

Article

Cytotoxic Sesterterpenoids from a Sponge *Hippospongia* sp.

Yu-Chia Chang^{1,2}, Shang-Wei Tseng^{1,3}, Li-Lian Liu^{4,5}, Yalan Chou⁴, Yuan-Shing Ho⁶,
Mei-Chin Lu^{1,3} and Jui-Hsin Su^{1,3,5,*}

¹ National Museum of Marine Biology & Aquarium, Pingtung 944, Taiwan;
E-Mails: jay0404@gmail.com (Y.-C.C.); fallboys2006@hotmail.com (S.-W.T.);
jinx6609@nmmba.gov.tw (M.-C.L.)

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

⁴ Institute of Marine Biology, National Sun Yat-sen University, Kaohsiung 804, Taiwan;
E-Mails: lilian@mail.nsysu.edu.tw (L.-L.L.); ylchou@gmail.com (Y.C.)

⁵ Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan

⁶ Eastern Marine Biology Research Center, Fisheries Research Institute, Taitung 961, Taiwan;
E-Mail: yuanho18@gmail.com

* Author to whom correspondence should be addressed; E-Mail: x2219@nmmba.gov.tw;
Tel.: +886-8-8825001 (ext. 3126); Fax: +886-8-8825087.

Received: 28 March 2012; in revised form: 18 April 2012 / Accepted: 24 April 2012 /

Published: 27 April 2012

Abstract: One new pentacyclic sesterterpene, hippospongide A (**1**), and one new scalarane sesterterpenoid, hippospongide B (**2**), along with six previously reported known scalarane-type sesterterpenes (**3–8**), were isolated from a sponge *Hippospongia* sp. The structures of these compounds were elucidated on the basis of their spectroscopic data and comparison of the NMR data with those of known analogues. These metabolites are the first pentacyclic sesterterpene and scalarane-type sesterterpenes to be reported from this genus. Compounds **3–5** exhibited significant cytotoxicity against DLD-1, HCT-116, T-47D and K562 cancer cell lines.

Keywords: sesterterpenoid; scalarane; sponge; *Hippospongia*

1. Introduction

In previous reports, scalarane sesterterpenoids have been identified from sponges and nudibranchs [1]. Research into the pharmacological properties of this class of natural products is of particular interest. In fact, many scalarane metabolites show a variety of biological activities, such as antimicrobial, cytotoxic, antifeedant, ichthyotoxic, anti-inflammatory, antitubercular, platelet aggregation inhibition, RCE-protease inhibition and nerve growth factor synthesis-stimulating [1]. Our investigation of the chemical constituents of a sponge *Hippospongia* sp. (Figure 1) yielded one new pentacyclic sesterterpene, hippospongide A (**1**), and one new scalarane sesterterpenoid, hippospongide B (**2**), along with six known sesterterpenoids, heteronemin (**3**) [2], heteronemin acetate (**4**) [3], hyrtiosin E (**5**) [4], 12-deacetoyscalarin 19-acetate (**6**) [5], hyrtiosal (**7**) [6] and scalarafuran (**8**) [7]. The cytotoxicity of metabolites **1–8** against human colon adenocarcinoma (DLD-1 and HCT-116), hormone-dependent breast cancer (T-47D) and human chronic myelogenous leukemia (K562) cell lines was evaluated.

Figure 1. Sponge *Hippospongia* sp.



2. Results and Discussion

The EtOAc extract of the freeze-dried specimen was fractionated by silica gel column chromatography and the eluted fractions were further separated utilizing normal phase HPLC to yield metabolites **1–8** (Chart 1).

Chart 1. Structures of metabolites **1–8**.

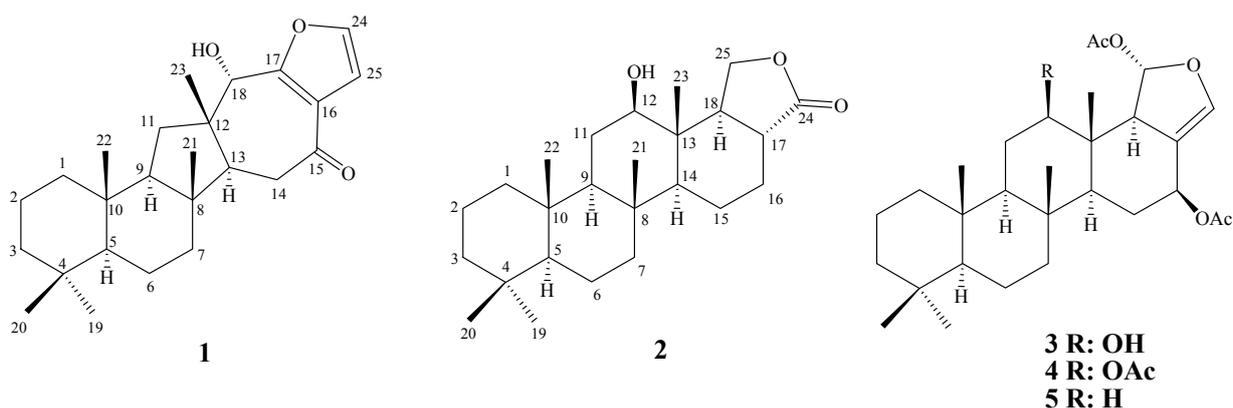
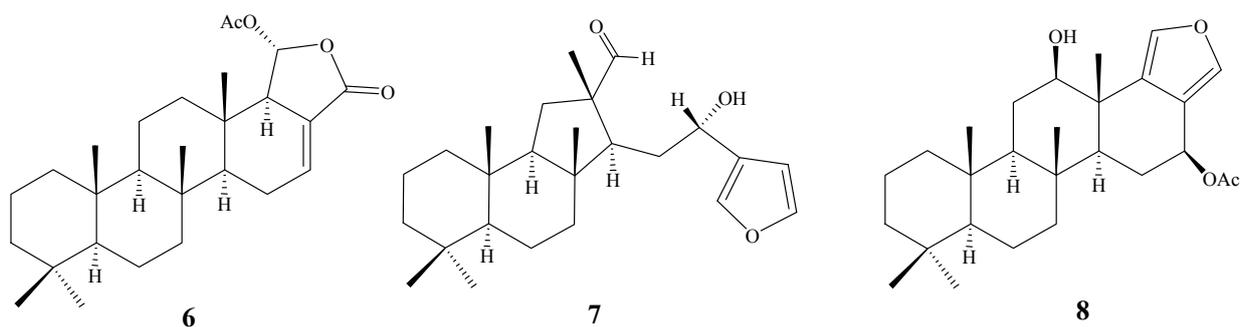


Chart 1. Cont.



The new metabolite hippospongide A (**1**) had a molecular formula of $C_{25}H_{36}O_3$ as determined by HRESIMS and NMR spectroscopic data. The IR spectrum of **1** showed absorption bands at 3386 cm^{-1} , suggesting the presence of a hydroxy group. The ^{13}C NMR data of **1** showed the presence of 25 carbons (Table 1): five methyls, seven sp^3 methylenes, four sp^3 methines (including one oxygenated carbon at δ 75.9), two sp^2 methines, and four sp^3 quaternary carbons. The remaining three signals appearing in the downfield region of the spectrum are due to the quaternary carbons of two olefinic carbons (δ 122.9 and 159.0) and one ketone carbonyl (δ 196.8). From the ^1H NMR (Table 1) spectrum of **1**, the ^1H NMR data revealed the presence of two olefinic methine protons (δ 7.33 Hz; d, $J = 1.5$ Hz; 6.76 Hz; d, $J = 1.5$ Hz). Furthermore, one oxygenated methine (δ 4.58, s) was also designated from the ^1H NMR signal. Careful analysis of the ^1H - ^1H COSY correlations observed for **1** led to the establishment of five partial structures, as shown in Figure 2. The molecular framework of **1** was further established by a HMBC experiment (Figure 2). The five rings and their connectivities were elucidated on the basis of the following key HMBC correlations: both methyls H_3 -19 and H_3 -20 to C-3, C-4 and C-5, H_3 -21 to C-7, C-8, C-9 and C-13, H_3 -22 to C-1, C-5, C-9 and C-10, H_3 -23 to C-11, C-12, C-13 and C-18, H-13 to C-15, H-14 to C-15 and C-16, H-18 to C-17 and C-16, and both olefinic methines H-24 and H-25 to C-16 and C-17. Thus, **1** was found to possess two double bonds at C-16/C-17 and C-24/C-25, one hydroxy group at C-18, and one ketone group at C-15. Linking all the above functional groups to the sesterterpene skeleton thus yielded the gross structure of **1**.

The relative configuration of **1**, elucidated mainly from the NOESY spectrum, was corroborated by MM2 force field calculations, which suggested the most stable conformation to be that shown in Figure 2. In the NOESY spectrum, H-9 showed NOEs with H-5 and H-13 but not with three methyls H_3 -21, H_3 -22 and H_3 -23. Thus, assuming an α -orientation of H-5, both H-9 and H-13 must also be on the α face whilst the three methyls H_3 -21, H_3 -22 and H_3 -23 must be located on the β face. Moreover, the NOE correlations of H_3 -23 with H-18 indicated the β -orientation of H-18. On the basis of the above findings and other detailed NOE correlations (Figure 3), the relative structure of **1** was determined. After determining the structure of **1**, we discovered that its molecular framework has been obtained as known sesterterpenoids salmahyrtisol A and similan A, which were isolated previously from sponges *Hyrtios erecta* [8] and *Hyrtios gumminae* [9], respectively.

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

Position	1		2	
	δ_{H} (J in Hz) ^a	δ_{C} (mult.) ^b	δ_{H} (J in Hz) ^a	δ_{C} (mult.) ^b
1	1.46 m; 0.98 m	40.2 (CH ₂) ^c	1.65 m	39.9 (CH ₂)
2	1.65 m; 1.40 m	18.4 (CH ₂)	1.54 m; 1.38 m	18.2 (CH ₂)
3	1.38 m; 1.19 m	42.5 (CH ₂)	1.36 m; 1.12 m	42.0 (CH ₂)
4		33.1 (C)		33.3 (C)
5	0.92 m	57.6 (CH)	0.80 m	56.5 (CH)
6	1.57 m; 1.38 m	18.8 (CH ₂)	1.61 m; 1.42 m	18.6 (CH ₂)
7	1.68 m; 1.10 m	40.1 (CH ₂)	1.74 m; 0.90 m	41.7 (CH ₂)
8		44.8 (C)		37.3 (C)
9	1.45 m	61.0 (CH)	0.88 m	58.9 (CH)
10		36.8 (C)		37.5 (C)
11	1.99 d (6.0); 1.43 m	35.0 (CH ₂)	1.70 m; 1.45 m	27.5 (CH ₂)
12		43.0 (C)	3.40 br d (10.5)	80.5 (CH)
13	2.20 dd (13.0, 2.5)	47.5 (CH)		42.0 (C)
14	2.64 dd (13.5, 13.0) 2.54 dd (13.5, 2.5)	39.6 (CH ₂)	0.80 m	58.1 (CH)
15		196.8 (C)	1.78 m; 1.36 m	20.0 (CH ₂)
16		122.9 (C)	2.20 m; 1.22 m	25.6 (CH ₂)
17		159.0 (C)	2.22 m	39.2 (CH)
18	4.58 s	75.9 (CH)	1.86 m	55.3 (CH)
19	0.85 s	33.5 (CH ₃)	0.84 s	33.2 (CH ₃)
20	0.84 s	21.3 (CH ₃)	0.80 s	21.3 (CH ₃)
21	0.85 s	16.2 (CH ₃)	0.84 s	17.3 (CH ₃)
22	0.87 s	15.6 (CH ₃)	0.84 s	16.3 (CH ₃)
23	1.14 s	23.4 (CH ₃)	0.91 s	9.8 (CH ₃)
24	7.33 d (1.5)	142.3 (CH)		177.8 (C)
25	6.76 d (1.5)	110.9 (CH)	4.38 dd (9.5, 7.0) 4.09 dd (11.0, 10.0)	70.0 (CH ₂)

^a 500 MHz in CDCl₃; ^b 125 MHz in CDCl₃; ^c Numbers of attached protons were deduced by DEPT experiments.

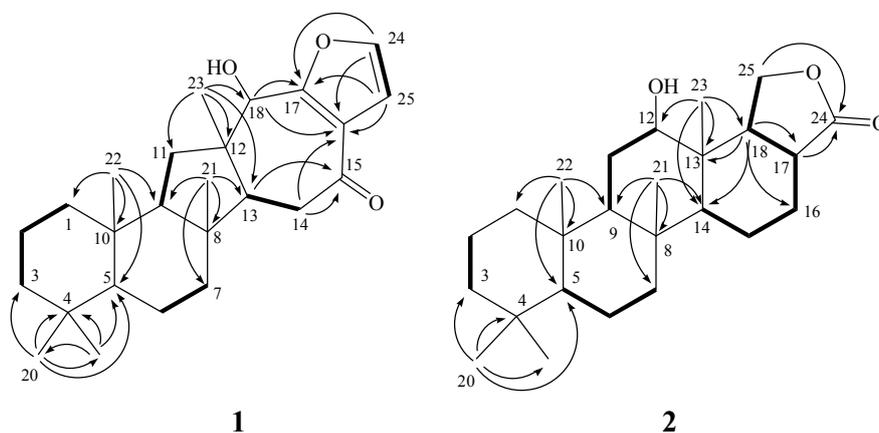
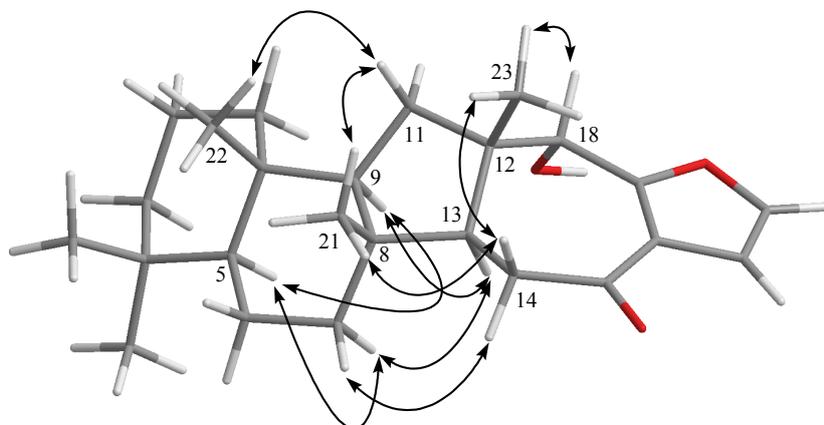
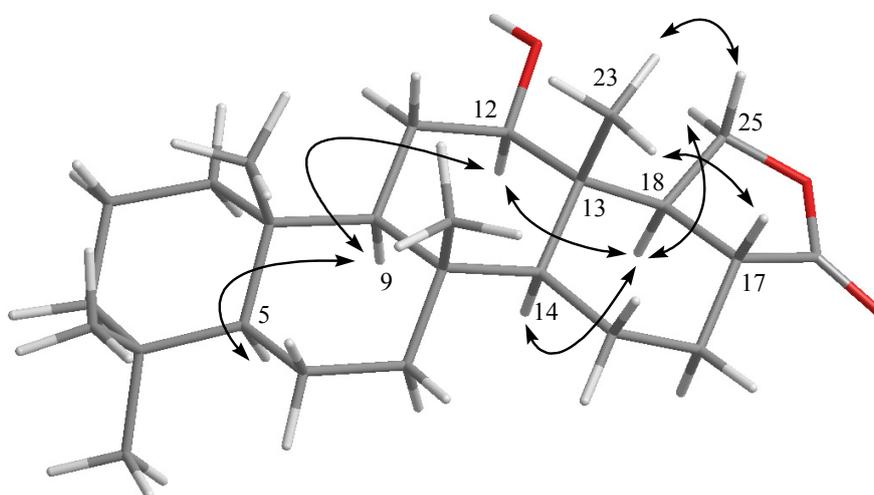
Figure 2. Selected ^1H - ^1H COSY (—) and HMBC (→) correlations of **1** and **2**.

Figure 3. Computer-generated model of **1** using MM2 force field calculations and key NOE correlations.



Hippospongide B (**2**) was isolated as a white powder with the molecular formula $C_{25}H_{40}O_3$, which possesses six degrees of unsaturation, as indicated by HRESIMS (m/z 411.2878, $[M + Na]^+$) and NMR spectroscopic data (Table 1). Moreover, it was found that the NMR data of the tricyclic skeleton (C-1 to C-14) of **2** were quite similar to those of **3** and **8**, indicating the same substitution and stereochemistry at C-5, C-8, C-9, C-10, C-12, C-13 and C-14. Furthermore, analysis of the 1H - 1H COSY and HMBC correlations established the remaining structure, including another two rings from C-13 to C-18 (Figure 2). Finally, the relative stereochemistries at C-17 and C-18 were resolved by careful interpretation of the NOE correlations (Figure 4). Key NOE correlations for **2** showed interactions between H-18 to H-12 and H-14. Thus, H-18 should be located on the α face. NOE correlations were also detected between H-17 and H₃-23, revealing the β -orientation of H-17, as suggested by a molecular model of **2**. After structural determination of **2**, we found that this compound had been obtained previously by hydrogenation of the natural product hydroxylactone IV [10]. In the original report, the authors gave a planar structure. However, our study led to the isolation of **2** for the first time from natural sources. In addition, we successfully elucidated the full structure of **2**. Moreover, our work also provides full assignment for the 1H and ^{13}C NMR spectral data of **2**.

Figure 4. Key NOE correlations of **2**.



The cytotoxicities of compounds **1–8** against DLD-1, HCT-116, T-47D and K562 cancer cells are shown in Table 2. The results showed that compounds **3–5** were found to exhibit cytotoxicity against all or part of the above carcinoma cell lines, while compound **3** (IC₅₀ values 0.001, 0.001, 0.001 and 0.001 μ M against the above carcinoma cell lines, respectively) was the most potent.

Table 2. Cytotoxicity (IC₅₀ μ M) of compounds **1–5**.

Compound	Cell Lines			
	DLD-1	HCT-116	T-47D	K562
1	– ^a	– ^a	– ^a	– ^a
2	– ^a	– ^a	– ^a	– ^a
3	0.001	0.001	0.001	0.001
4	2.4	2.7	0.3	0.05
5	1.1	8.0	0.7	0.7
6	– ^a	– ^a	– ^a	– ^a
7	– ^a	– ^a	– ^a	– ^a
8	– ^a	– ^a	– ^a	– ^a
Actinomycin D	1.9	0.2	0.6	0.03

^a IC₅₀ > 10 μ M.

3. Experimental Section

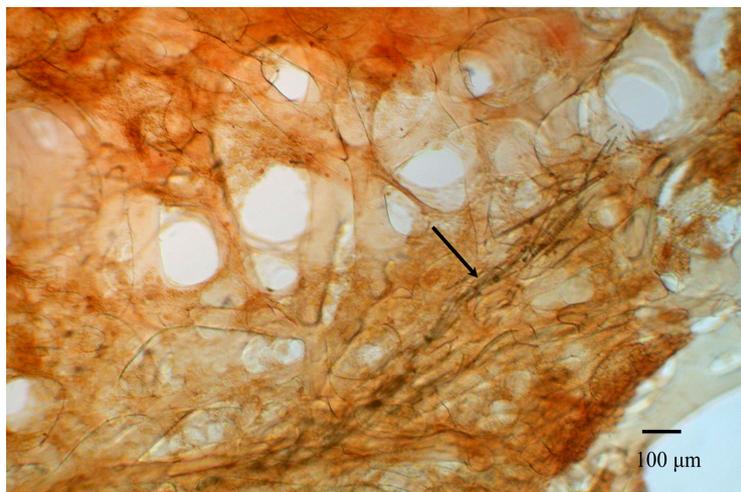
3.1. General Experimental Procedures

Optical rotation values were measured with a Jasco P-1010 digital polarimeter. IR spectra were recorded on a Varian Digilab FTS 1000 Fourier transform infrared spectrophotometer. The NMR spectra were recorded on a Varian Unity INOVA 500 FT-NMR instrument at 500 MHz for ¹H NMR and 125 MHz for ¹³C NMR, respectively, in CDCl₃. ESIMS data were obtained with a Finnigan LCQ ion-trap mass spectrometer. HRESIMS data were recorded on a LTQ Orbitrap XL mass spectrometer. Gravity column chromatography was performed on silica gel (230–400 mesh, Merck). TLC was carried out on pre-coated Kieselgel 60 F254 (0.2 mm, Merck) and spots were visualized by spraying with 10% H₂SO₄ solution followed by heating. High-performance liquid chromatography was performed using a system comprised of a Hitachi L-7100 pump and a Rheodyne 7725 injection port. A preparative normal phase column (250 \times 21.2 mm, 5 μ m) was used for HPLC.

3.2. Animal Material

The specimen of *Hippospongia* sp. was collected by scuba diving at a depth of 20 m from coral reefs off the coast of Tai-tung, Taiwan. Voucher specimen was deposited in the National Museum of Marine Biology and Aquarium, Taiwan (specimen No. 2011SP-1). This genus is often confused with *Hyattella* (Lendenfeld, 1888), whereas *Hippospongia* is more elastic and compressible with fewer primary fibers (Figure 5). Taxonomic identification was performed by Li-Lian Liu of the National Sun Yat-sen University, Kaohsiung, Taiwan.

Figure 5. Skeleton architecture of the *Hippospongia* sp. Arrow: foreign broken spicules in primary spongins.



3.3. Extraction and Separation

The frozen bodies of *Hippospongia* sp. (1.2 kg fresh wt) were collected and freeze-dried. The freeze-dried material (170 g) was minced and extracted exhaustively with EtOAc (5×1 L). The EtOAc extract (15.3 g) was chromatographed over silica gel by column chromatography and eluted with EtOAc in *n*-hexane (0–100%, stepwise), then with acetone in EtOAc (50–100%, stepwise) to yield 13 fractions. Fraction **3** (125.7 mg), eluted with *n*-hexane–EtOAc (10:1), was subjected to normal phase HPLC (*n*-hexane–EtOAc, 7:1) to afford four subfractions (A1–A4). Subfraction A4 (30.5 mg) was separated by normal phase HPLC using *n*-hexane–EtOAc (5:1) to afford **5** (5.9 mg, 0.039% dry wt. of extract) and **6** (2.1 mg, 0.014% dry wt. of extract). Fraction 4 (996 mg), eluting with *n*-hexane–EtOAc (8:1), was further purified by normal phase HPLC (*n*-hexane–EtOAc, 6:1) to afford five subfractions (B1–B5). Subfraction B1 (120 mg) was separated by normal phase HPLC using *n*-hexane–EtOAc (10:1) to afford **1** (1.7 mg, 0.011% dry wt. of extract), **7** (3.0 mg, 0.020% dry wt. of extract) and **8** (20.5 mg, 0.133% dry wt. of extract). Subfraction B2 (20 mg) was also purified by normal phase HPLC using *n*-hexane–EtOAc (7:1) to afford **4** (6.2 mg, 0.041% dry wt. of extract). Fraction 6 (10.5 g), eluting with *n*-hexane–EtOAc (3:1), was further separated by silica gel column chromatography with gradient elution (*n*-hexane–EtOAc, 3:1 to 1:1) to afford **3** (6 g, 39.2% dry wt. of extract). Fraction 8 (524 mg), eluted with *n*-hexane–EtOAc (2:1), was further separated by normal phase HPLC (*n*-hexane–EtOAc, 2:1) to yield six subfractions (C1–C6). Subfraction C3 was separated by normal phase HPLC using *n*-hexane–EtOAc (3:1) to afford **2** (0.8 mg, 0.005% dry wt. of extract).

Hippospongide A (**1**): white powder; mp 272–274 °C; $[\alpha]_D^{25} -66$ (*c* 0.1, CHCl₃); IR (neat) ν_{\max} 3386, 2922, 2854, 1715, 1642, 1455 and 1385 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; ESIMS *m/z* 407 (100, [M + Na]⁺); HRESIMS *m/z* 407.2560 (calcd for C₂₅H₃₆O₃Na, 407.2562).

Hippospongide B (**2**): white powder; mp 289–291 °C; $[\alpha]_D^{25} -3$ (*c* 0.05, CHCl₃); IR (neat) ν_{\max} 3436, 2927, 1753, 1461 and 1383 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; ESIMS *m/z* 411 (80, [M + Na]⁺); HRESIMS *m/z* 411.2878 (calcd for C₂₅H₄₀O₃Na, 411.2875).

Heteronmin (**3**): ^{13}C NMR (CDCl_3 , 100 MHz) data: δ 171.3 (C, OAc), 170.1 (C, OAc), 135.3 (C, C-17), 114.4 (CH, C-24), 101.6 (CH, C-25), 80.5 (CH, C-12), 69.3 (CH, C-16), 64.1 (CH, C-18), 58.7 (CH, C-9), 56.5 (CH, C-5), 54.6 (CH, C-14), 42.7 (C, C-13), 42.0 (CH_2 , C-3), 41.8 (CH_2 , C-7), 39.9 (CH_2 , C-1), 38.0 (C, C-10), 37.4 (C, C-8), 33.2 (CH_3 , C-19), 33.2 (C, C-4), 28.0 (CH_2 , C-15), 27.2 (CH_2 , C-11), 21.3 (CH_3 , OAc), 21.2 (CH_3 , OAc), 21.0 (CH_3 , C-20), 18.6 (CH_2 , C-6), 18.1 (CH_2 , C-2), 17.3 (CH_3 , C-21), 16.3 (CH_3 , C-22), 8.7 (CH_3 , C-23). Selective ^1H NMR (CDCl_3 , 400 MHz) data: δ 6.76 (1H, s, H-25), 6.16 (1H, s, H-24), 5.35 (1H, m, H-16), 3.42 (1H, d, $J = 11.6$ Hz, H-12), 2.43 (1H, s, H-18), 0.91 (3H, s, H_3 -21), 0.84 (6H, s, H_3 -19 and H_3 -22), 0.82 (3H, s, H-20).

Scalarafuran (**8**): ^{13}C NMR (CDCl_3 , 125 MHz) data: δ 171.2 (C, OAc), 139.0 (CH, C-24), 137.3 (CH, C-25), 134.5 (C, C-18), 120.9 (C, C-17), 79.6 (CH, C-12), 68.1 (CH, C-16), 58.6 (CH, C-9), 56.6 (CH, C-5), 54.0 (CH, C-14), 42.0 (CH_2 , C-3), 41.6 (CH_2 , C-7), 40.1 (C, C-13), 39.8 (CH_2 , C-1), 37.4 (C, C-10), 37.4 (C, C-8), 33.3 (CH_3 , C-19), 33.2 (C, C-4), 27.8 (CH_2 , C-11), 24.6 (CH_2 , C-15), 21.3 (CH_3 , OAc), 21.3 (CH_3 , C-20), 18.8 (CH_3 , C-23), 18.6 (CH_2 , C-6), 18.1 (CH_2 , C-2), 17.4 (CH_3 , C-21), 16.2 (CH_3 , C-22). Selective ^1H NMR (CDCl_3 , 500 MHz) data: δ 7.53 (1H, d, $J = 1.5$ Hz, H-25), 7.26 (1H, s, H-24), 5.76 (1H, dd, $J = 8.5, 8.0$ Hz, H-16), 3.60 (1H, d, $J = 10.5$ Hz, H-12), 1.26 (3H, s, H_3 -23), 0.91 (3H, s, H_3 -21), 0.85 (3H, s, H_3 -22), 0.84 (3H, s, H_3 -19), 0.81 (3H, s, H_3 -20).

3.4. Cytotoxicity Testing

Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of compounds **1–8** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method [11,12].

3.5. Molecular Mechanics Calculations

Implementation of the MM2 force field in Chem3D Pro software [13] was used to calculate the molecular models.

4. Conclusions

Previous chemical investigations of sponges of the genus *Hippospongia* have led to the isolation and identification of various metabolites [14–36]. Some of these have been found to possess several kinds of biological activities, such as isocitrate lyase (ICL) inhibitory [14], RCE protease inhibitory [15] and cytotoxic [16–21] activities. In the present study, two new sesterterpenoids, hippospongides A and B (**1** and **2**), together with six known scalarane sesterterpenoids were isolated from the sponge *Hippospongia* sp. Compounds **3–5** showed significant cytotoxicities against DLD-1, HCT-116, T-47D and K562 cell lines. However, the new compounds **1** and **2** and the other known compounds had no significant activity. Furthermore, it is worth mentioning that these compounds are the first pentacyclic sesterterpene and scalarane-type sesterterpenes to be reported from this genus. However, this genus is often confused with *Hyattella* and the sesterterpenoids are not likely to assist in chemical differentiation of the species.

Acknowledgements

This work was supported by grants from the Ministry of Education (00C030205) and National Museum of Marine Biology & Aquarium and the National Science Council (NSC 100-2320-B-291-001), Taiwan, awarded to J.-H. Su.

References

1. González, M.A. Scalarane sesterterpenoids. *Curr. Bioact. Comp.* **2010**, *6*, 178–206.
2. Kashman, Y.; Rudi, A. The ^{13}C NMR spectrum and stereochemistry of heteronemin. *Tetrahedron* **1977**, *33*, 2997–2998.
3. Crews, P.; Bescansa, P. Sesterterpenes from a common marine sponge, *Hyrtios erecta*. *J. Nat. Prod.* **1986**, *49*, 1041–1052.
4. Yu, Z.-G.; Bi, K.-S.; Gue, Y.-W. Hyrtiosins A–E, five new scalarane sesterterpenes from the South China Sea sponge *Hyrtios erecta*. *Helv. Chim. Acta* **2005**, *88*, 1004–1009.
5. Wonganuchitmeta, S.-N.; Yuenyongsawad, S.; Keawpradub, N.; Plubrukarn, A. Antitubercular sesterterpenes from the Thai sponge *Brachiaster* sp. *J. Nat. Prod.* **2004**, *67*, 1767–1770.
6. Iguchi, K.; Shimada, Y.; Yamada, Y. Hyrtiosal, a new sesterterpenoid with a novel carbon skeleton from the Okinawan marine sponge *Hyrtios erectus*. *J. Org. Chem.* **1992**, *57*, 522–524.
7. Walker, R.P.; Thompson, J.E.; Faulkner, D.J. Sesterterpenes from *Spongia idia*. *J. Org. Chem.* **1980**, *45*, 4976–4979.
8. Youssef, D.T.A.; Yamaki, R.K.; Kelly, M.; Scheuer, P.J. Salmahyrtisol A, a novel cytotoxic sesterterpene from the Red Sea sponge *Hyrtios erecta*. *J. Nat. Prod.* **2002**, *65*, 2–6.
9. Mahidol, C.; Prawat, H.; Sangpetsiripan, S.; Ruchirawat, S. Bioactive scalaranes from the Thai sponge *Hyrtios gumminae*. *J. Nat. Prod.* **2009**, *72*, 1870–1874.
10. Fattorusso, E.; Magno, S.; Santacroce, C.; Sica, D. Scalarin, a new pentacyclic C-25 terpenoid from the sponge *Cacospongia scalaris*. *Tetrahedron* **1972**, *28*, 5993–5997.
11. 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.
12. 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.
13. *Chem3D Ultra*, version 9.0.1; CambridgeSoft Corporation: Cambridge, MA, USA, 2005.
14. Lee, H.-S.; Lee, T.-H.; Yang, S.H.; Shin, H.J.; Shin, J.; Oh, K.-B. Sesterterpene sulfates as isocitrate lyase inhibitors from tropical sponge *Hippospongia* sp. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2483–2486.
15. Craig, K.S.; Williams, D.E.; Hollander, I.; Frommer, E.; Mallon, R.; Collins, K.; Wojciechowicz, D.; Tahir, A.; van Soest, R.; Andersen, R.J. Novel sesterterpenoid and norseseterterpenoid RCE-protease inhibitors isolated from the marine sponge *Hippospongia* sp. *Tetrahedron Lett.* **2002**, *43*, 4801–4808.

16. Liu, H.; Wang, G.; Namikoshi, M.; Kobayashi, H.; Yao, X.; Cai, G. Sesquiterpene quinones from a marine sponge *Hippospongia* sp. that inhibit maturation of starfish oocytes and induce cell cycle arrest with HepG2 cells. *Pharm. Biol.* **2006**, *44*, 522–527.
17. Shen, Y.-C.; Chen, C.-Y.; Kuo, Y.-H. New sesquiterpene hydroquinones from a Taiwanese marine sponge, *Hippospongia metachromia*. *J. Nat. Prod.* **2001**, *64*, 801–803.
18. Ishibashi, M.; Ohizumi, Y.; Cheng, J.-f.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. Metachromins A and B, novel antineoplastic sesquiterpenoids from the Okinawan sponge *Hippospongia* cf. *metachromia*. *J. Org. Chem.* **1988**, *53*, 2855–2858.
19. Musman, M.; Ohtani, I.I.; Nagaoka, D.; Tanaka, J.; Higa, T. Hipposulfates A and B, new sesterterpene sulfates from an Okinawan sponge, *Hippospongia* cf. *metachromia*. *J. Nat. Prod.* **2001**, *64*, 350–352.
20. Piao, S.-J.; Zhang, H.-J.; Lu, H.-Y.; Yang, F.; Jiao, W.-H.; Yi, Y.-H.; Chen, W.-S.; Lin, H.-W. Hippolides A–H, acyclic monoalide derivatives from the marine sponge *Hippospongia lachne*. *J. Nat. Prod.* **2011**, *74*, 1248–1254.
21. Oda, T.; Wang, W.; Fujita, A.; Mochizuki, M.; Ukai, K.; Namikoshi, M. Promotion of IL-8 production in PMA-stimulated HL-60 cells by sesquiterpene quinones from a marine sponge, *Hippospongia* sp. *J. Nat. Med.* **2007**, *61*, 434–437.
22. Madaio, A.; Piccialli, V.; Sica, D.; Corriero, G. New polyhydroxysterols from the dictyoceratid sponges *Hippospongia communis*, *Spongia officinalis*, *Ircinia variabilis*, and *Spongionella gracilis*. *J. Nat. Prod.* **1989**, *52*, 952–961.
23. Madaio, A.; Notaro, G.; Piccialli, V.; Sica, D. Minor 5,6-secoosterols from the marine sponge *Hippospongia communis*. Isolation and synthesis of (7Z,22E,24R)-24-methyl-5,6-secocholesta-7, 22-diene-3 β ,5 β ,6-triol. *J. Nat. Prod.* **1990**, *53*, 565–572.
24. Cimino, G.; de Stefano, S.; Minale, L. Furospongins-1, a new C-21 furanoterpene from the sponges *Spongia officinalis* and *Hippospongia communis*. *Tetrahedron* **1971**, *27*, 4673–4679.
25. Cimino, G.; de Stefano, S.; Minale, L. Minor C-21 furanoterpenes from the sponges *Spongia officinalis* and *Hippospongia communis*. *Tetrahedron* **1972**, *28*, 267–273.
26. Madaio, A.; Piccialli, V.; Sica, D. Hipposterol, a unique trihydroxylated 5,6-secoesterol from the marine sponge *Hippospongia communis*. *Tetrahedron Lett.* **1988**, *29*, 5999–6000.
27. Kobayashi, J.; Murayama, T.; Ohizumi, Y. Metachromin C, a new cytotoxic sesquiterpenoid from the Okinawan marine sponge *Hippospongia metachromia*. *J. Nat. Prod.* **1989**, *52*, 1173–1176.
28. Kobayashi, J.; Naitoh, K.; Saaski, T.; Shigemori, H. Metachromins D–H, new cytotoxic sesquiterpenoids from the Okinawan marine sponge *Hippospongia metachromia*. *J. Org. Chem.* **1992**, *57*, 5773–5776.
29. Kobayashi, J.; Shinonaga, H.; Shigemori, H.; Sasaki, T. Untenospongins C, a new C₂₁ furanoterpene from the Okinawan marine sponge *Hippospongia* sp. *Chem. Pharm. Bull.* **1993**, *41*, 381–382.
30. Rochfort, S.J.; Atkin, D.; Hobbs, L.; Capon, R.J. Hippospongins A–F: New furanoterpenes from a Southern Australian marine sponge *Hippospongia* sp. *J. Nat. Prod.* **1996**, *59*, 1024–1028.
31. Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Hirata, Y. Hippospongins, a novel furanosesterterpene possessing antispasmodic activity from the Okinawan marine sponge *Hippospongia* sp. *Tetrahedron Lett.* **1986**, *27*, 2113–2116.

32. Ohta, S.; Uno, M.; Tokumasu, M.; Hiraga, Y.; Ikegami, S. Hippospongiic acid A: An unusual triterpenoic acid from a marine sponge, *Hippospongia* sp., which inhibits gastrulation of starfish embryos. *Tetrahedron Lett.* **1996**, *37*, 7765–7766.
33. Guo, Y.W.; Trivellone, E. Ent-untenospongin A, a new C₂₁ furanoterpene from the Indian marine sponge *Hippospongia* sp. *Chin. Chem. Lett.* **2000**, *11*, 327–330.
34. Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, Y. Dictyoceratin-A and -B, novel antimicrobial terpenoids from the Okinawan marine sponge *Hippospongia* sp. *Tetrahedron* **1986**, *42*, 4197–4201.
35. Ishiyama, H.; Ishibashi, M.; Ogawa, A.; Yoshida, S.; Kobayashi, J. Taurospongin A, a novel acetylenic fatty acid derivative inhibiting DNA polymerase and HIV reverse transcriptase from sponge *Hippospongia* sp. *J. Org. Chem.* **1997**, *62*, 3831–3836.
36. Guo, Y.-W.; Trivellone, E. New hurgamides from a Red Sea sponge of the genus *Hippospongia*. *J. Asian Nat. Prod. Res.* **2006**, *2*, 251–256.

Samples Availability: Not available.

© 2012 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/>).