New antiproliferative cembrane diterpenes from the Red Sea sarcophyton species

Hassan, Hossam M.; Rateb, Mostafa E.; Hassan, Marwa H.; Sayed, Ahmed M.; Shabana, Samah; Raslan, Mai; Amin, Elham; Behery, Fathy A.; Ahmed, Osama M.; Bin Muhsinah, Abdullatif; Gulder, Tobias A.M.; Ramadan Abdelmohsen, Usama

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Abstract: The combination of liquid chromatography coupled to high resolution mass spectrometry (LC-HRESMS)-based dereplication and antiproliferative activity-guided fractionation was applied on the Red Sea-derived soft coral *Sarcophyton* sp. This approach facilitated the isolation of five new cembrane-type diterpenoids (1–5), along with two known analogs (6 and 7), as well as the identification of 19 further, known compounds. The chemical structures of the new compounds were elucidated while using comprehensive spectroscopic analyses, including one-dimensional (1D) and two-dimensional (2D) NMR and HRMS. All of the isolated cembranoids (1–7) showed moderate *in vitro* antiproliferative activity against a human breast cancer cell line (MCF-7), with IC$_{50}$ ranging from 22.39–27.12 µg/mL. This class of compounds could thus serve as scaffold for the future design of anticancer leads.

Keywords: LC-HRESMS; dereplication; cembranoids; *Sarcophyton*; antiproliferative

1. Introduction

Marine natural products are characterized by their diversity in chemical structures, along with biological activities [1]. The Red Sea is considered to be one of the most important marine spots comprising high biodiversity. About 40% of the soft corals that are identified worldwide are native to the Red Sea, however only a few species that have been chemically examined in the last decades [1–3]. The genus *Sarcophyton* (Phylum Cnidaria; Order Alcyonacea; Family Alcyoniidae) contains 46 species with typically mushroom- or toadstool-shaped appearance and it is considered one of the most
abundant Red Sea soft corals [1]. Sarcophyton sp. are well recognized as a rich source for a wide range of terpenoid metabolites. Macrocyclic cembrane-type diterpenoids and their derivatives represent the main chemical defense for Sarcophyton species against natural predators [3–5]. Previous studies concerning the biological activities of cembranoid analogues revealed that they exhibited a wide range of biological activities, including ichthyotoxic [6], anti-inflammatory [7], anti-bacterial [8], neuroprotective [9], and antitumor [10] properties. In addition, a number of Sarcophyton-derived cembranoid analogues (e.g., sarcophine and its hydroxylated derivatives) were investigated as potential competitive cholinesterase inhibitors [11], selective noncompetitive phosphofructokinase inhibitors [12], and a Na+, K+-ATPase inhibitors [13].

Cancer chemoprevention is based on chemical compounds that inhibit or reverse the development of cancer in normal or pre-neoplastic tissue [14]. A lot of novel marine metabolites were identified as anticancer leads during the past 20 years [15]. Our previous work on sarcophine (7) and its analog has illustrated the ability of this class of compounds to inhibit growth, proliferation, and migration of the prostate and breast metastatic cancer cell lines PC-3 and MDA-MB-231 [6].

Looking for new natural products with cytotoxic activity that is applicable in cancer therapy is of utmost importance. The discovery process has improved with the evolution of new spectroscopic techniques. Liquid chromatography coupled to high resolution mass spectrometry (LC-HRESMS) can generate information-rich data sets that can assist in the dereplication of previously reported natural products that are present in the crude extracts prior to a tedious isolation attempt. New biological material was collected and extracted in order to extend our previous investigation of the Red Sea Sarcophyton species [6]. Subsequently, bioactivity-guided isolation assisted by LC-HRESMS metabolic profiling led to the isolation of five new cembrane-type diterpenoids (1–5, Figure 1) that are structurally related to sarcophine (7) which was also isolated along with another known cembranoid, sinumaximol G (6). Testing of all isolated compounds against the MCF-7 breast cancer cell line showed a moderate in vitro growth inhibitory activity.

Figure 1. Structures of isolated compounds (1–7).
2. Results and Discussion

Extraction and fractionation on normal phase (NP) silica gel while using medium pressure liquid chromatography (MPLC) afforded seven major fractions. LC-HRESMS dereplication of these fractions using commercial and public databases led to the putative identification of 19 compounds, which were previously reported from Sarcophyton sp., in addition to 17 molecules with mass data showing no hits in MS databases, most of them in fractions 6 and 7 (Table S1). These LC-HRESMS findings, together with the in vitro cytotoxicity results against MCF-7 cell line using the acquired MPLC fractions, led to prioritization of fractions 6 and 7 for further chromatographic purification on silica gel and Sephadex LH-20 to isolate the active components. Preliminary $^1$H NMR spectroscopic analysis revealed that all the major compounds in fractions 6 and 7 had a cembranoid backbone [16,17], differing either in the degree of oxidation or the configuration of one or more chiral centers.

2.1. Identification of the Isolated Compounds

Compound 1 was obtained as colorless oil. Its molecular formula was established as C$_{22}$H$_{32}$O$_8$ that is based on HRESIMS data (Figure S1) that showed an [M + H]$^+$ ion at m/z 393.2264 (calcd m/z 393.2272). The $^1$H NMR spectrum (Tables 1 and 2), together with the multiplicity-edited HSQC (Figures S2–S5), were in harmony with a sarchophine (7) skeleton that was previously isolated from other Sarcophyton species [6,16,18]. $^1$H NMR and $^{13}$C NMR (Table 2), together with multiplicity-edited HMBC, showed the following characteristic resonances: (i) the presence of an $\alpha$-$\beta$-unsaturated-$\gamma$-lactone functionality: $\delta$C 122.9 (C-15), $\delta$C 161.4 (C-1), and $\delta$C 175.4 (C-16). (ii) Two olefinic moieties at $\delta$C 142.0 (C-4) and $\delta$C $^1$H 122.9/4.81 (C-3), as well as at $\delta$C $^2$H 124.5/ 5.48 (C-10) and $\delta$C $^3$H 140.0/5.47 (C-11). HMBC correlations confirmed the positions of the olefinic moieties (Figure 2 and Figures S6 and S7) H-5/C-4, H-18/C-3, H-9/C-10, H-9/C-11, H-10/C-8, and H-10/C-12. (iii) Three oxygen functionalities; an oxomethine at $\delta$C/ $\delta$C $^1$H 79.2/5.48 (C-2) and two oxygenated quaternary carbons at $\delta$C 74.3 (C-8) and $\delta$C 74.4 (C-12). (iv) Four methyl singlets at [ $\delta$H $^1$H 1.86 (H-17), $\delta$H $^1$H 1.85 (H-18), $\delta$H $^1$H 1.20 (H-19), and $\delta$H $^1$H 1.36 (H-20)], and five methylene groups [ $\delta$H $^1$H 2.14, 1.90 (H-5); $\delta$H $^1$H 1.90, 1.75 (H-6); $\delta$H $^1$H 2.28, 2.20 (H-9); $\delta$H $^1$H 1.70, 1.68 (H-13); and, $\delta$H $^1$H 2.18, 2.48 (H-14)]. HMBC correlations between H$_2$-14 and $\delta$C 161.4 (C-1), H-3 and $\delta$C 35.5 (C-5), H$_2$-5 and $\delta$C 142.0 (C-4) and H$_2$-6 and $\delta$C 35.5 (C-5) and H$_2$-6/H$_2$-5 and $\delta$C 74.4 (C-7) established the carbon linkages from C-14 to C-7. Furthermore, correlations between H$_2$-9 and $\delta$C 74.3 (C-8)/124.5 (C-10)/140.0 (C-11); H-10 and $\delta$C 74.3 (C-8)/74.4 (C-12); H$_2$-13 and $\delta$C 74.4 (C-12)/140.0 (C-11)/23.0 (C-14); and finally, H$_2$-14 and $\delta$C 161.4 (C-1)/79.2 (C-2) established the connectivity of the 14-membered ring. The positions of the methyl groups were recognized through HMBC correlations between $\delta$H $^1$H 1.85 (H$_3$-18, s) and C-3 and C-4; $\delta$H $^1$H 1.20 (H$_3$-19, s) and C-9; $\delta$H $^1$H 1.36 (H$_2$-20, s) and C-11 and C-12; and, $\delta$H $^1$H 1.86 (H$_3$-17, s) and C-1, C-15, and C-16. These NMR spectroscopic features were very similar to the reported spectroscopic data of the known compound sinumaximol G, which was previously isolated from the Soft Coral Sinularia maxima [16] and also isolated in the present work (Compound 6). The $^1$H NMR and $^{13}$C NMR spectroscopic data of compound 1 showed differences in the chemical shifts of C-7/$\delta$C 74.4 and H-7/$\delta$H $^1$H 4.82, which indicated changes of the substitution at C-7. In addition, the presence of additional resonances in the NMR spectral data for acetate moiety [ $\delta$C 170.3 (C-1'), $\delta$C $^3$H 21.1/2.08 (C-2')], along with the HMBC correlations of H$_2$-2'/C-1', H-7/C-1', and H-7/C-19, suggested that the OH group in sinumaximol G at C-7 has been acetylated in compound 1.

The relative configuration of 1 was determined on the basis of coupling constants and NOESY experiments (Figures S8 and S9). The vicinal coupling constant of 14.0 Hz between H-2 ($\delta$H $^1$H 5.48) and H-3 ($\delta$H $^1$H 4.81), together with the $^1$H-$^1$H NOESY correlations (Figure 3) between the methyl protons H$_3$-18 ($\delta$H $^1$H 1.85), and both H-2 and H-7 ($\delta$H $^1$H 4.82), suggested a $\beta$-orientation of both H-2 and H-7. Moreover, the $^1$H-$^1$H NOESY correlations between H-3 ($\delta$H $^1$H 4.81) and H-20 ($\delta$H $^1$H 1.36) suggested that H$_3$-20 were in the $\alpha$-orientation. Alterations in the $^1$H NMR chemical shift of H$_3$-19 ($\delta$H $^1$H 1.2), together with the $^1$H-$^1$H NOESY correlations between H$_3$-19 ($\delta$H $^1$H 1.2) and H$_3$-18 ($\delta$H $^1$H 1.85), indicated changes in the orientation of the methyl group at C-8 to be in the $\beta$-orientation. Therefore, compound 1 was assigned as a new natural product and given the name 7-acetyl-8-epi- sinumaximol G (1).
Table 1. $^1$H NMR spectral data of 1–5 (600 MHz, CDCl$_3$).

| Position | 1 | 2 | 3 | 4 | 5 |
|----------|---|---|---|---|---|
|          | $\delta_H$ (J in Hz) | $\delta_H$ (J in Hz) | $\delta_H$ (J in Hz) | $\delta_H$ (J in Hz) | $\delta_H$ (J in Hz) |
| 2        | 5.48,d,(14.0)  | 5.46,d,(14.0)  | 5.43,d,(14.0)  | 5.43,d(14.0)  | 5.44,d(15.0)  |
| 3        | 4.81,d,(14.0)  | 4.87,d,(14.0)  | 4.85,d,(14.0)  | 4.96,d(14.0)  | 4.95,d(15.0)  |
| 5        | 1.9,m;1.14,m  | 2.22,m;2.38,m  | 2.21,m;2.28,m  | 2.22,m;2.40,m  | 2.22,m;2.47,m  |
| 6        | 1.75,m;1.90,m  | 1.50,m; | 1.9,m  | 1.55,m;2.04,m  | 1.62,m;2.22,m  |
| 7        | 4.82,d,(10.0)  | 3.22,d,(10.0)  | 3.39,d,(10.0)  | 3.53,d,(10.0)  | -              |
| 9        | 2.20,m;2.28,m  | 2.25,m;2.41,m  | 2.53,m;2.68,m  | 2.20,m;2.62,m  | 1.98,m         |
| 10       | 5.48,m  | 5.65,m  | 5.61,m  | 5.76,m  | 2.44,m;2.20,m  |
| 11       | 5.47,d,(16.0)  | 5.53,d,(16.0)  | 5.57,d,(16.0)  | 5.68, d,(16.0)  | 4.73,(6,12.0)  |
| 13       | 1.68,m;1.70,m  | 1.70,m;1.81,m  | 1.75,m;2.23,m  | 1.78,m;1.80,m  | 1.95,m;1.81,m  |
| 14       | 2.18,m;2.48,m  | 2.10,m;2.38,m  | 2.00,m;2.28,m  | 2.20,m;2.32,m  | 2.21,m;2.44,m  |
| 17       | 1.86,s  | 1.87,s  | 1.82,s  | 1.84,s  | 1.77,s         |
| 18       | 1.85,s  | 1.83,s  | 1.82,s  | 1.78,s  | 1.89,s         |
| 19       | 1.20,s  | 1.34,s  | 1.54,s  | 1.31,s  | 1.26,s         |
| 20       | 1.36,s  | 1.34,s  | 1.56,s  | 1.38,s  | 1.53,s         |
| 2'       | 2.08,s  | 2.00,s  |            |            |                |

Table 2. NMR spectral data of 1–5 (150 MHz, CDCl$_3$).

| Position | 1 | 2 | 3 | 4 | 5 |
|----------|---|---|---|---|---|
|          | $\delta_C$, Type | $\delta_C$, Type | $\delta_C$, Type | $\delta_C$, Type | $\delta_C$, Type |
| 1        | 161.4, qC | 161.7 qC | 161.4, qC | 161.7, qC | 163.4, qC |
| 2        | 79.2, CH | 79.3, CH | 79.3, CH | 79.1, CH | 79.3, CH |
| 3        | 122.9, CH | 121.7, CH | 121.4, CH | 122.7, CH | 120.2, CH |
| 4        | 142.0, qC | 143.8, qC | 144.4, qC | 142.7, qC | 144.0, qC |
| 5        | 35.6, CH$_2$ | 35.5, CH$_2$ | 35.5, CH$_2$ | 36.1, CH$_2$ | 31.5, CH$_2$ |
| 6        | 25.3, CH$_2$ | 26.4, CH$_2$ | 27.8, CH$_2$ | 26.7, CH$_2$ | 34.2, CH$_2$ |
| 7        | 74.4, CH | 71.2, CH | 71.7, CH | 63.2, CH | 213.7, qC |
| 8        | 74.3, qC | 74.5, qC | 74.9, qC | 74.9, qC | 78.2, qC |
| 9        | 42.9, CH$_2$ | 42.9, CH$_2$ | 44.6, CH$_2$ | 42.8, CH$_2$ | 35.8, CH$_2$ |
| 10       | 124.5, CH | 124.8, CH | 125.3, CH | 122.7, CH | 25.7, CH$_2$ |
| 11       | 140.0, CH | 139.0, CH | 136.2, CH | 140.4, CH | 123.9, CH |
| 12       | 74.4, qC | 73.1, qC | 82.3, CH$_2$ | 73.1, CH | 135.1, qC |
| 13       | 39.7, CH$_2$ | 39.6, CH$_2$ | 36.8, CH$_2$ | 40.7, CH$_2$ | 39.4, CH$_2$ |
| 14       | 23.0, CH$_2$ | 23.7, CH$_2$ | 22.3, CH$_2$ | 23.8, CH$_2$ | 28.7, CH$_2$ |
| 15       | 122.9, qC | 121.7, qC | 123.4, qC | 122.7, qC | 124.0, qC |
| 16       | 175.4, qC | 175.2, qC | 175.2, qC | 175.4, qC | 175.1, qC |
| 17       | 9.1, CH$_3$ | 9.3, CH$_3$ | 9.3, CH$_3$ | 9.3, CH$_3$ | 8.8, CH$_3$ |
| 18       | 16.4, CH$_3$ | 16.3, CH$_3$ | 16.1, CH$_3$ | 15.5, CH$_3$ | 17.8, CH$_3$ |
| 19       | 24.7, CH$_3$ | 23.7, CH$_3$ | 25.7, CH$_3$ | 23.3, CH$_3$ | 28.7, CH$_3$ |
| 20       | 25.7, CH$_3$ | 27.8, CH$_3$ | 23.6, CH$_3$ | 28.9, CH$_3$ | 15.4, CH$_3$ |
| 1'       | 170.3, qC | 189.9, qC |            |            |                |
| 2'       | 21.1, CH$_3$ | 22.4, CH$_3$ |            |            |                |

The molecular formula of compound 2 was determined to be the same as that of sinumaximol G (6) (C$_{20}$H$_{30}$O$_5$); $m/z$ 351.2160 [M + H]$^+$ ion (calcd $m/z$ 351.2166) (Figure S10). The one-dimensional (1D) and two-dimensional (2D) NMR spectroscopic features (Tables 1 and 2; Figures S11–S15) also indicated that 2 had an almost identical structure as 1 and sinumaximol G (6) [16]. The difference between those two compounds was the deshielding of the H$_3$-19 methyl protons ($\delta_H$ 1.34), which indicated that the configuration of the methyl group at C-8 is altered to the $\beta$-configuration. The $^1$H-$^1$H NOESY correlation (Figure 3) between H$_3$-19 ($\delta_H$ 1.34) and H$_3$-18 ($\delta_H$ 1.83) further confirmed this change, and hence compound 2 was identified as a new natural product, which we named 8-epi-sinumaximol G (2).
Compound 3 was isolated as colorless oil. HRESIMS analysis (Figure S16) resulted in m/z 393.2264 [M + H]^+ ion (calcd m/z 393.2272), giving a molecular formula of C_{22}H_{32}O_6. The 1D NMR spectral analyses (Table 2) together with the multiplicity-edited HSQC spectrum of compound 3 showed remarkable similarity to that of compound 1 (Figures S17–S20). The difference between the two compounds was the change in the location of the acetyl moiety, which HMBC correlations confirmed (Figures 2 and S21) of H-3-20/C-1’ and H3-2'/C-12, and NOESY correlation (Figures 3 and S22) between H-3-20 (δ_H 1.56) and H3-2' (δ_H 2.0). Changes in the 1H NMR chemical shifts of H-7 (δ_H 4.85) and both H-7 (δ_H 3.99) and H3-2' (δ_H 2.00), indicated that H-7 and the acetyl moiety were both in α-orientation. In addition, agreement of the 1H NMR and 13C NMR chemical shifts at C-2 (δ_C 5.43, δ_C 79.3) and C-8 (δ_C 74.9) with that of 6 and the previously reported sinumaximol G (1), together with the NOESY correlations between H-3-18 (δ_H 1.54) and H-7 (δ_H 3.39), and between H3-18 (δ_H 1.82) and H-2 (δ_H 5.43), suggested a β-orientation of H-2 and α-orientation of H-19. Therefore, compound 3 was identified as a new natural product which we named 12-acetyl-7, 12-β-H-7 (NMR of C-12 (δ_C 82.3), along with the NOESY (Figure 3) correlations between H-3 (δ_H 3.39) and the previously reported sinumaximol G (Figures S17–S20). The difference between the two compounds was the change in the location of the acetyl moiety, which HMBC correlations confirmed (Figures 2 and S21) of H-3-20/C-1’ and H3-2'/C-12, and NOESY correlation (Figures 3 and S22) between H-3-20 (δ_H 1.56) and H3-2' (δ_H 2.0). Changes in the 1H NMR chemical shifts of H-7 (δ_H 4.85) and both H-7 (δ_H 3.99) and H3-2' (δ_H 2.00), indicated that H-7 and the acetyl moiety were both in α-orientation. In addition, agreement of the 1H NMR and 13C NMR chemical shifts at C-2 (δ_C 5.43, δ_C 79.3) and C-8 (δ_C 74.9) with that of 6 and the previously reported sinumaximol G (1), together with the NOESY correlations between H-3-18 (δ_H 1.54) and H-7 (δ_H 3.39), and between H3-18 (δ_H 1.82) and H-2 (δ_H 5.43), suggested a β-orientation of H-2 and α-orientation of H-19. Therefore, compound 3 was identified as a new natural product which we named 12-acetyl-7, 12-β-H-7 (NMR of C-12 (δ_C 82.3), along with the NOESY (Figure 3) correlations between H-3 (δ_H 3.39) and the previously reported sinumaximol G (Figures S17–S20). The difference between the two compounds was the change in the location of the acetyl moiety, which HMBC correlations confirmed (Figures 2 and S21) of H-3-20/C-1’ and H3-2'/C-12, and NOESY correlation (Figures 3 and S22) between H-3-20 (δ_H 1.56) and H3-2' (δ_H 2.0). Changes in the 1H NMR chemical shifts of H-7 (δ_H 4.85) and both H-7 (δ_H 3.99) and H3-2' (δ_H 2.00), indicated that H-7 and the acetyl moiety were both in α-orientation. In addition, agreement of the 1H NMR and 13C NMR chemical shifts at C-2 (δ_C 5.43, δ_C 79.3) and C-8 (δ_C 74.9) with that of 6 and the previously reported sinumaximol G (1), together with the NOESY correlations between H-3-18 (δ_H 1.54) and H-7 (δ_H 3.39), and between H3-18 (δ_H 1.82) and H-2 (δ_H 5.43), suggested a β-orientation of H-2 and α-orientation of H-19. Therefore, compound 3 was identified as a new natural product which we named 12-acetyl-7, 12-α-H-7 (NMR of C-12 (δ_C 82.3), along with the NOESY (Figure 3) correlations between H-3 (δ_H 3.39) and the previously reported sinumaximol G (Figures S17–S20). The difference between the two compounds was the change in the location of the acetyl moiety, which HMBC correlations confirmed (Figures 2 and S21) of H-3-20/C-1’ and H3-2'/C-12, and NOESY correlation (Figures 3 and S22) between H-3-20 (δ_H 1.56) and H3-2' (δ_H 2.0). Changes in the 1H NMR chemical shifts of H-7 (δ_H 4.85) and both H-7 (δ_H 3.99) and H3-2' (δ_H 2.00), indicated that H-7 and the acetyl moiety were both in α-orientation. In addition, agreement of the 1H NMR and 13C NMR chemical shifts at C-2 (δ_C 5.43, δ_C 79.3) and C-8 (δ_C 74.9) with that of 6 and the previously reported sinumaximol G (1), together with the NOESY correlations between H-3-18 (δ_H 1.54) and H-7 (δ_H 3.39), and between H3-18 (δ_H 1.82) and H-2 (δ_H 5.43), suggested a β-orientation of H-2 and α-orientation of H-19. Therefore, compound 3 was identified as a new natural product which we named 12-acetyl-7, 12-epi-sinumaximol G (3). Compound 4 was obtained as a colorless solid. Its molecular formula was deduced as C_{20}H_{28}O_4 based on the HRESIMS [M + H]^+ ion at m/z 333.2035 (calcd m/z 333.2066) (Figure S23). The 1H and 13C NMR spectral data (Tables 1 and 2), together with the 2D spectral data of compound 4 (Figures S24–S28),
was closely related to that of sarcophine (7) [18]. HMBC correlations (Figure 2) between H-10 (δH 5.76) and C-9 (δC 42.8)/C-11 (δC 140.4)/C-12 (δC 37.1), H-13 (δH 1.78, 1.80), and C-1 (δC 161.7)/C-11 (δC 140.4)/C-12 (δC 37.1)/C-14 (δC 23.8), and H-20 (δH 1.38) and C-11 (δC 140.4)/C-12 (δC 37.1)/C-13 (δC 40.7) revealed the presence of a double bond at C-10/C-11 and it also indicated the presence of an oxygenated quaternary carbon at C-12. The NOESY correlations (Figure 3 and Figure S29) between the methyl protons H3-18 (δH 1.78) and both H-2 (δH 5.43) and H-7 (δH 3.53), suggested a β-orientation of both H-2 and H-7. Similarly, NOESY correlations between H-7 (δH 3.53) and H3-19 (δH 1.31) indicated a β-orientation of the methyl group at C-8. Additionally, the NOESY correlations between H-3 (δH 4.96) and H3-20 (δH 1.38) suggested the methyl group at C-12 to be in α-orientation. Therefore, compound 4 was identified as a new natural product, to which we gave the name 12-hydroxysarcoph-10-ene (4).

Compound 5 was obtained as colorless oil. The molecular formula was deduced as C20H20O4 based on HRESIMS [M + H]+ ion at m/z 333.2035 (calcd m/z 333.2066) (Figure S30), which indicated seven degrees of unsaturation. The 1H and 13C NMR spectral data (Tables 1 and 2; Figures S31–S33) were in harmony with the skeleton of 8-epi-sarcophine that was previously isolated from the Soft Coral Sorocryptum glauca [19]. Considering 13C NMR chemical shifts, the following functional groups were identified: (i) an α,β-unsaturated-γ-lactone functionality: δC 124.0 (C-15), δC 163.4 (C-1) δC 175.1 (C-16). (ii) olefinic carbons at δC 120.2/144.0 (C-3/C-4) and δC 123.9/135.1 (C-11/C-12), and (iii) a ketone functional group at δC 213.7 (C-7) and alcohol functionalities at δC 79.3 (C-2) and δC 78.2 (C-8). The COSY spectrum (Figure S34) revealed the coupling of four hydrocarbon regions common to sarcophine skeletons: δH 5.44 (d, J = 12.0 Hz, H-2) and 4.95 (d, J = 12.0 Hz, H-3); δH 2.22/2.47 (m, H2-5) and 2.76/2.93 (m, H2-6); δH 1.98 (m, H2-9), 2.20/2.44 (m, H2-10) and 4.70 (br. t, J = 6 Hz, H-11) and δH 1.81/1.95 (m, H2-13) and 2.21/2.44 (m, H2-14). HMBC correlations (Figure 2 and Figure S35) between H-2 and δC 163.4 (C-1), H-2 and δC 120.2 (C-3), H-2 and δC 144.0 (C-4), H-3 and δC 17.8 (C-18), H3-18 and δC 120.2 (C-3), H3-18 and δC 31.5 (C-5), H2-5 and δC 144.0 (C-4), H2-5 and δC 34.2 (C-6) and H2-6/H2-5 and δC 213.7 (C-7); these correlations confirmed the linkage from C-1 to C-7. Additional correlations between H3-19 and δC 213.7 (C-7), H2-9, and δC 78.2 (C-8), H2-10 and δC 35.8 (C-9), H-11 and δC 25.7 (C-10), H2-20 and δC 123.9 (C-11), H2-10 and δC 135.1 (C-12), H2-20 and δC 39.4 (C-13), H2-13 and δC 163.4 (C-1), and H2-14 and δC 163.4 (C-1) confirmed the 14-membered ring skeleton. 1H-1H NOESY correlations (Figures 3 and S36) between H3-18 (δH 1.89) and H-2 (δH 5.44), and between H3-19 (δH 1.26) and both H-3 (δH 4.95) and H-20 (δH 1.53) suggested a β-orientation of H-2 and an α-orientation of H-19. The chemical shifts of H-3 at δH 4.95 and H-11 at δH 4.70 are very characteristic for E configuration at the C-11/C-12 and C-3-C-4 double bonds [6,16,20], which was further confirmed by the 1H-1H NOESY correlations of the two olefinic methyls at C-4 and C-12 with H-2 and H2-10, respectively. Based on these data, compound 5 was considered to be a new natural product, which was named 8-hydroxy-epi-sarcophine (5).

Compounds 6 and 7 were previously isolated from the Soft Corals Sinularia maxima and Sorocryptum glaucum, and identified as sinumaximol G (6) [16] and sarcophine (7) [18], respectively. Their structures were confirmed based on a comparison of their HRMS and NMR data with literate.

2.2. Antiproliferative Activity

Our previous in vitro screening results showed that, among several cancer cell lines, sarcophine (7), along with other analogs, were only active against breast (MDA-MB-231) and prostate (PC-3) cancer cell lines [6]. We consequently chose a breast carcinoma cell line (MCF-7) to assess the in vitro anticaner effect of the isolated compounds (1–7). All of the tested compounds induced dose-dependent cell death with IC50 values (Table 3) of (22.39–27.12 μg/mL). Although all of the tested compounds have significant cytotoxic potentials against MCF-7, they have slightly reduced (approx. two-fold) anticancer effects in comparison to the standard anticancer drug doxorubicin (IC50: 12.78 μg/mL). In the light of these results, along with the previous reports [6,16,19–21] on other membrane-type diterpenoids, we can conclude that the presence of an α, β-unsaturated-γ-lactone moiety is an essential feature for the
antiproliferative properties of these class of compounds. Additionally, changing in the orientation or acetylation of hydroxyl groups at C-7, C-8, and C-12 almost have no effect on their cytotoxicity.

Table 3. In-vitro antiproliferative activity of isolated metabolites (1–7) against MCF-7 cells.

| Tested Compound     | IC50 ± S.D. (µg/mL) a |
|---------------------|-----------------------|
| 1                   | 23.84 ± 0.2           |
| 2                   | 26.22 ± 0.1           |
| 3                   | 26.81 ± 0.2           |
| 4                   | 25.28 ± 0.3           |
| 5                   | 27.2 ± 0.5            |
| 6                   | 24.97 ± 0.3           |
| 7                   | 22.39 ± 0.2           |
| Doxorubicin         | 12.78 ± 0.3           |

a Values are a mean of 3 independent experiments.

3. Materials and Methods

3.1. General Experimental Procedures

Silica gel 60 (Natland, 63–200 µm) and solvent systems consisting of n-hexane-EtOAc (9:5:0.5 to 70:30) were used for column chromatography. Pre-coated silica gel plates (Merck, Darmstadt, Germany, Kieselgel 60 F254, 0.25 mm) were used for TLC analyses. 1% vanillin in concentrated H2SO4 was used as the visualizing reagent. LC/MS was conducted on a Thermo MS system (LTQ XL/LTQ Orbitrap Discovery) coupled to a Thermo Instruments HPLC system with Accela PDA detector: The data were recorded using Xcalibur 2.0.7. 1H and 13C NMR spectra were recorded in CDCl3 on a JEOL ECA-600 spectrometer (600 MHz for 1H and 150 MHz for 13C, respectively). All of the chemical shifts (δ) are given in ppm units with reference to TMS as an internal standard, and coupling constants (J) are reported in Hz.

3.2. Extraction and Fractionation

The soft coral Sarcophyton sp. was collected from the Egyptian Red Sea off the coast of Hurghada (GPS coordinates N 27°15048”, E 33°4903”) at depths of 5–7 m in March 2016 and then frozen for storage. A voucher specimen (NIOF404/2016) was reserved at the National Institute of Oceanography and Fisheries, Red Sea Branch, Invertebrates Department. The frozen soft coral (700 g) was extracted with MeCN aqueous MeCN. Further chromatographic separation of fractions 6 and 7 on silica gel while using n-hexane/EtOAc to afford seven fractions. Only fractions 6 and 7 exhibited in vitro cytotoxicity against MCF-7 cell lines. In addition, LC-HRESMS dereplication results were used to prioritize those fractions, as they showed several unknown molecular formulas.

7-Acetyl-8-epi-sinunaximol G (1): colorless oil; [α]D25 +4.8 (c 0.54, CH2Cl2); 1H NMR and 13C NMR data, see Tables 1 and 2; HR-ESI-MS [M + H]+ m/z 393.2262 (calc. 393.2272, C22H33O6).

8-epi-Sinunaximol G (2): colorless oil; [α]D25 +5.4 (c 0.50, CH2Cl2); 1H NMR and 13C NMR data, see Tables 1 and 2; HR-FAB-MS [M + H]+ m/z 351.2160 (calc. 351.2166, C20H30O5).

12-Acetyl-7, 12-epi- sinunaximol G (3): colorless oil; [α]D25 +1.7 (c 0.58, CH2Cl2); 1H NMR and 13C NMR data, see Tables 1 and 2; HR-ESI-MS [M + H]+ m/z 393.2264 (calc. 393.2272, C22H33O6).

12-Hydroxysarcoph-10-ene (4): colorless oil; [α]D25 +4.6 (c 0.46, CH2Cl2); 1H NMR and 13C NMR data, see Tables 1 and 2; HR-ESI-MS [M + H]+ m/z 343.2840 (calc. 343.2843, C20H38O4).

8-Hydroxy-epi-sarcophine (5): colorless oil; [α]D25 +5.1 (c 0.40, CH2Cl2); 1H NMR and 13C NMR data, see Tables 1 and 2; HR-ESI-MS [M + H]+ m/z 343.2849 (calc. 343.2843, C20H38O4).
Sinumaximol G (6): $[\alpha]_{D}^{25} = +4.1 \ (c=0.48, \ CH_2Cl_2)$; lit. $[\alpha]_{D}^{25} = +3.4 \ (c=0.52, \ CH_2Cl_2)$ [16].

Sarcophine (7): $[\alpha]_{D}^{25} = +79.6 \ (c=0.9, \ CH_2Cl_2)$; lit. $[\alpha]_{D}^{25} = +92 \ (c=1.0, \ CHCl_3)$ [6].

3.3. LC-HRESIMS Analysis and Dereplication

The following conditions were used for LC-HRESIMS analysis: capillary voltage 45 V, capillary temperature 260 °C, auxiliary gas flow rate 10–20 arbitrary units, sheath gas flow rate 40–50 arbitrary units, spray voltage 4.5 kV, and mass range 100–2000 amu (maximum resolution 30000). Gradient separation was achieved while using a SunFire C18 RP analytical HPLC column (5 µm, 4.6 × 150 mm, Waters) with a mobile phase of 0–100% MeOH over 30 min. at a flow rate of 1 mL/min. Multiple available databases (SciFinder: https://sso.cas.org/as/E9dPQ/resume/as/authorization.ping, MarinLit: http://pubs.rsc.org/marinlit/, Dictionary of Natural Products: http://dnp.chemnetbase.com/chemical/ChemicalSearch.xhtml, PubChem: https://pubchem.ncbi.nlm.nih.gov/, Chemspider: http://www.chemspider.com/) were used for the dereplication and annotation of known compounds.

3.4. In Vitro Antiproliferative Activity

The human breast cancer cell line (MCF-7) was obtained from the American Type Culture Collection (ATCC, Manassas, USA). They were seeded in 96 well microtiter plates at a concentration of 1000–2000 cells/well, 100 µL/well. After 24 h, the cells were incubated for 72 h with the compounds to be tested. Dulbecco’s Modified Eagle Medium (DMEM) with 10% foetal calf serum, sodium pyruvate, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C and 5% CO2 was used as culture medium. The medium was discarded at the end of the incubation. The cells were fixed with 150 µL cold trichloroacetic acid with 10% final concentration for 1 h at 4 °C. The plates were washed with distilled water (automatic washer Tecan, Germany) and then stained with 50 µL 0.4% Sulforhodamine B dissolved in 1% acetic acid for 30 min at room temperature in the dark. The plates were washed with 1% acetic acid to remove unbound dye and air-dried (24 h). The dye was solubilized with 150 µL/well of 10 mM tris base (pH 7.4) for 5 min. on a shaker at 1600 rpm. The optical density (OD) of each well was spectrophotometrically measured at 490 nm with an ELISA microplate reader. The mean background absorbance was automatically subtracted and the mean values of each tested compound and doxorubicin concentration was calculated. The experiment was repeated three times for each tested compound. The percentage of cell survival was calculated by using the following formula: surviving percent = [O.D. (treated cells)/O.D. (control cells)] x100. The IC50 values (the concentrations of compound required to produce 50% inhibition of cell growth) were also calculated.

4. Conclusions

The present study provides an additional chemical characterization of the Red Sea soft coral Sarcophyton. LC-HRESMS-based dereplication, in combination with biological activity-guided fractionation, allowed for the accelerated characterization of further new bioactive cembrane-type diterpenoids. All of the isolated compounds demonstrated moderate in vitro antiproliferative activity against breast cancer cell line MCF-7. This finding adds to our existing knowledge and understanding regarding the cytotoxic activity of cembranoid diterpenes, indicating that this class of compounds can be utilized as a potential scaffold for the future design of potent anticancer agents.

Supplementary Materials: The following are available online at http://www.mdpi.com/1660-3397/17/7/411/s1. Figures S1–S36: HRESIMS, 1D and 2D NMR spectra of isolated compounds.

Author Contributions: H.M.H. designed the experiments; H.M.H. and M.E.R. performed the experiments and isolated the compounds; H.M.H., A.M.S., M.H.H., M.R. and M.E.R. performed data acquisition and structure elucidation; O.M.A. performed the biological assays; H.M.H., A.B.M., M.E.R., S.S., M.R., E.A., F.A.B., T.A.M.G., U.R.A. drafted and revised the manuscript.

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