Highly Oxygenated Constituents from a Marine Alga-Derived Fungus Aspergillus giganteus NTU967

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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 by spectral analysis to be seven new highly oxygenated polyketides, namely aspergilmins A–G (1–7), along with previously reported patulin, deoxytryptoquivaline, tryptoquivaline and quinadoline B. Among these, aspergilmin C (3) and patulin displayed promising anticancer activities against human hepatocellular carcinoma SK-Hep-1 cells and prostate cancer PC-3 cells with IC50 values between 2.7–7.3 µM. Furthermore, aspergilmin 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; aspergilmin

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, etc. [5–7]. In addition, easy to scale-up and quality control of the fungal metabolites would be another major advantage 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* [9], 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 both the fermented products of *Aspergillus giganteus* NTU967 was performed, and this has resulted in the isolation and identification of seven previously unreported highly oxygenated polyketides 1–7 (Figure 1) together with four known compounds. This study describes the isolation and characterization of the new compounds together with their bioactivities (see Supplementary Materials).

![Chemical structures of compounds 1–7 and patulin.](image)

Figure 1. Chemical structures of compounds 1–7 and patulin.

2. Results and Discussion

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, patulin, deoxytryptoquivaline, tryptoquivaline and quinadoline B, 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 [10]. Recently, patulin in combination with oxaliplatin were found to exhibit synergism against human colorectal cancer [11]. Deoxytryptoquivaline, tryptoquivaline and quinadoline—three quinazolone-containing alkaloids—were identified by comparison of spectroscopic data with literatures [12,13]. In addition to be isolated from *Aspergillus* spp., a series of tryptoquivaline analogs have ever been obtained from a marine sea fan-derived fungus *Neosartorya siamensis* [14].

Compound 1, obtained as colorless oil, was determined to have a molecular formula of C9H12O5, as evidenced by its 13C NMR spectrum (Table 1) and a pseudomolecular ion [M + Na]+ at m/z 223.0574 (calcld. 223.0582 for C9H12O5Na) 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 (CD2OD, 400 MHz) of compound 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$_H$ 2.61 (d, $J = 6.2$ Hz, H$_2$-2), one oxygen-bearing methylene signal at δ$_H$ 4.55 (H$_2$-7), one oxygen-bearing methine signal at δ$_H$ 4.55 (H-3) and an olefinic methine signal at δ$_H$ 7.66 (s, H-6) (Table 2). The $^{13}$C NMR (CD$_3$OD, 125 MHz) coupled with phase-sensitive HSQC spectrum of compound 1 showed nine signals including two methoxyl carbons at δ$_C$ 57.7 (C-8) and 52.7 (C-9), one methylene carbon at δ$_C$ 36.7 (C-2), one oxymethylene carbon at δ$_C$ 75.9 (C-7), one oxygenated methine carbon at δ$_C$ 69.7 (C-3), one oxygenated olefinic carbon at δ$_C$ 166.1 (C-6), one nonprotonated olefinic carbon at δ$_C$ 116.2 (C-5), one ester carbonyl carbon at δ$_C$ 172.9 (C-1) and one conjugated ketone carbon at δ$_C$ 191.8 (C-4) (Table 1). On account of the molecular formula C$_9$H$_{12}$O$_5$, the double bond equivalent (DBE) of compound 1 was four including a double bond and two carbonyl groups. Thus, there should be one additional ring in compound 1. Further two dimensional NMR analysis including one cross-peak of δ$_H$ 2.61 (H$_2$-2)/δ$_H$ 4.55 (H-3) in the COSY spectrum of compound 1 in combination with key correlations of δ$_H$ 2.61 (H$_2$-2)/δ$_C$ 69.7 (C-3) and 191.8 (C-4); δ$_H$ 4.55 (H-3)/δ$_C$ 172.9 (C-1) and 191.8 (C-4); δ$_H$ 7.66 (H-6)/δ$_C$ 69.7 (C-3), 191.8 (C-4), 116.2 (C-5) and 75.9 (C-7); δ$_H$ 4.55 (H$_2$-7)/δ$_C$ 191.8 (C-4); δ$_H$ 3.35 (H$_3$-8)/δ$_C$ 75.9 (C-7); δ$_H$ 3.71 (H$_3$-9)/δ$_C$ 172.9 (C-1) in the HMBC spectrum of compound 1 (Figure 2), corroborated the gross structure of 1. The optical rotation value of compound 1 ([α]$_{27}^{20}$D $-0.36$) was close to zero, revealing that compound 1 could be a racemate.

![Figure 2](image.png)

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

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   | 119.7 d   | 119.9 d   |
| 3   | 69.7 d    | 111.7 s   | 147.5 s   | 147.6 s   | 156.5 s   | 163.9 s   | 163.9 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 CD$_3$OD (125 MHz); $^b$ Multiplicities were obtained from phase-sensitive HSQC experiments.
Table 2. $^1$H 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   | 2.61 d (6.2) | 3.14 s | 6.60 s | 6.05 s | 6.31 s | 6.12 s | 6.23 s |
| 2   | 4.55 $^c$ |        |        |        |        |        |        |
| 3   | 3.70 (4.7) | 6.02 m | 6.02 m | 5.85 t (6.9) | 1.91 m | 1.99 m |        |
| 5   | 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) | 2.38 dt (13.5, 2.1) | 2.34 d (13.2) |
| 6   | 4.55 $^c$ | 7.50 s | 5.68 s | 5.79 s | 5.52 s | 5.11 s | 5.52 s |
| 7   | 3.35 s | 3.45 s | 3.54 s | 3.71 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 CD$_3$OD (400 MHz); $^b$ Measured in CD$_3$OD (500 MHz); $^c$ Signals were overlapped and were picked up from HSQC spectrum.

The molecular formula of 2, C$_9$H$_{12}$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 $^1$H NMR spectrum of compound 2 coupled with phase-sensitive HSQC spectrum showed two methoxyl signals at δ$_H$ 3.45 (s, H$_3$-8) and 3.67 (s, H$_3$-9), one methylene signal at δ$_H$ 3.14 (s, H$_2$-2), one oxygenated methylene signal at δ$_H$ 4.49 (d, J = 4.7 Hz, H$_2$-6), one carboxyl methine signal at δ$_H$ 3.70 (t, J = 4.7 Hz, H-5), and one olefinic methine signal at δ$_H$ 7.50 (s, H-7) (Table 2). Nine carbon resonances including two methoxyl at δ$_C$ 58.7 (C-8) and 52.5 (C-9), one oxygenated methylene at δ$_C$ 72.3 (C-6), one methylene at δ$_C$ 31.1 (C-2), one olefinic methine at δ$_C$ 164.0 (C-7), one carboxyl methine at δ$_C$ 77.3 (C-5), one nonprotonated olefinic carbon at δ$_C$ 111.7 (C-3), one ester carbonyl carbon at δ$_C$ 173.6 (C-1), and one ketone carbon at δ$_C$ 191.3 were observed in the $^{13}$C NMR of compound 2 (Table 1), which were supported by phase-sensitive HSQC spectrum. On account of the molecular formula of 2, C$_9$H$_{12}$O$_5$, compound 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 compound 2 (Figure 2), one cross-peak of δ$_H$ 3.70 (H-5)/δ$_H$ 4.49 (H-6) in the COSY spectrum together with key cross-peaks of δ$_H$ 3.14 (H$_2$-2)/δ$_C$ 111.7 (C-3); δ$_H$ 4.49 (H$_2$-6)/δ$_C$ 191.3 (C-4); δ$_H$ 7.50 (H-7)/δ$_C$ 31.1 (C-2), 111.7 (C-3), 191.3 (C-4) and 72.3 (C-6); δ$_H$ 3.45 (H$_2$-8)/δ$_C$ 77.3 (C-5); δ$_H$ 3.67 (H$_2$-9)/δ$_C$ 173.6 (C-1) in the HMBC spectrum established the structure of 2.

The physical and NMR data of compound 3 and compound 4 were almost compatible with those of patulin except that an additional methyl and an additional ethyl were observed in the $^1$H and $^{13}$C NMR spectra of compound 3 and compound 4 (Tables 1 and 2), respectively. The pseudomolecular ion [M + H]$^+$ at m/z 169.0493 and 183.0655 in the HRESIMS of compounds 3 and 4, 14 and 28 Da more than that of patulin, confirmed that compound 3 and compound 4 were the methyl and ethyl derivatives of patulin, respectively. In the HMBC spectra of compounds 3 and 4, distinctive cross-peaks of δ$_H$ 3.54 (H$_3$-8)/δ$_C$ 95.9 (C-7) and δ$_H$ 3.71 and 3.91 (H$_2$-8)/δ$_C$ 94.8 (C-7) indicated the methyl and ethyl groups were attached to C-7 of compounds 3 and 4, respectively. The structures of compound 3 and compound 4 were thus elucidated to be as shown.

Compound 5, obtained as colorless oil, was determined to have a molecular formula of C$_{19}$H$_{14}$O$_{16}$, 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 $^1$H NMR data along with the HSQC spectrum of compound 5 showed two methyl signals at δ$_H$ 3.36 (s, H$_3$-10) and 1.24 (t, J = 7.1 Hz, H$_3$-10), two oxygenated methylene signals at δ$_H$ 4.40 (d, J = 6.9 Hz, H$_2$-6) and 3.63 (m, H$_2$-8), two olefinic methine signals at δ$_H$ 6.31 (s, H-2) and 5.85 (t, J = 6.9 Hz, H-5) and one dioxygenated methine signal at δ$_H$ 5.52 (s, H-7) (Table 2). The $^{13}$C NMR data of compound 5 coupled with its phase-sensitive HSQC assignments showed one methoxyl carbon at δ$_C$ 53.2 (C-10), one methyl carbon at δ$_C$ 15.4 (C-9), two oxygenated methylene carbons at δ$_C$ 62.8 (C-8) and 57.3 (C-6), two olefinic methine carbons at δ$_C$ 119.7 (C-2) and...
115.3 (C-5), one dioxygenated methine carbon at δC 97.6 (C-7) and three nonprotonated carbons at δC 169.6 (C-1), 156.5 (C-3) and 148.2 (C-4) (Table 1). Correlations of δH 5.85 (H-5)/δH 4.40 (H-6) and δH 3.63 (H-2)/δH 1.24 (H-3) in the COSY spectrum of compound 5 accompanied with key correlations including δH 6.31 (H-2)/δC 169.6 (C-1), 156.5 (C-3), 148.2 (C-4) and 97.6 (C-7); δH 5.58 (H-5)/δC 156.5 (C-3) and 148.2 (C-4); δH 3.63 (H-2)/δC 97.6 (C-7); δH 3.36 (H-10)/δC 97.6 (C-7) in the HMQC spectrum of compound 5 (Figure 2), established the gross structure of 5. The configuration of Δ⁴ in compound 5 was determined to be E form based on a key correlation of δH 4.40 (H-2)/δH 5.52 (H-7) in the ROESY of compound 5 (Figure 2). Since compound 5 had an acetal carbon at its C-7 and an optical rotation value close to zero ([α]27D +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 δH 6.02 (H-5) in compound 3 was substituted by a methylene group at δH 1.91 and 2.38 (H-5) in compound 6 and δH 1.99 and 2.34 (H-5) in compound 7 and an additional methoxyl functionality at δH 3.23 (H-9) and δH 3.19 (H-9) was observed in compound 6 and compound 7 (Table 2), respectively. These changes also reflected in their 13C NMR data (Table 1), in which two olefinic signals at δC 150.9 (C-4) and 109.3 (C-5) in compound 3 were replaced by a ketal carbon signal (δC-4 107.6 in 6; δC-4 107.2 in 7) along with a methylene carbon resonance (δC-5 40.3 in 6; δC-5 41.9 in 7). The additional methoxyl groups in compounds 6 and 7 were deduced to be attached at C-4 due to key cross-peaks of H-9/C-4 in the HMBC spectra of both compound 6 and compound 7 (Figure 2). In the ROESY spectra of compounds 6 and 7, a key correlation of δH 3.23 (H-9)/δH 5.11 (H-7) confirmed that two methoxyls in compound 6 were oriented in different phases, while a key cross-peak of δH 3.46 (H-3)/δH 3.19 (H-9) indicated that two methoxyls in compound 7 were located on the same side (Figure 2). The structures of compounds 6 and 7 were thus established as shown in Figure 1.

2.2. Anticancer and Anti-Angiogenic Assays of Secondary Metabolites

All eleven pure isolates were subjected to biological assays. Among these, compound 3 exerted promising anticancer activities against human hepatocellular carcinoma SK-Hep-1 cells and prostate cancer PC-3 cells with IC₅₀ values of 2.7 ± 0.2 and 7.3 ± 0.3 μM (Table 3). Paclitaxel, a well-known anticancer 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 ± 0.3 and 4.7 ± 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 3A,B). Furthermore, it was found that compound 3 and patulin did not induce the release of lactate dehydrogenase (LDH) in EPCs (Figure 3C), 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 C-4 of patulin could play crucial roles in the anticancer 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.
Table 3. Anticancer and anti-angiogenic activities of compound 3 and patulin.

| Compounds  | SK-Hep-1 IC₅₀(µM) | PC-3 IC₅₀(µM) | EPC IC₅₀(µM) |
|------------|-------------------|---------------|--------------|
| Compound 3 | 2.7 ± 0.2         | 7.3 ± 0.3     | 4.6 ± 0.3    |
| Patulin    | 2.9 ± 0.1         | 2.7 ± 0.1     | 4.7 ± 0.2    |
| Paclitaxel | 0.011 ± 0.002     | 0.013 ± 0.002 | –            |
| Sorafenib  | –                 | –             | 4.8 ± 0.3    |

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

Figure 3. Effects of compound 3 and patulin on tube formation and cytotoxicity of human EPCs.
(A) EPCs were treated with compound 3, patulin and sorafenib (10 µM) for 24 h. Tubular morphogenesis was recorded by the inverted phase contrast microscope. Representative images of EPCs’ tube formation were shown; (B) tube formation was quantified by measuring the length of tubes using ImageJ software; (C) Cells were treated with the indicated compounds for 24 h, then the cytotoxicity was determined using LDH assay. Data represent the mean ± S.E.M. of 4 independent experiments. * p < 0.05 compared with the control group.
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 Heλios α Spectrophotometer (Bellefonte, CA, USA), respectively. $^1$H and $^{13}$C NMR were acquired on Bruker AVIII HD 400 and Bruker AVIII-500 spectrometer (Ettlingen, Germany). Low and high resolution mass spectra were obtained using an API4000 triple quadrupole mass spectrometer (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, MD, USA), 1 g yeast extract (Becton, Dickinson and Company, Sparks, MD, USA), 10 g Dextrose (Becton, Dickinson and Company, Sparks, MD, USA) and 2.5 L deionized water. The fermentation was conducted with aeration at 25–30 °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, MD, USA), 1% sodium tartrate and 1% KH$_2$PO$_4$ 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 redissolved 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$_2$Cl$_2$-MeOH (10:1, v/v) for development, and dipping in vanillin–H$_2$SO$_4$ was used in the detection of compounds with similar skeletons. All the fractions were combined into four samples I–VI. Subsequently, sample III with antimicrobial activity was precoated 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$_2$O/MeOH in a stepwise gradient mode with a flow rate of 2.0 mL/min to obtain four subsamples I–IV. Subsample III eluted by 75% MeOH was rechromatographed on a semipreparative reversed-phase column (Phenomenex Luna 5 µm PFP, 10 × 250 mm) with 25% MeCN$_aq$ 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). Subsample III was further purified on a semipreparative reversed-phase column (Thermo Hypersil 5 µm C$_{18}$, 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$_2$O, 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 get 30 fractions (20.0 mL/fr.). All the fractions were combined into 6 samples as I–VI based on the results of TLC analysis and antimicrobial assay. Sample III with antimicrobial activity was precoated 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 H2O/MeOH in a stepwise gradient mode with a flow rate of 2.0 mL/min to get four subsamples I–IV. Subsample II eluted by 50% MeOH was rechromatographed on a semipreparative reversed-phase column (BIOSIL Pro-ODS-U 5 µ, 10 × 250 mm) with 15% MeOH as eluent, 2 mL/min, to obtain patulin (16.7 mg, tR = 20.1 min). Subsample IV eluted by 100% MeOH was further purified on a semipreparative reversed-phase column (Phenomenex Luna 5 µ PFP, 10 × 250 mm) with 75% MeOH as eluent, 2 mL/min, to afford quinodoline B (27.7 mg, tR = 11.8 min), deoxytryptoquivaline (21.0 mg, tR = 23.0 min) and tryptoquivaline (16.4 mg, tR = 36.2 min).

Aspergillosmin A (1): Colorless oil; [α]D −0.36 (c = 0.05, MeOH); IR (ZnSe) νmax: 2951, 1745, 1672, 1611, 1456, 1438, 1399, 1333, 1308, 1283, 1254, 1196, 1171, 1105, 1055, 1039 and 1009 cm−1; UV λmax (MeOH) (log e) 261 (3.9) nm; 1H and 13C NMR spectroscopic data: see Tables 1 and 2; HRESIMS [M + Na]+ at m/z 223.0574 (calcd. 223.0582 for C13H20O3Na).

Aspergillosmin B (2): Colorless oil; [α]D +1.22 (c = 0.05, MeOH); IR (ZnSe) νmax: 2947, 1737, 1673, 1619, 1443, 1406, 1344, 1291, 1257, 1157, 1097, 1056, 1043, 1024, 1011 and 854 cm−1; UV λmax (MeOH) (log e) 268 (3.9) nm; 1H and 13C NMR spectroscopic data: see Tables 1 and 2; HRESIMS [M + Na]+ at m/z 223.0573 (calcd. 223.0582 for C13H20O3Na).

Aspergillosmin C (3): Colorless oil; [α]D −3.52 (c = 0.05, MeOH); IR (ZnSe) νmax: 2955, 1780, 1536, 1443, 1406, 1344, 1210, 1065 and 868 cm−1; UV λmax (MeOH) (log e) 274 (4.0) nm; 1H and 13C NMR spectroscopic data: see Tables 1 and 2; HRESIMS [M + H]+ at m/z 169.0493 (calcd. 169.0501 for C8H14O4).

Aspergillosmin D (4): Colorless oil; [α]D −2.14 (c = 0.05, MeOH); IR (ZnSe) νmax: 2945, 1780, 1635, 1404, 1092 and 1019 cm−1; UV λmax (MeOH) (log e) 275 (4.0) nm; 1H and 13C NMR spectroscopic data: see Tables 1 and 2; HRESIMS [M + H]+ at m/z 183.0655 (calcd. 183.0657 for C9H13O4).

Aspergillosmin E (5): Colorless oil; [α]D +0.02 (c = 0.05, MeOH); IR (ZnSe) νmax: 3435, 2785, 1643, 1053 and 1008 cm−1; UV λmax (MeOH) (log e) 223 (3.7) and 270 (4.0) nm; 1H and 13C NMR spectroscopic data: see Tables 1 and 2; HRESIMS [M + H]+ at m/z 215.0915 (calcd. 215.0947 for C10H15O3).

Aspergillosmin F (6): Colorless oil; [α]D −0.53 (c = 0.05, MeOH); IR (ZnSe) νmax: 2951, 1768, 1643, 1456, 1396, 1207, 1163, 1082, 1022 and 972 cm−1; UV λmax (MeOH) (log e) 257 (4.1) nm; 1H and 13C NMR spectroscopic data: see Tables 1 and 2; HRESIMS [M + Na]+ at m/z 223.0579 (calcd. 223.0582 for C9H12O3Na).

Aspergillosmin G (7): Colorless oil; [α]D −1.86 (c = 0.05, MeOH); IR (ZnSe) νmax: 2971, 2842, 1766, 1456, 1435, 1358, 1329, 1266, 1205, 1196, 1171, 1129, 1041, 1034 and 915 cm−1; UV λmax (MeOH) (log e) 271 (4.0) nm; 1H and 13C NMR spectroscopic data: see Tables 1 and 2; HRESIMS [M + Na]+ at m/z 223.0574 (calcd. 223.0582 for C9H12O3Na).

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, USA) and maintained in DMEM medium containing 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 µg/mL). Cells were maintained in humidified air containing 5% CO2 at 37 °C. All cell culture reagents were purchased from Gibco-BRL life technologies (Grand Island, NY, USA). The isolation and maintenance of human CD34-positive endothelial progenitor cells (EPCs) were conducted using the standard method as previously described [15].
3.5. Biologic Assay for Anticancer Activity

SK-Hep-1 and PC-3 cancer cells were seeded onto 96-well plates in a density of $5 \times 10^3$ cells per well. Overnight, cells were treated with the tested compounds for 48 h. Then, anticancer activity was determined by the SRB assay according to previously described procedures [16].

3.6. Biologic Assay for Anti-Angiogenic Activity

For EPCs’ cell growth assay, EPCs were cultured in 96-well plates at a density of $5 \times 10^3$ cells in each well. Overnight, the culture medium was replaced with MV2 complete medium containing 2% FBS in the presence of the tested compounds for 48 h. The reaction was terminated after 48 h of incubation with 50% TCA. After the TCA fixation, every well was incubated for 15 min incubation with 0.4% sulforhodamine B in 1% acetic acid. The plates were then washed before the dye was dissolved by 10-mM Tris buffer. Absorbance density values were read by an enzyme-linked immunosorbent assay (ELISA) reader (515 nm).

For EPCs’ tube formation assay, EPCs were seeded with the density of $1.25 \times 10^4$ cells per well in Matrigel-coated 96-well plates and incubated in an MV2 complete medium containing 2% FBS and the tested compounds for 24 h. EPCs differentiation and capillary-like tube formation was taken with the inverted phase contrast microscope. The long axis of each tube was measured with MacBiophotonics Image J software in 3 randomly chosen fields per well.

For EPCs’ cytotoxicity assay, EPCs ($5 \times 10^3$ cells/well) were seeded onto 96-well plates and incubated with MV2 complete medium containing 2% FBS and the tested compounds for 24 h. Then, the quantification of LDH release in the medium was done with a cytotoxicity assay kit.

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 analog patulin exhibited promising anticancer as well as significant anti-angiogenic activities when compared with the clinically used drugs.

**Supplementary Materials:** The following are available online at [http://www.mdpi.com/1660-3397/18/6/303/s1](http://www.mdpi.com/1660-3397/18/6/303/s1), Figures S1–S38: All the NMR spectra of compounds 1–7. Figure S39: The structures of known compounds isolated in this study. Table S1: Cytotoxicities of aspergilsmins A-G (1–7), patulin, deoxytryptoquivaline, tryptoquivaline and quinadoline B against PC-3 and SK-Hep-1 cells.

**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.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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