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Isolation, Structural Elucidation, Optical Resolution, and Antineuroinflammatory Activity of Phenanthrene and 9,10-Dihydrophenanthrene Derivatives from *Bletilla striata*

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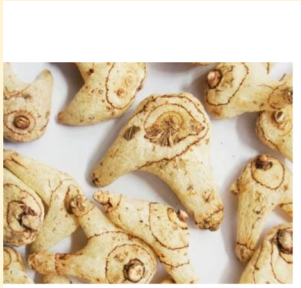
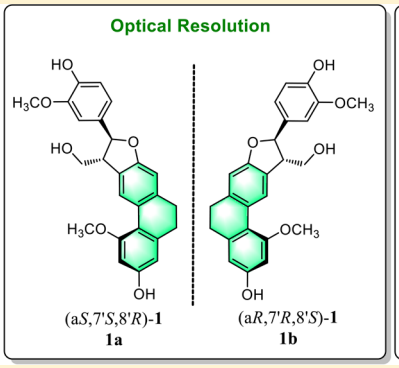
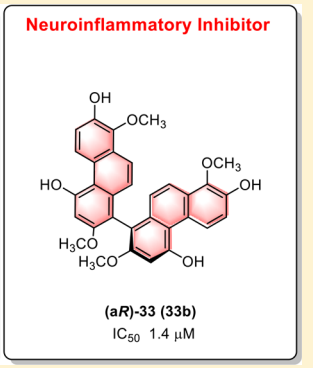
Supporting Information



Tubers of *Bletilla striata*





ABSTRACT: A phytochemical investigation of the aqueous EtOH extract of *Bletilla striata* tubers afforded 34 phenanthrene and 9,10-dihydrophenanthrene derivatives, including four new compounds, **1**−**4**. These compounds were identified using physicochemical analyses and various spectroscopic methods. Twelve of these compounds were resolved into their enantiomers, and the absolute configurations were determined by comparison of experimental and calculated ECD spectra. The antineuroinflammatory activities were evaluated by measuring the inhibition of nitric oxide production in lipopolysaccharide-stimulated BV-2 microglial cells. Compounds **7**, **32**, and **33** displayed inhibitory activities, with IC_{50} values of 1.9, 5.0, and 1.0 μM, respectively, suggesting that they should be subjected to development as potential inhibitors of neuroinflammation.

Phenanthrenes and 9,10-dihydrophenanthrenes are a class of natural products with limited distribution in the Orchidaceae, Dioscoreaceae, Juncaceae, and Combretaceae families.[1] These compounds display diverse and promising biological activities, including anti-inflammatory, antiproliferative, antimicrobial, spasmolytic, antiplatelet aggregative, antioxidant, and antiallergic activities.[2]

Bletilla striata (Thunb.) Reichb. f. (Orchidaceae) is a perennial herbaceous plant that is widely distributed in East Asia. The tubers have been applied in traditional Chinese medicine to relieve swelling, hematemesis, and traumatic bleeding, as well as to treat skin diseases, such as ulcers, sores, and chapped skin.[3] According to previous phytochemical studies, phenanthrenes and 9,10-dihydrophenanthrenes are characteristic constituents of *B. striata* that possess anti-inflammatory, antiproliferative, and antimicrobial activities.[2]

As part of our ongoing investigations aiming at discovery of natural inhibitors of neuroinflammation,[4,5] it was found that an extract from the tubers of *B. striata* exerted significant antineuroinflammatory activities on overactivated microglial cells. Thus, a chemical investigation was conducted and resulted in the isolation and structural elucidation of phenanthrene and 9,10-dihydrophenanthrene derivatives (**1**−**34**), including four new compounds (**1**−**4**). Twelve of these compounds were resolved into their enantiomers, and the absolute configurations were determined using experimental and calculated ECD data. The antineuroinflammatory activities of the isolated compounds were evaluated by measuring the inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated BV-2 microglial cells. The structure−activity relationship is also discussed.

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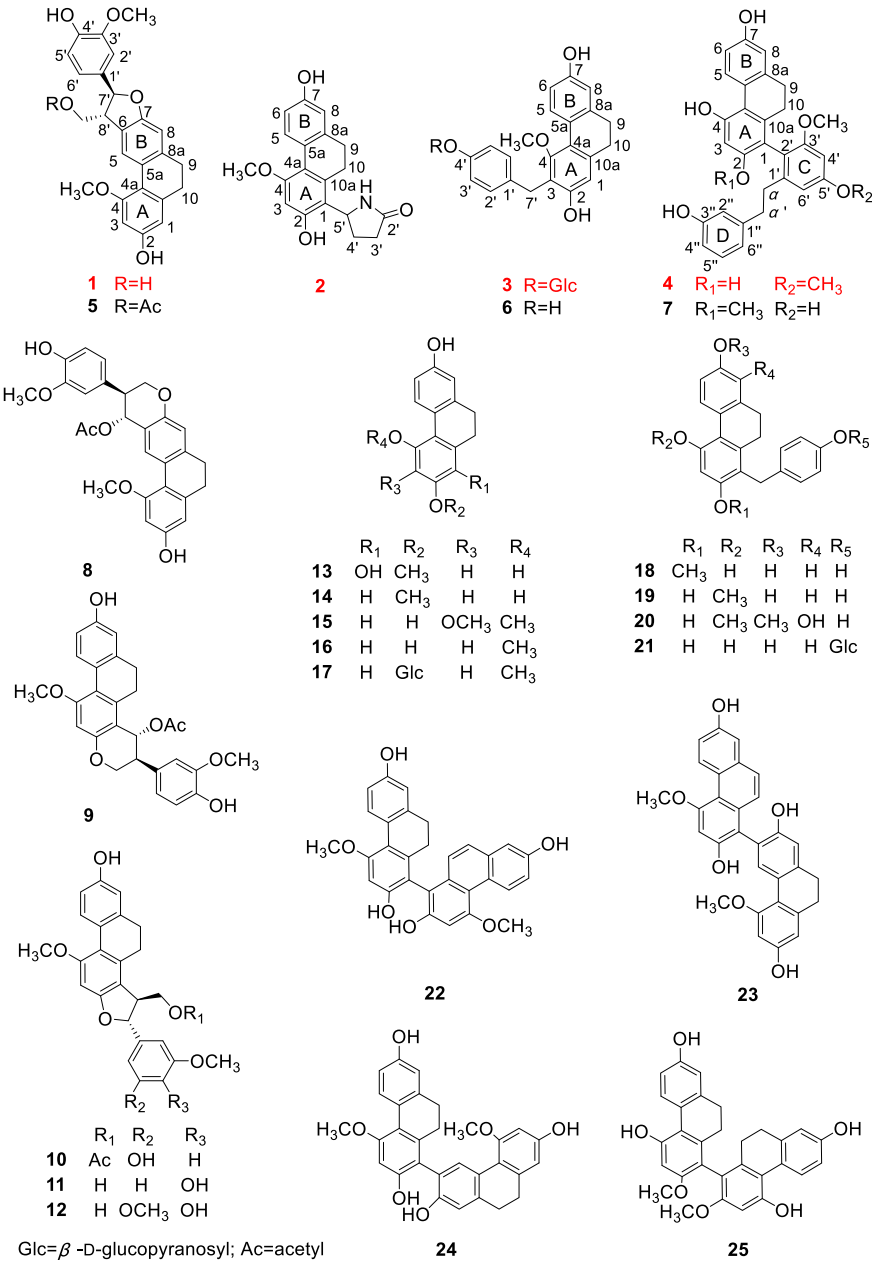

Figure 1. Structures of 9,10-dihydrophenanthrene derivatives.

RESULTS AND DISCUSSION

The tubers of *B. striata* were extracted by refluxing in 95% aqueous EtOH. The extract was concentrated and sequentially leached with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc fraction, which was enriched in phenanthrenes and 9,10-dihydrophenanthrenes, significantly decreased NO accumulation in LPS-stimulated BV-2 microglial cells (IC_{50} 72.7 μg/mL). Further isolation and purification were performed using multiple chromatographic methods to afford 34 compounds. The structures of the following known compounds were identified using comparison of observed and reported physicochemical data: shanciol H (**5**),[6] 3-(4-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol (**6**),[7] phochinenin K (**7**),[8] bletilol C (**8**),[9] bletilol B (**9**),[9] pleionesin C (**10**),[10] (2,3-*trans*)-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-10-methoxy-2,3,4,5-tetrahydrophenanthro[2,1-*b*]furan-7-ol (**11**),[11] cyrtonesin B (**12**),[12] 1,4,7-trihydroxy-2-methoxy-9,10-dihydrophenanthrene (**13**),[13] lusianthridin (**14**),[14] erianthridin (**15**),[15] coelonin (**16**),[16] shancigusin G (**17**),[17] shancidin (**18**),[18] 1-(4-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol (**19**),[19] 1-(4-hydroxybenzyl)-4,7-dimethoxy-9,10-dihydrophenanthrene-2,8-diol (**20**),[20] 2,7-dihydroxy-l-(4′-hydroxybenzyl)-9,10-dihydrophenanthrene-4′-*O*-glucoside (**21**),[9] blestriarene B (**22**),[21] 4,4′-dimethoxy-9,10-dihydro-(6,10-biphenanthrene)-2,2′,7,7′-tetraol (**23**),[11] gymconopin C (**24**),[12] blestriarene A (**25**),[21] flavanthrinin (**26**),[22] 4-methoxyphenanthrene-2,3,7-triol (**27**),[23] nudol (**28**),[24] 1-(4-hydroxybenzyl)-4,7-dimethoxyphenanthrene-2-ol (**29**),[25] 1-(4-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol (**30**),[22] 1-(4-hydroxybenzyl)-4,7-dimethoxyphenanthrene-2,8-diol (**31**),[25] bleformin F (**32**),[26] 4,8,4′,8′-tetramethoxy-(1,1′-biphenanthrene)-2,7,2′,7′-tetrol (**33**),[11] and monbarbatain A (**34**)[13] (Figures 1 and 2). Four new compounds (**1**−**4**) were named

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bletillatins A–D, respectively, and their detailed structures were elucidated as described below.

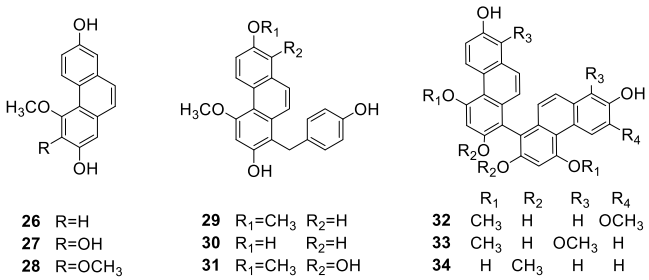

Figure 2. Structures of the phenanthrene derivatives.

Bletillatin A (**1**) was isolated as a brownish oil. The molecular formula $C_{25}H_{24}O_6$ was established based on the ^{13}C NMR data and a deprotonated molecular ion $[M - H]^-$ at m/z 419.1477 (calcd 419.1500 for $C_{25}H_{23}O_6$) in the HRESIMS data. The ^1H NMR spectrum displayed characteristic resonances for two methylene groups at δ_H 2.63 (2H, m, $H_{a,b}$-9) and 2.64 (2H, m, $H_{a,b}$-10), which revealed the presence of a 9,10-dihydrophenanthrene skeleton (Table 1). A pair of *meta*-coupled aromatic proton resonances at δ_H 6.31 (1H, d, J = 2.3 Hz, H-1) and δ_H 6.40 (1H, d, J = 2.3 Hz, H-3) was assigned to the A-ring based on the HMBC correlations from H-1 to C-10 and C-4a and from H-3 to C-4a (Figure 3). A pair of aromatic proton resonances at δ_H 6.67 (1H, s, H-8) and 8.06 (1H, s, H-5) was assigned to the tetrasubstituted B-ring based on the HMBC correlations from H-5 to C-4a and C-8a and from H-8 to C-5a and C-9. In addition, a set of ABX-type aromatic proton resonances was

Table 1. ^1H (600 MHz) and ^{13}C (150 MHz, Methanol-d_4) Data for Compounds 1–4

	1		2		3		4	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	6.31, d (2.3)	108.3		118.1	6.52, s	111.6		116.3
2		157.6		156.8		155.6		158.9
3	6.40, d (2.3)	99.3	6.46, s	99.6		121.8	6.56, s	99.1
4		159.0		158.2		158.1		154.9
4a		140.3		117.5		120.3		141.4
5	8.06, s	125.8	7.88, d (8.5)	130.4	8.04, d (9.1)	128.9	8.04, d (8.6)	130.3
5a		142.0		126.0		126.1		140.7
6		125.4	6.59, dd (8.5, 2.7)	113.5	6.64, dd (9.1, 2.8)	115.2	6.62, dd (8.6, 2.3)	113.5
7		159.1		156.2		156.6		156.0
8	6.67, s	109.0	6.62, d (2.7)	114.4	6.63, br s	114.3	6.58, d (2.3)	114.7
8a		127.3		140.6		140.6		117.3
9	2.63, m	31.6	2.59[a]	30.9	2.63[a]	31.2	2.57, m	28.4
10	2.66, m	31.8	2.79, dd (14.3, 6.5); 2.70, dd (14.3, 7.1)	27.2	2.63[a]	31.4	2.50, m	31.7
10a		116.9		140.3		139.8		126.6
1′		135.4		181.3		134.5		144.7
2′	6.94, d (1.9)	110.4			7.19, d (8.7)	130.5		117.3
3′		149.5	2.59;[a] 2.45[a]	32.3	6.95, d (8.7)	117.4		160.3
4′		147.4	2.45;[a] 2.28 m	27.5		157.1	6.36 d (2.9)	98.2
5′	6.76, d (8.2)	116.1	5.36, dd (8.7, 6.0)	53.2	6.95, d (8.7)	117.4		158.2
6′	6.83, dd (8.2, 1.9)	119.6			7.19, d (8.7)	130.5	6.37d (2.9)	109.2
7′	5.49, d (5.5)	88.7			3.96, s	27.4		
8′	3.46, dd (7.7, 5.5)	55.2						
9′	3.84, dd (11.0, 5.5); 3.76, dd (11.0, 7.7)	65.6			Glc			
1″					4.83, d (7.6)	102.5		145.2
2″					3.41, t (7.6)	75.0	6.39 d (2.9)	116.1
3″					3.42[a]	78.0		158.2
4″					3.36, m	71.4	6.49, dd (8.0, 2.0)	113.6
5″					3.38, m	78.1	6.93, t (8.0)	130.2
6″					3.85, dd (12.0, 2.0); 3.65, dd (12.0, 5.3)	62.5	6.38, dd (8.0, 2.9)	120.7
α							2.27, t (6.7)	38.1
α′							2.47, t (6.7)	37.7
3′-OCH_3	3.81, s	56.4					3.63, s	55.9
5′-OCH_3							3.89, s	56.0
4-OCH_3	3.82, s	55.9	3.79, s	55.8	3.28, s	60.3		

[a]Overlapped resonances.

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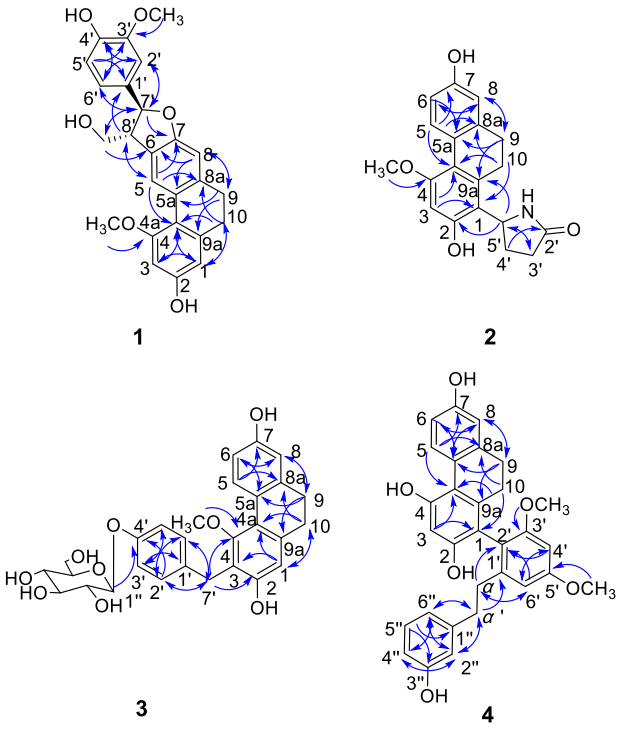

Figure 3. Key HMBC correlations of compounds **1**−**4**.

observed at δ_H 6.76 (1H, d, J = 8.2 Hz, H-5′), 6.83 (1H, dd, J = 8.2, 1.9 Hz, H-6′), and 6.94 (1H, d, J = 1.9 Hz, H-2″), as well as the resonances due to a three-carbon unit, including an oxymethine at δ_H5.49 (1H, d, J = 5.5 Hz, H-7′), a methine at δ_H 3.46 (1H, dd, J = 7.7, 5.5 Hz, H-8′), and an oxymethylene at δ_H 3.84 (1H, dd, J = 11.0, 5.5 Hz, H_a-9′) and δ_H 3.76 (1H, J = 11.0, 7.7 Hz, H_b-9′). The connection of the aromatic ring to C-7′ was deduced from the HMBC correlations from H-7′ to C-2′ and C-6′ and from H-8′ to C-1′. Furthermore, the connectivity of C-8′ and C-6 was deduced from the HMBC correlations from H-8′ to C-5 and C-7, and the connectivity of C-7′ and C-7 through an ether linkage was based on the HMBC correlations from H-7′ to C-7. Finally, two methoxy moieties that resonated at δ_H 3.81 (3H, s, 3′-OCH_3) and 3.82 (3H, s, 4-OCH_3) were assigned to C-3′ and C-4 by the NOESY correlations between 3′-$OC\underline{H}_3$/H-2′ and 4-$OC\underline{H}_3$/H-3, respectively. The HMBC correlations from 3′-$OC\underline{H}_3$(δ_H 3.81) and H-2′ (δ_H 6.95) to C-3′ (δ_C 149.5) and from 4-$OC\underline{H}_3$ (δ_H 3.82) and H-3 (δ_H 6.40) to C-4 (δ_C 159.0) were used to confirm the assignments. The 7′,8′-*trans* relative configuration was assigned via the NOESY correlations between H-7′/$H_{a,b}$-9′, H-8′/H-2′, and H-8′/H-6′ (Figure 4). The coupling constant ($J_{H\text{-}7',H\text{-}8'}$ = 5.5 Hz) also supported the conclusion.[24]

Compound **1** was a racemic mixture based on the specific rotation value ($[\alpha]^{20}_D$ = 0). Considering the axial chirality of the biphenyl bond, four enantiomerically pure compounds were possible, namely, (a*S*,7′*S*,8′*R*)-, (a*R*,7′*R*,8′*S*)-, (a*S*,7′*R*,8′*S*)-, and (a*R*,7′*S*,8′*R*)-**1** (Figure 5A). The resolution of compound **1** was achieved using chiral-phase column chromatography to afford two well-separated peaks (**1a** and **1b**) in a peak area ratio of 1:1 (Figure 5B). Compounds **1a** and **1b** are a pair of enantiomers since they showed opposite specific rotations and antipodal ECD curves. The absolute configurations of compounds **1a** and **1b** were defined by comparing the experimental and calculated ECD spectra (Figure 5C). The calculated ECD spectra of (a*S*,7′*S*,8′*R*)-**1** and (a*R*,7′*R*,8′*S*)-**1** closely matched the experimental data for compounds **1a** and **1b**, respectively, particularly the Cotton effects (CEs) in the region of 205−250 nm, which showed different patterns compared to their diastereomers. Based on the aforementioned evidence, bletillatin A (**1**) was elucidated as an enantiomeric mixture of (a*S*,7′*S*,8′*R*)- and (a*R*,7′*R*,8′*S*)-2-hydroxy-4-methoxy-7′-(4-hydroxy-3-methoxyphenyl)-8′-hydroxymethyl-7′,8′,9,10-tetrahydrofuro[2,3-*b*]phenanthrene.

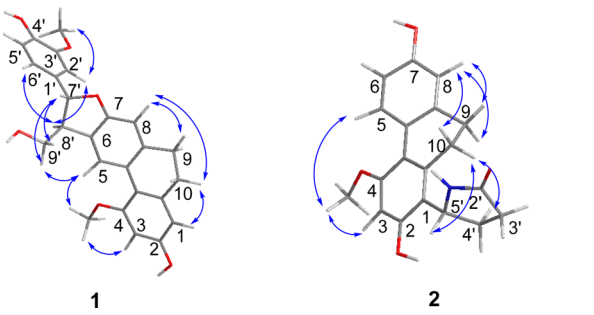

Figure 4. Key NOESY correlations of compounds **1** and **2**.

Bletillatin B (**2**) was isolated as a brownish oil, and the molecular formula was determined to be $C_{19}H_{19}NO_4$ by the protonated molecular ion peak at m/z 326.1375 $[M + H]^+$ observed in the HRESIMS data (calcd 326.1387 for $C_{19}H_{20}NO_4$) and the ^{13}C NMR data. Compound **2** was also a 9,10-dihydrophenanthrene derivate based on the characteristic resonances of two methylene groups at δ_H 2.59 (2H, m, $H_{a,b}$-9), 2.70 (1H, dd, J = 14.3, 7.1 Hz, H_a-10), and 2.79 (1H, dd, J = 14.3, 7.1 Hz, H_b-10). In the ^1H NMR data, an aromatic proton resonance at δ_H 6.46 (1H, s, H-3) assigned to the A-ring and a set of ABX-type aromatic proton resonances [δ_H 6.59 (1H, dd, J = 8.5, 2.7 Hz, H-6), 6.62 (1H, d, J = 2.7 Hz, H-8), 7.88 (1H, d, J = 8.5 Hz, H-5)] assigned to the B-ring were deduced from the HMBC correlations from H-3 to C-4a, H-5 to C-4a and C-8a, and H-8 to C-5a and C-9. A methoxy moiety was assigned to C-4 by the NOESY correlation between 4-$OC\underline{H}_3$ and H-3. In addition, HMBC correlations were observed from 4-OCH_3 (δ_H 3.79) to C-4 (δ_C 158.2). The presence of a 5′-substituted pyrrolidone moiety was suggested by the typical ^{13}C NMR resonances [δ_C 181.3 (C-2′), 32.3 (C-3′), 27.5 (C-4′), and 53.2 (C-5′)], and it was located at C-1 of the 9,10-dihydrophenanthrene moiety by the HMBC correlations from $H_{a,b}$-4′ to C-1 and from H-5′ to C-2 and C-9a. The specific rotation of compound **2** was zero, suggesting that it was a racemic mixture. Chiral-phase column chromatography yielded two well-separated peaks (**2a** and **2b**) in a ratio of approximately 1:1 (Figure 6A and B). The ECD spectrum of compound **2a** showed typical CEs for (a*S*)-9,10-dihydrophenanthrenes, namely, positive CEs in the region of 250−325 nm (Figure 6C).[8] Meanwhile, the antipodal ECD curves suggested the a*R* configuration for compound **2b**. The configuration of C-5′ could not be determined from either the NOESY correlations or calculated ECD spectra. Thus, the structure of bletillatin B (**2**) was elucidated as an enantiomeric mixture of (a*S*)- and (a*R*)-2,7-dihydroxy-4-methoxy-1-(2′-oxopyrrolidin-5′-yl)-9,10-dihydrophenanthrene.

Bletillatin C (**3**) was isolated as a brownish powder, and the molecular formula was determined to be $C_{28}H_{30}O_9$ by the ammonium adduct ion peak at m/z 528.2246 $[M + NH_4]^+$ observed in the HRESIMS data (calcd 528.2234 for $C_{28}H_{34}NO_9$). In the ^1H and ^{13}C NMR data, compound **3** displayed superimposable resonances assignable to the methyl-

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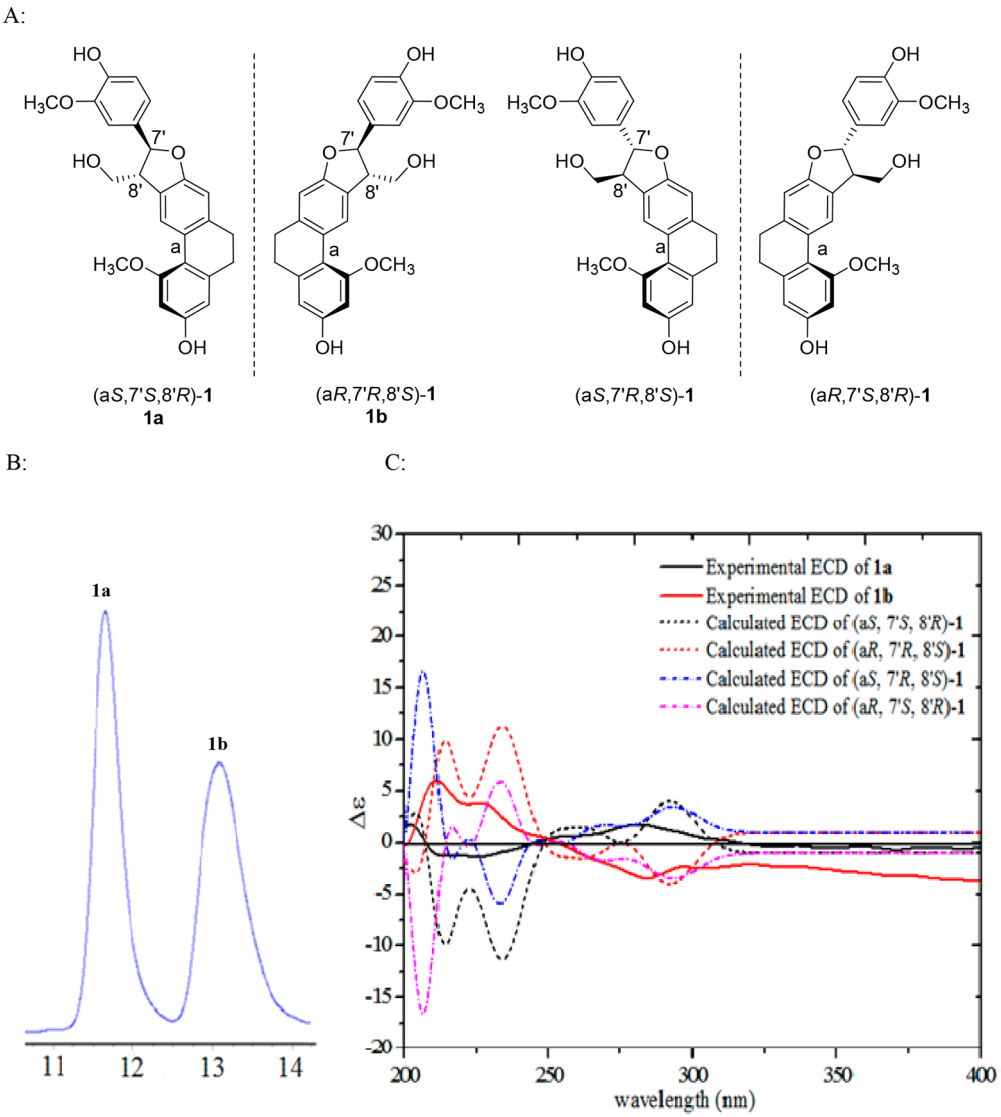

Figure 5. Chiral-phase resolution of compound **1**. (A) Four possible enantiomers. (B) Chiral-phase HPLC chromatograph. (C) Experimental and calculated ECD spectra.

ene groups and B-ring, similar to compound **2**, but differences were observed in the A-ring resonances.[6] An aromatic proton singlet at δ_H 6.52 (1H, s, H-1) was assigned to H-1 by the HMBC correlations from H-1 to C-4a and C-10. The presence of a 4-*O*-*β*-D-glucopyranosylbenzyl moiety was deduced from the resonances for a methylene [δ_H 3.96 (2H, s, H_2-7′), δ_C 27.4 (C-7′)], an AA′XX′-type aromatic ring [δ_H 6.95 (2H, d, J = 8.7 Hz, H-3′,5′), 7.19 (2H, d, J = 8.7 Hz, H-2′,6′)], and a *β*-glucopyranosyl moiety with the anomeric resonances at δ_H 4.83 (1H, d, J = 7.6 Hz, H-1″). Upon acid hydrolysis, the compound produced D-glucose, the absolute configurations of which were confirmed by an HPLC analysis of its thiocarbamate derivative and a comparison with the standard D-glucose.[28] The assignment of a methoxy group at C-4 was accomplished based on the HMBC correlations from 4-OC$\underline{H}_3$ and H-7′ to C-4. Thus, the structure of bletillatin C (**3**) was determined as 2,7-dihydroxy-3-(4-*O*-*β*-D-glucopyranosylbenzyl)-4-methoxy-9,10-dihydrophenanthrene.

Bletillatin D (**4**) was isolated as a brownish powder, and its molecular formula was established as $C_{30}H_{28}O_6$ based on the presence of a protonated molecular ion at m/z 485.1957 [M + H]$^+$ in the HRESIMS spectrum (calcd 485.1959 for $C_{30}H_{29}O_6$). The ^{1}H NMR data for compound **4** revealed resonances of four methylene groups [δ_H 2.27 (2H, t, J = 6.7 Hz, H_2-*α*), 2.47 (2H, t, J = 6.7 Hz, H_2-*α*′), 2.50 (2H, m, $H_{a,b}$-10), and 2.57 (2H, m, $H_{a,b}$-9)], suggesting a heterodimeric structure. The assignments of a proton singlet at δ_H 6.56 (1H, s, H-3) of the A-ring of the 9,10-dihydrophenanthrene moiety and a set of AMX-type proton resonances [δ_H 6.58 (1H, d, J = 2.3 Hz, H-8), 6.62 (1H, dd, J = 8.6, 2.3 Hz, H-6), and δ_H 8.04 (1H, d, J = 8.6 Hz, H-5)] of the B-ring were deduced from the HMBC correlations from H-3 to C-4a, from $H_{a,b}$-10 to C-1, from H-5 to C-4a and C-8a, and from H-8 to C-5a and C-9. On the other hand, the structure of the bibenzyl group was established by the presence of a set of *meta*-coupled aromatic protons [δ_H 6.36 (1H, d, J = 2.9 Hz, H-4′), 6.37 (1H, d, J = 2.9 Hz, H-6′)] (C ring) and a set of aromatic proton resonances [δ_H 6.38 (1H, dd, J = 8.0, 2.9 Hz, H-6″), 6.39 (1H, d, J = 2.9 Hz, H-2″), 6.49 (1H, dd, J = 8.0, 2.0 Hz, H-4″), 6.93 (1H, t, J = 8.0 Hz, H-5″)] for a *meta*-substituted phenyl moiety (D-ring), as well as the key HMBC correlations from H_2-*α* to C-6′ and C-2′, from H-4′ to C-2′ and C-6′, from H_2-*α*′ to C-2″,6″, from H-5″ to C-1″, and from H-4″ to C-2″ and C-6″. Two

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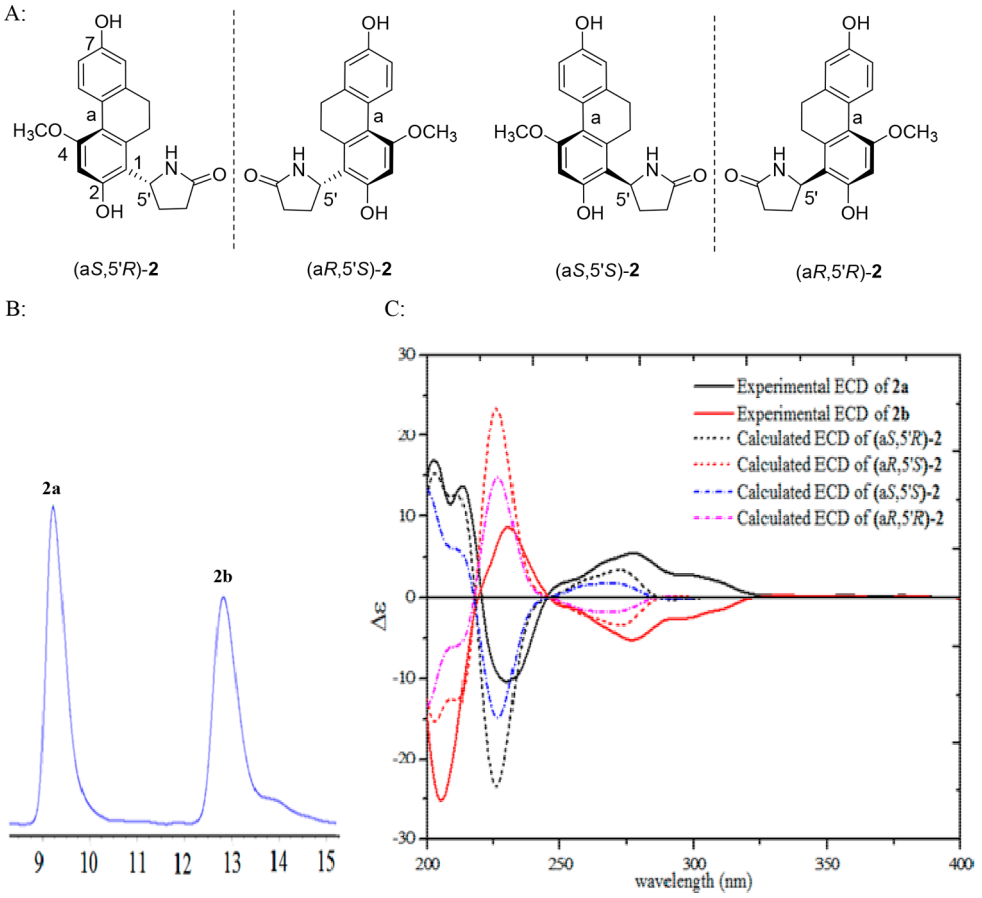

Figure 6. Chiral-phase resolution of compound **2**. (A) Four possible enantiomers. (B) Chiral-phase HPLC chromatograph. (C) Experimental and calculated ECD spectra.

methoxy groups were assigned to C-3′ and C-5′ by the HMBC correlations from H-4′ to C-3′ and C-5′; from H-6′ to C-2′ and C-5′, from 3′-OC$\underline{H}_3$ to C-3′, and from 5′-OC$\underline{H}_3$ to C-5′. Although HMBC correlations did not occur across the 9,10-dihydrophenanthrene and bibenzyl moieties, the connection of two quaternary carbons C-1 and C-2′ was reasonable by assignment of their positions using the HMBC correlations from H-3 and $H_{a,b}$-10 to C-1 and from H-4, H-6, and H-α' to C-2′. Thus, the structure of bletillatin D (**4**) was determined as 2,4,7-trihydroxy-1-{6-[2-(3-hydroxyphenyl)]ethyl-2,4-dimethoxy}-phenyl-9,10-dihydrophenanthrene.

In addition to compounds **1** and **2**, the chiral-phase resolution of compounds **5**, **8**−**11**, **13**, **22**, **25**, **33**, and **34** was also successfully achieved, and the absolute configurations of enantiopure compounds were determined using the experimental and calculated ECD spectra (Supporting Information).

The antineuroinflammatory activities of all isolated compounds were evaluated using Griess assays in overactivated BV-2 microglial cells stimulated with LPS. A known inhibitor of neuroinflammation, minocycline, was used as the positive control (IC_{50} 27.2 μM). The dimeric phenanthrene compound **33** showed the most potent antineuroinflammatory activity, with an IC_{50} value of 1.0 μM, followed by compound **32** (IC_{50} 5.0 μM). However, compound **34** exhibited no activity. The heterodimeric compound **7** also showed potent activity (IC_{50} 1.9 μM). A comparison of compounds **7** and **4** (IC_{50} 60.7 μM), the structures of which only differ in the presence of a methoxy group at C-5′ or C-2, revealed that compound **7** showed more potent activity than compound **4**. In addition, compounds **5**, **12**, **16**, **18**, **21**, **23**, and **24** exhibited weak inhibitory activities, with IC_{50} values ranging from 12.0 to 30.0 μM. Accounting for the chiralities of 9,10-dihydrophenanthrenes, enantiopure compounds (**33a** and **33b**, Figure 7) were evaluated for their

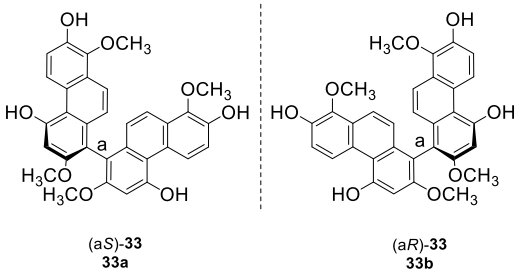

Figure 7. Chemical structures of **33a** and **33b**.

antineuroinflammatory activities. Results indicated that compounds **33a** and **33b** exhibited significant inhibitory effects with IC_{50} values of 6.9 and 1.4 μM, respectively (minocycline was also used as the positive control, with IC_{50} value at 35.7 ± 1.1 μM), and (a*R*)-**33** (compound **33b**) was more active than (a*S*)-**33** (compound **33a**). Based on these results, phenanthrenes and 9,10-dihydrophenanthrenes show potential for further development as neuroinflammatory inhibitors. Furthermore, the chiralities of bioactive isomers have an influence on antineuroinflammatory activities.

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EXPERIMENTAL SECTION

General Experimental Procedures. An MCP 200 polarimeter from Anton Paar (Graz, Austria) was used to measure optical rotations. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AVIII HD-600 spectrometer in δ (ppm) referenced to tetramethylsilane. HRESIMS data were collected using an Agilent HPLC 1290 Infinity and a 6540 UHD Accurate TOF-Q mass spectrometer. ECD spectra were recorded using a Bio-Logic Science MOS-450 spectrometer. The silica gel (SiO_2: 50−74 μm) used for column chromatography was produced by Qingdao Ocean Chemical Group Co. (Qingdao, People's Republic of China). The ODS (50 μm) used for column chromatography was produced by the YMC Company (Kyoto, Japan). HPLC separations were done by using a YMC ODS-A C_{18} column (5 μm, 250 mm × 20 mm) with a Shimadzu SPD-20A UV detector and a Shimadzu LC-20AR pumping system. Sigma-Aldrich (MO, USA) supplied the deuterium reagents, DMSO-d_6, and methanol-d_4. Chiral-phase separations were performed using the chiral-phase chromatographic column CHIRALPAK IF (Dacicel Chiral pak IF: CHIRALPAK IF analytic column, 4.6 × 250 mm, 4.6 μm particles, Daicel Chemical Industries, Tokyo, Japan). Tianjin DaMao provided all reagents for the chromatographic or analytical experiments (Tianjin, People's Republic of China).

Plant Material. Plant material was purchased from Shaanxi Tasly Plant Medicine LLC, collected from Shaanxi Province, People's Republic of China, in November 2016, and identified by Professor Yingni Pan (Shenyang Pharmaceutical University). A voucher specimen (20160911) is deposited in the herbarium of Shenyang Pharmaceutical University.

Extraction and Isolation. The dried tubers of *B. striata* (16 kg) were extracted using 95% aqueous EtOH (×3, for 3 h/extraction) using reflux and then successively leached with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc fraction (400 g) was subjected to silica gel column chromatography (CC) with a gradient of CH_2Cl_2−MeOH to produce 10 fractions (Fr.1−Fr.10). Fr.2 was loaded on the ODS column, eluted with MeOH−H_2O, and purified using HPLC to yield compounds **28** (12.3 mg), **26** (72.3 mg), **29** (21.6 mg), **15** (23.2 mg), and **20** (17.2 mg). Fr.3 was separated using silica gel CC with gradient elution using petroleum ether−EtOAc to yield Fr.3-1 and 3-2. These subfractions were separated on an ODS column and washed with a gradient of MeOH−H_2O to yield compounds **27** (15.1 mg), **6** (21.2 mg), **2** (16.1 mg), **30** (66.2 mg), and **31** (31.2 mg). Fr.4 was loaded onto an ODS column, eluted with MeOH−H_2O, and further purified by HPLC equipped with an ODS column to give compounds **10** (13.2 mg) and **5** (22.7 mg). Fr.6 was separated on an ODS column (MeOH−H_2O) and then purified using HPLC to afford compounds **32** (3.2 mg), **4** (22.2 mg), and **7** (31.2 mg) after elution with MeOH−H_2O. Fr.7 was subjected to silica gel chromatography with gradient elution using CH_2Cl_2−MeOH and purified by HPLC with MeOH−H_2O to give compounds **1** (12.6 mg), **9** (42.3 mg), **8** (33.2 mg), and **12** (26.5 mg). Fr.9 was isolated using an ODS column and eluted with MeOH−H_2O to produce five subfractions, which were further purified by RP-C_{18} HPLC to give compounds **14** (23.2 mg), **13** (61.4 mg), **16** (87.2 mg), **18** (67.3 mg), **19** (47.2 mg), **3** (14.7 mg), **21** (21.2 mg), **17** (29.3 mg), **22** (43.2 mg), and **23** (11.6 mg), with CH_3CN−H_2O. Fr.10 was subjected to an ODS column and purified by HPLC with CH_3CN−H_2O to give compounds **24** (22.5 mg), **25** (50.2 mg), **33** (31.5 mg), **34** (42.3 mg), and **11** (15.4 mg).

*Bletillatin A (**1**):* brownish oil (MeOH); $[\alpha]^{20}_D$ 0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 282 (3.30) nm, 210 (4.60) nm; ^{1}H and ^{13}C NMR data (see Table 1); HRESIMS *m*/*z* 419.1477 $[M-H]^-$ (calcd for $C_{25}H_{23}O_6$, 419.1500).

*Bletillatin B (**2**):* brownish oil (MeOH); $[\alpha]^{20}_D$ 0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 280 (2.61) nm, 212 (3.39) nm; ^{1}H and ^{13}C NMR data (see Table 1); HRESIMS *m*/*z* 326.1375 $[M + H]^+$ (calcd for $C_{19}H_{20}NO_4$, 326.1387).

*Bletillatin C (**3**):* brownish powder (MeOH); $[\alpha]^{20}_D$ −2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 282 (2.15) nm, 212 (3.60) nm; ^{1}H and ^{13}C NMR data (see Table 1); HRESIMS *m*/*z* 528.2246 $[M + NH_4]^+$ (calcd for $C_{28}H_{34}NO_9$, 528.2234).

*Bletillatin D (**4**):* brownish powder (MeOH); $[\alpha]^{20}_D$ 0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 280 (2.60) nm, 211 (3.45) nm; ^{1}H and ^{13}C NMR data (see Table 1); HRESIMS *m*/*z* 485.1957 $[M + H]^+$ (calcd for $C_{30}H_{29}O_6$, 485.1959).

Acid Hydrolysis of Compound 3. Compound **3** (1.0 mg) was dissolved in 4 M HCl (1 mL) and heated at 90 °C for 2 h. The mixture was extracted with CH_2Cl_2 after cooling. The aqueous layer was evaporated to dryness. The residue was added to 3.0 mg of L-cysteine methyl ester, dissolved in pyridine (1 mL), and heated at 60 °C for 1 h. *o*-Tolylisothiocyanate (5 μL) was added to the solution, and the mixture heated for an additional 1 h. The retention time of the reaction mixture (thiocarbamate derivative, 18.25 min) was confirmed by a comparison with authentic D-glucose using HPLC. The HPLC was equipped with a Shimadzu SPD-20A UV/visible detector and a Shimpack ODS (H) kit (4.6 × 250 mm, 5 μm particle size) and analyzed at 35 °C (detection wavelength: 250 nm; flow rate: 1 mL/min). The mobile phase was CH_3CN−H_2O (25:75).[27,28]

Chiral-Phase Resolution. The resolution was conducted using a chiral-phase column (Dacicel Chiral Pak IF) at 1 mL/min (detector: UV detection, λ: 210 nm). Chiral-phase separation of compound **1** using HPLC with *n*-hexane−EtOH (80:20) afforded compounds **1a** (1.0 mg, t_R = 11 min) and **1b** (0.9 mg, t_R = 13 min).

*(aS,7′S,8′R)-Bletillatin A (**1a**):* $[\alpha]^{20}_D$ −26 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 214 (−0.76), and 282 (+2.27) nm.

*(aR,7′R,8′S)-Bletillatin A (**1b**):* $[\alpha]^{20}_D$ +26 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 211 (+10.47), and 284 (+1.08) nm.

Similarity, chiral-phase separation of compound **2** using HPLC with *n*-hexane−EtOH (75:25) afforded compounds **2a** (1.2 mg, t_R = 9 min) and **2b** (1.1 mg, t_R = 12 min).

*(aS)-Bletillatin B (**2a**):* $[\alpha]^{20}_D$ −25 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 213 (+13.71), 230 (−10.41), and 277 (+5.49) nm.

*(aR)-Bletillatin B (**2b**):* $[\alpha]^{20}_D$ +25 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 205 (−25.19), 231 (+8.72), and 277 (−5.22) nm.

Using the same methods, compounds **5**, **8**−**11**, **13**, **22**, **25**, **33**, and **34** were optically resolved with HPLC using a chiral-phase chromatographic column. The $[\alpha]^{20}_D$ data and ECD data of these compounds were measured (Supporting Information).

Cell Viability and NO Production. Cell viability and NO production were tested using MTT and Griess assays, respectively, as described in our previous study.[4,5]

Table 2. Inhibitory Activities of the Compounds on NO Production in LPS-Activated BV-2 Microglial Cells (Mean ± SEM)

compound	IC_{50} (μM)	compound	IC_{50} (μM)
4	60.7 ± 1.6	**24**	12.0 ± 1.6
5	24.8 ± 1.9	**25**	39.4 ± 2.4
6	34.6 ± 1.3	**27**	30.0 ± 1.7
7	1.9 ± 1.9	**29**	82.0 ± 2.0
12	18.5 ± 2.1	**30**	30.2 ± 2.2
15	93.5 ± 1.4	**32**	5.0 ± 1.8
16	15.2 ± 2.2	**33**	1.0 ± 1.8
20	49.0 ± 2.2	**33a**[a]	6.9 ± 1.4
21	17.9 ± 1.6	**33b**[a]	1.4 ± 1.2
22	46.8 ± 1.7	minocycline[b]	27.2 ± 1.7
23	22.1 ± 1.8		

[a]The data were afforded by the supplementary experiment according to the reviewer's advice using the method described here. Minocycline was also used as the positive control with an IC_{50} value of 35.7 ± 1.1 μM. [b]Minocycline was used as the positive control.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00291.

1D and 2D NMR and HRESIMS spectra of compounds **1**−**4**; optical resolution and determination of the structures of compounds **5**, **8**−**11**, **13**, **22**, **25**, **33**, and **34** (PDF)

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Notes

The authors declare no competing financial interest.

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