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# Anti-inflammatory Alkaloid Glycoside and Quinoline alkaloid derivates from the stems of *Clausena lansium*

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Six new alkaloid glycosides, Clausenasides A-F (**1**-**6**), along with two new quinoline alkaloids, Clausenasides G-H (**7**-**8**), and ten known compounds (**9**–**18**) were obtained from the stems of *C. lansium*. The structures of new compounds were elucidated on the basis of their spectroscopic analysis, and the absolute configurations of **1**, **2**, **3** and **7** were confirmed by mosher's method, CD and ECD spectra, respectively. Compounds **4**, **6**, **9**, **17**, and **18** showed moderate inhibitory effects on LPS-induced NO production in murine microglial BV2 cells ($IC_{50}$ values < 10 $\mu$M).

## Introduction

*Clausena lansium* (Lour.) Skeels (Rutaceae), a fruit tree, was widely distributed in southern China. In traditional Chinese medicine, the leaves and roots of *C. lansium* were used for cough, asthma, dermatological disease, viral hepatitis, and gastro-intestinal diseases; the seeds are used to treat acute and chronic gastro-intestinal inflammation, ulcers, etc[1]. Various bioactive constituents including coumarins, carbazole alkaloids and amide alkaloids have been isolated and identified from this plant[2-4]. Previously, twenty new natural products including thirteen new carbazole alkaloids [5-6], four new coumarins[7], a new amide and a new megastigmane glucoside[8] from the leaves and skeels of *C. lansium* were reported by our research group, and some of these alkaloids showed selective neuroprotective effects. In order to continue research for microscale bioactive metabolites from *C. lansium*, 200 kg stems of *C. lansium* were extracted by EtOAc and n-BuOH. This paper reported further investigation of n-BuOH extract from the stems of *C. lansium* which led to the isolation and characterization of six new alkaloid glycosides (**1**-**6**), two new quinoline alkaloids (**7**-**8**) along with ten known compounds (**9**-**18**) from the stems of *C. lansium* (Figure 1). It was the first time to obtain these alkaloid glycosides from *C. lansium*. The inhibitory effects on LPS-induced NO production in a murine microglial cell lines of **1**-**18** were also evaluated. We present herein the isolation and structural characterization of Clausenasides A-H, as well as their bioactivities.

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†Electronic Supplementary Information (ESI) available: The spectra including 1D-, 2D-NMR, HRESIMS of compounds **1**-**8** as well as related original CD spectrum for Clausenasides A (**1**), B (**2**) and C (**3**), and ECD calculation data for Clausenaside G (**7**),). See DOI: 10.1039/b000000x/

## Results and discussion

Clausenaside A (**1**) was obtained as a white powder. Its molecular formula was assigned as $C_{22}H_{27}NO_8$ based on the $^{13}C$ NMR spectroscopic data and HRESIMS (*m/z* 456.1634 $[M + Na]^+$, calcd for $C_{22}H_{27}NO_8Na$, 456.1629), implying ten indices of hydrogen deficiency. The IR spectrum displayed absorptions characteristic of amino (3315 $cm^{-1}$), amide (1640 $cm^{-1}$) and aromatic ring (1618, 1579, and 1505 $cm^{-1}$) groups, and the UV spectrum showed absorptions at $\lambda_{max}$ 202 and 226 nm. A 1,4-disubstituted benzene ring at $\delta_H$ 7.32 (2H, d, $J$ = 8.8 Hz), 6.87 (2H, d, $J$ = 8.8 Hz) and a 1-substituted benzene ring at $\delta_H$ 7.79 (2H, d, $J$ = 7.2 Hz), 7.51 (1H, m), 7.44 (2H, t, $J$ = 7.6 Hz) were clearly observed in the $^1H$ NMR spectrum and were assigned with the aid of COSY correlations (Figure 2). In addition, an amide group $\delta_H$ 8.33 (1H, t, $J$ = 4.0 Hz, NH), a methoxy group [$\delta_H$ 3.73; $\delta_C$ 55.0], a methylene group [$\delta_H$ 3.69 (1H, m), 3.55 (1H, m); $\delta_C$ 44.5], an oxygenated methine group [$\delta_H$ 4.83 (1H, t, $J$ = 4.0 Hz); $\delta_C$ 78.8], and a $\beta$-glucopyranosyl moiety [4.42 (1H, d, $J$ = 7.6 Hz), $\delta_H$ 3.06-3.56 (5H, m); $\delta_C$ 102.9, 73.8, 76.5, 69.9, 76.8, 60.9] were clearly observed in the $^1H$ and $^{13}C$ NMR spectra along with HSQC correlations. On the basis of the NMR techniques, compound **1** was elucidated as an amide alkaloid glycoside. In the HMBC spectrum (Figure 2), the cross-peaks between H-3, H-5/ C-1 ($\delta_C$ 134.4), H-2, H-6/C-7 ($\delta_C$ 166.2) demonstrated the 1-substituted benzene ring attached to the carbonyl

group. H-8/C-7 ($\delta_C$ 166.2), N-H/C-7 ($\delta_C$ 166.2), C-9 ($\delta_C$ 78.8) indicated the methylene group attached to the N atom. H-9/C-10 ($\delta_C$ 132.5), C-11($\delta_C$ 127.9), H-1'/C-9 ($\delta_C$ 78.8) implying that 1,4-disubstituted benzene ring and $\beta$-glucopyranosyl unit were linked to C-9. The methoxy group resonated at C-13 ($\delta_C$ 158.5). Hydrolysis of **1** with snailase produced the aglycone (**1a**) and glucose. **1a** was identified as tembamide[9] by comparison of its $^1$H and $^{13}$C NMR, and HRESIMS. The absolute configuration o of **1a** was determined as 9*S* by comparison of the optical rotation of **1a** {$[\alpha]_D^{25}$ +54.8 (*c* 0.20 $CHCl_3$)} and (*S*)-(+)-tembamide[10] {$[\alpha]_D^{25}$ +56.9 (*c* 0.54 $CHCl_3$)}. Compound **1a** was treated with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) in anhydrous $CH_2Cl_2$[11] to afforded the **1a**-(*S*)-MTPA ester (**1aa**) and **1a**-(*R*)-MTPA ester (**1ab**), respectively. The $\Delta\delta_H^{SR}$ values were calculated as shown in Figure 3. Application of Mosher's rule[12] revealed that **1a** had the 9*S* configuration. The CD spectrum of **1a** exhibited negative Cotton effect at 235 nm (Figure 4), and the CD spectrum of **1** also showed negative Cotton effect at 238 nm (Figure 4), which indicated that the structure of **1a** had not changed in the process of enzymatic hydrolysis and the absolute configuration of **1** was elucidated as 9*S*. The D-glucose was identified by comparison of TLC and optical rotation data of D-glucose and the authentic sample[13]. Thus, the structure of **1** was defined as (*S*)-(+)-tembamide-9-*O*-*β*-D-glucopyranoside.

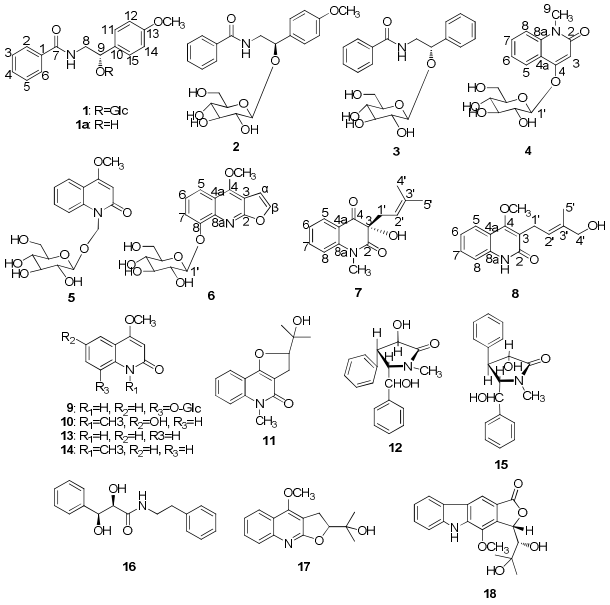


Figure 1. Alkaloid derivates (**1**-**18**) obtained from the stems of *C. lansium*

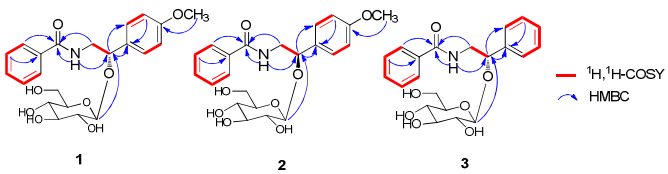


Figure 2. Key $^1$H, $^1$H-COSY and HMBC correlations of compounds **1**, **2** and **3**

Clausenaside B (**2**) was obtained as a white powder. Its molecular formula was established as $C_{22}H_{27}NO_8$ from the $^{13}$C NMR and positive-ion HRESIMS (*m*/*z* 456.1635 [M + Na]$^+$, calcd for $C_{22}H_{27}NO_8Na$, 456.1629), indicating ten indices of hydrogen deficiency. Comparison of spectroscopic data of **2** with those of **1** revealed that the planar structure of **2** was established the same as that of **1**. Therefore, Compound **2** was defined to be a stereo-isomer of **1**. The CD spectra of **2** which showed positive Cotton effect at 237 nm (Figure 4) was contrary to **1**. Thus, the structure of **2** was established as (*R*)-(-)-tembamide-9-*O*-*β*-D-glucopyranoside.

Table 1. $^1$H and $^{13}$C NMR Spectroscopic Data of Compounds **1**-**3** ($\delta$ in ppm, *J* in Hz)

| | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ [a] | $\delta_C$ [b] | $\delta_H$ [a] | $\delta_C$ [b] | $\delta_H$ [a] | $\delta_C$ [b] |
| 1 | | 134.4 s | | 134.6 s | | 134.8 s |
| 2, 6 | 7.79, br d (7.2) | 127.1 d | 7.77, br d (7.2) | 127.1 d | 7.79, br d (7.6) | 127.6 d |
| 3, 5 | 7.44, t (7.6) | 128.3 d | 7.43, t (7.6) | 128.4 d | 7.46, t (7.6) | 128.8 d |
| 4 | 7.51, m | 131.1 d | 7.50, m | 131.0 d | 7.52, m | 131.6 d |
| 7 | | 166.2 s | | 166.4 s | | 166.8 s |
| 8 | 3.69, m; 3.55, m | 44.5 t | 3.50, m | 45.6 t | 3.73, m; 3.57, m | 45.2 t |
| 9 | 4.83, t (4.0) | 78.8 d | 4.99, t (6.6) | 76.1 d | 4.90, m | 79.6 d |
| 10 | | 132.5 s | | 131.2 s | | 141.1 s |
| 11, 15 | 7.32, br d (8.8) | 127.9 d | 7.36, br d (8.8) | 128.2 d | 7.41, br d (7.6) | 127.1 d |
| 12, 14 | 6.87, br d (8.8) | 113.3 d | 6.90, br d (8.8) | 113.5d | 7.33, br d (7.4) | 128.4 d |
| 13 | | 158.5s | | 158.7 s | 7.26, t (7.2) | 127.8 d |
| Glc-1' | 4.42, d (7.6) | 102.9 d | 3.98, d (7.6) | 100.0 d | 4.46, d (7.6) | 103.5 d |
| Glc-2' | 3.07,[c] m | 73.8 d | 3.04,[c] m | 73.5 d | 3.10, m | 74.3 d |
| Glc-3' | 3.16, m | 76.5 d | 3.04,[c] m | 76.6 d | 3.18, m | 77.0 d |
| Glc-4' | 3.07,[c] m | 69.9 d | 3.04,[c] m | 70.2 d | 3.07,[c] m | 70.4 d |
| Glc-5' | 3.07,[c] m | 76.8 d | 2.96, m | 76.9 d | 3.08,[c] m | 77.4 d |
| Glc-6'a | 3.56, m | 60.9 t | 3.39, m | 61.2 d | 3.37, m | 61.4 t |
| Glc-6'b | 3.37, m | | 3.65, m | | 3.56, m | |
| NH | 8.33, t (4.0) | | 8.23, t (5.5) | | 8.35, t (5.4) | |
| $OCH_3$ | 3.73, s | 55.0 q | 3.74, s | 55.0 q | | |

[a] In DMSO-$d_6$ 400 MHz for **1**, **2**, **3**. [b] In DMSO-$d_6$ 100 MHz for **1**, **2**, **3**. Coupling constants (*J*) in Hz are given in parentheses. The assignments were based on HSQC and HMBC experiments. [c]signal overlapped.

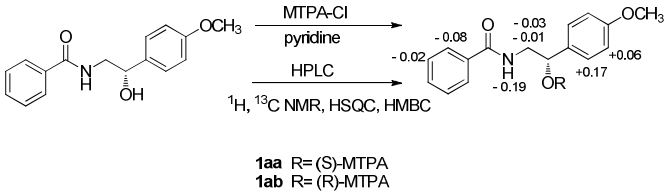


Figure 3. the mosher's method of **1a**

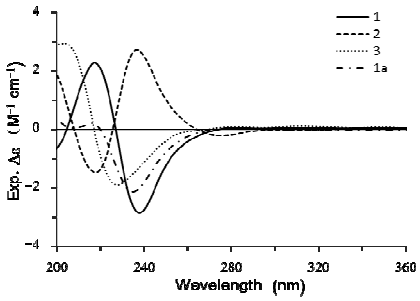


Figure 4. The experimental CD spectrum of **1**, **2**, **3** and **1a**

Clausenaside C (**3**) was obtained as a white powder. Its molecular formula was deduced as $C_{21}H_{25}NO_7$ on the basis of its $^{13}$C NMR and

HRESIMS at m/z 426.1524 $[M + Na]^+$, calcd for $C_{21}H_{25}NO_7Na$, 426.1523, implying ten indices of hydrogen deficiency. Comparison of the spectroscopic data of **3** with those of **1** revealed that compound **3** also was an amide alkaloid glycoside, with the primary difference being the absence of a methoxy group at C-13 ($\delta_C$ 127.8). Compound **3** showed similar CD Cotton effects to those of **1** (Figure 4), and the absolute configuration of **3** was 9*S*. Thus, the structure of **3** was defined as (*S*)-(+)-demethoxy-tembamide-9-*O*-*β*-D-glucopyranoside.

Clausenaside D (**4**) was obtained as a white powder. Its molecular formula was determined as $C_{16}H_{19}NO_7$ based on its $^{13}C$ NMR spectroscopic and HRESIMS at *m/z* 388.1232 $[M + H]^+$ (calcd for $C_{16}H_{19}NO_7$, 388.1234), corresponding with eight indices of hydrogen deficiency. The UV spectrum showed absorptions at 226, 270 and 322 nm. The $^1H$ NMR spectrum (Table 2) showed *o*-substituted bezene ring [$\delta_H$ 8.04 (1H, dd, *J* = 7.8, 1.8 Hz, H-5); 7.28 (1H, dt, *J* = 7.8, 0.6 Hz, H-6); 7.66 (1H, dt, *J* = 8.4, 1.8 Hz, H-7); 7.52 (1H, d, *J* = 8.4 Hz, H-8)], a bond group $\delta_H$ 6.19 (1H, s, H-3), a methyl group $\delta_H$ 3.57, and a *β*-glucopyranosyl moiety $\delta_H$ 5.09 (1H, d, *J* = 7.8 Hz, H-1'). The $^{13}C$ NMR spectrum (Table 2) exhibited 16 carbon signals including six in glucopyranosyl unit, a methyl group, and the remaining nine in the quinoline moiety. A comparison of the $^1H$ and $^{13}C$ NMR of **4** with those of integriquinolone[14] suggested that their structures are closely related, except for the presence of the *β*-glucopyranose unit signals in **4**. The glucose was confirmed to be located at C-4 from the HMBC correlation between H-1' ($\delta_H$ 5.09) and C-4 ($\delta_C$ 159.4) (Figure 5). D-glucose were isolated from the enzymatic hydrolysate of **4** and identified by TLC and specific rotation. Therefore, the structure of **4** was elucidated as integriquinolone-4-*O*-*β*-D-glucopyranoside.

Clausenaside E (**5**) was obtained as a white powder. The HRESIMS displayed *m/z* $[M + H]^+$ 368.1346 (calcd for $C_{17}H_{22}NO_8$, 368.1340), which was consistent with a molecular formula of $C_{17}H_{21}NO_8$ with eight indices of hydrogen deficiency. Compound **5** showed similar UV absorptions to those of **4**, which suggested that their structure were closely related except that the 4-methoxy group and 9-hydroxymethyl-*O*-*β*-D-glucopyranoside of **5** replaced 4-*O*-*β*-D-glucopyranoside group and the 9-methyl group of **4** respectively. The linkage of the *β*-D-glucose moiety to C-9 was supported by the HMBC correlation observed between H-1' ($\delta_H$ 4.42) and C-9 ($\delta_C$ 69.9) (Figure 5). D-glucose were isolated from the enzymatic hydrolysate of **5** and identified by TLC and specific rotation. Therefore, the structure of **5** was defined as 4-methoxy-1-hydroxymethyl-2(1H)-quinolinone-9-*O*-*β*-D-glucopyranoside.

Clausenaside F (**6**) was obtained as a white powder. Its molecular formula was assigned as $C_{18}H_{19}NO_8$ on the basis of its $^{13}C$ NMR and HRESIMS (*m/z* 378.1180 $[M + H]^+$, calcd for $C_{18}H_{19}NO_8$ 388.1234; 400.0999 $[M + Na]^+$, calcd for $C_{18}H_{19}NO_8$, 400.1003), implying ten indices of hydrogen deficiency. The $^1H$ and $^{13}C$ NMR of **6** displayed signals characteristic of quinoline alkaloid glycoside, which were similar to these reported for robustine[15]. The only difference was that the *β*-D-glucopyranose unit in **6** substituted the hydroxyl group in robustine. The D-glucose was localized at C-8, which was supported by the HMBC correlations (Figure 5) from H-1' ($\delta_H$ 5.04) to C-8 ($\delta_C$ 153.0). D-glucose were isolated from the enzymatic hydrolysate of **6** and identified by TLC and specific rotation. Therefore, the structure of **6** was elucidated as robustine-8-*O*-*β*-D-glucopyranoside.

Table 2. $^1H$ and $^{13}C$ NMR Spectroscopic Data of Compounds **4**-**6** ($\delta$ in ppm, *J* in Hz)

| | **4** | | **5** | | **6** | |
|---|---|---|---|---|---|---|
| position | $\delta_H$[a] | $\delta_C$[b] | $\delta_H$[a] | $\delta_C$[b] | $\delta_H$[c] | $\delta_C$[d] |
| 2 | | 162.3 s | | 163.3 s | | 164.8 s |
| 3 | 6.19, s | 99.4 d | 6.02, s | 96.7 d | | 105.1 s |
| 4 | | 159.7 s | | 163.5 s | | 159.2 s |
| 4a | | 115.4 s | | 116.4 s | | 120.9 s |
| 5 | 8.04, dd (7.8,1.8) | 123.2 d | 7.87, dd (7.8,1.2) | 123.2 d | 7.95, dd (8.4,1.2) | 117.0 d |
| 6 | 7.28, m | 121.4 d | 7.28, m | 122.9 d | 7.39, dd (8.4,7.8) | 125.0 d |
| 7 | 7.66, m | 131.5 d | 7.64, [e]m | 132.2 d | 7.48, dd (7.8,1.2) | 114.4 d |
| 8 | 7.52, br d (8.4) | 114.6 d | 7.62, [e]m | 116.3 d | | 153.0 s |
| 8a | | 139.5 s | | 139.5 s | | 137.7 s |
| 9 | 3.57, 3H, s | 28.7 q | 5.87, d (9.6); 5.75, d (9.6) | 69.9 t | | |
| Glc-1' | 5.09, d (7.8) | 99.5 d | 4.42, d (7.8) | 101.9 d | 5.04, d (7.8) | 103.0 d |
| Glc-2' | 3.90, m | 73.0 d | 2.95, m | 73.9 d | 3.74, m | 74.7 d |
| Glc-3' | 3.34, m | 76.1 d | 3.12, [e]m | 77.5 d | 3.59, m | 77.3 d |
| Glc-4' | 3.19, m | 69.5 d | 3.07, m | 70.6 d | 3.47, m | 71.6 d |
| Glc-5' | 3.46, m | 77.2 d | 3.10, [e]m | 77.9 d | 3.57, m | 78.5 d |
| Glc-6' | 3.48, m; 3.70, m | 60.6 t | 3.47, m; 3.66, m | 61.7 t | 3.98, m; 3.75, m | 62.6 t |
| $OCH_3$ | | | 3.96, s | 57.1 q | 4.52, s | 60.2 q |
| α | | | | | 7.86, d (2.4) | 145.4 d |
| β | | | | | 7.40, d (2.4) | 106.5 d |

[a] In DMSO-$d_6$ 600 MHz for **4** and **5**, [c] In DMSO-$d_6$ 125 MHz for **4** and **5**, [c] In MeOH-$d_6$ 600 MHz for **6**, [d] In MeOH-$d_6$ 125 MHz for **6**. Coupling constants (*J*) in Hz are given in parentheses. The assignments were based on HSQC and HMBC experiments. [e]signal overlapped.

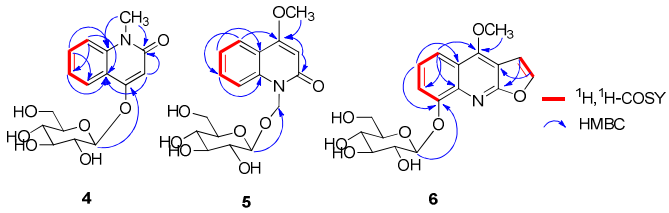


Figure 5. Key $^1H$, $^1H$-COSY and HMBC correlations of compounds **4**, **5** and **6**

Clausenaside G (**7**) was obtained as a yellow powder. The molecular formula was established as $C_{15}H_{17}NO_3$ by the HRESIMS at *m/z* 282.1102 $[M + Na]^+$, calcd for $C_{15}H_{17}NO_3$, 282.1101), indicating eight indices of hydrogen deficiency. The $^1H$ and $^{13}C$ NMR of **7** displayed signals characteristic of quinoline alkaloid, which was an analog of 3-hydroxyquinoline-2, 4(1H, 3H)-diones[16]. In the HMBC spectrum (Figure 6) of **7**, correlations of H-1' ($\delta_H$ 2.42) /C-2 ($\delta_C$ 171.9), C-3 ($\delta_C$ 82.0), C-4 ($\delta_C$ 195.1), C-2' ($\delta_C$ 116.3), C-3' ($\delta_C$ 135.2) and of H-4' ($\delta_H$ 1.49) and H-5' ($\delta_H$ 1.29) / C-2' ($\delta_C$ 116.3), C-3' ($\delta_C$ 135.2) suggested that the isopentene group was attached to C-2. The absolute configuration of **7** was defined as 3*S* by comparison of the experimental ECD spectra and calculated ECD data using the time-dependent density functional theory (TDDFT) method at the $B_3LYP$/6-31G (d) level[17]. The calculated ECD spectrum of 3*S* (**7a**) (Figure 7) matched the experimental spectrum very well. Thus, the structure of **7** was elucidated.

Table 3. $^{1}$H and $^{13}$C NMR Spectroscopic Data of Compounds **7** and **8** ($\delta$ in ppm, $J$ in Hz)

| | 7 | | 8 | |
|---|---|---|---|---|
| position | $\delta_H$ [a] | $\delta_C$ [b] | $\delta_H$ [a] | $\delta_C$ [b] |
| 2 | | 171.9 s | | 163.3 s |
| 3 | | 82.0 s | | 121.9 s |
| 4 | | 195.1 s | | 160.7 s |
| 4a | | 120.6 s | | 116.0 s |
| 5 | 7.76, dd (7.6,1.6) | 126.7 d | 7.69, dd (8.0,1.0) | 122.5 d |
| 6 | 7.20, t (7.6) | 122.8 d | 7.20, td (8.0,0.8) | 121.7 d |
| 7 | 7.70, td (7.0,1.6) | 136.0 d | 7.49, td (8.0,1.4) | 130.0 d |
| 8 | 7.33, d (8.3) | 115.5 d | 7.33, dd (8.0,1.4) | 115.3 d |
| 8a | | 142.6 s | | 137.7 s |
| 1' | 2.42, 2H, m | 39.4 t | 3.27, 2H, d (6.8) | 22.4 t |
| 2' | 4.91, t (7.7) | 116.3 d | 5.40, td (6.8, 1.2) | 120.7 d |
| 3' | | 135.2 s | | 135.8 s |
| 4' | 1.49, s | 25.8 q | 3.76, 2H, s | 66.2 t |
| 5' | 1.29, s | 17.4 q | 1.71, s | 13.7 q |
| 6' | | 171.9 s | | |
| NH | | | 11.72, br s | |
| N-$CH_3$ | 3.36, 3H, s | 29.7 q | | |
| $OCH_3$ | | | 3.87, s | 61.5 q |

[a] In DMSO-$d_6$ (600 MHz for **7**, 400 MHz for **8**), [b]In DMSO-$d_6$ (125 MHz for **7**, 100 MHz for **8**). Coupling constants ($J$) in Hz are given in parentheses. The assignments were based on HSQC and HMBC experiments.

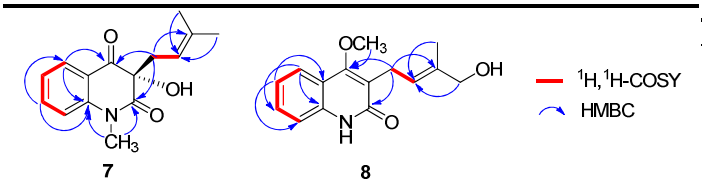


Figure 6. Key $^{1}$H, $^{1}$H-COSY and HMBC correlations of compounds **7** and **8**

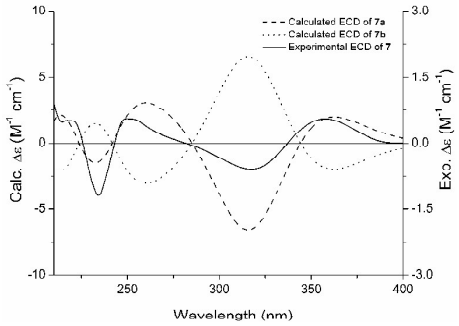


Figure 7. Calculated ECD spectra of **7**a (3*S*)- and **7**b (3*R*)-isomers and the experimental ECD spectrum of **7**.

Clausenaside H (**8**) was obtained as a white powder. Its molecular formula $C_{15}H_{17}NO_3$ was deduced from the HRESIMS (*m/z* 260.1280 $[M + H]^+$, calcd for $C_{15}H_{17}NO_3$, 260.1281; 282.1102 $[M + Na]^+$, calcd for $C_{15}H_{17}NO_3$, 282.1101) and $^{13}$C NMR spectroscopic data, corresponding with eight indices of hydrogen deficiency. The $^{1}$H and $^{13}$C NMR of **8** displayed signals characteristic of quinoline alkaloid, which were similar to these reported for atanine[18]. The only difference between **8** and atanine was that a methyl group in atanine is replaced by a hydroxymethyl group [$\delta_H$ 3.76 (2H, s, H-4'), $\delta_C$ 66.2] in **8**. This was supported further by the HMBC correlations (Figure 6) between H-4' ($\delta_H$ 3.76) and C-2' ($\delta_C$ 120.7) / C-3' ($\delta_C$ 135.8)/C-5' ($\delta_C$ 13.7). The NOE difference experiment displayed that a strong enhancement of H-2' was observed when H-4' was irradiated, while H-2' was no enhanced on irradiation of H-5', indicating an *E* configuration of the double bond. Thus, the structure of **8** was elucidated.

The ten known alkaloids were identified as 4-methoxy-8-*O*-β-D-glucopyranosyloxy-2(1*H*)-quinolinone (**9**)[19], integriquinolone (**10**)[20], araliopsine (**11**)[21], clausenamide (**12**)[22], 4-methoxy -2(1*H*)-quinolone (**13**)[23], 4-methoxy-1-methyl-2-quinolone (**14**)[24], neoclausenamide (**15**)[20], demethylsecoclausenamide (**16**)[25], ribalinine (**17**)[21], claulansine D (**18**)[5] based on the analysis and comparison with their spectroscopic data (NMR, UV and MS) with literature data.

Compounds **1-18** were tested for their inhibitory effects on LPS-induced NO production in murine microglial BV2 cells. In the anti-inflammatory assay, compounds **4**, **6**, **9**, **17**, **18** exhibited moderate inhibitory effect on LPS-stimulated NO production in murine microglial BV2 cells with curcumin as a positive control as shown in Table 4, whereas the other compounds were inactive in this assay ($IC_{50}$ value >10 $\mu$M).

Table 4. Inhibitory effects of Compounds **1-18** against LPS-Induced NO Production in Microglia BV2 Cells

| compound | $IC_{50}$ ($\mu$M) | compound | $IC_{50}$ ($\mu$M) |
|---|---|---|---|
| **1** | >10 | **10** | >10 |
| **2** | >10 | **11** | >10 |
| **3** | >10 | **12** | >10 |
| **4** | 7.58 | **13** | >10 |
| **5** | >10 | **14** | >10 |
| **6** | 6.28 | **15** | >10 |
| **7** | >10 | **16** | >10 |
| **8** | >10 | **17** | 5.70 |
| **9** | 5.03 | **18** | 5.93 |
| curcumin[a] | 5.59 | | |

[a] Positive control.

## Experimental

Optical rotations were measured on a JASCO P2000 automatic digital polarimeter. UV spectra were recorded on a JASCO V-650 spectrophotometer, CD spectra were measured on a JASCO J-815 spectropolarimeter. IR spectra were recorded on a Nicolet 5700 spectrometer using an FT-IR microscope transmission method. NMR spectra were acquired with Bruker AVIIIHD 600, VNS-600, and Mercury-400 spectrometers in DMSO-$d_6$ and MeOH-$d_6$. HRESIMS spectra were collected on an Agilent 1100 series LC/MSD ion trap mass spectrometer. MPLC system was composed of two C-605 pumps (Büchi), a C-635 UV detector (Büchi), a C-660 fraction collector (Büchi), and an ODS column (450 mm×60 mm, 50 μm, 400 g; YMC). Semi-preparative HPLC was conducted using a Shimadzu LC-6AD instrument with an SPD-20A detector and a Daicel Chiralpak AD-H column (250×10 mm, 5 μm). Preparative HPLC was also performed on a Shimadzu LC-6AD instrument with a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Column chromatography (CC) was performed with silica gel (200-300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China)， SF-PRP 512A (100-200 mesh, Beijing Sunflower and Technology Development Co., Beijing, People's Republic of China), ODS (50 μm, YMC, Japan), and Sephadex LH-20 (GE, Sweden). TLC was carried out on glass precoated silica gel GF254 plates.

Spots were visualized under UV light or by spraying with 10% sulfuric acid in EtOH followed by heating. Curcumin (99%, Sinopharm Chemical Reagent Company Limited).

**Plant materials.**

The stems of *C. lansium* were collected in Liuzhou, Guangxi, China, in March 2013 and identified by Engineer Guangri Long, Forestry of Liuzhou. A voucher specimen has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College (ID-S-2320).

**Extraction and isolation.**

Air-dried, powdered stems of *C. lansium* (200 kg) were extracted with 95% ethanol (1000 L × 2 h × 3). The residue was suspended in water and then partitioned with EtOAc (3× 40 L), and n-BuOH (3 × 40 L), successively. After removing the solvent, the n-BuOH-soluble portion (850 g) was fractionated via macroporous adsorbent resin (HPD-100) column with $H_2O$, 30% EtOH, 60% EtOH, and 95% EtOH to yield four corresponding fractions A-D.

Fraction B (180 g) was fractionated via silica gel column chromatography, eluting with $CHCl_3$-MeOH-$H_2O$ (10:1:0.05, 9:1:0.1, 8:2:0.2, 7:3:0.3, 6:4:0.4) to afford twelve fractions $B_1$-$B_{12}$ on the basis of TLC analysis. Fraction $B_4$ (5.9 g) was further separated by reversed-phase silica MPLC with 20% -50% MeOH (50 mL/min, 6 h) to afford $B_{4\text{-}1}$-$B_{4\text{-}47}$ fractions. Fractions $B_{4\text{-}38}$-$B_{4\text{-}42}$ were successively separated using preparative HPLC (detection at 210 nm, 18% $CH_3CN$, 8 mL/min) to yield **1** (29 mg, $t_R$ 83.45 min), **2** (5.0 mg, $t_R$ 73.87 min), **3** (19 mg, $t_R$ 67.02 min) and **6** (9 mg, $t_R$ 78.25 min). Fraction $B_6$ (8.3 g) was further separated by reversed-phase silica MPLC with 20% -45% MeOH (50 mL/min, 6 h) to afford fractions $B_{6\text{-}1}$-$B_{6\text{-}39}$. Fraction $B_{6\text{-}31}$-$B_{6\text{-}32}$ was successively separated using preparative HPLC (detection at 210 nm, 15% $CH_3CN$, 8 mL/min) to yield **5** (3 mg, $t_R$ 51.14 min). Fraction $B_7$ (36.8 g) was fractionated via silica gel column chromatography, eluting with $CHCl_3$-MeOH-$H_2O$ (10:1:0.5-8:2:0.3) to afford fractions $B_{7\text{-}1}$-$B_{7\text{-}18}$ on the basis of TLC analysis. Fraction $B_{7\text{-}6}$ (11.7 g) was further separated by reversed-phase silica MPLC with 20% -50% MeOH (50 mL/min, 8 h) to afford $B_{7\text{-}6\text{-}1}$-$B_{7\text{-}6\text{-}11}$ fractions. Fraction $B_{7\text{-}6\text{-}5}$ was successively separated using preparative HPLC (detection at 210 nm, 12% $CH_3CN$, 8 mL/min) to yield **4** (16 mg, $t_R$ 40.59 min). Fraction $B_{7\text{-}6\text{-}8}$ was successively separated using preparative HPLC (detection at 210 nm, 11% $CH_3CN$, 8 mL/min) to yield **9** (16 mg, $t_R$ 40.59 min).

Fraction C (220 g) was partitioned via kieselguhr eluting with $CHCl_3$, EtOAc, n-BuOH, acetone, and MeOH to afford five fractions $C_1$-$C_5$. After the solvent was removed, the $C_1$ fraction (43 g) was subjected to SF-PRP 512A resin eluting with 35%-70% MeOH to afford fractions $C_{1\text{-}1}$-$C_{1\text{-}6}$. $C_{1\text{-}2}$ (7.1 g) was further separated by reversed-phase silica MPLC with 30% -50% MeOH (50 mL/min, 6 h) to afford fractions $C_{1\text{-}2\text{-}1}$-$C_{1\text{-}2\text{-}11}$. Fraction $C_{1\text{-}2\text{-}4}$ was subjected to Sephadex LH-20 and preparative HPLC (detection at 210 nm, 19% $CH_3CN$, 8 mL/min) to yield **10** (8 mg, $t_R$ 39.04 min). Fraction $C_{1\text{-}2\text{-}5}$ was subjected to Sephadex LH-20 and preparative HPLC (detection at 210 nm, 19% $CH_3CN$, 8 mL/min) to yield **11** (7 mg, $t_R$ 32.04 min). Fraction $C_{1\text{-}2\text{-}8}$ was subjected to Sephadex LH-20 and preparative HPLC (detection at 210 nm, 25% $CH_3CN$, 8 mL/min) to yield **12** (153 mg, $t_R$ 21.99 min) and **13** (8 mg, $t_R$ 30.68 min). Fraction $C_2$ (14.6 g)was further separated by reversed-phase silica MPLC with 35% -55% MeOH (50 mL/min, 10 h) to afford fractions $C_{2\text{-}1}$– $C_{2\text{-}15}$. Fractions $C_{2\text{-}11}$ was subjected to Sephadex LH-20 and preparative HPLC (detection at 210 nm, 29% $CH_3CN$, 8 mL/min) to yield **7** (2 mg, $t_R$ 46.59 min), **14** (80 mg, $t_R$ 38.00 min) and **15** (54 mg, $t_R$ 38.20 min). Fractions $C_{2\text{-}12}$ was subjected to Sephadex LH-20 and preparative HPLC (detection at 210 nm, 30% $CH_3CN$, 8 mL/min) to yield **8** (10 mg, $t_R$ 28.20 min), **16** (84 mg, $t_R$ 45.00 min), **17** (40 mg, $t_R$ 41.81 min) and **18** (4 mg, $t_R$ 42.87 min).

**Structure characterization.**

Clausenaside A (**1**): white amorphous powder; $[\alpha]_D^{25}$ + 18.5 (*c* 0.1 MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 201.6 (4.31), 225.6 (4.14) nm; IR (microscope) $\nu_{max}$ 3314, 2919, 1640, 1617, 1540, 1515, 1246,1103, 1075, 1036, 828, 697 $cm^{-1}$; CD (MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 217.5 (+2.28), 238 (-2.86) nm; $^1$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz), see Table 1; HRESIMS *m/z* 456.1634 $[M + Na]^+$ (calcd for $C_{22}H_{27}NO_8Na$, 456.1629).

Clausenaside B (**2**): white amorphous powder; $[\alpha]_D^{25}$ – 8.5 (*c* 0.1 MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 201.8 (4.61), 226.2 (4.38) nm; IR (microscope) $\nu_{max}$ 3361, 2924, 1641, 1613, 1577, 1543, 1513, 1306, 1248, 1077, 1029, 833, 713 $cm^{-1}$; CD (MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 218 (-1.48), 237 (+2.71) nm; $^1$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz), see Table 1; HRESIMS *m/z* 456.1635 $[M + Na]^+$ (calcd for $C_{22}H_{27}NO_8Na$, 456.1629).

Clausenaside C (**3**): white amorphous powder; $[\alpha]_D^{25}$ + 8.2 (*c* 0.1 MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 201.6 (4.14) nm; IR (microscope) $\nu_{max}$ 3357, 2917, 1641, 1577, 1541, 1490, 1313, 1159,1076, 701 $cm^{-1}$; CD (MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 203.5 (+2.94), 228 (-1.90) nm; $^1$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz), see Table 1; HRESIMS at *m/z* 426.1524 $[M + Na]^+$ (calcd for $C_{21}H_{25}NO_7Na$, 426.1523).

Clausenaside D (**4**): white amorphous powder; $[\alpha]_D^{25}$ – 58.4 (*c* 0.1 MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 228.2 (4.39), 269.6 (3.48), 320.4 (3.34) nm; IR (microscope) $\nu_{max}$ 3501, 3362, 3273, 2930, 2882, 1640, 1615, 1574, 1109, 1080, 841, 755 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$, 600 MHz) and $^{13}$C NMR (DMSO-$d_6$, 150 MHz), see Table 2; HRESIMS *m/z* 388.1232 $[M + H]^+$ (calcd for $C_{16}H_{20}NO_7$, 388.1234).

Clausenaside E (**5**): white amorphous powder; $[\alpha]_D^{25}$ – 28.7 (*c* 0.1 MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 226.6 (4.38), 268 (3.50), 314.6 (3.22) nm; IR (microscope) $\nu_{max}$ 3391, 2923, 1647, 1581, 1463, 1400, 1326, 1245, 1113, 1071, 828, 775, 759 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$, 600 MHz) and $^{13}$C NMR (DMSO-$d_6$, 150 MHz), see Table 2; HRESIMS *m/z* 368.1346 $[M + H]^+$ (calcd for $C_{17}H_{22}NO_8$, 368.1340), 390.1161 $[M + Na]^+$ (calcd for $C_{17}H_{21}NO_8Na$, 390.1159).

Clausenaside F (**6**): white amorphous powder; $[\alpha]_D^{25}$ – 57.8 (*c* 0.1 MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 202.4 (4.24), 243.2 (4.53) nm; IR (microscope) $\nu_{max}$ 3342, 2935, 1589, 1516, 1460, 1397, 1373, 1267, 1094, 1034,748 $cm^{-1}$; 1H NMR (MeOH-$d_4$, 600 MHz) and $^{13}C$ NMR (MeOH-$d_4$, 150 MHz), see Table 2; HRESIMS *m/z* 378.1180 $[M + H]^+$ (calcd for $C_{18}H_{20}NO_8$, 388.1234), 400.0999 $[M + Na]^+$ (calcd for $C_{18}H_{19}NO_8Na$, 400.1003).

Clausenaside G (**7**): white amorphous powder; $[\alpha]_D^{25}$ – 38.3 (*c* 0.1 MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 201.8 (4.13), 234.8 (4.27) nm; IR (microscope) $\nu_{max}$ 3394, 2920, 2849, 1709, 1663, 1603, 1472, 1368, 1302, 1123, 1102, 759 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 600 MHz) and $^{13}C$ NMR (DMSO-$d_6$, 150 MHz), see Table 3; HRESIMS *m/z* 282.1102 $[M + Na]^+$ (calcd for $C_{15}H_{17}NO_3Na$, 282.1101).

Clausenaside H (**8**): white amorphous powder; $[\alpha]_D^{25}$ + 8.6 (*c* 0.1 MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 226.6 (4.41), 270.6 (3.74), 322.8 (3.71) nm; IR (microscope) $\nu_{max}$ 3295, 3174, 2997, 2945, 2898, 2853, 1659, 1612, 1571, 1501, 1434, 1379, 1267, 1094, 1001, 751 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 400 MHz) and $^{13}C$ NMR (DMSO-$d_6$, 100 MHz), see Table 3; HRESIMS *m/z* 260.1280 $[M + H]^+$ (calcd for $C_{15}H_{18}NO_3$, 260.1281), 282.1102 $[M + Na]^+$ (calcd for $C_{15}H_{17}NO_3Na$, 282.1101).

**Enzymatic hydrolysis of 1-6**

Compounds **1-6** (1.0 – 8.0 mg) were hydrolyzed in $H_2O$ (1 mL) with snailase at 37℃ for 4 h, then extracted by EtOAC (3×3 mL). The aqueous phase was dried by a stream of $N_2$, then chromatographed over silica gel eluting with $CH_3CN$-$H_2O$ (8:1), to yield glucose. The glucose (0.3 - 1.2 mg) from the hydrolysates of **1-6** gave retention factor ($R_f \approx 0.33$; $CH_3CN$-$H_2O$, 6:1) on TLC, $[\alpha]_D^{25}$ values + 43.1 to + 68.6 (*c* = 0.02 - 0.1, $H_2O$)], and $^1H$ NMR spectral data ($D_2O$) consistent with those of an authentic D-glucose. The EtOAC phase was concentrated under reduced pressure, and purified by RP-HPLC to obtain **1a** (3.14 mg) from **1**. (*S*)-(+)-tembamide (**1a**): white amorphous powder; $[\alpha]_D^{25}$ + 54.8 (*c* 0.2 $CHCl_3$); CD (MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 216 (+0.21), 235 (-2.14) nm; $^1H$ NMR (DMSO-$d_6$, 400 MHz) $\delta_H$ 8.46 (NH, t, *J* = 6.0 Hz), 7.83 (2H, d, *J* = 7.6 Hz), 7.50 (1H, m), 7.44 (2H, m), 7.28 (2H, d, *J* = 8.0 Hz), 6.89 (2H, d, *J* = 8.0 Hz), 5.41 (1H, d, *J* = 4.0 Hz), 4.72 (1H, m), 3.72 (3H, s), 3.43 (1H, m), 3.28 (1H, m); $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta_C$ 166.3, 158.3, 135.8, 134.5, 131.0, 128.2, 128.2, 127.2, 127.2, 127.1, 127.1, 113.4, 113.4, 70.7, 55.0, 47.7; HRESIMS at *m/z* 294.1109 $[M + Na]^+$ (calcd for $C_{16}H_{17}NO_3Na$, 294.1101).

**Preparation of (*R*)- and (*S*)-MTPA esters of 1a**

A solution of **1a** (1.09 mg) in dehydrated $CH_2Cl_2$ (2 mL) was treated with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*S*)-MTPA-Cl (10 mg)] in the presence of anhydrous pyridine, and the mixture was stirred at room temperature for 13 h. After cooling, the reaction mixture was poured into ice-water and extracted with EtOAC. The EtOAC extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $Na_2SO_4$ and filtered. The solvent was removed from the filtrate under reduced pressure to afford a residue[26]. The residue was purified by semi-preparative HPLC (C18 column, 3.0 ml/min, UV 210 nm, 58% $CH_3CN$-$H_2O$) to yield (*S*)-MTPA ester derivative of **1a** (compound **1aa** 1.12 mg). Using a similar procedure, (*R*)-MTPA ester derivative of **1a** (compound **1ab** 0.93 mg) was obtained from **1a** (1.04 mg).

Compound **1aa**: $^1H$ NMR (600 MHz, DMSO) $\delta_H$ 8.61 (1H, dd, *J* = 6.4, 4.5 Hz), 7.72 (2H, m), 7.53 (1H, m), 7.46 (2H, m), 7.39 (2H, d, *J* = 8.7 Hz), 7.36 (1H, m), 7.31 (2H, m), 7.23 (2H, dd, *J* = 8.4, 7.3 Hz), 7.00 (2H, d, *J* = 8.7 Hz), 6.25 (1H, dd, *J*=9.3, 4.5 Hz), 3.80 (1H, m), 3.77 (3H, s), 3.52 (1H, dt, *J* = 13.9, 4.5 Hz), 3.35 (3H, s); $^{13}C$ NMR (DMSO-$d_6$, 150 MHz) $\delta_C$ 166.4, 165.4, 159.6, 134.0, 131.3, 131.2, 129.8, 128.7, 128.7, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 127.1, 127.1, 127.1, 127.1, 114.1, 114.1, 75.9, 55.2, 55.2, 43.6.

Compound **1ab**: $^1H$ NMR (600 MHz, DMSO) $\delta_H$ 8.82 (1H, dd, *J* = 6.5, 4.3 Hz), 7.84 (2H, m), 7.55 (1H, m), 7.49 (2H, dd, *J* = 8.2, 6.8 Hz), 7.40 (1H, m), 7.25 (4H, m), 7.22 (2H, d, *J* = 8.7 Hz), 6.94 (2H, d, *J* = 8.7 Hz), 6.20 (1H, dd, *J* = 9.6, 4.1 Hz), 3.83 (1H, ddd, *J* = 14.0, 9.6, 6.6 Hz), 3.77 (3H, s), 3.53 (1H, dt, *J* = 14.0, 4.2 Hz), 3.40 (3H, s); $^{13}C$ NMR (DMSO-$d_6$, 150 MHz) $\delta_C$ 166.4, 165.1, 159.5, 133.9, 131.4, 129.8, 129.6, 128.7, 128.5, 128.4, 128.4, 128.4, 128.4, 128.2, 128.2, 127.1, 127.1, 127.0, 126.9, 126.9, 113.9, 113.9, 76.3, 55.3, 55.2, 43.9.

**Inhibition of nitric production assay**

The murine microglial BV2 cells, purchased from the Cell Culture Centre at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), at 37 °C atmosphere, 5% $CO_2$, and 100% relative humidity. The microglia cells were placed in 96-well cell culture plates (2× 104 cell/mL) and preincubated for 24 h. Then the cells were treated with various concentrations of isolated compounds in triplicate for 1 h and continuously incubated with LPS (Sigma-Aldrich) (0.3 μg/mL) for 24 h. Curcumin was used as the positive control. After incubation, the supernatants (100 μL) were added to a solution of 100 μL of Griess reagent (a 1:1 mixture of 0.1% naphthyl ethylene diamine and 1% sulfanilamide in 5% $H_3PO_4$) at room temperature for 20 min. NO concentration was quantified by a microplate reader at 540 nm for the amount of stable nitrite produced in the cell culture supernatants using the Griess assay[27].

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