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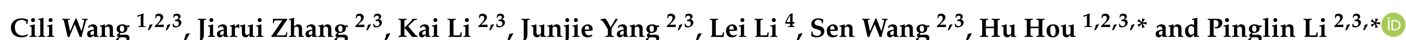
Terpenoids from the Soft Coral *Sinularia densa* Collected in the South China Sea



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Abstract: The chemical investigation of the South China Sea soft coral *Sinularia densa* has resulted in the isolation of seven new terpenoids, including two new meroterpenoids, namely sinudenoids F–G (**1**–**2**), and five new cembranes, namely sinudenoids H–L (**3**–**7**). Their structures and absolute configurations were elucidated based on extensive analyses of spectroscopic data, single-crystal X-ray diffraction, comparison with the literature data, and quantum chemical calculations. Among them, sinudenoid F (**1**) and sinudenoid G (**2**) are rare meroterpenoids featuring a methyl benzoate core. Sinudenoid H (**3**) possesses a rare carbon skeleton of 8, 19-bisnorfuranocembrenolide, which is the second reported compound with this skeleton. In a bioassay, sinudenoid H (**3**) exhibited better anti-inflammatory activity compared to the positive control indomethacin at 20 μM in $CuSO_4$-treated transgenic fluorescent zebrafish. Moreover, sinudenoid J (**5**) and sinudenoid L (**7**) exhibited moderate anti-thrombotic activity in arachidonic acid (AA)-induced thrombotic zebrafish at 20 μM.

Keywords: *Sinularia densa*; soft coral; terpenoids; anti-inflammatory activity; anti-thrombotic activity

Citation: Wang, C.; Zhang, J.; Li, K.; Yang, J.; Li, L.; Wang, S.; Hou, H.; Li, P. Terpenoids from the Soft Coral *Sinularia densa* Collected in the South China Sea. *Mar. Drugs* **2024**, *22*, 442. https://doi.org/10.3390/md22100442

Academic Editors: Concetta Imperatore, Eva Zubía and Chuanbin Shen

Received: 30 August 2024
Revised: 26 September 2024
Accepted: 26 September 2024
Published: 27 September 2024

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1. Introduction

Soft corals of the genus *Sinularia* (order Malacalcyonacea, family Sinulariidae) are one of the most widely distributed soft coral genera across the oceans. Since the first report of this genus in 1975 [1], more than 150 species of this genus have been identified, and more than 70 species have been chemically investigated [2]. As an important source of marine-derived natural products, the genus has yielded over 700 metabolites, such as diterpenes [3], norditerpenes [4], sesquiterpenes [5], steroids [6], and other types [7,8]. The majority of secondary metabolites isolated from the genus *Sinularia* are terpenoid derivatives [2], and have exhibited diverse biological activities, including anti-inflammatory [9], cytotoxic [10], antifouling [11], antibacterial [12], and anti-HBV activities [13]. The genus *Sinularia* deserves further investigation due to its chemical diversity and extensive biological activities.

In our recent chemical investigations of the Xisha soft coral *Sinularia densa*, which is the first systematic investigation for this genus, five C_{19}-Norcembranoid diterpenes with unusual scaffolds, named sinudenoids A–E, were discovered, showing the chemical diversity of this genus [14]. In order to find more bioactive metabolites, our ongoing research on the soft coral Xisha from *Sinularia densa* (Sinulariidae) has uncovered seven undescribed substances (**1**–**7**) (Figure 1). Here, we report the isolation, structure elucidation, and anti-inflammatory and anti-thrombotic effects of these isolated compounds.

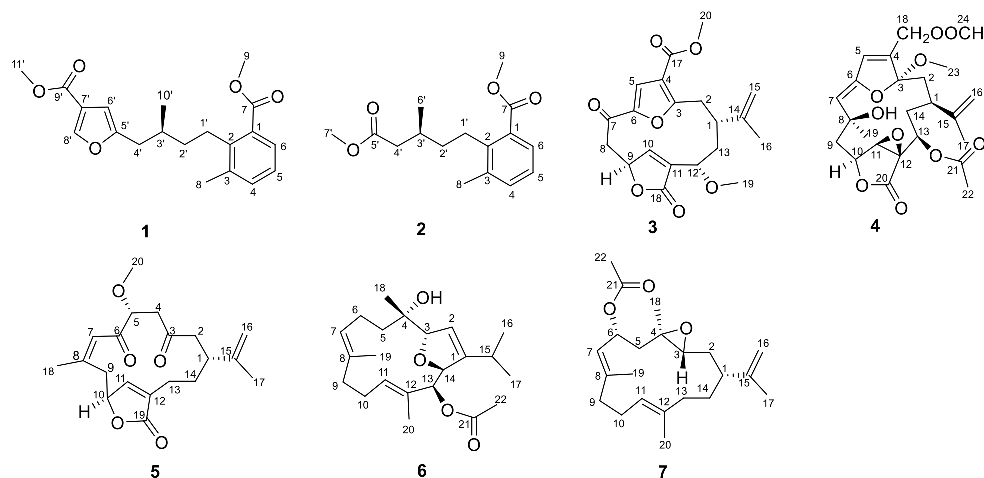

Figure 1. Structures of **1**–**7** from the soft coral *Sinularia densa*.

2. Results

Sinudenoid F (**1**) was isolated as a colourless oil, and its molecular formula was determined as $C_{20}H_{24}O_5$ by the HRESIMS ion peak at m/z 367.1511 [M + Na]$^+$, implying nine degrees of unsaturation. Analysis of the ^{1}H and ^{13}C NMR data of **1** (Table S1) revealed the presence of 20 carbons, including seven non-protonated carbons (five olefinic and two carbonyl), six methines (one sp^3 hybridized and five olefinic), three methylenes (all sp^3 hybridized), and four methyls (two linked to oxygen, one to a sp^3 carbon and one to an olefinic carbon).

The presence of an isopentyl group was confirmed by the ^{1}H–^{1}H COSY correlations from H-1′ to H-4′ and H-3′ to H$_3$-10′ (Figure 2). Subsequently, the presence of a trisubstituted benzene ring was supported by the HMBC correlations from H$_3$-8 to C-2 (δ_C142.0), C-3 (δ_C137.4), and C-4 (δ_C133.9); H-5 to C-1; and H-6 to C-7, together with the ^{1}H–^{1}H COSY correlation from H-4 to H-6. Furthermore, the HMBC correlation from H$_3$-9 to C-7 (δ_C169.0) indicated the presence of a methyl ester group at C-1 of the aromatic ring. Considering the aforementioned data, the HMBC correlations from H-1′ to C-1 and C-2 indicated the attachment of the isopentyl group to the trisubstituted benzene ring at C-2. Moreover, the presence of a furan ring located at C-4′ was inferred by the HMBC correlations from H-8′ to C-5′ and C-7′; H-6′ to C-5′ and C-7′; and H-4′ to C-5′, together with the downfield chemical shifts of C-5′ (δ_C157.0) and C-8′ (δ_C146.4). The HMBC correlation from H$_3$-11′ to C-9′ (δ_C164.1) indicated the presence of a methyl ester group, which was further confirmed by its molecular formula and the requirement of degrees of unsaturation. Due to the absence of the key HMBC correlation from either H-6′ to C-9′ or H-8′ to C-9′, the connection between C-7′ and C-9′ was established through a meticulous comparison of the 1D NMR data between **1** and 3-furancarboxylic acid [15], in conjunction with the absence of a substituent at C-7′. Consequently, the planer structure of **1** was established. Finally, the absolute configuration of **1** was defined as 3′*S* in the TDDFT-ECD calculations (Figure 3).

Sinudenoid G (**2**), a colourless oil, had a molecular formula of $C_{16}H_{22}O_4$, as established by the HRESIMS ion peak at m/z 301.1412 [M + Na]$^+$, which was 66 mass units less than that of **1**. The ^{1}H and ^{13}C NMR data of **2** (Table S2) resemble those of **1**, with the exception of the disubstituted furan ring at C-4′ in **1** replaced by a methyl ester group in **2**. This alteration was confirmed by the HMBC correlations (Figure 2) from H$_3$-7′ to C-5′ (δ_C173.6) and H-4′ to C-5′. Finally, the absolute configuration of **2** was defined as 3′*S* in the TDDFT-ECD calculations (Figure 3).

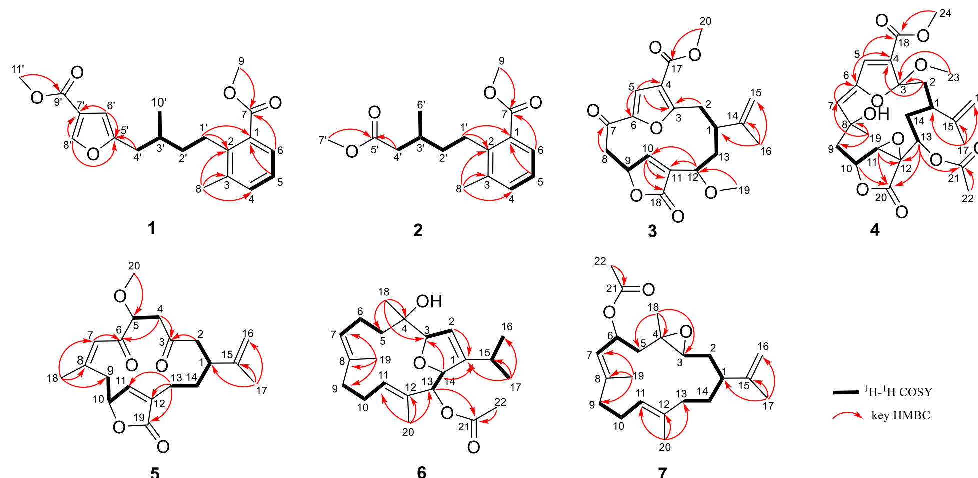

Figure 2. Selected ^{1}H–^{1}H COSY and HMBC correlations of **1**–**7**.

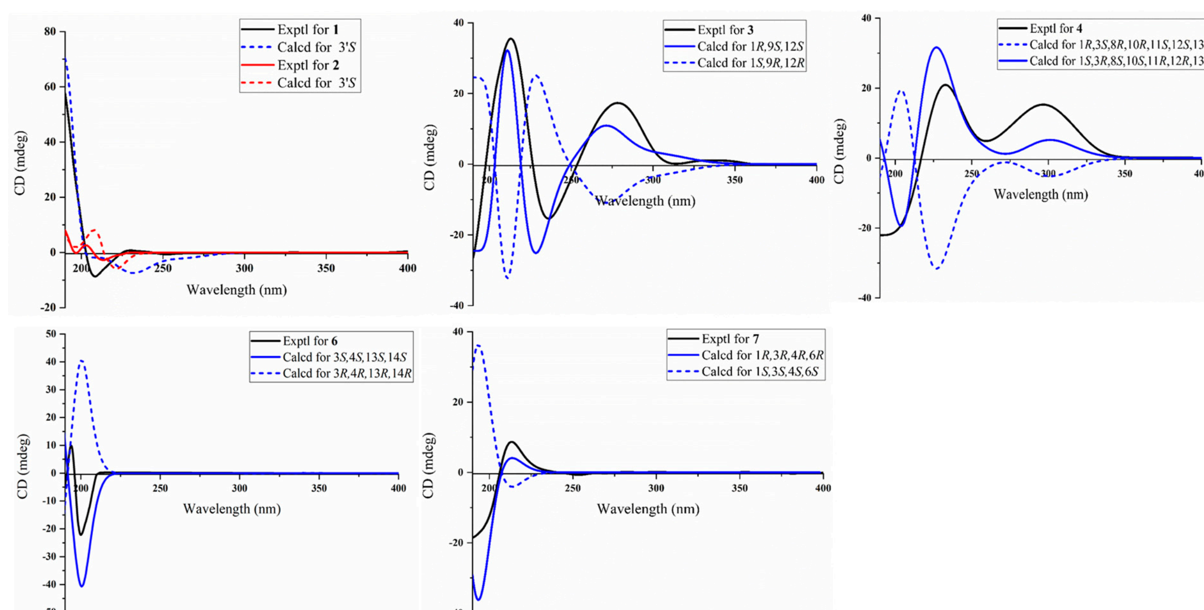

Figure 3. Experimental and calculated ECD spectra of **1**–**4** and **6**–**7**.

Sinudenoid H (**3**) was isolated as a colourless oil, with a molecular formula of $C_{20}H_{22}O_7$ deduced from the HRESIMS ion peak at m/z 375.1448 $[M + H]^+$, implying ten degrees of unsaturation. The 1D NMR data of **3** (Table S3) resemble those of sarcofuranocembrenolide A, a known 8, 19-bisnorfuranocembrenolide previously isolated from the soft coral *Sarcophyton* sp. [16]. In fact, the structure of **3** closely resembles sarcofuranocembrenolide A, differing in the presence of a methoxy group at C-12 in **3** instead of an ethoxy group in sarcofuranocembrenolide A (Figure S1). The HMBC correlation (Figure 2) from H_3-19 (δ_H 3.19) to C-12 confirmed these functional group disparities. In the NOESY spectrum of **3** (Figure 4), the correlation of H-12 (δ_H 4.08)/H-1 (δ_H 2.43) suggested their co-facial orientation. Due to the absence of definitive NOESY correlations, the configuration of C-9 was determined as S^* using the DP4$^+$ calculation (Supporting Information, Figures S2 and S3). Moreover, the TDDFT/ECD calculations also supported the 1*R*, 9*S*, 12*S*-configurations of **3** (Figure 3), instead of the 1*R*, 9*R*, 12*S* configurations (Figure S10).

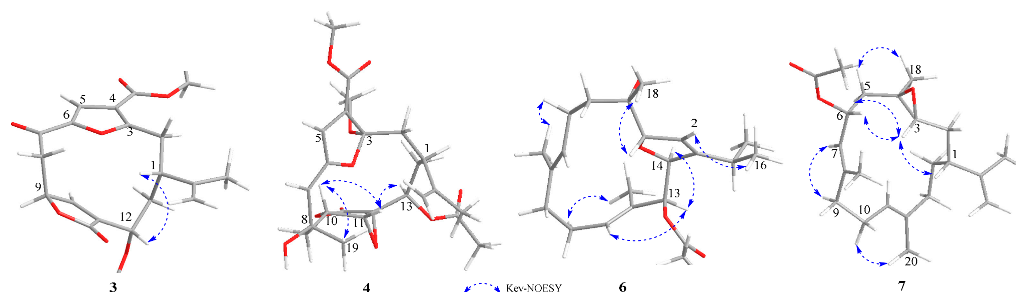

Figure 4. NOESY correlations of **3**–**4** and **6**–**7**.

Sinudenoid I (**4**) was isolated as a colourless oil. Its molecular formula was determined to be $C_{24}H_{30}O_{10}$ from the sodium adduct ion peak at *m*/*z* 501.1729 [M + Na]$^+$, implying ten degrees of unsaturation. Analysis of the 1D NMR data (Table S4) and HSQC spectrum of **4** suggested the presence of twenty-four carbons, including nine non-protonated carbons (three olefinic, three carbonyl, and three oxygenated), six methines (one sp^3 hybridized, two olefinic, and three oxygenated), four methylenes (three sp^3 hybridized and one olefinic), and five methyls (one linked to a carbonyl, one to an oxygenated sp^3 carbon, two to oxygen atoms, and one to an olefinic carbon). These dates indicated that **4** was a polycyclic diterpenoid.

Upon scrutinizing the NMR data of **4**, it was found that the 1D NMR data resemble that of sinumaximol C, a diterpenoid previously isolated from the soft coral *Sinularia maxima* [17]. In fact, the structure of **4** was truly similar to sinumaximol C (Figure S1), except for the presence of an additional acetoxy group at C-13 in compound **4** in comparison to sinumaximol C. This deduction was further proven by HMBC correlations (Figure 2) from H_3-22 (δ_H 2.01) to C-21 (δ_C 170.7), and H-13 (δ_H 4.84) to C-21. The 1D NOE correlations (Figure 4) from H-10 to H-11, H_3-19, along with the NOESY correlation between H-13 and H-11, indicated that H-10, H-11, H-13, and H-19 were positioned on the same face. Subsequently, the similar chemical shifts of carbons from C-3 to C-12 compared with sinumaximol C indicated the 8*R**, 10*R**, 11*S**, and 12*S** configurations in **4**, which was same as those of sinumaximol C. In the absence of definitive NOESY correlations, the relative configurations of C-1 and C-3 were determined as 1*R**, 3*S** by the DP4$^+$ calculation (Supporting Information, Figures S4 and S5). Finally, the absolute configurations of **4** were defined as 1*S*, 3*R*, 8*S*, 10*S*, 11*R*, 12*R*, and 13*R* using the TDDFT-ECD calculations (Figure 3).

Sinudenoid J (**5**) was purified as a colourless crystal, with a molecular formula of $C_{20}H_{26}O_5$ determined from its HRESIMS ion peak at *m*/*z* 369.1674 [M + Na]$^+$. The ^{1}H and ^{13}C NMR data (Table S5) of **5** resemble those of gyrosanolide D (Figure S1), a known cembranoid previously isolated from the soft coral *Sinularia gyrosa* [18]. The difference in the planar structures between **5** and gyrosanolide D was that the hydroxy group at C-5 in gyrosanolide D was replaced by a methoxy group in **5**. This deduction was further proven by the HMBC correlation (Figure 2) from H_3-20 (δ_H 3.41) to C-5 (δ_C 82.6). Due to the lack of effective NOESY correlations, the geometry of the double bond and the configurations of chiral centres in compound **5** could not be definitively determined. However, an X-ray crystallographic experiment with the Cu Kα radiation of **5** (Figure 5) confirmed the *Z*-geometry of the Δ^7 double bond. Subsequently, the absolute configurations of **5** were defined as 1*R*, 5*R*, and 10*S*, indicating that the geometry of the Δ^7 double bond and the configuration at C-5 in **5** differ from those of gyrosanolide D.

Sinudenoid K (**6**) was isolated as a colourless oil. Its molecular formula of $C_{22}H_{32}O_4$ was deduced from the sodium adduct ion peak at *m*/*z* 361.2374 [M + H]$^+$, indicating seven degrees of unsaturation. Analysis of the 1D NMR data (Table S6) and HSQC spectrum of **6** revealed the presence of twenty-two carbons, including five non-protonated carbons (three olefinic, one carbonyl, and sp^3 hybridized), seven methines (one sp^3 hybridized, three olefinic, and three oxygenated), four methylenes (all sp^3 hybridized), and six methyls (one linked to a carbonyl, one to an oxygenated sp^3 carbon, two to sp^3 carbons and two to olefinic carbons). These dates indicated that **6** possessed a cembrane nucleus.

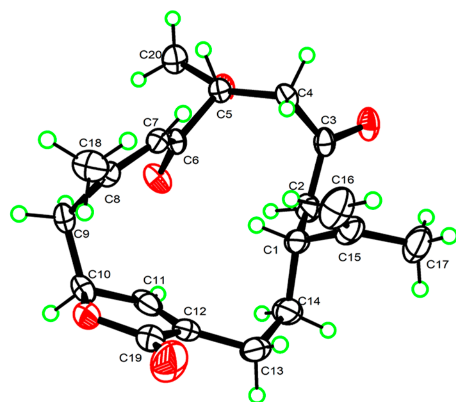

Figure 5. Perspective ORTEP drawings of the X-ray structures of **5** (displacement ellipsoids are drawn at the 50% probability level).

Upon analysis the NMR data of **6**, it was found that the 1D NMR data of **6** resemble that of klyflaccicembranols H, a cembranoid previously isolated from the soft coral *Klyxum flaccidum* [19]. In fact, the structure of **6** was truly similar to that of klyflaccicembranol H (Figure S1), except for the presence of the trisubstituted double bond at C-11, C-12, and C-20 in **6,** instead of a trisubstituted epoxide ring in klyflaccicembranol H. This deduction was further proven by the HMBC correlations (Figure 2) from the H_3-20 (δ_H 1.53) to C-11 (δ_C 133.3), C-12 (δ_C 129.2), and C-13 (δ_C 81.2).

In the NOESY spectrum of **6** (Figure 4), the correlations of H_3-19/H-6a (δ_H 2.42), H-11/H_3-13, and H_3-20/H-10a (δ_H 2.29) indicated that the Δ^7 and Δ^{11} double bonds exhibited *E*-geometry. The correlation of H-2/H_3-16 (δ_H 1.16) suggested that the Δ^2 double bond had a *Z*-geometry. Additionally, the NOESY cross-peak of H_3-18/H-3 suggested that these protons were co-facial. Furthermore, the correlation from H-14 to H-13 in the 1D-NOE difference spectrum indicated that H-13 and H-14 were on the same side. In the absence of the key NOESY correlations, the relative configurations of these chiral centres were determined as 3*S**, 4*S**, 13*S**, and 14*S** using the DP4$^+$ calculation (Supporting Information, Figures S6 and S7). Finally, the absolute configurations of **6** were defined through the TDDFT-ECD calculations (Figure 3).

Sinudenoid L (**7**) was isolated as a colourless oil, with a molecular formula of $C_{22}H_{34}O_3$, as indicated by its HRESIMS ion peak at m/z 369.2399 [M + Na]$^+$. The 1D NMR data of **7** (Table S7) resemble those of (7*E*,11*E*)-3,4-epoxy-7,11,15-cembratriene (Figure S1), a cembranoid previously isolated from a south Pacific soft coral [20]. The only difference was the replacement of one hydrogen atom of methylene at C-6 in the known cembranoid with an acetoxy group in **7**. The differentiation was supported by the HMBC correlations (Figure 2) from H_3-22 to C-21 (δ_C 170.1) and H-6 (δ_H 5.66) to C-21.

Then, the relative configurations of **7** were determined by the analysis of the NOESY spectrum (Figure 4). The NOESY correlations of H-7/H-9a (δ_H 1.98) and H_3-20/H-10a (δ_H 2.25) were used to establish the *E*-geometry of the Δ^7 and Δ^{11} double bonds. Additionally, the NOESY correlations of H-1/H-3 and 5a (δ_H 1.45)/H-3 indicated that these protons were all co-facial. Moreover, the NOESY correlations of H-5b/H_3-18 indicate these protons were also co-facial. By utilizing the ^{13}C NMR chemical shift calculations for the DP4$^+$ calculations (Supporting Information, Figures S8 and S9), in conjunction with the NOESY correlation of H-3/H-6, the configuration of C-6 was defined as *R**. Finally, the TDDFT/ECD calculations of **7** also supported the 1*R*, 3*R*, 4*R*, and 6*R*-configurations (Figure 3), instead of the 1*R*, 3*R*, 4*R*, and 6*S*-configurations (Figure S11).

In the bioassay, these new compounds (**1–7**) were evaluated for anti-inflammatory activity in $CuSO_4$-treated transgenic fluorescent zebrafish. The results (Figure 6) showed that **3** could reduce migration and decrease the number of macrophages surrounding the neuromasts in zebrafish, displaying better anti-inflammatory activity with an inhibition rate of 56.8% compared to the indomethacin positive control with an inhibition rate of

38.4% at 20 µM. Moreover, due to the limited availability, **5** and **7** were selectively evaluated for anti-thrombotic activity in arachidonic acid (AA)-induced thrombotic zebrafish. In arachidonic acid (AA)-induced thrombotic zebrafish, **5** and **7** enhanced the staining intensity of erythrocytes in the heart and inhibited the area of caudal vein thrombosis, demonstrating anti-thrombotic activity at 20 µM (Figure 7).

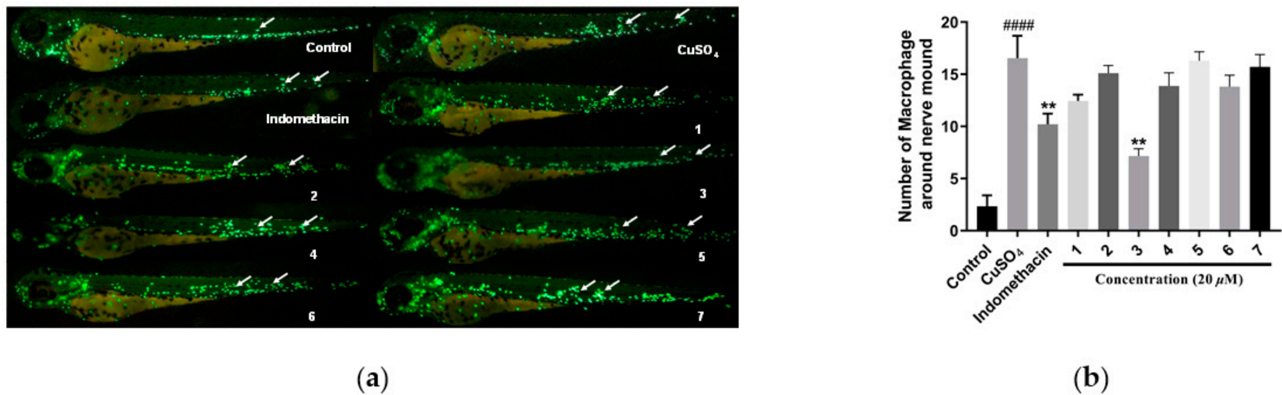

Figure 6. Anti-inflammatory assays of **1**–**7**. (**a**) Images of inflammatory sites in $CuSO_4$-treated transgenic fluorescent zebrafish (Tg:zlyz-EGFP) expressing enhanced green fluorescent protein (EGFP) treated with **1** through **7**, using indomethacin as a positive control. (**b**) Quantitative analysis of macrophages in the region of inflammatory sites in zebrafish treated with **1** through **7**. #### indicates that the $CuSO_4$ model group has a very significant difference compared with the control group ($p < 0.01$). ** indicates that sample groups have significant differences compared with the $CuSO_4$ model group ($p < 0.01$).

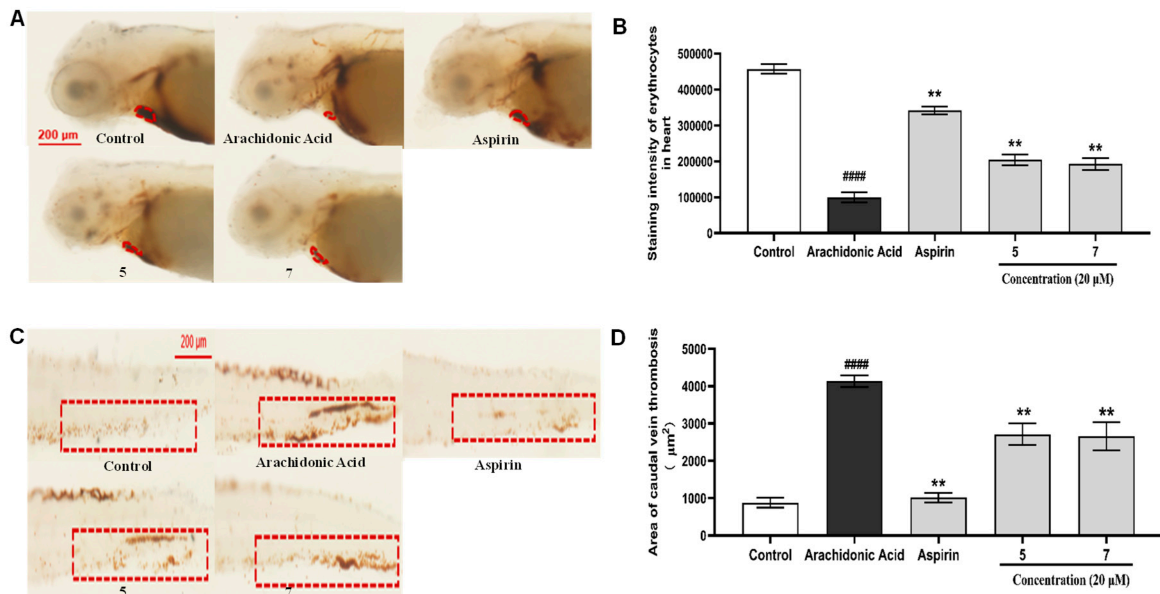

Figure 7. Anti-thrombotic assays of **5** and **7**. (**A**) Images of the staining intensity of erythrocytes in the heart in arachidonic acid (AA)-induced thrombotic zebrafish, treated with either **5** or **7**, using aspirin as a positive control. (**B**) Quantitative analysis of the staining intensity of erythrocytes in the heart in zebrafish treated with either **5** or **7**. (**C**) Images of areas of caudal vein thrombosis in arachidonic acid (AA)-induced thrombotic zebrafish, treated with either **5** or **7**, using aspirin as a positive control. (**D**) Quantitative analysis of the area of caudal vein thrombosis in zebrafish treated with either **5** or **7**. #### indicates that the arachidonic acid model group has a very significant difference compared with the control group ($p < 0.01$). ** indicates that sample groups have significant differences compared with the arachidonic acid model group ($p < 0.01$).

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were measured using a Jasco P-1020 digital polarimeter. Ultraviolet (UV) spectra were measured using a Beckman DU640 spectrophotometer. CD spectra were measured using a Jasco J-810 spectropolarimeter. NMR spectra were measured using an Agilent 500 MHz and a JEOL JNMECP 600 spectrometer. The 7.26 ppm (^{1}H) resonance of the residual $CHCl_3$ in $CDCl_3$ and the 77.16 ppm (^{13}C) resonance of $CDCl_3$ were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. HRESIMS spectra were measured using a Micromass Q-Tof Ultima GLOBAL GAA076LC mass spectrometer. The crystallographic data were measured on a Bruker D8 Venture diffractometer (Bruker, Beijing, China) equipped with graphite-monochromatized Cu Kα radiation. A semi-preparative HPLC was performed using a Waters 1525 pump 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) was used for the column chromatography.

3.2. Animal Material

The soft coral *Sinularia densa* (Sinulariidae) was collected from Xisha Island (Ganquan island) of the South China Sea in July 2018 (111°58′ E, 16°50′ N), and was frozen immediately after collection. The specimen was dispatched via express delivery and subsequently identified based on its morphology and sclerites by Prof Ping-Jyun Sung, Institute of Marine Biotechnology, National Museum of Marine Biology & Aquarium, Pingtung 944, Taiwan. The voucher specimen (No. xs-18-yg-99) has been 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 *Sinularia densa* (3.5 kg, wet weight) was homogenized and then exhaustively extracted with CH_3OH six times (for 3 days each time) at room temperature. The combined solutions were concentrated in vacuo and desalted by redissolving with CH_3OH to yield a residue (83.0 g). The crude extract was subjected to silica gel vacuum column chromatography eluted with a gradient of petroleum ether/acetone (from 200:1 to 1:1, *v*/*v*) and subsequently CH_2Cl_2/MeOH (from 10:1 to 1:1, *v*/*v*) to obtain fourteen fractions (Fr.1–Fr.14). Each fraction was detected by TLC. Fr.2 was subjected to silica gel vacuum column chromatography (petroleum ether/acetone, from 50:1 to 1:1, *v*/*v*) to give four subfractions, Fr.2.1–Fr.2.4. Fr.2.3 was separated by semi-preparative HPLC (ODS, 5 μm, 250 × 10 mm; MeOH/H_2O, 85:15, *v*/*v*; 1.5 mL/min) to afford **1** (6.3 mg, t_R = 24 min). Fr.2.4 was separated by semi-preparative HPLC (ODS, 5 μm, 250 × 10 mm; MeOH/H_2O, 85:15, *v*/*v*; 1.5 mL/min) to afford **2** (1.4 mg, t_R = 15 min). Fr.4 was subjected to silica gel vacuum column chromatography (petroleum ether/acetone, from 40:1 to 1:1, *v*/*v*) to give four subfractions, Fr.4.1–Fr.4.4. Fr.4.3 was separated by semi-preparative HPLC (ODS, 5 μm, 250 × 10 mm; MeOH/H_2O, 65:35, *v*/*v*; 1.5 mL/min) to afford **3** (1.0 mg, t_R = 42 min) and **6** (2.4 mg, t_R = 48 min). Fr.6 was subjected to silica gel vacuum column chromatography (petroleum ether /acetone, from 30:1 to 1:1, *v*/*v*) to give six subfractions, Fr.6.1–Fr.6.6. Fr.6.4 was separated by semi-preparative HPLC (ODS, 5 μm, 250 × 10 mm; MeOH/H_2O, 45:55, *v*/*v*; 1.5 mL/min) to afford **5** (2.2 mg, t_R = 48 min) and **4** (3.0 mg, t_R = 30 min). Fr.6.5 was separated by semi-preparative HPLC (ODS, 5 μm, 250 × 10 mm; MeOH/H_2O, 45:55, *v*/*v*; 1.5 mL/min) to afford **7** (2.3 mg, t_R = 48 min).

Sinudenoid **F** (**1**): Colourless oil; $[\alpha]^{25}_D$ +85.2 (*c* 0.1, MeOH); UV (MeOH) λmax (log ε) = 200 (2.22) nm, 233 (0.36) nm; IR (KBr) ν_{max} = 3420, 2980, 1740, 1635, 1033 cm^{-1}; HRESIMS m/z 345.1693 $[M + H]^+$ (calcd. for $C_{20}H_{25}O_5$, 345.1697) and HRESIMS *m*/*z* 367.1511 $[M + Na]^+$ (calcd. for $C_{20}H_{24}O_5Na$, 367.1516). For ^{1}H NMR and ^{13}C NMR data, see Table S1.

Sinudenoid **G** (**2**): Colourless oil; $[\alpha]^{25}_D$ −32.1 (*c* 0.1, MeOH); UV (MeOH) λmax (log ε) = 204 (1.20) nm, 242 (0.40) nm; IR (KBr) ν_{max} = 2924, 1719, 1615, 1384, 1269 cm^{-1}; HRESIMS *m*/*z* 279.1594 $[M + H]^+$ (calcd. for $C_{16}H_{23}O_4$, 279.1591) and HRESIMS *m*/*z*

301.1412 [M + Na]$^+$ (calcd. for $C_{16}H_{22}O_4Na$, 301.1410). For ^{1}H NMR and ^{13}C NMR data, see Table S2.

Sinudenoid **H** (**3**): Colourless oil; $[\alpha]^{25}{}_D$ +35.2 (*c* 0.5, MeOH); UV (MeOH) λmax (log ε) = 193 (1.50) nm, 280 (0.69) nm; IR (KBr) ν_{max} = 3420, 1756, 1718, 1683, 1592, 1384 cm^{-1}; HRESIMS *m/z* 375.1448 [M + H]$^+$ (calcd. for $C_{20}H_{23}O_7$, 375.1438). For ^{1}H NMR and ^{13}C NMR data, see Table S3.

Sinudenoid **I** (**4**): Colourless oil; $[\alpha]^{25}{}_D$ −124.0 (*c* 1.0, MeOH); UV (MeOH) λmax (log ε) = 192 (0.84) nm, 300 (0.92) nm; IR (KBr) ν_{max} = 2925, 1750, 1708, 1670, 1645, 1363 cm^{-1}; HRESIMS *m/z* 501.1729 [M + Na]$^+$ (calcd. for $C_{24}H_{30}O_{10}Na$, 501.1731). For ^{1}H NMR and ^{13}C NMR data, see Table S4.

Sinudenoid **J** (**5**): Colourless crystal; $[\alpha]^{25}{}_D$ +67.0 (*c* 0.3, MeOH); UV (MeOH) λmax (log ε) = 194 (0.89) nm; IR (KBr) ν_{max} = 1757, 1734, 1716 cm^{-1}; HRESIMS *m/z* 347.1859 [M + H]$^+$ (calcd. for $C_{20}H_{27}O_5$, 347.1853). For ^{1}H NMR and ^{13}C NMR data, see Table S5.

Sinudenoid **K** (**6**): Colourless oil; $[\alpha]^{25}{}_D$ −29.0 (*c* 0.5, MeOH); UV (MeOH) λmax (log ε) = 192 (0.04) nm, 214 (0.04) nm, 240 (0.03) nm; IR (KBr) ν_{max} = 3431, 2359, 1631, 1375 cm^{-1}; HRESIMS *m/z* 361.2374 [M + H]$^+$ (calcd. for $C_{22}H_{33}O_4$, 361.2384). For ^{1}H NMR and ^{13}C NMR data, see Table S6.

Sinudenoid **L** (**7**): Colourless oil; $[\alpha]^{25}{}_D$ +28.3 (*c* 0.1, MeOH); UV (MeOH) λmax (log ε) = 195 (1.68) nm; IR (KBr) ν_{max} = 3419, 2925, 1683, 1615, 1384 cm^{-1}; HRESIMS *m/z* 369.2399 [M+Na]$^+$ (calcd. for $C_{22}H_{34}O_3Na$, 369.2400). For ^{1}H NMR and ^{13}C NMR data, see Table S7.

3.4. X-ray Crystallographic Analysis

Sinudenoid J (**5**) was obtained as a colourless crystal in an EtOH-H_2O solvent system using the vapour diffusion method. Crystal data for Sinudenoid J (**5**): $C_{20}H_{26}O_5$, M = 346.41, a = 7.4110(3) Å, b = 12.6917(5) Å, c = 19.7025(8) Å, α = 90°, β = 90°, γ = 90°, V = 1853.18 (13) Å^3, T = 150 K, space group $P2_12_12_1$, Z = 4, μ (Cu Kα) = 0.720 mm^{-1}, 3717 independent reflections (R*int* = 0.0488). The final R_1 values were 0.0356 (I > 2σ(I)). The final wR_2 values were 0.0869 (I > 2σ(I)). The final R_1 values were 0.0370 (all data). The final wR_2 values were 0.0882 (all data). The goodness of fit on F^2 was 1.044. The Flack parameter was −0.02(7). The crystallographic data for **5** in this article have been deposited at the Cambridge Crystallographic Data Centre under supplementary publication number 2150265. The data can be obtained via https://www.ccdc.cam.ac.uk/ (accessed on 5 February 2022).

3.5. Bioassay

Three-day post-fertilization (dpf) healthy macrophage fluorescent transgenic zebrafish were employed as animal models to evaluate the anti-thrombotic and anti-inflammatory effects of the isolates. Healthy macrophage fluorescent transgenic zebrafish (Tg: zlyz-EGFP) were provided by the Biology Institute of Shandong Academy of Sciences (Jinan, China). Zebrafish larvae with $CuSO_4$-treated transgenic fluorescent (Tg:zlyz-EGFP) expressing enhanced green fluorescent protein (EGFP) were used to evaluate anti-inflammatory effects. Zebrafish larvae with arachidonic acid (AA)-induced thrombus were utilized to evaluate anti-thrombosis effects. Each zebrafish larva was imaged using a fluorescence microscope (AXIO, Zom.V16), and the number of macrophages around the nerve mound were calculated using Image-Pro Plus software (version 5.1). Statistical analysis was conducted using a one-way analysis of variance with GraphPad Prism 7.00 software.

4. Conclusions

In summary, two rare meroterpenoids (**1**–**2**) and five new cembranes (**3**–**7**) were isolated from the soft coral *Sinularia densa*. Sinudenoid F (**1**) and sinudenoid G (**2**) are rare meroterpenoids featuring a methyl benzoate core. While the skeleton of **1** has been obtained in synthetic studies, it represents the first instance of this natural product skeleton [21]. Sinudenoid H (**3**) possesses a rare carbon skeleton of 8, 19-bisnorfuranocembrenolide, and it is the second reported compound of this skeleton [15]. Sinudenoid J (**5**) is a new

furanobutenolide-derived C_{19}-norcembranoid diterpene, whose structure was confirmed by X-ray diffraction analysis. In the biological assays, **3** showed potent anti-inflammatory activity, while **5** and **7** demonstrated moderate anti-thrombotic activity in zebrafish models. The current study shows the potential of the cembranes as marine-derived anti-inflammatory and anti-thrombotic lead molecules, and provides a basis for developing new drugs.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/md22100442/s1, Tables S1–S7: NMR data of **1**–**7**; Figure S1: Structures of **3**–**7** and structurally related compounds of **3**–**7**; Tables S8–S11 and Figures S2–S9: The determination of the relative and absolute configurations for **3**, **4**, **6**, and **7**, respectively; Figures S10 and S11: Experimental and calculated ECD spectra of **3a** and **7b**, respectively; Table S12: Anti-inflammation assay of **1**–**7**; Table S13: Anti-thrombotic assay of **5** and **7**; Table S14 and Figures S12–S17: Computational details; Figures S18–S84: Spectra for **1**–**7**.

Author Contributions: Conceptualization, H.H. and P.L.; methodology, H.H., P.L. and C.W.; software, K.L.; validation, J.Y.; investigation, L.L. and S.W.; writing—original draft preparation, C.W. and J.Z.; writing—review and editing, H.H. and P.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (No. 42276088).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data are contained within the article or Supplementary Materials; further inquiries can be directed to the corresponding author.

Acknowledgments: We give special thanks to the Center for High Performance Computing and System Simulation (Pilot National Laboratory for Marine Science and Technology) for the support extended toward the computer calculations. Special thanks also go to the major scientific and technological innovation project in Shandong Province (No. 2022CXGC020414) and major scientific and technological innovation project of Qingdao West Coast New Area (No. ZDKC-2022-593 01) for the support extended toward the computer calculations.

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

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