Sarcoeleganolides C–G, Five New Cembranes from the South China Sea Soft Coral Sarcophyton elegans

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Abstract: Five new cembranes, named sarcoeleganolides C–G (1–5), along with three known analogs (6–8) were isolated from soft coral Sarcophyton elegans collected from the Yagong Island, South China Sea. Their structures and absolute configurations were determined by extensive spectroscopic analysis, QM-NMR, and TDDFT-ECD calculations. In addition, compound 3 exhibited better anti-inflammation activity compared to the indomethacin as a positive control in zebrafish at 20 µM.

Keywords: Sarcophyton elegans; sarcoeleganolides C–G; cembranes; anti-inflammation activity

1. Introduction

Soft corals have been recognized as a rich source of nature products with diverse chemical structures. Soft corals of the genus Sarcophyton (family Alcyoniidae) are widely regarded as an important source of cembranoids [1–8]. These marine secondary metabolites are featured by a 14-membered carbocyclic ring [3], and showed a broad spectrum of biological activities, such as anti-inflammatory [9], cytotoxic [10], antibacterial [11], antifouling [12], neuroprotective activities [13]. Due to their complex structures and multiple bioactivities, the level of interest in cembranoids from Sarcophyton soft corals has continued to grow over the years, and impressive achievements have been made. In previous studies, numbers of cembranoids such as sarcomililate A [14], 13-oxo-thunbergol [11], ximaoglauccumins A–F [15], ximaolides H–L [16], and trocheliophols A–S [17] were isolated from Sarcophyton soft corals.

Their fascinating structures and extensive biological activities make them attractive for further investigation. To pursue novel metabolites with bioactivities, a continuous search of the soft coral Sarcophyton elegans collected from the Yagong Island in the South China Sea led to the discovery of five new cembranoids, named sarcoeleganolides C–G (1–5), along with three known analogs, trocheliolide B (6) [18], (−)-sartrochine (7) [19], and 7α-hydroxy-Δ8(19)-deepoxysarcophine (8) [20], as shown in Figure 1. Herein, the isolation, structure elucidation and biological activity of these isolated compounds are reported.
Figure 1. Structures of compounds 1–8.

2. Results

Sarcoeenanolid C (1), which was isolated as a colorless oil, gave a molecular formula of C_{30}H_{40}O_{4} by its HRESIMS ion peak at m/z 317.2114 [M + H]^+\), implying seven degrees of unsaturation. The 1D NMR data (Table 1) and HSQC spectrum of 1 revealed the presence of 20 carbons belonging to four methyls (three olefinic, and one sp\(^3\) hybridized), six methylenes (all sp\(^3\) hybridized), four methines (two olefinic and two oxygenated), and six quaternary carbons (four olefinic, one sp\(^3\) hybridized, and one carbonyl). These data indicate that compound 1 was a cembrane-type diterpenoid.

Table 1. \(^1\)H and \(^{13}\)C NMR data of sarcoeenanolides C–G (1–5).

| No. | \(\delta^1\)H (J in Hz) | \(\delta^1\)C | \(\delta^1\)C (J in Hz) | \(\delta^1\)C_d | \(\delta^1\)C \(\delta^1\)C (J in Hz) | \(\delta^1\)C \(\delta^1\)C (J in Hz) | \(\delta^1\)C \(\delta^1\)C (J in Hz) | \(\delta^1\)C \(\delta^1\)C (J in Hz) |
|-----|-----------------|-------|-----------------|--------|-----------------|-----------------|-----------------|-----------------|
| 1   | 160.7, qC       | 158.3, qC | 160.0, qC       | 151.9, qC | 161.6, qC       |
| 2   | 4.94, m         | 79.1, CH | 108.3, qC       | 4.91, d | 78.4, CH        | 148.2, qC       | 5.45, d         | 79.9, CH        |
| 3   | 2.77, d, (4.2)  | 61.5, CH | 5.16, s         | 120.6, CH | 125.3, s        |
| 4   | 61.5, qC        | 143.8, qC | 139.0, qC       | 72.0, qC | 144.7, qC       |
| 5a  | 1.37, m         | 38.8, CH₂ | 2.20, m         | 40.2, CH₂ | 2.04, t         |
| 5b  | 2.08, m         | 2.20, m | 2.03, m         | 2.16, m | 2.32, m         |
| 6a  | 2.20, m         | 23.7, CH₂ | 2.35, m         | 24.6, CH₂ | 5.36, td        |
| 6b  | 2.08, m         | 2.14, m | 2.03, m         | 2.03, m | 2.20, CH₂       |
| 7   | 5.05, t, (7.2)  | 124.3, CH | 5.02, t         | 125.7, CH | 5.19, d         |
| 8   | 135.2, qC       | 134.3, qC | 141.9, qC       | 141.1, qC | 135.5, qC       |
| 9a  | 2.11, m         | 38.8, CH₂ | 2.29, m         | 37.0, CH₂ | 1.76, m         |
| 9b  | 2.18, m         | 2.03, m | 2.10, m         | 38.7, CH₂ | 2.03, m         |

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Figure 1. Structures of compounds 1–8.

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Table 1. \(^1\)H and \(^{13}\)C NMR data of sarcoeenanolides C–G (1–5).

| No. | \(\delta^1\)H (J in Hz) | \(\delta^1\)C | \(\delta^1\)C (J in Hz) | \(\delta^1\)C_d | \(\delta^1\)C \(\delta^1\)C (J in Hz) | \(\delta^1\)C \(\delta^1\)C (J in Hz) | \(\delta^1\)C \(\delta^1\)C (J in Hz) | \(\delta^1\)C \(\delta^1\)C (J in Hz) |
|-----|-----------------|-------|-----------------|--------|-----------------|-----------------|-----------------|-----------------|
| 1   | 160.7, qC       | 158.3, qC | 160.0, qC       | 151.9, qC | 161.6, qC       |
| 2   | 4.94, m         | 79.1, CH | 108.3, qC       | 4.91, d | 78.4, CH        | 148.2, qC       | 5.45, d         | 79.9, CH        |
| 3   | 2.77, d, (4.2)  | 61.5, CH | 5.16, s         | 120.6, CH | 125.3, s        |
| 4   | 61.5, qC        | 143.8, qC | 139.0, qC       | 72.0, qC | 144.7, qC       |
| 5a  | 1.37, m         | 38.8, CH₂ | 2.20, m         | 40.2, CH₂ | 2.04, t         |
| 5b  | 2.08, m         | 2.20, m | 2.03, m         | 2.16, m | 2.32, m         |
| 6a  | 2.20, m         | 23.7, CH₂ | 2.35, m         | 24.6, CH₂ | 5.36, td        |
| 6b  | 2.08, m         | 2.14, m | 2.03, m         | 2.03, m | 2.20, CH₂       |
| 7   | 5.05, t, (7.2)  | 124.3, CH | 5.02, t         | 125.7, CH | 5.19, d         |
| 8   | 135.2, qC       | 134.3, qC | 141.9, qC       | 141.1, qC | 135.5, qC       |
| 9a  | 2.11, m         | 38.8, CH₂ | 2.29, m         | 37.0, CH₂ | 1.76, m         |
| 9b  | 2.18, m         | 2.03, m | 2.10, m         | 38.7, CH₂ | 2.03, m         |
Table 1. Cont.

| No. | \( \delta^1H \) (J in Hz) | \( \delta^1C \) | \( \delta^2H \) (J in Hz) | \( \delta^2C \) | \( \delta^3H \) (J in Hz) | \( \delta^3C \) | \( \delta^4H \) (J in Hz) | \( \delta^4C \) | \( \delta^5H \) (J in Hz) | \( \delta^5C \) |
|-----|--------------------------|--------|-------------------------|--------|-------------------------|--------|-------------------------|--------|-------------------------|--------|
| 10a | 2.26, m                  | 24.4, m| 2.06, m                 | 24.2, m| 2.22, m                 | 24.3, m| 2.11, m                 | 24.3, m| 1.71, m                 | 34.4, m|
| 10b | 2.20, m                  | 1.34, m| 24.2, m                 | 2.20, m| 1.71, m                 | 21.0, m| 1.71, m                 | 17.1, m| 1.71, m                 | 17.1, m|
| 11  | 5.09, t, (6.6)           | 126.4, CH| 2.69, dd, (9.6, 3.3) | 61.5, CH| 2.30, dd, (10.5, 2.5) | 58.8, CH| 4.86, d, (4.0) | 125.9, CH| 3.98, t, (6.5) | 72.1, CH|
| 12  | 133.6, qC                | 61.6, qC| 59.9, qC                | 131.7, qC| 151.9, qC              | 174.9, qC| 174.9, qC              | 174.9, qC| 174.9, qC              | 174.9, qC|
| 13a | 2.04, m                  | 36.6, CH| 1.68, m                 | 34.0, CH| 1.09, m                 | 35.2, CH| 2.33, m                 | 36.2, CH| 2.24, m                 | 32.1, CH|
| 13b | 2.45, m                  | 1.89, m| 34.0, CH                | 1.63, m| 2.33, m                 | 23.3, m| 2.17, m                 | 21.7, m| 2.17, m                 | 21.7, m|
| 14a | 2.74, m                  | 24.9, CH| 2.45, m                | 23.4, CH| 1.74, m                 | 22.0, CH| 2.54, m                 | 22.5, CH| 2.26, m                 | 27.0, CH|
| 14b | 2.39, m                  | 1.24, m| 1.94, m                | 1.59, m| 1.94, m                 | 2.54, m| 2.46, m                 | 24.6, m| 2.46, m                 | 24.6, m|
| 15  | 124.0, qC                | 126.4, qC| 124.0, qC              | 124.0, qC| 123.2, qC              | 124.2, qC| 124.2, qC              | 124.2, qC| 124.2, qC              | 124.2, qC|
| 16  | 174.4, qC                | 172.2, qC| 173.9, qC              | 170.0, qC| 170.0, qC              | 174.9, qC| 174.9, qC              | 174.9, qC| 174.9, qC              | 174.9, qC|
| 17  | 1.83, s                  | 8.8, CH3| 1.90, s                 | 8.8, CH3| 1.61, s                 | 8.8, CH3| 1.93, s                 | 9.3, CH3| 1.87, s                 | 9.0, CH3|
| 18  | 1.53, s                  | 17.9, CH3| 1.57, s                | 15.9, CH3| 1.34, s                 | 18.3, CH3| 1.45, s                 | 32.9, CH3| 1.78, s                 | 15.9, CH3|
| 19  | 1.58, s                  | 16.1, CH3| 1.66, s                | 15.0, CH3| 1.46, s                 | 22.4, CH3| 1.66, s                 | 17.2, CH3| 1.64, s                 | 17.1, CH3|
| 20  | 1.68, s                  | 17.0, CH3| 1.29, s                | 16.6, CH3| 1.11, s                 | 17.3, CH3| 1.60, s                 | 17.1, CH3| 5.19, s                 | 110.9, CH2|
| 21  | 3.14, s                  | 50.2, CH3| 169.4, qC              | 3.21, s| 55.1, CH3               |        |                        |        |                        |        |
| 22  | 1.65, s                  | 20.9, CH3|                        |        |                        |        |                        |        |                        |        |

\( ^a \) Spectra recorded in chloroform -d4. \(^b\) Spectra recorded in benzene -d6. \(^c\) Spectra recorded at 600 MHz. \(^d\) Spectra recorded at 150 MHz. \(^e\) Spectra recorded at 500 MHz. \(^f\) Spectra recorded at 125 MHz.

The planar framework of 1 was elucidated by \(^1H-\(^1H\) COSY and HMBC spectra (Figure 2). Four spin systems were established by the \(^1H-\(^1H\) COSY correlations from \(H-2\) to \(H-3\); \(H-5\) to \(H-7\); \(H-9\) to \(H-11\), and \(H-13\) to \(H-14\). As previously reported, 3, 4-epoxy-cembranolides [21,22], a trisubstituted epoxide ring located at C-3 and C-4, were deduced by the downfield chemical shift of C-3 (\(\delta^1C 61.5\)) and C-4 (\(\delta^1C 61.5\)) and HMBC correlations from \(H_3-18\) to C-3, C-4, and C-5. Based on the above data, together with the key HMBC correlation from \(H_3-19\) to C-7, C-8, and C-9; \(H_3-20\) to C-11, C-12, and C-13; \(H-17\) to C-1, C-15, and C-16; \(H-14a\) (\(\delta^1H 2.74\)) to C-1, C-2, and C-15 the connection of the carbon skeleton was permitted. Thus, compound 1 was deduced as a cembranoid possessing a trisubstituted epoxide. In the NOESY spectrum of 1 (Figure 3), the correlations of \(H_3-19/H-6a\) (\(\delta^1H 2.20\), \(H_3-20/H-10a\) (\(\delta^1H 2.26\)) indicate that the \(\Delta^7\) and \(\Delta^{11}\) double bonds could be of an E-configuration. The NOESY correlation of \(H-2/H_3-18\) indicate that these protons were on the same side. In addition, considering the geometry of the 3-(\(E\))-olefin in co-isolates, the epoxide of 1 should be in an anti-relationship between H-3 and \(H_3-18\), which was further confirmed by the \(^13C\) NMR chemical shift calculation for the DP4 calculations (Supplementary Materials, Figures S1 and S2) [23]. Finally, the absolute configurations of 1 were defined as 2S, 3R, and 4R by TDDFT-ECD calculations (Figure 4).
The planar framework of 1 was elucidated by 1H–1H COSY and HMBC spectra (Figure 2). Four spin systems were established by the 1H-1H COSY correlations from H-2 to H-3; H-5 to H-7; H-9 to H-11, and H-13 to H-14. As previously reported, 3, 4-epoxy-cembranolides [21,22], a trisubstituted epoxide ring located at C-3 and C-4, were deduced by the downfield chemical shift of C-3 (δC 61.5) and C-4 (δC 61.5) and HMBC correlations from H3-18 to C-3, C-4, and C-5. Based on the above data, together with the key HMBC correlation from H3-19 to C-7, C-8, and C-9; H3-20 to C-11, C-12, and C-13; H3-17 to C-1, C-15, and C-16; H-14a (δH 2.74) to C-1, C-2, and C-15 the connection of the carbon skeleton was permitted. Thus, compound 1 was deduced as a cembranoid possessing a trisubstituted epoxide. In the NOESY spectrum of 1 (Figure 3), the correlations of H3-19/H-6a (δH 2.20), H3-20/H-10a (δH 2.26) indicate that the Δ7 and Δ11 double bonds could be of an E-configuration. The NOESY correlation of H-2/H-18 indicates that these protons were on the same side. In addition, considering the geometry of the 3-(E)-olefin in co-isolates, the epoxide of 1 should be in an anti-relationship between H-3 and H3-18, which was further confirmed by the 13C NMR chemical shift calculation for the DP4 + calculations (Supplementary Materials, Figures S1 and S2) [23]. Finally, the absolute configurations of 1 were defined as 2S, 3R, and 4R by TDDFT-ECD calculations (Figure 4).

Figure 2. Selected 1H–1H COSY and HMBC correlations of compounds 1–5.

Figure 3. Key NOESY and 1D-NOE correlations of 1–5.
1.89) suggests these protons were on the opposite side. Finally, the absolute configuration
(Figure 2) from the H$_3$-21/H-13a (δH 1.68) indicate that these protons were all co-facial. Moreover, the NOESY correlation of H$_3$-20/H-13b (δH 1.89) suggests these protons were on the opposite side. Finally, the absolute configuration of 2 was defined by TDDFT-ECD calculations (Figure 4).

Sarcoeleganolide D (2), a colorless oil, had a molecular formula of C$_{21}$H$_{30}$O$_4$ on the basis of its HRESIMS ion peak at m/z 347.2221 [M + H]$^+$, requiring seven degrees of unsaturation. The $^1$H and $^{13}$C NMR data of 2 (Table 1) resemble that of (−)-sartrochine (7), a known cembranoid previously isolated from the soft coral Sarcophyton trochilifrons. In fact, the structure of 2 was truly similar to 7, with the exception of a methoxyl at C-2 in 2 instead of the proton in 7. This deduction was further proven by the HMBC correlation (Figure 2) from the H$_3$-21 (δH 3.14) to C-2, along with the significant downfield shift observed for C-2 (δC 108.3). Then, the relative configurations of 2 were deduced on the basis of the NOESY experiment (Figure 3). The NOESY correlations of H-3/H$_2$-5 (δH 2.20 and δH 2.20), and H$_3$-19/H-6a (δH 2.35) established the E geometry of the Δ$^3$ and Δ$^7$ double bonds. The NOESY correlations of H$_3$-21/H-13a (δH 1.68), and H-11/13a (δH 1.68) indicate that these protons were all co-facial. Moreover, the NOESY correlation of H$_3$-20/H-13b (δH 1.89) requires seven degrees of freedom.

Sarcoeleganolide E (3), a colorless oil, possessed the molecular formula C$_{22}$H$_{30}$O$_5$, as indicated by its HRESIMS ion peak at m/z 397.1991 [M + Na]$^+$. The comparison of the 1D NMR data (Table 1) of 3 and 6 indicate similarities between them. The 2D NMR data of 3 (Figure S3 and Figure 2) indicate the plane structure was identical to 6, suggesting that 3 should be a stereoisomer of 6. The relative configurations of 3 were deduced by the NOESY spectrum (Figure 3). By the NOESY correlation of H$_3$-19/H-7, the geometry of the Δ$^7$ double bonds was assigned to be a Z-configuration, which was further confirmed by the downfield chemical shift of C-19 (δC 22.4), revealing the major difference in configurations between 3 and 6. The E geometry of the Δ$^3$ double bonds was established by the observed NOESY correlations of H-3/H$_5$-5a (δH 1.38) and H-2/H$_5$-18. Based on the above data, the NOESY correlations of H$_3$-18/H-5b (δH 2.04) indicate the inverse orientation of H-2 and H-3, which was further confirmed by the coupling constants (J$_{2,3}$ = 10.0 Hz). The diagnostic NOESY correlations of H-13a (δH 1.09)/H-11, and H-13a/H-2 assigned H-11 and H-2 were all co-facial. The NOESY correlations of H-6/H-3, H-6/H-9a (δH 2.61), and H$_3$-20/H-9a suggest that H$_3$-20, H-3, and H-6 were on the same side of the ring system. Hence, the relative configurations of 3 were deduced, and finally, the absolute configurations of 3 were defined by TDDFT-ECD calculations (Figure 4).
was established. The relative configurations of 4 were established by the \(^{13}\)C NMR chemical shift calculations for the DP4\(^+\) calculations (Supplementary Materials, Figures S3 and S4), the configurations were defined as \(4S\). A survey of the literature revealed that the 1D NMR data of compound 5 (Table 1) were similar to those of compound 8, a known cembrane diterpenoid isolated from the Red Sea soft coral *Sarcophyton glaucum*. In fact, compound 5 had the same functional groups as 8, except for the migration of the \(\Delta^8\) double bonds in 8 to the \(\Delta^{12}\) double bonds in 5, and the hydroxy group at the C-7 position in 8 to C-11 in 5. These variations of the functional groups were further proven by the HMBC correlations from H-20 to C-11, C-12, and C-13, and from H-3-19 to C-7, C-8, and C-9. Furthermore, other detailed HMBC correlations and \(^{1}H\)-\(^{1}H\) COSY correlations helped complete the planar framework of 5 (Figure 2). In the NOESY spectrum of 5 (Figure 3), the correlations of H-3/H-5a (\(\delta_H 4.30\)) and H-11/H-13 (\(\delta_H 2.33\)) established the \(E\) geometries of the \(\Delta^7\) and \(\Delta^{13}\) double bonds. By the NOESY cross-peaks of H-3/H-6 (\(\delta_H 4.30\)) and H-11/H-12 (\(\delta_H 2.54\)) the Z geometry of the \(\Delta^6\) double bonds. By the \(^{13}\)C NMR chemical shift calculations for the DP4\(^+\) calculations (Supplementary Materials, Figures S3 and S4), the configurations were defined as \(4R\) and \(6S\), which were further confirmed by the NOESY correlations of H-3/H-5a (\(\delta_H 2.16\)) and H-6/H-5a. Finally, the absolute configurations of 4 were defined by TDDFT-ECD calculations (Figure 4).

Sarcoeleganolide G (5) was isolated as a colorless oil with a molecular formula of C\(_{20}\)H\(_{26}\)O\(_{3}\), established by the HRESIMS ion peak at \(m/z\) 339.1928 [M + Na]\(^{+}\). A survey of the literature revealed that the 1D NMR data of compound 5 (Table 1) were similar to those of compound 8, a known cembrane diterpenoid isolated from the Red Sea soft coral *Sarcophyton glaucum*. In fact, compound 5 had the same functional groups as 8, except for the migration of the \(\Delta^8\) double bonds in 8 to the \(\Delta^{12}\) double bonds in 5, and the hydroxy group at the C-7 position in 8 to C-11 in 5. These variations of the functional groups were further proven by the HMBC correlations from H-20 to C-11, C-12, and C-13, and from H-3-19 to C-7, C-8, and C-9. Furthermore, other detailed HMBC correlations and \(^{1}H\)-\(^{1}H\) COSY correlations helped complete the planar framework of 5 (Figure 2). In the NOESY spectrum of 5 (Figure 3), the correlations of H-3/H-5a (\(\delta_H 2.20\)), H-2/H-13, and H-7/H-9 (\(\delta_H 2.03\)) indicate that the geometries of \(\Delta^7\) and \(\Delta^{13}\) double bonds were of an \(E\)-configuration. By the NOESY correlations of H-2/H-14a (\(\delta_H 2.21\)) and H-11/H-14a (\(\delta_H 2.25\)), the relative configurations were defined as \(2S\) and \(11R\). Finally, the absolute configurations of 5 were defined by TDDFT-ECD calculations (Figure 4).

Although the anti-inflammatory activity of cembranoids in zebrafish models has been reported previously [24], it is still not very common. Hence, we aimed to seek newer cembranoids with anti-inflammatory activity in zebrafish models. These new compounds (1–5) were evaluated for anti-inflammatory activity in CuSO\(_{4}\)-induced transgenic fluorescent zebrafish. CuSO\(_{4}\) can produce an intense acute inflammatory response in the neuromast and mechanosensory cells in the lateral line of zebrafish, stimulating the infiltration of macrophages [25–27]. Then the number of macrophages surrounding the neuromast in the zebrafish was observed and imaged under a fluorescence microscope (Supplementary Materials, Section 3). The results are shown in Figure 5. In CuSO\(_{4}\)-induced transgenic fluorescent zebrafish, compound 3 could alleviate migration and decreased the number of macrophages surrounding the neuromast in the zebrafish, showing stronger anti-inflammatory activity than the indomethacin, which was used as the positive control at 20 \(\mu\)M, while other compounds showed no anti-inflammatory activity, as shown in Figure 5.
3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were measured on a Jasco P-1020 digital polarimeter (Jasco, Tokyo, Japan). The UV spectra were recorded on a Beckman DU640 spectrophotometer (Beckman Ltd., Shanghai, China). The CD spectra were obtained on a Jasco J-810 spectropolarimeter (Jasco, Tokyo, Japan). The NMR spectra were measured by Agilent 500 MHz (Agilent, Beijing, China), JEOL JNMECP 600 spectrometers (JEOL, Beijing, China). The HRESIMS spectra were measured on Micromass Q-Tof Ultima GLOBAL GAA076LC mass spectrometers (Autospec-Ultima-TOF, Waters, Shanghai, China). Semi-preparative HPLC was performed using a Waters 1525 pump (Waters, Singapore) equipped with a 2998 photodiode array detector and a YMC C18 column (YMC, 10 × 250 mm, 5 µm). Silica gel (200–300 mesh, 300–400 mesh, and silica gel H, Qingdao Marine Chemical Factory, Qingdao, China) was used for column chromatography.

3.2. Animal Material

The soft coral Sarcophyton elegans was collected from Xisha Island (YaGong Island) in the South China Sea in 2018 and frozen immediately after collection. The specimen was identified by Ping-Jyun Sung, at the Institute of Marine Biotechnology, the National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan. The voucher specimen (No. xs-18-yg-114) was deposited at the State Key Laboratory of Marine Drugs, Ocean University of China, People’s Republic of China.

3.3. Extraction and Isolation

A frozen specimen of Sarcophyton elegans (7.2 kg, wet weight) was homogenized and then exhaustively extracted with CH3OH six times (3 days each time) at room temperature. The combined solutions were concentrated in vacuo and were then subsequently desalted by redissolving with CH3OH to yield a residue (178.0 g). The crude extract was subjected to silica gel vacuum column chromatography eluted with a gradient of petroleum/acetone (400:1–1:1, v/v) and subsequently eluted with a gradient of CH2Cl2/MeOH (20:1–1:1, v/v) to obtain fourteen fractions (Frs.1–Frs.14). Each fraction was detected by TLC. Frs.5

Figure 5. (a) Quantitative analysis of macrophages in the region of inflammatory sites in zebrafish treated with sarcoeleganolides C–G (1–5) in zebrafish at 20 µM. (b) Images of inflammatory sites in CuSO4-induced transgenic fluorescent zebrafish (Tg:zlyz-EGFP) expressing enhanced green fluorescent protein (EGFP) treated with sarcoeleganolides C–G (1–5), using indomethacin as a positive control. ### Indicates that the CuSO4 model group shows very significant differences compared to the control group (p < 0.01). ** Indicates that the sample groups show significant differences compared to the CuSO4 model group (p < 0.01).
was subjected to a silica gel vacuum column chromatography (petroleum/acetone, from 100:1 to 1:1, v/v) to give three subfractions Frs.5.1–Frs.5.3. Frs.5.1 was separated by semi-preparative HPLC (ODS, 5 µm, 250 × 10 mm; MeOH/H₂O, 70:30, v/v; 1.5 mL/min) to afford 1 (5.0 mg, t_R = 72 min). Frs.5.2 was separated by semi-preparative HPLC (ODS, 5 µm, 250 × 10 mm; MeOH/H₂O, 65:35, v/v; 1.5 mL/min) to afford 2 (3.7 mg, t_R = 70 min). Frs.6 was subjected to silica gel vacuum column chromatography (petroleum/acetone, from 100:1 to 1:1, v/v) to give two subfractions, Frs.6.1–Frs.6.2. Frs.6.2 was separated by semi-preparative HPLC (ODS, 5 µm, 250 × 10 mm; MeOH/H₂O, 65:35, v/v; 1.5 mL/min) to afford 4 (2.0 mg, t_R = 54 min) and 5 (3.5 mg, t_R = 27 min). Frs.7 was subjected to silica gel vacuum column chromatography (petroleum/acetone, from 50:1 to 1:1, v/v) to give six subfractions, Frs.7.1–Frs.7.6. Frs.7.4 was separated by semi-preparative HPLC (ODS, 5 µm, 250 × 10 mm; MeOH/H₂O, 65:35, v/v; 1.5 mL/min) to afford 3 (2.0 mg, t_R = 48 min).

Sarcoeleganolide C (1): colorless oil; [α]_25D +23.3 (c 1.0, MeOH); UV (MeOH) λ_max (log ε) = 200 (0.91) nm; HRESIMS m/z 317.2114 [M+H]^+ (calcd. for C_{20}H_{20}O_5^+, 317.2111). For ^1H NMR and ^13C NMR data, see Table 1.

Sarcoeleganolide D (2): colorless oil; [α]_25D −36.7 (c 1.0, MeOH); UV (MeOH) λ_max (log ε) = 200 (0.92) nm; HRESIMS m/z 347.2221 [M + H]^+ (calcd. for C_{21}H_{31}O_4^+, 347.2217). For ^1H NMR and ^13C NMR data, see Table 1.

Sarcoeleganolide E (3): colorless oil; [α]_25D +45.5 (c 0.5, MeOH); UV (MeOH) λ_max (log ε) = 197 (2.13) nm; HRESIMS m/z 397.1991 [M + Na]^+ (calcd. for C_{22}H_{30}O_5Na^+, 397.1985). For ^1H NMR and ^13C NMR data, see Table 1.

Sarcoeleganolide F (4): colorless oil; [α]_25D +66.2 (c 0.5, MeOH); UV (MeOH) λ_max (log ε) = 201 (2.25) nm, 280 (1.58) nm; HRESIMS m/z 364.2481 [M + NH_4]^+ (calcd. For C_{21}H_{31}O_4N^+, 364.2482) and 369.2034 [M + Na]^+ (calcd. For C_{21}H_{30}O_5Na^+, 369.2036). For ^1H NMR and ^13C NMR data, see Table 1.

Sarcoeleganolide G (5): colorless oil; [α]_25D +54.2 (c 0.5, MeOH); UV (MeOH) λ_max (log ε) = 195 (0.57) nm; HRESIMS m/z 339.1928 [M + H]^+ (calcd. for C_{20}H_{20}O_3Na^+, 339.1931). For ^1H NMR and ^13C NMR data, see Table 1.

3.4. Anti-Inflammatory Activity Assay

Healthy macrophage fluorescent transgenic zebrafish (Tg: zlyz-EGFP) was provided by the Biology Institute of the Shandong Academy of Science (Jinan, China). Zebrafish maintenance and the anti-inflammatory assay were carried out as previously described [26]. Each zebrafish larva was photographed by a fluorescence microscope (AXIO, Zom.V16), and the number of macrophages around the nerve mound was calculated using Image-Pro Plus 6.0 software (Rockville, MD, USA) [28]. One-way analysis of variance was performed using GraphPad Prism 7.00 software (San Diego, CA, USA) [29]. Sarcoeleganolides C–G (1–5) were tested for anti-inflammatory activities with zebrafish models. Three days post-fertilization (dpf) healthy macrophage fluorescent transgenic zebrafish were used as animal models to evaluate the anti-inflammatory effects of 1–5.

4. Conclusions

In our search for soft coral Sarcophyton elegans collected from the South China Sea, five new cembranes, named sarcoeleganolides C–G (1–5), and three known analogs, trocheliolide B (6), (−)-sartrochine (7), and 7α-hydroxy-Δ^{19} deepoxysarcophine (8), were isolated. In addition, their structures and absolute configurations (1–5) were determined by extensive spectroscopic analysis, QM-NMR, and TDDFT-ECD calculations. Among them, compound 3 showed better anti-inflammatory activity, compared to the indomethacin as the positive control at 20 µM in the zebrafish model. This research enriches the chemical libraries of soft coral Sarcophyton elegans and provides a basis for developing new drugs.
