Aspertetranones A–D, Putative Meroterpenoids from the Marine Algal-Associated Fungus Aspergillus sp. ZL0-1b14

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Aspertetranones A–D, Putative Meroterpenoids from the Marine Algal-Associated Fungus *Aspergillus* sp. ZL0-1b14

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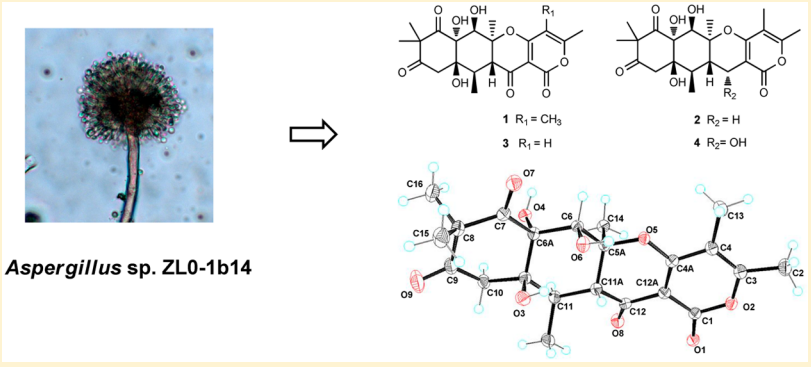
Supporting Information



ABSTRACT: Aspertetranones A–D (**1**–**4**), four new highly oxygenated putative rearranged triketide-sesquiterpenoid meroterpenes, were isolated from the marine algal-associated fungus *Aspergillus* sp. ZL0-1b14. On the basis of a comprehensive spectroscopic analysis, the planar structures of aspertetranones were determined to possess an unusual skeleton in the terpenoid part. The relative and absolute configurations of the aspertetranones were assigned on the basis of NOESY analysis, X-ray crystallography, and circular dichroism spectroscopy. Compounds **1**–**4** were evaluated for anti-inflammatory activity in LPS-stimulated RAW264.7 macrophages. Aspertetranone D exhibited an inhibitory effect against IL-6 production with 69% inhibition at 40 μM.

Fungi from specific habitats are prone to be outstanding producers of unusual chemical structures.[1−4] During our screening for fungi from unique niches, a marine algal-associated fungal strain, *Aspergillus* sp. ZL0-1b14, derived from the Jinjiang Dongshi salt pan, Fujian Province, China, exhibited halophilic properties, and the HPLC profile of its extract revealed the presence of diverse chemical entities. This initial analysis prompted the scale-up fermentation of this strain and the isolation of a series of new compounds from this strain. Here, we report the structure elucidation, biological evaluation, and a possible biosynthetic pathway to aspertetranones A–D (**1**–**4**),[5] four new highly oxygenated putative triketide-sesquiterpenoid meroterpenes with an unusual skeleton rearrangement in the terpenoid part.

RESULTS AND DISCUSSION

The strain *Aspergillus* sp. ZL0-1b14 was cultured on potato-dextrose agar (PDA) medium containing 20% NaCl at 28 °C for

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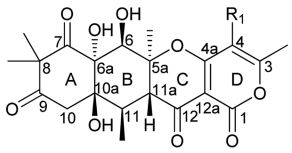
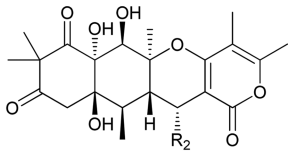
28 days. Sequential chromatographic methods as reported in the Experimental Section were applied to afford aspertetranones A–D (**1**–**4**).



1 $R_1 = CH_3$
3 $R_1 = H$



2 $R_2 = H$
4 $R_2 = OH$

Aspertetranone A (**1**) was obtained as colorless crystals from MeOH. The molecular formula was deduced as $C_{22}H_{26}O_9$ by HRESIMS. The ^1H, ^{13}C NMR and HSQC spectra (pyridine-d_5) of **1** display signals for six methyls, one methylene, three methines, and 12 nonprotonated carbons. The HMBC correlations from the protons of six methyls to the corresponding carbons resulted in three partial structures (rings A, B, and D). HMBC correlations from the protons of the only methylene H_2-10 [δ_H 3.19, 3.27, (d, J = 16.8 Hz)] to five carbons, C-11 (δ_C 35.7), C-10a (δ_C 76.8), C-9 (δ_C 210.6), C-8 (δ_C 56.8), and C-6a (δ_C 77.5), joined two previously deduced fragments together to form a 6/6 AB ring junction system, which was further supported by the HMBC correlations from the hydroxy proton OH-10a (δ_H 5.95, s) to C-10 (δ_C 46.9) and C-6a, and the OH-6a (δ_H 8.93) to C-10a. Moreover, one ketone carbonyl was connected to C-11a according to the HMBC correlations from H-11a to C-12 and C-11. Among the two unassigned carbon signals, the relatively shielded one, C-12a (δ_C 101.9), was assumed to be at the α-position of the carbonyl group C-12 (δ_C 191.3). The chemical shift of the other unassigned carbon, C-1 (δ_C 158.2), was similar as that of oxygen-substituted aromatic or vinyl carbons, suggesting the formation of an α-pyrone (ring D) with the remaining fragment. An oxygen atom was the linker between C-5a and C-4a on the basis of the molecular formula $C_{22}H_{26}O_9$ and the chemical shift of C-4a (δ_C 172.3). Thus, the planar structure of **1** was elucidated as shown in Figure 1 and named aspertetranone A.

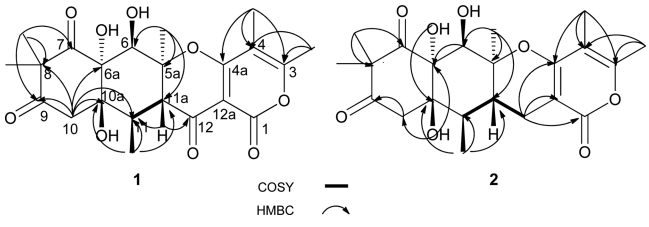


Figure 1. Selected COSY and HMBC correlations for asperteranones A (**1**) and B (**2**).

The relative configuration of **1** was established on the basis of NOESY spectra (Supporting Information, Figure S6). The observed strong NOESY cross-peaks at H_3-5a/H-11 and H-11/H-10α indicated a 1,3-diaxial orientation for H-11 and 5a-Me. NOESY cross-peaks of H-11a/OH-10a, H-11a/H_3-11, and H_3-5a/H-6 revealed a *syn* orientation between H-11a, OH-10a, 11-Me, and OH-6 (Figure 1).

The absolute configuration of **1** was determined by a single-crystal X-ray diffraction experiment using Cu Kα radiation. On the basis of the crystallographic analysis, the absolute configuration of **1** was assigned as 5a*S*, 6*R*, 6a*R*, 10a*R*, 11*R*, 11a*R* with the refined Flack parameter value [χ = 0.02(11)] (Supporting Information, Table S1). Thus, the absolute structure of **1** was unambiguously established (Figure 2).

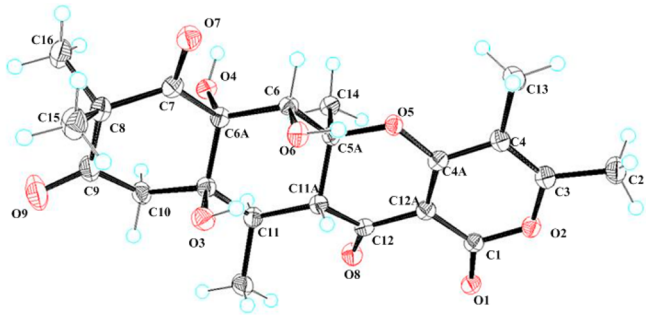


Figure 2. X-ray crystal structure of aspertetranone A (**1**).

Aspertetranone B (**2**) was obtained as a white, amorphous powder. The ^1H and ^{13}C NMR spectra of **2** are similar to those of **1** with the exception of the absence of the carbonyl group C-12 and the presence of a methylene (δ_H 2.62, 2.01 and δ_C 20.3) (Table 1). Further analysis of the HSQC and HMBC spectra of **2** revealed a shielded chemical shift for H-11a (δ_H 2.18) and a COSY correlation between H-11a and H-12 (δ_H 2.62, 2.01), which indicated that the carbonyl group at C-12 was replaced by a methylene (Figure 1). This deduction was also confirmed by the molecular formula of **2**, $C_{22}H_{28}O_8$, established on the basis of HRESIMS. Detailed examination of NOESY data revealed that H-11a, OH-10a, OH-6, and 11-Me were on the same side of the ring system, which was supported by cross-peaks at H-11a/OH-10a, H-11a/OH-6, and H-11a/H_3-11. Meanwhile, NOESY correlations between H-11/H_3-5a, H_3-5a/OH-6a, and H_3-5a/H-6 indicated H-11, 5a-Me, and OH-6a were located on the other side of this ring system (Figure 3). The same NOESY patterns of **1** and **2** and the same direction of their specific rotations indicated the configuration of **2** (5a*S*, 6*R*, 6a*R*, 10a*R*, 11*R*, 11a*R*) was consistent with **1**. Also the same biogenic origin of **1** and **2** further supported the absolute configuration of **2**.

Aspertetranone C (**3**) was purified as a white, amorphous powder. The ^1H NMR spectrum of **3** exhibited a pattern analogous to that of **1**. The ^{13}C and HSQC spectra revealed that one methyl group was missing in **3**. Further analysis of HMBC spectrum indicated the missing group was the 4-Me. This deduction was confirmed by the molecular formula of **3**, $C_{21}H_{24}O_9$, established by HRESIMS. Thus, the structure of aspertetranone C (**3**) was defined as the 4-demethyl derivative of aspertetranone A (**1**). Compounds **1** and **3** were expected to share the same relative configurations due to almost identical chemical shifts of atoms in rings A and B (Table 1). Considering the specific rotations of **1** and **3** were $[\alpha]^{20}_D$ +97.6 and $[\alpha]^{20}_D$ +88.0, respectively, we assume the absolute configuration of **3** was consistent with **1**. The similar ECD curves of **1** and **3** further support this conclusion (Figure S33).

Aspertetranone D (**4**) was obtained as a white, amorphous powder. The molecular formula was deduced as $C_{22}H_{28}O_9$ on the basis of HRESIMS. The ^1H and ^{13}C NMR spectra of **4** exhibited a pattern analogous to that of **2**. Detailed analysis of the 2D NMR spectra revealed the methylene (δ_H 2.62, 2.01 and δ_C 20.3) in compound **2** was replaced by an oxygenated methine (δ_H 4.71 and δ_C 58.9) at C-12 as shown in Figure 4. Compared to aspertetranones A–C (**1**–**3**), aspertetranone D had one more stereocenter at C-12. On the basis of the analysis of the NOESY spectrum, the correlations between H-12 and H_3-11 indicated they were cofacial, and the remaining configuration was the same

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Table 1. NMR Data for Aspertetranones A–D (1–4) in Acetone-d_6 (^{1}H at 600 MHz, ^{13}C at 150 MHz)

	1		2		3		4	
position	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)
1	156.2, C		163.0, C		156.5, C		163.3, C	
2								
3	163.7, C		155.3, C		168.3, C		157.1, C	
4	106.2, C		106.5, C		99.2, CH	6.02, s	106.6, C	
4a	171.0, C		162.2, C		171.7, C		162.2, C	
5								
5a	87.2, C		81.5, C		87.3, C		82.1, C	
6	73.2, CH	4.72, d (4.6)	73.6, CH	4.60,d (3.9)	72.7, CH	4.66, s	74.5, CH	4.54, d (3.7)
6a	75.7, C		75.9, C		75.2, C		75.9, C	
7	207.8, C		208.1, C		207.8, C		208.2, C	
8	54.9, C		55.0, C		55.0, C		54.9, C	
9	208.3, C		208.6, C		208.4, C		208.7, C	
10α	44.8, CH_2	2.83, d (17.1)	45.2, CH_2	2.82, d (16.7)	44.8, CH_2	2.84, d (17.0)	45.4, CH_2	2.88, d (16.9)
10β		2.75, d (17.1)		2.72,d (16.7)		2.75, d (17.0)		2.75, d (16.9)
10a	74.8, C		74.8, C		74.6, C		75.3, C	
11	34.0, CH	2.15, m	38.2, CH	1.90, m	34.0, CH	2.15, m	34.5, CH	2.32, m
11a	48.1, CH	3.32, d (11.7)	33.5, CH	2.18, m	48.4, CH	3.34, d (11.4)	39.2, CH	2.22, dd (11.3, 3.9)
12α	188.9, C		20.3, CH_2	2.62, dd (16.7, 5.2)	188.6, C		58.9, CH	4.71, d (3.9)
12β				2.01, dd (16.7, 12.4)				
12a	100.3, C		97.7, C		99.8, C		101.5, C	
3-Me	17.3, CH_3	2.26, s	16.3, CH_3	2.19, s	19.6, CH_3	2.26, s	16.5, CH_3	2.21, s
4-Me	8.6, CH_3	1.97, s	8.7, CH_3	1.89, s			8.7, CH_3	1.91, s
5a-Me	17.6, CH_3	1.53, s	17.7, CH_3	1.41, s	17.5, CH_3	1.54, s	20.2, CH_3	1.64, s
8-Meα	25.3, CH_3	1.31, s	25.2, CH_3	1.32, s	25.3, CH_3	1.32, s	25.2, CH_3	1.32, s
8-Meβ	22.7, CH_3	1.29, s	22.8, CH_3	1.29, s	22.7, CH_3	1.29, s	22.8, CH_3	1.29, s
11-Me	10.9, CH_3	1.28, d (6.5)	9.4, CH_3	1.08, d (6.7)	10.9, CH_3	1.28, d (6.5)	9.6, CH_3	1.21, d (6.7)
6-OH				5.63, d (4.7)				5.66, d (4.1)
6a-OH				5.72, s				5.69, s
10a-OH				4.72, d (2.3)				4.67, d (2.5)
12-OH								3.97, s

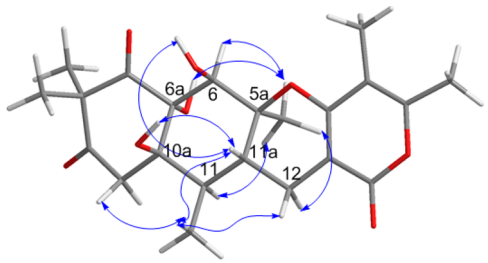

Figure 3. Key NOESY correlations of aspertetranone B (2).

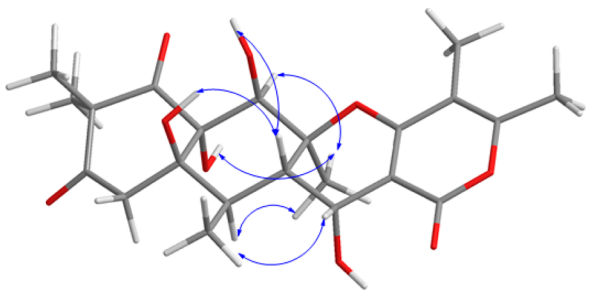

Figure 4. Key NOESY correlations of aspertetranone D (4).

as aspertetranone B (2). To determine the absolute configuration of 4, the theoretical calculated ECD spectra (Figure S34) of two possible enantiomers were obtained using time-dependent density functional theory (TDDFT). The overall pattern of the experimental ECD spectrum was in reasonable agreement with the calculated ECD spectrum of the 12*R* enantiomer. The calculated specific rotation also supported this conclusion (Experimental Section). Thus, we could determine the absolute configuration of 4 as 5a*S*, 6*R*, 6a*R*, 10a*R*, 11*R*, 11a*S*, 12*R*.

There are a number of examples of meroterpenoids with the same or similar polyketide portion as aspertetranones, attached to a modified diterpene or sesquiterpene, for example, the territrems,[6,7] pyripyropenes,[8] terreulactones,[9] and penicillipyrones.[2] However, the linear 6/6/6/6 tetracyclic ring architecture in the aspertetranones has not been reported. After thorough investigation of the literature regarding related structures, we found a very close compound, scapanin G,[10] a diterpenoid isolated from a liverwort, in which C6 delta-lactone in aspertetranone C was replaced by C5 at the five-membered-ring (highlighted in blue in Scheme 1). On the basis of structures of scapanin G and another analogue, scapanin B, we proposed a biogenetic pathway to aspertetranones A–D (1–4). As shown in Scheme 1, the farnesylated pyrone was cyclized to give the common drimane-type merosesquiterpene,[11] then oxidation and retro-Aldol/Aldol rearrangement[12] were required to produce the unique terpenoid part of aspertetranones. After nucleophilic attack and dehydration, the elaborate preaspertetranone was achieved.

Aspertetranones A–D (1–4) were evaluated for their anti-inflammatory effects using LPS-stimulated RAW264.7 macrophages as a model system. The pro-inflammatory cytokines

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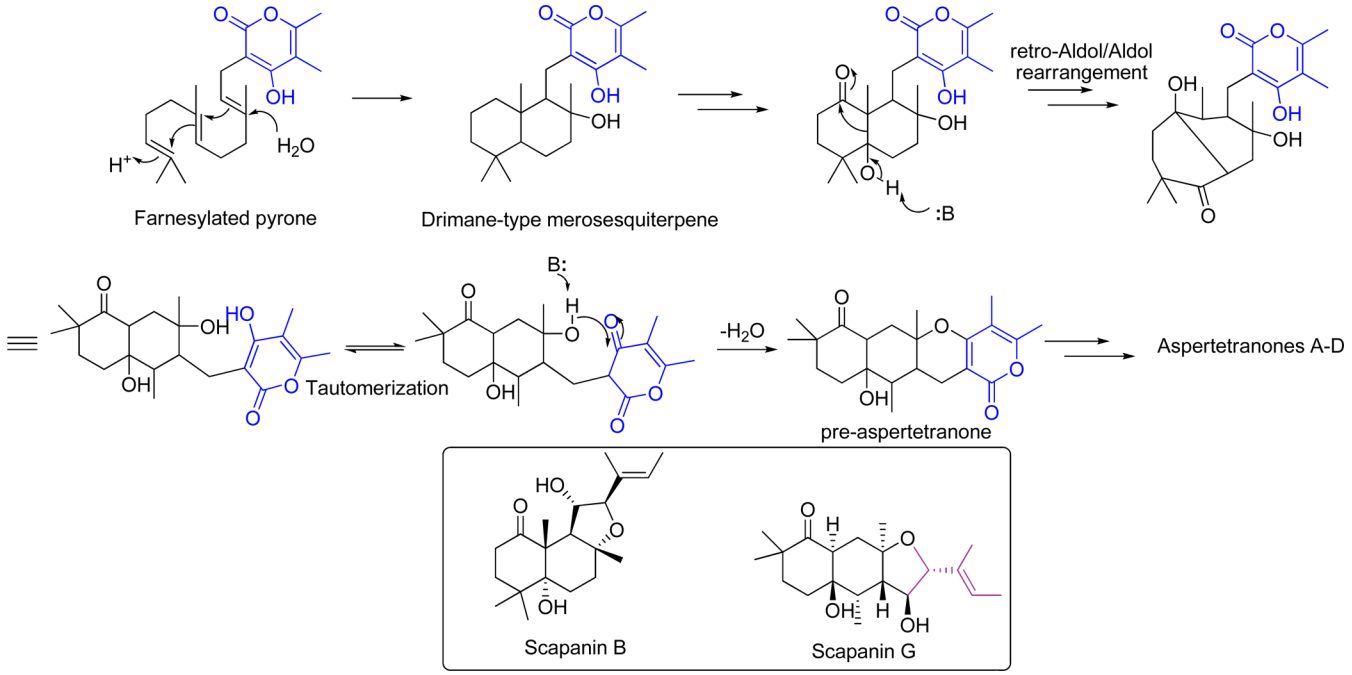
Scheme 1. Proposed Biogenesis of Aspertetranones



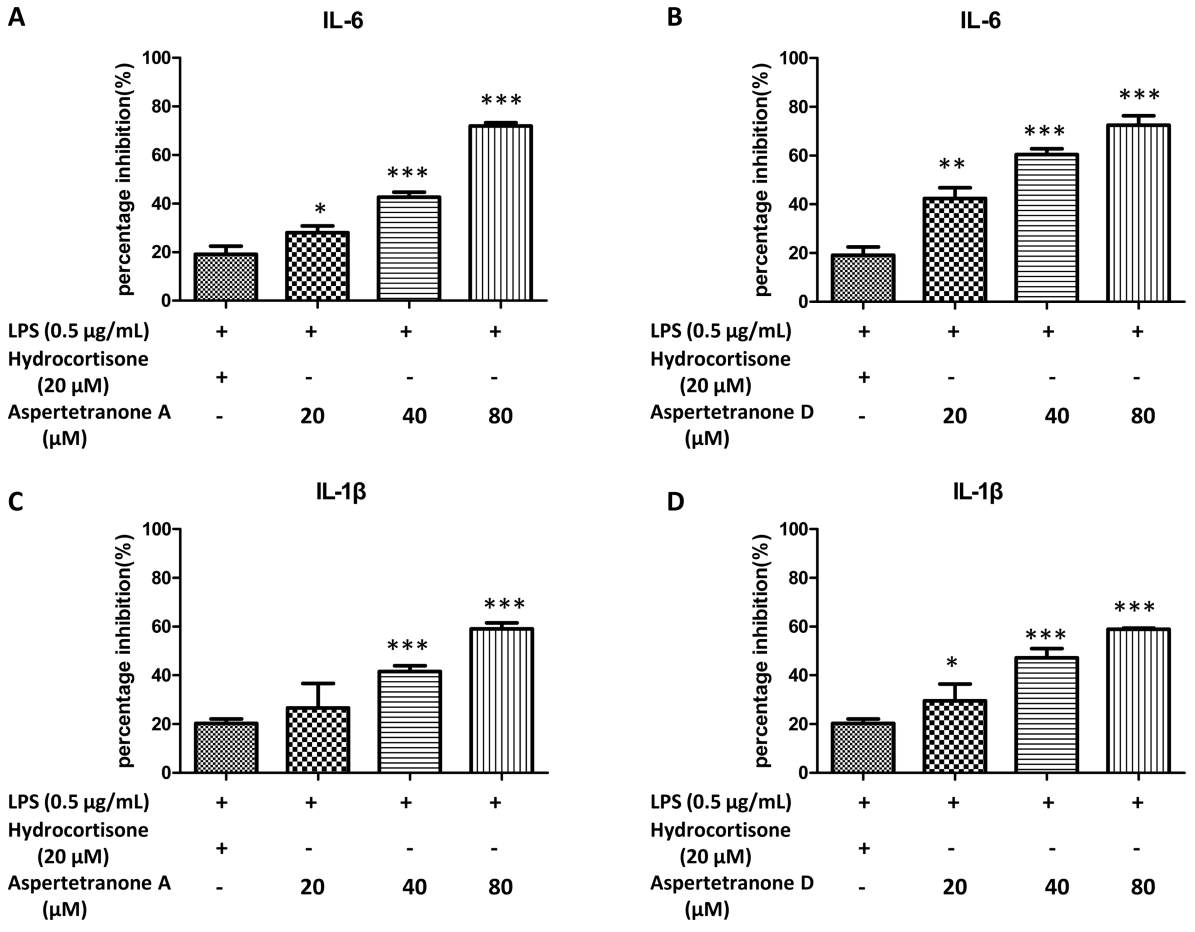


Figure 5. Aspertetranones A and D inhibit LPS-induced expression of IL-6 and IL-1β in RAW 264.7 macrophages. Cells were pretreated with **1** or **4** (0−80 μM) or hydrocortisone (20 μM) for 2 h followed by a further 22 h treatment with LPS. The IL-6 (A and B) and IL-1β (C and D) contents in the culture medium were determined by ELISA. Data are expressed as means ± SEM from three independent experiments. Statistical significance is indicated (*p < 0.05, **p < 0.01, and ***p < 0.001).

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(IL-6, IL-1β, TNF-α) and nitric oxide (NO) were measured in LPS-stimulated RAW 264.7 macrophages treated with aspertetranones A−D. No significant changes in cell viability were observed in RAW 264.7 cells after exposure to aspertetranones A−D for 24 h at a concentration gradient of 10−100 μM. Aspertetranones A (**1**) and D (**4**) suppressed the production of IL-6 and IL-1β in a dose-dependent manner (Figure 5), while only weak inhibitory effects were observed for aspertetranones B (**2**) and C (**3**) (data not shown). At a concentration of 40 μM, aspertetranones A and D exhibited inhibitions of 43 ± 2% and 69 ± 2% against IL-6 (Figure 5A and B), respectively. For IL-1β, their inhibitions were 42 ± 2% and 47 ± 4% (Figure 5C and D), respectively. At a concentration of 33.3 μM, aspertetranones A−D showed only weak inhibitory effects (less than 35% inhibition) for TNF-α and NO production. These data indicated that aspertetranones A−D exhibited differential anti-inflammatory profiles.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 341 polarimeter (PerkinElmer Inc.) using a 1 cm cell. UV spectra were acquired using a Jasco V-530 spectrophotometer. IR spectra were obtained on a PerkinElmer 552 spectrophotometer. ^{1}H, ^{13}C, and 2D NMR spectra were acquired on a Bruker Avance-600 spectrometer (600 MHz) using TMS as the internal standard. Electrospray ionization (ESI) low-resolution LC/MS data were acquired on a Thermo-Finnigan LCQ Advantage mass spectrometer. High-resolution electrospray ionization mass spectra (HRESIMS) was obtained on a Bruker LC-QTOF mass spectrometer. Semipreparative high-pressure liquid chromatography (HPLC) was performed on an Agilent 1200 using a XDB C_{18} column (10 × 250 mm, 5 μm, flow rate of 2 mL/min). TLC detection was carried out using precoated silica gel GF_{254} plates (10−40 μm, Qingdao Marine Chemical Plant). Column chromatography was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Plant), reversed-phase RP-18 (40−63 μm, Merck), and Sephadex LH-20 (Amersham Biosciences).

Fungal Material. The endophytic fungus *Aspergillus* sp. ZL0-1b14 was isolated from the marine green algal species of the genus *Enteromorpha* collected in Jinjiang Dongshi salt pan, Fujian Province, China. The green algal sample was rinsed in sterile water prior to grinding. Fungal identification was carried out by DNA amplification and sequencing of the ITS region. The sequence data obtained from the fungal strain have been deposited in GenBank (accession no. KT626003). A BLAST search result showed that the sequence was the most similar (99%) to the sequence of *Aspergillus ochraceopetaliformis* EIODSF003 (accession no. KJ173526.1). The strain ZL0-1b14 was deposited at China Center for Type Culture Collection (preservation no. CCTCC M 2014668).

Fermentation and Extraction. The strain *Aspergillus* sp. ZL0-1b14 was cultured on PDA medium containing 20% NaCl at 28 °C for 28 days. A total of 20 L of fungal solid culture was prepared. The fermented agar cakes were diced and extracted with EtOAc−MeOH−AcOH (v/v/v, 80/15/5, 3 × 4 L). After the removal of solvents under vacuum, the extract was suspended in EtOAc and washed with H_2O; then the EtOAc layer was concentrated and resuspended in MeOH and petroleum ether. The MeOH layer was concentrated to give the defatted extract (4.6 g).

Isolation of Aspertetranones A−D (1−4). The MeOH extract was fractionated by medium-pressure liquid chromatography over an RP-18 column (170 g) eluting with a MeOH−H_2O gradient (v/v, from 10% to 100% in 4 h, flow rate of 25 mL/min) to afford fractions F1−F6. The 30% MeOH fraction, F3, was sequentially subjected to Sephadex LH-20 (2.5 × 150 cm) eluting with MeOH to get two subfractions, the solid part (3A) and the supernatant part (3B). The supernatant part 3B was chromatographed using reversed-phase HPLC on an XDB C_{18} column (Agilent 1200, 5 μm, 250 × 10 mm) under isocratic conditions (45:55 MeOH−H_2O, UV detection at 254 nm, flow rate of 2 mL/min). Finally, aspertetranone C (**3**) (1 mg) was isolated as a pure compound at a retention time of 17.7 min. The solid part, 3A, was dissolved in acetone and subjected to Sephadex LH-20 chromatography (2.5 × 80 cm) eluting with acetone. Finally, aspertetranone D (**4**) (11.2 mg) was isolated as a pure compound (elution volume 42−54 mL). The 50% MeOH fraction, F4, was sequentially subjected to Sephadex LH-20 (2.5 × 150 cm) eluting with MeOH, Sephadex LH-20 (2.5 × 80 cm) eluting with acetone−MeOH (v/v, 4:1), and semipreparative HPLC eluting with 55% MeOH in 0.05% formic acid aqueous solution to give aspertetranone A (**1**) (20 mg, t_R 9.3 min) and aspertetranone B (**2**) (8 mg, t_R 13.3 min), respectively.

Aspertetranone A (1): colorless crystals (MeOH); mp 265.3 °C; $[\alpha]^{20}_D$ +97.6 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.95), 315 (3.79) nm; ECD (*c* 0.115 × 10^{-3} mol/L, MeOH) λ_{max} ($\Delta\varepsilon$) 218 (−1.46), 222 (+0.97), 248 (+0.38), 250 (−0.80), 260 (−0.27), 262 (+0.52) nm; IR (KBr) 1068, 1389, 1649, 1712, 2880, 2941, 2984, 3358 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 457.1453 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_9Na$ 457.1475).

Aspertetranone B (2): white, amorphous powder; $[\alpha]^{20}_D$ +67.4 (*c* 0.46, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.06), 290 (3.62) nm; IR (KBr) 1020, 1383, 1547, 1580, 1691, 3451 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 421.1862 $[M + H]^+$ (calcd for $C_{22}H_{29}O_8$, 421.1862).

Aspertetranone C (3): white, amorphous powder ; $[\alpha]^{20}_D$ +88.0 (*c* 0.1, MeOH); UV(MeOH) λ_{max} (log ε) 205 (4.02), 305 (3.56) nm; ECD (*c* 0.119 × 10^{-3} mol/L, MeOH) λ_{max} ($\Delta\varepsilon$) 207 (−2.60), 208 (+2.39), 238 (+0.22), 240 (−0.27), 257 (−0.27), 259 (+1.09) nm; IR (KBr) 1734, 1698, 1539, 1455, 1222 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 421.1504 $[M + H]^+$ (calcd for $C_{21}H_{25}O_9$, 421.1499).

Aspertetranone D (4): white, amorphous powder; $[\alpha]^{20}_D$ +65.6 (*c* 0.25, MeOH); UV(MeOH) λ_{max} (log ε) 205(4.13), 290 (3.91) nm; ECD (*c* 0.114 × 10^{-3} mol/L, MeOH) λ_{max} ($\Delta\varepsilon$) 211 (+3.86), 228 (+4.01), 255 (+0.69), 305 (+0.95); IR (KBr) 1684, 1569, 1428, 1257 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* $[M + Na]^+$ 459.1637 (calcd for $C_{22}H_{28}O_9Na$, 421.1631).

Single-Crystal X-ray Diffraction. Crystals of aspertetranone A (**1**) were obtained by dissolving the compounds in MeOH and allowing the solvent to evaporate at room temperature. Crystallographic diffraction data were collected with an Oxford Gemini S Ultra diffractometer using Cu radiation (λ = 1.541 84 Å). Data were reduced using the CrysalisPRO software. The temperature of the data collection was maintained at 100 K. The structure was solved by direct methods and difference Fourier synthesis, and it was refined on F^2 (SHELXL-97).[13] A thermal ellipsoid plot was generated using the program ORTEP-3[14] integrated within the WINGX program suite.[15] For fractional atomic coordinates and equivalent isotropic displacement parameters, see Table S2. $C_{22}H_{26}O_9$, *M* = 434.43, *T* = 100.0(2) K, λ = 1.54184 Å, monoclinic, space group $P2_1$, *a* = 6.58475(12) Å, *b* = 12.3394(2) Å, *c* = 13.1873(3) Å, *V* = 1050.67(3) $Å^3$, *Z* = 2, D_c = 1.373 g/cm^3 μ(Cu Kα) 0.901 mm^{-1}, *F*(000) = 460.0, crystal size 0.8 × 0.2 × 0.08 mm, 14 211 reflections collected, 3499 independent reflections (R_{int} = 0.0212), final *R* 0.0252 [$I > 2\sigma(I)$] and wR_2 0.0664 (all data), Flack parameter 0.02(11). Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre (CCDC 1404278), 12 Union Road, Cambridge, CB2 1EZ, UK (www.ccdc.cam.ac.uk/data_request/cif).

Computational Analysis. The ECD data were calculated using TDDFT(CAM-B3LYP) with the basis set 6-311+G(D,P) for all atoms. The ECD spectra were obtained by the following equation:

$$\Delta\varepsilon(E) = \frac{1}{2.297 \times 10^{-39}} \frac{1}{\sqrt{2\pi}\sigma} \sum_{A}^{i} \Delta E_i R_i e[-(E - \Delta E_i)^2/(2\sigma)^2]$$

where σ is the width of the band at 1/*e* height (fixed at 0.34 eV), and ΔE_i and R_i are the excitation energies and rotatory strengths for transition *i*, respectively.

The specific rotation was calculated using ωB97X-D in conjunction with the 6-311+G(D,P) basis set.[16] The calculated specific rotations ($|\alpha|_D$) of aspertetranone D with proposed configurations of (5a*S*, 6*R*, 6a*R*, 10a*R*, 11*R*, 11a*S*, 12*R*) and (5a*R*, 6*S*, 6a*S*, 10a*S*, 11*S*, 11a*R*, 12*S*) are +129.58 and −129.58, respectively. Combined with the experimental value of +65.6 and its relative configuration, the absolute configuration of C-12 in aspertetranone D is determined as *R*.

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Measurement of Nitric Oxide Content and Cell Viability. RAW264.7 cells were plated at a density of 2×10^6 cells per well in a 24-well plate. When 80% confluency was reached, the cells were pre-incubated in medium containing 20, 40, and 80 μM of test compounds for 2 h. The cells were then treated with 1 μg/mL of LPS for 22 h, and no production was measured as described ref 17. Cytotoxicity was determined using the MTS colorimetric assay, after 24 h incubation with the test compounds.

Measurement of TNF-α, IL-1β, and IL-6. These measurements were performed as described ref 17.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00487.

MS and NMR data of **1**−**4**; ECD spectra of **1**, **3**, and **4** (PDF)

X-ray data and CIF file for **1** (CIF)

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Notes

The authors declare no competing financial interest.

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