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Polyketide Derivatives, Guhypoxylonols A–D from a Mangrove Endophytic Fungus *Aspergillus* sp. GXNU-Y45 That Inhibit Nitric Oxide Production

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Abstract: Four undescribed compounds, guhypoxylonols A (**1**), B (**2**), C (**3**), and D (**4**), were isolated from the mangrove endophytic fungus *Aspergillus* sp. GXNU-Y45, together with seven previously reported metabolites. The structures of **1**–**4** were elucidated based on analysis of HRESIMS and NMR spectroscopic data. The absolute configurations of the stereogenic carbons in **1**–**3** were established through a combination of spectroscopic data and electronic circular dichroism (ECD). Compounds **1**–**11** were evaluated for their anti-inflammatory activity. Compounds **1**, **3**, **4**, and **6** showed an inhibitory activity against the production of nitric oxide (NO), with the IC_{50} values of 14.42 ± 0.11, 18.03 ± 0.14, 16.66 ± 0.21, and 21.05 ± 0.13 μM, respectively.

Keywords: *Aspergillus* sp.; mangrove endophytic fungus; guhypoxylonols A–D; anti-inflammatory

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1. Introduction

Marine-derived endophytic fungi have drawn considerable attention for drug discovery, and have been shown to produce various constituents, including sesquiterpenes, alkaloids, and polyketides [1]. Fungi are prolific producers of a variety of biologically active secondary metabolites, including anti-inflammatory, antibiotics, and cytotoxic compounds [1,2]. Lately, the investigation of the constituents of a fungus *Pleosporales* sp., isolated from diverse marine environments has led to the discovery of broad-spectrum cytotoxic secondary metabolites, such as dipleosporalones A and B [3]. In recent years, metabolites discovered from marine-derived fungi have been shown to display a broad range of promising biological activities [1–6]. Our group has reported a series of polyketides and structurally related polyketide derivatives from the culture of mangrove endophytic fungi [7–10].

As part of our ongoing project to discover anti-inflammatory polyketide derivatives from mangrove endophytic fungi, modifications of the composition of the culture medium were employed to reinvestigate the secondary metabolites of *Aspergillus* sp. GXNU-Y45, isolated from a fresh branch of the mangrove plant *Acanthus ilicifolius L*. Chemical investigation of its culture extracts resulted in the isolation of four undescribed polyketides, guhypoxylonols A (**1**), B (**2**), C (**3**), and D (**4**), together with seven previously reported metabolites (**5**–**11**) (Figure 1). Preliminarily screening of **1**–**11** in Supplementary Materials for their ability to prevent NO production of lipopolysaccharide (LPS)-stimulated RAW264.7 cells showed that **1**, **3**, **4**, and **6** have significant inhibitory potency. Herein

we report the details of isolation, structure elucidation, and anti-inflammatory activity evaluation of **1**, **3**, **4**, and **6**.

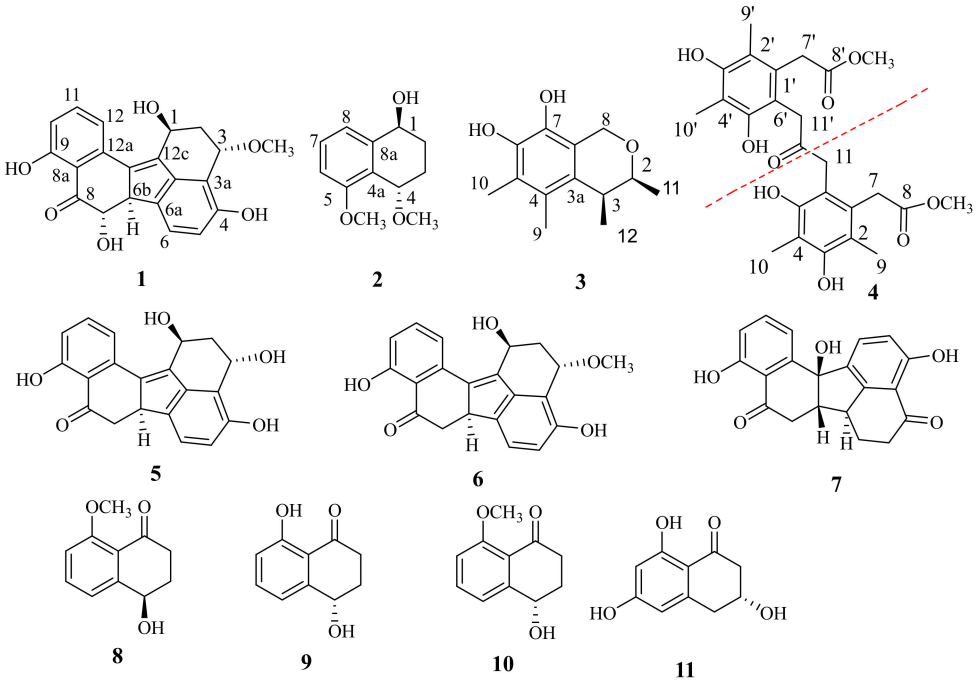

Figure 1. Structures of **1**–**11**.

2. Results and Discussion

2.1. Structure Elucidation of the Compounds

Compound (**1**) was obtained as a brown oil. The molecular formula $C_{21}H_{18}O_6$ was determined from the quasimolecular ion at *m*/*z* 389.1004 ([M + Na]$^+$, calcd for $C_{21}H_{18}O_6Na$, 389.1001) from a high resolution electrospray ionization mass spectrum (HRESIMS) and the ^{13}C NMR spectrum (Table 1). The ^{1}H NMR spectrum of **1** displayed two multiplets at δ_H 2.50 (1H, H-2α), and 1.68 (1H, H-2β), one multiplet at δ_H 5.22 (1H, H-1), one triplet at δ_H 4.74 (1H, H-3), two double doublets at δ_H 3.94 (1H, H-6b), and δ_H 3.78 (1H, H-7), five aromatic protons at δ_H6.71 (1H, H-5), 7.38 (1H, H-6), 6.84 (1H, H-10), 7.55 (1H, H-11), and 7.43 (1H, H-12), two phenolic hydroxyl protons at δ_H 9.54 (1H, H-4), and 12.32(1H, H-9). The ^{13}C NMR spectrum (Table 1) exhibited 21 carbon signals including one ketone carbonyl at δ_C 206.5, one methoxyl at δ_C 55.9, one sp^3 methylene at δ_C 39.7, four oxygenated methine sp^3 at δ_C76.4, 70.4, 62.5, and 56.1, five protonated sp^2 carbons at δ_C 136.1, 125.5, 121.5, 115.6, and 112.9, and eight non-protonated sp^2 carbons at δ_C161.4, 154.4, 134.4, 117.8, 114.0, 138.2, 134.2, 140.0, and 144.9. Analysis of the 2D-NMR spectra (Figure 2) revealed that the structure of **1** resembled that of the previously reported **6** [11] except for the chemical shift value of C-7 which appeared at δ_C 76.4 CH, indicating that C-7 is oxygen-bearing.

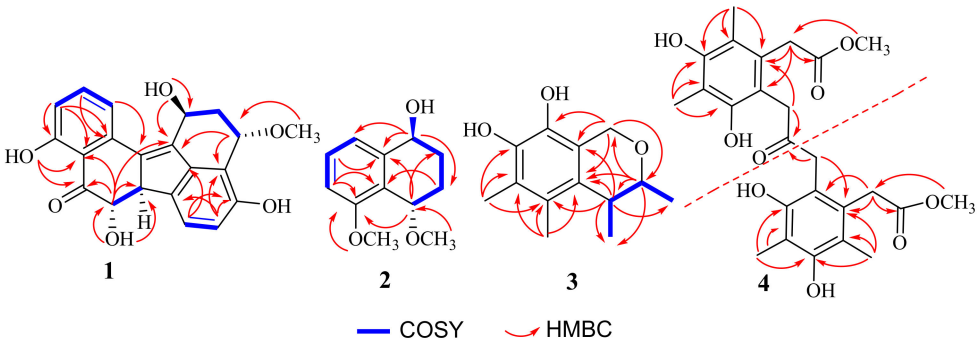

Figure 2. Key COSY of **1-3** and HMBC correlations of **1**–**4**.

Table 1. ^{1}H and ^{13}C NMR (DMSO-d_6, 600 and 150 MHz) and COSY and HMBC assignment of **1**.

Position	δ_C, Type	δ_H, (Mult., *J* in Hz)	COSY	HMBC
1	62.5, CH	5.22, m	H-2	
2α	39.7, CH_2	2.50, m	H-1, 3	
2β		1.68, m		
3	70.4, CH	4.74, t (3.0)	H-2	C-3a, 12c
4	154.4, C			
5	112.9, CH	6.71, d (8.0)	H-6	C-3a, 4, 6a
6	125.5, CH	7.38, d (8.0)	H-5	C-4, 12d
6a	134.4, C			
6b	56.1, CH	3.94, dd (12.4, 3.1)	H-7	
7	76.4, CH	3.78, dd (12.3, 5.6)	H-6b	C-6b, 8, 8a, 12c
8	206.5, C			
8a	114.0, C			
9	161.4, C			
10	115.6, CH	6.84, d (8.2)	H-11	C-8a, 9, 12a
11	136.1, CH	7.55, d (8.0)	H-10, 12	C-12a
12	121.5, CH	7.43, d (7.7)	H-11	C-12b
12a	138.2, C			
12b	134.2, C			
12c	140.0, C			
12d	144.9, C			
1-OH		5.06, d (7.8)		C-1, 12c
4-OH		9.54, s		
7-OH		6.17, d (5.9)		C-6b, 7
9-OH		12.32, s		C-8, 8a
3-OCH_3	55.9, CH_3	3.29, s		C-3

The relative configuration of **1** was determined by the NOESY spectrum (Figure 3) analysis. The NOESY correlations between H-1 (δ_H 5.22) and OCH_3-3 (δ_H 3.29), OCH_3-3 and H-6b (δ_H 3.94), and H-6b and OH-7 (δ_H 6.17) determined the relative configuration of **1** as 1*S**3*S**6b*R**7*S**. The experimental ECD spectrum of **1** was recorded (Figure 4) and the calculated ECD spectrum of 1*S*3*S*6b*R*7*S*-**1** fits well with the experimental ECD spectrum of **1**, as shown in Figure 4. Since **1** has not been previously reported, it was named guhypoxylonol A.

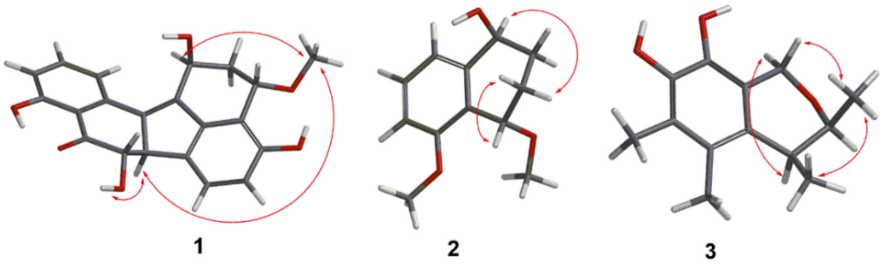

Figure 3. Key NOESY correlations in **1**–**3**.

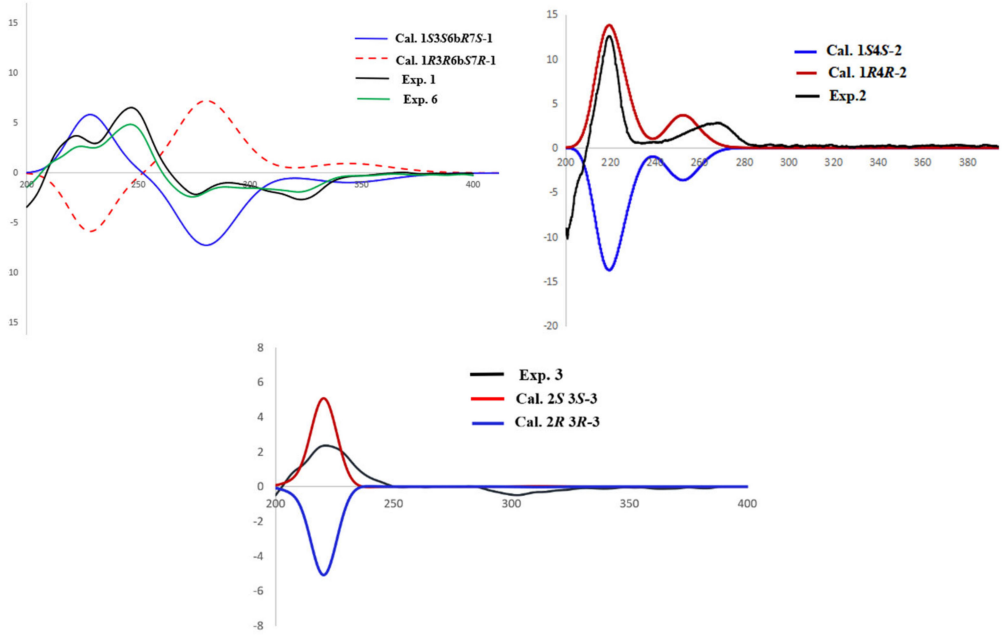

Figure 4. Experimental ECD and calculated ECD spectra of **1**–**3**.

Compound (**2**) was obtained as a colorless powder with a molecular formula of $C_{12}H_{16}O_3$ as deduced from the HRESIMS *m*/*z* 231.0998 $[M + Na]^+$ (cald 231.0997 for $C_{12}H_{16}O_3Na$), indicating six degrees of unsaturation. The ^{1}H-NMR (Table 2) showed two methoxyl singlets at δ_H 3.31 (3H, s, OCH_3-4), and 3.75 (3H, s, OCH_3-5), three aromatic protons at δ_H 7.24 (1H, d, J = 7.9 Hz, H-6), 7.14 (1H, d, J = 7.7 Hz, H-8), and 6.83 (1H, d, J = 8.1 Hz, H-7), two multiplets at δ_H 1.80 (2H, m, CH_2-2), and 1.51, 2.09 (2H, m, CH_2-3), and two multiplets at δ_H 4.41 (1H, m, H-1), and 4.35 (1H, m, H-4). The ^{13}C NMR spectrum (Table 2) showed 12 carbon signals comprising six aromatic carbons of a benzene ring (δ_C 157.5 C, 143.1 C, 128.5 CH, 124.8 C, 118.7 CH and 108.8 CH), two methoxyls (δ_C 55.7 and 56.7), two methylene sp^3 (δ_C 27.2 and 24.7), and two oxygenated methine sp^3 (δ_C 69.8 and 67.8). The COSY spectrum (Table 2) of **2** displayed two isolated proton spin systems (H-1/H_2-2/H_2-3/H-4, and H-6/H-7/H-8). The HMBC spectrum showed correlations from the proton sinal at δ_H 4.41 (1H, m, H-1) to δ_C 24.7 (C-3), 118.7 (C-8), and 143.1 (C-8a), from δ_H 4.35 (1H, t, J = 2.8 Hz, H-4) to δ_C 157.5 (C-5), 27.2 (C-2), and 143.1 (C-8a). The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of nodulisporol [12]. The main difference between **2** and nodulisporol was the replacement of a hydroxyl group with a methoxy group at C-4.

Table 2. ^{1}H and ^{13}C NMR (DMSO-d_6, 600 and 150 MHz) and COSY and HMBC assignment of **2**.

Position	δ_C, Type	δ_H (Mult., J in Hz)	COSY	HMBC
1	67.8, CH	4.41, m	H-2	C-3, 8, 8a
2	27.2, CH_2	1.80, m	H-1, 3	
3α	24.7, CH_2	2.09, m	H-2, 4	C-4a
3β		1.51, m		
4	69.8, CH	4.35, t (2.8)	H-3	C-2, 5, 8a
4a	124.8, C			
5	157.5, C			
6	128.5, CH	7.24, d (7.9)	H-7	C-4a, 5
7	108.8, CH	6.83, d (8.1)	H-6, 8	C-8a
8	118.7, CH	7.14, d (7.7)	H-7	
8a	143.1, C			
1-OH		5.28, s		
4-OCH_3	56.7, CH_3	3.31, s		C-4
5-OCH_3	55.7, CH_3	3.75, s		C-5

The relative configuration of **2** was determined from its NOESY spectrum, which showed correlations from H-1/H-3α (δ_H 2.09), and H-4/H-3β (δ_H 1.51) suggesting that H-1 and H-4 were on the opposite face. To establish the absolute configuration of C-1 and C-4, the ECD spectra of two simplified isomers (1*S*4*S*, and 1*R*4*R*) of **2** were calculated at the Cam-B3LYP/6-31+G(d,p) level of theory in methanol, and these calculated spectra were compared with the experimental spectrum of **2**. The experimental ECD spectrum of **2** showed an excellent fit with the calculated ECD spectrum of 1*S*4*S*-**2** (Figure 4), establishing the absolute configurations of C-1 and C-4 as 1*S*4*S*. Since **2** has never been reported, it was named guhypoxylonol B.

Compound (**3**) was obtained as a colorless powder with a molecular formula of $C_{13}H_{18}O_3$ as deduced from the HRESIMS *m*/*z* 223.1332 $[M + H]^+$ (cald 223.1334 for $C_{13}H_{19}O_3$), indicating five degrees of unsaturation. The ^{1}H NMR (Table 3), in combination with DEPT and HSQC spectra, displayed two doublets of methylene group at δ_H 4.65 (*J* = 15.8 Hz, H-8) and 4.58 (*J* = 15.8 Hz, H-8), two multiplets of methine groups at δ_H 3.86 (*J* = 6.6, 2.6 Hz, H-2) and 2.63 (*J* = 6.8, 2.6 Hz, H-3), two methyl doublets at δ_H 1.18 (*J* = 6.8 Hz, H-11) and δ_H 1.19 (*J* = 6.6 Hz, H-12), and two methyl singlets at δ_H 2.10 (H-9, H-10). The ^{13}C NMR (Table 3) spectrum, in combination with HMQC spectrum, of **3** revealed the presence of four methyl carbons at δ_C 21.0, 18.2, 9.1, and 11.1, one sp^3 methylene carbon at δ_C 60.8, two sp^3 methine carbons at δ_C 76.0 and 36.4, together with six non-protonated sp^2 carbons at δ_C 153.3, 149.6, 134.8, 115.9, 114.4, and 111.3. The COSY (Figure 2) correlations from H-2 to H-3 and H_3-11, and H-3 to H_3-12 suggest the existence of -CH(CH_3)CH(CH_3)O-. The HMBC (Figure 2) correlations from H-2 to δ_C 21.0 (C-11), 134.8 (C-3a), 36.4 (C-3), and 60.8 (C-8), from H-3 to δ_C 134.8 (C-3a), 115.9 (C-4), 114.4 (C-7a), 18.2 (C-12), and 21.0 (C-11), suggests that C-3 is connected to C-3a. The HMBC correlations from H-9 (δ_H 2.10) to C-4, C-5 (δ_C 111.3), and C-3a, from H-10 (δ_H 2.10) to C-4, C-5, C-6 (δ_C 153.3), indicate that the two methyl groups were on C-4 and C-5, respectively. Finally, the HMBC correlations from H-8 to C-3a, C-7a, C-2 (δ_C 76.0), and C-7 (δ_C 149.6), indicated that the remaining substructure of **3** was established as shown in Figure 1.

A NOSEY correlation observed between H-2 and H-3, suggests that the relative configuration of **3** is either 2*R**3*R** or 2*S**3*S** (Figure 3). The absolute configurations of C-2 and C-3 were established by comparing the experimental and calculated ECD spectra of 2*R*3*R*, and 2*S*3*S*. The experimental ECD spectrum of **3** matched very well with the calculated 2*S*3*S*-**3** ECD spectrum (Figure 4), calculated at the Cam-B3LYP/6-311+G (2d, p) level of theory in methanol. Therefore, the absolute configurations of C-2 and C-3 were determined to be 2*S*3*S*. Since **3** has never been reported, it was named guhypoxylonol C.

Table 3. ^{1}H and ^{13}C NMR (CD_3OD, 400 and 100 MHz) and COSY and HMBC assignment of **3**.

Position	δ_C, Type	δ_H (Mult., *J* in Hz)	COSY	HMBC
2	76.0, CH	3.86, qd (6.6, 2.6)	H-3, 11	C-3, 3a, 8, 12
3	36.4, CH	2.63, qd (6.8, 2.6)	H-2, 12	C-3a, 4, 7a, 11, 12
3a	134,8, C			
4	115.9, C			
5	111.3, C			
6	153.3, C			
7	149.6, C			
7a	114.4, C			
8	60.8, CH_2	4.65, d (15.2) 4.58, d (15.2)		C-2, 3a, 7, 7a
9	11.1, CH_3	2.10, s		C-3a, 4, 5
10	9.1, CH_3	2.10, s		C-4, 5, 6
11	21.0, CH_3	1.18, d (6.8)		
12	18.2, CH_3	1.19, d (6.6)		

Compound (**4**) was obtained as a white powder and the molecular formula $C_{25}H_{30}O_9$ was deduced from the HRESIMS m/z 473.1816 $[M - H]^-$ (cald 473.1812 for $C_{25}H_{29}O_9$), indicating 11 degrees of unsaturation. The ^{1}H NMR (Table 4) spectrum of **4** displayed two methyl singlets at δ_H 2.10 (H-9) and 2.07 (H-10), one methoxyl singlet at δ_H 3.67 (-OCH_3-8), and two singlets at δ_H 3.73 (H_2-7) and 2.50 (H_2-11). The ^{13}C NMR spectrum (Table 4), in combination with the HSQC spectrum of **4**, displayed one ketone carbonyl at δ_C 207.9 (C-12), one ester carbonyl at δ_C 173.8 (C-8), one methoxy at δ_C 52.5 (OCH_3), two methyls at δ_C12.1, and 9.0, and the two sp^3 methylene carbons at δ_C 36.5 (C-7) and 32.5 (C-11). The presence of six non-protonated sp^2 at δ_C123.1, 118.7, 155.3, 112.5, 157.5, and 130.6 is an indicative of the presence of a benzene ring. The HMBC correlations (Figure 2) from δ_H 3.73 (H-7) to C-8, 123.1 (C-6), 118.7 (C-1), and from δ_H 3.67 to C-8, confirm that a methyl acetate is connected to C-1. HMBC correlations from δ_H 2.07 (H-9) to C-1, 130.6 (C-2), and 157.5 (C-3), from δ_H 2.07 (H-10) to δ_C112.5 (C-4), 155.3 (C-5), and C-3, and from H-11 to C-1 and C-12, suggested that **4** contains methyl (3,5-dihydroxy-2,4-dimethyl pheny) acetate moiety, with -CH_2-C=O connected to C-6. Since the molecular formula of $C_{25}H_{30}O_9$, only a ketone carbonyl (δ_C207.9) is present in **4**. Therefore, the structure of **4** is a disubstituted acetone whose substituents are methyl (3,5-digydroxy-2,4-dimethylphenyl)acetate. Since **4** has never been reported, it was named guhypoxylonol D.

Table 4. ^{1}H and ^{13}C NMR (CD_3OD, 400 and 100 MHz) and HMBC assignment of **4**.

Position	δ_C, Type	δ_H (Mult., *J* in Hz)	HMBC
1 (1′)	118.7, C		
2 (2′)	130.6, C		
3 (3′)	157.5, C		
4 (4′)	112.5, C		
5 (5′)	155.3, C		
6 (6′)	123.6, C		
7 (7′)	36.5, CH_2	3.73, s	C-1 (1′), 6 (6′), 8 (8′)
8 (8′)	173.8, C		
9 (9′)	9.0, CH_3	2.10, s	C-1 (1′), 2 (2′), 3 (3′)
10 (10′)	12.1, CH_3	2.07, s	C-3 (3′), 4 (4′), 5 (5′)
11 (11′)	32.5, CH_2	2.50, s	C-1 (1′), 12
12	207.9, C		
8-OCH_3	52.5, CH_3	3.67, s	C-8 (8′)

The previously described **5**–**11** were identified based on the analysis of their NMR data, and compared with those reported in the literature and identified as hypoxylonol C (**5**) [11], hypoxylonol B (**6**) [11], daldinone C (**7**) [13], nodulisporol (**8**) [12], isosclerone (**9**) [14], xylarenone (**10**) [14], scytalone (**11**) [15], respectively.

2.2. Anti-Inflammatory Activity

Compounds **1**–**11** were evaluated for their anti-inflammatory effects on the production of the NO in the RAW 264.7 macrophage cell line exposed to the inflammatory stimulus by lipopolysaccharide (LPS) (Table 5). Compounds **1**, **3**, **4**, and **6** showed inhibitory activity against the production of NO, with the IC_{50} values 14.42 ± 0.11, 18.03 ± 0.14, 16.66 ± 0.21, and 21.05 ± 0.13 μM, respectively. Dexamethasone was used as a positive control with IC_{50} value of 16.12 ± 1.41 μM, while **2**, **5**, and **7**–**11** did not show any inhibitory activity under their safe concentrations.

Table 5. Inhibitory activities of **1**–**11** on NO production in LPS-induced RAW 264.7 cells [a].

Compounds	IC_{50} (μM)
1	14.42 ± 0.11
2	32.48 ± 0.19
3	18.03 ± 0.14
4	16.66 ± 0.21
5	>80
6	21.05 ± 0.13
7	>80
8	>80
9	>80
10	>80
11	>80
Dexamethasone [b]	16.12 ± 1.41 μM

[a] Values present mean ± SD of triplicate experiments. [b] Dexamethasone was used as a positive control.

3. Materials and Methods

3.1. General Experimental Procedures

NMR spectra were recorded on a AVANCE-400 spectrometer (Bruker, Bremen, Germany). The chemical shifts of ^{1}H and ^{13}C NMR spectra are given in δ (ppm) and referenced to the solvent signal (DMSO-d_6, δ_H 2.50 and δ_C 39.52, CD_3OD-d_4, δ_H 3.34 and δ_C 49.00). Coupling constants (*J*) are reported in Hz. The mass spectrometric (HRESIMS) data were acquired using a Micro Mass Q-TOF spectrometer (Waters Corporation, Milford, MA, USA). ECD data was recorded using a JASCO J-715 spectropolarimeter (Jasco, Tokyo, Japan). Semipreparative HPLC was performed on an ODS column (10 × 250 mm, 5 μm, 3 mL/min, YMC, Kyoto, Japan).

3.2. Fungal Material

The strain GXNU-Y45 was isolated from a leaf of a mangrove tree *Acanthus ilicifolius*, October 2019, in Beihai City, China. The fungal strain GXNU-Y45 was identified as *Aspergillus* sp. based on the sequence of its internal transcribed spacer region (ITS) and morphology. ITS-rDNA of GXNU-Y45 was submitted to GenBank and the accession number is MT626059.

3.3. Fermentation, Extraction, and Isolation

The fungus was cultured in 60 × 1000 mL Erlenmeyer flasks each containing 50 g cooked rice and 60 mL of water (30 g sea salt, per liter pure water) or 300 mL medium (liquid media, 20.0 g dextrose, 20.0 g potatoes, 30 g sea salt, per liter pure water). The fungus was cultured in the medium and incubated at room temperature for 35 days.

3.4. Extraction and Isolation

The fermented material was extracted three times with EtOAc to obtain 16.8 g crude extract (liquid medium) and 20.2 g (solid medium). The crude extract was subjected to a silica gel VLC column, eluting with a stepwise gradient of petroleum ether-EtOAc (10:1, 8:1, 6:1, 4:1, 2:1, 1:1, *v*/*v*) to yield six subfractions (Fr. 1–Fr. 6). Fr. 3 (3 g) was applied to ODS silica gel with gradient elution of MeOH-H_2O (3:7, 4:6, 5:5, 6:4, 7:3, 9:1, 0:1, *v*/*v*) to afford four subfractions (Fr. 3-1–Fr. 3-4). Fr. 3-2 (650 mg) was subjected to semipreparative HPLC (70% MeOH/H_2O; 3 mL/min) to obtain **1** (15.6 mg), **2** (7.5 mg), and **3** (4.4 mg). Fr. 3-3 (345 mg) was repurified by RP-18 CC (eluted with MeOH/H_2O from 3:7 to 10:0, *v*/*v*) and Sephadex LH-20 (eluted with CH_2Cl_2/MeOH, 5:5, *v*/*v*) to afford **5** (10.6 mg), **9** (3.3 mg), **10** (5.2 mg), and **11** (6.7 mg). Fr. 4 (1.1 g) was separated by ODS silica gel with gradient elution of MeOH-H_2O (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 9:1, 0:1, *v*/*v*) to yield four subfractions (Fr. 4-1–Fr. 4-4). Fr.4-3 (73 mg) was purified by Sephadex LH-20 eluted with CH_2Cl_2/MeOH (50:50) to give **4** (6.3 mg). Fr.4-4 (84 mg) was separated by semipreparative HPLC (80% MeCN/H_2O; 3 mL/min) to give **6** (5.6 mg), **7** (8.1 mg), and **8** (5.2 mg).

Guhypoxylonol A (**1**): was obtained as a brown oil; $[\alpha]_D^{20}$ + 63.2 (c0.6, MeOH); ^{1}H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m*/*z* 389.1004 ([M + Na]$^+$ (cald $C_{21}H_{18}O_6Na$, 389.1001).

Guhypoxylonol B (**2**): was obtained as a colorless powder; $[\alpha]_D^{20}$ + 8.5 (c0.6, MeOH); ^{1}H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m*/*z* 231.0998 [M + Na]$^+$ (cald 231.0997 for $C_{12}H_{16}O_3Na$).

Guhypoxylonol C (**3**): white powder; $[\alpha]_D^{20}$ + 80 (c0.6, MeOH); ^{1}H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m*/*z* 223.1332 [M + H]$^+$ (cald 223.1334 for $C_{13}H_{19}O_3$).

Guhypoxylonol D (**4**): white powder; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m*/*z* 473.1816 [M − H]$^-$ (cald 473.1812 for $C_{25}H_{30}O_9$).

3.5. Anti-Inflammatory Assay

The anti-inflammatory effects of compounds **1–11** were examined on the production of the NO in LPS-stimulated cells using a method described in the literature [16].

4. Conclusions

The chemical investigation of a marine-derived fungus *Aspergillus* sp. GXNU-Y45 resulted in the isolation of four undescribed compounds (**1–4**), and seven previously reported metabolites (**5–11**). Based on modifications of the culture medium strategy, the fungus *Aspergillus* sp. GXNU-Y45 was cultured in different media to stimulate a production of its metabolites. It was found that the fungus *Aspergillus* sp. GXNU-Y45 produced different metabolites in two culture media. The liquid medium can stimulate the fungus to produce a series of metabolites, **1**, **5**, **6**, **7**, **8**, **9**, **10**, and **2** (a new precursor of **1**). On the contrary the solid medium yeiled **3** and **4**. Different compositions of the culture media represented a powerful tool to induce new metabolites from microorganisms. Preliminarily screening of **1–11** for their ability to prevent NO production of LPS-induced RAW264.7 cells showed that **1**, **3**, **4**, and **6** exhibited significant inhibitory effects against NO release with IC_{50} values of 14.42 ± 0.11, 18.03 ± 0.14, 16.66 ± 0.21, and 21.05 ± 0.13 μM, respectively. The inhibition of NO production by **1** and **6** was stronger than **5** and **7**, which showed the same skeleton but differ only the presence of -OCH_3 at C-3. Compounds **2** and **8–11**, which are precursors of **1**, **5**, **6**, and **7**, did not exhibit inhibitory effects against NO release. Compounds **3** and **4** exhibited remarkable inhibitory effects against NO release suggesting that the fully substituted benzene ring was essential for inhibition of the production of NO release. In summary, this study revealed that **1**, **3**, **4**, and **6** could be considered as potential metabolites for further anti-inflammatory studies.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/md20010005/s1, NMR and HRESIMS spectra of **1–11**.

Author Contributions: R.Y. and X.H. conceived and designed the experiments. X.Q. performed the experiments. J.H., W.Z., D.Z., Y.Z., J.L. and X.H. analyzed the data. X.Q. and X.H. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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