Int. J. Mol. Sci. **2014**, *15*, 21865-21874; doi:10.3390/ijms151221865

OPEN ACCESS
International Journal of
Molecular Sciences
ISSN 1422-0067
www.mdpi.com/journal/ijms

Article

Krempfielins Q and R, Two New Eunicellin-Based Diterpenoids from the Soft Coral *Cladiella krempfi*

Chi-Jen Tai [1,2], Uvarani Chokkalingam [1], Yang Cheng [1], Shou-Ping Shih [3], Mei-Chin Lu [3,4], Jui-Hsin Su [3,4], Tsong-Long Hwang [5] and Jyh-Horng Sheu [1,2,6,7,*]

1 Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan; E-Mails: jean801023@hotmail.com (C.-J.T.); uvaranichem@gmail.com (U.C.); jack1991106@yahoo.com.tw (Y.C.)
2 Doctoral Degree Program in Marine Biotechnology, National Sun Yat-sen University and Academia Sinica, Kaohsiung 804, Taiwan
3 Graduate Institute of Marine Biotechnology, National Dong Hwa University, Pingtung 944, Taiwan; E-Mails: m6430005@hotmail.com (S.-P.S.); jinx6609@nmmba.gov.tw (M.-C.L.); x2219@nmmba.gov.tw (J.-H.S.)
4 National Museum of Marine Biology & Aquarium, Pingtung 944, Taiwan
5 Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan; E-Mail: htl@mail.cgu.edu.tw
6 Department of Medical Research, China Medical University Hospital, China Medical University, Taichung 404, Taiwan
7 Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan

* Author to whom correspondence should be addressed; E-Mail: sheu@mail.nsysu.edu.tw; Tel.: +886-75-252-000 (ext. 5030); Fax: +886-75-255-020.

External Editor: Chang Y. Lee

Received: 23 September 2014; in revised form: 12 November 2014 / Accepted: 21 November 2014 / Published: 27 November 2014

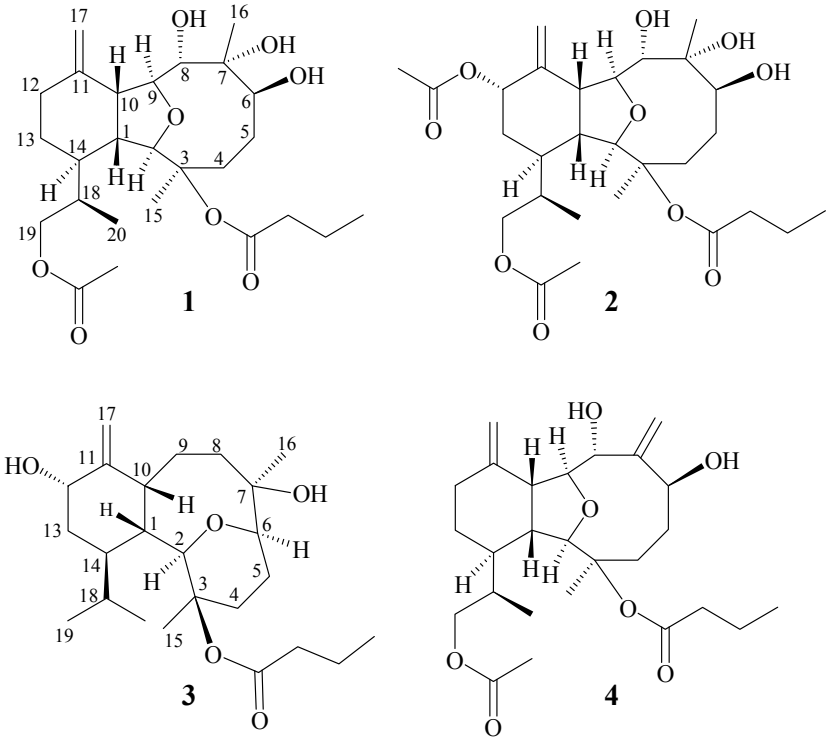
Abstract: Two new eunicellin-based diterpenoids, krempfielins Q and R (**1** and **2**), and one known compound cladieunicellin K (**3**) have been isolated from a Formosan soft coral *Cladiella krempfi*. The structures of these two new metabolites were elucidated by extensive spectroscopic analysis. Anti-inflammatory activity of new metabolites to inhibit the superoxide anion generation and elastase release in *N*-formyl-methionyl-leucyl phenylalanine/cytochalasin B (FMLP/CB)-induced human neutrophil cells and cytotoxicity of both new compounds toward five cancer cell lines were reported.

Keywords: *Cladiella krempfi*; eunicellin-based diterpenoid; anti-inflammatory activity

1. Introduction

Soft corals of the genus *Cladiella* have been known to be rich sources of eunicellin-type metabolites and several bioactivities of these compounds have been studied [1–18]. Our previous studies on the soft coral *Cladiella krempfi* have resulted in the isolation of a series of new eunicellin-based diterpenoids, krempfielins A–P [15–18]. Our contineous investigation on the chemical constituents of soft coral *C. krempfi* has afforded two new compounds, krempfielins Q and R (**1** and **2** in Chart 1), and one minor known compound cladieunicellin K (**3**) [11]. The molecular structures of **1** and **2**, including the relative configurations, were established by the detailed spectroscopic analysis and by comparison with related physical and spectral data of known compound, krempfielin E (**4**) [16]. For the anti-inflammatory activity of these two new compounds to inhibit the superoxide anion generation and elastase release in *N*-formyl-methionyl-leucyl phenylalanine/cytochalasin B (FMLP/CB)-induced human neutrophils and the cytotoxicity of them against five human cell lines, human T cell lymphoblast-like cell line (CCRF-CEM), human erythromyeloblastoid leukemia (K562), human acute lymphoblastic leukemia cell line (Molt 4), human ductal breast epithelial tumor cell line (T47D) and human colorectal adenocarcinoma cell line (DLD-1) were also evaluated.

Chart 1. Structures of metabolites **1**–**4**.

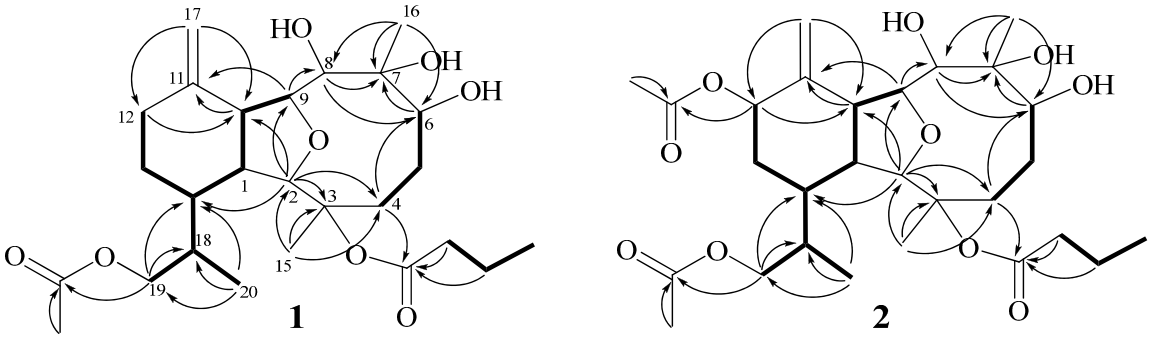


2. Results and Discussion

Krempfielin Q (**1**) showed the molecular ion peak $[M + Na]^+$ at *m*/*z* 505.2779 in the HRESIMS (Figure S1) and established a molecular formula of $C_{26}H_{42}O_8$, implying six degrees of unsaturation. The IR

absorption bands at ν_{max} 3445 and 1733 cm^{-1} revealed the presence of hydroxy and ester carbonyl functionalities, respectively. Its ^{13}C NMR spectrum (Figure S2) showed signals of 26 carbons (Table 1) which were assigned by the assistance of the distortionless enhancement by polarization transfer (DEPT) spectrum to five methyls (including one acetate methyl δ_C 21.1), seven sp^3 methylenes, one sp^2 methylene, eight sp^3 methines (including four oxymethines), two sp^3 and three sp^2 quaternary carbons (including two ester carbonyls). The NMR spectroscopic data of **1** (Figures S2,S3 and Table 1) displayed signals for 1,1-disubstituted double bond (δ_C 148.4 C, 110.7 CH_2; δ_H 4.91 and 4.81 s). Two ester carbonyls (δ_C 172.2 and 171.2) were assigned from the ^{13}C NMR spectrum and their signals were correlated with the methylene protons (δ_H 2.33, 2H, m) of an *n*-butyrate and protons of an acetate methyl (δ_H 2.08 s, 3H), respectively. Therefore, the remaining three degrees of unsaturation identified **1** as a tricyclic molecule. The ^1H-^1H correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) correlations (Figure 1) were further used for establishing the molecular skeleton of **1**. It was found that the COSY experiment showed the presence of three isolated consecutive proton spin systems. These evidences and the analysis of HMBC spectrum (Figure 1) suggested that **1** is an eunicellin-based diterpenoid. Furthermore, the acetoxy group attaching at C-19 was confirmed by the HMBC correlations from oxymethylene (δ_H 3.96 (H_2-19)) and acetate methyl protons (δ_H 2.08) to the ester carbonyl carbon appearing at δ 171.2 (C). Thus, the remaining *n*-butyryloxy group showed to be positioned at C-3, which was confirmed by an oxygen-bearing quaternary carbon resonating at δ 86.0 ppm. On the basis of above analysis, the planar structure of **1** was established.

Figure 1. Selected COSY (▬) and HMBC (→) correlations of **1** and **2**.



The relative configuration of **1** was deduced by the analysis of nuclear Overhauser effect (NOE) correlations, as shown in Figure 2. The observation of the NOE correlations of H-1 with H-10 and H_3-20 suggested that these protons had the same orientation and were assumed to be β-oriented. The NOE interactions found between the oxymethine proton H-8 with H-10 and H_3-16 assigned the α-orientation of the two hydroxy groups positioned at C-7 and C-8. The NOE correlations of H-2 with both H-14 and H_3-15, but not with H-1 and H-10; H-14 with both H-9 and H_2-19; and H-5α (δ_H 1.62) with both H-6 and H_3-15, permitted that H-2, H-6, H-9, H-14, and H_3-15 were assigned to be α-oriented. Furthermore, the configuration of C-18 was suggested to be *R* * on the basis of NOE correlations of H-1 with H_3-20, H-14 with H_2-19, and H-2 with H-18. The relative configuration of **1** was thus established. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **1** with those of its 7,16-dehydration derivative, krempfielin E (**4**) [16], further confirmed the structure of **1**.

Table 1. ^{13}C and ^{1}H NMR data for compounds **1**–**2**.

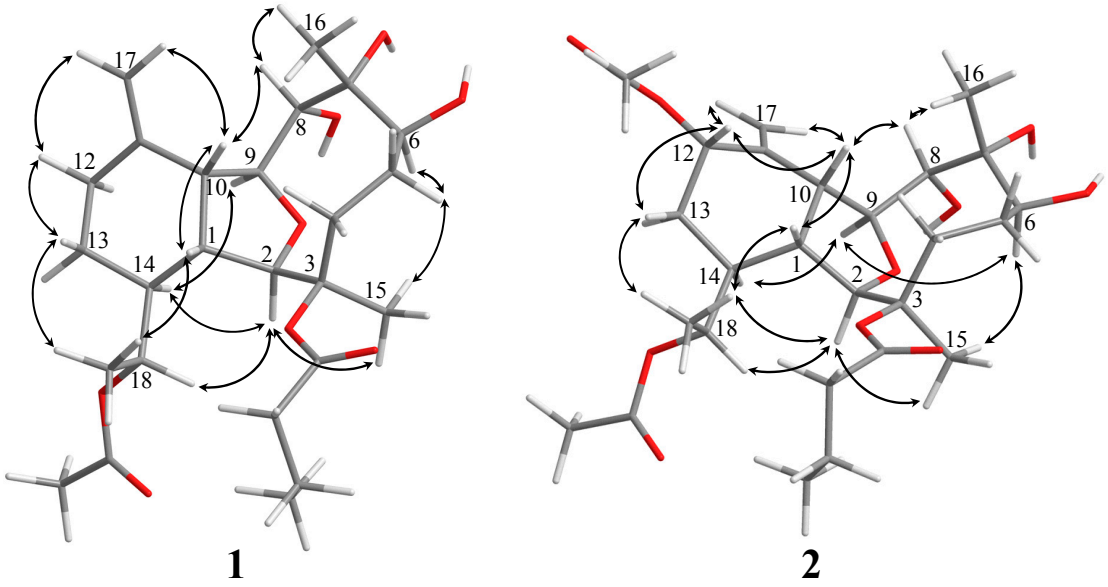
C	1 [a]		2 [b]	
	δ_C	δ_H	δ_C	δ_H
1	44.9 (CH) [c]	2.27 m [d]	43.5 (CH)	2.35 m
2	92.3 (CH)	3.54 br s	90.8 (CH) [e]	3.63 d (1.5)
3	86.0 (qC)		86.0 (qC)	
4	35.6 (CH_2)	1.84 dd (14.4, 10.4) [f] 2.61 dd (14.4, 9.2)	34.6 (CH_2) [e]	1.88 m 2.49 dd (14.0, 9.0)
5	29.5 (CH_2)	1.42 m 1.62 m	29.9 (CH_2)	1.43 m 1.70 m
6	77.6 (CH)	4.64 d (6.0)	77.0 (CH)	4.63 d (7.5)
7	79.6 (qC)		79.4 (qC)	
8	80.0 (CH)	3.60 br d (8.4)	79.1 (CH)	3.50 br t (8.0)
9	81.3 (CH)	3.85 dd (9.2, 6.4)	82.2 (CH)	4.05 dd (9.5, 5.5)
10	53.3 (CH)	3.34 t (6.8)	50.6 (CH)	3.35 t (6.5)
11	148.4 (qC)		143.0 (qC)	
12	31.4 (CH_2)	2.08 m 2.31 m	72.8 (CH)	5.44 d (4.5)
13	25.4 (CH_2)	1.17 dd (12.8, 2.8) 1.68 m	29.4 (CH_2)	1.50 m 1.88 m
14	38.9 (CH)	1.51 m	32.7 (CH)	1.90 m
15	23.1 (CH_3)	1.41 s	23.2 (CH_3)	1.46 s
16	17.7 (CH_3)	1.26 s	17.7 (CH_3)	1.26 s
17	110.7 (CH_2)	4.81 s 4.91 s	116.3 (CH_2) [e]	5.23 s
18	34.1 (CH)	1.92 m	34.0 (CH)	1.99 m
19	67.7 (CH_2)	3.96 d (7.2)	67.8 (CH_2)	3.90 dd (11.0, 7.0) 4.02 dd (11.0, 7.0)
20	11.0 (CH_3)	0.85 d (6.4)	11.4 (CH_3)	0.90 d (6.5)
3-OCOPr	172.2 (qC)		172.2 (qC)	
	37.3 (CH_2)	2.33 m	37.3 (CH_2)	2.30 m
	18.4 (CH_2)	1.68 m	18.3 (CH_2)	1.63 m
	13.7 (CH_3)	0.99 t (7.2)	13.6 (CH_3)	0.98 t (7.0)
12-OAc			170.4 (qC)	
			21.5 (CH_3)	2.08 s
19-OAc	171.2 (qC)		171.0 (qC)	
	21.1 (CH_3)	2.08 s	20.9 (CH_3)	2.07 s

[a] ^{13}C and ^{1}H spectra recorded at 100 and 400 MHz in $CDCl_3$; [b] ^{13}C and ^{1}H spectra recorded at 125 and 500 MHz in $CDCl_3$; [c] Deduced from DEPT; [d] Mutiplicity m deduced from HSQC; [e] Broad signal; and [f] *J* values (Hz) in parentheses.

Krempfielin Q (**2**) showed the molecular ion peak $[M + Na]^+$ at *m*/*z* 563.2835 in the HRESIMS (Figure S4) which established a molecular formFfigure sula of $C_{28}H_{44}O_{10}$, implying seven degrees of unsaturation for this compound. The IR absorptions at ν_{max} 3444 and 1732 cm^{-1} were consistent with the presence of hydroxy and ester carbonyl functionalities. The ^{13}C NMR spectrum of **2** showed signals of 28 carbons (Figure S5 and Table 1), which were differentiated by the DEPT spectrum as six methyls

(including two acetate methyls δ_C 21.5 and 20.9), six sp^3 methylenes, one sp^2 methylene, nine sp^3 methines (including five oxymethines), two sp^3 and four sp^2 quaternary carbons (including three ester carbonyls). The NMR spectroscopic data of **2** (Figures S5–S7 and Table 1) showed the presence of 1,1-disubstituted double bond (δ_C 143.0 C, 116.3 CH_2; δ_H 5.23 s, 2H). Three ester carbonyls (δ_C 172.2, 171.0 and 170.4) were assigned from the ^{13}C NMR spectrum and their signals were correlated with the methylene protons (δ_H 2.30, 2H, m) of an *n*-butyrate and protons of two acetate methyl (δ_H 2.08 s and 2.07 s, each 3H), respectively, indicated the presence of one *n*-butyrate and two acetoxy groups. The remaining three degrees of unsaturation identified **2** also a tricyclic diterpenoid. The molecular framework of this compound was also established by COSY and HMBC correlations (Figure 1). Comparison of the NMR data of **2** with those of the known compound krempfielin E (**4**) [16] revealed that **2** is the C-12 acetylated derivative of krempfielin E. The stereochemistry of compound **2** was determined by the NOESY correlations as shown in Figure 2.

Figure 2. Key NOESY (↔) correlations for **1** and **2**.



Many cytotoxic and anti-inflammatory eunicellin-based compounds have been discovered from soft corals [4–27]. Recently, we isolated several eunicellins with anti-inflammatory activity by significantly inhibiting superoxide anion generation and elastase release in human neutrophiles induced by FMLP/CB [17,18]. The same *in vitro* anti-inflammatory effects of the diterpenoids **1** and **2** also were tested. At a concentration of 10 μM, compound **2** exhibited some anti-inflammatory activity in reducing the generation of superoxide anion (13.17% ± 2.09% inhibition) and in inhibiting the elastase release (11.09% ± 5.55% inhibition), relative to the control cells stimulated with FMLP/CB only (Table 2). The cytotoxicity of **1** and **2** against five human carcinoma cell lines, CCRF-CEM, K562, Molt 4, T47D and DLD-1 were also evaluated by the MTT assay, and both compounds did not show activity against the proliferation of these cancer cell lines. The impurity of compound **2** might affect the bioactivity and the biological activities of **3** were not measured due to the paucity of this compound.

Table 2. Effects of compounds **1** and **2** on superoxide anion generation and elastase release in FMLP/CB-induced human neutrophils.

Compound	Superoxide Anion		Elastase	
	Inh %	IC_{50} (μM) [a]	Inh %	IC_{50} (μM)
1	5.46 ± 5.19	>10	2.99 ± 2.82	>10
2	13.17 ± 2.09 **	>10	11.09 ± 5.55	>10

Percentage of inhibition (Inh %) at 10 μM concentration. Results are presented as mean ± S.E.M. (the standard error of mean) (n = 3 or 4). ** $p < 0.01$ compared with the control value; and [a] Concentration necessary for 50% inhibition (IC_{50}).

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were measured on a JASCO P-1020 polarimeter (Jasco, Tokyo, Japan). IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer (Jasco). ESIMS were obtained with a Bruker APEX II mass spectrometer (Bruker Daltonics, Billerica, MA, USA). The NMR spectra were recorded in $CDCl_3$, either on a Varian UNITY INOVA-500 FT-NMR (Varian Inc., Palo Alto, CA, USA) or a Varian 400MR FT-NMR (Varian Inc.). Silica gel (Merck, Darmstadt, Germany, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Kieselgel 60 F-254, 0.2 mm, Merck) were used for TLC analysis (Merck). High-performance liquid chromatography (HPLC) was performed on a Hitachi L-2130 HPLC apparatus (Hitachi, Tokyo, Japan) equipped with Hitachi L-2455 diode array detector (Hitachi) and a Supelco C18 column (250 mm × 21.2 mm, 5 μm, Supelco, Bellefonte, PA, USA).

3.2. Animal Material

C. krempfi was collected by hand using scuba off the coast of Penghu islands of Taiwan in June 2008, at a depth of 5–10 m, and stored in a freezer until extraction. A voucher sample (specimen No. 200806CK) was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

3.3. Extraction and Separation

The octocoral (1.1 kg fresh wet weight) was collected and freeze-dried. The freeze-dried material was minced and extracted exhaustively with EtOH (3 × 10 L). The EtOH extract of the frozen organism was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble portion (14.4 g) was subjected to column chromatography on silica gel and eluted with EtOAc in *n*-hexane (0%–100% of EtOAc, stepwise) and then further with MeOH in EtOAc with increasing polarity to yield 41 fractions. Fraction 30, eluted with MeOH–EtOAc (1:10), was rechromatographed over a silica gel open column using acetone in *n*-hexane (0%–100% of acetone, stepwise) as the mobile phase to afford six subfractions (A1–A6). Subfraction A4 (eluted with *n*-hexane–acetone 1:1) was further separated by reverse phase HPLC (CH_3CN–H_2O, 1.5:1) to afford compound **3** (1.0 mg). Fraction 32, eluted with MeOH–EtOAc (1:10), was rechromatoraphed over a silica gel open column using acetone in *n*-hexane (0%–100% of acetone, stepwise) as the mobile phase to afford eight subfractions (B1–B8). Subfraction B2 (eluted with *n*-hexane–acetone 2:1) was

separated by reverse phase HPLC (CH_3CN–H_2O, 1:1) to afford compound **1** (2.2 mg). Subfraction B3 (eluted with *n*-hexane–acetone 1.8:1) was subjected to reverse phase HPLC (CH_3CN–H_2O, 1:1.6) and yielded compound **2** (2.0 mg).

3.3.1. Krempfielin Q (**1**)

Colorless oil; $[\alpha]_D^{23}$ = +84.7 (*c* 0.77, $CHCl_3$); IR (neat) ν_{max} 3445, 3076, 2963, 2932, 1733, 1646, 1455, 1373, 1237, 1183, and 1067 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; ESIMS *m*/*z* 505 $[M + Na]^+$; HRESIMS *m*/*z* 505.2779 $[M + Na]^+$ (calcd. for $C_{26}H_{42}O_8Na$, 505.2777).

3.3.2. Krempfielin R (**2**)

White powder; $[\alpha]_D^{25}$ = +82.0 (*c* 0.57, $CHCl_3$); IR (neat) ν_{max} 3444, 3038, 2963, 2930, 1732, 1650, 1456, 1373, 1239, 1182 and 1074 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; ESIMS *m*/*z* 563 $[M + Na]^+$; HRESIMS *m*/*z* 563.2835 $[M + Na]^+$ (calcd. for $C_{28}H_{44}O_{10}Na$, 563.2832).

3.4. Cytotoxicity Testing

Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of compounds **1** and **2** were performed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) colorimetric method [28,29].

3.5. In Vitro Anti-Inflammatory Assay–Superoxide Anion Generation and Elastase Release by Human Neutrophils

Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Measurements of superoxide anion generation and elastase release were carried out according to previously described procedures [30,31]. LY294002, a phosphatidylinositol-3-kinase inhibitior, was used as a positive control for inhibition of superoxide anion generation and elastase release with IC_{50} values of 1.88 ± 0.45 and 4.12 ± 0.92 μM, respectively. Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome c. Elastase release experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate [32].

4. Conclusions

Two new eunicellin-based diterpenoids **1** and **2** were isolated together with a known one from the continuing investigation of a soft coral *Cladiella krempfi.* Although both compounds were not cytotoxic towards a limited panel of cancer cell lines, **2** could inhibit the generation of superoxide anion and the release of elastase in FMLP/CB-induced human neutrophils.

Supplementary Materials

Supplementary figures can be found at http://www.mdpi.com/1422-0067/15/12/21865/s1.

Acknowledgments

This research was supported by grants from the National Science Council of Taiwan (NSC 102-2113-M-110-001-MY2), Aim for the Top University Program (03C030205) from Ministry of Education of Taiwan and National Sun Yat-sen University–Kaohsiung Medical University Joint Project (NSYSU–KMU 02C030117), awarded to Jyh-Horng Sheu.

Author Contributions

Jyh-Horng Sheu designed the experiment and contributed to manuscript preparation; Chi-Jen Tai, Uvarani Chokkalingam, Yang Cheng and Jui-Hsin Su carried out the experiment and wrote the manuscript; Shou-Ping Shih, Mei-Chin Lu and Tsong-Long Hwang performed and analyzed the bioassay.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2013**, *30*, 237–323.
2. Wei, W.-C.; Sung, P.-J.; Duh, C.-Y.; Chen, B.-W.; Sheu, J.-H.; Yang, N.-S. Anti-inflammatory activities of natural products isolated from soft corals of Taiwan between 2008 and 2012. *Mar. Drugs* **2013**, *11*, 4083–4126.
3. Williams, D.E.; Amlani, A.; Dewi, A.S.; Patrict, B.O.; van Ofwegen, L.; Mui, A.L.-F.; Andersen, R.J. Australin E isolated from the soft coral *Cladiella* sp. collected in pohnpei activates the inositol 5-phosphatase SHIP1. *Aust. J. Chem.* **2010**, *63*, 895–900.
4. Ahmed, A.F.; Wu, M.-H.; Wang, G.-H.; Wu, Y.-C.; Sheu, J.-H. Eunicellin-based diterpenoids, australins A–D, isolated from the soft coral *Cladiella australis*. *J. Nat. Prod.* **2005**, *68*, 1051–1055.
5. Chen, Y.-H.; Tai, C.-Y.; Hwang, T.-L.; Weng, C.-F.; Li, J.-J.; Fang, L.-S.; Wang, W.-H.; Wu, Y.-C.; Sung, P.-J. Cladielloides A and B: New eunicellin-type diterpenoids from an Indonesian octocoral *Cladiella* sp. *Mar. Drugs* **2010**, *8*, 2936–2945.
6. Chen, B.-W.; Chang, S.-M.; Huang, C.-Y.; Chao, C.-H.; Su, J.-H.; Wen, Z.-H.; Hsu, C.-H.; Dai, C.-F.; Wu, Y.-C.; Sheu, J.-H. Hirsutalins A–H, eunicellin-based diterpenoids from the soft coral *Cladiella hirsuta*. *J. Nat. Prod*. **2010**, *73*, 1785–1791.
7. Hassan, H.M.; Khanfar, M.A.; Elnagar, A.Y.; Mohammed, R.; Shaala, L.A.; Youssef, D.T.A.; Hifnawy, M.S.; El Sayed, K.A. Pachycladins A–E, prostate cancer invasion and migration inhibitory eunicellin-based diterpenoids from the Red Sea soft coral *Cladiella pachyclados*. *J. Nat. Prod.* **2010**, *73*, 848–853.
8. Chen, Y.-H.; Tai, C.-Y.; Su, Y.-D.; Chang, Y.-C.; Lu, M.-C.; Weng, C.-F.; Su, J.-H.; Hwang, T.-L.; Wu, Y.-C.; Sung, P.-J. Discovery of new eunicellins from an Indonesian octocoral *Cladiella* sp. *Mar. Drugs* **2011**, *9*, 934–943.

9. Cai, Y.-S.; Yao, L.-G.; Pascale, A.D.; Irace, C.; Mollo, E.; Taglialatela-Scafati, O.; Guo, Y.-W. Polyoxygenated diterpenoids of the eunicellin-type from the Chinese soft coral *Cladiella krempfi*. *Tetrahedron* **2013**, *69*, 2214–2219.
10. Chen, T.-H.; Lu, M.-C.; Chang, Y.-C.; Su, Y.-D.; Chen, Y.-H.; Lin, N.-C.; Fang, L.-S.; Wu, Y.-C.; Sung, P.-J. Discovery of new eunicellin-based diterpenoids from a Formosan soft coral *Cladiella* sp. *Mar. Drugs* **2013**, *11*, 4585–4593.
11. Shih, F.-Y.; Chen, T.-H.; Lu, M.-C.; Chen, W.-F.; Wen, Z.-H.; Kuo, Y.-H.; Sung, P.-J. Cladieunicellins K and L, new eunicellin-based diterpenoids from an octocoral *Cladiella* sp. *Int. J. Mol. Sci.* **2013**, *14*, 21781–21789.
12. Chen, B.-W.; Wang, S.-Y.; Huang, C.-Y.; Chen, S.-L.; Wu, Y.-C.; Sheu, J.-H. Hirsutalins I–M, eunicellin-based diterpenoids from the soft coral *Cladiella hirsuta. Tetrahedron* **2013**, *69*, 2296–2301.
13. Huang, T.-Z.; Chen, B.-W.; Huang, C.-Y.; Hwang, T.-L.; Dai, C.-F.; Sheu, J.-H. Eunicellin-based diterpenoids, hirsutalins N–R, from the Formosan soft coral *Cladiella hirsuta*. *Mar. Drugs* **2014**, *12*, 2446–2457
14. Chen, T.-H.; Chen, W.-F.; Wen, Z.-H.; Lu, M.-C.; Wang, W.-H.; Li, J.-J.; Wu, Y.-C.; Sung, P.-J. Cladieunicellins M–Q, new eunicellins from *Cladiella* sp. *Mar. Drugs* **2014**, *12*, 2144–2155.
15. Tai, C.-J.; Su, J.-H.; Huang, M.-S.; Wen, Z.-H.; Dai, C.-F.; Sheu, J.-H. Bioactive eunicellin-based diterpenoids from the soft coral *Cladiella krempfi*. *Mar. Drugs* **2011**, *9*, 2036–2045.
16. Tai, C.-J.; Su, J.-H.; Huang, C.-Y.; Huang, M.-S.; Wen, Z.-H.; Dai, C.-F.; Sheu, J.-H. Cytotoxic and anti-inflammatory eunicellin-based diterpenoids from the soft coral *Cladiella krempfi*. *Mar. Drugs* **2013**, *11*, 788–799.
17. Lee, Y.-N.; Tai, C.-J.; Hwang, T.-L.; Sheu, J.-H. Krempfielins J–M, new eunicellin-based diterpenoids from the soft coral *Cladiella krempfi*. *Mar. Drugs* **2013**, *11*, 2741–2750.
18. Lee, Y.-N.; Tai, C.-J.; Hwang, T.-L.; Sheu, J.-H. Krempfielins N–P, new anti-inflammatory eunicellins from a Taiwanese soft coral *Cladiella krempfi*. *Mar. Drugs* **2014**, *12*, 1148–1156.
19. Wu, S.-L.; Su, J.-H.; Wen, Z.-H.; Hsu, C.-H.; Chen, B.-W.; Dai, C.-F.; Kuo, Y.-H.; Sheu, J.-H. Simplexins A–I, eunicellin-based diterpenoids from the soft coral *Klyxum simplex*. *J. Nat. Prod.* **2009**, *72*, 994–1000.
20. Chen, B.-W.; Wu, Y.-C.; Chiang, M.Y.; Su, J.-H.; Wang, W.-H.; Fan, T.-Y.; Sheu, J.-H. Eunicellin-based diterpenoids from the cultured soft coral *Klyxum simplex*. *Tetrahedron* **2009**, *65*, 7016–7022.
21. Chen, B.-W.; Chao, C.-H.; Su, J.-H.; Wen, Z.-H.; Sung, P.-J.; Sheu, J.-H. Anti-inflammatory eunicellin-based diterpenoids from the cultured soft coral *Klyxum simplex*. *Org. Biomol. Chem.* **2010**, *8*, 2363–2366.
22. Wu, S.-L.; Su, J.-H.; Lu, Y.; Chen, B.-W.; Huang, C.-Y.; Wen, Z.-H.; Kuo, Y.-H.; Sheu, J.-H. Simplexins J–O, eunicellin-based diterpenoids from a Dongsha Atoll soft coral *Klyxum simplex*. *Bull. Chem. Soc. Jpn.* **2011**, *84*, 626–632.
23. Hsu, F.-J.; Chen, B.-W.; Wen, Z.-H.; Huang, C.-Y.; Dai, C.-F.; Su, J.-H.; Wu, Y.-C.; Sheu, J.-H. Klymollins A–H, bioactive eunicellin-based diterpenoids from the Formosan soft coral *Klyxum molle*. *J. Nat. Prod.* **2011**, *74*, 2467–2471.

24. Chen, B.-W.; Chao, C.-H.; Su, J.-H.; Tsai, C.-W.; Wang, W.-H.; Wen, Z.-H.; Huang, C.-Y.; Sung, P.-J.; Wu, Y.-C.; Sheu, J.-H. Klysimplexins I–T, eunicellin-based diterpenoids from the cultured soft coral *Klyxum simplex*. *Org. Biomol. Chem.* **2011**, *9*, 834–844.
25. Gómez-Reyes, J.F.; Salazar, A.; Guzmán, H.M.; González, Y.; Fernández, P.L.; Ariza-Castolo, A.; Gutiérrez, M. *seco*-Briarellinone and Briarellin S, two new eunicellin-based diterpenoids from the Panamanian octocoral *Briareum asbestinum*. *Mar. Drugs* **2012**, *10*, 2608–2617.
26. Lin, M.-C.; Chen, B.-W.; Huang, C.-Y.; Dai, C.-F.; Hwang, T.-L.; Sheu, J.-H. Eunicellin-based diterpenoids from the Formosan soft coral *Klyxum molle* with inhibitory activity on superoxide generation and elastase release by neutrophils. *J. Nat. Prod.* **2013**, *76*, 1661–1667.
27. Chang, F.-Y.; Hsu, F.-J.; Tai, C.-J.; Wei, W.-C.; Yang, N.-S.; Sheu, J.-H. Klymollins T–X, bioactive eunicellin-based diterpenoids from the soft coral *Klyxum molle*. *Mar. Drugs* **2014**, *12*, 3060–3071.
28. Alley, M.C.; Scudiero, D.A.; Monks, A.; Hursey, M.L.; Czerwinski, M.J.; Fine, D.L.; Abbott, B.J.; Mayo, J.G.; Shoemaker, R.H.; Boyd, M.R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res*. **1988**, *48*, 589–601.
29. Scudiero, D.A.; Shoemaker, R.H.; Paull, K.D.; Monks, A.; Tierney, S.; Nofziger, T.H.; Currens, M.J.; Seniff, D.; Boyd, M.R. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res*. **1988**, *48*, 4827–4833.
30. Yu, H.-P.; Hsieh, P.-W.; Chang, Y.-J.; Chung, P.-J.; Kuo, L.-M.; Hwang, T.-L. 2-(2-Fluorobenzamido) benzoate ethyl ester (EFB-1) inhibits superoxide production by human neutrophils and attenuates hemorrhagic shock-induced organ dysfunction in rats. *Free Radic. Biol. Med.* **2011**, *50*, 1737–1748.
31. Hwang, T.-L.; Wang, C.-C.; Kuo, Y.-H.; Huang, H.-C.; Wu, Y.-C.; Kuo, L.-M.; Wu, Y.-H. The hederagenin saponin SMG-1 is a natural FMLP receptor inhibitor that suppresses human neutrophil activation. *Biochem. Pharmacol*. **2010**, *80*, 1190–1200.
32. Hwang, T.-L.; Leu, Y.-L.; Kao, S.-H.; Tang, M.-C.; Chang, H.-L. Viscolin, a new chalcone from *Viscum coloratum*, inhibits human neutrophil superoxide anion and elastase release via a cAMP-dependent pathway. *Free Radic. Biol. Med.* **2006**, *41*, 1433–1441.