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## Unusual Anti-allergic Diterpenoids from the Marine Sponge *Hippospongia lachne*

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Hipposponlachnins A (1) and B (2), possessing an unprecedented tetracyclo [9.3.0.0<sup>2,8</sup>.0<sup>3,7</sup>] tetradecane ring system, and the probable biogenetic precursor [3, (1*R*\*,2*E*,4*R*\*,7*E*,10*S*\*,11*S*\*,12*R*\*)-10, 18-diacetoxydolabella-2,7-dien-6-one] of 1–2 were isolated from the South China Sea marine sponge *Hippospongia lachne*. The structures of the novel compounds were determined using integrated spectroscopic methods in combination with single-crystal X-ray diffraction analysis. Compounds 1–2 showed potent inhibitory activity on the release of  $\beta$ -hexosaminidase, a biomarker for degranulation, as well as the production of pro-inflammatory cytokine IL-4 and lipid mediator LTB<sub>4</sub> in DNP-IgE-stimulated RBL-2H3 cells.

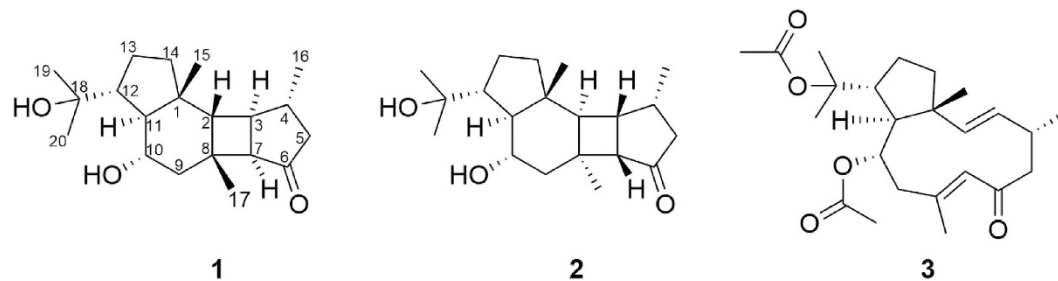
Allergy is an abnormal adaptive immune response against allergens, such as pollen, food, dust mites, cosmetics, mold spores, and animal hairs<sup>1,2</sup>.  $\beta$ -Hexosaminidase, typically released in addition to histamine by mast cells after stimulation with antigen, has been used as a biomarker for antigen-induced degranulation in rat basophilic leukemia (RBL-2H3) cells<sup>3</sup>. Thus, the release of  $\beta$ -hexosaminidase in RBL-2H3 cells has commonly been used as a reliable indicator of inhibitory activity against allergic reactions induced by natural products<sup>4</sup>. Natural products have been proved to be an important source for drug discovery and development. More than three hundreds natural compounds were reported to exhibit diverse biological properties, such as antitumor, antibacterial, anti-fungal, and a range of other bioactivities<sup>5</sup>. However, only a few natural compounds with anti-allergic activity have been isolated to date<sup>5</sup>. Therefore, there is a great opportunity in search for leads to treat allergy effectively.

Marine invertebrates such as sponges are well-known sources of bioactive natural products<sup>6</sup>. Sponges of the genus *Hippospongia* are reported to be rich sources of polyketides<sup>7</sup>, sesquiterpenes<sup>8</sup>, sesterterpenes and sulfates<sup>9</sup>, furanoterpenes<sup>10</sup>, triterpenic acids<sup>11</sup>, fatty acids<sup>12</sup>, and sterols<sup>13</sup>. These metabolites exhibited a broad spectrum of biological activities, including cytotoxic<sup>9</sup>, antifungal<sup>7</sup>, anti-inflammatory<sup>8</sup>, coronary vasodilating<sup>14</sup>, and RCE-protease inhibitory activities<sup>11</sup>. A literature survey revealed that no anti-allergic metabolites have been isolated from sponges belonging to this genus. As part of our searching for anti-allergic secondary metabolites from marine sponges, we investigated the sponge *Hippospongia lachne*, collected from the Xisha Islands of China in March 2013. The 95% ethanol extract of *H. lachne* showed promising  $\beta$ -hexosaminidase inhibitory activity with IC<sub>50</sub> value of 7.8  $\mu$ g/mL. Further bioactivity-guided fractionation of this extract led to the isolation of compounds 1–3 (Fig. 1). Of these three metabolites, hipposponlachnins A (1) and B (2) possessed a rare tetracyclo [9.3.0.0<sup>2,8</sup>.0<sup>3,7</sup>] tetradecane carbon skeleton, exemplifying the first diterpene scaffold obtained from marine source, and showed inhibitory activity on the release of  $\beta$ -hexosaminidase in anti-murine DNP-IgE-stimulated RBL-2H3 cells. Herein, we report the isolation, structure elucidation and anti-allergic activity of the two unprecedented diterpenoids. In addition, biogenetic relationship between these compounds was proposed, suggesting 1–2 may be obtained from 3 via an intramolecular [2 + 2] cycloaddition reaction.

### Results and Discussion

**Isolation and structure elucidation.** Air dried specimen of *H. lachne* were extracted with 95% ethanol and after evaporation under reduced pressure, the aqueous residue was extracted with EtOAc. The EtOAc

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**Figure 1.** Structures of compounds 1–3.

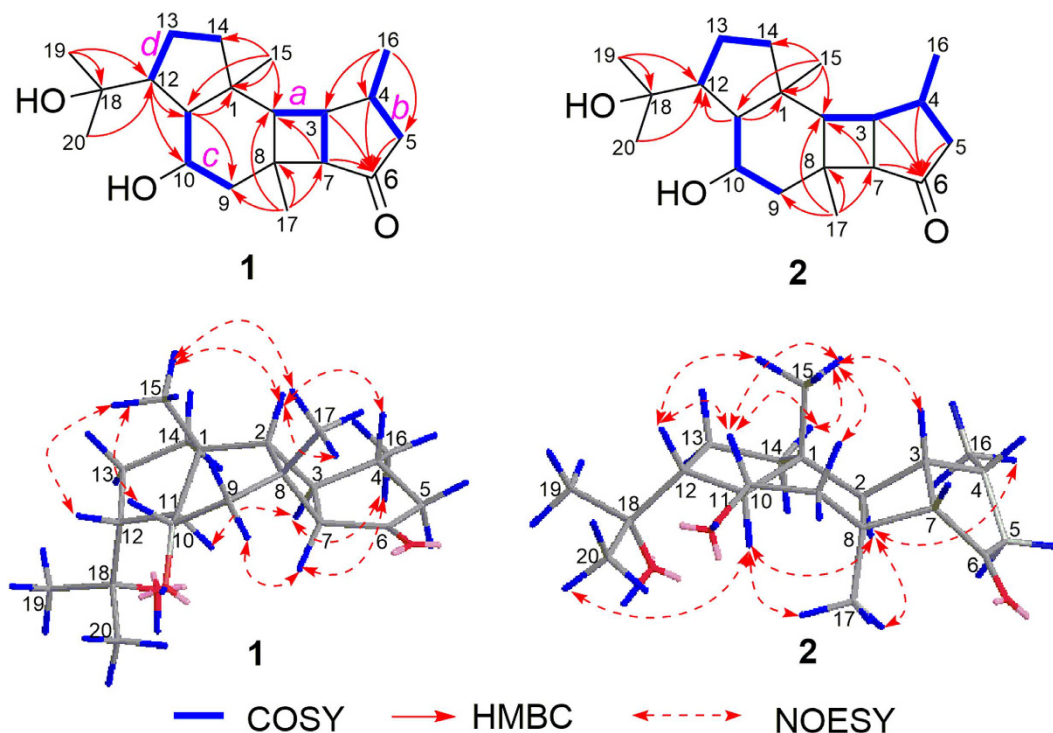
position	1		2	
	$\delta_C$ , type	$\delta_H$ , mult ( <i>J</i> in Hz)	$\delta_C$ , type	$\delta_H$ , mult ( <i>J</i> in Hz)
1	45.9, C		43.1, C	
2	54.01, CH	1.84, d (7.5)	50.4, CH	2.10, d (7.0)
3	41.0, CH	2.47, t (7.5)	39.2, CH	2.90, q (7.0)
4	34.2, CH	2.10, m	32.3, CH	2.33, m
5 $\alpha$	47.1, CH <sub>2</sub>	2.00, d (19.0)	46.0, CH <sub>2</sub>	2.17, m
5 $\beta$		2.75, dd (19.0, 7.5)		2.34, m
6	220.6, C		220.4, C	
7	53.98, CH	2.34, d (7.5)	56.1, CH	2.32, m
8	39.3, C		38.0, C	
9 $\alpha$	48.8, CH <sub>2</sub>	1.52, dd (21.0, 10.0)	40.9, CH <sub>2</sub>	1.65, d (15.0)
9 $\beta$		2.10, m		2.15, m
10	67.2, CH	3.72, td (10.0, 4.0)	70.2, CH	3.92, dd (9.5, 7.5)
11	50.3, CH	1.53, dd (10.0, 4.0)	49.4, CH	1.54, t (9.5)
12	51.2, CH	2.10, m	52.8, CH	2.03, m
13 $\alpha$	26.0, CH <sub>2</sub>	1.28, m	27.2, CH <sub>2</sub>	1.31, m
13 $\beta$		1.94, m		1.96, m
14 $\alpha$	33.1, CH <sub>2</sub>	1.28, m	42.3, CH <sub>2</sub>	1.22, d (11.0)
14 $\beta$		1.24, m		1.60, dd (11.0, 8.5)
15	22.7, CH <sub>3</sub>	0.85, s	19.6, CH <sub>3</sub>	0.93, s
16	21.1, CH <sub>3</sub>	0.97, d (7.0)	15.5, CH <sub>3</sub>	1.01, d (6.0)
17	26.5, CH <sub>3</sub>	1.11, s	26.2, CH <sub>3</sub>	1.12, s
18	72.7, C		73.2, C	
19	30.8, CH <sub>3</sub>	1.28, s	30.9, CH <sub>3</sub>	1.27, s
20	23.0, CH <sub>3</sub>	1.24, s	23.0, CH <sub>3</sub>	1.19, s

**Table 1.** <sup>1</sup>H and <sup>13</sup>C NMR Data for 1 and 2 (500, 125 MHz, CDCl<sub>3</sub>, TMS,  $\delta$  ppm).

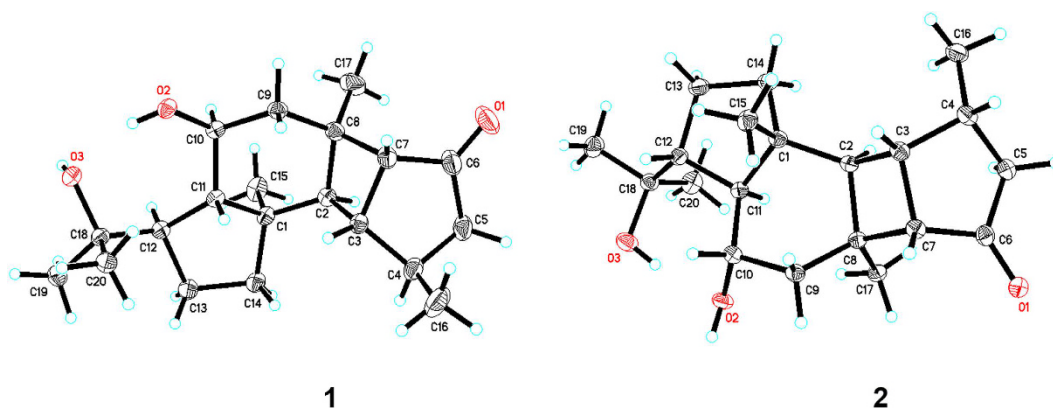
layer was repeatedly chromatographed on Sephadex LH-20, silica gel, ODS column and reversed phase high performance liquid chromatography (HPLC) to yield hipposponlachnin A (1), hipposponlachnin B (2), and (1*R*\*,2*E*,4*R*\*,7*E*,10*S*\*,11*S*\*,12*R*\*)-10,18-diacetoxydolabella-2,7-dien-6-one (3) (Fig. 1).

Hipposponlachnin A (1) was obtained as a colorless crystal. Its molecular formula was determined to be C<sub>20</sub>H<sub>32</sub>O<sub>3</sub> by HRESIMS (*m/z* 343.2251 [M + Na]<sup>+</sup>), indicating five degrees of unsaturation. The IR spectrum featured characteristic absorptions of hydroxyl (3280 cm<sup>-1</sup>) and carbonyl (1732 cm<sup>-1</sup>) groups. The <sup>1</sup>H NMR spectrum revealed the presence of four methyl singlets ( $\delta_H$  0.85, 1.11, 1.24, and 1.28), one methyl doublet ( $\delta_H$  0.97, d, *J* = 7.0 Hz), and one oxymethine proton ( $\delta_H$  3.72, td, *J* = 10.0, 4.5 Hz). The <sup>13</sup>C and DEPT 135 NMR spectra displayed 20 carbon resonances (Table 1), including five methyls, four methylenes, seven methines (one oxygenated), and four quaternary carbons (one oxygenated and one carbonyl). The aforementioned data accounted for one degree of unsaturation. The remaining four degrees of unsaturation suggested a tetracyclic core in 1.

The planar structure of 1 was elucidated via a detailed analysis of 2D NMR data (Fig. 2). The COSY spectrum readily revealed the presence of four isolated spin systems: (a) C-2–C-3–C-7, (b) C-5–C-4–C-16, (c) C-9–C-10–C-11, and (d) C-12–C-13–C-14. The observed HMBC correlations from H<sub>3</sub>-16 to C-3, C-4, and C-5 and from H-12 to C-11 revealed the connectivities of C-3 and C-4 and of C11 and C-12, respectively. The HMBC correlations from H-3, H-4, H-5, and H-7 to C-6 indicated that C-5 and C-7 were connected via the carbonyl carbon C-6. The additional HMBC correlations of H<sub>3</sub>-17/C-2, C-7, C-8, and C-9, H<sub>3</sub>-15/C-1, C-2, C-11, and C-14, and H-12/C-10, tethered the remaining three fragments a, c, and d by inserting the “loose ends” of the quaternary



**Figure 2.** Key HMBC and COSY and selected NOE correlations of **1** and **2**.

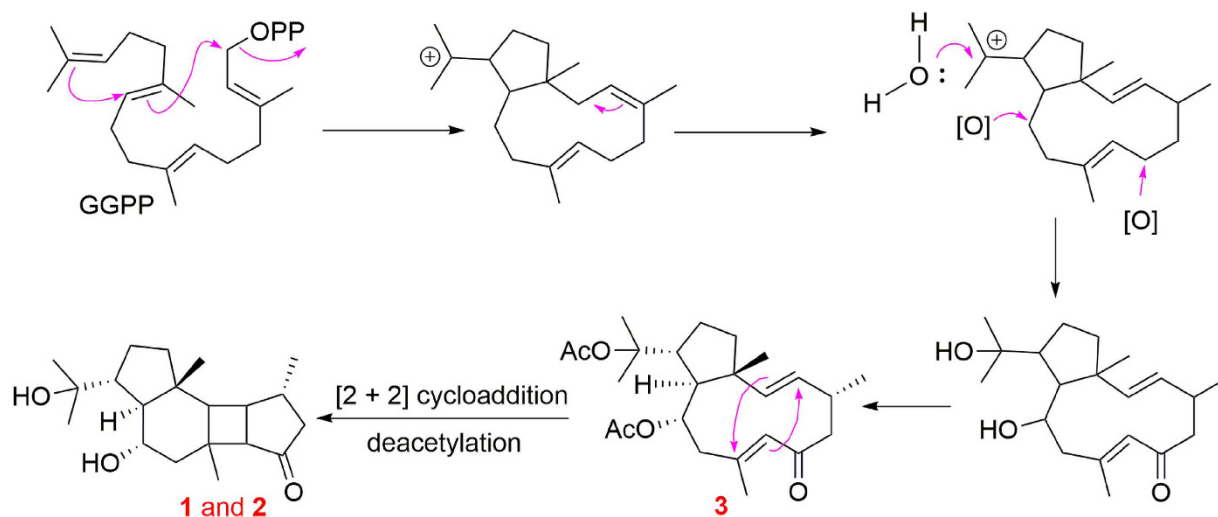


**Figure 3.** X-ray crystallographic structures of **1** and **2**.

carbons C-1 and C-8, and located the three methyl groups at C-1, C-4, and C-8, respectively, demonstrating a tetracyclo [9.3.0.0<sup>2,8</sup>.0<sup>3,7</sup>] tetradecane core in **1**. Moreover, the presence of a hydroxy isopropyl group at C-12 was supported by the HMBC correlations from the two remaining methyl groups, H<sub>3</sub>-19 and H<sub>3</sub>-20, to C-12 and the oxygenated quaternary carbon at  $\delta_C$  72.7 (C-18). The unassigned hydroxyl group was attached at the downfield-shifted carbon at ( $\delta_C$  67.2, C-10). Thus, the planar structure of hipposponlachnin A (**1**) was established as shown in Fig. 2.

The relative configuration of **1** was determined by NOESY data (Fig. 2). The NOESY correlations of H<sub>3</sub>-15/H-2, H<sub>3</sub>-15/H<sub>3</sub>-17, H<sub>3</sub>-15/H-10, H<sub>3</sub>-15/H-12, H-2/H-4, and H-2/H<sub>3</sub>-17 indicated that these protons and methyl groups were cofacially oriented, whereas the NOESY correlations of H-11/H-3, H-3/H<sub>3</sub>-16, H<sub>3</sub>-16/H-7, and H-7/H-9 $\alpha$  suggested that they were oriented in opposite directions. Therefore, the configuration of **1** was determined and confirmed by single-crystal X-ray diffraction analysis with Cu K $\alpha$  irradiation as shown in Fig. 3.

Hipposponlachnin B (**2**) was also obtained as a colorless crystal. Its molecular formula of C<sub>20</sub>H<sub>32</sub>O<sub>3</sub> was deduced from its HRESIMS data, consistent with that of **1**. The overall appearance of the NMR spectrum of **2** revealed close structural similarity between **1** and **2**, indicating the presence of the same tetracyclo [9.3.0.0<sup>2,8</sup>.0<sup>3,7</sup>] tetradecane skeleton in both compounds. Further analysis of 1D and 2D NMR spectra of **2** established the planar structure of **2**, which was identical to that of **1** (Fig. 2). Moreover, the almost mirror-image CD spectra of **1** and **2** (see Supplementary Fig. S21) indicated that these two compounds must be a pair of diastereoisomers with



**Figure 4. Proposed Biosynthetic Pathway of 1–3.**

a variation of configurations at the chiral centers around the carbonyl chromophore. Detailed analysis of the single-crystal X-ray diffraction patterns of **2** confirmed this hypothesis and established the absolute configuration of **2** as 1*R*,2*R*,3*S*,4*R*,7*S*,8*S*,10*S*,11*S*,12*R* (Fig. 3).

Compound **3** was identified as a known dolabellane diterpene, named (1*R*<sup>\*</sup>,2*E*,4*R*<sup>\*</sup>,7*E*,10*S*<sup>\*</sup>,11*S*<sup>\*</sup>,12*R*<sup>\*</sup>)-10,18-diacetoxydolabella-2,7-dien-6-one, by comparing its physical and spectroscopic features with the data reported in the literature<sup>15,16</sup>.

In addition, the biosynthetic pathway for **1–3** was proposed as shown in Fig. 4. **3** could be produced from geranylgeranyl diphosphate (GGPP) following a series of biosynthetic cyclization, migration, oxidation, and acetylation processes<sup>17–19</sup>. The four-membered ring in **1** and **2** might be formed from **3** via an intramolecular [2 + 2] cycloaddition reaction between two double bonds ( $\Delta^{2,3}$  and  $\Delta^{7,8}$ )<sup>7,20</sup>. The deacetylation could be carried out by an esterase<sup>21</sup>. What's more, compound **3** with 7, 8-*E* geometry rather than 7,8-*Z* isomer is the predicted precursor of **1** and **2**, because the high rotational barrier around the C<sub>7</sub>-C<sub>8</sub> bond in the intermediate of the 7, 8-*Z* isomer in the [2 + 2] cycloaddition process makes it difficult to form stable *trans*-cyclobutane products (**1** and **2**) (see Supplementary Fig. S26).

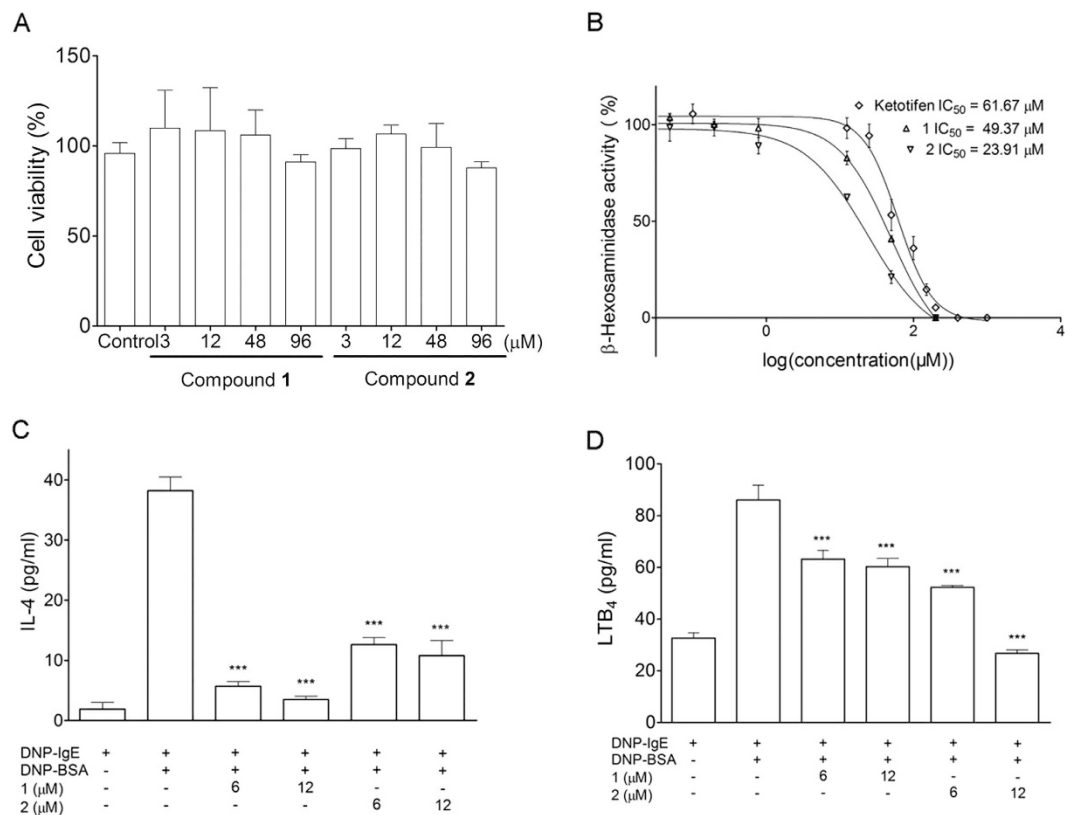
**Anti-allergic activity evaluation.** Subsequently, RBL-2H3 cells were used as a model system to evaluate the anti-allergic activity of **1–2**<sup>22</sup>. As indicated in Fig. 5A, no significant cytotoxicity was observed in RBL-2H3 cells after 24 h of treatment with **1** or **2**. **1** and **2** exhibited higher activity (IC<sub>50</sub> 49.37 and 23.91 μM, respectively) in the release of β-hexosaminidase inhibition (Fig. 5B), compared with the market-available anti-asthmatic drug, ketotifen fumarate (IC<sub>50</sub> = 63.88 μM). In addition, **1** and **2** suppressed IL-4 production in a dose-dependent manner (Fig. 5C) and significantly inhibited LTB<sub>4</sub> release in activated RBL-2H3 cells compared with untreated control (Fig. 5D). The results indicated that **1** and **2** are promising new anti-allergic lead compounds.

In summary, two novel tetracyclic diterpenes (**1–2**) were isolated from the marine sponge *H. lachne*, together with their probable biogenetic precursor (**3**). The structures of new compounds were elucidated by spectroscopic and single-crystal X-ray diffraction analysis. To our knowledge, only one diterpenoid with a similar skeleton, namely vulgarisin A, derived from the Chinese Medicinal Plant *Prunella vulgaris*, has been reported to date<sup>19</sup>. These two novel compounds represented an unprecedented tetracyclo [9.3.0.0<sup>2,8</sup>.0<sup>3,7</sup>] tetradecane ring system from marine source for the first time. Moreover, the potent inhibitory activity on the release of β-hexosaminidase for **1–2** suggested that these two bioactive diterpenoids can be the potential therapeutic agents for the treatment of allergy.

## Methods

**General Experimental Procedures.** Optical rotation data were recorded on a PerkinElmer model 341 polarimeter with a 10 cm length cell at room temperature. UV and IR (KBr) spectra were obtained on a Hitachi U-3010 spectrophotometer and Jasco FTIR-400 spectrometer, respectively. CD spectra were obtained on a Jasco J-715 spectropolarimeter in MeCN. NMR spectra including 1D and 2D spectra were acquired at room temperature on Bruker AMX-500 instrument. HRESIMS data were obtained on a Waters Q-ToF micro YA019 mass spectrometer. Reversed-phase HPLC was performed on YMC-Pack Pro C18 RS (5 μm) columns with a Waters 1525 separation module equipped with a Waters 2998 photodiode array detector. Purifications by column chromatography were performed on silica gel 60 (200–300 mesh; Yantai, China), Sephadex LH-20 (18–110 μm, Pharmacia Co.), and ODS (50 μm, YMC Co.). Analytical thin-layer chromatography was carried out using HSGF 254 plates and visualized by spraying with anisaldehyde-H<sub>2</sub>SO<sub>4</sub> reagent.

**Animal Material.** The marine sponge *H. lachne* was collected from the Xisha Islands in the South China Sea in March 2013, and identified by Prof. Jin-He Li (Institute of Oceanology, Chinese Academy of Sciences, China). The voucher specimens of *H. lachne* (RM-2013) is deposited at Research Center for Marine Drugs, State



**Figure 5.** (A) Cytotoxic effects of **1** and **2** on RBL-2H3 cells. (B) Inhibitory effects of **1** and **2** on the release of  $\beta$ -hexosaminidase in DNP-IgE-activated RBL-2H3 cells. (C) Inhibition of IL-4 production of **1** and **2** in RBL-2H3 cells. (D) Inhibitory activity on lipid mediator LTB<sub>4</sub> secretion of **1** and **2** in RBL-2H3 cells. Data are presented as the mean  $\pm$  SD values of triplicate determinations. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  versus DNP-BSA-treated group ( $n = 3$ ). Data are analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Post-Test (GraphPad Prism 5.0).

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**Extraction and Isolation.** The sponge *H. lachne* (1.2 kg, dry weight) was cut into small pieces and exhaustively extracted by percolation with 95% EtOH at room temperature to give 56.0 g extract, which was suspended in H<sub>2</sub>O and extracted sequentially with EtOAc to afford EtOAc-soluble extract (27.5 g). The EtOAc-soluble extract was dissolved in 90% aqueous MeOH, and extracted with petroleum ether to yield petroleum ether-soluble extract (15.2 g). The 90% aqueous MeOH phase was diluted to 60% MeOH with H<sub>2</sub>O, and extracted with CH<sub>2</sub>Cl<sub>2</sub> to yield CH<sub>2</sub>Cl<sub>2</sub>-soluble extract (10.0 g). The CH<sub>2</sub>Cl<sub>2</sub>-soluble extract was subjected to column chromatography on silica gel with a gradient elution of MeOH in CH<sub>2</sub>Cl<sub>2</sub> to give six fractions (A–F). Fraction C was subjected to column chromatography (CC) on Sephadex LH-20 eluting with CH<sub>2</sub>Cl<sub>2</sub>–MeOH (1:1) to afford three subfractions (C1–C3). Subfraction C2 was separated by CC on ODS to give nine subfractions (C2A–C2I) with 80% MeOH–H<sub>2</sub>O as elution. Fr. C2C was purified by reversed-phase semipreparative HPLC (65% MeCN/H<sub>2</sub>O, 2.0 mL/min, 250 nm) to obtain **3** (3.0 mg,  $t_R = 51.0$  min). Fr. C2D was further purified by reversed-phase semipreparative HPLC (50% MeCN/H<sub>2</sub>O, 2.0 mL/min, 210 nm) to obtain **1** (2.4 mg,  $t_R = 15.2$  min) and **2** (2.8 mg,  $t_R = 18.0$  min).

**Chemical structure data.** All compounds were  $\geq 98\%$  pure, which were supported by the NMR spectra of the compounds provided in the Supporting Information.

*Hipposponlachnin A (1).* Colorless crystal;  $[\alpha]_D^{30} + 49$  ( $c$  0.11, MeOH); UV (MeOH)  $\lambda_{max}$  ( $\log \epsilon$ ) 203 (3.37) nm; CD (MeCN)  $\lambda_{max}$  ( $\Delta \epsilon$ ) 200 (+0.28), 308 (+0.16) nm; IR (KBr)  $\nu_{max}$  3280, 3167, 2943, 2922, 2861, 1732, 1453, 1410, 1377, 1339, 1306, 1252, 1147, 1122, 1076, 1045, 1025, 962, 942, 905, 858, 806, 672  $cm^{-1}$ ; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1; HRESIMS  $m/z$  343.2251 [M + Na]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>32</sub>O<sub>3</sub>Na, 343.2249).

*Hipposponlachnin B (2).* Colorless crystal;  $[\alpha]_D^{30} - 44$  ( $c$  0.24, MeOH); UV (MeOH)  $\lambda_{max}$  ( $\log \epsilon$ ) 204 (3.47) nm; CD (MeCN)  $\lambda_{max}$  ( $\Delta \epsilon$ ) 208 (−0.13), 308 (−0.25) nm; IR (KBr)  $\nu_{max}$  3375, 3331, 2953, 2926, 2900, 1726, 1704, 1456, 1412, 1377, 1360, 1329, 1263, 1240, 1171, 1130, 1088, 1032, 1009, 982, 957, 906, 874, 839, 804  $cm^{-1}$ ; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1; HRESIMS  $m/z$  343.2247 [M + Na]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>32</sub>O<sub>3</sub>Na, 343.2249).

(1*R*\*,2*E*,4*R*\*,7*E*,10*S*\*,11*S*\*,12*R*\*)-10,18-diacetoxylolabella-2,7-dien-6-one (**3**). Colorless oil;  $[\alpha]_D^{30} = -23$  (c 0.15, MeOH);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 600 MHz)  $\delta$  6.05 (1H, s, H-7), 5.53 (1H, dd,  $J = 16.2$  and 6.0 Hz, H-3), 4.99 (1H, d,  $J = 16.2$  Hz, H-2), 4.81 (1H, dd,  $J = 10.2$  and 3.0 Hz, H-10), 3.13 (1H, m, H-12), 2.69 (1H, dd,  $J = 11.4$  and 3.6 Hz, H-5a), 2.55 (1H, m, H-4), 2.33 (3H, m, H-5b and H-9), 2.06 (3H, s, H-10-OAc), 2.01 (3H, br s, H-17), 1.95 (1H, m, H-13a), 1.92 (3H, s, H-18-OAc), 1.64 (1H, d,  $J = 10.2$  Hz, H-11), 1.61 (3H, s, H-19), 1.49 (2H, m, H-13b and H-14a), 1.44 (1H, m, H-14b), 1.39 (3H, s, H-20), 1.02 (3H, d,  $J = 6.6$  Hz, H-16), 0.86 (3H, s, H-15);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 150 MHz)  $\delta$  201.5 (C, C-6), 170.2 (C, C-10-OAc), 170.0 (C, C-18-OAc), 146.3 (C, C-8), 138.2 (CH, C-2), 132.1 (CH, C-3), 131.3 (CH, C-7), 84.7 (C, C-18), 71.9 (CH, C-10), 53.8 (CH, C-11), 51.1 (CH<sub>2</sub>, C-5), 47.8 (C, C-1), 44.9 (CH, C-12), 43.8 (CH<sub>2</sub>, C-9), 39.1 (CH<sub>2</sub>, C-14), 34.1 (CH, C-4), 26.7 (CH<sub>3</sub>, C-19), 26.2 (CH<sub>2</sub>, C-13), 23.3 (CH<sub>3</sub>, C-20), 23.0 (CH<sub>3</sub>, C-18-OAc), 21.5 (CH<sub>3</sub>, C-17), 21.0 (CH<sub>3</sub>, C-10-OAc), 18.3 (CH<sub>3</sub>, C-16), 17.5 (CH<sub>3</sub>, C-15); HRESIMS  $m/z$  427.2451  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{24}\text{H}_{36}\text{O}_5\text{Na}$ , 427.2460).

**X-ray Crystallographic Analysis of 1 and 2.** Hipposponlachnins A (**1**) and B (**2**) were crystallized from MeOH at room temperature. The X-ray crystallographic data of them were measured on a Bruker Apex-II CCD diffractometer employing graphite monochromated Cu-K $\alpha$  radiation ( $\lambda = 1.54178 \text{ \AA}$ ) at 140(2) K (operated in the  $\varphi$ - $\omega$  scan mode). The structures were solved by direct method using SHELXS-97 program and refined with full-matrix least-squares calculations on  $F^2$  using SHELXL-97. Crystallographic data for **1** and **2** have been deposited at the Cambridge Crystallographic Data Centre. Copies of these data can be obtained free of charge via the internet at [www.ccdc.cam.ac.uk/conts/retrieving.html](http://www.ccdc.cam.ac.uk/conts/retrieving.html) or on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Tel: (+44) 1223-336-408; Fax: (+44) 1223-336-033; E-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)].

**Crystallographic data of 1.** Colorless crystal,  $\text{C}_{20}\text{H}_{32}\text{O}_3$ ,  $M_r = 320.45$ , orthorhombic, space group  $P2_12_12_1$ ,  $a = 6.62210(10) \text{ \AA}$ ,  $b = 15.2395(3) \text{ \AA}$ ,  $c = 18.4069(3) \text{ \AA}$ ,  $\alpha = \beta = \gamma = 90^\circ$ ,  $V = 1857.58(6) \text{ \AA}^3$ ,  $T = 140 \text{ K}$ ,  $Z = 4$ ,  $D_{\text{calcd}} = 1.146 \text{ g/cm}^3$ , crystal size  $0.20 \times 0.12 \times 0.05 \text{ mm}^3$ ,  $\mu = 0.588 \text{ mm}^{-1}$ ,  $F(000) = 704$ , 8888 reflections measured ( $7.53^\circ < 2\theta < 139.318^\circ$ ), 3177 unique ( $R_{\text{int}} = 0.0311$ ,  $R_{\text{sigma}} = 0.0335$ ). The final  $R_1$  value is 0.0354 [ $I > 2\sigma(I)$ ] and  $wR_2 = 0.0953$  (all data). The goodness of fit on  $F^2$  was 1.060. Flack parameter = 0.13 (13). CCDC deposition number: 1501503.

**Crystallographic data of 2.** Colorless crystal,  $\text{C}_{20}\text{H}_{32}\text{O}_3$ ,  $M_r = 320.45$ , monoclinic, space group  $P2_1$ ,  $a = 8.17720(10) \text{ \AA}$ ,  $b = 9.71010(10) \text{ \AA}$ ,  $c = 11.2700(2) \text{ \AA}$ ,  $\alpha = \gamma = 90^\circ$ ,  $\beta = 92.8110(10)^\circ$ ,  $V = 893.78(2) \text{ \AA}^3$ ,  $T = 140 \text{ K}$ ,  $Z = 2$ ,  $D_{\text{calcd}} = 1.191 \text{ g/cm}^3$ , crystal size  $0.20 \times 0.10 \times 0.02 \text{ mm}^3$ ,  $\mu = 0.611 \text{ mm}^{-1}$ ,  $F(000) = 352$ , 6940 reflections measured ( $7.854^\circ < 2\theta < 139.132^\circ$ ), 2953 unique ( $R_{\text{int}} = 0.0248$ ,  $R_{\text{sigma}} = 0.0310$ ). The final  $R_1$  value is 0.0307 [ $I > 2\sigma(I)$ ] and  $wR_2 = 0.0818$  (all data). The goodness of fit on  $F^2$  was 1.066. Flack parameter =  $-0.03$  (9). CCDC deposition number: 1501504.

**Anti-allergic assay.** Cell viability were determined using the MTT method. RBL-2H3 cells were plated into a 96-well plate at  $5 \times 10^5$  cells per well ( $100 \mu\text{L/well}$ ) for 24 h. Subsequently, cells were incubated with hipposponlachnins A (**1**) and B (**2**) for 24 hours and medium was replaced with MTT solution ( $250 \mu\text{g/mL}$ ) and incubated at  $37^\circ\text{C}$  for 4 h. The medium was carefully discarded and formazan was resuspended in  $150 \mu\text{L}$  of dimethyl sulfoxide (DMSO). The absorbance was measured at 490 nm using a microplate reader. Values measured from untreated cells were considered to represent 100% viability. RBL-2H3 cells were seeded in a 24-well plate at  $5 \times 10^5$  cells per well, and sensitized with dinitrophenyl (DNP)-specific IgE (DNP-IgE) ( $1 \mu\text{g/mL}$ ) at  $37^\circ\text{C}$  overnight. DNP-IgE-sensitized cells were preincubated with hipposponlachnins A (**1**) and B (**2**) for 30 min, and then stimulated with DNP-BSA for 1.5 h. To measure the activity of  $\beta$ -hexosaminidase released from the cells, cultured media were centrifuged ( $17,000 \text{ g}$ , 10 min) at  $4^\circ\text{C}$ . The supernatant ( $50 \mu\text{L}$ ) was mixed with  $50 \mu\text{L}$  of 0.1 M sodium citrate buffer (pH 4.5) containing 10 mM 4-nitrophenyl  $N$ -acetyl- $\beta$ -D-glucosaminide in a 96-well plate, and then incubated for 90 min at  $37^\circ\text{C}$ . The absorbance was measured at 405 nm after terminating the reaction by 0.2 M glycine (pH 10.0). To measure Interleukin-4 (IL-4) and Leukotriene B<sub>4</sub> (LTB<sub>4</sub>) level in cultured media, all cultured media were centrifuged at  $4^\circ\text{C}$ , and hipposponlachnins A (**1**) and B (**2**) were stored at  $-80^\circ\text{C}$  until assay. IL-4 and LTB<sub>4</sub> were quantified using an ELISA kit according to the manufacturer's instructions. The data were analyzed using a one-way ANOVA followed by Dunnett's Multiple Comparison Test with GraphPad Prism software (GraphPad Prism version 5.01 for Windows, San Diego, CA, USA). The values are expressed as the means  $\pm$  SD. The differences with  $p < 0.05$  were considered significant.

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## Author Contributions

L.-L.H. conducted the experiments. L.-L.H. and H.-B.Y. wrote the manuscript; J.W. assisted extraction and isolation; W.-H.J. and B.-H.C. assisted the biological test; W.-H.J., F.Y., B.-B.G., and Y.-J. Z. assisted the structure elucidation and analysed the data. S.-J.S. and H.-W.L. revised and polished the manuscript. All authors reviewed the manuscript.

## Additional Information

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