

Article

Secosteroids and Norcembranoids from the Soft Coral *Sinularia nanolobata*

Yen-Ju Tseng¹, Shang-Kwei Wang^{2,3,*} and Chang-Yih Duh^{1,3,*}

¹ Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan; E-Mail: pit0424@yahoo.com.tw

² Department of Microbiology, Kaohsiung Medical University, Kaohsiung 807, Taiwan

³ Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan

* Authors to whom correspondence should be addressed; E-Mails: skwang@cc.kmu.edu.tw (S.-K.W.); yihduh@mail.nsysu.edu.tw (C.-Y.D.); Tel.: +886-7-312-1101 (ext. 2150) (S.-K.W.); +886-7-525-2000 (ext. 5036) (C.-Y.D.); Fax: +886-7-312-1101 (ext. 2151) (S.-K.W.); +886-7-525-5020 (C.-Y.D.).

Received: 25 July 2013; in revised form: 15 August 2013 / Accepted: 21 August 2013 /

Published: 27 August 2013

Abstract: Two new 9,11-secosteroids, 22 α -acetoxy-24-methylene-3 β ,6 α ,11-trihydroxy-9,11-seco-cholest-7-en-9-one (**1**) and 11-acetoxy-24-methylene-1 β ,3 β ,6 α -trihydroxy-9,11-seco-cholest-7-en-9-one (**2**), as well as two known norcembranoids, 5-*epi*-sinuleptolide (**3**) and sinuleptolide (**4**), were isolated from the soft coral *Sinularia nanolobata*. The structures of these metabolites were elucidated on the basis of extensive spectroscopic analysis. The anti-HCMV (human cytomegalovirus) activity of **1–4** and its cytotoxicity against selected cell lines were evaluated.

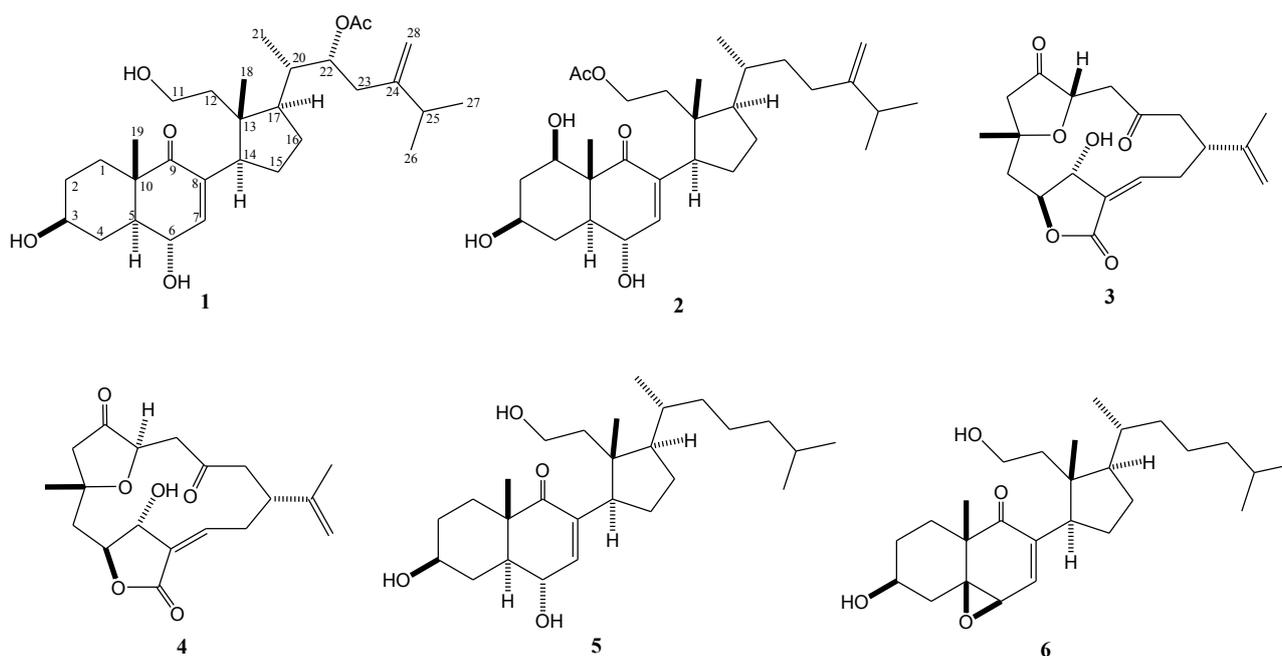
Keywords: soft coral; *Sinularia nanolobata*; secosteroid; cytotoxicity

1. Introduction

Soft corals have been proven to be a rich source of 9,11-secosterols [1–14] and C-4 norcembranoids [15–24]. 9,11-Secosteroids were found to possess cytotoxic [2,3,5,6,8,10–13] and anti-inflammatory activities [13]. C-4 Norcembranoids were found to possess cytotoxic [18–20,25], anti-inflammatory [23,24], and antiviral activities [23]. As part of the continuing search for bioactive metabolites from marine invertebrates, we explored the secondary metabolites of the soft coral *Sinularia*

nanolobata (Verseveldt, 1977) which was collected from Xiao-Liuqiu Island, Pingtung County, Taiwan. Chromatographic separation of the EtOAc extract of the soft coral resulted in the isolation of new secosteroids **1** and **2**, along with two known norcembranoids, 5-*epi*-sinuleptolide (**3**) and sinuleptolide (**4**) [21] (Figure 1). The structures of these metabolites were established by extensive spectroscopic analysis. The anti-HCMV (human cytomegalovirus) activity of **1–4** and cytotoxicity against P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma), and A-549 (human lung carcinoma) cancer cell lines were evaluated *in vitro*.

Figure 1. Structures of Metabolites **1–6**.



2. Results and Discussion

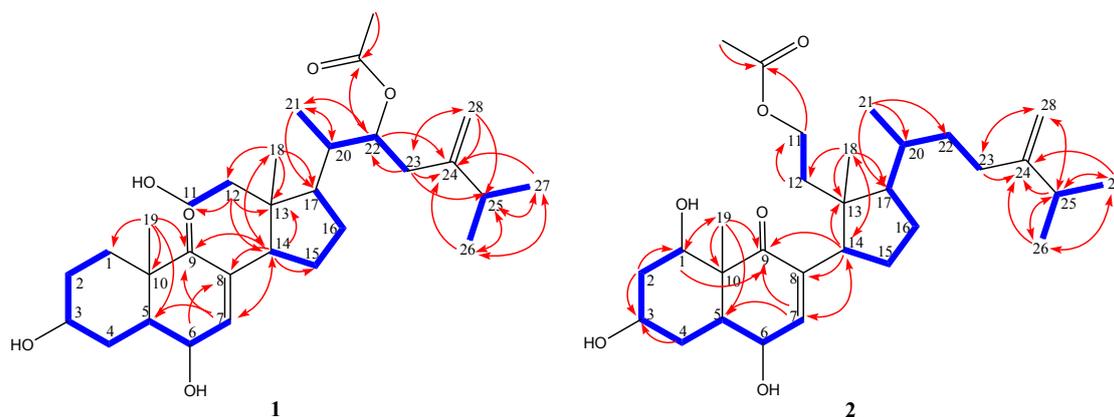
Metabolite **1** was isolated as colorless needles. Its molecular formula, $C_{30}H_{48}O_6$, was established by HRESIMS (m/z 527.3351, $[M + Na]^+$), implying seven degrees of unsaturation. The presence of hydroxyl groups was suggested by a strong absorption band at 3459 cm^{-1} in the IR spectrum. The ^{13}C NMR and DEPT (Table 1) spectroscopic data showed signals of six methyls, eight sp^3 methylenes (including one oxymethylene), one sp^2 methylene, eight sp^3 methines (including three oxymethines), one sp^2 methine, two sp^3 and four sp^2 quaternary carbons. The quaternary carbon signal at δ_C 205.4 combined with the chemical shifts of H_3 -18 (δ 0.67, s), H_3 -19 (δ 1.14, s), H_3 -21 (δ 1.02, d, $J = 6.8$ Hz), H_3 -26 (δ 1.03, d, $J = 6.8$ Hz), H_3 -27 (δ 1.03, d, $J = 6.8$ Hz), H-3 (δ 3.61, m), H-6 (δ 4.30, dd, $J = 10.0$, 1.6 Hz), H-7 (δ 6.61, d, $J = 1.6$ Hz), and H_2 -11 (each 1H, δ 3.70, m; δ 3.93, m) were found to be similar to those of 3 β ,6 α ,11-trihydroxy-9,11-seco-5 α -cholest-7-en-9-one (**5**) [4]. Moreover, the signals appearing at δ_C 170.7 (qC), 152.2 (qC), 108.8 (CH_2), 74.7 (CH), and 21.2 (CH_3) indicated the presence of an acetoxy and an exomethylene in **1**. This was further supported by the ^1H NMR signals of acetoxy and exomethylene protons at δ_H 1.99 (3H, s) and 4.71 and 4.80 (each 1H, s), respectively. From the COSY spectrum measured in $CDCl_3$, it was possible to establish five proton sequences from H-1 to H-7, H_2 -11 to H_2 -12, H-14 to H-17, H-21 to H_2 -23 through H-20, and H-25 to H_3 -26 and H_3 -27 (Figure 2).

Key HMBC correlations of H₂-4 to C-3 and C-5; H-7 to C-5, C-9 and C-14; H₂-12 to C-13 and C-14; H-14 to C-7, C-8, C-9, C-13, C-15 and C-18; H₃-18 to C-12, C-13, C-14, and C-17; H₃-19 to C-1, C-5, C-9, and C-10; H-20 to C-21; H₃-21 to C-17, C-20 and C-22; H-22 to C-24; H₂-23 to C-25; H₃-26 to C-24, C-25, and C-27; H₃-27 to C-24, C-25, and C-26; H₂-28 to C-23, C-24 and C-25; H₃-OAc to OAc-22 permitted the establishment of the secosterol-type skeleton of **1**.

Table 1. ¹H and ¹³C NMR Spectroscopic Data for compounds **1** and **2**.

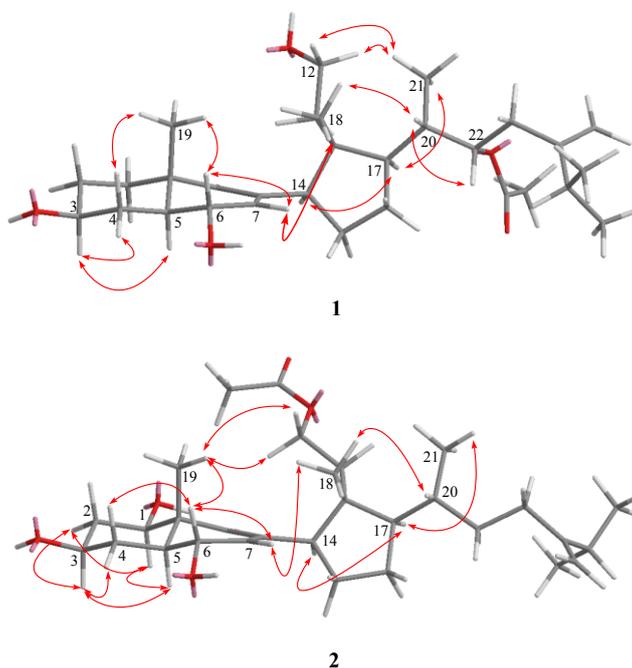
Position	1		2	
	δ_{H}^a	δ_{C}^b	δ_{H}^a	δ_{C}^b
1	1.48 m; 1.90 m	31.8, CH ₂ ^d	3.87 dd (12.0, 4.4)	70.7, CH
2	1.48 m; 1.94 m	30.5, CH ₂	1.52 m; 2.14 m	37.8, CH ₂
3	3.61 m	69.9, CH	3.71 m	67.4, CH
4	1.45 m; 2.31 m	32.8, CH ₂	1.43 m; 2.29 m	32.2, CH ₂
5	1.80 m	48.5, CH	1.70 m	46.4, CH
6	4.30 dd (10.0, 1.6) ^c	69.4, CH	4.38 dd (9.2, 2.0)	69.1, CH
7	6.61 d (1.6)	147.8, CH	6.58 d (1.6)	147.4, CH
8		136.5, qC		136.8, qC
9		205.4, qC		206.5, qC
10		44.9, qC		49.0, qC
11	3.70 m; 3.93 m	59.3, CH ₂	4.16 td (10.4, 6.4); 4.23 td (10.4, 5.2)	61.2, CH ₂
12	1.04 m; 1.64 m	40.7, CH ₂	1.15 m; 1.70 m	36.8, CH ₂
13		46.3, qC		46.0, qC
14	3.47 dd (9.6, 8.8)	42.2, CH	3.25 dd (10.8, 9.6)	42.2, CH
15	1.62 m	26.4, CH ₂	1.62 m	26.4, CH ₂
16	1.65 m; 1.91 m	27.0, CH ₂	1.45 m; 1.87 m	26.2, CH ₂
17	1.75 m	45.9, CH	1.67 m	50.1, CH
18	0.67 s	16.9, CH ₃	0.69 s	17.0, CH ₃
19	1.14 s	16.2, CH ₃	1.17 s	9.8, CH ₃
20	1.78 m	39.8, CH	1.46 m	34.7, CH
21	1.02 d (6.8)	11.8, CH ₃	1.02 d (6.4)	18.8, CH ₃
22	5.12 m	74.7, CH	1.18 m; 1.56 m	34.0, CH ₂
23	2.17 m	32.6, CH ₂	1.89 m; 2.11 m	31.6, CH ₂
24		152.2, qC		156.5, qC
25	2.20 m	33.4, CH	2.21 m	33.8, CH
26	1.03 d (6.8)	21.6, CH ₃	1.02 d (6.8)	21.8, CH ₃
27	1.03 d (6.8)	22.1, CH ₃	1.02 d (6.8)	22.0, CH ₃
28	4.71 s; 4.80 s	108.8, CH ₂	4.66 s; 4.73 s	106.2, CH ₂
OAc	1.99 s	21.2, CH ₃	2.01 s	21.1 CH ₃
		170.7, qC		171.2, qC

^a Spectra recorded at 400 MHz in CDCl₃; ^b Spectra recorded at 100 MHz in CDCl₃; ^c *J* values (in Hz) are in parentheses; ^d Carbon types are deduced by HSQC and DEPT experiments.

Figure 2. Selected ^1H - ^1H COSY (—) and HMBC (\rightarrow) correlations of **1** and **2**.

The relative configurations of eight chiral centers at C-3, C-5, C-6, C-10, C-13, C-14, C-17, and C-20 in **1** were found to be the same as those of $3\beta,6\alpha,11$ -trihydroxy- $9,11$ -seco- 5α -cholest- 7 -ene- 9 -one [4] (Figure 2). Key NOE correlations for **1** showed interactions between H-20/H-22. Thus, OAc-22 should be α -oriented. The above data established the structure of compound **1** as $22R$ -acetoxy- $3\beta,6\alpha,11$ -trihydroxy- 24 -methylene- $9,11$ -secocholestan- 9 -one.

Metabolite **2** possessed the same molecular formula ($\text{C}_{30}\text{H}_{48}\text{O}_6$) as that of **1**, as revealed from HRESIMS. The ^{13}C NMR spectral data of **2** were found to be close to those of **1**, except for the signals due to C-1, C-11, and C-22. COSY correlations from H-1 to H-2, H₂-11 to H₂-12, and H-20 to H₂-22, as well as HMBC correlations from H-1 to C-10, C-9, from H₂-11 to 11-OAc, and from Me-21 to C-17, C-20, C-22 confirmed the presence of a hydroxyl at C-1, an acetoxy at C-11, and the absence of the acetoxy at C-22 (Figure 2). Furthermore, the configuration of C-1 was deduced from the NOE correlations of H-1/H-5 and H-3/H-5 (Figure 3). Thus, the structure of compound **2** was established as 11 -acetoxy- 24 -methylene- $1\beta,3\beta,6\alpha$ -trihydroxy- $9,11$ -seco-cholest- 7 en- 9 -one.

Figure 3. Key NOESY Correlations for **1** and **2**.

Cytotoxicity of compounds 1–4 against the proliferation of a limited panel of cancer cell lines, including P-388, A549, and HT-29, were evaluated (Table 2). Metabolites 1–3 displayed weak cytotoxicity against P-388, with IC₅₀ of 10.2, 27.8, and 15.7 µg/mL, respectively. Compounds 1–4 was also examined for antiviral activity against human cytomegalovirus (HCMV) using a human embryonic lung (HEL) cell line. Compounds 4 showed anti-HCMV activity with ED₅₀ of 1.92 µg/mL (Ganciclovir showed anti-HCMV activity with ED₅₀ of 0.12 µg/mL).

Table 2. Cytotoxicity and anti- human cytomegalovirus (HCMV) activity of 1–4.

Compounds	IC ₅₀ (µg/mL)				Anti-HCMV
	A549	HT-29	P-388	HEL	
1	>50	>50	10.2	>50	>50
2	>50	>50	27.8	>50	>50
3	>50	>50	15.7	>50	>50
4	>50	>50	>50	>50	1.92

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were measured on a JASCO P1020 digital polarimeter. IR spectra were recorded on a JASCO FT/IR4100 infrared spectrophotometer. The NMR spectra were recorded on a Varian MR 400 FT-NMR at 400 MHz for ¹H and 100 MHz for ¹³C, in CDCl₃ using solvent peak as internal standard. LRMS and HRMS were obtained by ESI on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, Darmstadt, Germany, 230–400 mesh) and LiChroprep RP-18 (Merck, 40–63 µm) were used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F₂₅₄, 0.25 mm) and precoated RP-18 F_{254s} plates (Merck) were used for TLC analysis. High-performance liquid chromatography was carried out using a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 220 nm together with a semi-preparative reversed-phase column (Merck, Hibar LiChrospher RP-18e, 5 µm, 250 × 25 mm).

3.2. Animal Material

The octocoral *S. nanolobata* was collected by hand using scuba at the Xiao Liuqiu, Pingtung County, Taiwan, in November 2011, at a depth of 6 m. This soft coral was identified by Prof. Chang-Fong Dai, Institute of Oceanography, National Taiwan University. A voucher specimen (SL-10) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

3.3. Extraction and Separation

The frozen soft coral (1.5 kg) was chopped into small pieces and extracted with EtOAc (3L × 3) in a percolator at room temperature. The EtOAc extract of *S. nanolobata* was concentrated to a dark brown gum (12.3 g). The EtOAc residue was subjected to Si 60 CC using *n*-hexane-EtOAc-MeOH mixtures of increasing polarity for elution. Fraction 15~21, eluted with *n*-hexane-EtOAc (1:10) to EtOAc-MeOH (2:1), was further subjected to Si 60 CC EtOAc-MeOH (5:1) to give four subfractions. A subfraction

15~21-1, was purified by reverse-phase HPLC (MeOH-H₂O, 55:45) to afford **3** (50.0 mg) and **4** (35.0 mg). A subfraction 15~21-4, was purified by reverse-phase HPLC (MeOH-H₂O, 85:15) to afford **1** (2.5 mg) and **2** (3.1 mg).

22 α -acetoxy-24-methylene-3 β ,6 α ,11-trihydroxy-9,11-seco-cholest-7-en-9-one (**1**): White powder; $[\alpha]_D^{25} = +54$ (*c* 0.6, CHCl₃); IR (KBr) ν_{\max} 3459, 2956, 1733, 1031, 1009 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; ESIMS *m/z* 527 [M + Na]⁺; HRESIMS *m/z* 527.3351 (calcd for C₃₀H₄₈O₆Na, 527.3348).

11-acetoxy-24-methylene-1 β ,3 β ,6 α -trihydroxy-9,11-seco-cholest-7-en-9-one (**2**): White powder; $[\alpha]_D^{25} = +16$ (*c* 0.8, CHCl₃); IR (KBr) ν_{\max} 3444, 2925, 1741, 1260, 1036 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; ESIMS *m/z* 527 [M + Na]⁺; HRESIMS *m/z* 527.3345 (calcd for C₃₀H₄₈O₆Na, 527.3348).

3.4. Cytotoxicity Testing

Cytotoxicity was determined on P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma), and A-549 (human lung epithelial carcinoma) tumor cells using a modification of the MTT colorimetric method according to a previously described procedure [26,27]. The provision of the P-388 cell line was supported by J.M. Pezzuto, formerly of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago. HT-29 and A-549 cell lines were purchased from the American Type Culture Collection. To measure the cytotoxic activities of tested compounds, five concentrations with three replications were performed on each cell line. Mithramycin was used as a positive control.

3.5. Anti-HCMV Assay

To determine the effects of natural products upon HCMV cytopathic effect (CPE), confluent human embryonic lung (HEL) cells grown in 24-well plates were incubated for 1 h in the presence or absence of various concentrations of tested natural products with three replications. Ganciclovir was used as a positive control. Then, cells were infected with HCMV at an input of 1000 pfu (plaque forming units) per well of a 24-well dish. Antiviral activity was expressed as IC₅₀ (50% inhibitory concentration), or compound concentration required to reduce virus induced CPE by 50% after 7 days as compared with the untreated control. To monitor the cell growth upon treating with natural products, an MTT-colorimetric assay was employed [28].

4. Conclusions

In the previous studies, 5-*epi*-sinuleptolide (**3**) and sinuleptolide (**4**) both showed inhibition of iNOS protein by LPS stimulation [23], TNF- α production in a dose-dependent manner [24], and nitric oxide (NO) production [24]. 5-*Epi*-sinuleptolide (**3**) inhibited human skin cancer cells growth [25]. Sinuleptolide (**4**) exerted antiviral activity against HCMV (human cytomegalovirus) cells [23]. 9,11-Secosteroid **6** with epoxide at C-5 and C-6 exhibited cytotoxicity against HT-29 cells [29]. This investigation of soft coral *S. nanolobata* collected at Xiao-Liuqiu Island (Pingtung County, Taiwan) has led to the isolation of two new 9,11-secosteroids (**1** and **2**) and two known compounds, 5-*epi*-sinuleptolide (**3**) and sinuleptolide (**4**). Metabolites **1–3** displayed weak cytotoxicity and

selectivity against P-388, with IC₅₀ of 10.2, 27.8, and 15.7 µg/mL, respectively. Metabolite 4 exhibited antiviral activity against HCMV with ED₅₀ of 1.92 µg/mL.

Acknowledgements

This research was financially supported by grants from the National Science Council (NSC102-2320-B-110-003-MY3) and NSYSUKMU Joint Project (NSYSUKMU 2013-P018) awarded to C.-Y. Duh.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Kazlauskas, R.; Murphy, P.T.; Ravi, B.N.; Sanders, R.L.; Wells, R. Spermidine derivatives and 9,11-secosteroids from a soft coral (*Sinularia* sp.). *Aust. J. Chem.* **1982**, *35*, 69–75.
2. Koljak, R.; Pehk, T.; Järving, I.; Liiv, M.; Lopp, A.; Varvas, K.; Vahemets, A.; Lille, Ü.; Samel, N. New antiproliferative 9,11-secosterol from soft coral *Gersemia fruticosa*. *Tetrahedron Lett.* **1993**, *34*, 1985–1986.
3. Lopp, A.; Pihlak, A.; Paves, H.; Samuel, K.; Koljak, R.; Samel, N. The effect of 9,11-secosterol, a newly discovered compound from the soft coral *Gersemia-fruticosa*, on the growth and cell-cycle progression of various tumor-cells in culture. *Steroids* **1994**, *59*, 274–281.
4. Aknin, M.; Costantino, V.; Mangoni, A.; Fattorusso, E.; Gaydou, E.M. New 9,11-secosterols from gorgonia *Subergorgia suberosa* of the Indian Ocean. *Steroids* **1998**, *63*, 575–578.
5. Koljak, R.; Lopp, A.; Pehk, T.; Varvas, K.; Müürisepp, A.-M.; Järving, I.; Samel, N. New cytotoxic sterols from the soft coral *Gersemia fruticosa*. *Tetrahedron* **1998**, *54*, 179–186.
6. Morris, L.A.; Christie, E.M.; Jaspars, M.; van Ofwegen, L.P. A bioactive secosterol with an unusual A- and B-ring oxygenation pattern isolated from an Indonesian soft coral *Lobophytum* sp. *J. Nat. Prod.* **1998**, *61*, 538–541.
7. van Altena, I.A.; Butler, A.J.; Dunne, S.J. A new cyclized 9,11-secosterol enol-ether from the Australian sponge *Euryspongia arenaria*. *J. Nat. Prod.* **1999**, *62*, 1154–1157.
8. Naz, S.; Kerr, R.G.; Narayanan, R. New antiproliferative epoxysecosterols from *Pseudopterogorgia americana*. *Tetrahedron Lett.* **2000**, *41*, 6035–6040.
9. Anta, C.; González, N.; Rodríguez, J.; Jiménez, C. A new secosterol from the Indonesian octocoral *Pachyclavularia vioacea*. *J. Nat. Prod.* **2002**, *65*, 1357–1359.
10. Su, J.-H.; Tseng, Y.-J.; Huang, H.-H.; Ahmed, A.F.; Lu, C.-K.; Wu, Y.-C.; Sheu, J.-H. 9,11-Secosterols from the soft corals *Sinularia lochmodes* and *Sinularia leptoclados*. *J. Nat. Prod.* **2006**, *69*, 850–852.
11. Cheng, S.-Y.; Chen, H.-P.; Wang, S.-K.; Duh, C.-Y. Three new 9,11-secosterols from the Formosan soft coral *Sinularia leptoclados*. *Bull. Chem. Soc. Jpn.* **2011**, *84*, 648–652.

12. Chen, B.-W.; Chang, S.-M.; Huang, C.-Y.; Su, J.-H.; Wen, Z.-H.; Wu, Y.-C.; Sheu, J.-H. Hirsutosterols A–G, polyoxygenated steroids from a Formosan soft coral *Cladiella hirsute*. *Org. Biomol. Chem.* **2011**, *9*, 3272–3278.
13. Huang, C.-Y.; Su, J.-H.; Duh, C.-Y.; Chen, B.-W.; Wen, Z.-H.; Kuo, Y.-H.; Sheu, J.-H. A new 9,11-secosterol from the soft coral *Sinularia granosa*. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4373–4376.
14. Sica, D.; Musumeci, D. Secosteroids of marine origin. *Steroids* **2004**, *69*, 743–756.
15. Bowden, B.F.; Coll, J.C.; Mitchell, S.J.; Mulder, J.; Stokie, G.J. Studies of Australian soft corals. IX a novel nor-diterpene from the soft coral *Sinularia leptoclados*. *Aust. J. Chem.* **1978**, *31*, 2049–2056.
16. Sato, A.; Fenical, W.; Zheng, Q.-T.; Clardy, J. Norcembrene diterpenoids from Pacific soft-corals of the genus *sinularia* (Alcyonacea; octocorallia). *Tetrahedron* **1985**, *41*, 4303–4308.
17. Shoji, N.; Umeyama, A.; Arihara, S. A novel norditerpenoid from the Okinawan soft coral *Sinularia* sp. *J. Nat. Prod.* **1993**, *56*, 1651–1653.
18. Sheu, J.-H.; Ahmed, A. F.; Shiue, R.-T.; Dai, C.-F.; Kuo, Y.-H. Scabrolides A–D, four new norditerpenoids isolation from the soft coral *Sinularia scabra*. *J. Nat. Prod.* **2002**, *65*, 1904–1908.
19. Ahmed, A.F.; Su, J.-H.; Kuo, Y.-H.; Sheu, J.-H. Scabrolides E–G, three new norditerpenoids from the soft coral *Sinularia scabra*. *J. Nat. Prod.* **2004**, *67*, 2079–2082.
20. Ahmed, A.F.; Shiue, R.-T.; Wang, G.-H.; Dai, C.-F.; Kuo, Y.-H.; Sheu, J.-H. Five novel norcembranoids from *Sinularia leptoclados* and *S. parva*. *Tetrahedron* **2003**, *59*, 7337–7344.
21. Tseng, Y.-J.; Ahmed, A.F.; Dai, C.-F.; Chiang, M.Y.; Sheu, J.-H. Sinulochmodins A–C, three novel terpenoids from the soft coral *Sinularia lochmodes*. *Org. Lett.* **2005**, *7*, 3813–3816.
22. Tseng, Y.-J.; Ahmed, A.F.; Hsu, C.-H.; Su, J.-H.; Dai, C.-F.; Sheu, J.-H. New norcembranoids from the soft coral *Sinularia lochmodes*. *J. Chin. Chem. Soc.* **2007**, *54*, 1041–1044.
23. Cheng, S.-Y.; Chuang, C.-T.; Wen, Z.-H.; Wang, S.-K.; Chiou, S.-F.; Hsu, C.-H.; Dai, C.-F.; Duh, C.-Y. Bioactive norditerpenoids from the soft coral *Sinularia gyrosa*. *Bioorg. Med. Chem.* **2010**, *18*, 3379–3386.
24. Takaki, H.; Koganemaru, R.; Iwakawa, Y.; Higuchi, R.; Miyamoto, T. Inhibitory effect of norditerpenes on LPS-Induced TNF- α production from the Okinawan soft coral, *Sinularia* sp. *Biol. Pharm. Bull.* **2003**, *26*, 380–382.
25. Liang, C.-H.; Wang, G.-H.; Chou, T.-H.; Wang, S.-H.; Lin, R.-J.; Chan, L.-P.; So, E.-C.; Sheu, J.-H. 5-*epi*-Sinuleptolide induces cell cycle arrest and apoptosis through tumor necrosis factor/mitochondria-mediated caspase signaling pathway in human skin cancer cells. *Biochim. Biophys. Acta* **2012**, *1820*, 1149–1157.
26. Hou, R.-S.; Duh, C.-Y.; Chiang, M.Y.; Lin, C.-N. Sinugibberol, a new cytotoxic cembranoid diterpene from the soft coral *Sinularia gibberosa*. *J. Nat. Prod.* **1995**, *58*, 1126–1130.
27. Geran, R.I.; Greenberg, N.H.; MacDonald, M.M.; Schumacher, A.M.; Abbott, B.J. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother. Rep.* **1972**, *3*, 1–91.
28. Stevens, M.; Balzarini, J.; Tabarrini, O.; Andrei, G.; Snoeck, R.; Cecchetti, V.; Fravolini, A.; de Clercq, E.; Pannecouque, C. Cell-dependent interference of a series of new 6-aminoquinolone derivatives with viral (HIV/CMV) transactivation. *J. Antimicrob. Chemother.* **2005**, *56*, 847–855.

29. Duh, C.-Y.; Li, C.-H.; Wang, S.-K.; Dai, C.-F. Diterpenoids, norditerpenoids, and secosteroids from the Formosan soft coral *Cespitularia hypotentaculata*. *J. Nat. Prod.* **2006**, *69*, 1188–1192.

© 2013 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).