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Note

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Klymollins A−H, Bioactive Eunicellin-Based Diterpenoids from the Formosan Soft Coral *Klyxum molle*

Fang-Jung Hsu,[†] Bo-Wei Chen,[†] Zhi-Hong Wen,[†] Chiung-Yao Huang,[†] Chang-Feng Dai,[‡] Jui-Hsin Su,[§] Yang-Chang Wu,[⊥] and Jyh-Horng Sheu*[,†,#]

[†]Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

[‡]Institute of Oceanography, National Taiwan University, Taipei 112, Taiwan, Republic of China

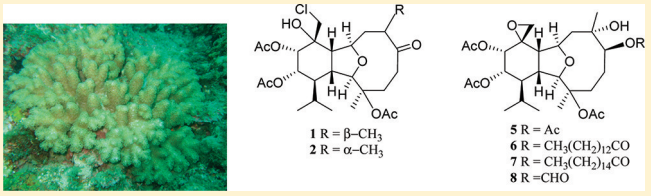
[§]Taiwan Coral Research Center, National Museum of Marine Biology & Aquarium, Checheng, Pingtung 944, Taiwan, Republic of China

[⊥]Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung 404, Taiwan, Republic of China

[#]Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

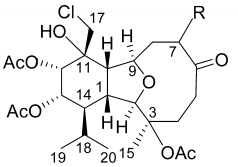
Supporting Information

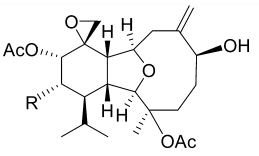
ABSTRACT: Eight new eunicellin-based diterpenoids, klymollins A−H (**1**−**8**), were isolated during the chemical investigation of the soft coral *Klyxum molle* from Taiwan waters. Their structures were elucidated by extensive spectroscopic analysis. The absolute configuration of **4** was determined by Mosher's method. Bioassays of the new metabolites showed that compounds **3**−**8** displayed significant *in vitro* anti-inflammatory activity by inhibiting the expression of the iNOS protein, and compounds **3**, **4**, and **6**−**8** also could effectively reduce the accumulation of COX-2 protein in LPS-stimulated RAW264.7 macrophage cells.

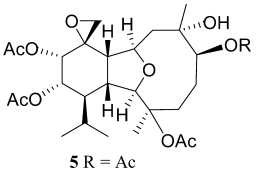


The eunicellin-based diterpenoids are secondary metabolites that possess a cladiellane skeleton with a C-2, C-9 ether bridge. Compounds of this type have been isolated recently from soft corals of the genera *Acalycigorgia*,[1] *Briareum*,[2] *Cladiella*,[3−8] *Klyxum*,[9] and *Pachyclavularia*.[10,11] Our previous chemical investigations on the soft corals of the genus *Klyxum* have afforded several eunicellin-based diterpenoids, of which some have been shown to exhibit interesting bioactivities.[12−16] In continuation of our effort to discover novel and bioactive substances from marine invertebrates, the chemical constituents of the soft coral *Klyxum molle*, which has been previously investigated as *Alcyonium molle*,[17] were studied. In this paper, we report the isolation, structure determination, and biological activity of eight eunicellin metabolites, klymollins A−H (**1**−**8**), from this soft coral. The structures of **1**−**8** were established by extensive spectroscopic analysis, including 2D NMR (^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY) spectroscopy. The abilities of **1**−**8** to inhibit up-regulation of the pro-inflammatory iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) proteins in LPS (lipopolysaccharide)-stimulated RAW264.7 macrophage cells were evaluated.

The octocoral was collected and frozen. The frozen material was minced and extracted exhaustively with EtOAc. The combined EtOAc-soluble fraction was concentrated under reduced pressure, and the residue was repeatedly purified by chromatography to yield metabolites **1**−**8**.







Klymollin A (**1**) was isolated as a colorless oil. The HRESIMS spectrum of **1** exhibited a $[M + Na]^+$ peak at m/z 553.2184 [3:1, $(M + Na)^+/(M + 2 + Na)^+$, ESIMS] and established a molecular formula of $C_{26}H_{39}ClO_9Na$, implying seven degrees of unsaturation. The IR spectrum of **1** revealed the presence of carbonyl functionalities from absorptions at 1741 and 1697 cm^{-1}. The ^{13}C NMR spectroscopic data of **1**

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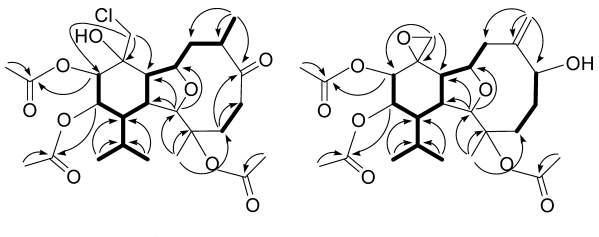
Table 1. ^{13}C NMR and ^{1}H Data for Compounds 1, 3, and 5

position	1[a]	1[b]	3[c]	3[d]	5[a]	5[b]
1	43.5, CH[e]	2.10, dd (11.5, 7.0)[f]	41.8, CH	2.39, t (9.2)	43.5, CH	2.30, m
2	91.2, CH	3.61, s	90.8, CH	3.80, s	91.6, CH	3.78, s
3	84.4, C		84.6, C		86.4, C	
4	33.1, CH_2	2.75, dd (14.5, 8.5)	27.7, CH_2	2.28, m	34.8, CH_2	2.55, dd (15.0, 7.0)
		2.03,, m		1.53, ddd (15.2, 11.2, 4.0)		2.08, m
5	30.1, CH_2	2.48, m	34.2, CH_2	2.17, m	29.0, CH_2	1.54, dd (14.0, 8.0)
		1.91, m		1.69, m		1.41, m
6	215.1, C		72.5, CH	4.29, dd (10.4, 4.8)	83.6, CH	5.62, d (5.5)
7	48.4, CH	2.66, m	149.9, C		75.3, C	
8	44.9, CH_2	2.04, m	40.7, CH_2	2.77, dd (12.8, 4.4)	47.7, CH_2	1.81, dd (15.0, 3.5)
		1.91, dd (13.0, 4.0)		2.47, d (12.8)		1.72, m
9	78.9, CH	4.58, ddd (11.5, 8.5, 4.0)	80.1, CH	4.90, m	77.0, CH	4.85, m
10	50.3, CH	2.86, t (7.5)	42.9, CH	2.26, t (9.2)	49.9, CH	2.13, m
11	72.3, C		55.2, C		55.7, C	
12	72.1, CH	5.36, d (2.0)	73.8, CH	4.90, br s	73.0, CH	4.94, br s
13	69.9, CH	4.87, dd (12.0, 2.0)	72.1, CH	4.93, d (11.2)	71.9, CH	4.96, d (14.5)
14	39.8, CH	2.00, m	40.7, CH	2.00, m	40.5, CH	2.00, m
15	22.4, CH_3	1.40, s	22.1, CH_3	1.65, s	23.2, CH_3	1.46, s
16	16.8, CH_3	1.06, d (7.5)	116.8, CH_2	5.37, s	23.5, CH_3	1.18, s
				4.99, s		
17	52.3, CH_2	3.72, d (12.0)	53.4, CH_2	2.86, d (4.8)	53.8, CH_2	2.62, d (5.0)
		3.62, d (12.0)		2.64, d (4.8)		2.89, d (5.0)
18	28.9, CH	1.70, m	27.4, CH	2.02, m	29.7, CH	1.77, m
19	23.6, CH_3	1.02, d (7.0)	24.0, CH_3	1.09, d (6.8)	23.8, CH_3	1.10, d (7.0)
20	15.3, CH_3	0.83, d (7.0)	15.5, CH_3	0.85, d (6.8)	15.9, CH_3	0.90, d (7.0)
3-OAc	21.0, CH_3	2.14, s	22.4, CH_3	1.92, s	23.2, CH_3	2.02, s
	170.9, C		169.4, C		170.2, C	
6-OAc					22.3, CH_3	2.08, s
					171.7, C	
12-OAc	22.4, CH_3	2.18, s	21.1, CH_3	2.14, s	21.1, CH_3	2.15, s
	169.4, C		169.9, C		170.2, C	
13-OAc	21.1, CH_3	2.02, s	21.1, CH_3	2.01, s	21.4, CH_3	2.02, s
	169.9, C		170.1, C		169.1, C	

[a]Spectrum recorded at 125 MHz in $CDCl_3$ at 25 °C. [b]Spectrum recorded at 500 MHz in $CDCl_3$ at 25 °C. [c]Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. [d]Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [e]Multiplicities deduced by DEPT. [f]J values (in Hz) in parentheses.

exhibited 26 carbon signals (Table 1), which were assigned by the assistance of a DEPT spectrum to seven methyls, four sp^3 methylenes, eight sp^3 methines (including four oxymethines), three sp^2 carbonyls, and two sp^3 oxygenated quaternary carbons. The ^{13}C NMR spectrum of **1** showed the presence of a ketone carbonyl (δ_C 215.1). Three ester carbonyls (δ_C 170.9, 169.9, and 169.4) were assigned from the ^{13}C NMR spectrum and were HMBC correlated with three acetate methyls (δ_H 2.18, 2.14, and 2.02 s). Therefore, the remaining three degrees of unsaturation identified compound **1** as a tricyclic compound. Proton signals (Table 1) resonating at δ_H 2.10 (1H, dd, J = 11.5, 7.0), 2.86 (1H, t, J = 7.5), 3.61 (1H, s), and 4.58 (1H, ddd, 11.5, 8.5, 4.0) and carbon signals appearing at δ_C 43.5, 50.3, 91.2, and 78.9 indicated the presence of the tetrahydrofuran structural unit of the eunicellins.[12−14] Also in the ^{1}H NMR spectrum, a doublet at δ_H 1.06 (3H, d, J = 7.5 Hz) represented H_3-16 and two doublets at δ_H 1.02 and 0.83 (each 3H, d, J = 7.0 Hz) represented two methyls of an isopropyl group. The CH_2-17 resonating at δ_C 55.4 was more shielded than C-11 (δ_C 72.3) and was HSQC correlated with the methylene signals at δ_H 3.72 and 3.62, suggesting the attachment of a chlorine and an oxygen at C-17 and C-11, respectively. The gross structure of metabolite **1** was further confirmed by analysis of ^{1}H−^{1}H COSY and HMBC correlations (Figure 1). From the ^{1}H−^{1}H COSY spectrum of **1**, it was possible to identify two structural units, which were



1 3

Figure 1. ^{1}H−^{1}H COSY (bold lines) and HMBC correlations (arrows) for **1** and **3**.

further assembled by key HMBC correlations from H-2 to C-1, C-9, and C-10; H_3-15 to C-2, C-3, and C-4; H_2-4 to C-6; H_3-16 to C-6, C-7, and C-8; H_2-17 to C-10, C-11, and C-12; and both H_3-19 and H_3-20 to C-14 and C-18. Moreover, the downfield chemical shift of the tertiary methyl CH_3-15 (δ_H 1.40) and the HMBC correlations of H-12 and H-13 to two acetate carbonyl carbons, respectively, showed the presence of three acetoxy groups at C-3, C-12, and C-13. In the NOESY spectrum of **1** (Figure 2), NOE correlations between H-10 and H_2-17 (δ

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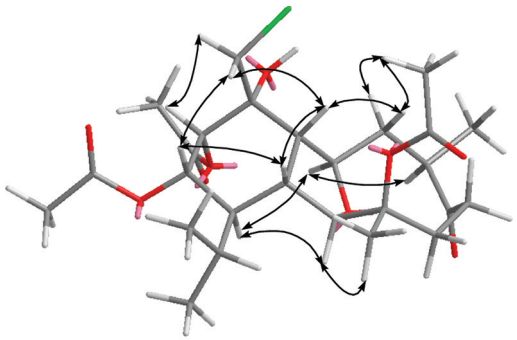


1

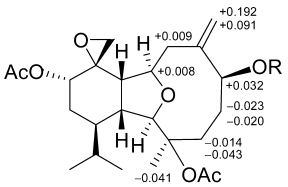
Figure 2. Key NOESY correlations of **1**.

3.62), H-13, H-8β (δ 1.91), and H-1; H_3-16 and H_2-8 (δ 2.04 and 1.91); H_2-17 (δ 3.72) and H-10, H-12, and H-13; and H-1 and both H_2-17 (δ 3.62) and H-12 suggested that H-1, H-10, H-12, H-13, H_3-16, and H_2-17 are β-oriented. Also, NOE interactions between H-2 and both H_3-15 and H-14 and between H-9 and both H-7 and H-14 suggested that H-2, H-7, H-9, H-14, and H_3-15 are α-oriented. Thus, the structure of diterpenoid **1** was established.

Klymollin B (**2**) was isolated as a colorless oil. HRESIMS and NMR spectroscopic data (Table 1) reveal that **2** has the same molecular formula, $C_{26}H_{39}ClO_9$, as that of **1**. By analysis of 2D NMR spectra, including ^{1}H−^{1}H COSY, HSQC, and HMBC, compound **2** was shown to possess the same molecular framework as that of **1**. Careful investigation of the NOESY spectrum of **2** revealed that H-9 showed an NOE interaction with H_3-16, revealing the α-orientation of H_3-16. Further analysis of other NOE interactions revealed that **2** possessed the same relative configurations at C-1, C-2, C-3, C-9, C-10, C-11, C-12, C-13, and C-14 as those of **1**. Therefore **2** was found to be the C-7 epimer of **1**.

Klymollin C (**3**) was obtained as a colorless oil that gave a pseudomolecular ion peak at m/z 517.2410 $[M + Na]^+$ in the HRESIMS spectrum, consistent with the molecular formula $C_{26}H_{38}O_9$ and implying eight degrees of unsaturation. The assignments of ^{1}H and ^{13}C NMR spectroscopic data of **3** were assisted by a series of 2D NMR (^{1}H−^{1}H COSY, HMQC, and HMBC) experiments (Supporting Information Figure S18). By comparison of the NMR data of **3** with those of **2** (Table 1 and Supporting Information Figures S20 and S21), it was found that a double bond was present between C-7 and C-16 in **3** and that the signal for the ketone carbonyl (δ_C 213.2, C, C-6) in **2** was replaced by that of an oxymethine (δ_C 72.5, CH, C-6) in **3**. Additionally, the NMR signals of C-17 and C-11 (δ_C 53.4 and 55.2) were assigned to an epoxy group in **3**. The relative configurations of all of the asymmetric centers of **3**, except C-6 and C-11, were confirmed to be identical with those of **2** by the NOE interactions (Supporting Information Figure S19) and NMR data. H-6 was found to exhibit an NOE interaction with H_3-15, revealing the β-orientation of the C-6 hydroxy group. In addition, the relative configuration of C-11 in **3** is R^*, as confirmed by NOE correlations between H_2-17 and both H-10 and H-12.

Klymollin D (**4**) was obtained as a colorless oil with a molecular formula of $C_{24}H_{36}O_7$, implying seven degrees of unsaturation, as established by the HRESIMS peak at m/z 459.2362 $[M + Na]^+$. By means of extensive 2D NMR experiments (COSY, HSQC, and HMBC), the structure of **4** was found to be close to that of **3** except that the ester group at C-13 in **3** was replaced by a hydrogen atom in **4**. Furthermore, the analysis of NOE correlations of **4** revealed the same relative configurations at C-1, C-2, C-3, C-6, C-10, C-11, and C-12 as those of **3** and the same β-orientation of the isopropyl substituent at C-14. Thus, the relative structure of diterpenoid **4** was established. The absolute configuration at C-6 was determined using Mosher's method.[18,19] The (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) esters of **4** (**4a** and **4b**, respectively) were prepared by using the corresponding *R*-(−)- and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chlorides, respectively. The values of $\Delta\delta$ [δ(*S*-MTPA ester) − δ(*R*-MTPA ester)] for H-8, H-9, and H_2-16 were positive, while the values of $\Delta\delta$ for H-4, H_2-5, and H_3-15 were negative, revealing the *S*-configuration at C-6 (Figure 3).



4a: R=(*S*)–MTPA
4b: R=(*R*)–MTPA

Figure 3. ^{1}H NMR chemical shift differences $\Delta\delta$ ($\delta_S - \delta_R$) in ppm for the MTPA esters of **4**.

The HRESIMS spectrum of **5** exhibited a pseudomolecular ion peak at m/z 577.2626 $[M + Na]^+$, consistent with a molecular formula of $C_{28}H_{42}O_{11}$ and implying eight degrees of unsaturation. A comparison of the NMR data of **5** (Table 1) with those of klysimplexin C and **3** showed that **5** has the same 10-membered ring as that of klysimplexin C and a very similar six-membered ring to that of **3**, as evidenced by COSY and HMBC correlations (Supporting Information Figure S18). The relative configurations of all stereogenic centers except that of C-7 in **5** were confirmed to be the same as those of **3** by comparison of the proton shifts, coupling constants, and NOE correlations. H_3-16 was found to exhibit an NOE correlation with H-5β and H_2-8 but not with H-6, revealing the β-orientation of the acetoxy group at C-6 and the α-orientation of the hydroxy group at C-7. Thus, the structure of diterpenoid **5** was established.

Klymollins F (**6**) and G (**7**) were also isolated as colorless oils. The molecular formulas of $C_{40}H_{66}O_{11}$ and $C_{42}H_{70}O_{11}$, 168 and 196 mass units higher than that of **5**, were determined by HRESIMS, respectively. The negative mode ionization of **6** and **7** by LC-ESI MS/MS fragmentation exhibited $[M - H]^-$ peaks at m/z 227.3 and 255.5, consistent with the molecular formulas $C_{14}H_{27}O_2$ and $C_{16}H_{31}O_2$, and indicated the presence of tetradecanoate and hexadecanoate esters in **6** and **7**, respectively. The ^{1}H and ^{13}C NMR spectroscopic data of **6** and **7** were found to be very close to those of **5** (Supporting Information Figures S20 and S22), indicating the very similar structures for these three metabolites. The relative configurations of **6** and **7** were suggested to be the same as that of **5** due to biogenetic considerations, NMR spectroscopic data, and the same sign of specific optical rotations. A structurally related metabolite, klymollin (**8**), was also isolated as a colorless oil with a molecular formula of $C_{27}H_{40}O_{11}$, implying eight degrees

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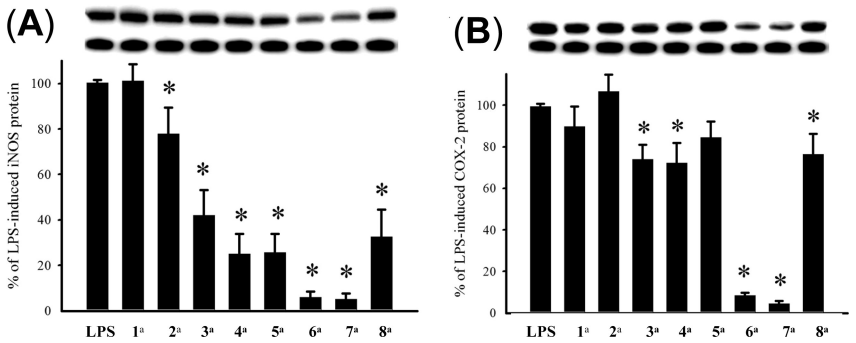

Figure 4. Effect of compounds **1**–**8** on iNOS and COX-2 protein expression of RAW264.7 macrophage cells by immunoblot analysis. (A) Immunoblots of iNOS and β-actin; (B) immunoblots of COX-2 and β-actin. The values are mean ± SEM (n = 6). Relative intensity of the LPS alone stimulated group was taken as 100%. *Significantly different from LPS alone stimulated group (*p < 0.05). [a]Stimulated with LPS in the presence of **1**–**8** (10 μM).

of unsaturation. NMR spectroscopic data of **8** (Supporting Information Figures S20 and S22) showed the presence of three acetoxy groups instead of four in **5**. The signal appearing at δ_H 8.14 (1H, br s) was assigned as the proton signal of a formate. Comparison of the NMR data of **8** with those of **5** revealed that the only difference between the two compounds arose from the replacement of the acetate moiety at C-6 in **5** by a formate in **8**.

The anti-inflammatory activities of **1**–**8** against the accumulation of pro-inflammatory iNOS and COX-2 proteins in RAW264.7 macrophage cells stimulated with LPS were evaluated using immunoblot analysis. At a concentration of 10 μM (Figure 4), compounds **3**–**8** were found to effectively reduce the levels of iNOS protein to 42.1 ± 11.5%, 25.1 ± 8.7%, 25.7 ± 8.0%, 6.0 ± 2.6%, 5.2 ± 2.5%, and 32.6 ± 11.8%, respectively, relative to control cells stimulated with LPS only. At the same concentration, metabolites **6** and **7** also significantly reduced COX-2 expression (8.5 ± 1.3% and 4.4 ± 1.3% relative to control cells) by LPS treatment. Thus, compounds **3**–**8**, and in particular **6** and **7**, could be promising anti-inflammatory agents and may warrant further study. The relatively weak anti-inflammatory activities of **1** and **2** suggest that the ring-opening of the 11,17-epoxy group of related eunicellins leads to a significant reduction of this activity.

The cytotoxicities of metabolites **1**–**8** toward a panel of cancer cell lines were evaluated. However, all of **1**–**8** did not show significant cytotoxicity toward human medulloblastoma (Daoy), human breast carcinoma (MCF-7), human cervical epitheloid (HeLa), and human laryngeal (Hep 2) carcinoma cells.

Although many eunicellin-type natural products have been discovered, eunicellins containing a 17-chloro (**1** and **2**) or an 11,17-epoxy group (**3**–**8**) were discovered for the first time. Biosynthetically, 17-chloroeunicellins (**1** and **2**) might be derived initially by the protonation of the corresponding 11,17-epoxyeunicellins, followed by S_N2 nucleophilic attack of chloride at C-17. Also, due to the shared biosynthetic pathway for the same chemotype from the same organism, compounds **1**–**3** and **5**–**8** should possess the same the absolute configurations at C-1, C-2, C-3, C-9, and C-10 as for **4**.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were recorded on a JASCO FT/IR-4100 infrared spectrophotometer. The NMR spectra were recorded on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C or on a Varian 400 MR FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively. ESIMS were obtained with a Bruker APEX II mass spectrometer. LC-ESI MS/MS spectrometry analysis was carried out using an Applied Biosystem API 4000 tandem quadrupole mass spectrometer. Silica gel (Merck, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography was performed on a Hitachi L-7100 HPLC apparatus with a C-18 column (250 × 21.2 mm, 5 μm).

Animal Material. The soft coral *Klyxum molle* was collected by hand using scuba along the coast of Peng-hu Islands, Taiwan, in June 2008 at a depth of 10 m, and was stored in a freezer until extraction. A voucher sample (PI-20080610) was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation. The frozen bodies of *K. molle* (1.3 kg, wet wt) were sliced and exhaustively extracted with EtOAc (3 × 10 L). The organic extract was concentrated to an aqueous suspension and was partitioned between EtOAc and H_2O. The EtOAc layer was dried with anhydrous Na_2SO_4. After removal of solvent *in vacuo*, the residue (22 g) was subjected to column chromatography on silica gel and eluted with EtOAc in *n*-hexane (0–100% of EtOAc, gradient) and further with MeOH in EtOAc of increasing polarity to yield 31 fractions. Fraction 17, eluted with *n*-hexane–acetone (5:1), was rechromatographed over a reversed-phase RP-18 column using CH_3CN and H_2O (0–100% of EtOAc, gradient) as the mobile phase to afford four subfractions (A1–A4). Subfractions A3 and A4 were separated by reversed-phase HPLC (CH_3CN–H_2O, 10:1 to 1:1) to afford compounds **3** (8.0 mg), **5** (7.5 mg), **6** (3.8 mg), **7** (4.1 mg), and **8** (4.8 mg), respectively. Fraction 20, eluted with *n*-hexane–EtOAc (4:1), was rechromatographed over a Sephadex LH-20 column, using acetone as the mobile phase, to afford four subfractions (B1–B4). Subfractions B2 and B3 were separated by reversed-phase HPLC (CH_3CN–H_2O, 1:1) to afford compounds **1** (1.6 mg), **2** (6.7 mg), and **4** (3.4 mg), respectively.

Klymollin A (1): $[\alpha]^{25}_D$ −184 (*c* 0.13, $CHCl_3$); IR (neat) ν_{max} 1741 and 1697 cm^{-1}; ^{13}C and ^{1}H NMR data (500 MHz; $CHCl_3$), see Table 1; ESIMS *m*/*z* 553 [M + Na]$^+$, 555 [M + 2 + Na]; HRESIMS *m*/*z* 553.2184 [M + Na]$^+$ (calcd for $C_{26}H_{39}ClO_9Na$, 553.2180).

Klymollin B (2): colorless oil; $[\alpha]^{25}_D$ −42 (*c* 0.65, $CHCl_3$); IR (neat) ν_{max} 1740 and 1698 cm^{-1}; ^{13}C and ^{1}H NMR data (500 MHz; $CHCl_3$), see S20 and S21; ESIMS *m*/*z* 555 [M + Na]$^+$, 555 [M + 2 + Na]; HRESIMS *m*/*z* 553.2176 [M + Na]$^+$ (calcd for $C_{26}H_{39}ClO_9Na$, 553.2180).

Klymollin C (3): colorless oil; $[\alpha]^{25}_D$ −41 (*c* 0.48, $CHCl_3$); IR (neat) ν_{max} 3480 and 1745 cm^{-1}; ^{13}C and ^{1}H NMR data (400 MHz; $CDCl_3$), see Table 1; ESIMS *m*/*z* 517 [M + Na]$^+$; HRESIMS *m*/*z* 517.2410 [M + Na]$^+$ (calcd for $C_{26}H_{38}O_9Na$, 517.2413).

Klymollin D (4): colorless oil; $[\alpha]^{25}_D$ −113 (*c* 0.31, $CHCl_3$); IR (neat) ν_{max} 3458 and 1732 cm^{-1}; ^{13}C and ^{1}H NMR data (400 MHz;

$CDCl_3$), see S20 and S21; ESIMS m/z 459 $[M + Na]^+$; HRESIMS m/z 459.2362 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_7Na$, 459.2359).

*Klymollin E (**5**):* colorless oil; $[\alpha]^{25}_D$ −48 (c 0.67, $CHCl_3$); IR (neat) ν_{max} 3504 and 1737 cm^{-1}; ^{13}C and ^1H NMR data (500 MHz; $CDCl_3$), see Table 1; ESIMS m/z 577 $[M + Na]^+$; HRESIMS m/z 577.2626 $[M + Na]^+$ (calcd for $C_{28}H_{42}O_{11}Na$, 577.2624).

*Klymollin F (**6**):* colorless oil; $[\alpha]^{25}_D$ −67 (c 0.32, $CHCl_3$); IR (neat) ν_{max} 3504 and 1739 cm^{-1}; ^{13}C and ^1H NMR data (500 MHz; $CDCl_3$), see S20 and S22; ESIMS m/z 745 $[M + Na]^+$; HRESIMS m/z 745.4507 $[M + Na]^+$ (calcd for $C_{40}H_{66}O_{11}Na$, 745.4503).

*Klymollin G (**7**):* colorless oil; $[\alpha]^{25}_D$ −62 (c 0.38, $CHCl_3$); IR (neat) ν_{max} 3503 and 1739 cm^{-1}; ^{13}C and ^1H NMR data (500 MHz; $CDCl_3$), see S20 and S22; ESIMS m/z 773 $[M + Na]^+$; HRESIMS m/z 773.4820 $[M + Na]^+$ (calcd for $C_{42}H_{70}O_{11}Na$, 773.4816).

*Klymollin H (**8**):* colorless oil; $[\alpha]^{25}_D$ −110 (c 0.14, $CHCl_3$); IR (neat) ν_{max} 1739 cm^{-1}; ^{13}C and ^1H NMR data (500 MHz; $CDCl_3$), see S20 and S22; ESIMS m/z 563 $[M + Na]^+$; HRESIMS m/z 563.2465 $[M + Na]^+$ (calcd for $C_{27}H_{40}O_{11}Na$, 563.2468).

Preparation of (*S*)- and (*R*)-MTPA Esters of 4. To a solution of **4** (0.5 mg) in pyridine (0.4 mL) was added *R*-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (25 μL), and the mixture was allowed to stand for 24 h at room temperature. The reaction was quenched by addition of 1.0 mL of H_2O, and the mixture was subsequently extracted with EtOAc (3 × 1.0 mL). The EtOAc-soluble layers were combined, dried over anhydrous $MgSO_4$, and evaporated. The residue was subjected to column chromatography over silica gel using *n*-hexane−acetone (3:1) to yield the (*S*)-MTPA ester, **4a** (0.3 mg, 20%). The same procedure was used to prepare the (*R*)-MTPA ester, **4b** (0.6 mg, 40%), from the reaction of (*S*)-MPTA chloride with **4** in pyridine. Selected ^1H NMR ($CDCl_3$, 400 MHz) of **4a**: δ 5.4735 (1H, m, H-6), 3.930 (1H, ddd, J = 13.2, 9.6, and 4.0 Hz, H-9), 3.515 (1H, t, J = 7.6 Hz, H-10), 2.481 (1H, m, H-12b), 1.530 (1H, m, H-18), 1.395 (1H, s, H-17), 0.721 (3H, d, J = 7.0 Hz, H-19), 0.529 (3H, d, J = 7.0 Hz, H-20). Selected ^1H NMR ($CDCl_3$, 400 MHz) of **4b**: δ 5.298 (1H, ddd, J = 15.2, 11.2, and 4.0 Hz, H-13), 3.892 (1H, ddd, J = 13.2, 9.2, and 3.6 Hz, H-9), 3.453 (1H, t, J = 7.6 Hz, H-10), 2.440 (1H, m, H-12b), 1.630 (1H, m, H-18), 1.391 (1H, s, H-17), 0.863 (3H, d, J = 7.0 Hz, H-19), 0.832 (3H, d, J = 7.0 Hz, H-20).

***In Vitro* Anti-inflammatory Assay.** Assay procedure was as previously reported.[20]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra of **1**−**8**, ^1H−^1H COSY and HMBC correlations for **4**−**8**, key NOESY correlations of **3**, ^{13}C NMR data for compounds **2**, **4**, **6**−**8** (S20), ^1H NMR data for compounds **2** and **4** (S21), ^1H NMR data for compounds **6**−**8** (S22), and cytotoxicity data are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 886-7-5252000, ext. 5030, Fax: 886-7-5255020. E-mail: sheu@mail.nsysu.edu.tw.

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