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Restricticin B from the Marine Fungus *Penicillium janthinellum* and Its Inhibitory Activity on the NO Production in BV-2 Microglia Cells

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Abstract: A new compound containing a triene, a tetrahydropyran ring and glycine ester functionalities, restricticin B (**1**), together with four known compounds (**2**-**5**) were obtained from the EtOAc extract of the marine fungus *Penicillium janthinellum*. The planar structure of **1** was determined by detailed analyses of MS, 1D and 2D NMR data. The relative and absolute configurations of **1** were established via analyses of NOESY spectroscopy data, comparison of optical rotation values with those of restricticin derivatives reported and electronic circular dichroism (ECD). All the compounds were screened for their anti-neuroinflammatory effects in lipopolysaccharide (LPS)-induced BV-2 microglia cells. Restricticin B (**1**) and N-acetyl restricticin (**2**) exhibited anti-neuroinflammatory effects by suppressing the production of pro-inflammatory mediators in activated microglial cells.

Keywords: restricticin; marine fungus; *Penicillium janthinellum*; BV-2 microglia cells; anti-inflammatory; rotamer

1. Introduction

The novel class of potent antifungal agents, restricticin and restricticinol, were first isolated from the fermentation broth of *Penicillium restrictum* [1, 2]. These compounds contain a tetrahydropyran ring and triene side chains in common and restricticin possesses a glycine unit linked to the tetrahydropyran ring [3]. The initial biological activity study of restrciticin derivatives displayed that only restricticin exhibited potent antifungal activity against both yeast and filamentous fungi [1, 4]. The very closely related compounds with a shorter polyene chain, lanomycin and lanomycinol, were isolated from *Pycnidiophora dispersa* in 1992 [5, 6]. Lanomycin was active against some *Candida* species and dermatophytes and not active against Gram-positive, Gram-negative bacteria and *Aspergillus fumigatus* [7]. Resrcticin and lanomycin were first reported as natural antifungal agents to inhibit cytochrome P_{450} lanosterol demethylase [6, 8]. Interestingly, restricticinol and lanomycinol

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did not show such antifungal activity [8]. Because of considerable interest for biological activity, several methods for total synthesis of the antifungal agents have been described [8-10].

Microglia, macrophages and representative of the innate immune system in the brain, have been implicated as active contributors to neuron damage and readily become activated in response to infection or injury [11, 12]. Activated microglia up-regulate pro-inflammatory and neurotoxic mediators including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and free radicals such as nitric oxide (NO) and superoxide [13]. Moreover, large numbers of activated microglia are present in CNS tissue of patients with chronic neurodegenerative diseases including Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis and prion disease [14, 15]. Therefore, the suppression of pro-inflammatory and neurotoxic molecules in activated microglia would be effective therapeutic approach to treat various neuronal diseases [16].

Over the past decade, the secondary metabolites from marine microbes have been researched for a novel chemical source for drug discovery and development [17, 18]. Among the marine-derived microbes, marine fungi produce bioactive compounds with a wide range of bioactivities such as antiviral, antibacterial, anticancer, antiplasmodial and anti-inflammatory [19]. As a part of our ongoing investigation for diverse secondary metabolites from marine microorganisms, we isolated the fungal 168CLC-17.1 strain from a sediment sample collected from Cu Lao Cham Island, Vietnam, and based on its 28S rRNA gene sequence, it was identified as *Penicillium janthinellum*. Further chemical investigation of the ethyl acetate extract of the fungal culture broth yielded a new restricticin derivative, named restricticin B (**1**), together with four known compounds, *N*-acetyl restricticin (**2**) [19], 3,3''-dihydroxy-6'- desmethyl terphenyllin (**3**) [20], fellutanine B (**4**) [21] and 10,23-dihydro-24,25-dehydro aflavinin (**5**) [22](Figure 1). Here, we describe the isolation, structure elucidation, and biological activities of these compounds.
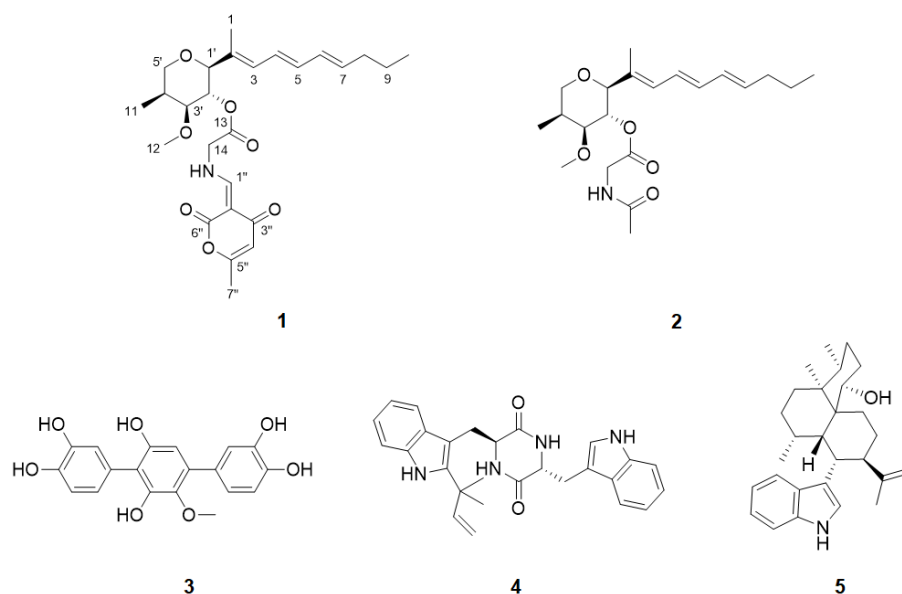

Figure 1. Structures of **1-5** isolated from *Penicillium janthinellum*.

2. Results and Discussion

Compound **1** was isolated as a pale brown oil and its molecular formula was determined to be $C_{26}H_{35}NO_7$ based on the HRESIMS ion at *m/z* 496.2306 ([M+Na]$^+$ calcd. 496.2311). In the ^{1}H NMR spectrum taken in CD_3OD (Figure S2), **1** appeared as a 3:2 mixture of rotamers. The NMR data of the major rotamer are shown in Table 1. The ^{1}H NMR spectrum of **1** indicated characteristic signals of seven olefinic protons (δ_H 8.20, 6.27, 6.21, 6.08, 5.98, 5.74, and 5.70), four methine protons (δ_H 5.00, 3.60, 3.52, and 2.33), four methylene protons (δ_H 4.32, δ_H 3.78, 3.63, δ_H 2.05, and δ_H 1.40) and five methyl protons (δ_H 3.33, 2.51, 1.74, 1.06, and 0.90). The ^{13}C NMR spectrum, in combination with the

HSQC spectrum, displayed the presence of 26 carbon signals, three carbonyl carbons (δ_C 184.1, 164.9, and 167.2), five pairs of olefinic carbons (δ_C 165.4, 162.7, 135.3, 134.4, 132.5, 130.6, 129.5, 125.3, 106.9, and 96.6), three oxygenated methine carbons (δ_C 84.9, 81.0, and 70.4), one methine carbon (δ_C 32.1), one oxygenated and one nitrogenated methylene carbons (δ_C 70.2 and 50.0, respectively), two methylene carbons (δ_C 34.5 and 22.0), one methoxy carbon (δ_C 55.0), and four methyl carbons (δ_C 18.7, 12.6, 10.4, and 9.5). The planar structure of **1** was elucidated by analysis of the 2D NMR data, including the COSY and HMBC spectra (Figure 2). The COSY correlations of H-3/H-4/H-5/H-6/H-7/ H$_2$-8/H$_2$-9/H$_3$-10 revealed the presence of an unsaturated side chain. The tetrahydropyran ring was confirmed by the COSY correlations of H-1'/H-2'/H-3'/H-4' /H$_2$-5'/H$_3$-11 and the HMBC correlations from H-5' to C-1', H$_3$-12 to C-3', and H$_3$-11 to C-3' and C-5'. The HMBC correlations from H$_3$-1 to C-1', C-2, and C-3, H-1' to C-2 and C-3, and H-2' to C-2 indicated that the triene side chain is connected to the tetrahydropyran ring. The geometry of the side chain was determined as all *E* by the large $^3J_{H,H}$ coupling constants for H-4/H-5 (14.5 ppm) and H-6/H-7 (15.0 ppm) and the NOESY correlations of H-3/H-5 and H$_3$-1/H-4. Afterward, a glycine moiety was confirmed by the HMBC correlations from H-2' and H$_2$-14 to C-13. Detailed analysis of 1D and 2D NMR spectra of **1** revealed that the partial structure was closely similar to that of restricticin. The HMBC correlations from H-1" to C-2", C-3", C-6", and C-14, H-4" to C-2" and C-5", and H-7" to C-4" and C-5" established the α-pyrone moiety linked to NH of the glycine unit. In addition, the NMR data in specific area showed the proton and carbon signals of the above-mentioned rotamers (Table S1). The ^{1}H NMR spectrum of **1** displayed a pair of olefinic singlet signals at H-1" (major: δ_H 8.20, minor: δ_H 8.32) and doublet methylene signals at H-14 (major: δ_H 4.27, 4.36, minor: δ_H 4.29, 4.38). Moreover, a pair of signals for the exchangeable NH proton (major: δ_H 11.8, minor: δ_H 10.1) was detected in the ^{1}H NMR spectrum in CDCl$_3$. The ^{13}C NMR spectrum also showed a doubling of signals in the glycine and α-pyrone residues, corresponding to three pairs of carbonyls, two pairs of olefinic carbons, two pairs of quaternary carbons, and a pair of a nitrogenated methylene carbon. We discussed here only the structure elucidation of the major rotamer for clarity.
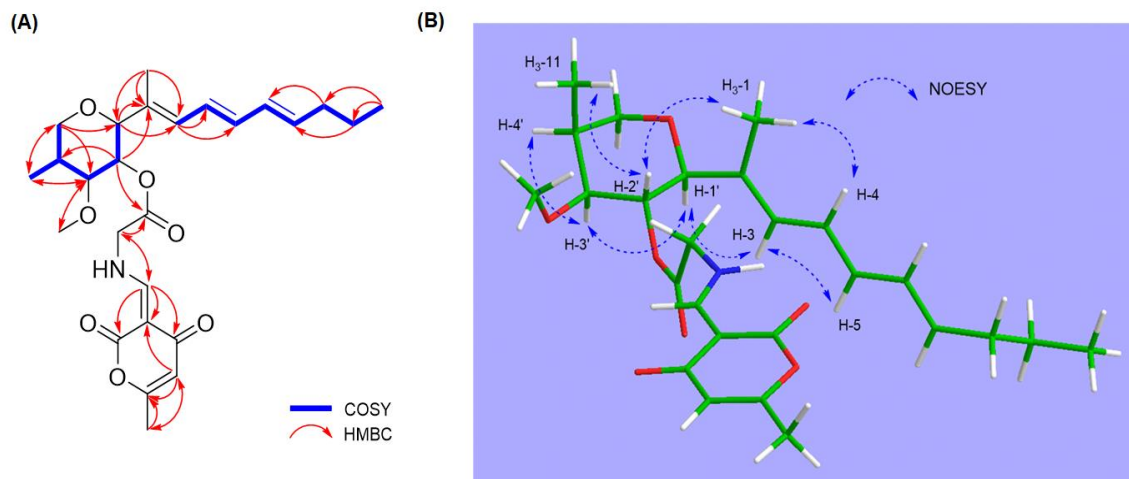

Figure 2. (**A**) Key COSY and HMBC correlations of **1**. (**B**) Key NOESY correlations of **1**.

The relative stereochemistry of **1** was determined by the analysis of NOESY spectra and the comparison of the optical rotation values with literatures. The strong NOESY correlations of H$_3$-1/H-2'/H$_3$-11 and H-1'/H-3'/H-4' suggested that H$_3$-1, H-10 and H$_3$-11 were on the same face, and H-1', H-3' and H-4' were on the opposite face. Finally, the absolute configuration of **1** was confirmed by comparison of the specific rotation value of **1** with those in the literature [8-10, 22](Figure 3) and electronic circular dichroism(ECD) method (Figure 4). The optical rotation value

Table 1. ^1H and ^{13}C NMR data for **1** and **2** at 500 MHz and 125 MHz in CD_3OD (δ in ppm, J in Hz)

Position	1			N-Acetyl restricticin (2)		
	δ_H (*J* in Hz)	Type	δ_C	δ_H (*J* in Hz)	Type	δ_C
1′	3.60 (d, 9.5)	CH	84.9	3.60(d, 9.5)	CH	85.0
2′	5.00 (dd, 9.5, 9.5)	CH	70.4	4.98 (dd, 9.5, 9.5)	CH	67.7
3′	3.52, m	CH	81.0	3.47, m	CH	81.3
4′	2.33, m	CH	32.1	2.30, m	CH	32.4
5′	3.63 (d, 12.0)	CH_2	70.2	3.64 (d, 12.0)	CH_2	70.3
	3.78 (d, 12.0)			3.75 (d, 12.0)		
1	1.74. s	CH_3	10.4	1.78. s	CH_3	10.5
2		C	132.5		C	132.5
3	5.98 (d, 10.5)	CH	129.5	5.99 (d, 10.5)	CH	129.4
4	6.27 (dd, 14.5, 10.0)	CH	125.3	6.27 (dd, 14.5, 10.5)	CH	125.6
5	6.21 (dd, 14.5, 10.0)	CH	134.4	6.23 (dd, 14.5, 10.5)	CH	134.0
6	6.08 (dd, 15.0, 10.5)	CH	130.6	6.10 (dd, 14.5, 10.5)	CH	130.7
7	5.70, (dt, 15.0, 7.0)	CH	135.3	5.72, (dt, 14.5, 7.0)	CH	135.0
8	2.05 (q, 7.0)	CH_2	34.5	2.07 (q, 7.0)	CH_2	34.5
9	1.40, m	CH_2	22.0	1.44, m	CH_2	22.1
10	0.90 (t, 7.5)	CH_3	12.6	0.91 (t, 7.0)	CH_3	12.6
11	1.06 (d, 7.0)	CH_3	9.5	1.06 (d, 7.5)	CH_3	9.6
12	3.33, s	OCH_3	55.0	3.34, s	OCH_3	55.4
13		C	167.2		C	169.1
14	4.27 (d, 18.0)	CH_2	50.0	3.73 (d, 18.0)	CH_2	40.5
	4.36 (d, 18.0)			3.94 (d, 18.0)		
NH^a	11.8, brs					
1″	8.20, s	CH	162.7		C	172.0
2″		C	96.6	1.95, s	CH_3	20.8
3″		C	184.1			
4″	5.74, s	CH	106.9			
5″		C	165.4			
6″		C	164.9			
7″	2.51, s	CH_3	18.7			

The assignments were aided by COSY, NOESY, HSQC, and HMBC NMR spectra. [a] Measured in $CDCl_3$

of **1** is in good agreement with all reported restrciticins, lanomycins and N-acetyl restricticin (**2**), suggesting that **1** has the same absolute configuration with its derivatives as shown in Figure 3. Additionally, ECD calculation of enantiomers (**1** : *1′S,2′R,3′S,4′S*, *ent*-**1** : *1′R,2′S,3′R,4′R*) was carried out at B3LYP/6-311 + G(d)1 level. The experimental CD spectrum showed a positive Cotton effect at 305 nm. Then, the calculated ECD spectrum of **1a** was in good agreement with the experimental CD spectrum, suggesting the absolute configurations of **1** are defined as *1′S,2′R,3′S,4′S*. Based on these results, the structure of **1** was determined and named restricticin B.
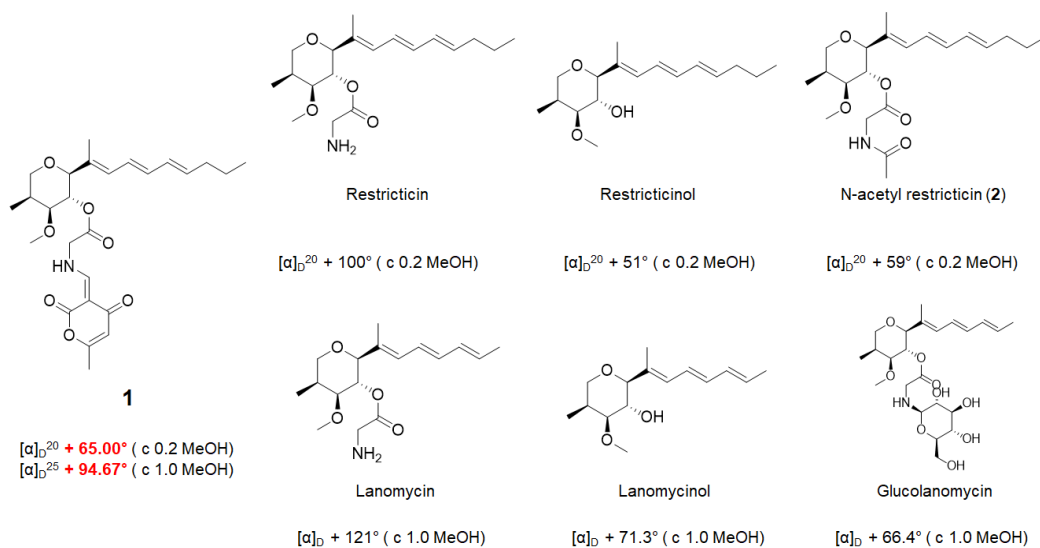
Figure 3. Comparison of optical rotation value of **1** with reference compounds.
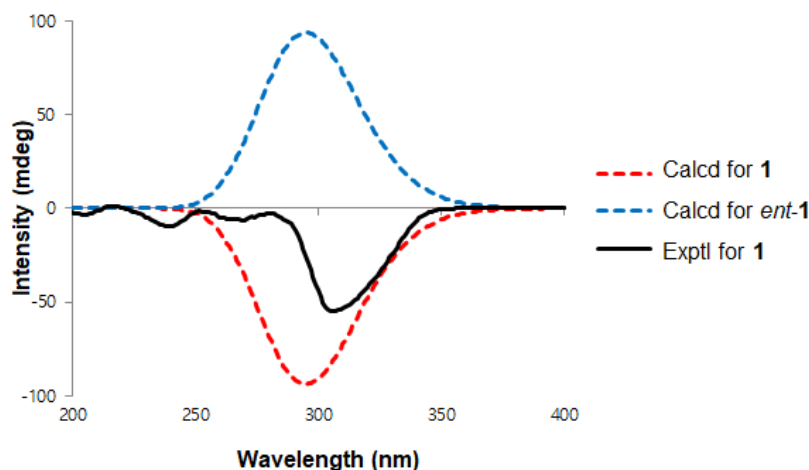
Figure 4. Comparison of experimental CD curve of **1** with calculated ECD spectra of **1** and *ent*-**1**.

The structures of the four known compounds were determined as *N*-acetyl restricticin (**2**), 3,3''-dihydroxy-6'-desmethyl terphenyllin (**3**), fellutanine B (**4**) and 10,23-dihydro-24,25-dehydro aflavinin (**5**) by comparing their ^{1}H, ^{13}C NMR and MS data with those reported in literature (Supporting information).

Compounds **1** and **2** were tested for their antimicrobial activity using fungi (*Penicillium italicum* KCTC 6437, *Rhizopus oryzae* KCTC 6944), yeast (*Candida albicans* KCTC 7678), Gram-positive bacteria (*Micrococcus lutes* KCTC1915, *Staphylococcus auresus* KCTC 1927, *Bacillus subtilis* KCTC 1021) and Gram-negative bacteria (*Salmonella typhimurium* KCTC 2515, *Klebsiella pneumoniae* KCTC 2690, *Escherichia coli* KCTC 2441) and the cytotoxicity against cancer cell lines (HCT-15, NUGC-3, NCI-H23, ACHN, PC-3 and MDA-MB-231). However, **1** and **2** showed no antibacterial activity and cytotoxicity against cancer cell lines. Along with the fact that restrictinol and lanomycinol have no antifungal activity in previous research, the results of this study suggested that the free amine of the glycine unit is important for the antifungal activity.
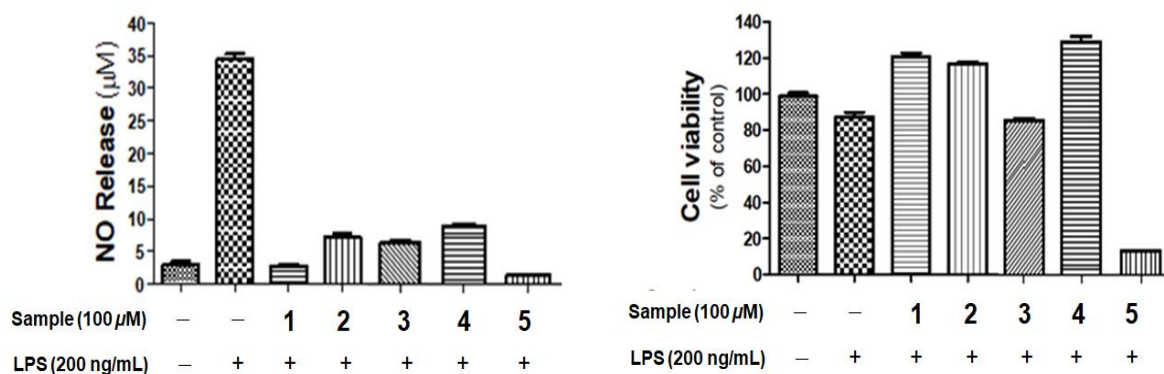

Figure 5. The effects of compounds **1-5** on NO production in LPS-stimulated BV-2 cells. Cells were pretreated with the indicated concentration of samples for 1 h and then stimulated with LPS (100 ng/mL) for 24 h. Cell viability was evaluated using MTT assay. Results are shown as percentage of control samples. Values are mean ± standard error.

All the isolated compounds (**1-5**) were evaluated for inhibitory activity on nitric oxide (NO) production in LPS-stimulated BV-2 microglial cells and for their cytotoxicity. The cells were initially treated with 100 μM concentration of each compounds and/or LPS (200 ng/mL) to measure the levels of NO and cytotoxicity using MTT assay. As shown in Figure 5, all the compounds inhibited

the LPS-induced NO production in BV-2 microglial cells. However, compounds **3** and **5** displayed weak and strong toxicity, respectively. Compounds **1**, **2**, and **4** decreased the production of NO without showing cytotoxicity at the treated concentrations. According to the results, restricticin derivatives have the most potent anti-inflammatory effect among the treated compounds without cytotoxicity. Therefore, restricticin B (**1**) and N-acetyl restricticin (**2**) were selected to further investigate the effects on LPS-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) mRNA and protein expression, and production of LPS-stimulated pro-inflammatory cytokines in BV-2 microglia cells.

Two restricticin compounds (**1** and **2**) at 50 μM and 100 μM concentrations inhibited the NO production in BV-2 cells in a dose dependent manner (Figure 6A). Compound **1** showed stronger inhibition of NO production than **2**. As shown in Figure 6B and 6C, LPS treatment (200 ng/mL) significantly upregulated iNOS and COX-2 expression, and the mRNA and protein expression levels of iNOS and COX-2 were inhibited by restricticins in a concentration-dependent manner. To investigate whether restricticins repress the production of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, RT-PCR was performed, and the results showed that restricticins reduced the secretion of proinflammatory cytokines. Interestingly, IL-1β was the most strongly inhibited by compound **1** at the 100 μM concentration. Consequently, these results indicated that restricticins have effective properties in neuroinflammation processes and suppress the expression of proinflammatory mediators at the transcriptional level.
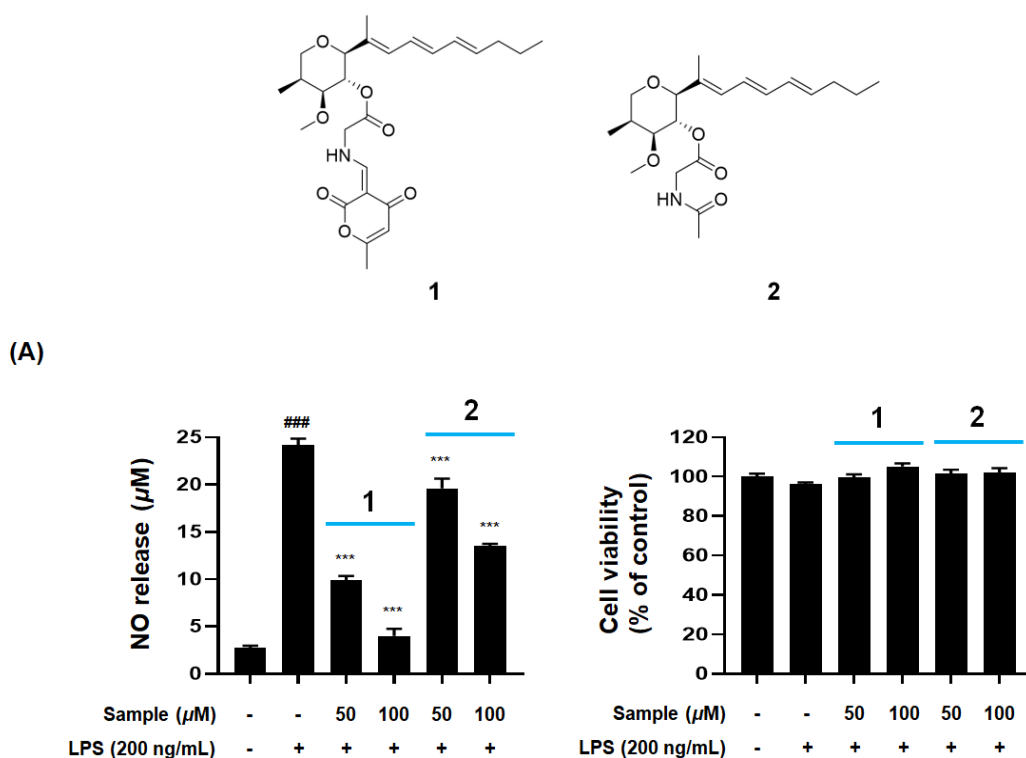

(A)
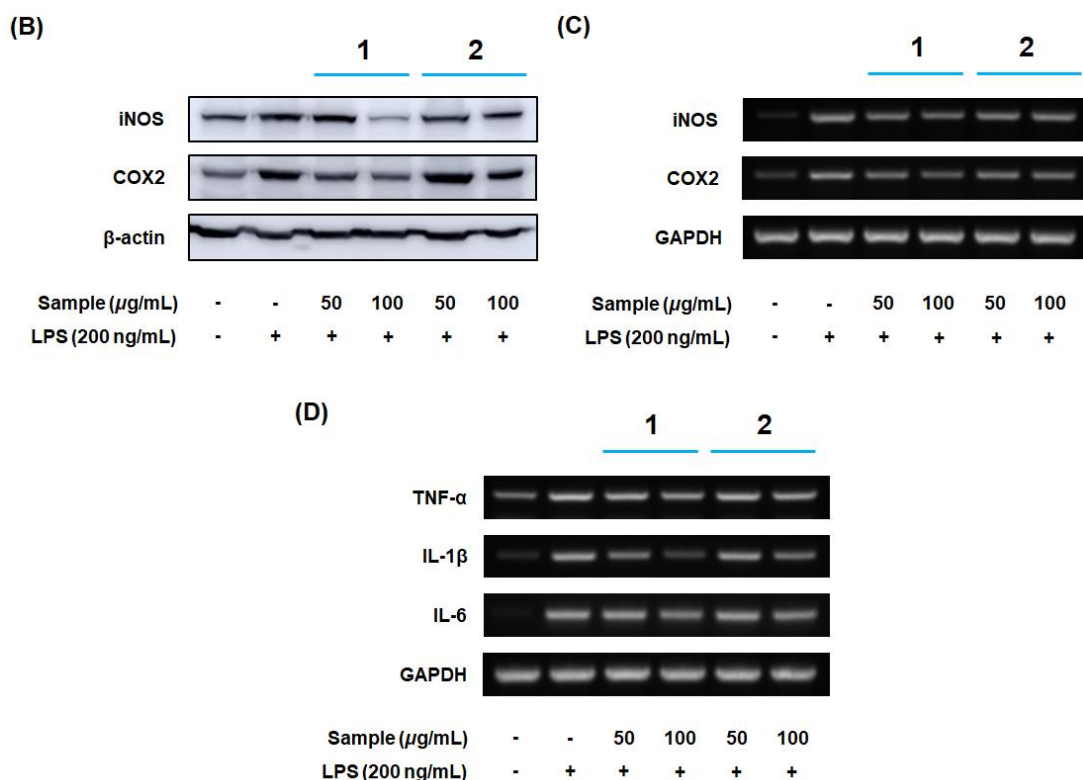

Figure 6. *Cont.*

Figure 6. (A) The nitrite levels were measured in the culture media using the Griess reaction. Cell viability was evaluated using the MTT assay. Results are shown as percentage of control samples. (B) Suppression of iNOS and COX-2 protein (C) and mRNA (D) and pro-inflammatory cytokines expression by compounds **1-2** in LPS-stimulated BV-2 cells. The corresponding quantification data were shown in the each panel respectively. GAPDH and β-actin were used as internal controls, respectively. The results are expressed as relative signal intensity for two independent experiments (B, C, and D). Values are mean ± standard error. ###$p < 0.001$, vs. control group and *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. LPS-treated group.

3. Materials and Methods

3.1. General Experimental Procedures

1D (^{1}H and ^{13}C) and 2D (COSY, HSQC, HMBC and NOESY) NMR spectra were measured on a Varian Unity 500 MHz and a Bruker 600 MHz spectrometer with TMS as an internal standard. Optical rotations were obtained on a Rudolph Research Analytical (Autopol III) polarimeter. CD spectra were measured on a JASCO J-1500 spectrometer. UV spectra were acquired on a Shimadzu UV-1650PC spectrophotometer. IR spectra were recorded on a JASCO FT/IR-4100 spectro-photometer. HRESIMS were measured on a hybrid ion-trap time-of-flight mass spectrometer (Shimadzu LC/MS-IT-TOF). HPLC system was composed of a PrimeLine Binary pump with RI-101(Shodex). Semi-preparative HPLC was performed using an ODS column (YMC-Pack-ODS-A, 250 × 10 mm i.d, 5 μm). Analytical HPLC was conducted on an ODS column (YMC-Pack-ODS-A, 250 × 4.6 mm i.d, 5 μm).

3.2. Reagents

Lipopolysaccharide (LPS), dimethyl sulfoxide (DMSO), 3-(3, 4-dimethylthiazole-2-yl)-2, 5-diphenyl-tetrazoliumbromide (MTT), sulfanilamide, H3PO4 and N-1(1-naphthyl)ethylene diamine dihydrochloride were obtained from Sigma-Aldrich. The 10 x RIPA buffer was obtained from Millipore. Protease inhibitor and phosphatase inhibitor cocktail tablets were purchased from Roche. Plastic wares (6-well, 24-well tissue culture plates and 100 mm culture dishes) were

purchased from SPL. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), 1x trypsin-EDTA (TE), and 100,000 U/ml penicillin-streptomycin (P-S) were obtained from Gibco/Invitrogen. Primary antibodies anti-COX-2 (1:1000) was obtained from Abcam and Calbiochem. Anti-β-actin (1:5000) and anti-iNOS (1:1000) were procured from Sigma-Aldrich and BD Biosciences, respectively. Secondary antibodies anti-mouse (1:2000) and anti-rabbit (1:2000) were obtained from Cell Signaling Technology and Bio-Rad.

3.3. BV-2 Microglial cell culture and Treatment

The BV-2 microglial cells were cultured in Dulbecco's modified Eagle medium supplemented with 5% fetal bovine serum and 1% penicillin/streptomycin (100 units/mL) at 37 ℃ in a humidified 5 % CO_2 incubator. The cell was seeded at a density of 2.5 x 10^5 cells/mL and pretreated same concentration of samples and followed by LPS incubation (200 ng/mL) [24].

3.4. Cell viability and Nitrite assay

The BV-2 microglial cells seeded at a density of 2.5 X 10^5 cells/well were pretreated with 100 μM single concentration of compounds **1-5** for 1 h, followed by LPS(200 ng/mL) for 24 h. 20 μL of MTT solution (2 mg/mL, conc.) was added to each well in 24 well. After 1h supernatant was sucked and dissolved the formazan crystals in viable cells from DMSO. Optical density was measured at 550 nm using a microplate reader and values were determined in comparison to control cells. For nitrite assay, the BV-2 microglial cells seeded at a density of 2.5 x 10^5 cells/well were pretreated with 100 μM single concentration of compounds **1-5** for 1 h, followed by LPS (200 ng/mL) for 24 h. 100 μL of supernatants collected, transferred to new microplate. After transfer, assayed for NO release using Griess reagent (1 vol. 0.1% naphthylethylenediamine and 1 vol. 1% sulfanilamide in 5% H_3PO_4) and a range of sodium nitrite dilutions was used to obtain a standard curve with the amount of nitrite in each sample. Absorbance was determined at 540 nm using a microplate reader [24].

3.5. Total RNA Extraction and Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from BV-2 microglial cells using Trizol reagent (Invitrogen Life Technologies) according to the manufacturer's instructions. RNA (2.5 μg) was reverse-transcribed using ReverTra Ace-α kit (Toyobo) according to the manufacturer's instruction. PCR amplification was performed using specific primers (Bioneer)[25].

3.6. Western Blot Analysis

Treated BV-2 cells (5 × 10^5 cells/well) was washed twice with PBS and lysed for 10 min using 1 x RIPA lysis buffer at 4 ℃. Cell lysates was centrifuged at 14,000 rpm at 4 ℃ and the corresponding supernatants were collected and separately stored for further analysis. The protein concentration of each sample was obtained using a DC Protein Assay kit (Bio-Rad). Equal amounts of protein (35 μg for cells) were separated electrophoretically by 10% sodium dodecyl sulfate-polyacrylamide electrophoresis, and the resolved proteins were transferred to polyvinylidene difluoride membranes. The membranes were incubated for 1 h with 5% Skim-milk in TBS buffer to block nonspecific binding. The blots were visualized by a PowerOpti-ECL kit obtained from the detection system (Animal Genetics Inc.)[25].

3.7. Fungal strain and Fermentation

The strain 168CLC-17.1 was isolated from a sediment sample, collected at Cu Lao Cham Island, Quang Nam, Vietnam in August 2016. The strain was identified by DNA amplification and sequencing of the ITS region (GenBank accession number AB293968.1) and named as *Penicillium janthinellum* 168CLC-17.1. The fungal strain was cultured on a potato dextrose agar (PDA) for a week and then the grow-well strain was inoculated on rice medium at 28℃ for 22 days in 80

Erlenmeyer flasks (500 mL), each containing rice (20.0 g), yeast extract (20.0 mg), KH_2PO_4 (10 mg), and natural sea water (40 mL) as reported previously [26].

3.8. Isolation of Compounds *1*-*5*

The mycelia and rice media were extracted with EtOAc and then concentrated to yield a crude extract (6 g). The crude extract was fractionated by flash column chromatography on ODS using a gradient of MeOH/ H_2O (1:4, 2:3, 3:2, 4:1 and 100% MeOH, each fraction 300 × 3). The third fraction eluted with 80% MeOH was purified with a semi-preparative reversed-phase HPLC (2.0 mL/ min, RI detector, 22% MeCN/H_2O) to obtain **1** (7.5 mg, t_R = 28 min) and **2** (3.2 mg, t_R = 18 min). The first fraction eluted with 40% MeOH was further separated into ten subfractions (Fr. A-H) by column chromatography on ODS eluting with a step gradient of MeOH/H_2O (20:80 to 40:60, v/v). Fr. B (82 mg) was subjected to a semi-preparative reversed-phase HPLC (YMC-Pack-ODS-A, 250 × 10 mm i.d, 5 μm, flow rate 3.0 mL/ min, RI detector) using isocratic elution with 25% MeOH in H_2O to yield **3** (62 mg, t_R = 14 min). Compound **4** (5.1 mg, t_R = 46 min) was isolated from the third fraction eluted with 60% MeOH by a semi-preparative reversed-phase HPLC (3.0 mL/min, RI detector, 55% MeOH/H_2O). The first fraction eluted with 100% MeOH was further separated into ten subfractions (Fr. A-J) by column chromatography on ODS eluting with a step gradient of MeOH/H_2O (90:10 to 100:0, v/v). Fr. C-F (182 mg) was purified by a semi-preparative reversed-phase HPLC (YMC-Pack-ODS-A, 250 × 10 mm i.d, 5 μm, flow rate 4.0 mL/ min, RI detector) using isocratic elution with 95% MeOH in H_2O to yield **5** (85 mg, t_R = 12 min).

Restricticin B (**1**): pale brown oil ; $[\alpha]_D^{20}$ +65 (c 0.2, MeOH); CD (MeOH), λ_{max} ($\Delta\varepsilon$) 305 (54.59) nm; IR ν_{max} 2933, 1727, 1653, 1617, 1458, 1328, 1201, 1109, 1035, 985 cm^{-1}; UV(MeOH) λ_{max} (log ε) 317 (3.39), 276 (3.77), 236 (3.47), 201 (3.53) nm; HRESIMS m/z 496.2306 [M + Na]$^+$ (calcd for 496.2311, $C_{26}H_{35}NO_7Na$); ^{1}H NMR (CD$_3$OD, 500 MHz) and ^{13}C NMR (CD$_3$OD, 125 MHz) see Table 1.

3.9. Calculation of ECD spectra

Conformational searches were performed by CONFLEX version 8.0 program (CONFLEX Corporation) with molecular mechanics force field (MMFF) within window of 3.0 kcal/mol. The conformers were further optimized by the B3LYP/6-311G (d,p). The theoretical calculations of the ECD data were conducted using the TD-DFT method at the B3LYP/6-311G (d,p), which were performed with Gaussian 16 software (Gaussian Inc.). The calculated ECD spectra data were averaged based on their Boltzmann populations.

4. Conclusions

Two restrciticin derivatives (**1** and **2**), along with three known compounds (**3-5**) were isolated from the rice medium cultures of the marine-derived fungus *Penicillium janthinellum* 168CLC-17.1. The structures of the isolated compounds were determined by analysis of their NMR and mass spectrometric data. The absolute configuration of restricticin B (**1**) was established by NOESY correlations, comparison of specific rotation values with those of restricticin derivatives reported and electronic circular dichroism (ECD). Interestingly, the new restricticin possessed an α-pyrine ring connected to NH of glycine moiety and showed signal duplication as a 3:2 mixture of rotamers. Two isolated restricticins exhibited inhibitory activity on NO production in LPS-stimulated BV-2 microglial cells. Moreover, the restricticins suppressed iNOS and COX-2 expression (both at the protein and mRNA levels), and also inhibited LPS-induced production of pro-inflammatory cytokines. Additionally, compound **1** possessing α-pyrone moiety linked to NH showed stronger activity than **2** containing N-acetyl group. To the best of our knowledge, this is the first report on the anti-neuroinflammatory activity of restrciticins.

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