Oxygenated Cembrene Diterpenes from *Sarcophyton convolutum*: Cytotoxic Sarcoconvolutum A–E

Tarik A. Mohamed 1, Abdelsamed I. Elshamy 2, Asmaa M. Abdel-Tawab 3, Mona M. AbdelMohsen 1, Shinji Ohta 4, Paul W. Pare 5,* and Mohamed-Elamir F. Hegazy 1,6,*

Abstract: The soft coral genus *Sarcophyton* contains the enzymatic machinery to synthesize a multitude of cembrene-type diterpenes. Herein, highly oxygenated cembrenoids, sarcoconvolutum A–E (1–5) were purified and characterized from an ethyl acetate extract of the red sea soft coral, *Sarcophyton convolutum*. Compounds were assembled according to spectroscopic methods including FTIR, 1D- and 2D-NMR as well as HRMS. Metabolite cytotoxicity was tested against lung adenocarcinoma, cervical cancer, and oral-cavity carcinoma (A549, HeLa and HSC-2, respectively). The most cytotoxic compound, (4) was observed to be active against cell lines A549 and HSC-2 with IC50 values of 49.70 and 53.17 µM, respectively.

Keywords: *Sarcophyton convolutum*; sarcoconvolutum A–E; cembrenoids; cytotoxicity

1. Introduction

Natural products and structural analogues are key for drug discovery, especially for pharmacotherapies for cancers and infectious diseases [1–5]. Such biologically active metabolites are characterized by scaffold diversity and structural complexity. For example, within *Sarcophyton* soft coral, isolated metabolites include cembrenoids [2,6–9], diterpene dimers [10,11], sesquiterpenes [12,13], ceramides [14], steroids [15,16], and prostaglandins [17,18]. Cembrenoids in particular exhibit a significant number of sp3 carbon centers and oxygen atoms with no nitrogen or halogen atoms. Higher numbers of H-bond acceptors and donors confer hydrophilicity and ring structures provide significant molecular rigidity. Cembrenoids also exhibit promising biological activities including anti-cancer [6–8], anti-inflammatory [8], antifouling [19,20], ichthyotoxic [21], antifeedant [12], antiviral [22], and neuroprotective [23] properties.

In a pursuit of novel metabolites with biological activity, polyoxygenated cembrene-type diterpenoids (Figure 1) have been isolated from the Red Sea soft coral *S. convolutum*. Metabolite antiproliferative activity was assayed against a non-small-cell lung adenocarcinoma, a cervical cancer, and an oral-cavity squamous-cell carcinoma, A549, HeLa, and HSC-2, respectively.
Figure 1. Sarcoconvolutum A–E (1–5) isolated from S. convolutum.

2. Results and Discussion

Sarcoconvolutum A (1) was isolated as yellow oil with a positive optical rotation (+10.6) in a MeOH solvent. The HRCIMS molecular ion peak at m/z 367.2122 [M + H]⁺ was assigned predicting a molecular formula of C₂₀H₃₀O₆ (calcd. 367.2121, [M + H]⁺) with six degrees of unsaturation suggesting a bicyclic skeleton. FTIR spectrum exhibited bands at 3465 cm⁻¹ and 1765 cm⁻¹ corresponding to hydroxyls and carbonyl groups. From ¹H NMR spectrum (Table 1), two oxygenated protons at δH 3.26 br d (J = 10.8 Hz) and 5.53 d (J = 10.4 Hz), three olefinic protons at δH 4.84 br d (J = 10.4 Hz), 5.48 br d (J = 16.3 Hz), and 5.50 m, in addition to four methyls were at δH 1.28 s, 1.37 s, 1.83 s, and 1.87 s were characterized. Twenty carbon resonances were predicted from ¹³C NMR (Table 1) and categorized into 6 quaternary (including carbonyl at δC 175.3; three olefins at δC 123.1, 143.9 and 162.3; alongside of two oxygenated carbons at δC 74.5 and 84.1), five methines (comprising two oxygenated carbons at δC 71.6 and 79.4; as well as three olefinic at δC 121.1, 127.8, and 134.8), five methylenes, four methyls (δC 8.8, 16.5, 20.3, and 24.2) depending upon DEPT and HSQC experiments. The above analyses of 1D NMR of 1 revealed the cembrene-based diterpenoid [6,7,24]. The 1D NMR of 1 was similar to 12-hydroperoxylsarcoph-10-ene isolated previously from S. glaucum [24]. Observed signal differences for the newly isolated compound included a down field shift of H-7 and C-7 by 0.73 and 12.1 ppm, respectively; a down field shift of 15.3 ppm for C-8; and a down field shift of 6 ppm for Me-19 consistent with the substitution of a hydroxyl group at C-7 instead of an epoxide ring coupling C-7 and C-8. The proposed structure was confirmed by ¹H ¹H COSY and HMBC spectral analysis. H-2 [δH 5.53 d (J = 10.4 Hz)]/C-4 (δC 143.9, 7), and ¹H ¹H COSY of H-2/H-3 [δH 4.84 br d (J = 10.4 Hz)] indicated the location of Δ¹⁰(11). The HMBC correlation of H₃-18 (δH 1.87 s) and C₁₁₂-5 (δC 35.2, 7) followed by ¹H ¹H COSY correlations of H₂-5 [δH 2.18 br d (J = 12.7 Hz)]/H-6 (δH 1.77 br d (J = 13.4 Hz)); H-6/H-7 (δH 3.26 br d (J = 10.8 Hz) confirmed a hydroxyl group at C-7. HMBC correlation of H₃-19 (δH 1.28 s)/C-7 (δC 71.6, 7), H₃-19/C-8 (δC 74.5, 7), and H-7/C-8 (J) established hydroxylation of the C-8 quaternary carbon. Subsequently, HMBC correlation of H₃-19/C₁₁₂-9 (δC 42.9, 7), H₃-20 (δH 1.37 s)/C₁₁₂-11 (δC 134.8, 7), in addition to ¹H ¹H COSY of H₂-9 [δH 2.34 m]/H-10 (δH 5.50 m), H-10/H-11 [(δH 5.48 br d (J = 16.3 Hz)] established Δ¹⁰(11). The down field
shift of C-12 by ca. 7 ppm [24] as well as the HMBC correlation of H-11/C-12 (δC 84.1, J3), H3-20/C-12 (J2), and H3-20/C-13 (δC 34.4 J3) indicated the presence of a hydroperoxide group at C-12. The C-1/C-2 included lactone ring was established via HMBC of H-2/C-16 (keto group, δC 175.3, J3), H-2/C-15 (δC 123.1, J3), H-3 (δH 1.83 s) and H3-16 (J3), H3-17/C-1 (δC 162.3, J3), and H3-17/C-15 (Figure 2).

The relative configuration of 1 is based on coupling constants and NOESY data (Figure 3). From the coupling constant of H-2 (10.4 Hz) along with the vicinal coupling of H-3 (10.4 Hz), the cis orientation and α configuration of H-2 was established [7,24,25]. Starting from this point, the NOESY correlations of H-2α [δH 1.41 ddd (J = 16.1 Hz), H3-19 (δH 3.32 s), H-11/ H-13 (δH 5.53 d (J = 10.8 Hz)] established an α orientation for H-7. NOESY correlations of H-6β [δH 1.41 ddd (J = 13.7, 12.7, 10.9 Hz) H-3-17/C-1 (δH 2.34 m), H-9β (δH 2.34 m) H-11/ H-13β (δH 1.72 br t (J = 13.2 Hz)], H-13β H-20 [δH 1.37 s], H-11/ H-20 indicated β configurations for both Me-19 and Me-20. Based on the described spectral analysis, 1 was identified as 7β,8α-dihydroxy-12α-hydroperoxide-16-keto-cembr-1E,3E,10E-triene (sarcoconvolutum A).

![Figure 2. Key HMBC and 1H-1H COSY of sarcoconvolutum A–E (1–5).](image)

Sarcoconvolutum B (2) was isolated as yellow oil with a positive optical rotation (+43.1) in MeOH. From the HRCIMS molecular ion peak at m/z 367.2122 [M + H]+, the molecular formula of C20H30O6 (calcd. 367.2121, [M + H]+) a bicyclic skeleton with six degrees of unsaturation was predicted. The FTIR broad bands corresponding to hydroxyls and carbonyl groups were detected at 3462 cm−1 and 1768 cm−1. The 1H NMR spectrum (Table 1) displayed two oxygenated protons at δH 3.32 d (J = 10.9 Hz) and 5.45 d (J = 10.2 Hz), three olefinic protons at δH 4.88 d (J = 10.1 Hz), 5.50 d (J = 16.1 Hz), and 5.57 dt (J = 16.1, 7.2 Hz), along with four methyls were at δH 1.28 s, 1.42 s, 1.82 s, 1.85 s. From the observed twenty signals in the 13C NMR spectrum (Table 1), 6 quartenary (including carbonyl) at δC 174.8; three olefinics at δC 123.5, 143.5 and 161.9; alongside of two oxygenated carbons at δC 74.5 and 84.0), 5 methenes (comprising two oxygenated carbons at δC 78.7 and 71.1, as well as three olefinic signals at δC 121.6, 126.9, 134.6), 5 methylenes, 4 methyls (δC 8.9, 16.1, 23.6, and 24.1) were identified based on DEPT and HSQC signals. A down field shift of δC 84.0 by ca. 7 ppm suggested the presence of a hydroperoxyl unit at C-12 [24] which was consistent with mass spectral data. A 13C NMR comparison of the two isolated compounds indicated a down field shift of Me-20 for 2. The 2D NMR comparisons showed otherwise
similar $^1$H $^1$H COSY and HMBC signals (Figure 2). A $^{13}$C NMR down field shift of 3.3 ppm for Me-20 indicated the opposite stereochemistry at C-12.

Relative stereochemistry was determined based on coupling constants and NOESY data (Figure 3). The coupling constant of H-2 (10.2 Hz) in addition to the vicinal coupling of H-3 (10.1 Hz) suggested a cis orientation and α configuration of H-2 [7,24,25]. The NOESY correlations of H-2α [δ$_H$ 5.45 d (J = 10.2 Hz)]/ H$_{18}$-18 [δ$_H$ 1.82 s], H-18/ H-5 [δ$_H$ 2.37 td (J = 13.2t, 2.8 Hz)], H-18/ H-6 [δ$_H$ 1.49 td (J = 13.7t, 3.6 Hz)], H-5α/ H-6, H-6/ H-7 [δ$_H$ 3.32 br d (J = 10.9 Hz)], H-7/ H-19 [δ$_H$ 1.28 s] and H-13β [δ$_H$ 1.95 td (J = 13.1, 4.1 Hz)]/ H$_3$-19 indicated a H-7a Me-19 and Me-20 orientation. Based upon these spectral data, 2 was identified as 7β,8α-dihydroxy-12β-hydroperoxide-16-keto-cembra-1E,3E,10E-triene (sarcoconvolutum B).

Sarcoconvolutum C (3) was isolated as yellow oil with a positive optical rotation (+33.1, c 0.003, CH$_2$OH). The molecular formula was deduced as C$_{24}$H$_{32}$O$_6$ (calcd. 364.2250, [M-OH + H]$^+$) based on a HREIMS molecular ion peak at m/z 364.2258 [M-OH + H]$^+$ indicating a bicyclic skeleton with six degrees of unsaturation. FTIR broad bands corresponded to hydroxyls and carbonyl groups were detected at 3457 cm$^{-1}$ and 1761 cm$^{-1}$. From $^1$H NMR spectrum (Table 1), two oxygenated protons at δ$_H$ 3.27 br d (J = 10.9 Hz) and δ$_H$ 5.49 d (J= 9.9 Hz), three olefinic protons at δ$_H$ 4.86 br d (J = 9.9 Hz), δ$_H$ 5.44 br d (J = 16.1 Hz) and δ$_H$ 5.56 dt (J = 15.6, 8.1 Hz), four methyls as well as one methyl of methoxy group at δ$_H$ 3.23 s were assigned. Based on the $^{13}$C NMR (Table 1), twenty-one carbon resonances were observed and were categorized by DEPT and HSQC analysis as 6 quaternary carbons (including carbonyl at δ$_C$ 174.8; three olefinics at δ$_C$ 123.3, 144.2 and 161.4; alongside of two oxygenated carbons at δ$_C$ 78.3 and 84.4), 5 methines (comprising two oxygenated carbons at δ$_C$ 78.3 and 71.9; as well as three olefinic at δ$_C$ 121.2, 128.2, 134.0), 5 methylenes, 5 methyls (δ$_C$ 9.0, 16.2, 18.2, 21.2, and 49.3). As with 1 and 2, down field shift at δ$_C$ 84.4 ppm of ca. 7 ppm along with a mass shift of [M-H$_2$O], suggested the addition of a hydroperoxy group [24]. The compound is similar to 1 and 2 except for a down field shift of δ$_H$ 78.3 by 3.8 ppm associated with C-8 and a new methoxyl signal at δ$_H$ 3.23 s and δ$_C$ 49.3. HMBC correlations of a methyl proton at δ$_H$ 3.23 and the C-12 at δ$_H$ 78.3

Figure 3. Significant NOESY of sarcoconvolutum A–E (1–5).
confirm the localization of the methoxylation to C-12 (Figure 2). Thus, 3 was identified as 7-hydroxy-8-methoxy-12-hydroperoxide-16-keto-cembra-1E,3E,10E-triene.

Table 1. $^1$H and $^{13}$C NMR (CDCl$_3$, 500 Hz) of sarcoconvolutum A–E (1–5).

| No | Sarcoconvolutum A (1) | Sarcoconvolutum B (2) | Sarcoconvolutum C (3) | Sarcoconvolutum D (4) | Sarcoconvolutum E (5) |
|----|------------------------|------------------------|------------------------|------------------------|------------------------|
|    | $^1$H NMR (f Hz)       | $^1$H NMR (f Hz)       | $^1$H NMR (f Hz)       | $^1$H NMR (f Hz)       | $^1$H NMR (f Hz)       |
|----|------------------------|------------------------|------------------------|------------------------|------------------------|
| 1  | 1.28 s                  | 1.28 s                  | 1.28 s                  | 1.19 s                  | 1.26 s                 |
| 2  | 24.2                    | 24.1                    | 18.2                    | 24.8                    |                        |
| 3  | 5.45 d (10.4)           | 5.45 d (10.9)           | 5.45 d (9.9)            | 5.45 d (9.4) *          | 5.47 d (10.1)          |
| 4  | 78.9                    | 79.0                    | 121.1                   | 121.1                   | 121.1                  |
| 5a | 5.28 d (12.7)           | 5.32 d (13.2, 3.4)      | 5.35 d (13.4, 3.4)      | 5.35 d (13.2, 7.4)      | 5.36 d (13.0, 5.1)     |
| 5b | 2.33 d (13.2, 2.8)      | 2.37 d (13.4, 2.8)      | 2.37 d (13.4, 7.4)      | 2.34 td (13.1, 5.7)     | 2.35 dt (13.0, 7.6)    |
| 6a | 1.77 br d (13.4)        | 1.49 td (13.7, 3.6)     | 1.67 td (13.4, 3.6)     | 1.60 m                   | 1.75 m *               |
| 6b | 1.51 ddd (13.7, 12.7, 10.9) | 1.83 m *                 | 1.44 td (15.3, 4.3)     | 1.82 m *                 | 1.25 m *               |
| 7  | 7.16                    | 7.11                    | 7.19                    | 7.19                    | 7.19                   |
| 8  | 7.45                    | 7.45                    | 7.83                    | 7.83                    | 7.83                   |
| 9a | 2.34 m *                | 2.44 dd (13.7, 7.8)     | 2.34 m *                | 3.72                    | 3.90                   |
| 9b | 42.9                    | 43.4                    | 43.4                    | 3.72                    | 3.90                   |
| 10a | 5.50 m                  | 5.56 dt (16.1, 8.1)     | 5.56 dt (15.6, 8.1)     | 5.56 dt (15.6, 8.1)     | 5.56 dt (15.6, 8.1)    |
| 10b | 128.7                   | 128.2                   | 128.2                   | 128.2                   | 128.2                  |
| 11 | 5.48 br d (16.3)        | 5.50 br d (16.1)        | 5.44 br d (16.1)        | 5.44 br d (16.1)        | 5.44 br d (16.1)       |
| 12 | 134.8                   | 134.6                   | 134.6                   | 134.6                   | 134.6                  |
| 13a | 84.0                    | 84.1                    | 84.1                    | 84.1                    | 84.1                   |
| 13b | 5.32 m *                | 5.49 m *                | 5.49 m *                | 5.49 m *                | 5.49 m *               |
| 14a | 2.01 br t (13.2)        | 2.06 br t (13.7)        | 2.05 br t (13.4)        | 2.05 br t (13.4)        | 2.05 br t (13.4)       |
| 14β | 2.23 br t (13.2)        | 2.26 td (13.7, 4.0)     | 2.27 td (13.2, 5.5)     | 2.27 td (13.2, 5.5)     | 2.27 td (13.2, 5.5)    |
| 15 | 123.1                   | 123.1                   | 123.1                   | 123.1                   | 123.1                  |
| 16 | 178.3                   | 178.4                   | 178.4                   | 178.4                   | 178.4                  |
| 17 | 1.83 s                  | 1.85 s                  | 1.85 s                  | 1.86 s                  | 1.86 s                 |
| 18 | 16.5                    | 16.1                    | 16.1                    | 16.2                    | 16.2                   |
| 19 | 1.28 s                  | 1.28 s                  | 1.28 s                  | 1.28 s                  | 1.28 s                 |
| 20 | 20.3                    | 20.3                    | 20.3                    | 20.3                    | 20.3                   |
| OMe | 3.25 s                  | 3.25 s                  | 3.25 s                  | 3.25 s                  | 3.25 s                 |

* overlapped.

The relative configuration of 3 was deduced via the coupling constants and NOESY analysis (Figure 3). The coupling constant of H-2 (9.9 Hz) and the vicinal coupling of H-3 (9.9 Hz) identified an $\alpha$ and cis orientation for H-2 [7,24,25]. The NOESY experiments exhibited the same configuration of 1 that elucidated the $\alpha$ orientation of H-7 and $\beta$ and both methyls, Me-19 and Me-20. Based on these spectral observations, 3 was identified as 7β-hydroxy-8$\alpha$-methoxy-12α-hydroperoxide-16-keto-cembra-1E,3E,10E-triene (sarcoconvolutum C).

Sarcoconvolutum D (4) was obtained as dark yellow oil with a positive optical rotation ($\delta^{+}$ 37.2, c 0.003, CH$_3$OH). The HREIMS molecular ion peak at $m/z$ 348.1939 [M$^+$] that predicted a molecular formula of C$_{20}$H$_{25}$O$_5$ (calcd. 348.1937, [M$^+$]$^+$. A bicyclic skeleton and seven degrees of unsaturation. The corresponding FTIR broad bands to hydroxyls and carbonyl groups at 3456 cm$^{-1}$ and 1763 cm$^{-1}$ were assigned. The $^1$H NMR (Table 1) revealed two oxygenated protons at $\delta_H$ 2.65 dd ($J$ = 6.8, 3.6 Hz), and 5.45 d ($J$ = 9.4 Hz), three olefinic protons at $\delta_H$ 5.00 d ($J$ = 9.4 Hz), 5.42 m, and 5.47 m, in addition to four methyls at $\delta_H$ 1.31 s, 1.41 s, 1.86 s, and 1.87 s. From $^{13}$C NMR, twenty carbon resonances were characterized and categorized to six quartenary (consisting of carbonyl at C-3, C-7 hydroxy-8-methoxy-12-hydroperoxide-16-keto-cembra-1E,3E,10E-triene).
The relative stereochemistry was identified via coupling constants [7,24,25] and NOESY analysis (Figure 3). Compound 4 exhibited almost same NOESY correlations as I except the correlations of Me-19/H-7, and H-6β/H-7 that elucidated the β orientation of H-7. These data confirmed 4 as 12α-hydroperoxide-16-keto-cembra-1E,3E,10E-triene-7α, 8α-epoxide (sarcoconvolutum E).

Sarcoconvolutum E (5) was obtained as yellow oil exhibiting a positive optical rotation (+61.7, c 0.003, CH3OH). The mass spectroscopy exhibited a HREIMS molecular ion peak at m/z 367.2130 [M + H]+ that was identified as C20H30O6 (calcld. 367.2042, [M + H]+) with a bicyclic skeleton and six degrees of unsaturation. The FTIR broad bands corresponding to hydroxyls and carbonyl groups were detected at 3464 cm⁻¹ and 1759 cm⁻¹. ¹H NMR (Table 1) displayed three oxygenated protons at δH 3.45 br d (J = 10.5 Hz), 4.43 dd (J = 9.0, 5.0 Hz), and 5.47 d (J = 10.1 Hz), one olefinic proton at δH 5.07 s, and 5.13 s, and three methyls at δH 1.26 s, 1.86 s (6H). The ¹³C NMR showed twenty carbon resonances. Six quaternary (including carbonyl at δC 174.9; four olefinics at δC 124.3, 145.9, 146.0 and 161.8; and one oxygenated carbon at δC 73.9), four methenes (comprising two oxygenated carbons at δC 70.0 and 89.3; and one olefinic carbon (at δC 119.2), 7 methylenes (comprising exomethylene carbon at δC 113.4), three methyls (δC 8.9, 16.4, and 24.8) that were characterized via DEPT-135 and HSQC experiments. NMR spectral data of 5 were similar to 1 and 2 except for the following signals: (i) a down-field shift of δC 75.0, by ca. 3.5 ppm associated with C-7; (ii) an downfield shift of δH 3.45 brd (J = 10.5 Hz) by 0.2 ppm associated with H-7; and (iii) an up-field shift of δH 4.43 dd (J = 9.0, 5.0 Hz) by 1 ppm and a shift of ca. 45 ppm of δC 134.0 to 89.0 associated with C-11 indicating the oxygenation of this carbon; (iv) the appearance of δH 1.32 (m, 1.57) associated with H-10 and C-10 (at δC 25.0) indicating a disappearance of δH[10]¹³C); and (v) the presence of δC 146.0 associated with C-12 and signals δH 5.07 s, 5.13 s and δC 113.4 associated with an exomethylene carbon at C-20 indicating the presence of δH[12,20]. These new functional group assignments based on spectral data were consistent with 2D HMBC spectral signals including H3-19 [δH 1.26 s]/C-7 [δC 75.0, δF], H3-19/C-7 [δC 73.9, δF] confirmed the hydroxylation of both C-7 and C-8. The HMBC correlations of H2-20 [at δH 5.07 s, 5.13 s]/C-12 [at δC 146.0, δF], H2-20/C-11 [at δC 89.0, δF], and H2-20/C-13 [at δC 27.8, δF], H11 [at δH 4.43 dd (J = 9.0, 5.0 Hz)]/C-12 [δF], H11/C-13 [δF] confirmed the Δ¹²(20) and the oxygenation of C-11 (Figure 2). Consistent with the mass spectral data and the observed up-field shift of C-11, the oxygenation of C-11 was identified as a hydroperoxide group.

The relative stereochemistry assignment was based the coupling constant of H-2 (10.1 Hz) and the vicinal coupling of H-3 (10.1 Hz) the indicated a α and cis orientation of H-2 [7,24,25]. NOESY correlations (Figure 3) of H-2α [δH 5.47 d (J = 10.1 Hz)]/ H3-18 [δH 1.86 s], H-3α [δH 4.92 br d (J = 10.1 Hz)]]/ H-5α [δH 2.35 dt (J = 13.0, 7.6 Hz)], and H-5α/H-6 [δH 1.75 m], H3-18/H-6, H-5/H-7 [δH 3.45 br d (J = 10.5 Hz)], H-6/H-7, H-7/H3-19 [δH 1.26 s], H3-19/H-9β [δH 1.51 m], H-9β/H-11 [δH 4.43 dd (J = 9.0, 5.0 Hz)], H-13β [δH 2.25 m]/H-identified a β orientation for H-7, H-11 and Me-19. Therefore, 5 was assigned as 7α,8α-dihydroxy-11α-hydroperoxide-16-keto-cembra-1E,3E,12(20)-triene (sarcoconvolutum E). 1D, 2D-NMR, HRMS and FTIR for all isolated secondary metabolites (1–5) were deposited in the supplementary files (see Figures S1–S50).

The initial screening of I–5 (Figure 4) for cytotoxic activity was performed using three cancer cell lines (A549, HeLa and HSC-2) with concentrations of 100, 10 and 1 µM (Figure 4A). Compound 4 showed cytotoxic activity against cell lines A549 (Figure 4B) and HSC-2 (Figure 4C). The dose-dependent toxicity with concentrations range (0.001–100 µg/mL) was examined against A549 and HSC-2 cell lines (Figure 4). Compound 4 showed IC₅₀ values of 49.70 and 53.17 µM against A549 and HSC-2 cells, respectively (Table 2, Figure 4A,B).
values of 49.70 and 53.17 μM against A549 and HSC-2 cells, respectively (Table 2, Figure 4A,B).

Figure 4. Cytotoxic activity: (A) Screening cytotoxic activity against A549, HeLa and HSC2 cell lines; (B) Dose-dependent toxicity with concentrations in a range from 0.001 to 100 μM for compounds 4 against A549; (C) Dose-dependent toxicity with concentrations in a range from 0.001 to 100 μM for compound 4 against HSC2.

Table 2. Cytotoxicity of 1-5 against cancer cell lines.

| Cell Lines | 1    | 2    | 3    | 4          | 5    | Doxorubicin |
|------------|------|------|------|------------|------|-------------|
| A549       | >100 | >100 | >100 | 49.70 ± 0.05 | >100 | 0.42 ± 0.3  |
| HeLa       | >100 | >100 | >100 | 91.98 ± 0.15 | >100 | 1.35 ± 0.16 |
| HSC2       | >100 | >100 | >100 | 53.17 ± 0.03 | 91.39 ± 0.17 | 0.50 ± 2.6 |

3. Materials and Methods

3.1. General Experimental Procedures

A JASCO P-2300 polarimeter (Tokyo, Japan) and shimazu FTIR-8400S instrument (Columbia, MD 21046, USA) was operated for optical rotation and IR spectra, respectively. Then, 1D and 2D NMR spectra were recorded on a Bruker 600 or 500 Hz NMR spectrometer (MA, USA). HR-MS spectra were obtained on a JEOL JMS-700 instrument (Tokyo, Japan). For Chromatography: column chromatography (CC) [silica gel 60 (Merck, 230–400 mesh, Merck, Darmstadt, Germany)]; TLC analysis: [precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm, Merck, Darmstadt, Germany)]. High-performance liquid chromatography (HPLC) equipped with a Jasco PU-980 pump, a Jasco UV-970 intelligent UV/VIS detector at 210 nm and a semi preparative reversed-phase column (Cosmosil C18 column 250 × 10 mm, 5 μm).

3.2. Coral Material

The animal soft coral *Sarcophyton convolutum* was collected from the Egyptian Red Sea (the coast of Hurghada) in March 2017 and was recognized by M Al-Hammady with a voucher specimen (08RS1071). It was deposited in the National Institute of Oceanography and Fisheries, marine biological station, Hurghada, Egypt.

3.3. Extraction and Separation

The frozen soft coral (2.5 kg, total wet weight) was chopped into small pieces and extracted with ethyl acetate at room temperature (3 L × 5 times). The collective extracts were concentrated *in vacuo* to a brown gum. The dried material (95 g) was subjected to a silica gel column (6 × 120 cm) eluting with *n*-hexane (2000 mL), followed by a gradient of
n-hexane-EtOAc up to 100% EtOAc and EtOAc–MeOH up to 50% MeOH (3000 mL each of the solvent mixture). Fractions were collected (SC1-SC6) and monitored via TLC. Fraction (SC3; 1.4 g) was eluted step gradient with n-hexane-EtOAc over silica gel column afforded three main sub fractions (SC3A-C). Sub-fraction SC3A was re-purified by reversed-phase HPLC using MeOH/H2O (6.5:3.5), 3.5 mL/min, to afford 1 (6.1 mg, tR = 27 min), and 4 (11.6 mg, tR = 23 min). The sub-fraction SC3B was subjected to reversed-phase HPLC using MeOH/H2O (3:2), 3.5 mL/min, afforded 2 (10 mg, tR = 28 min). Fraction (SC4; 1.2 g) was further fractionated over silica gel column chromatography eluted by n-hexane/EtOAc step gradient afforded two main sub fractions (SC4A and B). Sub-fraction SC4A was eluted with MeOH/H2O (1:1) over reversed-phase HPLC, 3 mL/min, to afford 3 (7.3 mg, tR = 31 min), and 5 (9.6 mg, tR = 32 min).

3.4. Spectral Data of Sarcoconvolutum A–E (1–5)

7,8-Dihydroxy-12-hydroxy-16-keto-cembra-1E,3E,10E-triene (Sarcoconvolutum A, 1): yellow oil; [α]D25 +43.0 (c 0.03, CH3OH); FT-IR (KBr) νmax: 3465, 2961, 1765, 1435, and 987 cm−1; 1H and 13C NMR data (CDCl3, 500 Hz), see Table 1; HRCIMS m/z 367.2122 (100, [M+H]+); (calcd. 367.2121, for C28H31O6).

7,8-Dihydroxy-12-hydroxy-16-keto-cembra-1E,3E,10E-triene (Sarcoconvolutum B, 2): yellow oil; [α]D25 +10.5 (c 0.03, CH3OH); FT-IR (KBr) νmax: 3461, 2957, 1759, 1439, and 991 cm−1; 1H and 13C NMR data (CDCl3, 500 Hz), see Table 1; HRCIMS m/z 367.2122 (100, [M+H]+); (calcd. 367.2122, for C28H31O6).

7β-Hydroxy-8α-methoxy-12α-hydroperoxide-16-keto-cembra-1E,3E,10E-triene (Sarcoconvolutum C, 3): yellow oil; [α]D25 +37.2 (c 0.03, CH3OH); FT-IR (KBr) νmax: 3456, 2951, 1763, 1446, and 993 cm−1; 1H and 13C NMR data (CDCl3, 500 Hz), see Table 1; HRCIMS m/z 364.2258, [M-OH+H]+; (calcd. 364.2250, [M-OH+H]+, for C27H30O6).

12α-Hydroperoxide-16-keto-cembra-1E,3E,10E-triene-7α,8α-epoxide (sarcoconvolutum D, 4): yellow oil; [α]D25 +37.2 (c 0.03, CH3OH); FT-IR (KBr) νmax: 3456, 2958, 1763, 1442, and 996 cm−1; 1H and 13C NMR data (CDCl3, 500 Hz), see Table 1; HREIMS m/z 348.1939 [M]+; (calcd. 348.1937, for C20H26O6).

12α-Hydroperoxide-11α-hydroperoxide-16-keto-cembra-1E,3E,12(20)-triene (sarcoconvolutum E, 5): yellow oil; [α]D25 +10.5 +61.7 (c 0.03, CH3OH); FT-IR (KBr) νmax: 3464, 2955, 1759, 1433, and 997 cm−1; 1H and 13C NMR data (CDCl3, 500 Hz), see Table 1; HREIMS m/z 367.2130 (100, [M+H]+); (calcd. 367.2042, for C20H31O6).

3.5. Cell Culture and Treatment Conditions

Human cancer cell lines of non-small cell lung adenocarcinoma (A549), squamous cell carcinoma of the oral cavity (HSC-2), and human cervical cancer cell (HeLa). All cell lines were purchased from American Type Culture Collection (ATCC®) and were maintained as monolayer culture in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS, 4 mM l-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Monolayers were passaged at 70–90% confluence using a trypsin-EDTA solution. All cell incubations were maintained in a humidified CO2 incubator with 5% CO2 at 37 °C. All materials and reagents for the cell cultures were purchased from Lonza (Verviers, Belgium).

3.6. Cytotoxicity Assay

A modified MTT (3-[4,5-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay was used for cytotoxicity investigation were performed using based on a previously published method [26]. A549, HeLa and HSC2 cells (5000–10,000 cells/well) were seeded onto 96-well plates and incubated for 24 h with 5% CO2 at 37 °C. The prepared tested compounds (1–5) were screened and the dose-dependent toxicity with concentrations range (0.001–100 µg/mL) were investigated according to our protocol [26]. Doxorubicin (2 mg/mL) was used as positive control.
3.7. Anti-Proliferation Quantitative Analysis

GraphPad Prism® v6.0 software (GraphPad Software Inc., San Diego, CA, USA) were used to calculate the concentration-response curve fit to the non-linear regression for IC_{50} values.

4. Conclusions

Five compounds, sarcocovolutum A–E were isolated from an organic extract of Sarcophyton convolutum tissue. Chemical structures were elucidated based upon spectroscopic analyses. The cytotoxic activity of the isolated compounds was screened against three cancer cell lines (A549, HeLa and HSC2) and 4 was found to be the most active compound against cell lines A549 and HSC-2 with IC_{50} values of 49.70 and 53.17 µM, respectively.

Supplementary Materials: The following are available online https://www.mdpi.com/article/10.390/md19090519/s1, Figures S1–S50: spectra for compounds 1–5.

Author Contributions: Conceptualization, T.A.M., A.I.E., P.W.P. and M.-E.F.H.; Data curation, T.A.M., A.I.E., A.M.A.-T. and M.-E.F.H.; Formal analysis, T.A.M., A.I.E., M.M.A., P.W.P. and S.O.; Investigation, T.A.M., A.I.E. and A.M.A.-T. Methodology, M.-E.F.H.; Project administration, T.A.M. and M.-E.F.H.; Resources, T.A.M., A.I.E., A.M.A.-T. and M.-E.F.H.; Software, T.A.M. and M.-E.F.H.; Supervision, M.-E.F.H.; Visualization, T.A.M. and M.-E.F.H.; Writing—original draft, A.I.E. and M.M.A.; Writing—review and editing, T.A.M., A.I.E., A.M.A.-T., M.M.A., S.O., P.W.P. and M.-E.F.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Research Centre, Giza, Egypt under the project title “Secondary metabolites from Egyptian Soft corals: Chemical and biological investigation against cancer cells” grant number 12060120/2019-2022.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in Supplementary Material.

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

References
1. Ahmed, A.A.; Hegazy, M.-E.F.; Hassan, N.M.; Wojcinska, M.; Karchesy, J.; Pare, P.W.; Mabry, T.J. Constituents of Chrysothamnus viscidiflorus. Phytochemistry 2006, 67, 1547–1553. [CrossRef] [PubMed]
2. Hegazy, M.E.F.; Mohamed, T.A.; Alhammady, M.A.; Shaheen, A.M.; Reda, E.H.; Elshamy, A.I.; Aziz, M.; Paré, P.W. Molecular architecture and biomedical leads of terpenes from red sea marine invertebrates. Mar. Drugs 2015, 13, 3154–3181. [CrossRef] [PubMed]
3. Khalifa, S.A.M.; Elias, N.; Farag, M.A.; Chen, L.; Saeed, A.; Hegazy, M.-E.F.; Moustafa, M.S.; El-Wahed, A.; Al-Mousawi, S.M.; Musharraf, S.G. Marine natural products: A source of novel anticancer drugs. Mar. Drugs 2019, 17, 491. [CrossRef] [PubMed]
4. Nweze, J.A.; Mbaoji, F.N.; Li, Y.-M.; Yang, L.-Y.; Huang, S.-S.; Chigor, V.N.; Eze, E.A.; Pan, L.-X.; Zhang, T.; Yang, D.-F. Potentials of marine natural products against malaria, leishmaniasis, and trypanosomiasis parasites: A review of recent articles. Infect. Dis. Poverty 2021, 10, 1–19. [CrossRef]
5. Hegazy, M.-E.F.; Mohamed, T.A.; Abdel-Latif, F.F.; Alsaid, M.S.; Shahat, A.A.; Pare, P.W. Trochelioid A and B, new cembranoid diterpenes from the Red Sea soft coral Sarcophyton trocheliophorum. Phytochem. Lett. 2013, 6, 383–386. [CrossRef]
6. Hegazy, M.-E.F.; Elshamy, A.I.; Mohamed, T.A.; Hamed, A.R.; Ibrahim, M.A.A.; Ohta, S.; Paré, P.W. Cembrene diterpenoids with ether linkages from Sarcophyton ehrenbergi: An anti-proliferation and molecular-docking assessment. Mar. Drugs 2017, 15, 192. [CrossRef]
7. Hegazy, M.-E.F.; Mohamed, T.A.; Elshamy, A.I.; Hamed, A.R.; Ibrahim, M.A.A.; Ohta, S.; Umeyama, A.; Paré, P.W.; Effert, T. Sarcoehrenbergilides D–F: Cytotoxic cembrene diterpenoids from the soft coral Sarcophyton ehrenbergi. RSC Adv. 2019, 9, 27183–27189. [CrossRef]
8. Lin, W.-Y.; Su, J.-H.; Lu, Y.; Wen, Z.-H.; Dai, C.-F.; Kuo, Y.-H.; Sheu, J.-H. Cytotoxic and anti-inflammatory cembranoids from the Dongsha Atoll soft coral Sarcophyton crassocaule. Bioorg. Med. Chem. 2010, 18, 1936–1941. [CrossRef] [PubMed]
9. Liu, X.; Zhang, J.; Liu, Q.; Tang, G.; Wang, H.; Fan, C.; Yin, S. Bioactive cembranoids from the South China Sea soft coral Sarcophyton elegans. Molecules 2015, 20, 13324–13335. [CrossRef]
10. Jia, R.; Guo, Y.-W.; Chen, P.; Yang, Y.-M.; Mollo, E.; Gavagnin, M.; Cimino, G. Biscembranoids and their probable biogenetic precursor from the Hainan soft coral Sarcophyton tortuosum. *J. Nat. Prod.* 2007, 70, 1158–1166. [CrossRef]

11. Liang, L.F.; Guo, Y.W. Terpenes from the soft corals of the genus Sarcophyton: Chemistry and biological activities. *Chem. Biodivers.* 2013, 10, 2161–2196. [CrossRef] [PubMed]

12. Yasumoto, M.; Mada, K.; Ooi, T.; Kusumi, T. New terpenoid components from the volatile oils of the soft corals Clavularia viridis and Sarcophyton acutangulum. *J. Nat. Prod.* 2000, 63, 1534–1536. [CrossRef]

13. Anjaneyulu, A.S.R.; Rao, V.L.; Sastry, V.G.; Rao, D.V. Trocheliophorin: A novel rearranged sesquiterpenoid from the Indian Ocean soft coral Sarcophyton trocheliophorum. *J. Asian Nat. Prod. Res.* 2008, 10, 597–601. [CrossRef] [PubMed]

14. Cheng, S.-Y.; Wen, Z.-H.; Chiu, S.-F.; Tsai, C.-W.; Wang, S.-K.; Hsu, C.-H.; Dai, C.-F.; Chiang, M.Y.; Wang, W.-H.; Duh, C.-Y. Ceramide and cerebrosides from the octocoral Sarcophyton ehrenbergi. *J. Nat. Prod.* 2009, 72, 465–468. [CrossRef]

15. Anjaneyulu, A.S.R.; Krishna Murthy, M.V.R.; Gowri, P.M. Novel epoxy steroids from the Indian ocean soft coral Sarcophyton crassocaule. *J. Nat. Prod.* 2000, 63, 112–118. [CrossRef]

16. Wang, Z.; Tang, H.; Wang, P.; Gong, W.; Xue, M.; Zhang, H.; Liu, T.; Liu, B.; Yi, Y.; Zhang, W. Bioactive polyoxygenated steroids from the South China sea soft coral, Sarcophyton sp. *Mar. Drugs* 2013, 11, 775–787. [CrossRef]

17. Cheng, Z.-B.; Deng, Y.-L.; Fan, C.-Q.; Han, Q.-H.; Lin, S.-L.; Tang, G.-H.; Luo, H.-B.; Yin, S. Prostaglandin derivatives: Nonaromatic phosphodiesterase-4 inhibitors from the soft coral Sarcophyton ehrenbergi. *J. Nat. Prod.* 2014, 77, 1928–1936. [CrossRef]

18. Sekhar, V.C.; Rao, C.B.; Ramana, H.; Kuwar, M.M.K.; Rao, D.V. New Prostaglandins from the soft coral Sarcophyton ehrenbergi marengellier of Andaman and Nicobar Islands of Indian ocean. *Asian J. Chem.* 2010, 22, 5353–5358.

19. Li, Y.-X.; Wu, H.-X.; Xu, Y.; Shao, C.-L.; Wang, C.-Y.; Qian, P.-Y. Antifouling activity of secondary metabolites isolated from Chinese marine organisms. *Mar. Biotechnol.* 2013, 15, 552–558. [CrossRef]

20. Sun, X.-P.; Wang, C.-Y.; Shao, C.-L.; Li, L.; Li, X.-B.; Chen, M.; Qian, P.-Y. Chemical constituents of the soft coral Sarcophyton influndibuliforme from the South China Sea. *Nat. Prod. Commun.* 2010, 5, 1171–1174. [CrossRef]

21. Iwagawa, T.; Nakamura, S.; Masuda, T.; Okamura, H.; Nakatani, M.; Siro, M. Irregular cembranoids containing a 13-membered carbocyclic skeleton isolated from a soft coral, Sarcophyton sp. *Tetrahedron* 1995, 51, 5291–5298. [CrossRef]

22. Badria, F.A.; Guirguis, A.N.; El-Naggar, W.A. Antibacterial and antifungal agents from Egyptian marine organisms. *Int. J. Pharmacogn.* 1997, 35, 284–287. [CrossRef]

23. Badria, F.A.; Guirguis, A.N.; Perovic, S.; Steffen, R.; Müller, W.E.G.; Schröder, H.C. Sarcophytolide: A new neuroprotective compound from the soft coral Sarcophyton glaucum. *Toxicology* 1998, 131, 133–143. [CrossRef]

24. Hegazy, M.-E.F.; Eldeen, A.M.G.; Shahat, A.A.; Abdel-Latif, F.F.; Mohamed, T.A.; Whittlesey, B.R.; Paré, P.W. Bioactive hydroperoxyxyl cembranoids from the Red Sea soft coral Sarcophyton glaucum. *Mar. Drugs* 2012, 10, 209–222. [CrossRef] [PubMed]

25. Elkhateeb, A.; El-Beih, A.A.; Gamal-Eldeen, A.M.; Alhammady, M.A.; Ohta, S.; Paré, P.F.; Hegazy, M.-E.F. New terpenes from the Egyptian soft coral Sarcophyton ehrenbergi. *Mar. Drugs* 2014, 12, 1977–1986. [CrossRef] [PubMed]

26. Swapana, N.; Tominaga, T.; Elshamy, A.I.; Ibrahim, M.A.A.; Hegazy, M.-E.F.; Singh, C.B.; Suenaga, M.; Imagawa, H.; Noji, M.; Umeyama, A. Kaemgalangol A: Unusual seco-isopimarane diterpenoid from aromatic ginger Kaempferia galanga. *Fitoterapia* 2018, 129, 47–53. [CrossRef] [PubMed]