Cembranoids with 3,14-Ether Linkage and a Secocembrane with Bistetrahydrofuran from the Dongsha Atoll Soft Coral Lobophytum sp.

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Abstract: Four new cembranoids, lobophylins A–D (1–4), and one novel secocembrane, lobophylin E (5) were isolated from a soft coral Lobophytum sp. The structures of new metabolites were elucidated on the basis of extensive spectroscopic methods. Among these metabolites, 1–4 are rarely found cembranoids possessing a tetrahydrofuran moiety with a 3,14-ether linkage. In addition, 5 is the first secocembrane possessing two tetrahydrofuran moieties with 3,14- and 4,7-ether linkages.

Keywords: soft coral; secocembrane; Lobophytum; tetrahydrofuran

1. Introduction

Soft corals have proven to be important sources of secondary metabolites with interesting biological activities [1]. In the investigation of the secondary metabolites from soft corals in Taiwan waters, a series of bioactive cembranoids have been isolated from octocorals (Alcyonaceae) belonging to the genera Sinularia [2–7], Lobophytum [8–10], Sarcophyton [11–16] and Pachyclavularia [17,18]. Some
of these metabolites have been shown to exhibit significant cytotoxic activity against the growth of various cancer cell lines [10,15–17], and/or anti-inflammatory activity [3,6,8,10,15,16]. Our previous chemical investigation on Dongha Atoll soft coral Lobophytum sarcophytoides has led to the isolation of bioactive cembranoids [19]. In our continuing search for bioactive metabolites from Dongsha Atoll soft corals of the genus Lobophytum, we investigated the chemical constituents of Lobophytum sp. and succeeded in the isolation of four new cembranoidal lobophyllins A–D (1–4) and a novel seccembrane, lobophylin E (5) (Chart 1). The structures of these compounds have been established by extensive spectroscopic analysis. The cytotoxicity of compounds 1–5 against four human cancer cell lines was investigated, however, none of these was found to possess useful biological activity.

Chart 1. Structures of metabolites 1–5.

2. Results and Discussion

The new metabolite lobophylin A (1) exhibited a protonated molecule peak in the HRESIMS at m/z 343.2251 [M + Na]+, establishing the molecular formula C_{20}H_{32}O_{3} and five degrees of unsaturation. The IR spectrum suggested the presence of hydroxy group (ν_{max} 3460 cm^{-1}) in 1. The ^{13}C NMR spectrum of 1 measured in CDCl_{3} (Table 1) showed the presence of twenty carbon signals, which were assigned by the assistance of DEPT spectrum to four methyls, six sp^{3} methylenes, one sp^{2} methylene, four sp^{3} methines (including three oxymethines), one sp^{2} methine, and two sp^{3} quaternary and two sp^{2} quaternary carbons. From the ^{1}H NMR spectroscopic data of 1 (Table 2), the presence of two hydroxy protons resonating at δ 3.98 (dd, J = 9.6, 4.4 Hz) and 4.37 (ddd, J = 12.0, 3.6, 3.6 Hz) were observed. Moreover, the ^{1}H NMR spectrum revealed the presence of two olefinic methylene protons at δ 4.87 (d, J = 1.6 Hz) and 4.81 (s) and one olefinic methine proton at δ 5.09 (t, J = 6.8 Hz). A proton signal appearing at δ 3.27 (^{1}H, d, J = 6.8 Hz) and correlating with a carbon signal at δ 64.7 in the HMQC spectrum was due to the proton of the trisubstituted epoxide. The planar structure and all of the assignments of ^{1}H and ^{13}C NMR data of 1 were determined by the assistance of 2D NMR studies, including ^{1}H–^{1}H COSY and HMBC experiments (Figure 1). ^{1}H–^{1}H COSY spectrum revealed proton sequences from H-1 to H-3 and H-13 to H-1; H_{2}-5 to H-7; H_{2}-9 to H-11, as shown by the bold lines in
Figure 1. Key HMBC correlations of H-3 to C-4; H-7 to C-8; H2-13 to C-11 and C-12; H2-16 to C-1 and C-15; H3-17 to C-1, C-15 and C-16; H4-18 to C-3, C-4 and C-5; H3-19 to C-7, C-8 and C-9; and H3-20 to C-11, C-12 and C-13, permitted the connection of the carbon skeleton. Furthermore, the HMBC cross-peak from H-14 to C-3 suggested that C-3 and C-14 were linked through an oxygen to form a tetrahydrofuran ring. Thus, 1 was revealed as a cembranoid possessing a 3,14-ether linked tetrahydrofuran ring, on the basis of the above analysis.

Table 1. 13C NMR data for compounds 1–5.

| C# | 1a | 2a | 3a | 4b | 5b |
|----|----|----|----|----|----|
| 1  | 50.2 (CH) | 49.0 (CH) | 49.3 (CH) | 49.3 (CH) | 49.8 (CH) |
| 2  | 29.1 (CH3) | 27.4 (CH3) | 26.7 (CH3) | 26.7 (CH3) | 30.9 (CH3) |
| 3  | 77.5 (CH) | 77.6 (CH) | 77.8 (CH) | 77.6 (CH) | 82.2 (CH) |
| 4  | 74.5 (C) | 74.2 (C) | 74.6 (C) | 74.7 (C) | 86.6 (C) |
| 5  | 39.1 (CH3) | 38.6 (CH3) | 42.5 (CH3) | 43.3 (CH3) | 31.9 (CH3) |
| 6  | 23.8 (CH2) | 21.5 (CH2) | 118.9 (CH) | 121.8 (CH) | 33.3 (CH3) |
| 7  | 64.7 (CH) | 126.6 (CH) | 142.7 (CH) | 141.5 (CH) | 105.6 (CH) |
| 8  | 60.3 (C) | 132.8 (C) | 73.6 (C) | 72.6 (C) | 208.9 (C) |
| 9  | 38.1 (CH2) | 38.2 (CH2) | 44.4 (CH2) | 43.7 (CH2) | 43.7 (CH3) |
| 10 | 23.9 (CH2) | 24.4 (CH2) | 23.5 (CH2) | 22.2 (CH2) | 22.5 (CH2) |
| 11 | 126.5 (CH) | 127.1 (CH) | 129.4 (CH) | 129.6 (CH) | 124.2 (CH) |
| 12 | 133.0 (C) | 131.9 (C) | 130.9 (C) | 130.8 (C) | 134.2 (C) |
| 13 | 40.2 (CH2) | 39.3 (CH2) | 38.9 (CH2) | 38.8 (CH2) | 39.7 (CH2) |
| 14 | 78.5 (CH) | 76.7 (CH) | 76.0 (CH) | 76.0 (CH) | 80.3 (CH) |
| 15 | 141.6 (C) | 142.4 (C) | 142.2 (C) | 142.3 (C) | 144.0 (C) |
| 16 | 111.3 (CH2) | 111.0 (CH2) | 111.2 (CH2) | 111.1 (CH2) | 112.2 (CH2) |
| 17 | 25.0 (CH3) | 23.5 (CH3) | 23.5 (CH3) | 23.5 (CH3) | 22.5 (CH3) |
| 18 | 24.6 (CH3) | 23.1 (CH3) | 21.6 (CH3) | 21.9 (CH3) | 24.2 (CH3) |
| 19 | 19.8 (CH3) | 16.3 (CH3) | 29.6 (CH3) | 28.3 (CH3) | 29.9 (CH3) |
| 20 | 17.3 (CH3) | 15.4 (CH3) | 15.4 (CH3) | 15.5 (CH3) | 16.5 (CH3) |

OMe 54.3 (CH3)

a Spectra recorded at 100 MHz in CDCl3; b Spectra recorded at 125 MHz in CDCl3; c Attached protons were deduced by DEPT experiments.

Table 2. 1H NMR data for compounds 1–5.

|    | 1a | 2a | 3a | 4b | 5b |
|----|----|----|----|----|----|
| 1  | 2.77 dt (8.8, 8.8) | 2.73 dt (11.2, 7.2) | 2.73 dt (8.0, 8.8) | 2.74 dt (9.0, 8.5) | 2.78 dt (7.5, 8.5) |
| 2  | 2.16 m; 1.92 m | 2.08 m; 1.90 m | 2.04 m; 1.86 m | 2.05 m; 1.86 m | 1.96 m; 1.91 m |
| 3  | 3.98 dd (9.6, 4.4) | 3.97 dd (9.6, 4.5) | 3.82 dd (10.0, 4.8) | 3.82 dd (9.5, 4.5) | 3.98 dd (7.5, 7.5) |
| 5  | 1.97 m; 1.70 m | 1.94 m; 1.53 m | 2.40 dd (14.0, 10.0) | 2.40 dd (14.0, 10.0) | 2.40 dd (14.0, 10.0) |
| 6  | 2.05 m; 1.31 m | 2.25 m; 2.06 m | 5.60 ddd (15.2, 10.0, 5.2) | 5.51 ddd (15.5, 10.0, 5.0) | 2.02 m; 1.94 m |
| 7  | 3.27 d (6.8) | 5.17 dd (6.0, 6.0) | 5.70 d (15.6) | 5.75 d (15.5) | 5.00 d (4.5) |
| 9  | 1.86 m; 1.52 m | 2.14 m; 1.96 m | 1.92 m; 1.58 m | 1.95 m; 1.58 m | 2.45 dd (8.0, 7.0) |
| 10 | 2.21 m; 1.88 m | 2.32 m; 2.04 m | 2.19 m; 2.10 m | 2.56 m; 1.96 m | 2.27 dd (7.5, 7.5) |
Table 2. Cont.

|   | Signal |  δ (ppm) |  J (Hz) |
|---|--------|---------|---------|
| 11 | 5.09 t | 6.8     |         |
| 13 | 1.95 m; 1.68 m | 4.96 d | 10.0 |
| 14 | 4.37 ddd | 4.36 ddd | 4.33 ddd |
| (12.0, 3.6, 3.6) | (11.6, 5.2, 4.8) | (11.6, 6.0, 5.2) | (12.0, 6.0, 5.5) |
| 16 | 4.87 d (1.6); 4.81 s | 4.85 d (1.2); 4.78 s | 4.86 d (1.6); 4.80 s |
| 17 | 1.77 s | 1.75 s | 1.76 s |
| 18 | 1.15 s | 1.09 s | 1.11 s |
| 19 | 1.24 s | 1.65 s | 1.28 s |
| 20 | 1.61 s | 1.57 s | 1.67 s |
| OMe | 3.34 s |         |         |

* Spectra recorded at 400 MHz in CDCl₃; † Spectra recorded at 500 MHz in CDCl₃; ‡ J values (in Hz) in parentheses.

**Figure 1.** Selected ¹H-¹H COSY (▬) and HMBC (→) correlations of 1, 3 and 5.

The relative configuration of 1 elucidated mainly by NOESY spectrum was compatible with that of 1 offered by using the MM2 force field calculations which suggested the most stable conformations as shown in Figure 2. In the NOESY spectrum, it was found that H-1 (δ 2.77, dt, J = 8.8, 8.0 Hz) showed NOE interactions with H-14 and H-2-18 (δ 1.15, s); therefore, assuming the β-orientation of H-1, H-14 and H-18 should also be positioned on the β face. One of the methylene protons at C-2 (δ 1.92) exhibited NOE correlations with H-1 and was characterized as H-2β, while the other (δ 2.16) was assigned as H-2α. NOE correlations observed between H-2α and H-3 (δ 3.98, dd, J = 9.6, 4.4 Hz), and H-3 and H-7 (δ 3.27, d, J = 6.8 Hz), reflected the α-orientations of both protons H-3 and H-7. Also, H-3-19 was found to interact with H-2-6, but not with H-7, revealing the trans geometry of the
trisubstituted epoxide. Furthermore, the NOE correlations observed \( H_3-20 \) and \( H-10 (\delta 2.21) \), but not with \( H-11 \), reflected the \( E \) geometry of double bond at \( C-11 \). On the basis of the above findings and other detailed NOE correlations (Figure 2), the relative structure of \( 1 \) was determined.

**Figure 2.** Computer-generated model for \( 1 \) using MM2 force field calculations and key NOE correlations.

HRESIMS analysis of lobophylin B (2) provided a molecular formula of \( C_{20}H_{32}O_2 \) ([M + Na]\(^+\) \( m/z \) 327.2301). The \(^1\)H and \(^13\)C NMR spectroscopic data of 2 were very close to those of 1 (Tables 1 and 2), except for the replacement of the two carbon signals of the epoxide moiety in 1 by the signals of a trisubstituted double bond in 2 (\( \delta 126.6 \), CH, C-7 and 132.8, C, C-8). This double bond was positioned at C-7/C-8 due to the \(^1\)H-\(^1\)H COSY correlation found between the H-6 and H-7, the HMBC correlations observed from the olefinic methyl protons at \( \delta 1.65 \) (3H, s) to C-7, C-8 and C-9. Furthermore, the \( E \) geometry of the 7,8-double bond was deduced from the NOE correlation of \( H_3-19 \) with \( H_2-6 \) and not with \( H-7 \). Thus, the structure of 2 was determined unambiguously. Literature review revealed a known compound similar to compound 2 but possessing a rare 3,13-bridged tetrahydropyran ring [20].

Lobophylin C (3) showed a protonated molecule peak [M + Na]\(^+\) at \( m/z \) 343.2248 in the HRESIMS, corresponding to the molecular formula \( C_{20}H_{32}O_3 \) and five degrees of unsaturation. The IR spectrum showed the presence of hydroxy (3377 cm\(^{-1}\)) group. \(^1\)H and \(^13\)C NMR spectroscopic data (Tables 1 and 2) of 3 showed the structural unit of a 3,14-oxa-bridged tetrahydrofuran, too. \(^1\)H-\(^1\)H COSY and HMBC (Figure 1) further revealed that 3 possesses a 1,2-disubstituted double bond (\( \delta 118.9 \) and 142.7, each CH) at C-6 and C-7 and a quaternary oxycarbon at C-8 (\( \delta 73.6 \), C). On the basis of the above observations, and by the assistance of additional 2D NMR (\(^1\)H-\(^1\)H COSY and HMBC) correlations, it was possible to establish the planar structure of 3 as illustrated in Figure 1. The relative configurations of the five chiral centers at C-1, C-3, C-4, C-8 and C-14 in 3 were thus determined on the basis of NOE correlations (Figure 3). By careful inspection on the NOESY spectrum of 3, it was found that one proton (\( \delta 2.40 \)) of H2-5 showed NOE interaction with both H3-18 and H7-7, and H-7 was NOE correlated with H3-19. Therefore, H3-18 and H3-19 are situated on the same \( \beta \)-face. Furthermore,
NOESY spectrum showed correlation of H₃-20 with one proton (δ 2.19) of CH₂-10, but not with H-11, revealing the E-configurations of the 11,12-trisubstituted double bond. The above finding, together with J values for both H-6 (15.2 Hz) and H-7 (15.6 Hz), confirmed the E-configuration of the 6,7-double bond. Further NOE analysis revealed that 3 possessed the same configurations at C-1, C-3, C-4 and C-14, as in compound 1 (Figure 3). Based on the above results, the structure of 3 was established.

The HRESIMS spectrum of lobophylin D (4) showed a molecular formula of C₉₀H₁₃₂O₁₁, the same as that of 3. By analysis 2D NMR spectra, including ¹H-¹H COSY, HMQC and HMBC, 4 was shown to possess the same molecular framework as that of 3. Furthermore, it was found that the NMR data of 4 were very similar to those of 3 (Tables 1 and 2), revealing that 4 might be an isomer of 3. However, the significant downfield shift at C-6 (ΔδC +2.9 ppm) and the upfield shift at C-7 (ΔδC −1.2 ppm), C-8 (ΔδC −1.0 ppm) and C-19 (ΔδC −1.3 ppm), relative to those of 3 (Table 2), suggested that 4 might be the C-8 epimer of 3. From NOESY spectrum, it was found that one proton (δ 2.56, m) of H₂-10 of 4 showed NOE correlations with H-7 (δ 5.75, d, J = 15.5 Hz) and H₃-20 (δ 1.70, s), while H-6 (5.51, ddd, J = 15.5, 10.0, 5.0 Hz) was NOE correlated with H₃-19 (δ 1.37, s) (Figure 3). Therefore, both H-7 and H₃-20 are situated on the β-face, and in contrast, H-6 and H₃-19 should be positioned on the α-face. This inferred the R* configuration at C-8. Further analysis of other NOE interactions revealed that 4 possessed the same relative configurations at C-1, C-3, C-4 and C-14 as those of 3 (Figure 3). Therefore, 4 was found to be the C-8 epimer of 3.

**Figure 3.** Computer-generated model for 3 and 4 using MM2 force field calculations and key NOE correlations.

Lobophylin E (5) was assigned a molecular formula of C₂₁H₃₄O₄, according to the HRESIMS and NMR spectroscopic data (Tables 1 and 2). The IR absorption band at 3444 cm⁻¹ revealed the presence of hydroxy group. By the analysis of ¹³C and DEPT spectroscopic data, the carbons signals were assigned into five methyls (including one methoxy methyl resonating at δC 54.3), six sp³ methylenes, one sp² methylene, four sp³ methines (including two monooxygenated carbons resonating at δC 82.2 and 80.3 and an acetal carbon resonating at δC 105.6), one sp² methine, one sp³ quaternary carbons
and three sp² quaternary carbons (including a normal ketone resonating at δC 208.9). From the ¹H-¹H COSY spectrum of 5, it was possible to identify three different structure units, which were assembled with the assistance of an HMBC experiment. Key HMBC correlations between H-3 to C-4; H₂-9 and H₂-10 to C-8 (carbonyl carbon); H-11 to C-13; H₂-16 to C-1 and H₃-17 to C-1, C-15 and C-16; H₃-18 to C-3, C-4 and C-5; H₃-19 to C-8 and C-9; and H₃-20 to C-11, C-12 and C-13 permitted the connection of the carbon skeleton (Figure 1). Furthermore, the HMBC correlation observed from the methoxy protons (δ 3.34, 3H, s) to the carbon resonating at δ 105.6 positioned a methoxy group at C-7. In considering the degrees of unsaturation and molecular formula, two oxa-bridged ether linkages were placed between C-3/C-14 and C-4/C-7 by HMBC correlations from H-14 to C-3 and H-7 to C-4. The relative configuration of 5 was determined by the interpretation of the NOESY correlations (Figure 4). It was found that H₃-18 showed NOE interactions with H-1, H-3 and methoxy protons (H₃-21). Thus, by considering a molecular model as shown in Figure 4 and assuming the β-orientation of H₃-18, all of H-1, H-3 and methoxy group should be positioned on the β face. The NOE correlation observed between H-1 and H-14 also reflected the β-orientation of H-14. Furthermore, NOESY spectrum showed NOE interaction of H₃-20 with H-10, but not with H-11, revealing the E geometry of the C-11/C-12 double bond. From the above evidence and the other NOE correlations (Figure 4) the relative configurations at chiral centers of 5 was assumed to be 1R*, 3R*, 4R*, 7R* and 14S*. On the basis of the above analysis, the structure of 5 was established.

**Figure 4.** Computer-generated model for 5 using MM2 force field calculations and key NOE correlations.

It is worth noting that metabolites 1–4 are rare cembranoids possessing a tetrahydrofuran moiety with a 3,14-ether linkage, which has been discovered previously in the soft coral *Sinularia gibberosa* [5,21]. In addition, 5 is the first secocembrane possessing two tetrahydrofuran moieties with 3,14- and 4,7-ether linkages. Our study thus adds the structure diversity of cembranoidal natural compounds.

The cytotoxicity of compounds 1–5 against the proliferation of a limited panel of cancer cell lines, including K562 (human chronic myelogenous leukemia), DLD-1 (human colon adenocarcinoma) and
HepG2 and Hep3B (human liver carcinoma), was studied. The results showed that 1–5 are not cytotoxic toward the above cancer cells (IC\textsubscript{50} > 20 \textmu g/mL).

### 3. Experimental Section

#### 3.1. General Experimental Procedures

The melting points were determined using a Fisher-Johns melting point apparatus. Optical rotation values were measured with a JASCO P-1010 digital polarimeter. IR spectra were recorded on a VARIAN DIGLAB FT-1000 Fourier transform infrared spectrophotometer. The NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR (or Varian Unity INOVA 500 FT-NMR) instrument at 400 MHz (or 500 MHz) for \textsuperscript{1}H NMR and 100 MHz (or 125 MHz) for \textsuperscript{13}C NMR, respectively, in CDCl\textsubscript{3}. ESIMS were recorded on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) and precoated RP-18 F254S plates (Merck, 1.05560) were used for TLC analysis. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 210 nm. A semipreparative reversed-phase column (250 × 10 mm, 5 \textmu m) and a preparative normal phase column (250 × 21.2 mm, 5 \textmu m) was used for HPLC.

#### 3.2. Animal Material

The soft coral Lobophytum sp. was collected by hand using SCUBA off the coast of Dongsha Atoll, in April, 2007, at a depth of 10 m, and stored in a freezer until extraction. A voucher specimen (Specimen No. DA2007-04-20) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

#### 3.3. Extraction and Separation

The frozen soft coral (1.5 kg, fresh wt) was minced and extracted exhaustively with EtOAc (5 × 1 L). The organic extract was evaporated to yield a residue (21.9 g), which was fractionated by open column chromatography on silica gel using n-hexane–EtOAc and EtOAc–MeOH mixtures of increasing polarity to yield 16 fractions. Fraction 5, eluting with n-hexane–EtOAc (15:1), was further separated by silica gel column chromatography with gradient elution (n-hexane–EtOAc, 15:1 to 5:1) to yield five subfractions (5A–5E). Subfraction 5C was subjected to normal phase HPLC (n-hexane–EtOAc, 15:1) to obtain compound 2 (2.5 mg). Fractions 7 and 8, eluting with n-hexane–EtOAc (5:1), were combined and further separated over silica gel column chromatography (n-hexane–EtOAc, gradient elution, 5:1 to 1:1) to give four subfractions (7A–7D). Subfraction 7A was further purified by RP-18 HPLC (CH\textsubscript{3}CN–H\textsubscript{2}O, 3:2) to yield compound 5 (2.2 mg). In the same manner, compound 1 (4.2 mg) was obtained from subfraction 7B using RP-18 HPLC (CH\textsubscript{3}CN–H\textsubscript{2}O, 5:2). Fraction 11, eluting with n-hexane–EtOAc (1:1), was further separated by silica gel column chromatography with gradient elution (n-hexane–EtOAc, 1:1 to 1:5) to yield five subfractions (11A–11E). Subfraction 11C was further purified by RP-18 HPLC (CH\textsubscript{3}CN–H\textsubscript{2}O, 1:1) to yield compounds 3 (3.0 mg) and 4 (2.5 mg).
Lobophyllin A (1): colorless oil; [α]_D^{25} = −39 (c 0.3, CHCl₃); IR (neat) ν_max 3460, 2926, 1649, 1458, 1381 and 1215 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 343 [100, (M + Na)⁺]; HRESIMS m/z 343.2251 (calcd for C₂₀H₄₁₂O₂Na, 343.2249).

Lobophyllin B (2): colorless oil; [α]_D^{25} = −35 (c 0.3, CHCl₃); IR (neat) ν_max 3445, 2926, 1649, 1456, 1376 and 1265 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 327 [100, (M + Na)⁺]; HRESIMS m/z 327.2301 (calcd for C₂₀H₄₁₂O₂Na, 327.2300).

Lobophyllin C (3): white powder; mp 76–78 °C; [α]_D^{25} = +30 (c 0.1, CHCl₃); IR (neat) ν_max 3377, 2927, 1649, 1459, 1377 and 1269 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 343 [100, (M + Na)⁺]; HRESIMS m/z 343.2248 (calcd for C₂₀H₄₁₂O₂Na, 343.2249).

Lobophyllin D (4): white powder; mp 68–70 °C; [α]_D^{25} = +22 (c 0.2, CHCl₃); IR (neat) ν_max 3425, 2924, 1640, 1455, 1379 and 1240 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 343 [100, (M + Na)⁺]; HRESIMS m/z 343.2246 (calcd for C₂₀H₄₁₂O₂Na, 343.2249).

Lobophyllin E (5): colorless oil; [α]_D^{25} = +19 (c 0.2, CHCl₃); IR (neat) ν_max 3444, 2929, 1715, 1640, 1454, 1374 and 1214 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS m/z 373 [100, (M + Na)⁺]; HRESIMS m/z 373.2356 (calcd for C₂₁H₄₂O₂Na, 373.2355).

3.4. Cytotoxicity Testing

Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of compounds 1–5 were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method [22].

3.5. Molecular Mechanics Calculations

Implementation of the MM2 force filed in Chem3D Pro software from Cambridge Soft Corporation, Cambridge, MA, USA (ver. 9.0, 2005), was used to calculate molecular models.

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