Small Molecule Constituents of *Periplaneta americana* and Their IL-6 Inhibitory Activities

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Abstract

Two new glycosides, periplanosides A (1) and B (2), 3 compounds reported from a natural source for the first time (3 – 5), and 6 known compounds 6 – 11 were isolated from the ethanol extract of *Periplaneta americana* (Linnaeus). Their structures, including absolute configurations, were unambiguously identified by comprehensive spectroscopic and chemical methods. Compound 3 is a racemate whose enantiomers were purified by chiral high-performance liquid chromatography. The biological evaluation results showed that compound 7 (0 – 20 μM) did not affect the viability of RAW264.7 cells and could effectively inhibit the production of interleukin-6 stimulated by lipopolysaccharide in a concentration-dependent manner, indicating the potential to develop novel agents against inflammation-related diseases.

Keywords

*Periplaneta americana*, periplanosides A and B, ethanol extract, anti-inflammatory, IL-6

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*Periplaneta americana* (Linnaeus) is a medicinal insect traditionally used for the treatment of stomach diseases, ulcers, and burns.\textsuperscript{3} Pharmacological studies showed that *P. americana* could promote tissue repair and regeneration,\textsuperscript{2} and possess anticancer and anti-inflammatory activities as well.\textsuperscript{3} “Kangfuxinye,” with *P. americana* as the only ingredient, is a well-known drug in the Chinese market to treat various ulcers and wounds.\textsuperscript{3} Chemical investigations of the insect have disclosed the presence of amino acids,\textsuperscript{5} cyclic dipeptides,\textsuperscript{6} sex pheromones,\textsuperscript{6} carnosine,\textsuperscript{7} and isocoumarin. We are interested in small-molecule compounds of *P. americana* and our previous studies have identified the existence of some phenolic derivatives,\textsuperscript{9} dopamine derivatives,\textsuperscript{9} and dihydrocoumarin glucosides.\textsuperscript{10} Continuing with our study of *P. americana*, the present research records the isolation of 11 small molecules, including 2 new compounds, periplanoside A (1) and B (2), 3 compounds (3 – 5) which were isolated from a natural source for the first time, and 6 known compounds (6 – 11) (Figure 1).

Results and Discussion

Compound 1 was obtained as a yellowish gum. Its molecular formula was determined as C_{16}H_{17}NO_{8} on the basis of its high-resolution electrospray ionization mass spectrometry (HRESIMS) ion at \textit{m}/\textit{z} 374.0843 [M + Na]\textsuperscript{+} (calcd for C_{16}H_{17}NO_{8}Na, 374.0850), and \textsuperscript{13}C nuclear magnetic resonance (NMR) and distortionless enhancement by polarization transfer (DEPT) spectra, with the molecular formula indicating 9° of unsaturation. The \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopic data (Table 1) showed the presence of a glucose moiety in the structure. Its \textit{β}-form was indicated by the anomeric proton (\textit{δ}_{H} 5.16, d, J = 7.8 Hz, H-1'). The \textsuperscript{13}C NMR and DEPT spectra contained 11 carbon resonances attributed to 5 sp\textsuperscript{2} methines and 5 quaternary carbons. The \textsuperscript{1}H–\textsuperscript{1}H COSY spectrum (Figure 2) showed the correlations of H-3/H-4, H-5/H-6 and H-6/H-7. The HMBC spectrum presented the correlations of H-3/C-2,
Figure 1. Chemical formulas of compounds 1–11.

Table 1. $^1$H (600 MHz) and $^{13}$C Nuclear Magnetic Resonance (NMR) (150 MHz) Spectroscopic Data of 1 and 2 in Methanol-$d_4$ ($\delta$ in ppm, $J$ in Hz).

| Position | $\delta_1$ | $\delta_C$ | $\delta_1$ | $\delta_C$ |
|----------|------------|------------|------------|------------|
| 2        | 5.16 d (7.8)| 148.7, C   | 6.83 b (1.5)| 129.8, C   |
| 3        | 3.73 overlap| 74.6, CH   | 6.99 d (5.7)| 116.6, CH  |
| 4        | 3.54 overlap| 77.6, CH   | 4.62 d (5.7)| 141.3, C   |
| 5        | 3.46 overlap| 71.4, CH   | 4.12 q (3.0)| 146.8, C   |
| 6        | 3.56 overlap| 78.5, CH   | 4.31 d (3.4)| 116.6, CH  |
| 7        | 6.10 d (7.3, 5.1)| 59.3, CH | Ha: 4.28 dd (12.3, 7.3) | 62.9, CH$_2$ |
| 8        | 3.73 overlap| 77.6, CH   | 6.77 brd (7.9, 1.5)| 116.2, CH  |
| 9        | 3.73 overlap| 77.6, CH   | 6.76 brd (7.9)| 120.3, CH  |
| 10       | 3.73 overlap| 77.6, CH   | 6.10 d (7.3, 5.1)| 59.3, CH   |
| 11       | 3.73 overlap| 77.6, CH   | Ha: 4.28 dd (12.3, 7.3) | 62.9, CH$_2$ |

Natural Product Communications
C-11, H-4/C-2, C-9, C-10, H-5/C-4, C-10, C-9, H-6/C-8, C-10, and H-7/C-5, C-8, C-9. Considering these data and the downfield chemical shifts of C-2 (δ_C 148.7) and C-9 (δ_C 140.2), the molecular composition, and the requirement of unsaturation degrees, it can be found that 1 contains a quinoline ring with a carboxylic acid group at C-2. The connection of glucose with the aglycone was supported by the HMBC observation of H-1'/C-8 (δ_C 154.4). As a result, the planar structure of 1 was deduced. As for the sugar moiety of 1, the configuration was determined by acid hydrolysis followed by derivatization and comparison with a reference standard. Given that the standard retention times (min) of D-glucose and L-glucose derivatives were 12.43 and 14.04, respectively, and the retention time (min) of derivative 1 was 12.25, the glucose of 1 was determined as the D-form. Therefore, the structure of 1 was finally determined and named as periplanoside A.

Compound 2 was also obtained as a yellowish gum. Its molecular formula was determined as C_18H_20N_4O_8 on the basis of its HRESIMS ion at m/z 443.1175 [M + Na]^+ (calcd for C_18H_20N_4O_8Na, 443.1170), indicating 11° of unsaturation. The ^1H NMR spectrum of compound 2 (Table 1) showed two signals at δ_H 8.33 (s, 1H) and δ_H 8.26 (s, 1H), an ABX (1,2,4- or 1,3,4-trisubstituted) spin system (δ_H 6.76 [brd, J = 7.9 Hz, 1H], δ_H 6.83 [brd, J = 1.5 Hz, 1H], and δ_H 6.77 [brd, J = 7.9 and 1.5 Hz, 1H]). Besides, there are signals at δ_H 6.10 (dd, J = 7.3, 5.1 Hz, 1H, H-7’’), δ_H 5.99 (d, J = 5.7 Hz, 1H, H-1’), and five characteristics of the presence of a ribose. The ^13C NMR and DEPT spectra of 2 contained 18 carbon resonances attributed to 2 methylenes, 10 methines, and 6 quaternary carbons with a carbonyl. These diagnostic signals, in consideration of the previously isolated compounds from insects, prompted us to conclude the presence of a guanosine moiety. In addition, the other signals were identified as a phenylethyl alcohol group, evidenced from the observation of an ABX spin system, ^1H-^1H COSY correlation of H-7’’/H-8’’, and HMBC correlation of H-7’’/C-1’’, C-2’’, and C-6’’. The chemical shifts of C-3’’ (δ_C 141.3) and C-4’’ (δ_C 146.8) indicated that they were oxygenated. The phenylethyl alcohol group was connected with guanosine via C-7’’–N-5, supported by the HMBC correlations of H-7’’/C-4 and C-6. Thus far, the planar structure of 2 was deduced. As for the sugar moiety of 2, its configuration was determined by acid hydrolysis followed by derivatization and comparison with the reference standards. The standard retention times (min) of D-ribose and L-ribose derivatives were 17.10 and 13.30, respectively. The retention time (min) of derivative 2 was 17.00, indicating that the ribose was the D-form. However, it is challenging to assign the configuration at C-7’’. To clarify this, electronic circular dichroism (ECD) calculations were utilized. The results showed that the ECD spectrum of 7’R agreed well with the experimental CD of 2 (Figure 3), leading to the unambiguous assignment of the absolute configuration at C-7’’. The structure of 2 was determined and named as periplanoside B.

Compound 3 has a molecular formula of C_{11}H_{11}NO_4 by analysis of its positive HRESIMS ion at m/z 244.0581 [M + Na]^+ (calcd for C_{11}H_{11}NO_4Na, 244.0580), with 7° of unsaturation. The ^1H NMR spectrum of 3 (Table 2) showed a 1,2,3-trisubstituted benzene ring (δ_H 5.84 [dd, J = 7.5, 1.4 Hz, H-5], δ_H 5.93 [t, J = 6.35, H-6], and δ_H 5.88 [dd, J = 6.5, 1.4 Hz, H-7]). The ^13C NMR and DEPT spectra displayed 1 methyl group, 1 methylene, 4 methines, and 5 quaternary carbons (2 carbonyl groups). The NMR data of 3 were similar to those of jineol-8-sulfate, which is a quinoline derivative. Thus, it could be inferred that 3 was an analog of jineol-8-sulfate. The differences between 3 and jineol-8-sulfate were that compound 3 lacked a sulfonic acid group and 1 hydroxy group, but had 2 more carbonyl groups, according to the chemical shifts of C-2 (δ_C 167.5) and C-11 (δ_C 172.1). In addition, the ^1H-^1H COSY correlations of H-3'/H-4 and the HMBC correlations (Figure 2) of H-4/C-2, C-5, H-2'/C-10, and C-11 were sufficient to prove that the two carbonyl groups were located on C-2 and C-4, respectively. Further, the HMBC correlation of

Figure 2. The key COSY (–) and HMBC (—) correlations of compounds 1–4.

Figure 3. Comparison of B3LYP/6-311 g (d, p) calculated electronic circular dichroism (ECD) spectrum for (7’R)-2 with the experimental spectrum of 2 in MeOH. σ = 0.3 eV, shift = +21 nm.
H-12/C-11 ($\delta$C 172.1) suggested that the methoxy was located at C-11. Chiral high-performance liquid chromatography (HPLC) analysis indicated that 3 was a racemic mixture, which was further separated by chiral HPLC to afford two enantiomers (3a and 3b). The absolute configurations of these enantiomers were determined to be 4R for 3a and 4S for 3b by comparing the calculated ECD curves with the experimental CD spectra (Figure 4). After searching the database, it was found that 3 was commercially available, but no literature was found. Therefore, compound 3, as a naturally occurring substance, is reported for the first time. Here, we tentatively named it as methyl 8-hydroxy-2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylate.

Compound 4 has a molecular formula of C$_8$H$_{17}$NO$_2$ by analysis of its HRESIMS ion at $m/z$ 182.1150 [M+Na]$^+$ (calcd for C$_8$H$_{17}$NO$_2$Na, 182.1150), with 1° of unsaturation. The $^1$H NMR spectrum of 4 (Table 2) exhibited 1 methyl (0.91, t, $J$ = 7.2 Hz, H-6) and 6 sp$_3$ methylenes (2 hetero-atom bearing). The $^{13}$C NMR and DEPT spectra displayed resonances for 8 carbons including 1 methyl, 6 methylenes (1 oxygenated), and 1 amide carbonyl. The $^1$H–$^1$H COSY spectrum (Figure 2) showed correlations of H-2/H-3/H-4/H-5/H-6 and H-1'/H-2', suggesting 2 spin systems, which were assembled via HMBC correlations (Figure 2) of H-2, H-3, and H-1'/C-1 ($\delta$C 176.6). Apart from the above fragment, the remaining signal corresponded to an amino group on the basis of the chemical composition of 4 and the chemical shift of C-1' ($\delta$C 42.9). Therefore, the structure of 4 was determined.

Compound 4 had been previously synthesized,$^{13}$ but this was the first record of it as a natural product.

Compound 5 was isolated as white solid and its molecular formula was determined as C$_{10}$H$_{11}$NO$_3$. The NMR data of 5 were in accordance with those of a compound previously synthesized by Martínez-Gudiño.$^{14}$ However, it was characterized from a natural source for the first time. The absolute configuration of 5 was assigned as 8S by comparing the experimental CD curve with the calculated one in Figure 5. By comparing their spectroscopic data with those in the literature, 6 known compounds were identified as trans-4-hydroxy-2-nonenal (6),$^{15}$ (1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (7),$^{16}$ 6-(2-formyl-1-pyrrolyl)-L-norleucin (8),$^{17}$ (±)-1,2,3,4-tetrahydro-2-oxo-4-quino linecarboxylic acid (9),$^{18}$ phenylalanine (10),$^{19}$ and proline (11).$^{20}$
As illustrated in Figure 6, the cell counting kit-8 (CCK-8) assay showed that compound 7 did not affect the viability of RAW264.7 cells when using any of the examined concentrations. The enzyme-linked immunosorbent assay (ELISA) results showed that compound 7 could effectively suppress the production of interleukin-6 (IL-6) in RAW264.7 cells stimulated by lipopolysaccharide (LPS) in a concentration-dependent manner. Thus, the present data suggested that compound 7 might act as a lead compound for the development of novel agents against diseases resulting from pathogenic expression or activation of IL-6.

**Experimental**

**General**

HRESIMS were collected by a Shimazu LC-20AD AB SCIEX triple TOF X500R mass spectrometer (MS) spectrometer (Shimadzu Corporation). Ultraviolet (UV) and circular dichroism (CD) spectra were measured on a Jasco J-815 CD spectrometer (JASCO), and optical rotations on an Anton Paar MCP-100 digital polarimeter. MCI gel CHP 20P (75-150 μm, Mitsubishi Chemical Industries), macroporous adsorbents (Rohm Haas Amberlite TM XAD 16N), RP-18 (40-60 μm; Daiso Co.), YMC gel ODS-A-HG (40-60 μm; YMC Co.), and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography. For semipreparative HPLC, a Saipuruisi chromatograph with a YMC-Pack ODS-A column (25 mm × 10 mm, i.d., 5 μm) was used. For preparative HPLC, a Chuangxin-Tongheng Chromatograph was used, equipped with a Thermo Hypersil GOLD-C18 column (250 mm × 21.2 mm, i.d., 5 μm). Racemic compounds were purified by chiral HPLC on a Daicel Chiralpak AD-H column (250 mm × 4.6 mm, i.d., 5 μm) or a Phenomenex column (OOG-4762-E0 LUX i-Amylose-1, 250 mm × 4.6 mm, i.d., 5 μm). NMR spectra were recorded on either a Bruker AV-500 or an AV-600 spectrometer (Bruker) with tetramethylsilane (TMS) as an internal standard.

**Insect Material**

*P. americana* was bought from the Breeding Base of He-Yuan-Mei-Lian in Midu County, Dali Autonomous Prefecture, Yunnan Province, PR China, in December 2017. A voucher specimen (CHYX-0627) was deposited at the School of Pharmaceutical Sciences, Shenzhen University, PR China.

**Extraction and Isolation**

Dried and powdered *P. americana* (50.0 kg) were extracted with 70% EtOH to afford a crude extract. This was suspended in water and then extracted with an equal volume of ethyl acetate, three times, to obtain ethyl acetate and water extracts, respectively. The water-soluble extract was divided into 6 parts (Fraction [Fr.] A–Fraction F) using macroporous adsorbent resin and eluting with a gradient of aqueous ethanol (1%-100%). Fr. A (1.0 kg) was divided into 10 parts (Fr. A.1–Fr. A.10) by using a MCI gel CHP 20P column and eluting with a gradient of aqueous MeOH (3%-100%). Fr. A.6 (7.0 g) was gel filtered over Sephadex LH-20 (aqueous
MeOH, 65%) to yield Fr. A.6.1 to Fr. A.6.4. Fr. A.6.2 (3.0 g) was applied to a RP-18 column and eluted with a gradient of aqueous MeOH (2%-100%) to produce 4 fractions (Fr. A.6.2.1-Fr. A.6.2.4). Fr. A.6.2.2 (650.0 mg) was gel filtered over Sephadex LH-20 (MeOH) to give Fr. A.6.2.1 to Fr. A.6.2.4. Fr. A.6.2.2 (200.0 mg) was purified by preparative HPLC (aqueous MeOH, 3%-100%, flow rate: 8 mL/min) to obtain Fr. A.6.2.2.1 to Fr. A.6.2.2.4. Fr. A.6.2.2.2 (18.0 mg) was separated by semipreparative HPLC (aqueous ACN, 11%) to obtain compound 1 (1.2 mg, t_R = 18.4 min, flow rate: 3 mL/min). Fr. A.6.2.3 (100.0 mg) was purified by preparative HPLC (aqueous MeOH, 3%-100%, flow rate: 8 mL/min) to obtain Fr. A.6.2.3.1 to Fr. A.6.2.3.2. Fr. A.6.2.3.1 (15.0 mg) was separated by semipreparative HPLC (aqueous MeOH, 11%) to obtain compound 2 (2.5 mg, t_R = 16.3 min, flow rate: 3 mL/min) and compound 5 (4.0 mg, t_R = 22.3 min, flow rate: 3 mL/min). Fr. A.6.1.1 (3.7 g) was subjected to an RP-18 column (aqueous MeOH, 3%-100%) to produce 3 portions (Fr. A.6.1.1.1-Fr. A.6.1.1.3). Among them, Fr. A.6.1.1.3 (600.0 mg) was separated by Sephadex LH-20 (MeOH) to yield 4 fractions (Fr. A.6.1.1.3.1-Fr. A.6.1.1.3.4). Fr. A.6.1.1.3.4 (30.0 mg) was further treated by HPLC (aqueous MeOH, 11%) to afford compound 6 (3.0 mg, t_R = 19.2 min, flow rate: 3 mL/min). Fr. A.6.1.1 (3.7 g) was fractionated on an MCI gel CHP 20P column eluted with a gradient of aqueous MeOH (5%-100%) to provide 3 parts (Fr. A.6.1.1-Fr. A.6.1.3). Fr. A.6.1.3 (502.0 mg) was chromatographed over Sephadex LH-20 (MeOH) to produce 5 portions (Fr. A.6.1.3.1-Fr. A.6.1.3.5). Among them, Fr. A.6.1.3.5 was submitted to semipreparative HPLC (aqueous MeOH, 27%), followed by semipreparative HPLC (aqueous ACN, 12%) to afford compound 11 (3.6 mg, t_R = 31.0 min, flow rate: 3 mL/min). Fr. B (200.0 g) was divided into 5 parts (Fr. B.1–Fr. B.5) by using an MCI gel CHP 20P column eluted with a gradient of aqueous MeOH (3%-100%). Then, Fr. B.3 (30.0 g) was divided into 9 portions (Fr. B.3.1-Fr. B.3.9) by RP-18 (aqueous MeOH, 5%-100%). Fr. B.3.8 (1.9 g) was gel filtered through Sephadex LH-20 (MeOH) to give Fr. B.3.8.1 to Fr. B.3.8.5. Fr. B.3.8.5 (450.0 mg) was subjected to preparative HPLC (aqueous MeOH, 3%-100%, flow rate: 8 mL/min) to provide eight portions (Fr. B.3.8.5.1-Fr. B.3.8.5.8). Purification of Fr. B.3.8.5.1 (960.0 mg) with aqueous ACN (16%) afforded compound 3 (3.0 mg, t_R = 16.6 min, flow rate: 3 mL/min). Fr. B.3.4 (3.7 g) was separated on a Sephadex LH-20 column (MeOH) to give Fr. B.3.4.1 to Fr. B.3.4.3, and Fr. B.3.4.3 (1.5 g) on a RP-18 column (aqueous MeOH, 3%-100%) to produce 5 portions (Fr. B.3.4.3.1-Fr. B.3.4.3.5). Among them, Fr. B.3.4.3.3 (700.0 mg) was submitted to a Sephadex LH-20 column (MeOH) to yield 4 fractions (Fr. B.3.4.3.3.1-Fr. B.3.4.3.3.4). Fr. B.3.4.3.3.4 (40.0 mg) after semipreparative HPLC (aqueous MeOH, 33%), afforded compound 7 (5.0 mg, t_R = 14.1 min, flow rate: 3 mL/min). Fr. B.3.4.3.3 (70.0 mg), following semipreparative HPLC (aqueous ACN, 22%), afforded compounds 8 (3.0 mg, t_R = 16.6 min, flow rate: 3 mL/min) and 9 (8.0 mg, t_R = 22.4 min, flow rate: 3 mL/min).

### Compound Characterization Data

**Periplanoside A (1):** yellowish gum; UV (MeOH) _λ_{max} (log ε)_: 203 (4.10), 248 (4.12) nm; ([α]_{D}^{20} + 18.5 [ε 0.05, MeOH]; HRESIMS (positive) _m/z_ 374.0843 [M + Na]^+ (calcd for C_{16}H_{22}NO_{5}Na, 374.0850); for 1H and 13C NMR data, see Table 1.

**Periplanoside B (2):** yellowish gum; UV (MeOH) _λ_{max} (log ε)_: 203 (4.60), 225 (4.08), 244 (4.03), 263 (3.80), 280 (3.85) nm; ([α]_{D}^{20} + 17.3 [ε 0.05, MeOH]; CD [MeOH] Δε_{231} + 2.58, Δε_{231} – 1.89, Δε_{231} + 1.72, Δε_{284} + 2.68); HRESIMS (positive) _m/z_ 443.1175 [M + Na]^+ (calcd for C_{18}H_{23}NO_{4}Na, 443.1170); for 1H and 13C NMR data, see Table 1.

**Methyl-8-hydroxy-2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylate (3):** yellowish solid; UV (MeOH) _λ_{max} (log ε)_: 211 (4.03), 240 (1.54), 249 (3.56), 269 (2.87), 295 (3.44) nm; ([α]_{D}^{20} + 33.3 [ε 0.03, MeOH]; CD [MeOH] Δε_{206} + 4.9, Δε_{226}– 7.63, Δε_{251} + 3.74; 3α); ([α]_{D}^{20} – 28.1 [ε 0.03, MeOH]; CD [MeOH] Δε_{206}– 6.10, Δε_{226} + 6.83, Δε_{251}– 4.01; 3β); HRESIMS (positive) _m/z_ 244.0581 [M + Na]^+ (calcd for C_{11}H_{13}NO_{3}Na, 244.0580); for 1H and 13C NMR data, see Table 2.

**N-(Hexanoyl)ethanolamine (4):** yellowish gum; UV (MeOH) _λ_{max} (log ε)_: 200 (3.60), 254 (2.55) nm; ([α]_{D}^{20} + 33.3 [ε 0.09, MeOH]; HRESIMS (positive) _m/z_ 182.1150 [M + Na]^+ (calcd for C_{8}H_{17}NO_{2}Na, 182.1150); for 1H and 13C NMR data, see Table 2.

**N-Formylphenylalanine (5):** white solid; UV (MeOH) _λ_{max} (log ε)_: 196 (4.05) nm; ([α]_{D}^{20} + 37.9 [ε 0.04, MeOH]; CD [MeOH] Δε_{199} + 8.24, Δε_{213} + 1.89, Δε_{218} + 1.63, Δε_{233}– 0.13); HRESIMS (positive) _m/z_ 216.0636 [M + Na]^+ (calcd for C_{11}H_{17}NO_{2}Na, 216.0630); for 1H and 13C NMR data, see Table 2.

### Acid Hydrolysis and Preparation of Sugar Derivatives of Compounds 1 and 2

Compounds 1 and 2 (each 0.5 mg) were separately dissolved in 6 N HCl (0.8 mL) and heated at 65 °C for 1.5 h. L-cysteine methyl ester in 1 mL pyridine was added to the concentrated mixture and heated at 65 °C for 1 h. Using the same method, ribose (D/L) and glucose (D/L) were also derivatized by using L-cysteine methyl ester. L-cysteine methyl ester was added to the reaction mixtures and heated at 65 °C for 1 h. The reaction mixtures were concentrated and analyzed using chiral HPLC (Daicel Chiralpak IC column [250 mm × 4.6 mm, i.d., 5 μm]); n-hexane/ethanol, 80:20; flow rate: 1 mL/min; compound 1, D-glucose, and L-glucose) with a UV detector and RP-18 HPLC (YMC-Pack ODS-A column, [250 mm ×
4.6 mm, i.d., 5 μm]; MeOH/H2O [0.05% CF3COOH], 40:60; flow rate: 1 mL/min; compound 2, D-ribose, and L-ribose). The standard retention times (min) of the derivatives were as follows: D-glucose (12.43), L-glucose (14.04), D-ribose (17.10), and L-ribose (13.30). By comparing the retention times in our experiment with these standards, both glucose in compound 1 and ribose in compound 2 were determined to be in the D-form (see Supplemental Material).

Biological Evaluation

To obtain enough cells for analysis, a mouse macrophage line of RAW264.7 (Procell Life Science & Technology Co.), was cultured in high-glucose Dulbecco’s modified Eagle’s medium (DMEM) (C11995500BT, Gibco), which was supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum (2094468CP, Gibco) in a humidified environment containing 5% CO2 at 37 °C. To examine the cytotoxic effect of compounds on RAW264.7 cells, 2×104 cells/mL were seeded into 96-well plates with DMEM for overnight culture. Then, the cells were treated with either dimethyl sulfoxide (DMSO) or various concentrations of compounds for 24 h, following by the addition of 10 μL CCK-8 (Beyotime) into each well for 1 h at 37 °C. The absorbance of each well was recorded at 450 nm using a microplate reader (BioTek). To investigate whether compounds possess biological activities, different concentrations of compounds were used to pretreat RAW264.7 cells (2×104 cells/mL, 100 μL medium/well) for 2 h followed by stimulation with LPS (1 μg/mL) for another 4 h. According to the instructions from the manufacturer, the collected culture supernatant from each well was assayed with an IL-6 ELISA Kit (Jiangsu Yutong Biotech Co. Ltd).

Declaration of Conflicting Interests

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Supplemental Material

Supplementary material relating to this article is available online.

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