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# Stilbenes from the tubers of *Bletilla striata* with potential anti-neuroinflammatory activity

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ABSTRACT

Neuroinflammation are involved in the pathogenesis of many neurodegenerative disorders. In our screening of natural effective neuroinflammatory inhibitors from natural products, stilbenes, such as resveratrol and its analogues, have received considerable attention over the last several decades as anti-neuroinflammatory agents. Then, *Bletilla striata* attracted our attention due to its abundant stilbenes portion, PE fraction. So, three new stilbenes: dusuanlansin E1 (**23a**), dusuanlansin E2 (**23b**), 3-hydroxy-5-methoxybibenzyl-3′-*O*-*β*-D-glucopyranoside (**27**), and 30 known stilbene compounds were isolated from *B. striata*. These structures of the compounds were established on the basis of extensive spectroscopic analysis including 1D and 2D NMR and circular dichroism (CD) data. Furthermore, all the isolated components were tested *in vitro* for their inhibitory effects on the nitric oxide generation in LPS-stimulated BV2 cells. As a result, compounds **2**, **5**, **6**, **16**, **17** can greatly inhibit the NO production without cytotoxicity. In addition, SARs between stilbenes and anti-neuroinflammation effects were discussed briefly. In conclusion, stilbenes were characteristic constituents of the tubers of *B. striata* with potential anti- neuroinflammatory effects.

## 1. Introduction

Neurodegenerative diseases are a group of chronic, progressive disorders characterized by the gradual loss of neurons in discrete areas of the central nervous system (CNS) [1]. Neuroinflammatory processes are involved in the pathogenesis of many neurodegenerative disorders, such as Alzheimer's disease (AD) [2]. To overcome the limitations of current therapeutics for neurodegenerative diseases, extensive research is underway to identify new targets, together with new drugs that are effective and free of undesirable side effects. Some stilbenes with antioxidant and anti-inflammatory properties have received considerable attention as alternative candidates for neurodegenerative diseases prevention or therapy [3-5].

Stilbenes are widely found in nature, have been paid extensive attention for their various functions about healthy in human diet and medical treatments, such as antioxidative, anticancer activities. They represent a class of compounds with a common 1, 2-diphenylethylene backbone that have shown extraordinary potential in the biomedical fields, especially in the treatment of neuroinflammation [6]. As the most well-known example, resveratrol proved to have anti-aging effects. Recently, resveratrol's analogues, pterostilbene, have gained a significant amount of attention due to their potent antioxidant, anti-inflammatory properties [7,8]. Furthermore, the stilbene scaffold is a basic element for synthetic compounds, and it is considered as a privileged structure [8]. Therefore, stilbenes are of significant interest for drug research and synthetic fields.

Traditional Chinese medicines not only hold economic value of healthcare system, but also possess unique and great potential for new drug development [9]. *Bletilla striata*, is a perennial herb which is widely distributed in China, North Korea, Japan and Burma, belonging to Orchidaceae family. As a well-known Chinese folk herb medicine, its tubers have been employed to treat hemostasia, detumescence, healing and enhancement of bodily function [10,11]. Previous phytochemical studies on *Bletilla* species have led to the isolation of many stilbene compounds. Based on the traditional applications and structures types, stilbenes may be the material basis of anti-neuroinflammatory effects. As part of our search for new and bioactive stilbenes constituents, the petroleum ether (PE)-soluble fraction of 95% EtOH extract of was phytochemically studied, which furnished three new stilbenes and thirty known components. The structures of these compounds were

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elucidated mainly by NMR spectroscopic and mass spectroscopic methods. Furthermore, the anti-neuroinflammatory effects of the extract and purified constituents were performed by NO assays in LPS-stimulated BV2 cells [12].

## 2. Experimental

### 2.1. General experimental procedures

NMR spectra were recorded on Bruker ARX-400 and 600 M AVIII spectrometers, using TMS as an internal standard. Silica gel (200–300 mesh) for chromatography was produced by Qingdao Ocean Chemical Group Co. of China. Optical rotations were measured using MCP 200 polarimeter from Anton Paar GmbH (Graz, Austria). Sephadex LH-20 was purchased from Pharmacia Company (Uppsala, Switzerland). ODS (50 μm) for column chromatography was afforded by YMC Co. (Ltd) in Japan. HPLC separations were performed on a YMC-pack Prep-ODS column (250 × 20 mm) equipped with a shimadzu RID-20A UV detector and a shimadzu LC-6AD series pumping system (Tokyo, Japan). Dimethyl sulfoxide (DMSO), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), $CDCl_3$, DMSO-$d_6$ and $CD_3OD$ were obtained from Sigma-Aldrich Company (St. Louis, MO, USA). All the chromatographic and analytical grade reagents were obtained from Tianjin DaMao Chemical Company (Tianjin, China).

### 2.2. Plant material

The tubers of *B. striata* were obtained from Shaanxi Tasly Plant Medicine Limited Liability Company, China, in November 2016. The plant material was identified by Professor Yingni Pan (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University). A voucher specimen (No. 20161123) is deposited in School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University.

### 2.3. Extraction and isolation

The dried and powdered tubers of *B. striata* (16 kg) were refluxed with 95% EtOH (3 times, 3 h for each). The extract was evaporated to dryness under vacuum to afford a crude residue (1.82 kg) (11.38%), which was partitioned successively with petroleum ether (PE), ethyl acetate (EtOAc) and *n*-BuOH.

The PE portion was evaporated under reduced pressure to afford a crude extract. The effective PE extract (200.0 g) was subjected to silica gel column ($CH_2Cl_2$: MeOH, 100:0–0:100). Then similar fractions were collected together on the basis of similarity in $R_f$ values and afforded 10 fractions (Fr. 1-Fr. 10). Fr. 1 was chromatographed on open ODS column (100 × 3.5 cm) and eluted with MeOH-$H_2O$ (0: 100–100:0) to yield sub-fractions Fr. 3.1. This fraction was purified by HPLC with ODS column (250 mm × 10 mm, 3 mL/min) to yield compounds **1** (25.5 mg), **8** (17.8 mg), **15** (22.3 mg) and **19** (29.4 mg) using MeOH/$H_2O$ (68: 32) as mobile phase. Similarly, Fr.2 was separated by HPLC by ODS column with elution phase MeOH/$H_2O$ (68: 32) to provide compound **21** (37.1 mg). Fr.3 was firstly isolated by silica gel column chromatography (50 × 3 cm) with gradient elution of PE/EtOAc (0:100–100:0) to get Fr. 3–1 and 3–2, and then these subfractions were further eluted by MeOH/$H_2O$ with ODS column to gain compounds **3** (30.2 mg), **4** (12.0 mg), **14** (18.8 mg), **16** (22.6 mg), **17** (27.4 mg), **18** (20.2 mg), **22** (20.1 mg). And Fr. 4 was loaded on a ODS column (75 × 3.5 cm), eluting with MeOH and further purified by HPLC with ODS column (250 mm × 10 mm, 3 mL/min) to yield compounds **6** (32.0 mg), **12** (123.2 mg) and **7** (10.2 mg) using MeOH/$H_2O$ (64:36) as eluting solvent. Fr. 6 was subjected to ODS column (MeOH-$H_2O$, 0:100–100:0) and similar fractions were pooled together followed by TLC analysis. Among them, 30% part was chromatographed over HPLC and eluted with MeOH-$H_2O$ (55:45) to yield compounds **2** (20.2 mg), **5** (73.2 mg), **13** (120.2 mg), **20** (35.2 mg) and **23** (16.6 mg). Using the same method, 45% part and 55% part were separated by HPLC to obtain compounds **24** (22.8 mg) and **25** (37.7 mg), compounds **26** (12.3 mg), **27** (17.4 mg), **28** (45.9 mg), **29** (25.2 mg) and **30** (17.0 mg), respectively.

**Table 1**
$^1H$ (600 MHz) and $^{13}C$ (150 MHz) spectral data of compounds **23** and **27**.

| No. | **23**[a] | | No. | **27**[b] | |
|---|---|---|---|---|---|
| | $\delta_H$, *J* (Hz) | $\delta_C$ | | $\delta_H$, *J* (Hz) | $\delta_C$ |
| 1 | | 143.3 | 1 | | 143.4 |
| 2 | | 120.0 | 2 | 6.21, m | 108.2 |
| 3 | | 161.4 | 3 | | 160.3 |
| 4 | 6.33, d, 2.4 | 99.2 | 4 | 6.13, m | 98.9 |
| 5 | | 159.0 | 5 | | 160.3 |
| 6 | 6.23, d, 2.4 | 109.7 | 6 | 6.21, m | 104.3 |
| α | 2.74, m | 36.7 | α | 2.81–2.88, m | 37.2 |
| α′ | 2.89, m | 39.7 | α′ | 2.81–2.88, m | 36.7 |
| 1′ | | 144.3 | 1′ | | 143.1 |
| 2′ | 6.62, m | 120.9 | 2′ | 6.83, m | 113.5 |
| 3′ | | 158.5 | 3′ | | 157.5 |
| 4′ | 6.60, m | 114.0 | 4′ | 6.89, m | 116.3 |
| 5′ | 7.06, t, 7.8 | 130.4 | 5′ | 7.17, t, 7.8 | 129.0 |
| 6′ | 6.58, m | 116.4 | 6′ | 6.84, m | 121.8 |
| 2″ | | 181.5 | 1″ | 4.80, d, 7.2 | 100.4 |
| 3″ | 2.50, m; 2.39, m | 32.2 | 2″ | 3.21, t, 7.2 | 73.3 |
| 4″ | 2.32, m; 2.06, m | 27.4 | 3″ | 3.26, t, 7.2 | 76.7 |
| 5″ | 5.03, dd, 9.0, 5.0 | 53.4 | 4″ | 3.15, t, 7.2 | 69.7 |
| 3- $OCH_3$ | 3.75, s | 55.9 | 5″ | 3.30, m | 77.0 |
| | | | 6″ | 3.67, m; 3.46, m | 60.7 |
| | | | 5-$OCH_3$ | 3.65, s | 54.7 |

[a] Measured in $CD_3OD$.
[b] Measured in DMSO-$d_6$.

#### 2.3.1. Dusuanlansin E1 (23a)

Brownish powder; [α] − 30 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log$\varepsilon$) 210 (4.60) nm; $^1H$ NMR (600 MHz, $CD_3OD$) and $^{13}C$ NMR (150 MHz, $CD_3OD$) data see Table 1; HRESIMS *m/z*: 328.1539 $[M+H]^-$, calcd for $C_{19}H_{22}NO_4$, 328.1543.

#### 2.3.2. Dusuanlansin E2 (23b)

Brownish powder; [α] + 30 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log$\varepsilon$) 210 (−4.60) nm; $^1H$ NMR (600 MHz, $CD_3OD$) and $^{13}C$ NMR (150 MHz, $CD_3OD$) data see Table 1; HRESIMS *m/z*: 328.1539 $[M+H]^-$, calcd for $C_{19}H_{22}NO_4$, 328.1543.

#### 2.3.3. 3-Hydroxy-5-methoxybibenzyl-3′-O-β-D-glucopyranoside (27)

Brownish powder; [α] − 19 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log$\varepsilon$) 210 (- 3.20) nm; $^1H$ NMR (600 MHz, DMSO-$d_6$) and $^{13}C$ NMR (150 MHz, DMSO-$d_6$) data see Table 1; HRESIMS *m/z*: 405.1554 $[M-H]^-$, calcd for $C_{21}H_{25}O_8$, 405.1555.

### 2.4. Acid hydrolysis of compound 27 and HPLC analysis for sugar residues

Compound **27** (1.0 mg) was heated in 4 M HCl (2 mL) for 3 h in a $H_2O$ bath at 90 °C. After cooling, these mixture were extracted by $CH_2Cl_2$ and get $CH_2Cl_2$ extract. Then aqueous layer was evaporated to dryness. The water layer and D-glucose (1.0 mg) were added in 3.0 mg L-cysteine methyl ester and were dissolved in pyridine (1 mL) and heated at 60 °C for 1 h and then *o*-tolyl isothiocyanate (5 μL) was added to the mixture and heated further for 1 h. The reaction mixture was analyzed by HPLC and detected at 250 nm, $t_R$ (min): D-glucose (18.73). The HPLC analysis was carried out on a shimadzu SPD-20A (UV/visible detector) using a shimpack ODS (H) KIT (5 μm particle size, 4.6 × 250 mm) at 35 °C with a flow rate of 1 mL/min. Mobile phase was 25% $CH_3CN/H_2O$ (0.1% formic acid of water).

### 2.5. Nitrite assays of compounds 1–30 in LPS-induced BV2 cells

Using the griess method, accumulation of nitrite ($NO_2^-$), an indicator of NO synthase activity, in culture medium was measured. BV2 cells ($3 \times 10^5$ cells/well) were plated in 96-well microtiter plates and treated with extract (0, 1, 10, 30, 100 μg/mL) and each compounds (0, 1, 10, 30, 100 μM) in presence of lipopolysacchride (LPS; 1 μg/mL) for 24 h. Fifty microliter culture medium supernatants were mixed with 50 μL griess reagent at room temperature for 15 min. The optical density of each well was measured at 540 nm.

### 2.6. Cytotoxicities assay of compounds 1–30

Cell viability was evaluated by MTT assay. Briefly, BV2 microglial cells were seeded at $3 \times 10^5$ cells/well in 96-well microtiter plates. After overnight incubation, the cells were treated with LPS (1 μg/mL) in the absence or presence of the test extracts (0, 1, 10, 30, 100 μg/mL) and each compounds (0, 1, 10, 30, 100 μM) for 24 h, the medium was removed and the cells were incubated with MTT (0.25 mg/mL) for 4 h at 37 °C. The formazan crystals in the cells were dissolved in DMSO. The absorbance was measured at 490 nm by a microplate reader.

## 3. Results and discussion

### 3.1. Isolation of compounds from the PE extract of the tubers of B. striata

Bio-guided fractionation and isolation of PE-soluble fraction of the 95% ethanol extract of the tubers of *B. striata* afforded 33 compounds (Fig. 1) by means of chromatographic methods and recrystallization [13]. Finally, their structures were determined as follow: (*E*)-3,3′,5-trimethoxystilbene (**1**) [15], dihydropinosylvi (**2**) [16], gigantol (**3**) [17], 3,3′,5-trimethoxybibenzyl (**4**) [18], batatasin III (**5**) [19], 5-[2-(3-methoxyphenyl)ethyl]-1,3-benzenediol (**6**) [20], 3,3′,4-trihydroxybibenzyl (**7**) [21], 3- hydroxy-5-methoxybibenzyl (**8**) [22], 3′-*O*-methylbatatasin III (**9**) [22], 3-hydroxy-5- methoxybibenzyl (**10**) [22], shancigusin D (**11**) [22], 3,3′-dihydroxy-2-(4-hydroxyl benzyl)-5-methoxy-bibenzyl (**12**) [23], 3′,5-dihydroxy-2-(4-hydroxybenzyl)-3-methoxy-bibenzyl (**13**) [24], bulbocol (**14**) [25], 3′-hydroxy-2-(4-hydroxybenzyl)- 3,5-dimethoxy-bibenzyl (**15**) [26], gymconopin D (**16**) [27], 5-hydroxy-2-(*p*- hydroxybenzyl)-3-methoxybibenzyl (**17**) [28], 4-hydroxy-5-(*p*-hydroxybenzyl)-2- methoxybibenzyl (**18**) [26], arudinan (**19**) [26], 3,3′-dihydroxy-4-(4-hydroxybenzyl)-5-methoxybibenzyl (**20**) [29], 5-hydroxy-4-(*p*-hydroxybenzyl)-3′,3-dimethoxy bibenzyl (**21**) [30], 3′-dihydroxy-4-(4-hydroxybenzyl)-3,5-dimethoxybibenzyl (**22**) [26], dusuanlansin E1 (**23a**), dusuanlansin E2 (**23b**), dusuanlansin A (**24a**) [31], dusuanlansin B (**24b**) [31], dusuanlansin C (**25a**) [31], dusuanlansin D (**25b**) [31], 3,3′-dihydroxy-2′,6′-bis(*p*-hydroxybenzyl)-5-methoxybibenzyl (**26**) [32], 3-hydroxy- 5-methoxybibenzyl-3′-*O*-*β*-D-glucopyranoside (**27**), 3′,5-dimethoxybibenzyl-3-*O*- *β*-D-glucopyranoside (**28**) [33], batatsin III-3-*O*-glucoside (**29**) [33], 5-methoxy- bibenzyl-3,3′-di-*O*-*β*-D-glucopyranoside (**30**) [34]. Among them, compounds **1**, **7**, **15**, **17**–**18**, **22**, **24**–**25**, and **30** were isolated from this species for the first time (Fig. 1).

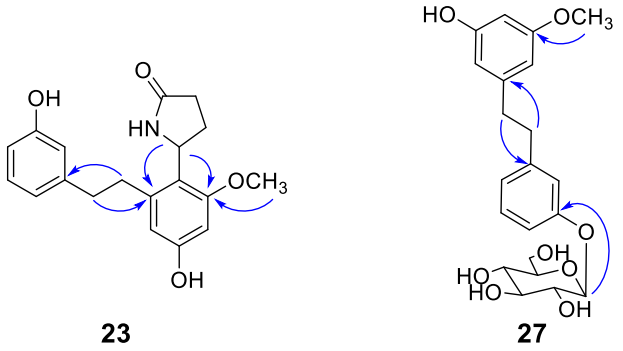


**Fig. 2.** The key HMBC (↷) correlations of compounds **23** and **27**.

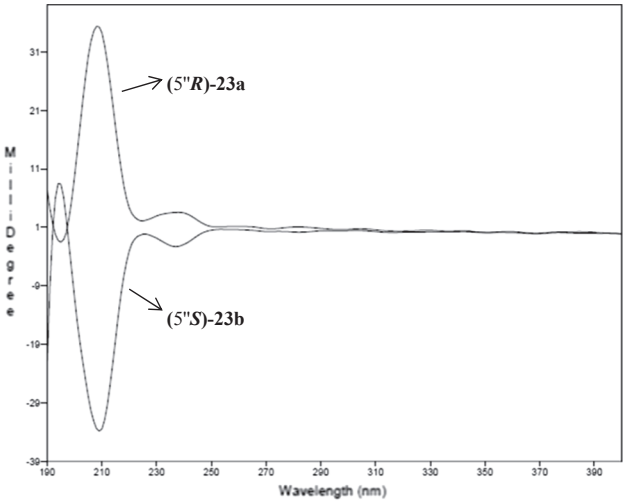


**Fig. 3.** Comparison of experimental ECD spectra of compounds **23a** and **23b** (in MeOH).

### 3.2. Structural elucidation of new compounds

The molecular formula of compound **23**, $C_{19}H_{21}NO_4$, with 10 degrees of unsaturation, was evidenced by the $[M+H]^+$ ion at *m/z* 328.1539 (calcd. 328.1543 for $C_{19}H_{22}NO_4$) in HRESI-MS. Analysis of the NMR data (Table 1) revealed the presence of a disubstituted [$\delta_H$ 7.06 (1H, t, $J$ = 7.8 Hz, H-5′), 6.60 (1H, m, H-4′), 6.58 (1H, m, H-6′), 6.62 (1H, m, H-2′)] and a tetrasubstituted [6.33 (1H, d, $J$ = 2.4 Hz, H-4), 6.23 (1H, d, $J$ = 2.4 Hz, H-6)] phenyl groups, four methylenes, a methine, a methoxy and a carboxylic carbon ($\delta_C$ 181.5). The signals of two phenyl groups combined with a pair of methylenes [$\delta_H$ 2.74–2.89 (4H, m, H-*α*, *α*′)] suggest the existence of a bibenzyl skeleton, which is similar to batatasin III. Additionally, the methoxy group [$\delta_H$ 3.75 (3H,

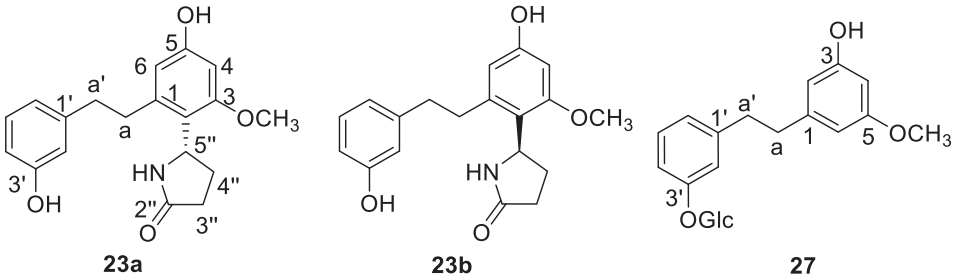


**Fig. 1.** The structures of new compounds.

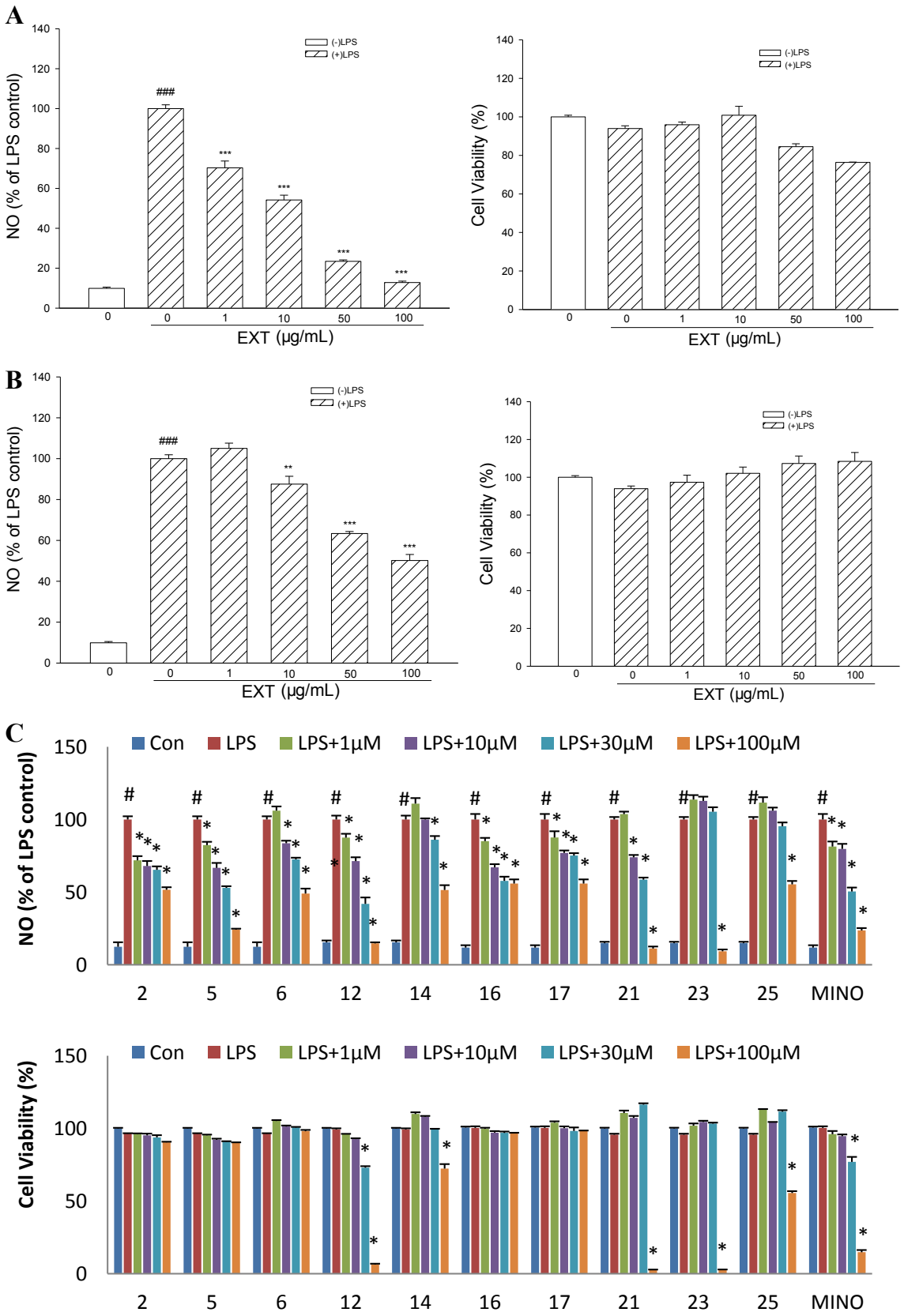


**Fig. 4.** Anti-neuroinflammatory activities and cytotoxicities of ethanol extract, PE extract and isolated compounds on LPS-induced NO production in BV2 microglial cells. A: Anti-neuroinflammatory effect and cytotoxicity of ethanol extract; B: Anti-neuroinflammatory effect and cytotoxicity of PE extract; C: Anti-neuroinflammatory effect and cytotoxicities of compounds **2**, **5**, **6**, **12**, **14**, **16**, **17**, **21**, **23**, **25**. (Each bar represents the means ± SE of three independent experiments. Significance: $^{*}P < 0.001$ compared to LPS groups. $^{\#}P < 0.001$ compared to control groups; Ext-1: ethanol extract; Ext-2: PE extract; Con: control, NO: nitric oxide, LPS: lipopolysaccharide, MINO: minocycline).

**Table 2**
Effects of extracts and identified compounds from *B. striata* on NO production by LPS-activated BV2 microglia cells (Mean ± SEM).[d]

| Sample name | $IC_{50}$[a] | Sample name | $IC_{50}$[a] |
|---|---|---|---|
| Ext-1[b] | 4.3 ± 1.6 | **5** | 31.8 ± 2.1 |
| Ext-2[b] | 75.3 ± 2.1 | **6** | 66.1 ± 2.4 |
| Ext-3[b] | 72.7 ± 1.1 | **16** | 61.1 ± 2.6 |
| Ext-4[b] | > 100 | **17** | 58.8 ± 2.6 |
| **2** | 96.0 ± 2.6 | Minocycline[c] | 18.1 ± 2.8 |

[a] $IC_{50}$ (μg/mL for extracts and μM for compounds).
[b] Ext-1: total ethanol extract of *B. striata*; Ext-2: PE extract of ethanol extract of *B. striata*; Ext-3: EtOAc extract of ethanol extract of *B. striata*; Ext-4: *n*-BuOH extract of ethanol extract of *B. striata*.
[c] Minocycline was used as a positive control.
[d] Compounds **1**, **3**, **4**, **7**, **8**, **9**, **10**, **11**, **13**, **15**, **18**, **19**, **20**, **22**, **24**, **26**, **27**, **28**, **29** and **30** showed no inhibitory activity at tested concentrations (1, 10, 30, 100 μM), compounds **12**, **14**, **21**, **23**, **25** showed toxicities at 30 or 100 μM.

s), $\delta_C$ 55.9] was deduced to be linked at C-3 from the HMBC correlation of $\delta_H$ 3.75 (3-$OCH_3$) and 161.4 (C-3). A pyrrolidone moiety was established by 5.03 (1H, dd, $J$ = 9.0, 5.0 Hz, H-5″), 2.39 (1H, m, H-3″a), 2.50 (1H, m, H-3″b), 2.06 (1H, m, H-4″a) and 2.32 (1H, m, H-4″b), as well as the HMBC correlations of H-3″/C-5″, H-4″/C-2″ (C═O), H-5″/C-3″, and C-2″ (see Fig. 2). The HMBC correlations from $\delta_H$5.03 (H-5″) to $\delta_C$ 143.3 (C-1) and 161.4 (C-3), and from H-4″ to C-2 allowed the attachment of the 2-oxopyrrolidin-5-yl group at C-2 (See Fig. 1 and Table 1). Therefore, structure **23** was determined as dusuanlansin E (Fig. 2).

Due to negligible or weak optical rotations and Cotton effects in circular dichroism (CD) spectrum of compound **23**, chiral analysis and optical resolution of **23** were achieved by HPLC with a Chiral pack IF chiral column (*n*-hexane/EtOH, 85:15) at 1.0 mL/min, which afforded compounds **23a** and **23b**. The relative peak area ratio of **23a** to **23b** was approximately 1:1, the absolute configuration of **23a** was confirmed to be 5′*R*, and the absolute configuration of **23b** was confirmed as 5′*S* by combining the CD spectrum of **23a** (Fig. 3) [31]. Thus, the structures of **23a** and **23b** were assigned and named dusuanlansin E1 and dusuanlansin E2 (Fig. 3).

Compound **27** was obtained as a brownish powder. High resolution ESI-MS analysis of **27** yielded a quasi-molecular ion peak at *m/z* 405.1554 $[M-H]^-$ (calcd. 405.1555 for $C_{21}H_{25}O_8$), in accordance with the molecular formula, $C_{21}H_{26}O_8$. Its $^1$H NMR and $^{13}$C NMR spectral characteristics were very similar to those of analogous compound batatasin III. However, signals of 4.80 (H-1″) in $^1$H NMR spectrum indicated the presence of a glucose substituent. This conclusion was also supported by signals of 100.4 (C-1″), 73.3 (C-2″), 76.7 (C-3″), 69.7 (C-4″), 77.0 (C-5″), 60.7 (C-6″). And glycosyl unit was shown to be located at C-3′ according to the long range correlations between 4.80 (1H, d, $J$ = 7.2 Hz, H-1″) and 157.5 (C-3′). According to the large coupling constant, the configuration was confirmed to be *β*. Acid hydrolysis of **27** produced glucose as the sole sugar identified on the basis of derivatization by comparing with an authentic sugar sample [14]. The structure of **27** was elucidated as 3-hydroxy-5-methoxybibenzyl-3′-*O*-*β*-D-glucopyranoside.

### 3.3. Neuroinflammatory activities of compounds 1–30 in LPS-induced BV2 cells

To investigate the anti-inflammatory effects of the extracts and isolated components (**1**–**30**), the inhibitory activities were evaluated by NO assay in LPS-induced BV2 cells. In order to avoid that the inhibitory activities exhibited by tested samples were due to their cytotoxicities, the viabilities of BV2 cells were measured using MTT methods before NO assays. Concentration higher than 10 μM used in our present study is mainly based on other reports in which the highest concentration of compounds was set to 100 μM or higher to investigate their anti-inflammation effect in BV2 cells [35,36]. Moreover, we also want to investigate compounds cytotoxic activities comprehensively in a wider range, thus we set the highest dose to 100 μM. As you mentioned the compounds are toxic at 100 μM which should not be used for anti-inflammation evaluation.

As shown in Fig. 4A, B and Table 2, the ethanol extract displayed remarkable inhibitory effects against NO production, with $IC_{50}$ value of 4.3 μg/mL and cytotoxicity was not observed at the all tested concentrations (1, 3, 10, 30, and 100 μg/mL). Of note, stilbenes-rich fraction, PE extract could decrease the production of NO with $IC_{50}$ value at 75.3 μg/mL without cytotoxicity observed in the experiment [the tested results of other portions, EtOAc ($IC_{50}$, 72.7 μg/mL) and *n*-BuOH extract ($IC_{50}$ > 100 μg/mL)]. Therefore, PE and ethanol extracts may have potential anti-inflammation activity, however PE is less active compared to ethanol extract (Fig. 4).

As shown in Fig. 4C, compounds **12**, **14**, **21**, **23**, **25** were toxic to cells at a concentration of 30 μM or 100 μM, which may affect their inhibitory effects on LPS-induced NO release. Taking the anti-inflammatory and cytotoxic activities into consideration, we found that compounds **2**, **5**, **6**, **16**, **17** can greatly inhibit the production of NO without showing cytotoxicity, with $IC_{50}$ values at 96.0, 31.8, 66.1, 61.1, 58.8 μM, respectively. Previous studies also reported that the effect of compound on NO release can reflect its anti-inflammation activity. However, this single assay may not be sufficient to indicate anti-inflammation activity of a compound. In future, we will further investigate the level of other pro-inflammatory mediators such as TNF-α, IL-6 and IL-1β, and explore the effect of the compound on signaling pathways related with inflammatory response to clarify its anti-inflammatory activity comprehensively.

On the corresponding references data (only some compounds): compounds 5 (50.2 μM), 14 (80.2 μM), and 25 (> 100 μM) [31,37]. And these results are also very close to our measured values. But there are few reports in the literature about SAR. Combining the activities with structures of the isolated components, brief structure-activity relationships could be suggested as follows. Firstly, the glycosidations of stilbenes were considered to be the negative factor for their anti-inflammatory activities, such as compounds **5** vs **27**, **29**, **30** (31.8 μM vs > 100 μM). Secondly, the presence of pyrrolidone moiety was detrimental to the inhibitory effects and increased the cytotoxicities, such as **5** vs **23** (31.8 μM vs 67.7 μM). In addition, as to compounds **2** and **6**, the presence of 3-methoxy was beneficial to the activity of dihydropinosylvi (**2**) with $IC_{50}$ values of 96.0 and 66.1 μM, respectively (Fig. 5).

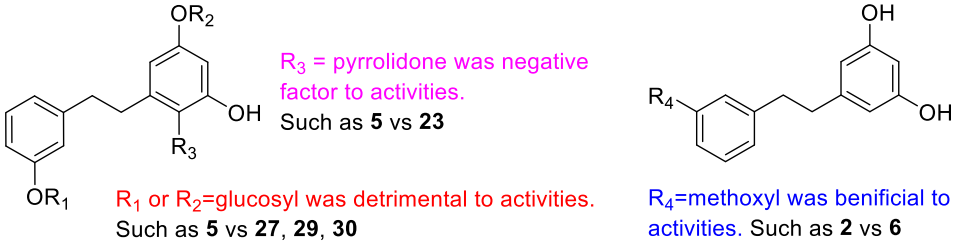


**Fig. 5.** The relationships of.

## 4. Conclusions

In conclusion, stilbenes were the characteristic components of the tubers of *B. striata* and the effective material basis of anti-neuroinflammatory effects. Among the isolated compounds **2**, **5**, **6**, **16**, **17** can greatly inhibit the production of NO without showing cytotoxicity. Moreover, the possible action mechanisms of anti-neuroinflammation need to be further studied.

## Declaration of Competing Interest

The authors declare no competing financial interests.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.103715. These data include the 1D, 2D NMR data and the spectra data of known compounds.

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