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

Xiaoya Qin 1, Jiguo Huang 2, Dexiong Zhou 1, Wenxiu Zhang 1, Yanjun Zhang 3, Jun Li 1, Ruiyun Yang 1,* and Xishan Huang 1,*

1 State Key Laboratory for Chemistry and Molecular Engineering of Medicinal Resources, Collaborative Innovation Center for Guangxi Ethnic Medicine, College of Chemistry and Pharmaceutical Sciences, Guangxi Normal University, Guilin 541005, China; qinxiaoya6536@163.com (X.Q.); zhoudexiong3@163.com (D.Z.); wenxiuz912@163.com (W.Z.); lijun0993@gxnu.edu.cn (J.L.)
2 School of Chemical Engineering and Technology, Guangdong Industry Polytechnic, Guangzhou 510300, China; huangjiguo@126.com
3 Guangxi Key Laboratory of Green Chemical Materials and Safety Technology, Beibu Gulf University, Qinzhou 535011, China; Zhangyj201608@163.com
* Correspondence: yang_rui_yun@163.com (R.Y.); huangxishan13@foxmail.com (X.H.);
Tel.: +86-77-3212-0958 (X.H.)

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

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). Preliminary 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]^+), calcld for C_{21}H_{18}O_{6}Na, 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 \(\delta_H\) 2.50 (1H, H-2\(\alpha\)), and 1.68 (1H, H-2\(\beta\)), one multiplet at \(\delta_H\) 5.22 (1H, H-1), one triplet at \(\delta_H\) 4.74 (1H, H-3), two double doublets at \(\delta_H\) 3.94 (1H, H-6\(\beta\)), and \(\delta_H\) 3.78 (1H, H-7), five aromatic protons at \(\delta_H\) 6.71 (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 \(\delta_H\) 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 \(\delta_C\) 206.5, one methoxyl at \(\delta_C\) 55.9, one sp\(^3\) methylene at \(\delta_C\) 39.7, four oxygenated methine sp\(^3\) at \(\delta_C\) 76.4, 70.4, 62.5, and 56.1, five protonated sp\(^2\) carbons at \(\delta_C\) 136.1, 125.5, 121.5, 115.6, and 112.9, and eight non-protonated sp\(^2\) carbons at \(\delta_C\) 161.4, 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 \(\delta_C\) 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 | $\delta_C$, Type | $\delta_H$, (Mult., $J$ in Hz) | COSY | HMBC |
|----------|------------------|-------------------------------|-------|-------|
| 1        | 62.5, CH         | 5.22, m                       |       |       |
| 2a       | 39.7, CH$_2$     | 2.50, m                       | H-2   |       |
| 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 ($\delta_H$ 5.22) and OCH$_3$-3 ($\delta_H$ 3.29), OCH$_3$-3 and H-6b ($\delta_H$ 3.94), and H-6b and OH-7 ($\delta_H$ 6.17) determined the relative configuration of 1 as 1S*3S*6bR*7S*. The experimental ECD spectrum of 1 was recorded (Figure 4) and the calculated ECD spectrum of 1S3S6bR7S-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 guhypoxyylonol A.

Figure 3. Key NOESY correlations in 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_{3}Na), indicating six degrees of unsaturation. The \(^1\)H-NMR (Table 2) showed two methoxyl singlets at \(\delta_H 3.31\) (3H, s, OCH\(_3\)-4), and 3.75 (3H, s, OCH\(_3\)-5), three aromatic protons at \(\delta_H 7.24\) (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 \(\delta_H 1.80\) (2H, m, CH\(_2\)-2), and 1.51, 2.09 (2H, m, CH\(_2\)-3), and two multiplets at \(\delta_H 4.41\) (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 (\(\delta_C\) 157.5 C, 143.1 C, 128.5 CH, 124.8 C, 118.7 CH and 108.8 CH), two methoxyls (\(\delta_C\) 55.7 and 56.7), two methylene sp\(^3\) (\(\delta_C\) 27.2 and 24.7), and two oxygenated methine sp\(^3\) (\(\delta_C\) 69.8 and 67.8). The COSY spectrum (Table 2) of 2 displayed two isolated proton spin systems (H-1/H-2/H-3/H-4, and H-6/H-7/H-8). The HMBC spectrum showed correlations from the proton signal at \(\delta_H 4.41\) (1H, m, H-1) to \(\delta_C\) 24.7 (C-3), 118.7 (C-8), and 143.1 (C-8a), from \(\delta_H 4.35\) (1H, t, \(J = 2.8\) Hz, H-4) to \(\delta_C\) 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 | \(\delta_C\), Type | \(\delta_H\) (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 |      |
| 3\(\alpha\) | 24.7, CH\(_2\)  | 2.09, m              | H-2, 4 | C-4a |
| 3\(\beta\) | 24.7, CH\(_2\)  | 1.51, m              | H-3  | C-2, 5, 8a |
| 4        | 69.8, CH          | 4.35, t (2.8)        | H-3  | C-2, 5, 8a |
| 4\(a\)  | 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  |      |
| 8\(a\)  | 143.1, C          |                      |      |      |
| 1-OH    |                   | 5.28, s              |      | C-4  |
| 4-OCH\(_3\) | 56.7, CH\(_3\) | 3.31, s              |      |      |
| 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 (δH 2.09), and H-4/H-3β (δH 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 (1S4S, and 1R4R) 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 1S4S-2 (Figure 4), establishing the absolute configurations of C-1 and C-4 as 1S4S. Since 2 has never been reported, it was named guhypoxylonol B.

Compound (3) was obtained as a colorless powder with a molecular formula of C13H18O3 as deduced from the HRESIMS m/z 223.1332 [M + H]+ (cald 223.1334 for C13H19O3), indicating five degrees of unsaturation. The 1H NMR (Table 3), in combination with DEPT and HSQC spectra, displayed two doublets of methylene group at δH 4.65 (J = 15.8 Hz, H-8) and 4.58 (J = 15.8 Hz, H-8), two multiplets of methine groups at δH 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 δH 1.18 (J = 6.8 Hz, H-11) and 1.19 (J = 6.6 Hz, H-12), and two methyl singlets at δH 2.10 (H-9, H-10). The 13C NMR (Table 3) spectrum, in combination with HMQC spectrum, of 3 revealed the presence of four methyl carbons at δC 21.0, 18.2, 9.1, and 11.1, one sp3 methylene carbon at δC 60.8, two sp3 methine carbons at δC 76.0 and 36.4, together with six non-protonated sp2 carbons at δC 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 to H-11 suggest the existence of -CH(CH3)CH(CH3)O-. The HMBC (Figure 2) correlations from H-2 to δC 21.0 (C-11), 134.8 (C-3a), 36.4 (C-3), and 60.8 (C-8), from H-3 to δC 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 (δH 2.10) to C-4, C-5 (δC 111.3), and C-3a, from H-10 (δH 2.10) to C-4, C-5, C-6 (δC 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 (δC 76.0), and C-7 (δC 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 2R*3R* or 2S*3S* (Figure 3). The absolute configurations of C-2 and C-3 were established by comparing the experimental and calculated ECD spectra of 2R3R, and 2S3S. The experimental ECD spectrum of 3 matched very well with the calculated 2S3S-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 2S3S. Since 3 has never been reported, it was named guhypoxylonol C.

### Table 3. 1H and 13C NMR (CD3OD, 400 and 100 MHz) and COSY and HMBC assignment of 3.

| Position | δC, Type | δH (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, CH2 | 4.65, d (15.2)      | C-2, 3a, 7, 7a |
| 9        | 11.1, CH3 | 2.10, s             | C-3a, 4, 5  |
| 10       | 9.1, CH3  | 2.10, s             | C-4, 5, 6  |
| 11       | 21.0, CH3 | 1.18, d (6.8)       |      |      |
| 12       | 18.2, CH3 | 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 $\delta$H 2.10 (H-9) and 2.07 (H-10), one methoxyl singlet at $\delta$H 3.67 (-OCH$_3$-8), and two singlets at $\delta$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 $\delta$C 207.9 (C-12), one ester carbonyl at $\delta$C 173.8 (C-8), one methoxyl at $\delta$C 52.5 (OCH$_3$), two methyls at $\delta$C 12.1, and 9.0, and the two sp$^3$ methylene carbons at $\delta$C 36.5 (C-7) and 32.5 (C-11). The presence of six non-protonated sp$^2$ at $\delta$C 123.1, 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 $\delta$H 3.73 (H-7) to C-8, 123.1 (C-6), 118.7 (C-1), and from $\delta$H 3.67 to C-8, confirm that a methyl acetate is connected to C-1. HMBC correlations from $\delta$H 2.07 (H-9) to C-1, 130.6 (C-2), and 157.5 (C-3), from $\delta$H 2.07 (H-10) to $\delta$C 112.5 (C-4), 155.3 (C-5), and C-3, and from H-11 to C-11 and C-12, suggested that 4 contains methyl (3,5-dihydroxy-2,4-dimethyl phenyl) 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 ($\delta$C 207.9) 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$_3$OD, 400 and 100 MHz) and HMBC assignment of 4.

| Position | $\delta$C, Type | $\delta$H (Mult., J in Hz) | HMBC |
|----------|-----------------|---------------------------|------|
| 1 (1’)   | 118.7, C        |                           |      |
| 2 (2’)   | 150.6, 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$_3$OD-$d_4$, 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$_2$O (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$_2$O; 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$_2$O from 3:7 to 10:0, v/v) and Sephadex LH-20 (eluted with CH$_2$Cl$_2$/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$_2$O (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$_2$Cl$_2$/MeOH (50:50) to give 4 (6.3 mg). Fr.4-4 (84 mg) was separated by semipreparative HPLC (80% MeCN/H$_2$O; 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; [α]_{D}^{20} + 63.2 (c0.6, MeOH); ¹H and ¹³C NMR data (see Tables 1 and 2); HRESIMS m/z 389.1004 ([M + Na]⁺ (cald C_{21}H_{18}O_{6}Na, 389.1001).

Guhypoxylonol B (2): was obtained as a colorless powder; [α]_{D}^{20} + 8.5 (c0.6, MeOH); ¹H and ¹³C NMR data (see Tables 1 and 2); HRESIMS m/z 231.0998 [M + Na]⁺ (cald 231.0997 for C_{12}H_{16}O_{3}Na).

Guhypoxylonol C (3): white powder; [α]_{D}^{20} + 80 (c0.6, MeOH); ¹H and ¹³C 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; ¹H and ¹³C 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. Prelimarily 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](https://www.mdpi.com/article/10.3390/md20010005/s1), NMR and HRESIMS spectra of 1–11.

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**Conflicts of Interest:** The authors declare no conflict of interest.
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