Dysivillosins A–D, Unusual Anti-allergic Meroterpenoids from the Marine Sponge Dysidea villosa

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Four unusual meroterpenoids, dysivillosins A–D (1–4), were isolated from an organic extract of the marine sponge Dysidea villosa collected from the South China Sea. Their planar structures were determined by 1D and 2D NMR and HRESIMS techniques, while the relative and absolute configurations were elucidated by NOESY experiments and comparison between the calculated and experimental ECD spectra. To the best of our knowledge, dysivillosins A–D are the first examples of terpene-polyketide-pyridine hybrid metabolites from the nature. Anti-allergic activity evaluation showed that compounds 1–4 potently inhibited the release of β-hexosaminidase, a marker of degranulation, in a dose-dependent manner with IC₅₀ values of 8.2–19.9 μM. Additionally, the four meroterpenoids could downregulate the production of lipid mediator leukotrienes B₄ (LTB₄) and pro-inflammatory cytokine interleukin-4 (IL-4) in the antigen-stimulated RBL-2H3 mast cells. Further biological investigations revealed that dysivillosin A (1) could suppress the phosphorylation of Syk and PLCγ₁ in IgE/FcεRI/Syk signaling pathway, which resulted in the inhibition of degranulation and the downregulation of LTB₄ and IL-4 production in mast cells.

Mast cells have long been considered as important effector cells in immunoglobulin E (IgE)-associated immune responses. IgE binds to mast cells via its high-affinity receptor, FcεRI, and cross-linking of FcεRI-bound IgE molecules by allergen triggers a cascade of signaling events which leads to mast cell degranulation and the release of allergic mediators characteristic of type I hypersensitivity reactions. FcεRI aggregation initiates Src family kinases, and subsequently activates another key tyrosine kinase, Syk, which is most essential for the activation of various downstream signaling molecules, such as GTPase, Ras, PI3K, and PLCγ₁. Therefore, FcεRI-proximal kinase, Syk, has recently been proposed as an excellent therapeutic target for the treatment of allergic diseases.

Marine sponges are rich sources of diverse bioactive metabolites with unusual chemical scaffolds. As part of our search for new anti-allergic compounds from the South China Sea marine sponges, several sponge specimens have been collected and screened for their inhibitory activity against β-hexosaminidase, a degranulation indicator of mast cells. Among them, the MeOH extract of Dysidea villosa showed the most potent inhibitory activity against β-hexosaminidase with an IC₅₀ value of 5.3 μg/mL. Detailed bioassay-guided fractionation of the active organic extract led to the isolation of four unusual meroterpenoids, dysivillosins A–D (1–4), as depicted in Fig. 1. To the best of our knowledge, this is the first report of terpene-polyketide-pyridine hybrid metabolites from the nature. All the four compounds were evaluated for their anti-allergic activity, they could significantly inhibit β-hexosaminidase and the production of LTB₄ and IL-4 in antigen-stimulated RBL-2H3 mast cells. Further investigations showed that anti-allergic activity of dysivillosin A was triggered by suppressing Syk/PLCγ₁ signaling pathway.

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Results and Discussion

Dysivillosin A (1) was isolated as an amorphous powder ([α]D26 + 26.5) and gave a molecular formula of C26 H33 NO3 with 11 degrees of unsaturation, as determined by HRESIMS data. The IR spectrum indicated the presence of hydroxy (3185 cm−1), carbonyl (1713 and 1662 cm−1), and benzene (1591 and 1440 cm−1) groups7, 8, whereas the UV absorption maxima at 216, 255, and 303 nm were indicative of a 2-pyridone unit9. The 1H NMR spectrum of dysivillosin A (1) (Table 1) displayed the resonances for four methyls at δH 0.94, 1.03, 1.20, and 1.45, two singlet aromatic protons at δH 7.59 and 7.74, three mutually coupling olefinic protons at δH 7.17 (1 H, dd, J = 7.8, 4.8 Hz), 8.13 (1 H, dd, J = 7.8, 1.8 Hz), and 8.46 (1 H, dd, J = 4.8, 1.8 Hz), along with a phenolic hydroxy proton at δH 11.65. The 13C NMR and HSQC spectra revealed the presence of one conjugated carbonyl (δC 163.5), four aromatic quaternary carbons [δC 120.8, 128.4, 147.8 (oxygenated), and 153.9 (oxygenated)], two olefinic quaternary carbons (δC 117.0 and 143.6), two aromatic methines (δC 106.1 and 114.9), four olefinic methines (δC 118.4, 120.4, 129.1, and 145.4), two aliphatic quaternary carbons (δC 38.0 and 41.8), two aliphatic methines (δC 36.0 and 45.9), five methylenes (δC 19.5, 26.3, 27.4, 35.6, and 37.8), and four methyls (δC 17.3, 17.4, 17.6, and 19.6). These above NMR spectroscopic features were characteristic of a sesquiterpene hydroquinone containing a 2-pyridone unit10.

The COSY correlations of H-2-1/H-10, H-2-1/H-2, H-2-6/H-7, H-7/H-8, H-8/H-3, H-23/H-24, and H-24/H-25 coupled with HSQC spectrum revealed the presence of three isolated spin systems: (a) C-10–C-1–C-2–C-3, (b) C-6–C-7–C-8–C-13, and (c) C-23–C-24–C-25, as shown in Fig. 2. The elongation from C-3 to C-11 was evident from the allylic COSY correlation of H-3/H-11. The connections of the three spin systems with the other carbons were mainly determined by analysis of the HMBC spectrum. The long-ranged HMBC
correlations of H3-11/C-3, C-4, and C-5 and H3-12/C-4, C-5, C-6, and C-10 connected the two partial structures a and b via C-5 in rings A and B and also allowed the assignment of the two methyls at C-4 and C-5, respectively. Moreover, the HMBC correlations of H3-13/C-7 and C-9 and H3-14/C-8, C-9, C-10, and C-15 established a tetramethyl decalin sesquiterpene moiety with a methylene CH2-15 tethered at C-9. This assignment was supported by the HMBC correlations of H-10/C-4, C-5, C-6, C-9, C-14 and C-15 and H2-15/C-8, C-9, C-10, and C-14. In the down field, the aromatic proton H-18 showed HMBC correlations with C-16 and the oxygenated aromatic carbon C-20, meanwhile H-21 showed HMBC correlations with C-19 and oxygenated aromatic carbon C-17, which indicated the presence of a 1,4-hydroquinone unit (ring C) in 1. The HMBC correlations from the phenolic hydroxy proton at δH 11.65 to C-16, C-17, and C-18 assigned the phenolic hydroxy group at C-17. Additionally, HMBC correlations of H2-15/C-16, C-17, and C-21 and H-21/C-15 suggested that the sesquiterpene fragment was linked to hydroquinone unit through the carbon bond C-15–C-16 (Fig. 2)10. What’s more, HMBC correlations of H-24/C-22 and H-23/C-27, and H-25/C-27 coupled with the remaining conjugated amide carbonyl C-27 (δC 163.5) indicated the presence a 2-pyridone substructure (ring D). Finally, HMBC correlations of H-18/C-22 and H-23/C-19 determined that the 2-pyridone unit was tethered at C-19 in the hydroquinone, supported by the NOESY correlation of H-18/H-23.

Once the planar structure of 1 was established, its relative configuration was addressed by NOESY experiments, as shown in Fig. 2. The large coupling constant value of H-10 (J = 12.0 Hz) and the chemical shift of CH3-12 (δC 19.6, δH 1.03) suggested the trans-fusion of the bicyclic system (rings A/B)11,12, which was confirmed by the NOESY correlations of H3-12/H-6α and H-10/H-6α. The NOESY correlations of H-8/H-10 and H-6α/H-10 indicated the α-orientation of the three protons, meanwhile the correlations of H3-12/H3-14 and H2-13/H2-14 assigned the three methyl groups as β-orientation. Thus, the relative configuration of 1 was determined as 5S*, 8S*, 9R*, 10S*, as depicted in Fig. 2. Additionally, the aromatic proton H-18 in the hydroquinone unit showed NOESY correlations with H-23 in the 2-pyridone moiety, which suggested the presence of two possible configurations (a and b) of the axial chiral center C-19–C-22 (Fig. 2).

Despite of the best efforts, the crystallization of 1 was finally unsuccessful. In order to determine the whole absolute configuration of 1, quantum chemical ECD calculation methods were employed to determine the absolute configuration of 113. Firstly, a hydroquinone-2-pyridone model structure was used to investigate the configuration of the axial chirality in the structure of 1 by HF/6-31G(d) method in Gaussian0914. Only two equilibrium conformers (a and b) were found using molecular mechanics (ΔE = 0–5 kcal/mol), as shown in Fig. 3, which is identical with the deduction form the NOESY experiment. The Boltzmann population ratios of the two conformers are 51.85% (a) and 48.15% (b) at the temperature of 298.15 K, and their dihedral angels are 37.5° and 37.6°, respectively. It was interesting to find that the Cotton effects (CEs) in the calculated ECD spectrum of a were opposite to those of b, as shown in Fig. 4. The majority of axial chiral compounds have a rotational barrier
sufficiently high to prevent racemization of the atropisomers at room temperature, and the separation of stereoisomers at room temperature requires energy barriers of at least 22 kcal/mol15. However, conformer a could easily convert to b with the energy barriers of less than 5 kcal/mol, which suggested that the axial chiral center in compound 1 could isomerize from M to P atropisomer at room temperature. Therefore, the whole absolute configuration of 1 was mainly determined by the configurations of four chiral centers (C-5, C-8, C-9 and C-10) in the sesquiterpene moiety. Subsequently, two optimized stereoisomers, (5R, 8R, 9S, 10R)-1 and (5S, 8S, 9R, 10S)-1, were built based on the relative configuration of 1, their ECD spectra were calculated at the level of PBE0/6-311+ +G (2d, 2p) with MeOH as the solvent 16, 17. The positive CE at 210 nm in the calculated spectrum of the 5S, 8S, 9R, 10S enantiomer matched well with the CE observed in the experimental ECD spectrum of 1 (Fig. 5), which determined the absolute configuration of 1 as 5S, 8S, 9R, 10S, identical with that of avarol.

Dysivillosin B (2) was assigned the same molecular formula C26H33NO3 as that of 1 by HRESIMS data. The 1H and 13C NMR data of 2 corresponded closely to those of 1 (Table 1), except for three subunits, a methylene CH2-3 (δC 32.6/δH 2.36, 2.11), an olefinic quaternary carbon C-4 (δC 159.4), and an exomethylene CH2-11 (δC 102.6/δH 4.46, 4.38), which implied that the avarol sesquiterenoid fragment in 1 was replaced by an neoavarol fragment in 218. This assignment was further supported by detailed analyses of COSY and HMBC correlations (Figure S1). Similar NOESY correlations and CE in the ECD spectrum (Fig. 6) showed that dysivillosin B shared the same relative and absolute configurations with those of 1.

Dysivillosin C (3) was also obtained as an isomer of 1, based on the HRESIMS data. Comprehensive analysis of the 1H and 13C NMR data of 3 (Table 1) showed that the only difference between 1 and 3 was the position of 2-pyridone, which tethered at C-18 in 3 rather than at C-19 in 1. This assignment was supported by HMBC correlations of H-19/C-22 and H-23/C-18 as well as the NOESY correlation of H-19/H-23 (Figure S1). Subsequent analyses of the NOESY and ECD spectra of 3 disclosed that its relative and absolute configurations were both consistent with those of 1 (Fig. 6).

The HRESIMS data indicated that dysivillosin D (4) was an isomer of 1–3. Detailed comparison of the NMR data of 4 with those of 2 indicated that the main difference was the position of 2-pyridone in the hydroquinone unit (Table 1). Key HMBC correlations of H-19/C-22 and H-23/C-18 positioned the 2-pyridone at C-18 (Figure S1). Identical NOESY correlations and similar CE in the ECD spectrum showed that dysivillosin D shared the same relative and absolute configurations with those of 1–3, as shown in Fig. 6.
There are a large number of naturally occurring derivatives of avarol/avarone and meroterpenoids where the hydroquinone/quinone is substituted by amines and amino acids. Recently, Anderson et al. reported the structure of avinosol, a meroterpenoid-nucleoside conjugate. However, dysivillosins A–D (1–4) are the first example of terpene-polyketide-pyridine hybrid compounds from the nature. The proposed biosynthesis of 1–4 could be involved with the biosynthetic pathways of three precursors: terpenoid, acetate, and pyridine. The four compounds could be biogenetically related to two meroterpenoids, avarol and neoavarol, which were both isolated from marine sponges belonging to the Dysidea genus. As shown in Fig. 7, 2-pyridone might be produced by L-lysine via successive decarboxylation, amidation, and dehydrogenation reactions. Subsequent nucleophilic attack from the 2-pyridone to the hydroquinone unit in avarol could finally result in the formation of dysivillosin A (1).

The four compounds (1–4) from D. villosa were screened for their anti-allergic activity on rat basophilic leukemia (RBL-2H3) cells. All of them showed no cytotoxicity on RBL-2H3 cells at the concentrations of 6 and 12 μM, as depicted in Fig. 8a. Subsequently, the inhibitory activity of 1–4 on the degranulation in DNP-IgE-activated RBL-2H3 mast cells was examined, using ketotifen as a positive control. Dysivillosins A–D were found to potently inhibit the release of β-hexosaminidase, a marker of degranulation, in a dose-dependent manner with

| Position | 1 | 2 | 3 | 4 |
|----------|---|---|---|---|
| δc       | δh (J in Hz) | δc | δh (J in Hz) | δc | δh (J in Hz) |
| 1v       | 19.5, CH2 | 2.33, m | 22.8, CH2 | 2.35, m | 19.1, CH2 | 2.39, m | 22.5, CH2 | 2.41, m |
| 1i       | 1.63, m | 1.55, dd (12.0, 2.4) | 1.57, m | 1.53, dd (12.0, 3.6) |
| 2a       | 26.3, CH2 | 2.38, m | 28.0, CH2 | 1.93, m | 26.1, CH2 | 2.23, m | 27.9, CH2 | 1.92, m |
| 2b       | 2.14, m | 1.59, m | 2.07, m | 1.35, m |
| 3v       | 120.4, CH | 5.16, brs | 32.6, CH2 | 2.36, m | 120.4, CH | 4.98, brs | 32.5, CH2 | 2.26, td (15.0, 4.8) |
| 3i       | 2.11, m | 1.96, m |
| 4        | 143.6, C | 159.4, C | 143.1, C | 159.0, C |
| 5        | 38.0, C | 39.9, C | 37.8, C | 39.8, C |
| 6v       | 35.6, CH2 | 0.89, td (13.2, 3.6) | 36.4, CH2 | 1.29, dd (13.2, 3.6) | 35.5, CH2 | 0.75, td (13.2, 3.6) | 36.3, CH2 | 1.14, td (13.2, 3.6) |
| 6i       | 1.49, dt (12.6, 3.0) | 1.45, m | 1.41, dt (12.6, 3.6) | 1.35, m |
| 7v       | 27.4, CH2 | 1.32, m | 27.4, CH2 | 1.42, m | 27.0, CH2 | 1.20, dd (13.2, 3.6) | 27.0, CH2 | 1.41, m |
| 7i       | 1.37, td (12.6, 1.2) | 1.39, m | 1.30, td (13.2, 3.0) | 1.35, m |
| 8        | 36.0, CH | 1.81, m | 36.3, CH | 1.76, m | 35.9, CH | 1.69, m | 36.2, CH | 1.63, m |
| 9        | 41.8, C | 42.2, C | 41.3, C | 41.6, C |
| 10       | 45.9, CH | 1.55, d (12.0) | 48.2, CH | 1.36, dd (12.0, 2.4) | 45.9, CH | 1.39, dd (12.6, 1.2) | 48.3, CH | 1.20, dd (12.0, 1.8) |
| 11a      | 17.6, CH3 | 1.45, br s | 102.6, CH2 | 4.46, s | 17.5, CH | 1.35, br s | 102.6, CH2 | 4.32, s |
| 11b      | 11b | 43.8, s | 4.28, s |
| 12       | 19.6, CH3 | 1.03, s | 20.0, CH2 | 1.06, s | 19.5, CH2 | 0.96, s | 20.0, CH2 | 0.99, s |
| 13       | 17.3, CH3 | 1.20, d (6.6) | 17.4, CH3 | 1.20, d (6.6) | 16.6, CH2 | 1.09, d (6.6) | 16.8, CH2 | 1.10, d (6.6) |
| 14       | 17.4, CH3 | 0.94, s | 17.3, CH3 | 0.93, s | 17.3, CH3 | 0.87, s | 17.2, CH3 | 0.88, s |
| 15a      | 37.8, CH2 | 3.13, s | 37.7, CH2 | 3.09, d (14.4) | 37.2, CH2 | 3.20, d (14.4) | 37.3, CH2 | 3.12, d (14.4) |
| 15b      | 3.04, d (14.4) | 2.93, d (14.4) | 2.88, d (14.4) |
| 16       | 128.4, C | 128.2, C | 124.1, C | 123.9, C |
| 17       | 153.9, C | 153.8, C | 147.9, C | 149.7, C |
| 18       | 106.1, CH | 7.74, s | 106.1, CH | 7.69, s | 122.3, C | 7.63, d (3.0) |
| 19       | 120.8, C | 120.8, C | 105.0, CH | 7.68, d (2.4) | 105.1, CH | 7.63, d (3.0) |
| 20       | 147.8, C | 147.8, C | 154.3, C | 154.2, C |
| 21       | 114.9, CH | 7.59, s | 114.6, CH | 7.53, s | 120.0, CH | 7.26, d (2.4) | 119.7, CH | 7.20, d (3.0) |
| 22       | 117.0, C | 117.0, C | 117.3, C | 117.2, C |
| 23       | 129.1, CH | 8.13, dd (7.8, 1.8) | 129.1, CH | 8.10, dd (7.8, 1.8) | 129.5, CH | 8.21, dd (7.2, 1.8) | 129.4, CH | 8.18, dd (7.8, 1.8) |
| 24       | 118.4, CH | 7.17, dd (7.8, 4.8) | 118.4, CH | 7.16, dd (7.8, 4.8) | 118.6, CH | 7.21, dd (7.2, 4.8) | 118.5, CH | 7.16, dd (7.8, 4.8) |
| 25       | 145.4, CH | 8.46, dd (4.8, 1.8) | 145.4, CH | 8.45, dd (4.8, 1.8) | 145.8, CH | 8.52, dd (4.8, 1.8) | 145.8, CH | 8.50, dd (4.8, 1.8) |
| 27       | 163.5, C | 163.5, C | 163.3, C | 163.3, C |
| 17-OH    | 11.65, br s | 11.63, br s | 11.67, br s | 11.63, br s |

Table 1. $^1$H (600 MHz) and $^{13}$C (150 MHz) NMR Data for Dysivillosins A–D (1–4) in Pyr-$d_5$.
LTB4 and IL-4 in mast cells are triggered by cross-linking of FcεRI-bound IgE/FcγRI/Syk signaling pathway, because both degranulation and subsequent production of LTB4 and IL-4 was investigated as well. As depicted in Fig. 8c and d, all the four compounds could dose dependently downregulate the production of LTB4 and IL-4 in the antigen-stimulated RBL-2H3 mast cells at the concentrations of 6 and 12 μM. Among the four compounds, dysivillosin A (1) showed the most potent anti-allergic activity, and thus was selected for further inhibitory activity evaluation on the antigen-specific IgE/FcγRI/Syk signaling pathway, because both of the degranulation and subsequent production of LTB4 and IL-4 in mast cells are triggered by cross-linking of FcγRI-bound IgE molecules31, 32.

Interestingly, we found that dysivillosin A (1) could significantly inhibit the activation of Syk as well as its downstream signaling molecule, PLCδ1, as shown in Fig. 9a and b. This finding indicated that dysivillosin A could inhibit mast cell activation by suppressing the phosphorylation of Syk and PLCδ1, which subsequently inhibited the degranulation and downregulated the production of LTB4 and IL-4. These results highlight the potential of dysivillosin A (1) as a new chemotherapeutic scaffold targeting Syk-associated allergy.

Methods

General experimental procedures. Optical rotations were measured with an Autopol I-30575 (l = 10 cm) and UV spectra on a Hitachi U-3010 spectrophotometer; IR spectra (KBr) were obtained with a Jasco FTIR-410 spectrophotometer. NMR spectra were obtained with a Bruker Avance NMR spectrometer at 600 MHz for 1H and 150 Hz for 13C using Pyr-d6 as solvent. HRESIMS data were obtained on an Agilent 6210 LC/MSD TOF mass spectrometer.

Collection, extraction, and isolation. Samples of Dysidea villosa were collected from the coast of Yongxing Island in South China sea on May 7th, 2011. The voucher sample of D. villosa (XD110507A) was maintained at the Research Center for Marine Drugs, State Key Laboratory of Oncogenes and Related Genes, Department of Pharmacy, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University. The animals (520 g, wet weight) was extracted by MeOH repeatedly to give 9.2 g extract, which showed potent inhibitory activity on β-hexosaminidase (IC50 = 5.3 μg/mL). The bioactive extract was dissolved in 500 mL water, and partitioned with the same volume of CH2Cl2 four times to yield 8.7 g CH2Cl2-soluble fraction, which was subjected to a silica gel chromatography column eluted with gradient CH3CN, to give dysivillosin A (1, 2.3 mg, tR 58.1 min), dysivillosin B (2, 2.7 mg, tR 79.1 min), dysivillosin C (3, 6.4 mg, tR 62.3 min), and dysivillosin D (4, 2.7 mg, tR 58.1 min).

Dysivillosin A (1): colorless solid; [α]D+26 + 26.5 (c 0.1, MeOH); UV (MeOH) λmax (logε) 216 (4.12), 255 (3.90), 303 (4.08), 331 (3.65) nm; CD (MeOH) λ (Δε) 212 (+8.51) nm; IR (KBr) νmax 3185, 2958, 2924, 2855, 1713, 1662, 1591, 1444, 1261, 1166, 1094, 1021, 799 cm−1; 1H and 13C NMR data see Table 1; HRESIMS m/z 388.2274 [M - H2O - H]+ (calcd for C26H30NO2, 388.2277).

Dysivillosin B (2): colorless solid; [α]D+10 + 6.7 (c 0.1, MeOH); UV (MeOH) λmax (logε) 202 (3.54), 301 (3.74), 326 (3.34) nm; CD (MeOH) λ (Δε) 209 (+5.56) nm; IR (KBr) νmax 2958, 2924, 2855, 1735, 1665, 1634, 1592, 1444, 1378, 1260, 1162, 1093, 1023, 800 cm−1; 1H and 13C NMR data see Table 1; HRESIMS m/z 388.2275 [M - H2O - H]+ (calcd for C26H30NO2, 388.2277).

Dysivillosin C (3): colorless solid; [α]D+26 + 4.2 (c 0.3, MeOH); UV (MeOH) λmax (logε) 216 (4.11), 254 (3.70), 297 (3.91), 326 (3.33) nm; CD (MeOH) λ (Δε) 210 (+9.21) nm; IR (KBr) νmax 3194, 2957, 2925, 2856, 1713, 1664, 1620, 1594, 1461, 1403, 1379, 1253, 1171 cm−1; 1H and 13C NMR data see Table 1; HRESIMS m/z 388.2279 [M - H2O - H]+ (calcd for C26H30NO2, 388.2277).

Dysivillosin D (4): colorless solid; [α]D+26 + 8.5 (c 0.1, MeOH); UV (MeOH) λmax (logε) 205 (4.08), 244 (3.60), 297 (3.85), 326 (2.82) nm; CD (MeOH) λ (Δε) 207 (+11.03) nm; IR (KBr) νmax 3203, 2959, 2925, 2855, 1737, 1666, 1615, 1595, 1454, 1261, 1174, 1093, 1022, 800 cm−1; 1H and 13C NMR data see Table 1; HRESIMS m/z 388.2279 [M - H2O - H]+ (calcd for C26H30NO2, 388.2277).
Energy Minimization and ECD Calculations. The molecule of 1 was converted into SMILES code before its initial 3D structure was generated with CORINA version 3.4. The conformer database of 1 was generated using CONFLEX version 7.0 with an energy window for acceptable conformers (ewindow) of 5 kcal mol\(^{-1}\) above the ground state using the modified version of the MMFF94 force field. Each conformer was optimized with the HF/6-31G(d) method in Gaussian09. Further optimization at the B3P86/6-31G(d) level led to the observed dihedral angles. The optimized conformers were taken for the ECD calculations, which were performed with Gaussian09 (B3P86/6-311++G(2d,p))\(^{13}\). The solvent effects were taken into account by the conductor-like polarizable continuum model (MeOH as the solvent)\(^{14,16}\). To gain ECD curves from the output files that were suited for comparison with the experimental data, the length representation of the oscillator and rotatory strengths was always selected and the spectra were overlaid with Gaussian profiles that were in turn summed to give, after Boltzmann weighting of the individual spectra, a calculated ECD spectrum.

Cell Culture. Rat basophilic leukemia (RBL-2H3) cells from the cell bank of Shanghai Science Academy were cultured in Dulbecco’s modified Eagle’s medium (DMEM), which contains 10% (v/v) FBS, 100 U/mL penicillin, and 100 \(\mu\)g/mL streptomycin, at 37 °C in a humidified atmosphere of 5% CO\(_2\).

Cell Viability. MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assay was conducted to examine cell viability. RBL-2H3 cells (5 \(\times\) 10\(^5\) cells/well, 100 \(\mu\)L/well) were plated into a 96-well plate. After 24 h of incubation, cells were incubated with compounds 1–4 for 3 hours and the medium was replaced by MTT solution (250 \(\mu\)g/mL) and incubated at 37 °C for 4 h. The medium was then carefully discarded and the formazan was resuspended in 150 \(\mu\)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.

\(\beta\)-hexosaminidase Release Activity. RBL-2H3 cells were seeded in a 24-well plate (5 \(\times\) 10\(^5\) cells/well), and were sensitized with dinitrophenyl (DNP)-specific IgE (DNP-IgE) (1 \(\mu\)g/mL) at 37 °C for overnight.
DNP-IgE-sensitized cells were preincubated with sponge extracts or dysivillosins A–D (1–4) for 30 min, and then stimulated with DNP-BSA for 1.5 h. To measure the activity of β-hexosaminidase released from the cells, the cultured media were centrifuged (17,000 g, 10 min) at 4 °C. The supernatant (50 µL) was mixed with 50 µL of 0.1 M sodium citrate buffer (pH 4.5) containing 10 mM 4-nitrophenyl N-acetyl-β-D-glucosaminide in a 96-well plate, and then was incubated at 37 °C for 90 min. The absorbance was measured at 405 nm after terminating the reaction by 0.2 M glycine (pH 10.0).

**Determination of IL-4 and LTB4.** To measure Interleukin-4 (IL-4) and Leukotriene B4 (LTB4) level in the cultured media, all cultured media were centrifuged at 4 °C, and the test compounds were stored at −80 °C until assay. IL-4 and LTB4 were quantified using an ELISA kit according to the manufacturer’s instructions.

**Immunoblot Analysis.** After cell collection and extraction with the protein extraction solution, the protein samples were subjected to 10% SDS–polyacrylamide gel electrophoresis (PAGE). The protein spots were electrotransferred to a PVDF membrane, and then the membrane was incubated with blocking buffer (Tris-buffered saline (TBS) containing 0.05% Tween-20 and 5% w/v nonfat dry milk) for 1 h and was washed by TBST (TBS containing 0.05% Tween-20) three times, each time for 5 min, followed by a diluted solution of anti-p-syk, anti-syk, anti-p-PLCγ1, and anti-PLCγ1 antibodies (Cell Signaling Technology) overnight at 4 °C. In addition, the blots probed with a 1:1000 diluted solution of anti-β-actin antibody (Abcam) were used as internal control to keep each well loading with the same amount of protein. The membrane was washed by TBST for three times, each time for 10 min, shaken in a solution of horseradish peroxidase (HRP)-linked anti-rabbit or anti-mouse IgG secondary antibody, and washed by TBST for another three times, each time for 10 min. The expressions of proteins were detected by enhanced chemiluminescent (ECL) reagent (Thermo Scientific), followed by exposure of the membranes to photographic film (Fujifilm Corporation). The intensity of blots were analyzed and quantitated with ImageJ software.

**Statistical Analysis.** 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 ± SD. The differences with p < 0.05 were considered significant.

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**Author Contributions**

Wei-Hua Jiao designed and conducted the main experiments, data analyses, and wrote the manuscript. Bao-Hui Cheng performed the anti-allergic activity of the four compounds. Guo-Hua Shi extracted the marine sponge samples and purified the four compounds. Guo-Dong Chen conducted the ECD calculation for the compound 1. Bin-Bin Gu conducted the ECD calculation for the compounds 2–4. Yong-Jun Zhou revised the biogenetic pathway of compound 1. Li-Li Hong measured the optical values of the four compounds. Fan Yang assisted the sample collection. Zhi-Qiang Liu assisted the anti-allergic activity evaluation. Shu-Qi Qiu assisted the identification of the sample species. Zhi-Gang Liu revised the biological part of the manuscript. Ping-Chang Yang helped design the anti-allergic activity. Hou-Wen Lin helped design the chemical experiments.

**Additional Information**

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