *74*, 73-80.
(14) Gauvin, A.; Smadja, J.; Aknin, M. *Can. J. Chem*. **2000**, *78*, 986-992.
(15) Bu, M.; Yang, B. B.; Hu, L. *Med. Chem*. **2014**, *4*, 709-716.
(16) Jayatilake, G. S.; Thornton, M. P.; Leonard, A. C. *J. Nat. Prod.* **1996**, *59*, 293-296.
(17) Cimmino, A.; Puopolo, G.; Perazzolli, M.; Andolfi, A.; Melck, D.; Pertot, I.; Evidente, A. *Chem. Heterocycl. Compds.* **2014**, *50*, 290-295.
(18) Borthwick, A. D. *Chem. Rev.* **2012**, *112*, 3641-3716.
(19) Zhang, D.; Noviendri, D.; Nursid, M.; Yang, X.; Son, B. W. *Nat. Prod. Sci.* **2007**, *13*, 251-254.
(20) Puopolo, G.; Cimmino, A.; Palmieri, M. C. Giovannini, O.; Evidente, A.; Pertot, I. *J. Appl. Microbiol.* **2014**, *117*, 1168-1180.

Received August 6, 2015
Revised October 21, 2015
Accepted October 22, 2015