Eremophilane-Type Sesquiterpenes from a Marine-Derived Fungus Penicillium Copticola with Antitumor and Neuroprotective Activities †

Jianping Zhang 1, Dong Liu 1, Aili Fan 1*, Jian Huang 1 and Wenhan Lin 1,2, *

1 State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China
2 Institute of Ocean Research, Ningbo Institute of Marine Medicine, Peking University, Ningbo 315010, China
* Correspondence: whlin@bjmu.edu.cn; Tel.: +86-10-82806188
† Dedication to Peter Proksch for his 70th birthday.

Abstract: Chemical examination of a marine sponge-associated Penicillium coticola fungus resulted in the isolation of ten undescribed eremophilanes, namely copteremophilanes A–J (1–10), along with two new glycosides, 5-glycopenostatin F (11) and 5-glucopenostatin I (12). Their structures were determined by extensive spectroscopic data, in association with ECD data and chemical conversions for configurational assignments. Analogs 1, 2, and 10 represent a group of uncommon skeletons of eremophilanes with an aromatic ring and a methyl migration from C-5 to C-9, and analogs 11 and 12 are characteristic of a PKS scaffold bearing a glucose unit. The incorporation of a chlorinated phenylacetic unit in 3–9 is rarely found in nature. Analog 7 showed neuroprotective effect, whereas 8 exhibited selective inhibition against human non-small cell lung cancer cells (A549). This study enriched the chemical diversity of eremophilanes and extended their bioactivities to neuroprotection.

Keywords: marine fungus; Penicillium coticola; copteremophilanes A–J; structure elucidation; antitumor activity; neuroprotection

1. Introduction
Farnesyl diphosphate (FPP) biogenetically generates sesquiterpenes with diverse scaffolds via various ring rearrangements. Eremophilanes are one class of sesquiterpenes characterized by the presence of a bicyclic backbone with irregular rule to assemble FPP in association with a methyl migration from C-10 to C-5 [1,2]. Eremophilane analogues extensively distribute in nature. Apart from the eremophilanes derived by plants of which the genus Ligularia is dominated to produce eremophilanes [3], terrestrial- or marine-originated fungi emerge as the additional sources to produce relevant analogs. It is noteworthy that most fungal eremophilanes are enantiomeric to the corresponding entities from plants [1], and marine-associated fungi have the potential to generate structurally unique analogs [4–6]. Eremophilanes possess wide range of bioactivities, such as phytotoxins, antimicrobials, protein inhibitors, immunomodulators and cytotoxins. In marine-derived fungi, eremophilanes with chemical diversity are widely distributed in fungal genera of Acremonium [6], Penicillium [7–9], Cochliobolus [10], Phomopsis [11], and Cryptosphaeria [12]. These findings imply marine-derived fungi as a potential source to generate structurally unique and bioactive eremophilanes. With the aim to continue our discovery of bioactive natural products from marine-associated organisms, a marine sponge (Xestospongia testudinaria)-associated fungus strain Penicillium coticola WZXY-m122-9 was selected for chemical examination. The LC-MS/MS data of the EtOAc extract of the cultured fungus were processed into a molecular network using MZmine and the GNPS platform (http://gnps.ucsd.edu, accessed on 20 June 2022), which allowed the formation of the spectral nodes into clusters (Figure S103). Annotation of the nodes in a cluster with m/z values of 200 to 300 by GNPS MS/MS spectral library matched eremophilanes,
including dehydropetasol (m/z 233 [M + H]+), dihydroporogen AO-1 (m/z 251 [M + H]+), hydroxyphomenone (m/z 267 [M + H]+), sporogen AO-1 (m/z 248 [M + H]+) and penicillerephomenal X (m/z 279 [M + H]+). In addition, a cluster with the nodes ranging from m/z 430 to 480 presenting chlorine feature ([M]+/[M + 2]+ = 3:1) did not hit in the database, suggests a group of untapped metabolites. The scaled-up fermentation and extensive chromatographic separation of the EtOAc extract resulted in the isolation of ten new eremophilane-type sesquiterpenes, along with two new PKS glucosides (Figure 1). Herein, we report the structural determination of the new compounds (Figures S1–S102) and the bioactivities of antitumor cell lines and neuroprotection.

Figure 1. Structures of new compounds.

2. Results
2.1. Structure Elucidation of New Compounds

Copteremophilane A (1) has a molecular formula of C_{14}H_{18}O_{2} as determined by the HRESIMS and NMR data, requiring six degrees of unsaturation. The 13C NMR and DEPT data afforded a total of 14 carbon resonances, including six aromatic carbons for a phenyl group, a ketone, as well as seven alkyl carbons, of which three were classified into methyl groups. A tetra-substituted aromatic ring was recognized by the presence of two meta-coupled aromatic protons, H-6 (δ_{H} 7.61, brs) and H-8 (δ_{H} 7.55, brs). The substitution of a methyl group at C-9 (δ_{C} 136.4) and an acetyl group at C-7 (δ_{C} 140.8) was confirmed by the HMBC correlations from H-3-14 (δ_{H} 2.24, s) to C-8 (δ_{C} 126.8), C-9, and C-10 (δ_{C} 134.8), and both H-6 and H-8 to carbonyl carbon C-11 (δ_{C} 198.3), along with H-3-12 (δ_{H} 2.53, s) to C-7 (δ_{C} 140.8) and C-11. The COSY correlations established a segment in ring A from C-1 to C-4, and this unit was fused to C-5 (δ_{C} 141.1) and C-10 positions of the aromatic ring to form a cyclohexene ring due to the HMBC correlations of C-5 and C-10 to both H_{2}-1 (δ_{H} 2.62, 2.69) and H-4 (δ_{H} 2.78, dq, J = 2.6, 7.2 Hz). In addition, a methyl group at C-4 and a hydroxy group at C-3 were confirmed by the COSY correlations from H-4 to H-3 (δ_{H} 3.66, dt, J = 2.6, 4.9 Hz) and H_{3}-15 (δ_{H} 1.23, d, J = 7.2 Hz), along with the HMBC correlations from H_{3}-15 to C-3 (δ_{C} 69.8), C-4 (δ_{C} 41.2), and C-5. The NOE correlation between H-3-15 and H-3 was indicative of a trans-orientation of H_{3}-15 toward OH-3. Based on the modified Mosher method, the (R)-MPA and (S)-MPA esters of 1 were synthesized. Calculation of the Δ3 (δ_{R} – δ_{S}) values resulted in 3R configuration (Figure 2). Thus, a 4R configuration was suggested with the help of the NOE data. The similar data of the experimental ECD compared to that calculated for (3R, 4R)-1 (Figure 3A) further supported the configurational assignment.
The same relative configuration of both molecules was suggested with the help of the NOE data. The similar data of the experimental ECD spectra of 3R, 4R-2 were consistent with those calculated for (3R, 4R)-2. The molecular formula (C_{13}H_{20}O_2) of copteremophilane B (2) was afforded by the HRESIMS and NMR data. The NMR data of both 1 and 2 (Table 1) were comparable, except for the distinction of the substituent at C-7 (δC 136.3). A hydroxyisopropene unit was identified by the NMR resonances for two olefinic carbons and a hydroxymethyl group, in association with the HMBC correlations from the hydroxymethyl protons H2-13 (δH 4.28 (s) to C-11) and between H-3 and H-15. The similar ECD data suggested the same absolute configuration for both 1 and 2. This was supported by the calculated ECD data (Figure 3B), in which the experimental ECD data were consistent with those calculated for (3R, 4R)-2.
Table 1. $^1$H and $^{13}$C NMR data of 1, 2 and 10 in DMSO-$d_6$.

|   | $\delta_H$       | $\delta_C$ | $\delta_H$       | $\delta_C$ | $\delta_H$       | $\delta_C$ |
|---|------------------|------------|------------------|------------|------------------|------------|
| 1 | 2.62, dt (4.0, 12.0) | 23.7       | 2.50, dt (3.5, 12.0) | 23.3       | 2.55, dt (4.0, 12.0) | 22.4       |
|   | 2.69, td (6.0, 12.0) |           | 2.63, td (5.0, 12.0) |           | 2.62, td (4.0, 12.0) |           |
| 2 | 1.80, m; 1.95, m   | 27.1       | 1.71, m; 1.90, m  | 27.7       | 1.90, m; 1.98, m | 23.9       |
|   | 3.66, dt (2.6, 4.9) | 69.8       | 3.60, dt (3.0, 5.0) | 70.3       | 4.87, dt (4.4, 6.3) | 74.6       |
| 3 | 2.78, dq (2.6, 7.2) | 41.2       | 2.67, dq (3.0, 7.2) | 41.2       | 2.91, dq (4.4, 7.2) | 38.1       |
| 4 | 5.22, d (1.7)     | 110.1      | 1.36, s          |           | 20.1             |
| 5 | 2.24, s           | 19.8       | 2.16, s          | 21.3       | 2.17, s          | 20.1       |
| 6 | 1.23, d (7.2)     | 21.4       | 1.12, d (7.2)    | 19.9       | 1.21, d (7.2)    | 21.7       |
| 7 | 141.1             | 140.5      | 138.0            |           | 134.9            |
| 8 | 7.61, brs         | 127.1      | 7.06, brs        | 124.7      | 7.12, s          | 123.8      |
| 9 | 136.4             | 135.6      | 134.9            |           | 131.4            |
| 10| 134.8             | 134.1      | 131.4            |           | 73.9             |
| 11| 198.3             | 148.3      | 73.9             |           | 140.3            |
| 12| 2.53, s           | 27.1       | 4.28, s          | 63.0       | 3.35, dd (4.0, 12.0) | 71.0       |
| 13| 5.22, d (1.7)     | 110.1      | 1.36, s          |           | 26.6             |
| 14| 2.24, s           | 19.8       | 2.16, s          | 21.3       | 2.17, s          | 20.1       |
| 15| 141.1             | 140.3      | 140.3            |           | 146.9            |
| 16| 6.63, d (1.9)     | 115.6      | 4.01             |           | 171.4            |
| 17| 3.45, s           |           | 40.1             |           |                 |
| 18| 4.59, t (4.0)     |           |                 |           |                 |

Copteremophilane C (3) was determined to have a molecular formula of C$_{23}$H$_{25}$ClO$_7$ by the HRESIMS and NMR data, requiring 11 degrees of unsaturation. Diagnostic 2D NMR data revealed 3 to be assembled by two moieties. Based on the 2D NMR data, one of them was identified as a phomenone unit, a co-isolated eremophilane-type sesquiterpene which was structurally characterized by the presence of an $\alpha$,$\beta$-unsaturated ketone with an epoxy group at ring B [13]. The second moiety contained six aromatic carbons for a phenyl unit, a methylene and a carbonyl carbon, along with two $meta$-coupling aromatic protons H-2' ($\delta_H$ 6.70, d, $J = 2.1$ Hz) and H-6' ($\delta_H$ 6.67, d, $J = 2.1$ Hz) and the methylene protons H$_2$-7' ($\delta_H$ 3.50, brs). The HMBC correlations from H$_2$-7' to a carbonyl carbon at C-8' ($\delta_C$ 171.2), C-2' ($\delta_C$ 121.0) and C-6' ($\delta_C$ 115.5) and from H-2' and H-6' to the aromatic carbons...
established a tri-substituted phenylacetic segment. Additional HMBC correlations from two phenol protons OH-4′ (δ_H 9.01, brs) and OH-5′ (δ_H 9.76, brs) to the aromatic carbons, respectively, in association with the molecular composition, allowed the assignment of a 3-chloro-4,5-dihydroxyphenylacetic moiety. The linkage of the acyl unit to C-3 for an ester formation was evident from the HMBC correlation between H-3 (δ_H 4.82, dt, J = 4.4, 11.2 Hz) and C-3′. The same relative configuration of 3 as phenomone was identified by the NOE relationships of both H-14 (δ_H 1.22, s) and H-15 (δ_H 0.90, d, J = 6.8 Hz) with H-3 and H-6 (δ_H 3.40, s) and between H-6 and H-13 (δ_H 5.08, 5.20) (Figure 4). Comparison of the experimental and calculated ECD data (Figure 3C) suggested 3 possessing R configuration for C-3, C-4, C-5, C-6, and C-7.

![Figure 4. Key NOE correlations of 3 and 7–9.](Image)

The molecular composition of copteremophilane D (4) showed the same as that of 3 according to the HRESIMS data. The NMR data of both compounds (Tables 2 and 3) were very similar, and the 2D NMR data established the gross structure of 4 also containing two segments which were identical to those of 3. The distinction was attributed to the 3-chloro-4,5-dihydroxyphenylacetic moiety location. The chemical shifts of C-3 (δ_C 69.0 vs. δ_C 73.2 of 3) and C-12 (δ_C 64.9 vs. δ_C 62.1 of 3) in addition to the HMBC correlation between H-12 (δ_H 4.70, 4.71) and a carbonyl carbon C-8′ (δ_C 171.1) allowed the location of the acyl group at C-12. The similar NOE data of both 3 and 4, along with the comparable ECD data of 4 to those calculated for (3R, 4R, 5R, 6R, 7R)-4 (Figure 3D), assigned the same configuration of both 3 and 4.

The molecular formula of copteremophilane E (5) was established as C_{23}H_{25}ClO_6 from the HRESIMS (m/z 431.1259 [M – H]−, calcd 431.1261) data, containing 11 degrees of unsaturation. The NMR data of 5 (Tables 2 and 3) were comparable to those of 4, and the NMR resonances for phenomone moiety of both compounds were identical. The acyl moiety was identified as 3-chloro4-hydroxyphenylacetic group based on the presence of an ABX spin system for the aromatic protons at H-3′ (δ_H 6.88, d, J = 8.2 Hz), H-2′ (δ_H 6.97, dd, J = 1.5, 8.2 Hz), and H-6′ (δ_H 7.18, d, J = 1.5 Hz), and the HMBC correlations from the methylene protons H-2′ (δ_H 3.54, s) to C-1′ (δ_C 126.1), C-2′ (δ_C 131.0), C-6′ (δ_C 129.4) and the carbonyl carbon C-8′ (δ_C 171.2), along with the correlations of H-2′ to the nonprotonated carbons C-3′ (δ_C 119.8) and C-4′ (δ_C 152.5). The similar NOE and ECD data (Figure 3E) suggested the same configuration for both 4 and 5.
Table 2. $^1$H NMR data of 3-9 in DMSO-$d_6$.

|    | 3          | 4          | 5          | 6          | 7          | 8          | 9          |
|----|------------|------------|------------|------------|------------|------------|------------|
| 1  | 2.63, dt (2.0, 14.5) | 2.28, td (3.0, 12.0) | 2.26, td (3.2, 14.1) | 2.25, td (3.4, 11.2) | 2.30, m     | 2.30, td (3.4, 12.0) | 2.07, m     |
|    | 2.36, td (2.0, 14.5) | 2.54, dt (3.0, 12.0) | 2.51, dt (3.2, 14.1) | 2.50, dt (3.4, 11.2) | 2.33, m     | 2.39 dt (3.4, 11.2)  | 2.29, m     |
| 2  | 1.36, m     | 1.25, m     | 1.19, m     | 1.20, m     | 1.31, m     | 1.29, m     | 1.27, m     |
|    | 2.04, m     | 1.99, m     | 1.98, m     | 1.98, m     | 2.00, m     | 2.01, m     | 1.97, m     |
| 3  | 4.82, dt (4.4, 11.2) | 3.44, dt (5.0, 12.0) | 3.42, dt (5.0, 10.5) | 3.43, dt (5.0, 10.5) | 3.49, m     | 3.46, td (5.4, 10.6)| 4.73, m     |
| 4  | 1.82, dq (6.8, 11.2) | 1.53, dq (6.8, 12.0) | 1.54, dq (6.8, 10.5) | 1.54, dq (6.8, 10.5) | 1.29, dq     | 1.37, dq     | 1.44, dq     |
|    | 3.08, d (15.0) | 3.08, d (15.0) | 3.08, d (15.0) | 3.08, d (15.0) | 1.68, d (12.6)| 1.69, d (12.6)|            |
| 6  | 3.40, s     | 3.38, s     | 3.38, s     | 3.41, s     | 3.41, s     | 3.41, s     |            |
|    | 3.38, s     | 3.38, s     | 3.38, s     | 3.38, s     |            |            |            |
| 7  | 3.19, dd    | (4.0, 11.0) |            |            |            |            |            |
| 8  |            |            |            |            |            |            | 3.60, brd (4.5) |
| 9  | 5.75 d (1.7) | 5.71, d (1.7) | 5.70, d (1.7) | 5.68, d (1.7) | 5.78, d (1.3) | 5.82, d (1.3) | 5.40, d (4.5) |
| 12 | 4.05, dd (4.0, 12.0) | 4.70, d (12.0) | 4.71, d (12.0) | 4.74, d (12.0) | 4.76, d (12.0) | 1.60, s     | 1.80, d (1.5) | 1.76, s     |
| 13 | 5.08, d (1.5) | 5.20, d (1.5) | 5.29, d (0.9) | 5.28, d (1.1) | 5.38, d (1.1) | 4.68, brs    | 4.83, brs    | 4.76, d (1.0) |
|    |            |            | 5.36, d (1.1) | 5.36, d (1.1) |            |            |            |            |
| 14 | 1.22, s     | 1.12, s     | 1.12, s     | 1.11, s     | 4.19, d (11.5)| 4.43, d (11.5)| 4.15, s     | 1.08, s     |
| 15 | 0.90, d (6.8) | 1.11, d (6.8) | 1.11, d (6.8) | 1.11, d (6.8) | 0.98, brd (6.9) | 1.06, d (6.8) | 0.79, d (6.8) |
| 2' | 6.70, d (2.1) | 6.64, d (2.0) | 6.97, dd (15.8,2) | 6.79, d (19) | 6.61, d (1.6) | 6.61, d (1.9) | 6.68, d (1.9) |
| 3' |            |            |            |            | 6.88, d (8.2) |            |            |            |
| 6' | 6.67, d (2.1) | 6.62, d (2.0) | 7.18, d (1.5) | 6.77, d (19) | 6.58, d (1.6) | 6.57, d (1.9) | 6.65, d (1.9) |
| 7' | 3.50, s     | 3.47, s     | 3.54, s     | 3.55, s     | 3.45, s     | 3.40, s     | 3.47, s     |
| OH-3 |            |            |            |            | 4.70, br    |            |            |            |
| OH-12 | 4.83 t (4.0) |            |            |            |            |            |            |            |
| OH-4' | 9.01, brs   | 9.02, s     | 10.0, brs   | 9.00, s     | 8.99, s     |            |            |            |
| OH-5' | 9.76, brs   | 9.74, s     |            | 9.80, s     | 9.69, s     |            |            |            |
| MeO |            |            |            | 3.78, s     |            |            |            |            |

The structure of copteremophilane F (6) was determined to be a 5′-methoxylated analog of 4 due to the comparable NMR data of both analogs, with the exception of an additional methoxy group in 6. The methoxy protons at δH 3.78 (s) showed HMBC correlation to C-5′ (δC 148.4) and the NOE relationship with H-6′ (δH 6.77, d, J = 1.9 Hz) confirmed a 3-chloro-4-hydroxy-5-methoxyphenylacetic unit, which was linked to C-12 (δC 64.5) on the basis of the HMBC correlation between H-2′-12 (δH 4.70, 4.76) and the carbonyl carbon C-8′ (δC 170.6). The similar NOE data of 6 as those of 4 and 5 in association with the comparable ECD data of 6 to those calculated for (3R, 4R, 5R, 6R, 7R)-6 identified the same configuration of 6 as 4.

Copteremophilane G (7) has a molecular formula of C$_{23}$H$_{27}$ClO$_6$ as determined by the HRESIMS data. The NMR data demonstrated 7 possessing two moieties. Analyses of 2D NMR data (Figure S104) revealed the sesquiterpene unit being identical to JBIR-27 [9], while the second unit was consistent with a 3-chloro-4,5-dihydroxyphenylacetic group. The linkage of the acyl unit to nucleus moiety at C-14 (δC 66.6) was deduced by the HMBC correlation between H-2′-14 (δH 4.19, 4.43) and the carbonyl carbon C-8′ (δC 171.2). The NOE relationships from H-2′-14 to H-15 (δH 0.98, d, J = 6.9 Hz) and H-7 (δH 3.19, dd, J = 4.0, 11.0 Hz) and between H-3′-15 and H-3 (δH 3.49, m) (Figure 4) suggested the same relative configuration of the nucleus moiety as JBIR-27 [9]. The comparable ECD data of 7 to those calculated for (3R, 4R, 5S, 7S)-7 (Figure 3G) agreed the absolute configuration of 7 as R for C-3 and C-4 and S for C-5 and C-7.
The molecular formula of copteremophilane H (8) was the same as 7 due to the same HRESIMS data, requiring ten degrees of unsaturation. The NMR data of 8 were comparable to those of 7. The distinction was found in ring B, where two methyl groups were recognized for H-3-12 (δH 1.80, d, J = 1.5 Hz) and H-3-13 (δH 2.02, d, J = 1.5 Hz). The HMBC correlations of H-3-12/H-3-13 to C-7 (δC 127.3) and C-11 (δC 142.9) indicated 8 to be a stereoisomer of 7 with an olefinic transformation from C-11/C-13 to C-7/C-11. The NOE correlations from H-3-15 (δH 1.06, d, J = 6.8 Hz) to H-2-14 (δH 4.15, s) and H-3 (δH 3.46, td, J = 5.4, 10.6 Hz) clarified the same relative configuration of 8 and 7. Comparison of the experimental ECD data to those calculated for (3R, 4R, 5S)-8 (Figure 3H) established 3R, 4R, and 5S configurations for 8.

The molecular formula of copteremophilane I (9) was determined as C_{23}H_{29}ClO_{6} by the HRESIMS data, requiring nine degrees of unsaturation. The NMR data of 9 resembled those of 3 but distinguished by ring B of sesquiterpene unit. Hydroxylations at C-7 (δC 73.7) and C-8 (δC 68.2) were clarified by the COSY coupling between H-8 (δH 3.60, brd, J = 4.5 Hz) and H-9 (δH 5.40, brd, J = 4.5 Hz) together with the HMBC correlations from H-2-6 (δH 1.68, 1.69) to C-5 (δC 38.6), C-7, C-8, C-11 (δC 150.9) and C-14 (δC 20.2). The NOE correlations from H-3 (δH 4.73, m) to H-3-14 (δH 1.08, s) and H-15 (δH 0.79, d, J = 6.8 Hz) and from H-12 (δH 1.76, s) to H-14 and H-8 suggested the same face of both hydroxy groups. The absolute configuration of 9 was assigned by the similar ECD data of 9, compared to those calculated for (3R, 4R, 5R, 7S, 8S)-9.

Copteremophilane J (10) was determined to have a molecular formula of C_{23}H_{27}ClO_{6} by the HRESIMS data, containing ten degrees of unsaturation. The NMR data featured a homolog of 3, and the 2D NMR data assigned a 3-chloro-4,5-dihydroxyphenylacetic group at C-3 as the case of 3. In regard to the sesquiterpene moiety, a phenyl ring was assigned to ring B due to the presence of six aromatic carbons as well as the HMBC correlations of the aromatic protons H-6 (δH 7.12, brs) and H-8 (δH 7.06, brs) to the aromatic carbons. The HMBC correlations of methyl protons H-3-14 to C-8, C-9, and C-10 in association with the NOE correlation between H-2-1 and H-3-14 substituted a methyl group at C-9. The remaining NMR resonances were attributed to a 1,2-dihydroxypropane unit, which was deduced by

### Table 3. $^{13}$C NMR data of 3-9 in DMSO-$d_6$.

|   | 3  | 4  | 5  | 6  | 7  | 8  | 9  |
|---|---|---|---|---|---|---|---|
| 1 | 29.9 | 30.9 | 30.9 | 30.3 | 31.4 | 31.2 | 30.1 |
| 2 | 31.7 | 35.8 | 35.8 | 35.4 | 35.7 | 35.8 | 32.9 |
| 3 | 73.2 | 69.0 | 69.2 | 68.4 | 69.5 | 69.6 | 74.7 |
| 4 | 41.9 | 44.8 | 44.8 | 44.3 | 50.7 | 49.3 | 47.9 |
| 5 | 41.2 | 41.2 | 41.2 | 40.7 | 43.1 | 44.8 | 38.6 |
| 6 | 68.3 | 69.2 | 68.9 | 68.6 | 39.1 | 38.3 | 40.6 |
| 7 | 67.7 | 67.2 | 67.2 | 60.7 | 50.7 | 127.3 | 73.7 |
| 8 | 192.2 | 192.1 | 192.2 | 191.6 | 198.0 | 190.2 | 68.2 |
| 9 | 120.7 | 120.0 | 120.0 | 119.4 | 126.4 | 128.6 | 121.2 |
| 10 | 163.7 | 166.2 | 166.6 | 165.6 | 164.3 | 163.1 | 143.0 |
| 11 | 145.3 | 139.3 | 139.3 | 138.8 | 144.0 | 142.9 | 150.9 |
| 12 | 62.1 | 64.9 | 64.8 | 64.5 | 20.3 | 22.6 | 19.4 |
| 13 | 111.9 | 117.1 | 117.2 | 116.8 | 114.3 | 23.0 | 110.4 |
| 14 | 18.1 | 18.4 | 18.4 | 17.9 | 66.6 | 66.6 | 20.2 |
| 15 | 11.3 | 11.7 | 11.8 | 11.2 | 11.4 | 11.9 | 11.0 |
| 1′ | 126.0 | 125.6 | 126.1 | 125.1 | 125.5 | 125.5 | 126.2 |
| 2′ | 121.0 | 120.3 | 131.0 | 122.1 | 120.9 | 121.0 | 121.0 |
| 3′ | 120.3 | 121.0 | 119.8 | 119.5 | 120.4 | 120.4 | 120.3 |
| 4′ | 141.2 | 141.3 | 152.5 | 141.6 | 141.3 | 141.2 | 141.1 |
| 5′ | 146.9 | 146.9 | 116.9 | 148.4 | 146.9 | 146.9 | 146.9 |
| 6′ | 115.5 | 115.6 | 129.4 | 111.8 | 115.5 | 115.6 | 115.4 |
| 7′ | 39.9 | 39.6 | 39.2 | 39.7 | 39.0 | 40.0 | 40.0 |
| 8′ | 171.2 | 171.1 | 171.2 | 170.6 | 171.2 | 171.2 | 171.3 |
| OMe | | | | | | | 56.6 |
the HMBC correlations from H2-12 (δH 3.37, s) to C-11 (δC 73.9) and C-13 (δC 22.6). The location of this unit at C-7 (δC 145.0) was confirmed by additional HMBC correlations from H3-13 (δH 1.36, s) and H2-12 to C-7. The NOE relationships between H-3 (δH 4.87, ddd, J = 2.3, 4.4, 6.3 Hz) and H3-15 (δH 1.21, d, J = 7.2 Hz) suggested a cis-orientation of H-3 toward H3-15. The absolute configuration of C-12 was determined as S on the basis of the Mo(AcO)4 induced ECD data (ICD) (Figure 3J), in which a positive Cotton effect at 330 nm was observed.

Compound 11 has a molecular formula of C28H42O8 as established by the HRESIMS data. The DEPT 13C NMR spectrum exhibited a total of 28 carbon resonances, involving 6 olefinic carbons, a ketone carbon, 7 sp3 oxygenated carbons, and 14 other alkyl carbons. Analyses of the 2D NMR data established a nucleus structure which was identical to penostatin F [13]. This assignment was evident from the COSY correlations along with the HMBC correlations to establish a 4-methylcycloocta-3,5-dien-1-one (Figure 5). Additional COSY relationship between H-7 and H-8 in association with the HMBC correlations from H-7 to C-2 and C-3 and from H-2 to C-1 and C-9 fused a cyclohexene across C-1 and C-8. A spin system from H2-4 to H-7 via H-5 and H2-6 along with the HMBC correlation between H-5 and C-3 clarified a cyclopentane ring fused to C-3 and C-7, and C-5 (δC 78.7) was oxygenated. The location of an n-heptane unit at C-14 was based on additional COSY and HMBC data. The remaining six oxygenated sp3 carbons were attributed to a sugar unit, which was identified as glucose according to the comparison of the NMR data with those of penostatin F [13]. This assignment was evident from the COSY correlations along with the HMBC correlations to establish a 4-methylcycloocta-3,5-dien-1-one (Figure 5). Additional COSY relationship between H-7 and H-8 in association with the HMBC correlations from H-7 to C-2 and C-3 and from H-2 to C-1 and C-9 fused a cyclohexene across C-1 and C-8. A spin system from H2-4 to H-7 via H-5 and H2-6 along with the HMBC correlation between H-5 and C-3 clarified a cyclopentane ring fused to C-3 and C-7, and C-5 (δC 78.7) was oxygenated. The location of an n-heptane unit at C-14 was based on additional COSY and HMBC data. The remaining six oxygenated sp3 carbons were attributed to a sugar unit, which was identified as glucose according to the comparison of the NMR data with those of penostatin F [13].

Compound 12 has the same molecular formula as 11 according to the HRESIMS data. The NMR data of 12 (Table 4) were almost superimposed to those of 11, suggesting the structural similarity of both compounds. Analysis of 2D NMR data established the same planar structure of 11 and 12. The similar NOE data with the exception of the correlation between H-5 and H-7 instead of between H-7 and H-1’ of 11 suggested the stereoisomers of both compounds (Figure 6). However, the opposite rotation ([α]20D −10 for 11 and [α]20D +12 for 12) and the opposite Cotton effects (Figure 7) suggested an enantiomeric form with the exception of the chiral center at C-5, implying the nucleus part of 12 to be consistent with penostatin F [13].
Figure 6. Key NOE correlations of 11 and 12.

Figure 7. ECD spectra of 11 and 12 and their hydrolyzed products 11a and 12a.

In addition, the phenylacetic acids, including sporogen AO-1 [14], phomenone [15], JBIR28 [9], JBIR27 [9], 3-chloro-4,5-dihydroxyphenylacetic acid, 3-chloro-4-hydroxyphenylacetic acid, and 3-chloro-4-hydroxy-5-methoxyphenylacetic acid [16], were isolated and identified on the basis of spectroscopic data.

2.2. Bioassays

Fungus-derived eremophilanes gain potential activities against cancer cell lines for specific aspects in cancer therapies [17–18]. To evaluate the relevant effects of the isolated compounds, an MTT method was performed to detect the cytotoxic activities of compounds toward human non-small cell lung cancer cells (A549), human colon cancer cells (HCT-8) and human breast cancer cells (MCF-7). As shown in Table 5, eremophilane analogs possessing specific selection to inhibit tumor cell lines were observed. Copterecemophilane H (8) with an acyl unit at C-14 selectively inhibited A549 cells with IC₅₀ value of 3.23 μM. However, analog 7 with an olefinic rearrangement from C-7/C-11 of 8 to C-11/C-13 totally attenuated the inhibitory effect. Analogs 4 and 5 with a phenylacetic unit at C-12 of phomenone showed selective inhibition against HCT-8 cell line. It is noteworthy that 4 with a 3-chloro-4,5-dihydroxy-phenylacetic unit exhibited higher activity than 5 which possesses a 3-chloro-4-hydroxyphenylacetic unit, whereas 6 with a 5-methoxy-4-hydroxy-3-chlorophenylacetic unit was inactive toward the three tumor cell lines.

Table 4. ¹H and ¹³C NMR data of 11 and 12 in DMSO-d₆.

| No | δ_C   | δ_H      | δ_C   | δ_H      |
|----|-------|----------|-------|----------|
| 1  | 82.1  | 5.50, br d (2.6) | 82.3  | 5.50, br d (2.6) |
| 2  | 124.9 | 2.46, m        | 125.3 | 2.27, m    |
| 3  | 146.1 | 2.60, m        | 144.9 | 2.68, m    |
| 4  | 37.9  | 4.38, t (5.0)  | 37.7  | 4.34, m    |
| 5  | 78.7  | 1.44, m        | 78.1  | 1.45, m    |
| 6  | 39.6  | 2.43, m        | 39.5  | 2.57, m    |
| 7  | 48.6  | 3.11, m        | 48.5  | 2.79, m    |
| 8  | 50.4  | 2.86, t (5.6)  | 50.4  | 2.91, m    |
| 9  | 211.2 |                  | 210.6 |          |
| 10 | 128.3 | 5.58, dd (0.9, 6.4) | 128.2 | 5.61, dd (0.9, 6.4) |
| 11 | 129.4 |                  | 129.5 |          |
| 12 | 130.2 | 5.67, d (11.5)  | 130.3 | 5.68, d (11.5) |
| 13 | 133.9 | 5.60, dd (9.1, 11.5) | 133.9 | 5.34, dd (9.2, 11.5) |
| 14 | 43.3  | 2.57, m        | 42.7  | 2.53, m    |
| 15 | 28.4  | 1.55, m        | 28.2  | 1.53, m    |
| 16 | 28.0  | 1.09, m        | 27.8  | 1.08, m    |
| 17 | 29.6  | 1.22, m        | 29.2  | 1.20, m    |
| 18 | 29.1  | 1.22, m        | 29.0  | 1.20, m    |
| 19 | 31.7  | 1.22, m        | 31.7  | 1.21, m    |
| 20 | 22.5  | 1.25, m        | 22.5  | 1.25, m    |
| 21 | 14.4  | 0.87, t (7.1)  | 14.4  | 0.85, t (7.1) |
| 22 | 25.6  | 1.75, s        | 25.6  | 1.74, s    |
| 1' | 102.1 | 4.22, d (7.9)  | 102.5 | 4.20, d (7.7) |
| 2' | 73.8  | 2.93, m        | 73.8  | 2.89, m    |
| 3' | 77.2  | 3.16, m        | 77.3  | 3.13, m    |
| 4' | 70.6  | 3.05, m        | 70.5  | 3.03, m    |
| 5' | 77.4  | 3.08, m        | 77.4  | 3.03, m    |
| 6' | 61.6  | 3.44, dt (5.7, 11.8) | 61.6  | 3.43, m    |
In addition, the phenylacetic acids, including sporogen AO-1 [14], phomenone [15], JBIR28 [9], JBIR27 [9], 3-chloro-4,5-dihydroxyphenylacetic acid, 3-chloro-4-hydroxyphenylacetic acid, and 3-chloro-4-hydroxy-5-methoxyphenylacetic acid [16], were isolated and identified on the basis of spectroscopic data.

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In previous work, we even reported eremophilanes possessing the inhibitory effects against LPS-induced NO production in macrophages [19]. Oxidative stress has been implicated in the pathology of Alzheimer’s disease (AD), and accumulation of β-amyloid (Aβ) causes oxidative stress [20,21]. Aβ is considered as a major pathological factor to induce Alzheimer’s disease [22,23]. To evaluate the neuroprotective effects of analogs, we extend the bioassay of analogs to Aβ25-35-stimulated PC12 cell line. PC12 cells are the pheochromocytoma-derived cell line which maintains a differentiated neuroendocrine phenotype [24]. Firstly, the Aβ25-35-induced PC12 cell injury was detected by the CCK8 assay. Aβ25-35 induced cell death in a dose-dependent manner, and around 50% inhibition of PC12 cells was observed after the treatment of Aβ25-35 (40 µM) for 24 hrs. Thus, Aβ25-35 (40 µM) was selected as an optimal dose for subsequent experiments. Alternatively, eremophilanes (50 µM) alone maintained normal PC12 cell morphology and cell numbers, suggesting low cytotoxicity. Most analogs showed inhibitory activities against Aβ25-35-induced cell death, while 7 is the most active to increase the viability of Aβ25-35-induced PC12 cells (Table 6). Lactate dehydrogenase (LDH) as a stable enzyme in the cytosol is quickly released into the medium upon damage of plasma membrane, and malondialdehyde (MDA) as an essential product of lipid peroxidation tends to elevate free radical-mediated myocardial cell injury [25,26]. Thus, low levels of LDH and MDA can protect PC12 cells against oxidative stress. In the Aβ25-35 induced PC12 cell model, LDH release was significantly increased compared to the control group. Analog 7 significantly reduced LDH release dose-dependently. Meanwhile, the intracellular level of MDA, a marker of lipid peroxidation, was also reduced (Figure 8).

### Table 5. Inhibitory effects of 1-10 against tumor cell lines.

| Comp | A549 (µM) | HCT-8 (µM) | MCF-7 (µM) |
|------|-----------|------------|------------|
| 1    | >10       | >10        | >10        |
| 2    | >10       | >10        | >10        |
| 3    | >10       | >10        | >10        |
| 4    | >10       | 5.4 ± 0.1  | >10        |
| 5    | >10       | 7.3 ± 0.1  | >10        |
| 6    | >10       | >10        | >10        |
| 7    | >10       | >10        | >10        |
| 8    | 3.2 ± 0.1 | >10        | >10        |
| 9    | >10       | >10        | >10        |
| 10   | >10       | >10        | >10        |
| taxol | 0.2 ± 0.1 | 0.7 ± 0.3  | 0.2 ± 0.1  |

### Table 6. Aβ25-35-induced PC12 cell viability treated by analogs.

| µM    | 0    | 5.0 | 10.0 | 20.0 | 30.0 | 40.0 |
|-------|------|-----|------|------|------|------|
| Aβ25-35 | 100  | 92.5| 81.0 | 74.6 | 64.8 | 49.5 |
| Aβ25-35 + 3 | 50.0 | 53.7| 55.8 | 62.1 | 63.7 | 65.5 |
| Aβ25-35 + 4 | 50.0 | 55.2| 52.4 | 67.8 | 72.2 | 75.5 |
| Aβ25-35 + 5 | 50.0 | 50.0| 50.0 | 50.0 | 50.0 | 50.0 |
| Aβ25-35 + 6 | 50.0 | 51.0| 53.0 | 55.0 | 57.0 | 59.2 |
| Aβ25-35 + 7 | 50.0 | 56.2| 60.5 | 74.6 | 78.6 | 84.3 |
| Aβ25-35 + 8 | 50.0 | 52.6| 55.5 | 58.4 | 62.7 | 65.5 |
| Aβ25-35 + 9 | 50.0 | 50.0| 50.0 | 50.0 | 50.0 | 50.0 |
was isolated from this fungus. 12-Hydroxylation of sporogen-OA1 followed by various acylation generates 3-6. By the similar manner, 14-hydroxylation of petasol and then acylation derives 7, which follows olefinic migration affords 8 (Scheme 1).

![Figure 8](image)

**Figure 8.** Analog 7 reduced the expression of MDA and LDH in Aβ25-35-induced PC12 cells. Statistical significance values are indicated as *, # p < 0.05, **, ## p < 0.01.

### 3. Discussion

Biogenetically, petasol is considered as a precursor to derive relevant analogs. Dehydrogenation of petasol generates 6-dehydropetasol as an intermediate, which follows olefinic rearrangement and methyl migration and subsequent oxidation to afford the aromatic analogs 1, 2 and 10. Epoxidation 6-dehydropetasol derives sporogen-OA1, which was isolated from this fungus. 12-Hydroxylation of sporogen-OA1 followed by various acylation generates 3-6. By the similar manner, 14-hydroxylation of petasol and then acylation derives 7, which follows olefinic migration affords 8 (Scheme 1).

![Scheme 1](image)

**Scheme 1.** Biogenetic relationships of the isolated eremophilanes.

### 4. Materials and Methods

#### 4.1. General Experimental Procedures

Optical rotations were recorded on an Autopol-III automatic polarimeter (Rudolph Research Co., Ltd., Hackettstown, NJ, USA). UV spectra were recorded on a Cary 300 spectrometer. IR spectra were measured on a Thermo Nicolet Nexus 470 FT-IR spectrometer. ECD spectra were measured on a JASCO J-815 spectropolarimeter. 1H and 13C NMR spectra together with 2D NMR spectra were measured on Bruker Advance NMR spectrometers (400 MHz or 600 MHz for 1H and 100 MHz or 125 MHz for 13C, respectively). Chemical shifts are expressed in δ referenced to the solvent peaks of DMSO-d_6 (1H at δ_H 2.50 and for 13C at δ_C 39.5). HRESIMS spectra were obtained on a Bruker APEX IV 70 eV FT-MS spectrometer and on a Waters Xevo G2 Q-TOF spectrometer fitted with an ESI source (acquisition range:100–1000, acquisition: start time 0 and end time 4, voltages: ESI+ 2 kV and ESI− 1.5–2 kV, external standard: HCOONa). DAD HPLC was performed on Waters e2695 separations module with Waters 2998 photodiode array detector and Thermo BDS column (250 × 4.6 mm, 5 μm). Column chromatography (CC) was performed using ODS (50 μm, Daiso), silica gel (200–300 mesh, Qingdao Marine Chemistry Co., Ltd., Qingdao, China), and Sephadex LH-20 (Amersham Pharmacia Biotech AB, Staffanstorp, Sweden). Precoated silica gel plates (Qingdao Marine Chemistry Co., Ltd.) were used for TLC analyses. Solvents used for isolation are all analytical grade. Semipreparative HPLC was performed on an Alltech 426 pump using a Uvis-201 detector, and the Prevail C18 column (semipreparative, 5 μm) was used for separation.
4.2. Fungal Strain and Identification

Fungal strain WZXY-m122-9 was isolated from the sponge of *Xestospongia testudinaria*, which was collected from Weizhou island in May 2016. The fungal identification was based on the ITS gene sequence which showed 100% similarity to a *Penicillium copticola* clone using Blast. The ITS gene sequence was deposited in GenBank. The strain is preserved at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

4.3. Fermentation and Extraction

The fungal strain WZXY-m122-9 was cultured on flasks (120 × 500 mL) in rice medium with 45 g rice and 40 mL of 3.3% sea-salt in each flask at 25 °C for 4 days to obtain fresh mycelia and spores. They were then inoculated in 250 mL Erlenmeyer flasks (×10) containing 50 mL PDB medium to obtain seed medium after culture in a rotary shaker set to 120 rpm at 25 °C for 3 days. The seed culture was inoculated in 600 × 250 mL Erlenmeyer flasks, each containing 30 g rice and 30 mL of distilled artificial seawater. After 16 days under static conditions at 25 °C, the fermented culture was extracted with EtOAc (2 × 250 mL) twice. The organic solvent was evaporated to obtain an extract (20.0 g), which was suspended in 10% H2O of MeOH, and then extracted with cyclohexane to remove lipids. The MeOH solution was evaporated under reduced pressure to obtain an extract (10.0 g). This extract was dissolved in H2O and then extracted by EtOAc to yield EtOAc extract (4.8 g) after concentrated under vacuum. The EtOAc extract was fractionated upon silica gel column (3 mm) with 45 g rice and 40 mL of 3.3% sea-salt in each flask at 25 °C with 45 g rice and 40 mL of 3.3% sea-salt in each flask at 25 °C to afford three fractions: FA, FB (0.5 g), and FC (1.2 g). Fraction FA (2.4 g) was fractionated upon C18 (5.5 mg, Rf = 25.5 min), and JBIR27 (13.5 mg, Rf = 29.4 min). Subfraction FA2 (195 mg) was separated by semi-preparative HPLC (YMC-packed C18, 5 µm, 250 × 10 mm, 2 mL/min, UV detection at 210 nm) with MeCN-H2O (1:4, v/v) as a mobile phase to yield sporgen AO-1 (125 mg, Rf = 25.5 min), phenomenone (18.0 mg, Rf = 25.5 min), JBIR28 (4.0 mg, Rf = 23.6 min), and JBIR27 (13.5 mg, Rf = 29.4 min). Subfraction FA2 (195 mg) was separated by semi-preparative HPLC (YMC-packed C18, 5 µm, 250 × 10 mm, 2 mL/min, UV detection at 210 nm) eluting with MeCN-H2O (1:3, v/v) to obtain 1 (10.5 mg, Rf = 24.8 min), 2 (5.5 mg, Rf = 29.1 min), 11 (6.9 mg, Rf = 28.5 min), 12 (5.5 mg, Rf = 28.7 min), 3 (9.2 mg, Rf = 30.2 min), 4 (5.8 mg, Rf = 31.1 min), and 5 (10.5 mg, Rf = 30.4 min). Subfraction FA3 (55 mg) was purified by a semi-preparative HPLC (YMC-packed C18, 5 µm, 250 × 10 mm, 2 mL/min, UV detection at 210 nm) eluting with MeOH-H2O (45:55, v/v) to afford 6 (7.9 mg, Rf = 22.4 min), 7 (9.2 mg, Rf = 23.6 min), 8 (6.2 mg, Rf = 27.0 min), 9 (7.5 mg, Rf = 25.5 min), and 10 (4.1 mg, Rf = 26.0 min). FA1 (140 mg) was separated upon C18 ODS column (C18, 10 µm, 25 × 30 cm), eluting with 3-chloro-4,5-dihydroxyphenylacetic acid (7.6 mg), 3-chloro-4-hydroxyphenylacetic acid (5.5 mg), and 3-chloro-4-hydroxy-5-methoxyphenylacetic acid (3.1 mg). Copteremophilane A (1): yellow amorphous; [α]20D +62 (c = 0.2, MeOH); IR (KBr) vmax 3384, 2933, 1731, 1673, 1453, 1384, 1208 cm−1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 219.1380 [M + H]+ (calcd for C14H16O2, 219.1385).

(R)-MPA ester of 1: white powder; 1H NMR (400 MHz, DMSO-d6) δ 2.61 (2H, m, H-1), 1.94–2.08 (2H, m, H-2), 4.93 (1H, H, H-3), 2.85 (1H, m, H-4), 7.50 (1H, s, H-6), 7.58 (1H, s, H-8), 3.28 (3H, s, H-12), 2.25 (3H, s, H-13), 1.11 (1H, d, J = 6.5 Hz, H-14).

(S)-MPA ester of 1: white powder; 1H NMR (400 MHz, DMSO-d6) δ 2.48 (2H, m, H-1), 1.75–2.92 (2H, m, H-2), 4.99 (1H, H, H-3), 3.02 (1H, m, H-4), 7.58 (1H, s, H-6), 7.61 (1H, s, H-8), 3.28 (3H, s, H-12), 2.14 (3H, s, H-13), 1.23 (1H, d, J = 6.5 Hz, H-14).

Copteremophilane B (2): yellow amorphous; [α]20D +26 (c = 0.2, MeOH); IR (KBr) vmax 3396, 2931, 1685, 1604, 1453, 1384, 1208 cm−1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 233.1543 [M + H]+ (calcd for C15H22O2, 233.1542).

Copteremophilane C (3): yellow amorphous; [α]20D +90 (c = 0.3, MeOH); IR (KBr) vmax 3283, 2929, 1731, 1673, 1596, 1499, 1436, 1208, 1204 cm−1; 1H and 13C NMR data, see Tables 2 and 3; HRESIMS m/z 449.1369 [M + H]+ (calcd for C23H26ClO7, 449.1367).
Copteremophilane D (4): yellow amorphous; $[\alpha]_{D}^{20} +28$ (c = 0.2, MeOH); IR (KBr) $\nu_{\text{max}}$ 3260, 2941, 1732, 1670, 1596, 1500, 1436, 1205 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Tables 2 and 3; HRESIMS $m/z$ 447.1208 [M − H]$^-$ (calcd for C$_{23}$H$_{24}$ClO$_7$, 447.1211).

Copteremophilane E (5): yellow amorphous; $[\alpha]_{D}^{20} +28$ (c = 0.2, MeOH); IR (KBr) $\nu_{\text{max}}$ 3291, 2927, 1735, 1669, 1596, 1510, 1425, 1384, 1153 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Tables 2 and 3; HRESIMS $m/z$ 431.1259 [M − H]$^-$ (calcd for C$_{24}$H$_{24}$ClO$_6$, 431.1261).

Copteremophilane F (6): yellow amorphous; $[\alpha]_{D}^{20} +46$ (c = 0.2, MeOH); IR (KBr) $\nu_{\text{max}}$ 3315, 2926, 1735, 1650, 1384, 1285, 1145 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Tables 2 and 3; HRESIMS $m/z$ 461.1363 [M − H]$^-$ (calcd for C$_{24}$H$_{25}$ClO$_7$, 461.1367).

Copteremophilane G (7): yellow amorphous; $[\alpha]_{D}^{20} +60$ (c = 0.2, MeOH); IR (KBr) $\nu_{\text{max}}$ 3268, 2939, 1725, 1674, 1499, 1436, 1291, 1206 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Tables 2 and 3; HRESIMS $m/z$ 433.1415 [M − H]$^-$ (calcd for C$_{23}$H$_{24}$ClO$_6$, 433.1418).

Copteremophilane H (8): yellow amorphous; $[\alpha]_{D}^{20} +102$ (c = 0.2, MeOH); IR (KBr) $\nu_{\text{max}}$ 3256, 2928, 1685, 1436, 1383, 1297, 1209 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Tables 2 and 3; HRESIMS $m/z$ 433.1421 [M − H]$^-$ (calcd for C$_{23}$H$_{25}$ClO$_6$, 433.1418).

Copteremophilane I (9): yellow amorphous; $[\alpha]_{D}^{20} +54$ (c = 0.2, MeOH); IR $\nu_{\text{max}}$ (cm$^{-1}$): 3386, 2928, 1720, 1436, 1384, 1292, 1206, 1150, 1024; $^1$H and $^{13}$C NMR data, see Tables 2 and 3; HRESIMS $m/z$ 435.1578 [M − H]$^-$ (calcd for C$_{23}$H$_{25}$ClO$_6$, 435.1574).

Copteremophilane J (10): yellow amorphous; $[\alpha]_{D}^{20} +54$ (c = 0.2, MeOH); IR (KBr) $\nu_{\text{max}}$ 3432, 2926, 2913, 1661, 1436, 1406, 1312 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; HRESIMS $m/z$ 457.1395 [M + Na]$^+$ (calcd for C$_{23}$H$_{27}$ClO$_6$Na, 457.1390).

5-Glycopenostatin F (11): yellow amorphous; $[\alpha]_{D}^{20} +12$ (c 0.2, MeOH); IR $\nu_{\text{max}}$(KBr) cm$^{-1}$: 3402, 2926, 2855, 1716, 1674, 1384, 1205; $^1$H and $^{13}$C NMR data, see Table 4; HRESIMS $m/z$ 507.2958 [M + H]$^+$ (calcd for C$_{28}$H$_{43}$O$_{16}$, 507.2958).

5-Glycopenostatin I (12): yellow amorphous; $[\alpha]_{D}^{20} -10$ (c 0.2, MeOH); IR $\nu_{\text{max}}$(KBr) cm$^{-1}$: 3372, 2926, 1716, 1456, 1383, 1341, 1218; $^1$H and $^{13}$C NMR data, see Table 4; HRESIMS $m/z$ 507.2958 [M + H]$^+$ (calcd for C$_{28}$H$_{43}$O$_{16}$, 507.2958).

4.4. Hydrolysis of 11 and 12

Compound 11 (1.5 mg) was dissolved in HCl (2 M, 10 mL) to stir at 100 °C for 4 h monitored by TLC. After reaction time, the acidic solution was extracted by 5 mL EtOAc. EtOAc was concentrated under vacuum to yield 0.7 mg hydrolyzed product (11a), and the acidic solution was freeze-dried and solubilized in MeOH for further analysis. Compound 12 (1.3 mg) was hydrolyzed by the same protocol as for 11.

4.5. Mosher Reaction

Compound was dissolved in anhydrous CHCl$_3$, and then R-MPA (equal mol), DMAP and DCC were added to react at room temperature for 12 hrs. The product was purified by silica gel column eluting with petroleum ether-EtOAc (2:1) to yield R-MPA ester. S-MPA ester of compound was synthesized by the same protocol as for R-MPA ester. Calculation of the chemical shift difference ($\Delta \delta = \delta_S - \delta_R$) allowed the assignment of configuration of the stereogenic center of secondary alcohol [27].

4.6. Snatzke Method

Compound was weighted to two portions (0.2 mg for each). One portion was dissolved in 0.5 mL DMSO for the detection of ECD curve at wavelength 250–500 nm, and the second portion was dissolved in 0.5 mL DMSO with 0.5 mg/mL Mo(OAc)$_4$ for the detection of the ECD curve within 30 min [28].

4.7. Cytotoxic Detection

Cell viability was evaluated using the MTT assay according to the manufacturer’s instruction. In brief, cells were plated at a density of 1.0 × 10$^4$ cells per well in 96-well plates and allowed to attach overnight. Then, the cells were treated with or without compound at the indicated concentration. After 48 h, MTT solution containing 1 mg/mL and MTT 100 µL
was added into each well and incubated for 2 h at 37 °C. Then, the medium was changed with the same volume of DMSO. After incubation, absorbance is read at 570 nm for MTT by a spectrophotometer and the quantity of formazan product is directly proportional to the number of living cells in culture.

4.8. Cell Viability Assay

Rat pheochromocytoma (PC12) cells were cultured in Dulbecco’s modified Eagle medium (DMEM), and supplemented with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin in a humidified atmosphere at 37 °C with 5% CO₂. The cells were passaged every 3 days. PC12 cells (1 × 10⁴) were cultured for 24 h, and then the medium was replaced with serum-free DMEM medium. The PC12 cells were divided into three groups. In the model group, PC12 cells were treated using Aβ25-35 with different concentrations. Then, the cells were incubated for 12 h, 24 h and 48 h until there was a cell viability of up to 50%. PC12 cells without pretreatment were set up as control. The third group of PC12 was treated with different concentrations of compounds to detect the cell viability by CCK8 method. The model groups (Aβ25-35 induced PC12 cells) were treated with compounds in different concentrations, and the viability of PC12 cells was measured using cell counting kit-8 (Dojindo, Japan), according to the manufacturer’s instructions. Then, the medium was aspirated and cells in each group were incubated with 10 µL of CCK8 at 37 °C for 2 h. Afterwards, the absorbance was determined at a wavelength of 450 nm with a microplate reader (Thermo, MuLtiSKAN MK3, Waltham, MA USA).

4.9. Measurement of Malondialdehyde (MDA)

Cultured PC12 cells were initially seeded in 6-well plates for 24 h. The cells were then pre-incubated with or without compound, followed by incubation with Aβ25-35 (40 µM) for 24 h. The cultures were washed with ice-cold PBS and homogenized. The homogenate was centrifuged at 4 °C. The protein concentration in each sample was determined by the MDA Protein Assay Kit as a reference standard. The levels of MDA were determined according to the manufacturer’s instructions. Concentrations were normalized to the protein concentration expressed as a percentage of control samples.

4.10. Lactate Dehydrogenase (LDH) Release Assay

Cell injury was assessed through measuring the LDH activity in the supernatant of PC12 cells using an LDH kit according to the manufacturer’s protocol. In brief, double-distilled H₂O, 0.2 µM pyruvic acid, matrix buffer and coenzyme I buffer were added in sequence at 48 h after the treatment by compound. The supernatant was collected after incubation for 30 min at room temperature. The absorbance at 450 nm was then measured with a microplate reader.

4.11. Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Biostatistical analyses were conducted with SPSS 16.0 software. Statistical differences among groups were assessed by one-way analysis of variance (ANOVA). Differences were considered to be statistically significant at p < 0.05.

5. Conclusions

In summary, this work reports a chemical examination of the marine sponge-derived *P. copticola* fungus to afford 12 undescribed natural products, of which 10 are identified as eremophilanes and 2 are determined as glucosides. Eremophilanes 1, 2 and 10 are characteristic of the eremophilanes with a phenyl moiety in ring B accompanying a methyl migration to C-9, which are uncommonly found in nature. Eremophilanes 3–9 feature the esterification of a chlorinated phenylacetic unit in backbone, and this is the second isolation of relevant analogs from fungi. Analogs 11 and 12 are characteristic of an unprecedented PKS scaffold bearing a glucose unit. The bioassay results revealed the inhibitory effects
of the isolated eremophilanes against tumor cell lines related to the structure variation. Analog 8 only inhibited human non-small cell lung cancer cells (A549), and analogs 4 and 5 selectively inhibited HCT-8. The eremophilane nuclei or acyl moietyes showed inactivity towards tumor cell lines. These findings suggest the incorporation of chlorinated phenylacetic units significantly enhances the antitumor effects of eremophilanes, and the substituted positions and the acyl compositions are sensitive for the selective inhibition. In addition, the nontoxic analogs, such as 7, showed a neuroprotective effect. This study implies that eremophilanes have potential for development as antitumor or neuroprotective agents after structure modification.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/md20110712/s1, Figures S1–S102: spectra of IR, 1H and 13C NMR, COSY, HSQC, HSBC, and NOESY. Figure S103: Part node clusters of EtOAc extract by molecular networking. Figure S104: Key HMBC and COSY correlations of 1–10.

**Author Contributions:** Fungal fermentation and chromatography, J.Z.; compound purification, D.L.; bioactive detection, J.H.; biogenetic hypothesis, A.F.; structure elucidation and manuscript editing, W.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by COMRA DY135-B-05 and NSFC (81991525, 21861142006, 81872793, 81630089), 2022Z144 and 2021ZD06.

**Data Availability Statement:** The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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

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