Penigrisacids A–D, Four New Sesquiterpenes from the Deep-Sea-Derived Penicillium griseofulvum

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Abstract: Four new (penigrisacids A–D, 1–4) and one known (5) carotane sesquiterpenoids were isolated from the deep-sea-derived fungus Penicillium griseofulvum, along with four known compounds (6–9). The planar structures and relative configurations of the new compounds were determined by extensive analysis of the NMR and HRESIMS data. The absolute configurations were established by comparison of the experimental and calculated ECD (electronic circular dichroism) spectra or OR (optical rotation) value. Compound 9 exhibited potent anti-food allergic activity with IC_{50} value of 28.7 μM, while 4 showed weak cytotoxicity against ECA-109 tumor cells (IC_{50} = 28.7 μM).

Keywords: deep-sea-derived fungus; Penicillium griseofulvum; carotanes; sesquiterpenes; anti-food allergy

1. Introduction

Carotanes (also called daucanes) are bicyclic sesquiterpenes mainly isolated from the plants of the Umbelliferae family and the fungus Aspergillus terreus [1–4]. Some of these compounds showed significant biological activities, for example, aspterric acid was found to inhibit Arabidopsis pollen development at meiosis [5]. More recently, studies showed aspterric acid could be used as a herbicide [6]. In our current investigations on the bioactive secondary metabolites from the deep-sea-derived microorganisms [7–10], the crude extract of Penicillium griseofulvum, a fungus isolated from the Indian Ocean, showed potent in vitro anti-food allergic activity. Accordingly, a systematic isolation was conducted, which led to the isolation of five carotanes (1–5, Figure 1) and four other compounds (5–9). Herein, we report the isolation, structure elucidation, as well as anti-allergic and anti-tumor bioactivities of these compounds.

![Chemical structures of 1-5, isolated from Penicillium griseofulvum.](image)

Figure 1. Chemical structures of 1–5, isolated from Penicillium griseofulvum.

2. Results and Discussion

Compound 1 was obtained as a colorless oil. Its molecular formula was established as C_{15}H_{22}O_{4} on the basis of the sodium adduct ion peak at m/z 289.1411 [M + Na]^+ in its HRESIMS spectrum, indicating...
five degrees of unsaturation. The $^1$H NMR spectrum (Table 1) showed the presence of three methyl singlets ($\delta_H$ 1.18, s, Me-15; 1.82, s, Me-12; 1.63, s, Me-13) and one oxymethine ($\delta_H$ 4.48, d, $J = 7.5$ Hz, H-2). The $^{13}$C NMR spectrum, in association with the DEPT and HSQC spectra, indicated 15 carbon signals (Table 2), including three methyls ($\delta_C$ 20.1, 21.3, and 23.5, for Me-15, 12, and 13, respectively), five methylenes ($\delta_C$ 27.7, C-5; 28.5, C-4; 33.3, C-9; 40.4, C-1; 41.6, C-8), one oxygenated methine ($\delta_C$ 82.6, C-2), and six non-protonated carbons, including one carboxyl ($\delta_C$ 178.0, C-14), two olefinic ($\delta_C$ 135.6, C-10; $\delta_C$ 131.6, C-11), two oxygenated ($\delta_C$ 74.4, C-3; 93.9, C-6); and one aliphatic ($\delta_C$ 54.0, C-7) carbons. Since one carboxyl and two olefinic carbons accounted for two degrees of unsaturation, 1 was assumed to be a tricyclic molecule. The COSY correlations showed three isolated spin systems from H-2 ($\delta_H$ 2.01) to H$_2$-1 ($\delta_H$ 1.90), H$_2$-4 ($\delta_H$ 2.18, 1.69) to H$_2$-5 ($\delta_H$ 2.74, 1.55), and H$_2$-8 ($\delta_H$ 1.63, 1.55) through H$_2$-9 ($\delta_H$ 2.38) to Me-12 and Me-13. The HMBC spectrum displayed correlations from Me-15 to C-1/C-6/C-7/C-8, H-2 to C-3/C-4/C-6/C-14, and H$_2$-5 to C-6/C-7/C-10. Taking together the COSY and HMBC correlations, the planar structure of 1 was established (Figure 2), which was very similar to asperric acid except that the ether bond was between C-2 and C-6 in 1, instead of C-2 and C-15 in asperric acid. In the NOESY spectrum, strong correlations were observed from H-1a ($\delta_H$ 1.90) to Me-15, H-1b ($\delta_H$ 2.01) to H-2, Me-15 to H-5a ($\delta_H$ 1.55), suggesting the configurations of C-2 and C-6 at the epoxy moiety were opposite to that of Me-15. Accordingly, the relative structure of 1 was assigned as 10,11-dehydro-3α-hydroxy-2α,6α-epoxycaroten-14-oic acid.

![Figure 2. Key COSY ( ), HMBC ( ), and NOESY ( ) correlations of 1.](image-url)

| No. | 1 | 2 | 3 | 4 | 5 |
|-----|---|---|---|---|---|
| 1.00, dd (12.3, 7.6) | 2.14, br d (14.2) | 1.85, d (12.7) | 2.00, d (13.0) | 2.40, overlap |
| 1.90, d (13.2) | 1.93, overlap | 1.20, m | 1.33, m | 2.28, m |
| 2.18, dd (13.5, 6.4) | 2.18, dd (13.8, 7.6) | 2.42, dd (13.7, 7.5) | 2.26, dd (13.1, 8.4) | 2.03, overlap |
| 1.69, m | 1.25, d (13.2) | 1.43, overlap | 1.45, dd (13.6, 7.6) | - |
| 4.48, d (7.5) | 4.13, d (8.4) | 4.28, d (8.4) | 4.31, d (8.4) | 2.32, m |
| 4.48, d (8.0) | 4.62, d (8.0) | 3.34, d (7.9) | 3.70, d (8.0) | 1.73, br s |
| 3.35, d (8.7) | 3.64, d (8.0) | 4.62, d (8.0) | 3.70, d (8.0) | - |
| 2.10, m | 1.65, m | 1.84, m | 1.67, m | - |
| 2.18, m | 1.78, m | 1.41, overlap | 2.10, m | - |
| 1.26, dd (11.1, 7.6) | 1.56, m | 1.61, m | 2.18, m | - |
| 2.74, dt (12.6, 6.2) | 1.93, overlap | 1.43, overlap | 1.45, dd (13.6, 7.6) | 1.84, m |
| 1.55, overlap | 1.25, d (13.2) | 1.43, overlap | 1.45, dd (13.6, 7.6) | 1.73, br s |
| 1.59, t (10.5) | 1.42, overlap | 1.72, m | 2.26, m | - |
| 1.63, m | 1.41, m | 1.56, m | 1.84, m | 2.26, m |
| 1.55, overlap | 1.26, dd (11.1, 7.6) | 1.65, m | 2.10, m | - |
| 2.38, m | 1.78, m | 1.41, overlap | 2.10, m | 2.18, m |
| 1.68, m | 1.56, m | 1.61, m | 1.69, m | 1.73, br s |
| - | 1.93, overlap | 1.58, m | - | - |
| 1.82, br s | 1.19, s | 1.03, s | 1.76, s | 1.73, br s |
| 1.63, br s | 3.45, br s | 1.05, s | 5.01, s | 1.60, br s |
| 0.79, s | 3.64, d (8.0) | 4.62, d (8.0) | 3.70, d (8.0) | 3.35, d (8.7) |
| 3.18, d (8.1) | 3.34, d (7.9) | 3.35, d (8.7) | 3.35, d (8.7) | 3.35, d (8.7) |

* Recorded in CD$_3$OD.  * Recorded in DMSO-d$_6$. 

Table 1. $^1$H (400 MHz) NMR spectroscopic data of 1–5 ($\delta$ in ppm, $J$ in Hz within parentheses).

Mar. Drugs 2019, 17, 507
To determine the absolute configuration, a theoretical calculation on its ECD spectrum was performed. The calculated ECD spectra of 2,3,5R,6S,7R-1 (1a) and 2R,3S,6R,7S-1 (1b) were obtained by time-dependent density functional theory (TD-DFT) at the B3LYP/6-31+G(d,p) level in ACN (acetonitrile). As shown in Figure 3, the calculated ECD spectrum of 1a fits well with the experimental one. From the above-mentioned evidences, the structure of 1 was then established as (2S,3R,6S,7R)-10,11-dehydro-2,6-epoxy-3-hydroxy-14-carotanoic acid, and was named penigrisacid A.

Table 2. 13C (100 MHz) NMR spectroscopic data of 1-5.

| No. | 1a | 2a | 3b | 4a | 5a |
|-----|----|----|----|----|----|
| 1   | 40.4 CH₂ | 42.3 CH₂ | 35.8 CH₂ | 38.9 CH₂ | 35.2 CH₂ |
| 2   | 82.6 CH | 142.8 CH | 82.8 CH | 84.0 CH | 84.9 CH |
| 3   | 74.4 C  | 135.5 C  | 77.6 C  | 79.6 C  | 79.5 C  |
| 4   | 28.5 CH₂ | 28.5 CH₂ | 34.3 CH₂ | 35.3 CH₂ | 33.0 CH₂ |
| 5   | 27.7 CH₂ | 27.0 CH₂ | 24.8 CH₂ | 19.8 CH₂ | 36.8 CH₂ |
| 6   | 93.9 C  | 55.8 CH  | 52.3 CH  | 58.4 CH  | 56.7 CH  |
| 7   | 54.0 C  | 43.5 C  | 52.7 C  | 52.7 C  | 54.2 C  |
| 8   | 41.6 CH₂ | 42.4 CH₂ | 33.6 CH₂ | 33.3 CH₂ | 24.9 CH₂ |
| 9   | 33.3 CH₂ | 24.6 CH₂ | 26.8 CH₂ | 40.0 CH₂ | 35.1 CH₂ |
| 10  | 135.6 C | 50.1 CH | 51.8 CH | 86.0 C | 136.7 C |
| 11  | 131.6 C | 76.3 C | 71.5 C | 150.7 C | 125.2 C |
| 12  | 21.3 CH₃ | 22.9 CH₃ | 26.6 CH₃ | 19.7 CH₃ | 21.0 CH₃ |
| 13  | 23.5 CH₃ | 69.5 CH₂ | 29.2 CH₃ | 110.3 CH₂ | 23.4 CH₃ |
| 14  | 178.0 C | 172.0 C | 175.8 C | 177.9 C | 177.9 C |
| 15  | 20.1 CH₃ | 19.2 CH₃ | 74.5 CH₂ | 76.2 CH₂ | 76.5 CH₂ |

a Recorded in CD₂OD. b Recorded in DMSO-d₆.

Figure 3. Experimental and calculated ECD spectra of 1.

Compound 2 was also obtained as a colorless oil. The molecular formula C₁₅H₂₄O₄ was established according to its positive HRESIMS spectrum at m/z 291.1563 (calcld for C₁₅H₂₄O₄Na, 291.1572), indicating four degrees of unsaturation. The 1H NMR spectrum exhibited two methyl singlets (δH 0.79, s, Me-12; 1.19, s, Me-15), one oxygenated methylene (δH 3.45, s, H₂-13), and one olefinic proton (δH 7.07, dt, J = 8.6, 3.1 Hz, H-2). The 13C NMR spectrum displayed 15 carbons signals which, in combination with DEPT and HSQC spectra, could be categorized as two methyls (δC 19.2, Me-15; 22.9, Me-12), one oxymethylene (δC 69.5, C-13), five sp³ methylene (δC 24.6, C-9; 27.0, C-5; 28.5, C-4; 42.3, C-1; 42.4, C-8), two methines (δC 50.1, C-10; 55.8, C-6), one protonated sp² (δC 142.8, C-2), and four non-protonated carbons, including one carboxyl (δC 172.0, C-14), one olefinic (δC 136.5, C-3) and one oxygenated (δC 76.3, C-11) carbons. Since one carboxyl and two olefinic carbons accounted for two degrees of unsaturation, 2 was deduced to be a bicyclic molecule. The COSY spectrum exhibited...
correlations from H-2 (δH 7.07) to H2-1 (δH 2.41, dd, J = 14.9, 9.1 Hz, H-1a; 2.14, br d, J = 14.2 Hz, H-1b) and the overlapped proton at δH 1.93; H-5a (δH 1.25, d, J = 13.2 Hz) to H-4a (δH 2.99, dd, J = 15.8, 5.8 Hz) and H-6 (δH 1.59, t, J = 10.5 Hz); H2-9 (δH 1.78 m, 1.68 m) to H2-8 (δH 1.41, m) and the overlapped proton at δH 1.93. The HMBC spectrum showed correlations from Me-12 to C-10, C-11, C-13, from Me-15 to C-1, C-6, C-7, C-8, from H2-4 to C-2, C-3, C-14, and from H2-9 to C-6, C-10, C-11. Taking together the molecular formula, the COSY, and HMBC spectra, the planar structure of 2 was then established. The NOESY spectrum showed correlations from Me-15 to H-1a (δH 2.41)/H-5a (δH 1.25)/H-9a (δH 1.78), from H-1b (δH 2.14) to H-6, and from H2-13 to H-5b (δH 1.93)/H-6/H-9b (δH 1.68)/H-10 (Figure 4). Therefore, H-6, H-7, and H2-13 were on the same face which was opposite to that of Me-15.

To determine the absolute configuration of the stereogenic carbons of 2, a theoretical calculation of its ECD spectrum in ACN was performed. As shown in Figure 5, the calculated ECD spectrum of (6S,7R,10S,11S)-2 (2a) fit well with that of the experimental one. Accordingly, 2 was elucidated as (6S,7R,10S,11S)-2,3-dehydro-11,13-dihydroxy-14-carotanoic acid, and was named penigrisacid B.

Compound 3 showed a molecular formula C15H24O5, as established by its positive HRESIMS spectrum at m/z 307.1523 (calcd for C15H24O5Na, 307.1521), indicating four degrees of unsaturation. The 13C NMR spectrum showed 15 carbon signals which, according to DEPT and HSQC spectra, could be classified as two methyls (δC 26.6, C-12; 29.2, C-13), five methylenes (δC 24.8, C-5; 26.8, C-9; 33.6, C-8; 34.3, C-4; and 35.8, C-1), one oxymethylene (δC 74.5, C-15), two methines (δC 51.8, C-10; 52.3, C-6), one oxymethine (δC 82.8, C-2), and four non-protonated carbons including one carboxyl group (δC 175.8), two oxyquaternary carbons (δC 77.6 and 71.5). The general aspect of the 13C NMR spectrum of 3 was similar to that of aspterric acid, except that the signals of the olefinic carbons (C-10/C-11) in aspterric acid were replaced by the signals of one methine (δH 1.58, m, H-10; δC 51.8, C-10) and one oxyquaternary carbon (δC 71.5, C-11) in 3, respectively. This was confirmed by the HMBC correlations from Me-12 and Me-13 to C-10 and C-11. The NOESY spectrum showed a correlation from H-10 (δH 1.58, m) to H-15a (δH 3.64, d, J = 8.0 Hz), indicating H-10 is in the β position. Further by comparison
of the experimental OR ([α]D 25 −49.4) and the calculated one for (2R,3R,6S,7S,10R)-3 ([α]D 25 −52.9),
the full structure of 3 was then established as (10R)-10,11-dihydro-11-hydroxyaspterric acid, and was
named penigrisacid C.

The molecular formula of 4 was assigned as C15H22O5 on the basis of its positive HRESIMS
spectrum at m/z 305.1365 (calcd for C15H22O5Na, 305.1359), indicating five degrees of unsaturation.
The 1H NMR spectrum exhibited one methyl singlet (δH 1.76, Me-12), one sp² oxymethylene (δH 4.62,
d, J = 8.0 Hz; 3.34, d, J = 7.9 Hz, H-15), one olefinic methylene (δH 5.01, s; 4.84, s, H-2), and one
oxymethine (δH 4.28, d, J = 8.4, H-2). The 13C NMR spectrum in association with the DEPT (distortion
less enhancement by polarization transfer) and HSQC (heteronuclear single-quantum correlation) spectra
indicated 15 carbon signals ascribed to one methyl (δC 19.7, Me-12); five aliphatic (δC 19.8, C-5; 33.3,
C-8; 35.3, C-4, 38.9, C-1; 40.0, C-9), one oxygenated (δC 76.2, C-15), and one olefinic (δC 110.3, C-13)
methylene; one aliphatic (δC 58.4, C-6) and one oxygenated (δC 84.0, C-2) methines; one aliphatic
(δC 52.7, C-7), two oxygenated (δC 79.6, C-3; 86.0, C-10), one olefinic (δC 150.7, C-11), and one carbonyl
(δC 179.9, C-12) quaternary carbons (Table 2). These signals were very similar to those of 3, except
that the hydroxy moiety at the C-11 in 3 was located at the C-10 position in 4, while the methyl group at the
C-11 position was displaced by a terminal double bond. This was evidenced by the COSY correlations
from H-2 (δH 1.45) to H-2a (δH 2.20), from H2-5a (δH 1.45) to H2-4a (δH 2.24) and H-6 (δH 1.72),
from H2-8a (δH 1.84) to H2-9a (δH 2.10), and from Me-12 (δH 1.76) to H2-13 (δH 5.01, 4.84); as well as
the HMBC cross peaks of Me-12 (δH 1.76 s) to C-10 (δC 86.0 s), C-11 (δC 150.7 s), and C-13 (δC
110.3 t). Since H-13a (δH 5.01 s) was correlated to H-6 (δH 1.72 m) in the NOESY spectrum, the hydroxy
group at the C-10 position was deduced to be β-orientation. Therefore, compound 4 was established as
10,11-dihydro-10β-hydroxy-11,13-dehydroaspterric acid, and was named penigrisacid D. All detailed
data of theoretical calculations, NMR, and HRESIMS spectra of 1–4 can be found in the Supplementary
Materials Figures S1–S30 Tables S1 and S2.

By comparison of the NMR and MS data with those in references, the structures of
five known compounds were identified as aspterric acid (5) [11], aspermytin A (6) [12],
1-propanone,3-hydroxy-1-(1,2,4a,5,6,7,8,8a-octahydro-2,5-dihydroxy-1,2,6-trimethyl-1-naphthalenyl)
(7) [13], craterellone D (8) [14], and 3,4-dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone (9) [15].

All the nine isolates were tested for anti-food allergic activity. Compound 9 exhibited potent effect
with an IC50 value of 28.7 μM, compared to 91.6 μM of the positive control, loratadine. While compound
6 showed moderate effect with an IC50 value of 97.3 μM. Therefore, 9 could be a promising lead
compound for anti-food allergic agent. Moreover, 1–9 were also subjected to cytotoxicity assay against
five different cancer cells, i.e., BLU-87, Bel-7402, ECA-109, Hela-S3, and PANC-1. However, only 4
showed weak effect on ECA-109 tumor cells with IC50 value of 28.7 μM.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were obtained from an MCP 100 polarimeter (Anton Paar Trading Co. Ltd.,
Shanghai, China). HRESIMS spectra were conducted on a Xevo G2 Q-TOF mass spectrometer (Waters
Corporation, Milford, MA, USA). NMR spectra were recorded on a Bruker 400 MHz spectrometer
(Bruker, Fällanden, Switzerland). UV data were recorded from a UV-8000 UV/Vis spectrophotometer
(Shanghai Metash instrument Co., Ltd., Shanghai, China). ECD spectra were measured with a Chirascan
spectropolarimeter (Applied Photophysics, Beverly, MA, USA). Materials for column chromatography
involved silica gel, ODS (octadecylsilyle), and Sephadex LH-20.

3.2. Fermentation and Extraction

The fungus was isolated from the deep-sea-sediment (~1420 m) of the Indian Ocean in 2005 by
Prof. De-Zan Ye of the Third Institute of Oceanography. It was purchased from the Marine Culture
Collection of China with the accession number of MCCC 3A00225. The fungus was cultured on a PDA
plate at 25 °C for 3 days. The fresh mycelia and spores were inoculated to 10 × 250 mL Erlenmeyer flasks containing 120 mL of the ISP medium 2 (1.0 L contains 4.0 g yeast extract, 10.0 g malt extract, 4.0 g dextrose, and 20.0 g agar), which were incubated in a 180 rpm rotary shaker at 28 °C for 5 days. Then the spore cultures were used to inoculate 100 × 1 L Erlenmeyer flasks containing corn medium (100 g corn and 120 mL tap water for each flask) to perform the large-scale fermentation. After 62 days, the fermentation broth was extracted with EtOAc three times to provide a crude extract (55.4 g).

3.3. Isolation and Purification

The extract was fractionated into six fractions (Fr.1–Fr.6) by column chromatography (CC) on silica gel using a gradient CH2Cl2-MeOH (0→100%). Fr.4 (4.9 g) was subjected to CC (26 mm × 310 mm) over ODS using H2O-MeOH (10→100%) and Sephadex LH-20 (MeOH), followed by purification using preparative TLC (CH2Cl2-acetone, 2:1) to provide 1 (7.8 mg) and 6 (25.9 mg). Fr.5 (40.0 g) was chromatographed over ODS (H2O-MeOH, 5→80%, 49 mm × 460 mm) to get 15 subfractions (Fr.5.1–Fr.5.15), which were further chromatographed on a Sephadex LH-20 (MeOH). Compounds 8 (4.5 mg) and 9 (9.0 mg) were obtained from fractions Fr.5.3 and Fr.5.2 by prep. TLC using EtOAc-MeOH (20:1) and CH2Cl2-MeOH (10:1), respectively. Fraction Fr.5.4 was subjected to CC over Sephadex LH-20 (CH2Cl2-MeOH, 1:1), subsequent purification by prep. TLC (PE-EtOAc, 1:2) provided 3 (29.5 mg) and 7 (19.5 mg). Fr.5.5 was subjected to HPLC (MeOH-H2O, 20→40%), followed by prep. TLC (CH2Cl2-MeOH, 10:1) to give 4 (21.4 mg). By prep. TLC using CH2Cl2-MeOH (10:1), 2 (6.0 mg) and 5 (9.3 mg) were obtained from fractions Fr.5.11 and Fr.5.10, respectively.

Penigrisacid A (1): colorless oil; [α]25°D +66.2 (c 0.14, EtOH); UV (MeOH) λmax (log ε) 211 (3.29) nm; ECD (ACN) Δε299 +0.32; 1H and 13C NMR data, see Table 1; Table 2; HRESIMS m/z 289.1411 [M + Na]+ (calcd. for C15H22O4Na, 289.1410).

Penigrisacid B (2): colorless oil; [α]25°D −29.4 (c 0.11, EtOH); UV (MeOH) λmax (log ε) 209 (3.94) nm; ECD (ACN) Δε221 −0.24; 1H and 13C NMR data, see Tables 1 and 2; HRESIMS m/z 291.1563 [M + Na]+ (calcd. for C15H24O4Na, 291.1572).

Penigrisacid C (3): colorless oil; [α]25°D −49.4 (c 0.16, EtOH); UV (MeOH) λmax (log ε) 201 (2.98) nm; ECD (ACN) Δε207 −0.68; 1H and 13C NMR data, see Tables 1 and 2; HRESIMS m/z 307.1523 [M+Na]+ (calcd. for C15H23O3Na, 307.1521).

Penigrisacid D (4): colorless oil; [α]25°D −30.9 (c 0.41, EtOH); UV (MeOH) λmax (log ε) 209 (3.51) nm; ECD (ACN) Δε198 +0.44; 1H and 13C NMR data, see Tables 1 and 2; HRESIMS m/z 305.1365 [M + Na]+ (calcd. for C15H22O3Na, 305.1359).

3.4. Theoretical Calculations

As reported previously [7], the preliminary conformational analyses were carried out using RDKit Toolkit [16] by Genetic algorithm at MMFF94 force field. Subsequently, the dominating conformers were re-optimized using density functional theory (DFT) at the B3LYP/6-31+G(d) level. Further calculation in the same level with PCM were conducted for ECD in ACN and OR in MeOH. The ECD spectra of different conformers were simulated by the overlapping Gaussian function [17]. The final spectrum was averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG).

3.5. Anti-Allergic Experiment

As reported previously [18], anti-allergic bioassay was conducted on RBL-2H3 cells. In brief, RBL-2H3 cells were seeded into 96-well cell culture plates to incubate with dinitrophenol specific IgE overnight. IgE-sensitized RBL-2H3 cells were pre-treated with tested compounds for 1 h and stimulated with dinitrophenyl-bovine serum albumin. Phosphate-buffered saline (PBS) buffer and loratadine were used as negative and positive controls, respectively. The bioactivity was quantified by measuring the fluorescence intensity of the hydrolyzed substrate in a fluorometer.
3.6. Cytotoxicity Assay

The in vitro antiproliferative assay was performed using MTT method according to the previously reported protocol [19]. Five different cancer cell lines (BIU-87, Bel-7402, ECA-109, Hela-S3, and PANC-1) were seeded into 96-well cell culture plates. After 24 h, different concentrations of tested compounds were added, and the incubation was continued for another 48 h. Then 20 µL MTT solution were added and cell viability was evaluated by measuring the absorbance at 570 nm.

4. Conclusions

From the deep-sea-derived fungus *Penicillium griseofulvum*, four new (penigrisacids A–D) and five known compounds (aspterric acid, aspermytin A, 1-propanone,3-hydroxy-1-(1,2,4a,5,6,7,8,8a-octahydro-2,5-dihydroxy-1,2,6-trimethyl-1-naphthalenyl), craterellone D, 3,4-dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone) were obtained. 3,4-Dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone, showed potent anti-food allergic activity (IC\(_{50}\) = 28.7 µM), whereas penigrisacid D showed weak cytotoxicity against esophageal cancer cell line ECA-109 tumor cells (IC\(_{50}\) = 28.7 µM).

**Supplementary Materials:** The following are available online at http://www.mdpi.com/1660-3397/17/9/507/s1, Figures S1–S30 and Tables S1–S2: The theoretical calculation data for 1–3, 1D and 2D NMR as well as HRESIMS spectra of 1–4.

**Author Contributions:** X.-W.Y. designed and coordinated the project; C.-P. X. conducted fungal fermentation as well as compound isolation and characterization. C.-L. X. assisted C.-P. X.’s experiments. J.-M. X. contributed to the structure identification of isolated compounds. Q.-M. L. and G.-M. L. conducted anti-food allergic experiments. W.-X. L. contributed to the extraction and isolation. D.-Z. Y. isolated the fungus and deposited it to MCCC. C.-P. X. and X.-W. Y. wrote the paper, while critical revision of the publication was performed by all authors.

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