Sarglamides A–E, Indolidinoid-Monoterpenoid Hybrids with Anti-Neuroinflammatory Activity from a *Sarcandra* Species

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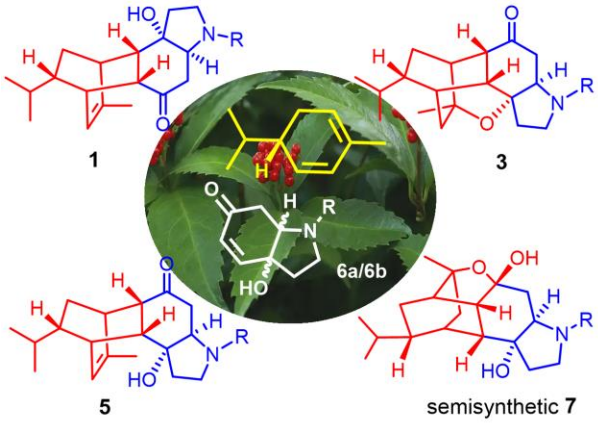
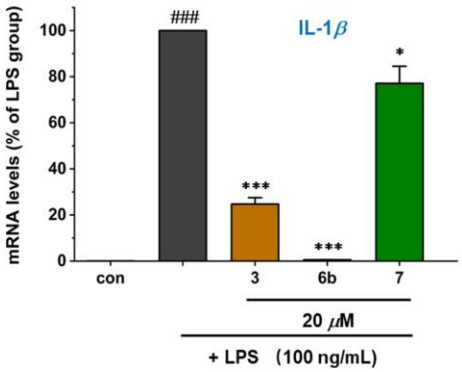
ABSTRACT: Sarglamides A–E (**1**−**5**), representing the first example of heterodimers of a *trans*-*N*-cinnamoylindolidinoid and an α-phelladrene derivatives, were isolated from *Sarcandra glabra* subsp. *brachystachys*. Particularly, compounds **4** and **5** possess unprecedented cage-like 6/6/5/6/5- and 6/6/6/6/5-fused pentacyclic scaffolds, respectively. Their structures were established by spectroscopic analysis, X-ray crystallography, quantum chemical calculations, and chemical conversions. Plausible biosynthetic pathways of **1**−**5**, involving the co-isolated enantiomers **6a** and **6b** were proposed. Compounds **3**–**7** showed inhibitory activity against lipopolysaccharide (LPS) induced inflammation in BV-2 microglial cells.



indolidinoid-monoterpenoid hybrids



anti-neuroinflammation

Keywords: *Sarcandra glabra* subsp. *brachystachys*; Indolidinoid-monoterpenoid hybrids; Novel skeletons; Structural elucidation; Anti-neuroinflammatory activity

Indolidinoids represent a class of structurally interesting alkaloids featuring a 6/5-fused bicyclic scaffold that commonly amidated to a cinnamoyl moiety.[1-3] These indolidinoids were reported to exist as pairs of enantiomers, probably because their biosynthesis from tetraketide precursors involves non-stereoselective cyclization processes.[1,3] The small group of indolidinoids were identified mainly from plants of family Annonaceae, showing moderate antimicrobial activity.[1-3]

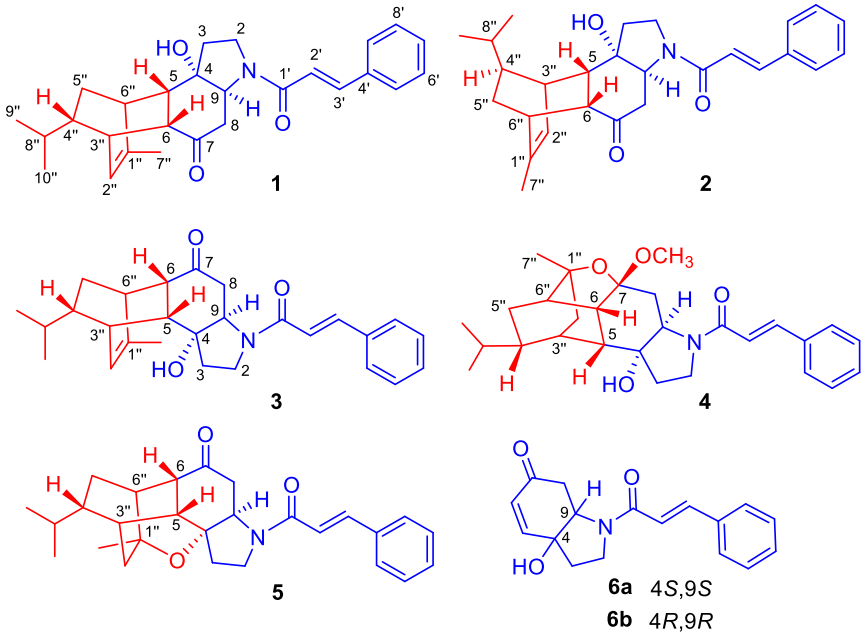


Figure 1. Structures of compounds **1**–**6**.

The plants of genus *Sarcandra* have long been used in Chinese folk medicine for treatments of inflammation and traumatic injuries, which are abundant with monoterpenoids, sesquiterpenoids, and dimeric sesquiterpenoids.[4-9] As a continued investigation on *Sarcandra* species for bioactive components,[4,8,9] five indolidinoid-monoterpenoid hybrids, sarglamides A–E (**1**–**5**), were isolated from *S. glabra* subsp. *brachystachys* (Figure 1). Compounds **1**–**5** are unprecedented conjugates biosynthetically formed of (*S*)-α-phelladrene and toussaintine C (**6**) by a [4 + 2] addition reaction and subsequent modifications. Their structures were established by spectroscopic analysis, X-ray

crystallography, quantum chemical calculations, and chemical conversions. Herein, we present a full account of the isolation, structural elucidation, biosynthetic pathways, chemical transformations, and anti-neuroinflammatory activity of these compounds.

■ RESULTS AND DISCUSSION

Sarglamide A (**1**), colorless crystals, presented a molecular formula of $C_{27}H_{33}NO_3$, according to the HRMS (ESI) ion at *m/z* 420.2544 $[M + H]^+$ and the ^{13}C NMR data, requiring 12 indices of hydrogen deficiency (IHDs). The characteristic NMR signals (Table S1) of one amide carbonyl group (δ_C 164.6), two *trans* disposed olefinic protons (δ_H 6.44 and 7.61, both d, J = 15.6 Hz), and one monosubstituted phenyl moiety, were indicative of a *trans*-*N*-cinnamoyl moiety in **1**.[2] Additionally, one keto carbonyl (δ_C 215.0) and one trisubstituted double bond (δ_C 123.4 and 143.2; δ_H 5.81) were verified based on the chemical shifts. These functionalities accounted for eight IHDs, suggesting compound **1** to be tetracyclic. An in-depth examination of the 1D and 2D NMR data revealed the planar structure of **1** was composed of subunits A and B. In unit A, three spin-spin coupling fragments of CH_2-2–CH_2-3, CH-5–CH-6, and CH_2-8–CH-9 were established by the ^1H–^1H COSY correlations (Figure 2), which were connected to a keto carbonyl, an oxygenated tertiary carbon, and a nitrogen atom, forming the indolidinoid structural fragment,[2] based on the HMBCs (Figure 2) from H-9 (δ_H 4.38) to C-2 (δ_C 43.7), C-3, C-4 (δ_C 76.7), C-5, and C-7 (δ_C 215.0); and H-6 and H_2-8 to C-7. The indolidinoid fragment amidated to the cinnamoyl moiety by the HMBC correlation of H-9/C-1′, constructing unit A as a *trans*-*N*-cinnamoylindolidinoid derivative. The remaining 10 carbons were assembled by the COSY correlations of H-2″/H-3″/H-4″/H_2-5″/H-

6″, H-4″/H-8″/H$_3$-9″, and H-8″/H$_3$-10″, and the HMBC correlations from H$_3$-7″ to C-1″ (δ_C 143.2), C-2″ (δ_C 123.4), and C-6″. These correlations furnished unit B as a cyclic monoterpenoid, structurally related to α-phelladrene.[10] Finally, COSY correlations of H-6/H-3″ and H-5/H-6″ indicated a doubly bonded linkage of C-5–C-6″ and C-6–C-3″ between units A and B, which was supported by the HMBCs from H-6 to C-2″, and H-5 to C-1″ and C-5″. The planar structure of **1** was thereby established as an unprecedented conjugate of an α-phelladrene and a *trans*-*N*-cinnamoylindolidinoid, featuring a novel 6/6/6/5-fused architecture.

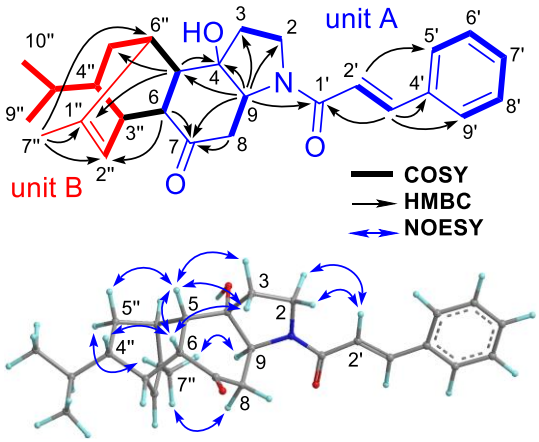


Figure 2. Selected 2D correlations of **1**.

The relative configuration of **1** was assigned by the NOESY data. The correlations (Figure 2) of H$_3$-7″/H-8α, H-9, and H-5″α indicated those protons to be co-facial, and they were assigned randomly as α-oriented. The olefin bridge at $\Delta^{1''}$ was thereby assigned as α-oriented. Accordingly, the NOESY correlations of H-6/H-4″, H-6/H-5, and H-5/H-5″β revealed these protons as being β-oriented. The hydroxy group at C-4 was α-oriented from the cross-peak of H-6/H-3. Finally, qualified crystals of **1** were obtained in MeOH, which established unequivocally its absolute configuration as 4*S*,5*S*,6*R*,9*S*,3″*S*,4″*S*,6″*S* [Figure 3, absolute structure parameter: –0.02 (13)].

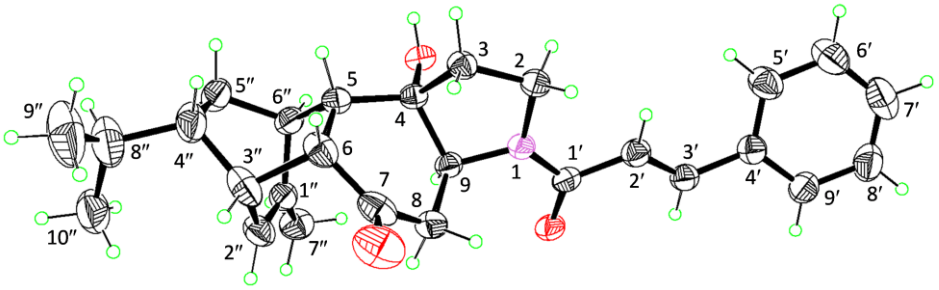


Figure 3. X-ray ORTEP drawing of **1**.

Sarglamide B (**2**) shared the same molecular formula of $C_{27}H_{33}NO_3$ as **1**, as inferred from the HRMS (ESI) and ^{13}C NMR data. Comparison of the ^{1}H and ^{13}C NMR data (Table S1) indicated **2** was a structural analogue of **1**. Units A and B of **2** were assigned individually as a *trans-N*-cinnamoylindolidinoid and an α-phelladrene derivatives, structurally identical to their counterparts in **1** by scrutiny of the 1D and 2D NMR data. The HMBCs (Figure S4) from H-3″ to C-4 (δ_C 77.0), and H-6 to C-1″ (δ_C 142.0) and C-5″, in combination with the COSY correlations (Figure S4) of H-6″/H-6 and H-3″/H-5, established the doubly linked bonds of C-6–C-6″ and C-5–C-3″ between units A and B. The NOESY correlations (Figure S4) of H-3β/H-6, H-5″β/H-6, and H-5/H-8″ assigned randomly those protons in a β-orientation. Accordingly, the cross-peaks of H-8α/H_3-7″, H-9/H-2″, and H-2″/H-4″ revealed they were α-oriented. The absolute configuration of **2** was assigned as shown, based on its closely compatible ECD data with that of **1** (Figure S8).

An initial interpretation of the NMR (Table S1) and MS data, indicated sarglamide C (**3**) was a structural congener of **2**. The planar structure of **3**, a hetero dimer of a *trans-N*-cinnamoylindolidinoid (unit A) and an α-phelladrene derivative (unit B) via bonds C-6–C-6″ and C-5–C-3″, was assigned the same as that of **2** by an in-depth investigation of the 1D and 2D NMR

data (Figure S5). The relative configuration of **3** was deduced from its NOESY data, of which the correlations (Figure S5) of H-8α/H_3-7″, H-9/H-2″, and H-2″/H-8″ assigned arbitrarily those protons in an α-orientation. The NOESY cross-peaks of H-4″/H-5 and H-6/H-3β revealed they were β-oriented. The closely mirror symmetric ECD curves of **3** (Figure S8) and **2**, suggested the absolute configuration of **3** to be 4*R*,5*R*,6*S*,9*R*,3″*S*,4″*S*,6″*S*.

Sarglamide D (**4**) was assigned a molecular formula of $C_{28}H_{37}NO_4$ with 11 IHDs, as deduced by the HRMS (ESI) and ^{13}C NMR data. The unit A of **4** was structurally related to the counterpart in **3**, with major differences occurring at C-7. Compound **4** contained a ketal/hemiketal carbon (δ_C 103.5) instead of the keto carbonyl in **3**, which was assignable to C-7 by the HMBCs (Figure S6) from H-5 and H-8 to C-7 (δ_C 103.5), and O$\underline{CH_3}$ (δ_H 3.19) to C-7. In unit B, a spin system of CH_2-2″–CH-3″–CH-4″–CH-8″ (CH_2-5″–CH-6″)–CH_3-9″ (CH_3-10″) was drawn with bold bonds from the COSY correlations (Figure S6). The fragment was connected to C-1″ and C-7″ with the aid of the HMBC correlations from H_3-7″ to C-1″, C-2″, and C-6″, furnishing the monocyclic monoterpenoid scaffold. Two linkage bonds of C-6–C-6″ and C-5–C-3″ between units A and B, were achieved from the HMBC correlations of H-5/C-2″ and H-6/C-1″, and the COSY correlations of H-6″/H-6 and H-3″/H-5. Finally, an extra ether bond between C-1″ (δ_C 82.8) and C-7 (δ_C 103.5), connecting the remaining two "loose ends", was the only possibility to satisfy the requirement for the remaining IHD, which was consistent with the chemical shifts and biogenetic consideration. Herein, the planar structure of **4** was established as a cage-like 6/6/5/6/5-fused pentacyclic complex of a *trans*-*N*-cinnamoylindolidinoid and an α-phelladrene derivative. The relative configuration of **4** was assigned shown, from the NOESY cross-peaks (Figure S6) of H-9/H-8α

and H-2″a; H-6/H-5″β and H-3β; H-5/H-4″; C$\underline{H_3}$O-7/H-6 and H-6″; and H_3-10″/H-2″b. Finally, the absolute configuration, 4*R*,5*R*,6*S*,7*R*,9*R*,1″*S*,3″*R*,4″*S*,6″*S* of compound **4**, was determined unequivocally by the comparison between the experimental ECD curve with the time-dependent density functional theory (TDDFT)[11] calculated results (Figure 4a).

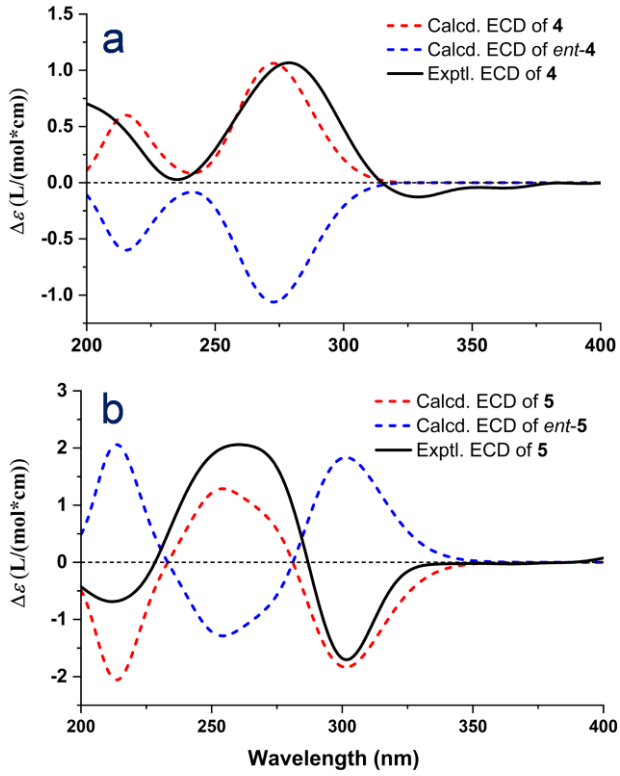


Figure 4. Experimental and calculated ECD spectra of **4** and **5**.

Comparison of the NMR data (Tables S1 and S2) indicated the structural components, units A and B of sarglamide E (**5**), were closely related to their counterparts in **3**, with the major differences being the presence of an extra oxygenated tertiary carbon and the lack of one double bond. The oxygenated tertiary carbon was assignable to C-1″ based on the HMBCs (Figure S7) from H_3-7″ and H-3″ to C-1″ (δ_C 75.7). The same doubly-linked bonds of C-6–C-6″ and C-5–C-3″ between units A and B in compound **5** as that of **3**, was deduced from the comparable HMBC and COSY

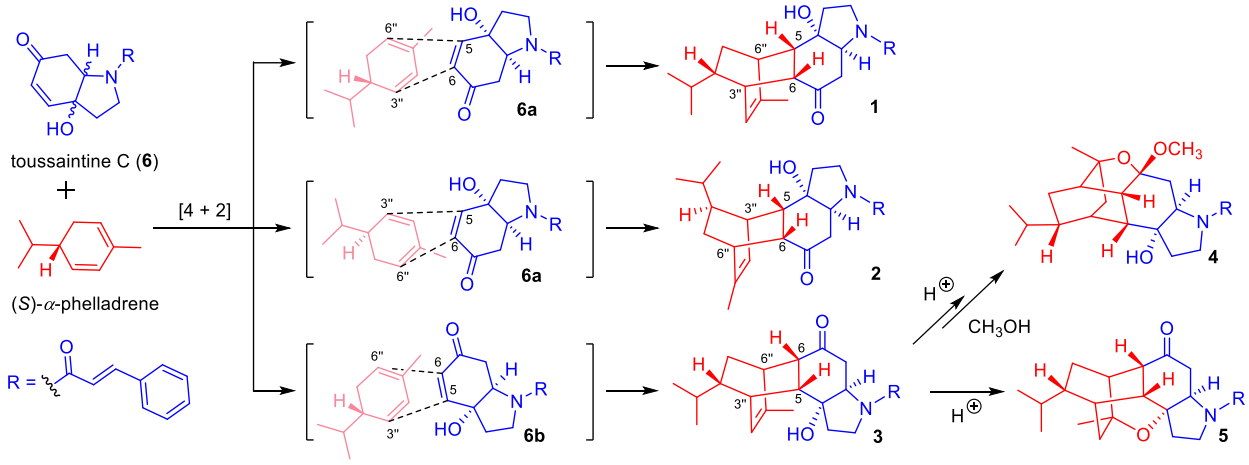
correlations (Figure S7). Finally, a pyran ring was formed by connecting C-1″ (δ_C 75.7) and C-4 (δ_C 81.8) through an oxygen atom, which was supported by the chemical shifts and molecular formula. Herein, the planar structure of **5** was established as a cage-like 6/6/6/6/5-fused pentacyclic conjugate of a *trans*-*N*-cinnamoylindolidinoid and an α-phelladrene derivatives. The relative configuration of **5** was deduced as depicted, by the NOESY correlations (Figure S7) of H-5/H-2β, 3β, H-8β, and H-4″; H-9/H-3α; H-2″b/H-3β; and H-5″β/H-6. Finally, the absolute configuration of **5** was determined by TDDFT-ECD calculations,[11] to be 4*R*,5*R*,6*S*, 9*R*,1″*S*,3″*R*,4″*S*,6″*S* (Figure 4b).

Toussaintine C (**6**)[1] was separated as optically pure **6a** and **6b** by chiral-phase HPLC, and their absolute configurations were established as 4*S*,9*S* and 4*R*,9*R*, respectively, based on X-ray crystallography (Figure S10, CCDC 1575725) and ECD data (Figure S9).[3] Notably, the ^{1}H NMR spectra of **1**–**6** ($CDCl_3$) showed doubling peaks with ratios ranging from 1:1 to 9:1 (Figures S12, S22, S32, S42, S52, and S62), which was likely caused due to the partial double-bond characteristic C–N bond of the *N*-cinnamoylindolidinoid and its resulting restricted bond rotation (Figure S1).[12-15] Take compound **6** as an example, its ^{1}H NMR spectra showed doubling peaks in the ratios of 5:4 (DMSO-d_6), 2:1 (pyridine-d_5), and 5:2 ($CDCl_3$) (Figure S2) at RT, respectively. An elevated-temperature NMR experiment (DMSO-d_6) was attempted to generate one average set of signals. When the temperature was set at 363 K, the two sets of signals were coalesced (Figure S3), which supported the above-mentioned conclusion.

Compounds **1**–**5** are unprecedented conjugates biosynthetically formed of (*S*)-α-phelladrene and toussaintine C (**6**) by Diels-Alder (DA) reactions, which differ in the configuration of

monomeric unit, DA selectivity, and subsequent modifications (Scheme 1). Compounds **1**–**3** are isomers originated due to DA regioselectivity and different precursors, while they showed good stereoselectivity that all of them are the *endo*-*syn*-HO-4 (H-9) adducts. Regioisomers **1** and **2** were determined respectively as the *ortho*- and *meta*-DA adducts of (*S*)-α-phelladrene and **6a**, while compound **3** was the *meta*-product by DA cycloaddition of (*S*)-α-phelladrene and **6b**. Compound **4** would be derived from **3** by an acetalization of the C-7 carbonyl group with methanol, followed by an electrophilic attack of the C-7 carbonyl oxygen to C-1″ of the $\Delta^{1''}$moiety. Compound **5** was converted from **3** by an acid induced electrophilic addition of the HO-4 group to C-1″.

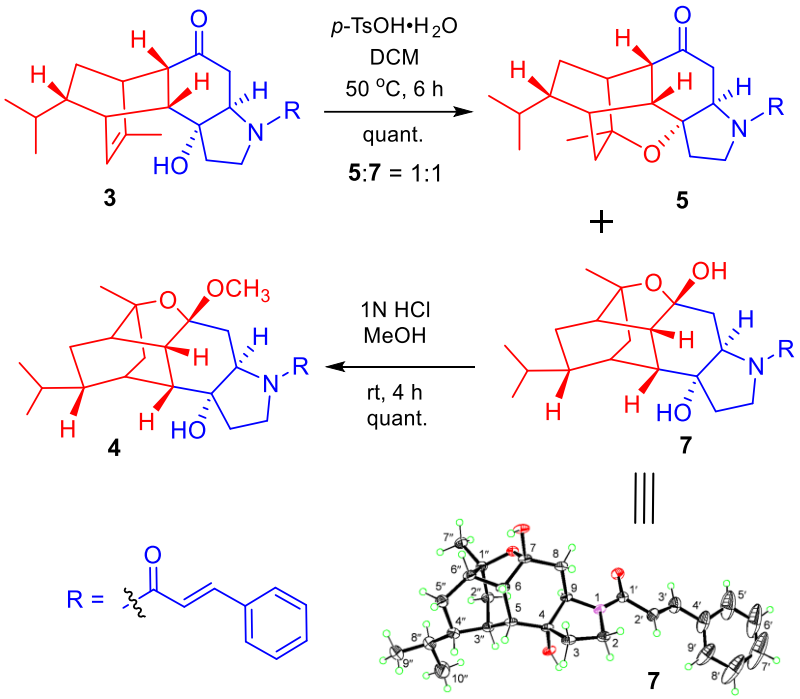
Scheme 1. Proposed Biosynthetic Pathway for Compounds 1–5



To corroborate the structures of compounds **4** and **5** and supported the above-mentioned biosynthetic hypothesis, as well as provide sufficient samples for further biological evaluations, biomimetic approaches were designed to convert compounds **4** and **5** from compound **3** (Scheme 2). Treatment of **3** with catalytic *p*-toluenesulfonic acid monohydrate in dichloromethane at 50 ºC for 6 hours afforded quantitatively compound **5** and new product **7** (ratio ca. 1:1). The structure of **7** was established by a detailed investigation of the MS, and 1D and 2D NMR data (Figures S68–

S74, Table S8), to be the hemiketal derivative of compound **4**. The absolute configuration of **7**, 4*R*,5*R*,6*S*,7*R*,9*R*,1″*S*,3″*R*,4″*S*,6″*S*, was determined based on the crystallographic study, with an absolute structure parameter of 0.12 (6) being observed (Scheme 2 and Figure S11, CCDC 2184006). Compound **7** was converted to **4**, quantitatively, by treating with 1N HCl in methanol at RT for 4 hours.

Scheme 2. Biomimetic Syntheses of Compounds 4, 5, and 7



The anti-neuroinflammatory activity of compounds **1**, **3**–**7** was evaluated in vitro against lipopolysaccharide (LPS) induced inflammation-related BV-2 microglial cells, with resveratrol as the positive control. Compounds **6a** and **6b** at 5 μM, **3** and **7** at 10 μM, and **4** and **5** at 20 μM displayed obvious inhibitory activity on NO production (> 30%) against LPS-induced inflammation-related BV-2 microglial cells and showed no cytotoxicity on BV-2 cells (cell viability > 80%) (Figure 5a and Table S10). Compound **1** at 10 μM exhibited noticeable

cytotoxicity on BV-2 cells, with cell viability of 48.6% (Table S10). In addition, compounds **3**, **6b**, and **7** at 20 μM reduced the mRNA levels of the pro-inflammatory cytokines IL-1β and IL-6 in LPS-stimulated BV-2 microglial cells (Figures 5b and 5c), which demonstrated further their anti-neuroinflammatory effects.

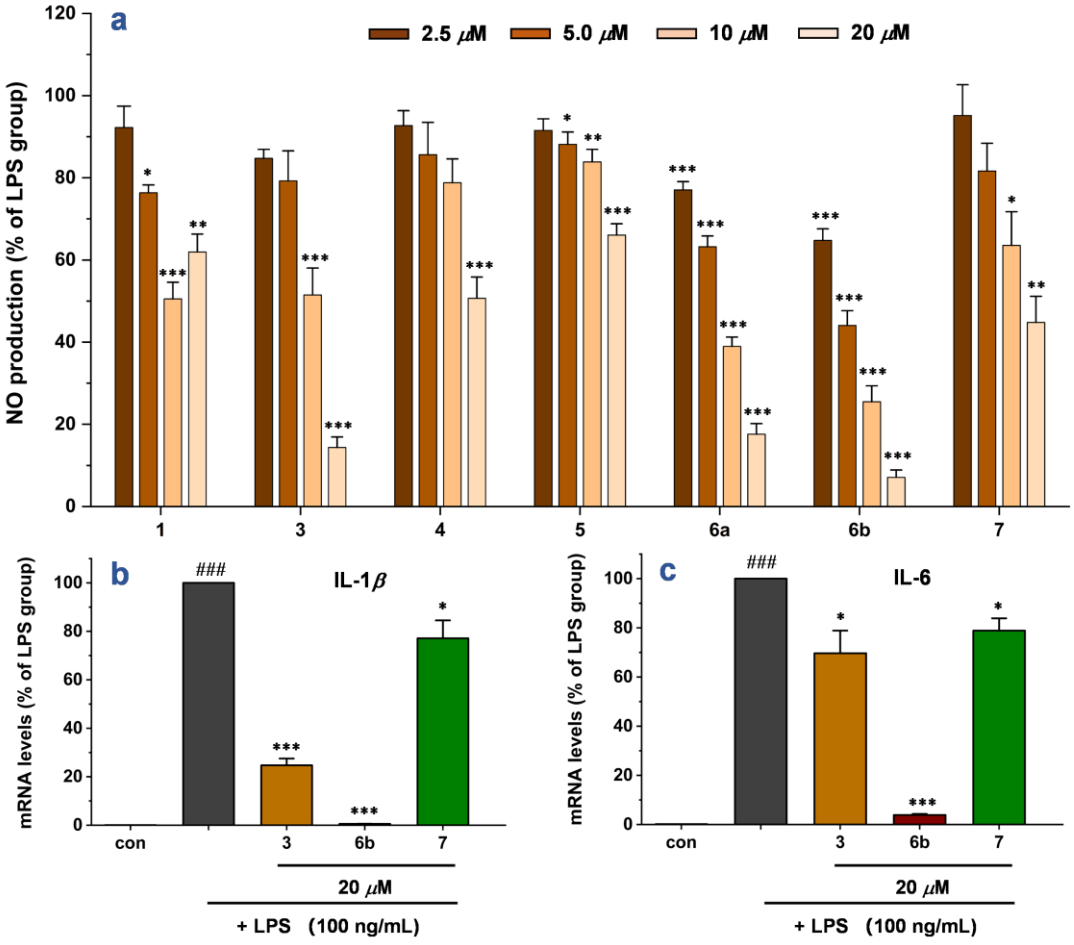


Figure 5. (a) The inhibitory effects of compounds **1**, **3**–**7** on LPS-induced NO over-production in BV-2 microglial cells. Compounds **3**, **6b**, and **7** reduced the mRNA levels of (b) IL-1β and (c) IL-6 in LPS-stimulated BV-2 microglial cells. Data were normalized by the mean value of the LPS group (which was set to 100%), and expressed as Means ± SEM (n = 3). $^{###}p < 0.001$ vs the control group, *P < 0.05, **P < 0.01, ***P < 0.001 vs the LPS group. con: control group.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at xxx

Experimental section, computational section, synthesis section, biological evaluation part, spectroscopic data including IR, MS, and NMR spectra for compounds **1**−**7** (PDF)

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Notes

The authors declare no competing financial interest.

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