

Article

Highly Oxygenated Constituents from A Marine Alga-derived Fungus *Aspergillus giganteus* NTU967

Jih-Jung Chen ^{1,†}, Shih-Wei Wang ^{2,3,†}, Yin-Ru Chiang ⁴, Ka-Lai Pang ⁵, Yueh-Hsiung Kuo ^{6,7,8}, Tsai-Yen Shih ⁹, Tzong-Huei Lee ^{9,*}

¹ Department of Pharmacy, National Yang-Ming University, Taipei 11221, Taiwan; chenjj@ym.edu.tw

² Department of Medicine, Mackay Medical College, New Taipei City 25245, Taiwan; shihwei@mmc.edu.tw

³ Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

⁴ Biodiversity Research Center, Academia Sinica, Taipei 11529, Taiwan; yinru915@gate.sinica.edu.tw

⁵ Institute of Marine Biology and Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung 20224, Taiwan; klpang@ntou.edu.tw

⁶ Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung 40447, Taiwan; kuoyh@mail.cmu.edu.tw

⁷ Department of Biotechnology, Asia University, Taichung 41354, Taiwan

⁸ Chinese Medical Research Center, China Medical University, Taichung 40447, Taiwan

⁹ Institute of Fisheries Science, National Taiwan University, Taipei 10617, Taiwan; r05b45006@ntu.edu.tw

* Correspondence: thlee1@ntu.edu.tw; Tel.: +886-2-3366-1828

† Equal contribution to this article

Received: date; Accepted: date; Published: date

Abstract: Agar-based disc diffusion antimicrobial assay has shown that the ethyl acetate extract of the fermented broth of *Aspergillus giganteus* NTU967 isolated from *Ulva lactuca* exhibited significant antimicrobial activity in our preliminary screening of bioactive fungal strains. Therefore, column chromatography of the active principles from liquid- and solid-state fermented products of the fungal strain was carried out, and which had led to isolation of eleven compounds. Their structures were determined to be seven new highly oxygenated polyketides, namely aspergilsmins A–G (1–7), along with previously reported patulin, deoxytryptoquivaline, tryptoquivaline, and quinadolone B by spectral analysis. Among these, aspergilsmin C (3) and patulin displayed promising anti-cancer activities against human hepatocellular carcinoma SK-Hep-1 cells and prostate cancer PC-3 cells with IC₅₀ values between 2.7–7.3 μ M. Furthermore, aspergilsmin C (3) and patulin exhibited significant anti-angiogenic functions by impeding cell growth and tube formation of human endothelial progenitor cells without any cytotoxicity.

Keywords: *Aspergillus giganteus*; Trichocomaceae; bioactive natural products; Polyketides; aspergilsmin

1. Introduction

The so called marine-derived fungi have been isolated from a wide array of marine organisms such as mangroves, algae, sponges, and corals, whose habitats distribute from deep sea to intertidal zone. Among these, algae-derived fungi have been reported to be the largest source of secondary metabolites with diversified bioactivities [1,2], and that can be exploited



potentially as lead compounds for new drug development. It was reported that by employing one of the post-genomic strategies, one strain many compounds (OSMAC), on the cultivation of the fungal strains could enhance the quantity and diversity of fungal secondary metabolites [3,4]. The OSMAC approach usually involved the manipulation of culturing parameters, such as media formulation, temperature, agitation, luminosity, aeration, and so on [5–7]. In addition, easy to scale-up and quality control of the fungal metabolites would be another major advantages that made fungi to be one of the best options for natural product research and new lead discovery.

Taiwan is an island located at tropical and subtropical region with highly diversified marine algal species [8], indicating an abundant resource of the fungal endophytes. However, the chemical investigation on the local algae-derived fungal strains are still rare so far. Thus, an efficient agar-based disc diffusion assay was applied for the preliminary biological screening against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Cryptococcus neoformans*, the ethyl acetate extracts of fermented broths of *Aspergillus giganteus* NTU967 derived from the green alga *Ulva lactuca* were found to exhibit significant inhibition zone against *S. aureus* and *C. neoformans*. Therefore, chemical investigation on the fermented products of *Aspergillus giganteus* NTU967 was performed, and that has resulted in the isolation and identification of seven previously unreported highly oxygenated polyketides **1–7** (Figure 1) together with four known compounds. This paper described the isolation and characterization of the new compounds together with their bioactivities.

2. Results and Discussion

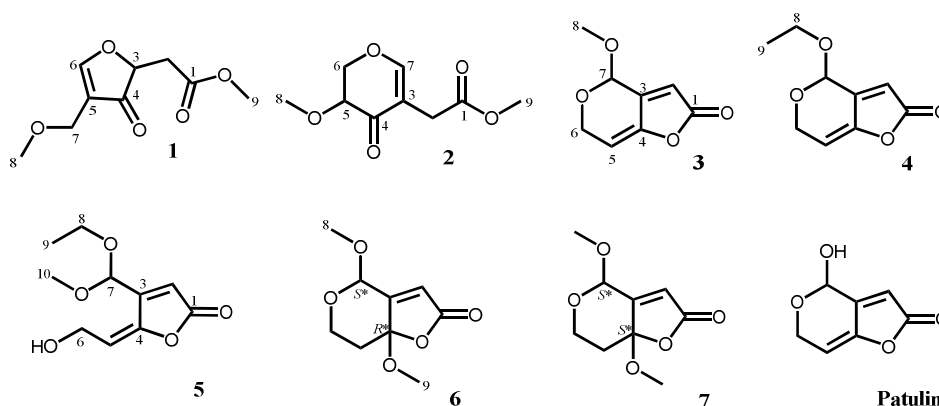


Figure 1. Chemical structures of compounds **1–7** and patulin isolated in this study.

2.1. Isolation and Characterization of Secondary Metabolites

In this study, the green alga *Ulva lactuca*-derived fungal strain *Aspergillus giganteus* NTU967 was cultured in both solid- and liquid-state culturing conditions in order to enrich the diversity of the fungal secondary metabolites, and eleven chemical entities including seven new compounds **1–7** and four previously reported compounds were obtained from the fermented products. Of the known compounds isolated, patulin, a highly oxygenated C₇ mycotoxin, with a hemiacetal functionality is apt to racemize naturally to form a pair of enantiomers, and was first characterized in 1943 under the name of tercinin as a potential antimicrobial agent [9]. Recently, patulin in combination with oxaliplatin were found to exhibit synergism against human colorectal cancer [10]. Deoxytryptoquivaline, tryptoquivaline, and quinadoline, three quinazalone-containing alkaloids, were identified by comparison of spectroscopic data with literatures [11,12]. In addition to be isolated from *Aspergillus* spp., a series of tryptoquivaline analogues have ever been obtained from a marine sea fan-derived fungus *Neosartorya siamensis* [13].



Compound **1**, obtained as colorless oil, was determined to have a molecular formula of $C_9H_{12}O_5$, as evidenced by its ^{13}C NMR spectrum (Table 1) and a quasi-molecular ion $[M + Na]^+$

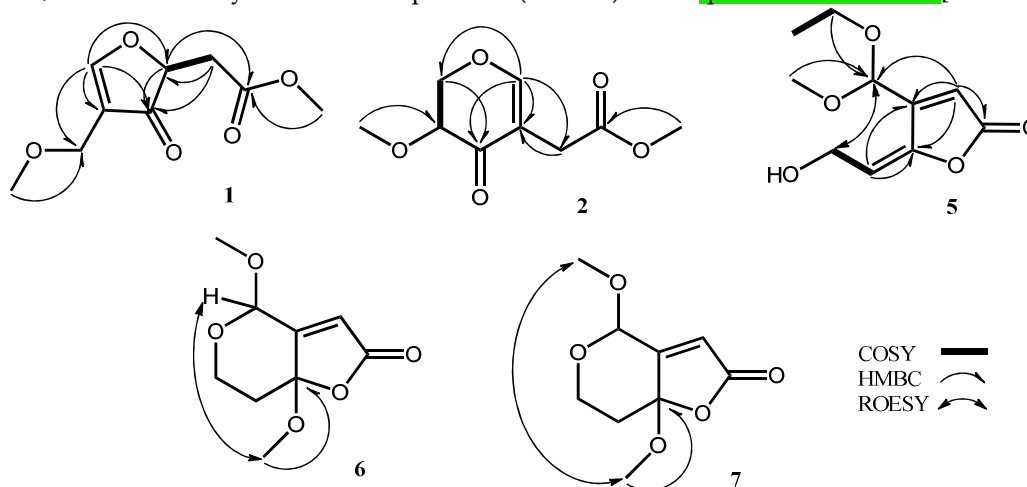


Figure 2. COSY and key HMBC and ROESY correlations of compounds **1**, **2**, **5**, **6**, and **7**.

at m/z 223.0574 (calcd. 223.0582 for $C_9H_{12}O_5Na$) in the positive mode of HRESIMS analysis. The IR absorptions at 1745 and 1672 revealed the presence of an ester carbonyl and a conjugated ketone functionality, respectively. The ^1H NMR (MeOH- d_4 , 400 MHz) of **1** revealed two oxygen-bearing three-proton signals at δ_H 3.35 (H_{3-8}) and 3.71 (H_{3-9}), one methylene signal at δ_H 2.60 (d, $J = 6.3$ Hz, H_{a-2}) and 2.62 (d, $J = 6.0$ Hz, H_{b-2}), one oxygen-bearing methylene signal at δ_H 4.55 (s, H_2-7), one oxygen-bearing methine signal at δ_H 4.55 (dd, $J = 6.3, 6.0$ Hz, $H-3$), and an olefinic methine signal at δ_H 7.66 (s, $H-6$) (Table 2). The ^{13}C NMR (MeOH- d_4 , 125 MHz) coupled with phase-sensitive HSQC spectrum of **1** showed nine signals including two methoxyl carbons at δ_C 57.7 (C-8) and 52.7 (C-9), one methylene

Table 1. ^{13}C NMR spectroscopic data for compounds **1–7** (δ in ppm, mult.).

No.	1 ^{a,b}	2 ^{a,b}	3 ^{a,b}	4 ^{a,b}	5 ^{a,b}	6 ^{a,b}	7 ^{a,b}
1	172.9 s	173.6 s	170.6 s	170.6 s	169.6 s	170.6 s	170.6 s
2	36.7 t	31.1 t	111.9 d	111.1 d	119.7 d	117.1 d	119.9 d
3	69.7 d	111.7 s	147.5 s	147.6 s	156.5 s	163.9 s	160.9 s
4	191.8 s	191.3 s	150.9 s	151.3 s	148.2 s	107.6 s	107.2 s
5	116.2 s	77.3 d	109.3 d	109.4 d	115.3 d	40.3 t	41.9 t
6	166.1 d	72.3 t	60.0 t	60.0 t	57.3 t	62.3 t	58.3 t
7	75.9 t	164.0 d	95.9 d	94.8 d	97.6 d	98.8 d	97.4 d
8	57.7 q	58.7 q	56.5 q	65.6 t	62.8 t	57.3 q	55.5 q
9	52.7 q	52.5 q		15.4 q	15.4 q	51.2 q	52.2 q
10					53.2 q		

^a Measured in MeOH- d_4 (125 MHz). ^b Multiplicities were obtained from phase-sensitive HSQC experiments.

**Table 2.** ^1H NMR spectroscopic data for compounds 1–7 (δ in ppm, mult., J in Hz).

No.	1 ^a	2 ^b	3 ^b	4 ^b	5 ^b	6 ^b	7 ^b
1							
2	2.60 d (6.3)	3.14 s	6.60 s	6.05 s	6.31 s	6.12 s	6.23 s
	2.62 d (6.0)						
3	4.55 dd (6.3, 6.0)						
4							
5		3.70 t (4.7)	6.02 m	6.02 m	5.85 t (6.9)	1.91 m	1.99 m
						2.38 dt (13.5, 2.1)	2.34 d (13.2)
6	7.66 s	4.49 d (4.7)	4.37 dd (17.5, 4.4)	4.36 dd (17.3, 4.4)	4.40 d (6.9)	3.76 td (12.2, 2.1)	3.71 m
			4.56 dd (17.5, 2.5)	4.56 dd (17.3, 2.6)		4.01 m	4.10 td (12.0, 2.0)
7	4.55 s	7.50 s	5.68 s	5.79 s	5.52 s	5.11 s	5.52 s
8	3.35 s	3.45 s	3.54 s	3.71 m 3.91 m	3.63 m	3.60 s	3.46 s
9	3.71 s	3.67 s		1.26 t (7.1)	1.24 t (7.1)	3.23 s	3.19 s
10					3.36 s		

^a Measured in MeOH- d_4 (400 MHz). ^b Measured in MeOH- d_4 (500 MHz).

carbon at δ 36.7 (C-2), one oxymethylene carbon at δ 75.9 (C-7), one carbinoyl carbon at δ 69.7 (C-3), one oxygenated olefinic carbon at δ 166.1 (C-6), one non-protonated olefinic carbon at δ 116.2 (C-5), one ester carbonyl carbon at δ 172.9 (C-1), and one conjugated ketone carbon at δ 191.8 (C-4) (Table 1). On account of the molecular formula $C_9H_{12}O_5$, the double bond equivalent (DBE) of **1** was four including a double bond and two carbonyl groups. Thus, there should be one additional ring in **1**. Further two dimensional NMR analysis including one cross-peak of ^1H 2.60 and 2.62 (H₂-2)/ ^1H 4.55 (H-3) in the COSY spectrum of **1** in combination with key correlations of ^1H 2.60 and 2.62 (H₂-2)/ δ 69.7 (C-3) and 191.8 (C-4); ^1H 4.55 (H-3)/ δ 172.9 (C-1) and 191.8 (C-4); ^1H 7.66 (H-6)/ δ 69.7 (C-3), 191.8 (C-4), 116.2 (C-5), and 75.9 (C-7); ^1H 4.55 (H₂-7)/ δ 191.8 (C-4); ^1H 3.35 (H₃-8)/ δ 75.9 (C-7); ^1H 3.71 (H₃-9)/ δ 172.9 (C-1) in the HMBC spectrum of **1** (Figure 2), corroborated the gross structure of **1**. The optical rotation value of **1** ($[\alpha]^{27D}$ -0.36) was close to zero, revealing that **1** could be a racemate.

The molecular formula of **2**, $C_9H_{12}O_5$, was deduced through analysis of its ^{13}C NMR and HRESIMS data. Its IR spectrum revealed the presence of an ester carbonyl (1737 cm^{-1}) and a conjugated ketone group (1673 cm^{-1}). The ^1H NMR spectrum of **2** coupled with phase-sensitive HSQC spectrum showed two methoxyl signals at ^1H 3.45 (s, H₃-8) and 3.67 (s, H₃-9), one methylene signal at ^1H 3.14 (s, H₂-2), one oxygenated methylene signal at ^1H 4.49 (d, J = 4.7 Hz, H₂-6), one carbinoyl methine signal at ^1H 3.70 (t, J = 4.7 Hz, H-5), and one olefinic methine signal at ^1H 7.50 (s, H-7) (Table 2). Nine carbon resonances including two methoxyl at δ 58.7 (C-8) and 52.5 (C-9), one oxygenated methylene at δ 72.3 (C-6), one methylene at δ 31.1 (C-2), one olefinic methine at δ 164.0 (C-7), one carbinoyl methine at δ 77.3 (C-5), one non-protonated olefinic carbon at δ 111.7 (C-3), one ester carbonyl carbon at δ 173.6 (C-1), and one ketone carbon at δ 191.3 were observed in the ^{13}C NMR of **2** (Table 1), which were supported by phase-sensitive HSQC spectrum. On account of the molecular formula of **2**, $C_9H_{12}O_5$, **2** would contain a ring in addition to a double bond, a ketone, and an ester carbonyl to fit the unsaturation number. Further comprehensive analysis of two dimensional NMR spectra of **2** (Figure 2), one cross-peak of ^1H 3.70 (H-5)/ ^1H 4.49 (H₂-6) in the COSY spectrum together with key cross-peaks of ^1H 3.14 (H₂-2)/ δ 111.7 (C-3); ^1H 4.49 (H₂-6)/ δ 191.3 (C-4); ^1H 7.50 (H-7)/ δ 31.3 (C-2), 111.7 (C-3), 191.3 (C-4), and 72.3 (C-6); ^1H 3.45 (H₃-8)/ δ 77.3 (C-5); ^1H 3.67 (H₃-9)/ δ 173.6 (C-1) in the HMBC spectrum established the structure of **2**.

The physical and NMR data of **3** and **4** were almost compatible with those of patulin except that an additional methyl and an additional ethyl were observed in the ^1H and ^{13}C NMR spectra of **3** and **4** (Tables 1 and 2), respectively. The pseudo-molecular ion $[M + H]^+$ at m/z 169.0493 and 183.0655 in the HRESIMS of **3** and **4**, 14 and 28 Da more than that of patulin, confirmed that **3** and **4** were the methyl and ethyl derivative of patulin, respectively. In the HMBC spectra of **3** and **4**, distinctive cross-peaks of ^1H 3.54 (H₃-8)/ δ 95.9 (C-7) and ^1H 3.71 and 3.91 (H₂-8)/ δ 94.8 (C-7) indicated the methyl and ethyl groups were attached to C-7 of **3** and **4**, respectively. The structures of **3** and **4** were thus elucidated to be as shown.

Compound **5**, obtained as colorless oil, was determined to have a molecular formula of $C_{10}H_{14}O_5$, as evidenced by its HRESIMS analysis and ^{13}C NMR spectrum (Table 1). It contained a hydroxy, a γ -lactone carbonyl, and a double bond due to the IR absorption bands at 3435, 1768, and 1643 cm^{-1} , respectively. Interpretations of the ^1H NMR data along with the HSQC spectrum of **5** showed two methyl signals at ^1H 3.36 (s, H₃-10) and 1.24 (t, J = 7.1 Hz, H₃-10), two oxygenated methylene signals at ^1H 4.40 (d, J = 6.9 Hz, H₂-6) and 3.63 (m, H₂-8), two olefinic methine signals at ^1H 6.31 (s, H-2) and 5.85 (t, J = 6.9 Hz, H-5), and one dioxygenated methine signal at ^1H 5.52 (s, H-7) (Table 2). The ^{13}C NMR data of **5** coupled with its phase-sensitive HSQC assignments showed one methoxyl carbon at δ 53.2 (C-10), one methyl carbon at δ 15.4 (C-9), two oxygenated methylene carbons at δ 62.8 (C-8) and 57.3 (C-6), two olefinic methine carbons at δ 119.7 (C-2) and 115.3 (C-5), one dioxygenated methine carbon at δ 97.6 (C-7), and three non-protonated carbons at δ 169.6 (C-1), 156.5 (C-3), and 148.2 (C-4) (Table 1). Correlations of ^1H 5.85 (H-5)/ ^1H 4.40 (H₂-6) and ^1H 3.63 (H₂-8)/ ^1H 1.24 (H₃-9) in the COSY spectrum of **5** accompanied with key correlations including ^1H 6.31 (H-2)/ δ 169.6 (C-1), 156.5 (C-3), 148.2 (C-4), and 97.6 (C-7); ^1H 5.58 (H-5)/ δ 156.5 (C-3) and 148.2 (C-4); ^1H 3.63 (H₂-8)/ δ 97.6 (C-7); ^1H 3.36 (H-10)/ δ 97.6 (C-7) in the HMBC spectrum of **5** (Figure 2), established the gross structure of **5**. The configuration of ^{4} in **5** was determined to be *E* form based on a key

correlation of ^1H 4.40 (H₂-6)/ ^1H 5.52 (H-7) in the ROESY of **5** (Figure 2). Since **5** had an acetal carbon at its C-7 and an optical rotation value close to zero ($[\alpha]^{27\text{D}} +0.02$), compound **5** was deduced to be an acetal racemate.

The ^1H NMR data of compounds **6** and **7** were almost identical with that of compound **3** except that an olefinic proton at ^1H 6.02 (H-5) in **3** was substitute by a methylene group at ^1H 1.91 and 2.38 (H₂-5) in **6** and ^1H 1.99 and 2.34 (H₂-5) in **7**, and an additional methoxyl functionality at ^1H 3.23 (H₃-9) and ^1H 3.19 (H₃-9) was observed in **6** and **7** (Table 2), respectively. These changes also reflected in their ^{13}C NMR data (Table 1), in which two olefinic signals at ^1C 150.9 (C-4) and 109.3 (C-5) in **3** were replaced by a ketal carbon signal (^1C 107.6 in **6**; ^1C 107.2 in **7**) along with a methylene carbon resonance (^1C 40.3 in **6**; ^1C 41.9 in **7**). The additional methoxyl groups in **6** and **7** were deduced to be attached at C-4 due to key cross-peaks of H₃-9/C-4 in the HMBC spectra of both **6** and **7** (Figure 2). In the ROESY spectra of **6** and **7**, a key correlation of ^1H 3.23 (H₃-9)/ ^1H 5.11 (H-7) confirmed that two methoxyls in **6** were oriented in different phases, while a key cross-peak of ^1H 3.46 (H₃-8)/ ^1H 3.19 (H₃-9) indicated that two methoxyls in **7** were located in the same phase (Figure 2). The structures of **6** and **7** were thus established as shown in Figure 1.

169 2.2. Anti-cancer and Anti-angiogenic Assays of Secondary Metabolites

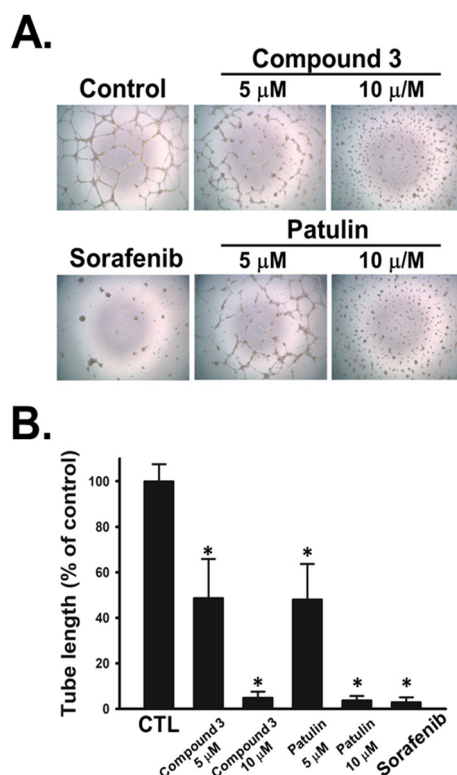


Figure 3. Effects of compound 3 and patulin on tube formation of human EPCs. Cells were treated with compound 3, patulin, and sorafenib for 24 h. Capillary-like structure formation was examined by tube formation assay (A). Tube formation of EPCs was quantified by measuring the length of tubes using ImageJ software (B). Data represent the mean $\pm$ S.E.M. of 4 independent experiments. * $p < 0.05$ compared with the control group.

Table 3. Anti-cancer and anti-angiogenic activities of compound 3 and Patulin.

Compounds	Anti-cancer (IC ₅₀ , μM)		Anti-angiogenesis (IC ₅₀ , μM)
	SK-Hep-1 ^a	PC-3 ^b	EPC ^c
Compound 3	2.7 $\pm$ 0.2	7.3 $\pm$ 0.3	4.6 $\pm$ 0.3
Patulin	2.9 $\pm$ 0.1	2.7 $\pm$ 0.1	4.7 $\pm$ 0.2
Paclitaxel ^d	0.011 $\pm$ 0.002	0.013 $\pm$ 0.002	–
Sorafenib ^d	–	–	4.8 $\pm$ 0.3

^a Hepatocellular carcinoma cells; ^b Prostate cancer cells; ^c Endothelial progenitor cells; ^d Positive control.

All eleven pure isolates were subjected to biological assays. Among these, compound 3 exerted promising anti-cancer activities against human hepatocellular carcinoma SK-Hep-1 cells and prostate cancer PC-3 cells with IC₅₀ values of 2.7 $\pm$ 0.2 and 7.3 $\pm$ 0.3 μM (Table 3). Paclitaxel, a well-known anti-cancer agent, was used as the positive control. Additionally, we evaluated the anti-angiogenic activities of all the pure isolates against human endothelial progenitor cells (EPCs). As shown in Table 3, compound 3 and patulin exhibited most potent anti-angiogenic activities by suppressing EPCs growth with IC₅₀ values of 4.6 $\pm$ 0.3 and 4.7 $\pm$ 0.2 μM, respectively. Since capillary-like tubules are the essential characteristic of angiogenesis, we next performed tube formation assay to validate the anti-angiogenic effects of compound 3 and patulin in EPCs with sorafenib as a positive control. The results showed that compound 3 and patulin concentration-dependently inhibited capillary tube formation of EPCs (Figure 3). Furthermore, it was found that compound 3 and patulin did not induce

the release of lactate dehydrogenase (LDH) in EPCs (Figure 4), suggesting that these two compounds display anti-angiogenesis property without the cytotoxic fashion. Compound **4** bearing an ethoxyl group at its C-7 instead of a hydroxy and a methoxyl at C-7 of patulin and **3**, respectively, reduced its bioactivity significantly. It was thus speculated that the size of the functional group attached at C-7 and an olefinic functionality at ⁴ of patulin could play crucial roles in the anti-cancer and anti-angiogenic activities. These findings provide evidences that both compound **3** and patulin may serve as the potential natural products to block tumor angiogenesis for cancer treatment.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations and UV were measured on a JASCO P-2000 polarimeter (Tokyo, Japan) and Thermo UV-Visible He λ ios α Spectrophotometer (Bellefonte, CA, USA), respectively. ¹H and ¹³C NMR were acquired on a Bruker AVIII-500 spectrometer (Ettlingen, Germany). Low and high resolution mass spectra were obtained using an API4000 triple quadrupole mass spectrometer

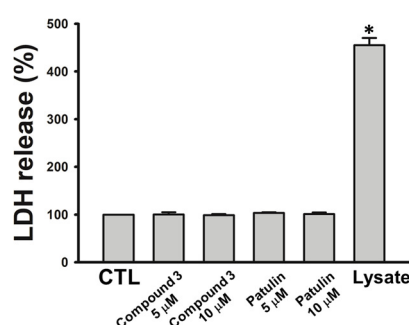


Figure 4. Effects of compound **3** and patulin on cytotoxicity of human EPCs. Cells were treated with compound **3** and patulin for 24 h. Then, cytotoxic effect was analyzed by the LDH assay. Data represent the mean $\pm$ S.E.M. of 4 independent experiments. * p < 0.05 compared with the

(Applied Biosystems, Foster City, CA, USA) and Q Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany), respectively. IR spectra were recorded on a JASCO FT/IR 4100 spectrometer (Tokyo, Japan). Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) and Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan) was used for open column chromatography. An HPLC pump L-7100 (Hitachi, Japan) equipped with a refractive index detector (Bischoff, Leonberg, Germany) was used for compound purification. All the organic solvents were purchased from Merck (Darmstadt, Germany).

3.2. Fungal Strain and Culture

Aspergillus giganteus NTU967 was isolated from the marine green alga *Ulva lactuca* collected from the northeast coast of Taiwan, and was identified by sequencing of the internal transcribed spacer regions of the rDNA (ITS) and β -tubulin gene. A BLAST search of the ITS sequence (GenBank accession no. MH250052) was not conclusive and led to the best matches as *Aspergillus clavatoanicus*, *A. clavatus*, *A. giganteus* and *A. longivesica* (query coverage 94–100%, identity 98–99%) while a BLAST search of the β -tubulin gene resulted the best matches as *A. giganteus* (query coverage 97–100%, identity 98.48–99.63%). For liquid culture, the mycelium of *Aspergillus giganteus* NTU967 was inoculated into 5 L serum bottles, each containing 2 g Peptone (Becton, Dickinson and Company, Sparks, USA), 1 g Yeast Extract (Becton, Dickinson and Company, Sparks, USA), 10 g Dextrose (Becton, Dickinson and Company, Sparks, USA), and 2.5 L deionized water. The fermentation was conducted with aeration at 25–30 $^{\circ}$ C for 16 days. For solid culture, the mycelium of *Aspergillus giganteus* NTU967 was inoculated into 500 mL flasks, each containing 50 g brown rice (Santacruz,

Taiwan), 2% Yeast Extract (Becton, Dickinson and Company, Sparks, USA), 1% sodium tartrate, and 1% KH_2HPO_4 in 20 mL deionized water. The solid culture was conducted at 25–30 °C for 30 days.

3.3. Extraction and Isolation of Secondary Metabolites

For liquid culture, the filtered fermented broth (15.0 L) of *Aspergillus giganteus* NTU967 was partitioned three times with 30 L EtOAc, then concentrated in vacuum to dryness (8.0 g). Subsequently, the crude extract was re-dissolved in 20 mL MeOH, then applied onto a Sephadex LH-20 column (2.5 cm i.d. × 68 cm) eluted with MeOH at a flow rate of 2.5 mL/min. Each fraction (20 mL) collected was checked for its compositions by TLC using CH_2Cl_2 -MeOH (10:1, v/v) for development, and dipping in vanillin- H_2SO_4 was used in the detection of compounds with similar skeletons. All the fractions were combined into four portions I–VI. Subsequently, portion III with antimicrobial activity was pre-coated with 15.0 g Diaion HP-20 gel, then applied onto a Diaion HP-20 column (4.5 cm i.d. × 30 cm) eluted with mixtures of H_2O /MeOH in a stepwise gradient mode with a flow rate of 2.0 mL/min to obtain four subportions I–IV. Subportion III eluted by 75% MeOH was rechromatographed on a semi-preparative reversed-phase column (Phenomenex Luna 5 μ PFP, 10 × 250 mm) with 35% MeOH_{aq} as eluent, 2 mL/min, to afford **1** (30.4 mg, t_R = 13.5 min), **2** (15.2 mg, t_R = 16.9 min), and **3** (27.4 mg, t_R = 27.5 min). Subportion III was further purified on a semi-preparative reversed-phase column (Thermo Hypersil 5 μ C₁₈, 10 × 250 mm) with 25% MeCN_{aq} as eluent, 2 mL/min, to give **4** (22.8 mg, t_R = 35.1 min), **5** (18.4 mg, t_R = 23.4 min), **6** (16.6 mg, t_R = 26.1 min), and **7** (35.6 mg, t_R = 27.6 min). For solid-state culture, the fermented products were lyophilized, ground into powder (750 g), and extracted three times with equal volumes of methanol. Extracts were first partitioned with *n*-hexane, and the methanol layers suspended in deionized H_2O , then partitioned with ethyl acetate and concentrated to obtain dried ethyl acetate extract (7.0 g). For compound separation, the ethyl acetate extract was subjected to Sephadex LH-20 column chromatography (2.5 i.d. × 68.0 cm), using methanol as the eluent at a flow rate of 2.5 mL/min to give 30 fractions (20.0 mL/fr.). All the fractions were combined into 6 portions as I–VI based on the results of TLC analysis and antimicrobial assay. Portion III with antimicrobial activity was pre-coated with 20.0 g Diaion HP-20 gel, then applied onto a Diaion HP-20 column (4.5 cm i.d. × 30 cm) eluted with mixtures of H_2O /MeOH in a stepwise gradient mode with a flow rate of 2.0 mL/min to get four subportions I–IV. Subportion II eluted by 50% MeOH was rechromatographed on a semi-preparative reversed-phase column (BIOSIL Pro-ODS-U 5, 10 × 250 mm) with 15% MeOH_{aq} as eluent, 2 mL/min, to obtain patulin (16.7 mg, t_R = 20.1 min). Subportion IV eluted by 100% MeOH was further purified on a semi-preparative reversed-phase column (Phenomenex Luna 5 μ PFP, 10 × 250 mm) with 75% MeOH_{aq} as eluent, 2 mL/min, to afford quinadoline B (27.7 mg, t_R = 11.8 min), deoxytryptoquivaline (21.0 mg, t_R = 23.0 min), and tryptoquivaline (16.4 mg, t_R = 36.2 min).

3.3.1. Aspergilsmin A (**1**)

Colorless oil; $[\alpha]_D^{25} -0.36$ (c = 0.05, MeOH); IR (ZnSe) ν_{max} : 2951, 1745, 1672, 1611, 1456, 1438, 1399, 1333, 1308, 1283, 1254, 1196, 1171, 1105, 1055, 1033, and 1009 cm^{-1} ; UV λ_{max} (MeOH) (log ϵ) 261 (3.9) nm; ^1H and ^{13}C NMR spectroscopic data: see Tables 1 and 2; HRESIMS $[\text{M} + \text{Na}]^+$ at m/z 223.0574 (calcd. 223.0582 for $\text{C}_9\text{H}_{12}\text{O}_5\text{Na}$).

3.3.2. Aspergilsmin B (**2**)

Colorless oil; $[\alpha]_D^{25} +1.22$ (c = 0.05, MeOH); IR (ZnSe) ν_{max} : 2947, 1737, 1673, 1619, 1443, 1406, 1344, 1291, 1257, 1157, 1097, 1056, 1043, 1024, 1011, and 854 cm^{-1} ; UV λ_{max} (MeOH) (log ϵ) 268 (3.9) nm; ^1H and ^{13}C NMR spectroscopic data: see Tables 1 and 2; HRESIMS $[\text{M} + \text{Na}]^+$ at m/z 223.0573 (calcd. 223.0582 for $\text{C}_9\text{H}_{12}\text{O}_5\text{Na}$).

3.3.3. Aspergilsmin C (**3**)

Colorless oil; $[\alpha]^{27D} -3.52$ ($c = 0.05$, MeOH); IR (ZnSe) $\nu_{\max}$: 2955, 1780, 1536, 1443, 1406, 1344, 1210, 1065, and 868 cm^{-1} ; UV $\nu_{\max}$ (MeOH) (log ϵ) 274 (4.0) nm; ^1H and ^{13}C NMR spectroscopic data: see Tables 1 and 2; HRESIMS $[\text{M} + \text{H}]^+$ at m/z 169.0493 (calcd. 169.0501 for $\text{C}_8\text{H}_9\text{O}_4$).

3.3.4. *Aspergilsmin D* (4)

Colorless oil; $[\alpha]^{27D} -2.14$ ($c = 0.05$, MeOH); IR (ZnSe) $\nu_{\max}$: 2945, 1780, 1635, 1404, 1092, and 1019 cm^{-1} ; UV $\nu_{\max}$ (MeOH) (log ϵ) 275 (4.0) nm; ^1H and ^{13}C NMR spectroscopic data: see Tables 1 and 2; HRESIMS $[\text{M} + \text{H}]^+$ at m/z 183.0655 (calcd. 183.0657 for $\text{C}_9\text{H}_{11}\text{O}_4$).

3.3.5. *Aspergilsmin E* (5)

Colorless oil; $[\alpha]^{27D} +0.02$ ($c = 0.05$, MeOH); IR (ZnSe) $\nu_{\max}$: 3435, 1768, 1643, 1053, and 1008 cm^{-1} ; UV $\nu_{\max}$ (MeOH) (log ϵ) 223 (3.7) and 270 (4.0) nm; ^1H and ^{13}C NMR spectroscopic data: see Tables 1 and 2; HRESIMS $[\text{M} + \text{H}]^+$ at m/z 215.0915 (calcd. 215.0947 for $\text{C}_{10}\text{H}_{15}\text{O}_5$).

3.3.6. *Aspergilsmin F* (6)

Colorless oil; $[\alpha]^{27D} -0.53$ ($c = 0.05$, MeOH); IR (ZnSe) $\nu_{\max}$: 2951, 1768, 1643, 1456, 1396, 1207, 1163, 1082, 1022, and 972 cm^{-1} ; UV $\nu_{\max}$ (MeOH) (log ϵ) 257 (4.1) nm; ^1H and ^{13}C NMR spectroscopic data: see Tables 1 and 2; HRESIMS $[\text{M} + \text{Na}]^+$ at m/z 223.0579 (calcd. 223.0582 for $\text{C}_9\text{H}_{12}\text{O}_5\text{Na}$).

3.3.7. *Aspergilsmin G* (7)

Colorless oil; $[\alpha]^{27D} -1.86$ ($c = 0.05$, MeOH); IR (ZnSe) $\nu_{\max}$: 2971, 2842, 1766, 1456, 1435, 1358, 1329, 1266, 1205, 1196, 1171, 1129, 1041, 1034, and 915 cm^{-1} ; UV $\nu_{\max}$ (MeOH) (log ϵ) 271 (4.0) nm; ^1H and ^{13}C NMR spectroscopic data: see Tables 1 and 2; HRESIMS $[\text{M} + \text{Na}]^+$ at m/z 223.0574 (calcd. 223.0582 for $\text{C}_9\text{H}_{12}\text{O}_5\text{Na}$).

3.4. Cell Culture

The human hepatocellular carcinoma cell line SK-Hep-1 and hormone refractory prostate cancer cell line PC-3 were purchased from the American Type Cell Culture Collection (Manassas, VA), and maintained in DMEM medium containing 10% fetal bovine serum (FBS), penicillin (100 units/ml) and streptomycin (100 $\mu\text{g}/\text{mL}$). Cells were maintained in humidified air containing 5% CO_2 at 37 $^\circ\text{C}$. All cell culture reagents were purchased from Gibco-BRL life technologies (Grand Island, NY). The isolation and maintenance of human CD34-positive endothelial progenitor cells (EPCs) were conducted using the standard method as previously described [14].

3.5. Anti-cancer Assay

SK-Hep-1 and PC-3 cancer cells were seeded onto 96-well plates in a density of 5×10^3 cells per well. Overnight, cells were treated with the tested compounds for 48 h. Then, anti-cancer activity was determined by the SRB assay according to previously described procedures [15].

3.6. Cell Growth Assay

The growth of human endothelial progenitor cells (EPCs) was measured according to an established protocol [16].

3.7. Tube Formation Assay

The capillary tube formation assay was carried out on Matrigel-coated 48-well plates. Measurement of tube formation was performed to determine the differentiation and formation of capillary-like tubules on EPCs as described previously [14].

3.8. Cytotoxicity Assay

Cytotoxicity of human EPCs was analyzed by the method based on our previous work [14].

4. Conclusions

In this report, we have identified seven new polyketides **1–7** along with four known compounds from a marine algicolous fungal strain *Aspergillus giganteus* NTU967. Of the compounds identified, compound **3** and its known analogue patulin exhibited promising anti-cancer as well as significant anti-angiogenic activities when compared with the clinically used drugs.

Supplementary Materials: The following are available online at XXX, Figure S1–S38: All the NMR spectra of compounds **1–7**. Figure S39: The structures of known compounds isolated in this study

Author Contributions: J.-J.C. drafted the manuscript initially. S.-W.W. performed the biological assays. Y.-R.C. analyzed and interpreted the data. K.-L.P. identified the fungal strain. Y.-H.K. revised the manuscript and gave suggestions. T.-Y.S. isolated and purified all the secondary metabolites. T.-H.L. designed and conceived the whole study. All authors checked and approved the final manuscript.

Funding: This work was supported by a grant from the Ministry of Science and Technology (MOST107-2320-B-002-017-MY3) of Taiwan to T.-H.L.

Acknowledgment: We thank Ms. S.-L.H. and Ms. S.-Y.S. in the Instrumentation Center of the College of Science, National Taiwan University for the NMR and MS data acquisition, respectively.

Conflict of Interest: The authors declare no conflict of interest.

References

1. Rateb, M.E.; Ebel, R. Secondary metabolites of fungi from marine habitats. *Nat. Prod. Rep.* **2011**, *28*, 290–344.
2. Xu, L.; Meng, W.; Cao, C.; Wang, J.; Shan, W.; Wang, Q. Antibacterial and antifungal compounds from marine fungi. *Mar. Drugs* **2015**, *13*, 3479–3513.
3. Li, W.; Ding, L.; Wang, N.; Xu, J.; Zhang, W.; Zhang, B.; He, B.; Wu, B.; Jin, H. Isolation and characterization of two new metabolites from the sponge-derived fungus *Aspergillus* sp. LS34 by OSMAC approach. *Mar. Drugs* **2019**, *17*, 283.
4. Pan, R.; Bai, X.; Chen, J.; Zhang, H.; Wang, H. Exploring structural diversity of microbe secondary metabolites using OSMAC strategy: A literature review. *Front. Microbiol.* **2019**, *10*, 294.
5. Hoang, T.P.T.; Roullier, C.; Boumard, M.C.; Robiou du Pont, T.; Nazih, H.; Gallard, J.F.; Pouchus, Y.F.; Beniddir, M.A.; Grovel, O. Metabolomics-driven discovery of meroterpenoids from a mussel-derived *penicillium ubiquestum*. *J. Nat. Prod.* **2018**, *81*, 2501–2511.
6. Selegato, D.M.; Freire, R.T.; Pilon, A.C.; Biasetto, C.R.; de Oliveira, H.C.; de Abreu, L.M.; Araujo, A.R.; da Silva Bolzani, V.; Castro-Gamboa, I. Improvement of bioactive metabolite production in microbial culture – A systems approach by OSMAC and deconvolution-based ¹H NMR quantification. *Magn. Reson. Chem.* **2019**, *57*, 458–471.
7. Wang, W.J.; Li, D.Y.; Li, Y.C.; Hua, H.M.; Ma, E.L.; Li, Z.L. Caryophyllene sesquiterpenes from the marine-derived fungus *Ascotricha* sp. ZJ-M-5 by the one strain-many compounds strategy. *J. Nat. Prod.* **2014**, *77*, 1367–1371.
8. Huang, S.F. Seaweeds of Northeastern Taiwan. National Taiwan Museum, Taipei, Taiwan, 2000; pp. 6–7.
9. Raistrick, H. Patulin in the common cold collaborative research on a derivative of *Penicillin patulum* Bainier. *Lancet* **1943**, *242*, 625.
10. Alam, N.; Yu, J.Q.; Beale, P.; Hug, F. Dose and sequence dependent synergism from the combination of oxaliplatin with emetine and patulin against colorectal cancer. *Anticancer Agents Med. Chem.* **2019**, *20*, 264–273.
11. Buechi, G.; Luk, K.C.; Kobbe, B.; Townsend, J.M. Four new mycotoxins of *Aspergillus clavatus* related to tryptoquivaline. *J. Org. Chem.* **1977**, *42*, 244–246.
12. Koyama, N.; Inoue, Y.; Sekine, M.; Hayakawa, Y.; Homma, H.; Omura, S.; Tomoda, H. Relative and absolute stereochemistry of quinadoline B, an inhibitor of lipid droplet synthesis in macrophages. *Org. Lett.* **2008**, *10*, 5273–5276.

13. Prata-Sena, M.; Ramos, A.A.; Buttachon, S.; Castro-carvalho, B.; Marques, P.; Dethoup, T.; Kijjoa, A.; Rocha, E. Cytotoxic activity of secondary metabolites from marine-derived fungus *Neosartorya siamensis* in human cancer cells. *Phytother. Res.* **2016**, *30*, 1862–1871.
14. Lee, M.S.; Wang, G.J.; Pang, K.L.; Lee, C.K.; Kuo, Y.H.; Lin, R.K.; Lee, T.H. Angiogenesis inhibitors and anti-inflammatory agents from *Phoma* sp. NTOU4195. *J. Nat. Prod.* **2016**, *79*, 2983–2990.
15. Thakur, R.S.; Ahirwar, B. A steroidal derivative from *Trigonella foenumgraecum* L. that induces apoptosis *in vitro* and *in vivo*. *J. Food Drug Anal.* **2019**, *27*, 231–239.
16. Koh, Y.C.; Ho, C.T.; Pang, M.H. Recent advances in cancer chemoprevention with phytochemicals. *J. Food Drug Anal.* **2020**, *28*, 14–37.