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Synthesis and Anti-inflammatory Evaluation of (*R*)-, (*S*)-, and (±)-Sanjuanolide Isolated from *Dalea frutescens*

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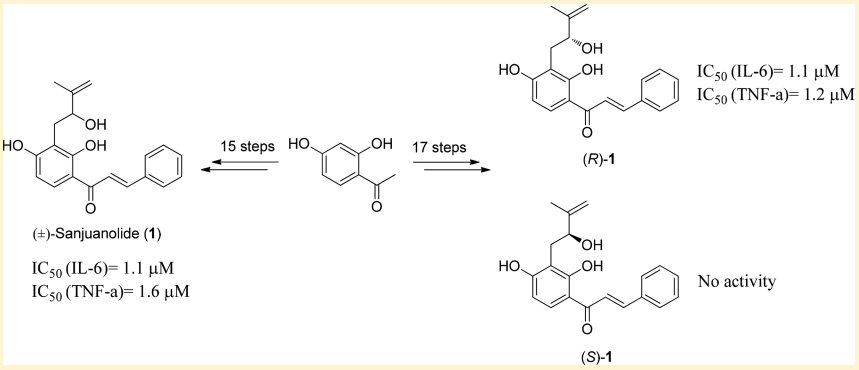
Supporting Information



ABSTRACT: The known chalcone (±)-sanjuanolide (**1**) can be isolated from *Dalea frutescens*. This study presents a convergent strategy for the first total synthesis of (*R*)-, (*S*)-, and (±)-sanjuanolide (**1**). The key step for synthesizing (*R*)- and (*S*)-**1** was a Corey−Bakshi−Shibata enantioselective carbonyl reduction to construct the C-2″ configuration. (*R*)-**1** efficiently inhibited the lipopolysaccharides (LPS)-induced expression of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), while (*S*)-**1** produced no significant anti-inflammatory effect. (*R*)-**1** also effectively inhibited the mRNA expression of several inflammatory cytokines after the LPS challenge in vitro. The synthesis and biological properties of these compounds have confirmed (*R*)-sanjuanolide and (±)-sanjuanolide as promising new leads for developing anti-inflammatory agents.

Naturally occurring chalcones have been isolated from several plant species and usually have significant and diverse bioactivities. They have also proven to be a prolific source of small-molecular chemical entities for developing clinical drugs.[1] The incorporation of isoprenyl moieties in the skeleton usually leads to a wide spectrum of biological properties, including anti-inflammatory, anti-tumor, and anti-diabetic activities.[2] Sanjuanolide (**1**, Figure 1) possesses an isoprenylated chalcone skeleton and was isolated in 2016 from the extracts of *Dalea frutescens* A. Gray (Leguminosae).[3] Further extensive spectroscopic studies have revealed the (2″-*R*) absolute configuration. However, the specific rotation data of (*R*)-**1** indicated racemization after several weeks of frozen storage.[3]

In biological assays, (*R*)-**1** exhibited a modest antiproliferative action on PC-3 and DU 145 prostate cancer cells, with IC_{50} values of 11.0 and 7.0 μM, respectively.[3] Despite extensive research on its isolation, biological activities, and mechanistic studies, the total synthesis of sanjuanolide (**1**) has not yet been reported. Since sanjuanolide (**1**) might be a valuable lead compound suitable for further modification during drug development,[1a] a new and efficient synthetic strategy has been developed for the first total synthesis of (*R*)-**1** and (*S*)-**1** using the commercially available 2,4-dihydroxyacetophenone as the starting material. In this approach, the C-2″ configuration was established using the Corey−Bakshi−Shibata enantioselective carbonyl reduction as a key step. This paper pursues the first total synthesis of (±)-sanjuanolide (**1**) and both of its enantiomers [(*R*)-**1**, (*S*)-**1**] and the evaluation of their in vitro anti-inflammatory activities.

The retrosynthetic analysis for synthesizing (*R*)-**1** is presented in Scheme 1. The target molecule, (*R*)-**1**, could be constructed through an aldol condensation between the protected acetophenone (*R*)-**2** and benzaldehyde,[4] followed by the deprotection of both phenolic groups. The chirality in (*R*)-**2** would be installed unambiguously using the Corey−Bakshi−Shibata stereospecific reduction of **3** with (*S*)-(+)-2-

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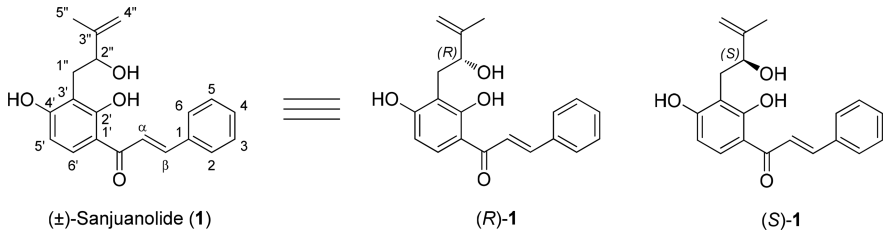
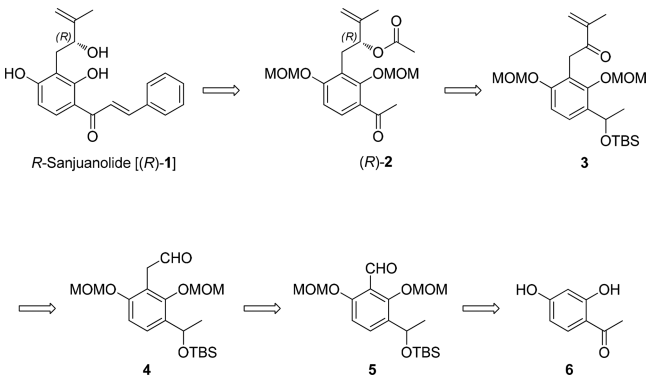


Figure 1. Structures of sanjuanolide (**1**) and its enantiomers.

Scheme 1. Retrosynthetic Analysis of (*R*)-1

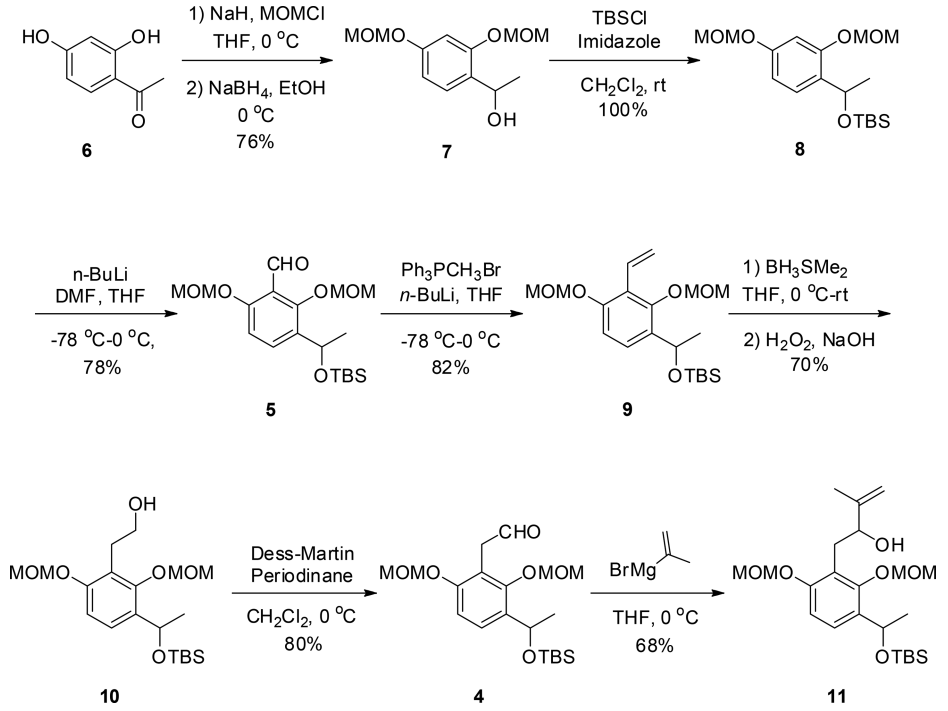


methyloxazaborolidine, while compound **3** could be derived from phenylacetaldehyde (**4**) via a Grignard reaction followed by oxidation.[5] Compound **4** would be derived from a protected benzaldehyde (**5**), through olefination, hydroboration−oxidation, and Dess−Martin oxidation.[6] The protected aldehyde **5** could be prepared from MOM (methoxymethyl ether)-protected 2,4-dihydroxyacetophenone (**6**), using Vilsmeier−Haack formylation.[4b,7]

The procedure for the synthesis of **11** from 2,4-dihydroxyacetophenone (**6**) is illustrated in Scheme 2. MOM protection of the phenolic groups of **6**, followed by $NaBH_4$ reduction, provided 1-[2,4-bis(methoxymethoxy)phenyl]-ethanol (**7**).[8] The resulting alcohol (**7**) was protected with *tert*-butyldimethylsilyl chloride (TBSCl) using a catalytic amount of imidazole to obtain a 76% overall yield of compound **8** in three steps. Treating **8** with *n*-butyllithium (*n*-BuLi) followed by dimethylformamide (DMF) at −78 °C provided benzaldehyde **5** in 78% yield.[4b] A Wittig olefination of aldehyde **5** with methyltriphenylphosphonium bromide in the presence of *n*-BuLi gave the anticipated phenylethylene **9**.[9] When phenylethylene (**9**) was subjected to hydroboration using $BH_3{\cdot}SMe_2$ at 0 °C, it afforded the primary alcohol **10** after an oxidative workup in 70% overall yield.[10] The primary alcohol **10** was oxidized using Dess−Martin periodinane in CH_2Cl_2, which smoothly generated the phenylacetaldehyde **4** in 80% yield. The addition of isopropenylmagnesium bromide provided the key secondary alcohol **11** in a yield of 68%.

With the key intermediate **11** in hand, the chirality in ketone **3** was introduced as depicted in Scheme 3. Thus, Dess−Martin oxidation of **11** in CH_2Cl_2 afforded ketone **3** in 79% yield. (*S*)-(+)-2-Methyloxazaborolidine was used as reagent due to its ability to provide defined configuration with a high enantiomeric excess (ee).[11] In this case, reduction with $BH_3{\cdot}SMe_2$ in the presence of catalytic (*S*)-(+)-2-methyloxazaborolidine provided (*R*)-**12** in a yield of 72%.

Scheme 2. Synthesis of the Key Intermediate 11 from 2,4-Dihydroxyacetophenone (6)



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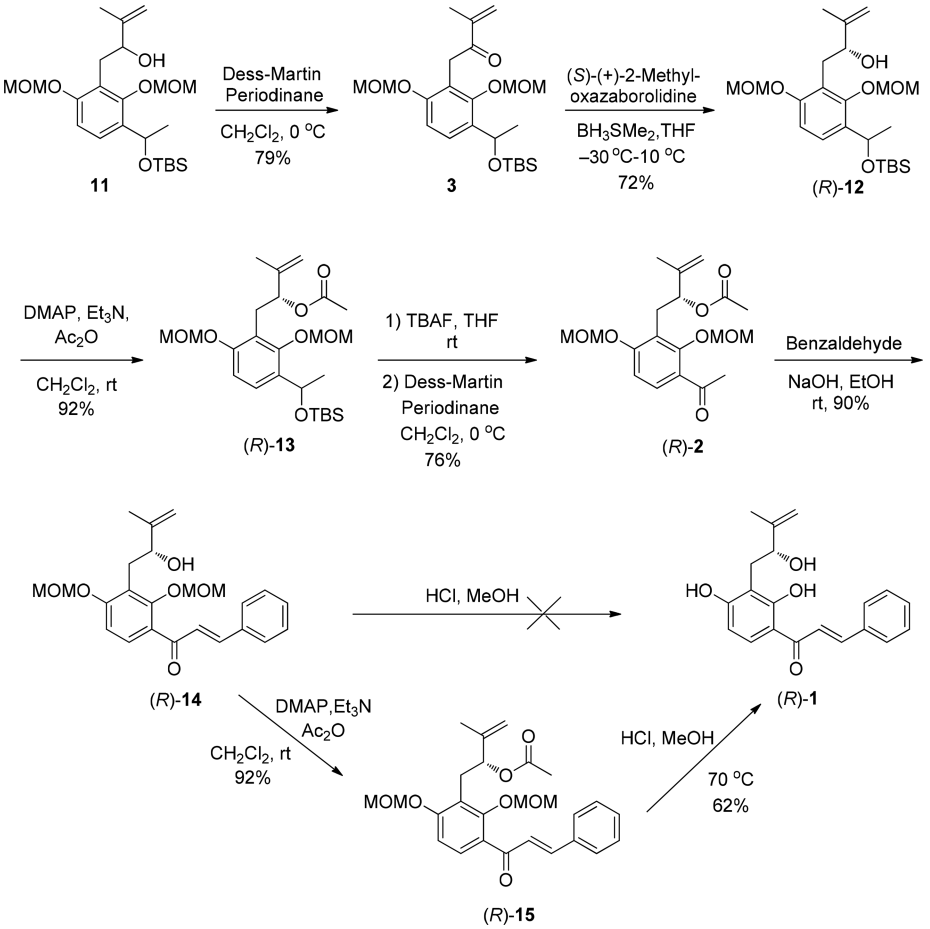
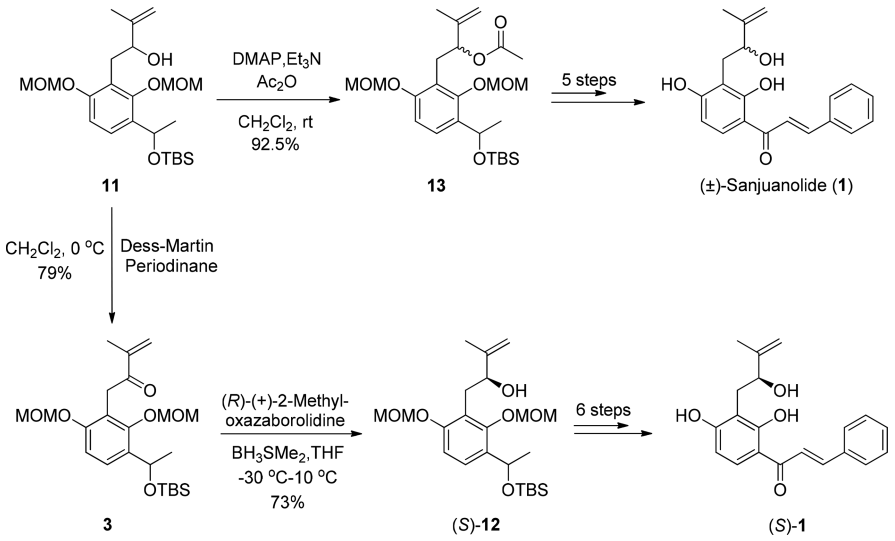
Scheme 3. Synthesis of (*R*)-1 from Intermediate 11



Scheme 4. Synthetic Routes of (±)-Sanjuanolide and (*S*)-1



Acetylation of (*R*)-**12** with Ac_2O/DMAP/Et_3N in CH_2Cl_2 efficiently gave intermediate (*R*)-**13** in a yield of 92%. TBS deprotection of (*R*)-**13** in the presence of tetrabutylammonium fluoride (TBAF) proceeded smoothly, and subsequent Dess−Martin oxidation produced the key acetophenone (*R*)-**2** in an overall yield of 76%.[12] Aldol condensation of the acetophenone (*R*)-**2** and benzaldehyde in ethanolic NaOH solution afforded chalcone (*R*)-**14** in a yield of 90%. It was significant that hydrolysis of the MOM protecting groups in (*R*)-**14** under standard conditions (HCl in MeOH) failed to give the target product. However, treating (*R*)-**14** with Ac_2O and catalytic amounts of DMAP under basic conditions afforded the acetylated intermediate (*R*)-**15**, which was effectively deprotected with HCl in MeOH to give target (*R*)-**1** (ee = 90.2%) in an overall yield of 57% in two steps.

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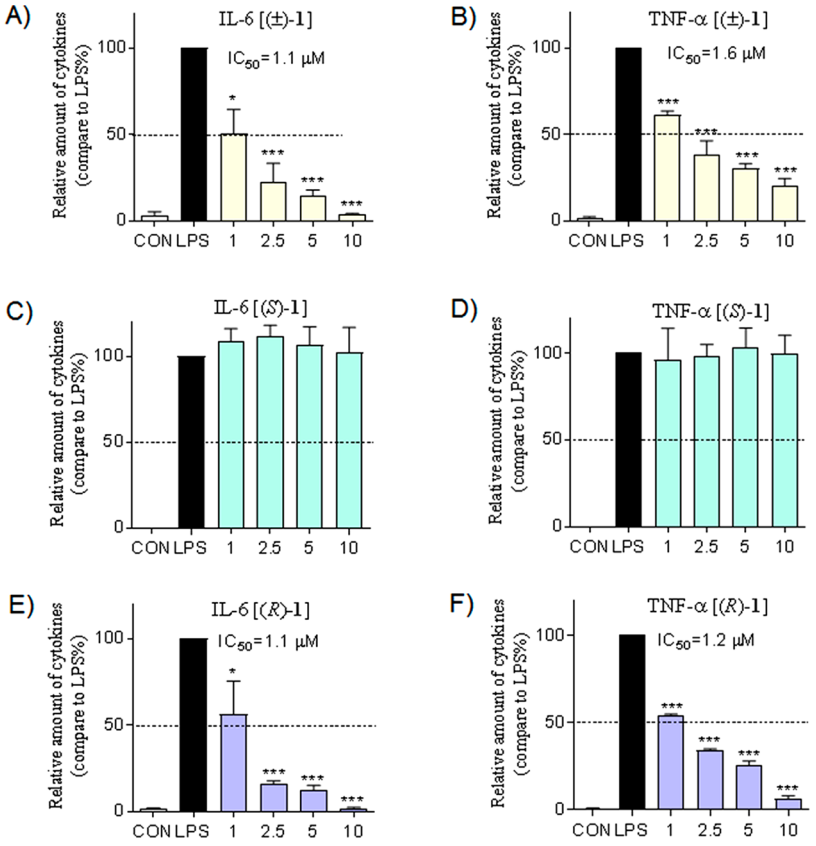

Figure 2. IC_{50} values (μM) of synthetic compounds for LPS-induced IL-6 and TNF-α. (A) IL-6 [(±)-**1**], (B) TNF-α [(±)-**1**], (C) IL-6 [(*S*)-**1**], (D) TNF-α [(*S*)-**1**], (E) IL-6 [(*R*)-**1**], (F) TNF-α [(*R*)-**1**]. Cells (5×10^5/plate) were pretreated with vehicle or compounds at indicated concentrations (1, 2.5, 5, and 10 μM) for 30 min. Then, cells were incubated with LPS (0.5 μg/mL) for 24 h. The inflammatory cytokine production in the supernatants was measured by ELISA. The significance of the difference in mean values relative to the LPS group is indicated as $^*p < 0.05$, $^{***}p < 0.001$ vs LPS vehicle.

The NMR data of the synthetic (*R*)-**1** (Table S1) and optical rotation data {observed $[\alpha]^{20}_D = -25.0$ (*c* 0.2, MeOH); reported $[\alpha]_D = -27.0$ (*c* 0.05, MeOH)} agreed well with those reported for the natural product.[3] Significantly, the racemization reported for the naturally isolated (*R*)-**1** (stored in powder form) was not observed in the synthetic sample.[3] However, the specific rotation for (*S*)-**1**, dissolved in MeOH, fell to almost zero after two months of frozen storage. Thus, the presence of the polar protic solvent may be responsible for its autoracemization.

A similar synthetic strategy was used to prepare (*S*)-**1** (ee = 86.8%) but with (*R*)-(−)-2-methyloxazaborolidine as the chiral auxiliary. Identical synthetic protocols were followed for the successful synthesis of (±)-sanjuanolide (**1**) in six steps from **11** to give an overall yield of 7.3% (Scheme 4).

Pro-inflammatory cytokines, such as IL-6 and TNF-α, have been recognized as critical regulators in inflammatory diseases[13] such as rheumatoid arthritis,[14] inflammatory bowel disease,[15] asthma,[16] and diabetic complications.[17] Inhibiting the release of pro-inflammatory cytokines is an important mode of action for anti-inflammatory drugs. The reported potent biological activity and anti-inflammatory potential of isoprenylated chalcones[18] therefore prompted the assessment of the anti-inflammatory activities of synthesized (±)-sanjuanolide (**1**) and its enantiomers. The bioassay results are shown in Figure 2. These results demonstrated that (±)-sanjuanolide (**1**) markedly reduced the production of lipopolysaccharide (LPS)-induced IL-6 and TNF-α with IC_{50} values of 1.1 and 1.6 μM, respectively (Figure 2A,B). Unexpectedly, (*S*)-**1** exhibited no inhibitory activity with these two cytokines (Figure 2C,D), and its enantiomer (*R*)-**1** exhibited similar activities to (±)-sanjuanolide (**1**) with IC_{50} values of 1.1 μM (for IL-6) and 1.2 μM (for TNF-α) (Figure 2E,F).

To further confirm the anti-inflammatory actions of these synthetic compounds, the potency of (*R*)-**1** for inhibiting inflammatory gene expression at the mRNA level was evaluated. Macrophages were treated with LPS (1.0 μg/mL) for 6 h, then examined for the expression of pro-inflammatory genes by a quantitative reverse transcription PCR (RT-qPCR) study. Figure 3 shows that LPS induced a significant increase in the mRNA expression of the pro-inflammatory cytokines, including TNF-α, IL-6, IL-1β (interleukin-1β), ICAM-1 (intercellular cell adhesion molecule-1), MACP-1 (mitochondrial anion carrier proteins-1), and COX-2 (cyclooxygenase-2), while pretreatment with (*R*)-**1** and (±)-sanjuanolide (**1**) significantly and effectively down-regulated mRNA expressions of TNF-α (Figure 3A), IL-6 (Figure 3B), IL-1β (Figure 3C), ICAM-1 (Figure 3D), MACP-1 (Figure 3E), and COX-2 (Figure 3F) in LPS-stimulated macrophages. These data indicated that (*R*)-**1** and (±)-sanjuanolide (**1**) were potent inhibitors of LPS-induced mRNA overexpression of inflammatory genes in LPS-stimulated macrophages, thus indicating that the configuration of (*R*)-**1** plays a key role in the observed anti-inflammatory activity.

In conclusion, a common route for the first total synthesis of (±)-sanjuanolide (**1**), (*R*)-**1**, and (*S*)-**1** has been established in

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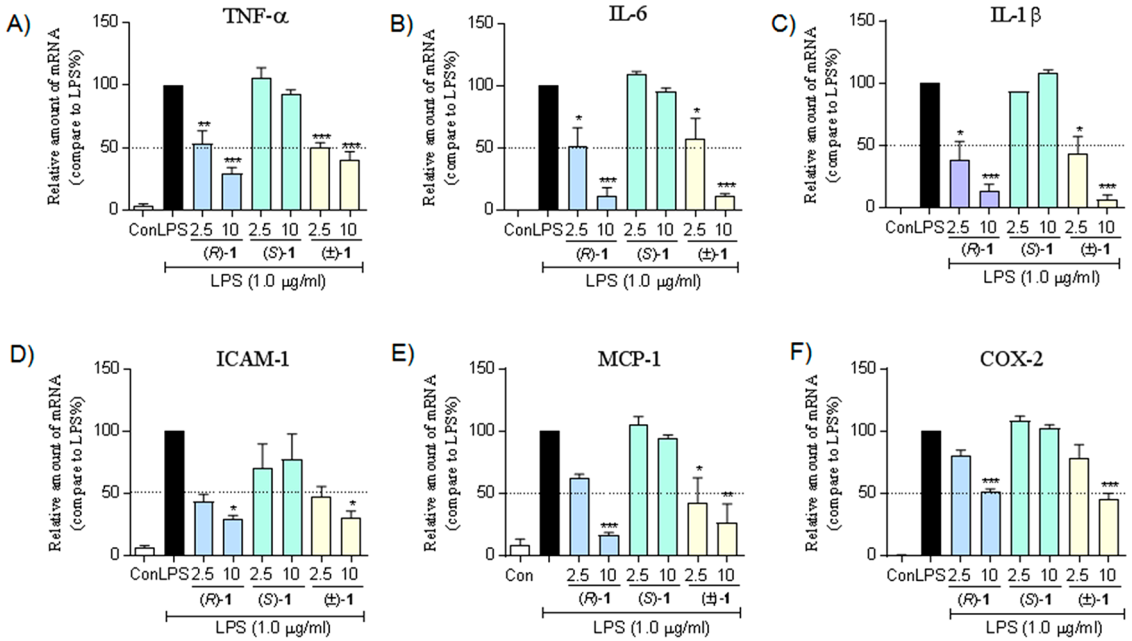

Figure 3. Inhibitory effects of compounds on the expression of inflammatory genes induced by LPS-stimulated macrophages. (A) TNF-α, (B) IL-6, (C) IL-1β, (D) ICAM-1, (E) MCP-1, (F) COX-2. The cells were plated at a density of 7.0×10^5/plate, then incubated overnight at 37 °C in a 5% CO_2 atmosphere. The macrophages were pretreated with 2.5 and 10 μM compounds for 30 min, then incubated with LPS (1.0 μg/mL) for 24 h. The cells were collected, and the total RNA was extracted. The mRNA levels of the inflammatory cytokines were detected by qPCR. The results are presented as the percentage of the LPS control. Each bar represents the mean ± SEM from three independent experiments. The significance of the difference in mean values relative to the LPS group are indicated as $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs LPS vehicle.

15 (racemic) and 17 (enantiomers) steps with overall yields of 3.8%, 4.2%, and 7.3%, respectively. Biological studies have shown that the (2″*R*)-configuration is important for anti-inflammatory activity. These results could be particularly useful for further pharmaceutical development to treat LPS-induced inflammatory damage. Further structural modifications and evaluation of their biological potential are ongoing and will be reported in due course.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO P-2000 digital polarimeter at ambient temperature. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker instrument at 400 or 500 MHz, and peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard. *J* values are given in Hz. ^{1}H NMR data are reported in the order chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and/or multiple resonances), number of protons, and coupling constant in Hz. High-resolution measurements were made with a Synapt HDMS (Waters, UK) instrument. Analytical HPLC analyses were performed at ambient temperature on an Agilent 1260 liquid chromatograph and equipped with a G1314A VWD detector. Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Reaction flasks were dried at 100 °C. Air- and moisture-sensitive reactions were performed under an argon atmosphere. TLC was conducted on Kieselgel 60 F_{254} plates, and flash column chromatography (MPLC) purifications were performed using Merck silica gel 60 (230−400 mesh ASTM) (Merck KGaA, Darmstadt, Germany).

*(±)-1-[2,4-Bis(methoxymethoxy)phenyl]ethanol (**7**).* To a solution of 2,4-dihydroxyacetophenone (**6**) (6.0 g, 24.6 mmol) in anhydrous tetrahydrofuran (THF) (50 mL) was slowly added NaH (3.8 g, 157.7 mmol) at 0 °C. Chloromethyl methyl ether (6.4 g, 78.9 mmol) was added, and the mixture stirred at room temperature for 4 h. The reaction progress was monitored by TLC. After completion of the reaction, the resulting mixture was quenched with ice water and diluted with EtOAc (50 mL). The organic layer was washed with H_2O (3 × 50 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc = 15:1) to provide 7.2 g (76%) of a colorless oil.

To a cooled (0 °C) solution of the oil (7.0 g, 29.1 mmol) in EtOH (50 mL) was added slowly $NaBH_4$ (2.2 g, 58.3 mmol). The mixture was warmed to room temperature and stirred for 3 h. The mixture was quenched with saturated NH_4Cl solution, and the EtOH was removed in vacuo. The aqueous layer was extracted with EtOAc (3 × 20 mL), and the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc = 5:1) to provide 7.1 g (100%) of **7** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, J = 8.5 Hz, 1H), 6.80 (d, J = 2.3 Hz, 1H), 6.71 (dd, J = 8.5, 2.3 Hz, 1H), 5.21 (s, 2H), 5.15 (s, 2H), 5.10 (q, J = 6.5 Hz, 1H), 3.49 (s, 3H), 3.47 (s, 3H), 1.49 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.5, 155.0, 128.0, 126.7, 108.9, 103.5, 94.6, 94.6, 65.7, 56.3, 56.0, 23.1; HRMS(ESI): calcd for $C_{12}H_{18}O_5Na$ $[M + Na]^+$ m/z 265.1052, found m/z 265.1017.

*(±)-{1-[2,4-Bis(methoxymethoxy)phenyl]ethoxy}(tert-butyl)-dimethylsilane (**8**).* To a solution of **7** (3.0 g, 12.4 mmol) in dichloromethane (DCM) (30 mL) was added imidazole (1.7 g, 24.8 mmol) at room temperature followed by TBSCl (3.7 g, 24.8 mmol), and the mixture was stirred at this temperature for 6 h. The reaction was quenched with saturated aqueous NH_4Cl (30 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc = 30:1) to give 3.6 g (100%) of **8** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, J = 8.5 Hz, 1H), 6.74 (s, 1H), 6.70 (d, J = 8.5 Hz, 1H), 5.18 (s, 2H), 5.17−5.13 (m, 3H), 3.49 (s, 3H), 3.48 (s, 3H), 1.35 (d, J = 6.1 Hz, 3H), 0.91 (s, 9H), 0.05 (s, 3H), −0.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.9, 153.5, 129.7, 126.8, 108.8, 102.6, 94.7, 94.3, 65.0, 56.0, 56.0, 26.0, 25.9 × 3, 18.3, −4.9, −4.9; HRMS(ESI) calcd for $C_{18}H_{32}O_5SiNa$ $[M + Na]^+$ m/z 379.1917, found m/z 379.1884.

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*(±)-3-{1-[(tert-Butyldimethylsilyl)oxy]ethyl}-2,6-bis-(methoxymethoxy)benzaldehyde (**5**).* To a solution of **8** (1.0 g, 2.8 mmol) in dry THF (15 mL) was added *n*-BuLi (2.5 M in *n*-hexane, 1.6 mL, 3.9 mmol) slowly at −78 °C under argon. After 30 min, the solution was allowed to warm to 0 °C and dry DMF (0.3 mL, 4.5 mmol) was then added dropwise. The mixture was stirred at room temperature for 2 h. After completion of the reaction, the reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc (3 × 20 mL). The organic layer was washed with H_2O (3 × 10 mL), dried over Mg_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc = 25:1) to provide 0.8 g (78%) of **5** as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.46 (s, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.01 (d, J = 8.8 Hz, 1H), 5.25−5.28 (m, 3H), 5.12 (d, J = 6.7 Hz, 1H), 5.00 (d, J = 6.7 Hz, 1H), 3.58 (s, 3H), 3.52 (s, 3H), 1.37 (d, J = 6.3 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 3H), −0.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.4, 159.6, 154.6, 134.8, 133.4, 118.4, 111.0, 102.3, 95.1, 65.1, 57.3, 56.6, 26.1, 25.8 × 3, 18.2, −4.9, −5.0; HRMS(ESI) calcd for $C_{19}H_{32}O_6SiNa$ $[M + Na]^+$ m/z 407.1866, found m/z 407.1847.

*(±)-{1-[2,4-Bis(methoxymethoxy)-3-vinylphenyl]ethoxy}(tert-butyl)dimethylsilane (**9**).* To a suspension of Ph_3PCH_3Br (2.8 g, 6.5 mmol) in THF (25 mL) was added *n*-BuLi (2.5 M in *n*-hexane, 3.3 mL, 8.3 mmol) slowly at −78 °C under argon. The mixture was stirred at 0 °C for 1 h. A solution of **5** (1.0 g, 2.6 mmol) in THF (5 mL) was added dropwise to the reaction mixture. After stirring for 2 h, the reaction was quenched with saturated aqueous NH_4Cl (30 mL) and extracted with Et_2O (3 × 30 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography (hexanes/EtOAc = 15:1) to afford **9** (0.8 g, 82%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (d, J = 8.5 Hz, 1H), 6.92 (d, J = 8.5 Hz, 1H), 6.79 (dd, J = 18.0, 12.0 Hz, 1H), 5.99 (dd, J = 18.0, 2.4 Hz, 1H), 5.50 (dd, J = 12.0, 2.4 Hz, 1H), 5.26−5.16 (m, 3H), 4.97 (d, J = 5.9 Hz, 1H), 4.91 (d, J = 5.8 Hz, 1H), 3.57 (s, 3H), 3.49 (s, 3H), 1.39 (d, J = 6.3 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 3H), −0.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.9, 152.4, 134.2, 128.5, 125.9, 120.3, 119.6, 111.1, 99.9, 94.9, 65.6, 57.3, 56.2, 26.3, 25.9 × 3, 18.2, −4.9, −5.0; HRMS (ESI) calcd for $C_{20}H_{34}O_5SiNa$ $[M + Na]^+$ m/z 405.2074, found m/z 405.2053.

*(±)-2-{3-{1-[(tert-Butyldimethylsilyl)oxy]ethyl}-2,6-bis-(methoxymethoxy)phenyl}ethanol (**10**).* To a solution of **9** (1.0 g, 2.6 mmol) in dry THF (15 mL) was added BH_3/SMe_2 (2.0 M in Et_2O, 2.6 mL, 5.2 mmol) slowly at 0 °C under argon. The reaction mixture was allowed to warm to room temperature and stirred for 4 h before 2 N NaOH (2.6 mL) and 30% H_2O_2 (1.0 mL) were added. Stirring was continued for 3 h, the mixture was diluted with saturated NH_4Cl (12 mL) and extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether/EtOAc = 15:1 to 8:1) to afford **10** (0.4 g, 70%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (d, J = 8.7 Hz, 1H), 6.92 (d, J = 8.7 Hz, 1H), 5.19 (d, J = 4.5 Hz, 2H), 5.12 (d, J = 6.3 Hz, 1H), 4.98 (d, J = 5.0 Hz, 1H), 3.84 (t, J = 6.3 Hz, 2H), 3.62 (s, 3H), 3.48 (s, 3H), 3.04−2.95 (m, 3H), 1.38 (d, J = 6.3 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 3H), −0.05 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.2, 153.7, 133.8, 125.5, 120.9, 110.6, 100.4, 94.7, 65.8, 62.6, 57.1, 56.2, 28.1, 26.3, 25.9 × 3, 18.2, −4.8, −4.9; HRMS(ESI) calcd for $C_{20}H_{36}O_6SiNa$ $[M + Na]^+$ m/z 423.2179, found m/z 423.2165.

*(±)-2-{3-{1-[(tert-Butyldimethylsilyl)oxy]ethyl}-2,6-bis-(methoxymethoxy)phenyl}acetaldehyde (**4**).* To a solution of **10** (1.0 g, 2.5 mmol) in CH_2Cl_2 (20 mL) was slowly added Dess−Martin periodinane (2.1 g, 5.0 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The mixture was quenched with saturated $NaHCO_3$ (20 mL) and NaS_2O_3 (2 mol/L, 4.0 mL). The aqueous layer was extracted with Et_2O (3 × 40 mL). The combined organic layers were washed with $NaHCO_3$ (40 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether/EtOAc = 30:1) to give **4** (0.8 g, 80%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.66 (s, 1H), 7.44 (d, J = 8.7 Hz, 1H), 6.96 (d, J = 8.7 Hz, 1H), 5.22−5.07 (m, 3H), 4.90−4.87 (m, 2H), 3.72 (d, J = 7.7 Hz, 2H), 3.56 (s, 3H), 3.44 (s, 3H), 1.39 (d, J = 6.3 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 3H), −0.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.3, 155.1, 153.7, 133.9, 126.7, 115.7, 110.4, 100.5, 94.6,65.7, 57.2, 56.2, 39.9, 26.3, 25.9 × 3, 18.2, −4.8, −4.9; HRMS(ESI) calcd for $C_{20}H_{34}O_6SiNa$ $[M + Na]^+$ m/z 421.2023, found m/z 421.2004.

*(±)-1-{3-{1-[(tert-Butyldimethylsilyl)oxy]ethyl}-2,6-bis-(methoxymethoxy)phenyl}-3-methylbut-3-en-2-ol (**11**).* To a solution of **4** (0.5 g, 1.3 mmol) in dry THF (8 mL) was slowly added isoprorenylmagnesium bromide (0.5 M solution in THF, 3.7 mL, 1.9 mmol) at −30 °C under argon. The mixture was stirred at 0 °C for 2 h. After completion of the reaction, the reaction was quenched with saturated ice water (25 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layer was washed with brine (25 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc = 20:1 to 5:1) to give **11** (0.4 g, 68%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, J = 8.6 Hz, 1H), 6.93 (d, J = 8.6 Hz, 1H), 5.19 (d, J = 2.4 Hz, 2H), 5.10 (q, J = 6.2 Hz, 1H), 5.03−4.99 (m, 3H), 4.82 (s, 1H), 4.35−4.30 (m, 1H), 3.62 (s, 3H), 3.49 (s, 3H), 3.03−2.99 (m, 1H), 2.94−2.89 (m, 1H), 1.85 (s, 3H), 1.38 (t, J = 6.3 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 3H), −0.05 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.1, 153.7, 148.1, 133.8, 125.6, 121.1, 110.7, 109.9, 100.4, 94.8, 75.4, 65.8, 57.2, 56.2, 31.5, 26.3, 25.9 × 3, 18.2, 18.1, −4.8, −4.9; HRMS(ESI) calcd for $C_{23}H_{40}O_6SiNa$ $[M + Na]^+$ m/z 463.2492, found m/z 463.2489.

*(±)-1-{3-{1-[(tert-Butyldimethylsilyl)oxy]ethyl}-2,6-bis-(methoxymethoxy)phenyl}-3-methylbut-3-en-2-one (**3**).* To a solution of **11** (0.4 g, 0.9 mmol) in CH_2Cl_2 (8 mL) was slowly added Dess−Martin periodinane (1.0 g, 2.4 mmol) at 0 °C. After stirring for 4 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL) and NaS_2O_3 (2 mol/L, 2.5 mL). The aqueous layer was extracted with Et_2O (3 × 25 mL). The combined organic layers were washed with $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether/EtOAc = 25:1) to afford **3** (0.3 g, 79%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, J = 8.7 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 6.05 (s, 1H), 5.76 (s, 1H), 5.16 (q, J = 6.2 Hz, 1H), 5.10 (q, J = 6.6 Hz, 2H), 4.85 (s, 2H), 4.08 (d, J = 4.3 Hz, 2H), 3.52 (s, 3H), 3.40 (s, 3H), 1.90 (s, 3H), 1.38 (d, J = 6.3 Hz, 3H), 0.89 (s, 9H), 0.04 (s, 3H), −0.05 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.4, 154.8, 153.3, 144.4, 133.7, 125.8, 123.8, 118.5, 110.4, 100.2, 94.6, 65.7, 57.0, 56.0, 34.8, 26.4, 25.9 × 3, 18.2, 17.9, −4.8, −5.0; HRMS(ESI) calcd for $C_{23}H_{38}O_6SiNa$ $[M + Na]^+$ m/z 461.2336, found m/z 461.2322.

*(2R)-1-{3-{1-[(tert-Butyldimethylsilyl)oxy]ethyl}-2,6-bis-(methoxymethoxy)phenyl}-3-methylbut-3-en-2-ol, (R)-**12**.* To a solution of *(S)-(+)-2-methyloxazaborolidine* (*S*-CBS,1.0 M solution in toluene, 0.1 mL, 27.7 mmol) in dry THF (2 mL) was added BH_3/SMe_2 (2.0 M in Et_2O, 0.3 mL, 0.7 mmol) dropwise at 0 °C under argon. After stirring for 30 min, a solution of **3** (0.2 g, 0.4 mmol) in dry THF was slowly added dropwise to the mixture at −30 °C. The reaction solution was allowed to warm gradually to 0 °C in 30 min. After 2 h, the reaction was quenched with MeOH (10 mL) and diluted with saturated NH_4Cl (20 mL). The mixture was extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography (petroleum ether/EtOAc = 15:1 to 8:1) to afford (*R*)-**12** (0.1 g, 72%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, J = 8.6 Hz, 1H), 6.93 (d, J = 8.6 Hz, 1H), 5.19 (d, J = 2.4 Hz, 2H), 5.10 (q, J = 6.3 Hz, 1H), 5.03−4.96 (m, 3H), 4.82 (s, 1H), 4.35−4.30 (m, 1H), 3.62 (s, 3H), 3.49 (s, 3H), 3.03−2.98 (m, 1H), 2.94−2.88 (m, 1H), 1.85 (s, 3H), 1.38 (t, J = 6.3 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 3H), −0.05 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.1, 153.7, 148.1, 133.8, 125.6, 121.0, 110.7, 109.9, 100.4, 94.8, 75.4, 65.8, 57.2, 56.2, 31.5, 26.3, 25.9 × 3, 18.2, 18.1, −4.8, −4.9; HRMS(ESI) calcd for $C_{23}H_{40}O_6SiNa$ $[M + Na]^+$ m/z 463.2492, found m/z 463.2489.

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The synthetic procedures, ^{1}H and ^{13}C NMR, and HRMS (ESI) data for (*S*)-**12** were identical with those of (*R*)-**12** described above. (*S*)-**12**: yield 73%, as a colorless oil.

*(2R)-1-{3-{1-[(tert-Butyldimethylsilyl)oxy]ethyl}-2,6-bis-(methoxymethoxy)phenyl}-3-methylbut-3-en-2-ylacetate, (R)-**13**.* To a solution of (*R*)-**12** (0.5 g, 1.1 mmol) in CH_2Cl_2 (10 mL) were added Et_3N (0.5 mL, 3.4 mmol), Ac_2O (0.2 mL, 2.3 mmol), and DMAP (0.02 g, 0.1 mmol). The mixture was stirred at room temperature for 2 h. The reaction was quenched with a saturated NH_4Cl solution (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc = 25:1) to provide 0.5 g (92%) of (*R*)-**13** as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (d, *J* = 8.7 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 1H), 5.62−5.57 (m, 1H), 5.20−5.13 (m, 3H), 4.99 (q, *J* = 6.5 Hz, 1H), 4.95 (s, 1H), 4.83−4.79 (m, 2H), 3.61 (s, 3H), 3.50 (s, 3H), 3.10−2.95 (m, 2H), 1.93 (s, 3H), 1.79 (d, *J* = 4.4 Hz, 3H), 1.36 (t, *J* = 5.6 Hz, 3H), 0.88 (s, 9H), 0.03 (s, 3H), −0.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 155.4, 153.6, 143.7, 133.8, 125.6, 119.5, 112.1, 110.3, 100.3, 94.8, 76.2, 65.7, 57.1, 56.2, 28.8, 26.4, 25.9 × 3, 21.0, 18.2, 18.2, −4.8, −5.0; HRMS(ESI) calcd for $C_{25}H_{42}O_7SiNa$ $[M + Na]^+$ *m*/*z* 505.2598, found *m*/*z* 505.2582.

The synthetic procedures and ^{1}H and ^{13}C NMR and HRMS (ESI) data for (*S*)-**13** and racemic **13** were identical with those of (*R*)-**13** described above. (*S*)-**13**: yield 95%, as a colorless oil. Racemic **13**: yield 92.5%, as a colorless oil.

*(R)-1-[3-Acetyl-2,6-bis(methoxymethoxy)phenyl]-3-methylbut-3-en-2-ylacetate, (R)-**2**.* To a solution of (*R*)-**13** (0.4 g, 0.8 mmol) in THF (6 mL) was slowly added TBAF (0.7 mL, 2.6 mmol) at room temperature for 5 h. After completion of the reaction, it was quenched with ice water (10 mL) and diluted with EtOAc (3 × 10 mL). The organic layer was washed with H_2O (30 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc = 10:1) to provide a colorless oil. To a solution of the oil (0.4 g, 0.9 mmol) in CH_2Cl_2 was added Dess−Martin periodinane (1.2 g, 2.9 mmol) slowly at 0 °C. After stirring for 4 h, the reaction was quenched with saturated $NaHCO_3$ (10 mL) and the aqueous layer was extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with brine (60 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether/EtOAc = 20:1 to 5:1) to give (*R*)-**2** (0.3 g, 76%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.7 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 5.59 (t, *J* = 6.5 Hz, 1H), 5.24 (s, 2H), 4.97 (dd, *J* = 6.5 Hz, 5.9 Hz, 2H), 4.88 (s, 1H), 4.84 (s, 1H), 3.53 (s, 3H), 3.50 (s, 3H), 3.16−3.14 (m, 1H), 3.07−3.05 (m, 1H), 2.55 (s, 3H), 1.95 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.3, 170.0, 159.5, 156.6, 143.5, 129.7, 127.4, 121.1, 112.4, 109.3, 101.5, 94.5, 76.0, 57.8, 56.3, 29.9, 28.3, 21.0, 18.2; HRMS(ESI) calcd for $C_{19}H_{26}O_7Na$ $[M + Na]^+$ *m*/*z* 389.1577, found *m*/*z* 389.1549.

The synthetic procedures and ^{1}H and ^{13}C NMR and HRMS (ESI) data for (*S*)-**2** and racemic **2** were identical with those of (*R*)-**2** described above. (*S*)-**2**: yield 81%, as a colorless oil. Racemic **2**: yield 82.5%, as a colorless oil.

*(R)-3′-(2″-Hydroxy-3″-methylbut-3″-en-1-yl)-2′,4′-bis-(methoxymethoxy)chalcone, (R)-**14**.* To a solution of (*R*)-**2** (0.2 g, 0.5 mmol) and benzaldehyde (0.1 g, 1.1 mmol) in EtOH was added KOH (0.05 g, 0.9 mmol) slowly. The reaction mixture was stirred at room temperature for 6 h, diluted with H_2O (10 mL), and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether/EtOAc = 15:1 to 3:1) to afford (*R*)-**14** (0.2 g, 90%) as a bright yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, *J* = 16.0 Hz, 1H), 7.61−7.58 (m, 2H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.41−7.39 (m, 3H), 7.29 (d, *J* = 16.0 Hz, 1H), 7.00 (d, *J* = 8.7 Hz, 1H), 5.28 (s, 2H), 5.03 (s, 1H), 4.99 (q, *J* = 6.3, 2H), 4.87 (s, 1H), 4.40−4.37 (m, 1H), 3.51 (s, 6H), 3.08−3.04 (m, 2H), 1.89 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.0, 159.0, 156.7, 148.0, 144.0, 134.8, 130.4, 129.7, 128.9 × 2, 128.4 × 2, 127.4, 126.2, 122.3, 110.0, 109.8, 101.7, 94.5, 75.1, 57.8, 56.4, 31.0, 18.2; HRMS(ESI) calcd for $C_{24}H_{28}O_6$ $[M + Na]^+$ *m*/*z* 435.1784, found *m*/*z* 435.1763.

The synthetic procedures and ^{1}H and ^{13}C NMR and HRMS (ESI) data for (*S*)-**14** and racemic **14** were identical with those of (*R*)-**14** described above. (*S*)-**14**: yield 89%, as a colorless oil. Racemic **14**: yield 92%, as a colorless oil.

*(R)-3′-(2″-Acetoxyl-3″-methylbut-3″-en-1-yl)-2′,4′-bis-(methoxymethoxy)chalcone, (R)-**15**.* To a solution of (*R*)-**14** (0.2 g, 0.5 mmol) in CH_2Cl_2 (8 mL) were added Et_3N (0.2 mL, 1.5 mmol), Ac_2O (0.1 mL, 1.0 mmol), and DMAP (0.01 g, 0.1 mmol) at room temperature. After 2 h, the reaction was quenched with a saturated NH_4Cl solution and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layer was washed with brine (30 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc = 10:1) to provide 0.2 g (92%) of (*R*)-**15** as a bright yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, *J* = 16.0 Hz, 1H), 7.61−7.59 (m, 2H), 7.50 (d, *J* = 8.7 Hz, 1H), 7.41−7.39 (m, 3H), 7.29 (d, *J* = 16.0 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 5.65 (dd, *J* = 8.2, 5.8 Hz, 1H), 5.26 (s, 2H), 4.96−4.92 (m, 3H), 4.87 (s, 1H), 3.53 (s, 3H), 3.47 (s, 3H), 3.20 (dd, *J* = 13.4 Hz, 8.6 Hz, 1H), 3.09 (dd, *J* = 13.4, 5.7 Hz, 1H), 1.98 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.3, 170.1, 159.2, 156.5, 143.8, 143.6, 135.0, 130.4, 129.8, 128.9 × 2, 128.4 × 2, 127.6, 126.4, 120.8, 112.4, 109.6, 101.5, 94.5, 76.2, 57.9, 56.4, 28.3, 21.1, 18.2; HRMS(ESI) calcd for $C_{26}H_{30}O_7Na$ $[M + Na]^+$ *m*/*z* 477.1890, found *m*/*z* 477.1873.

The synthetic procedures and ^{1}H and ^{13}C NMR and HRMS (ESI) data for (*S*)-**15** and racemic **15** were identical with those of (*R*)-**15** described above. (*S*)-**15**: yield 93%, as a colorless oil. Racemic **15**: yield 92%, as a colorless oil.

*(R)-2′,4′-Dihydroxy-3′-(2″-hydroxy-3″-methylbut-3″-en-1-yl)-chalcone, (R)-**1**.* To a solution of (*R*)-**15** (0.2 g, 0.4 mmol) in MeOH (6 mL) was added dropwise HCl (4.0 mol/L, 1.0 mL). The reaction mixture was stirred at 70 °C for 1.5 h, and the reaction was quenched with a saturated aqueous NH_4Cl solution (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (petroleum ether/EtOAc = 5:1) to afford target compound (*R*)-**1** (0.09 g, 62%) as a bright yellow powder: $[\alpha]^{20}_D$ = −25 (*c* 0.2, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.83 (s, 1H), 7.87 (d, *J* = 15.5 Hz, 1H), 7.77 (d, *J* = 8.9 Hz, 1H), 7.65−7.62 (m, 2H), 7.61 (d, *J* = 15.5 Hz, 1H), 7.43−7.42 (m, 3H), 6.54 (d, *J* = 8.9 Hz, 1H), 5.01 (s, 1H), 4.89 (s, 1H), 4.42 (d, *J* = 8.0 Hz, 1H), 3.23 (dd, *J* = 15.0, 1.6 Hz, 1H), 2.89 (dd, *J* = 15.0, 8.4 Hz, 1H), 1.88 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.0, 164.5, 163.5, 146.8, 144.1, 135.0, 130.5, 130.0, 129.0 × 2, 128.5 × 2, 120.7, 113.7, 113.2, 110.4, 109.1, 77.6, 28.6, 18.5; HRMS(ESI) calcd for $C_{20}H_{20}O_4Na$ $[M + Na]^+$ *m*/*z* 347.1260, found *m*/*z* 347.1225.

The synthetic procedures and ^{1}H and ^{13}C NMR and HRMS (ESI) data for (*S*)-**1** and racemic **1** [(±)-sanjuanolide] were identical with those of (*R*)-**1**. (*S*)-**1**: yield 62.5%, as a colorless oil; $[\alpha]^{20}_D$ = +26 (*c* 0.2, MeOH). Racemic **1**: yield 61%, as a colorless oil.

Cells and Reagents. Lipopolysaccharide were purchased from Sigma (St. Louis, MO, USA). Saline was prepared as a 0.9% NaCl solution. eBioscience, Inc. (San Diego, CA, USA) was the source of the mouse IL-6 enzyme-linked immunosorbent assay (ELISA) and mouse TNF-α ELISA kits. Mouse RAW 264.7 macrophages were obtained from the American Type Culture Collection (ATCC, U.S.). RAW 264.7 macrophages were incubated in Dulbecco's modified Eagle's medium (DMEM) medium (Gibco, Eggenstein, Germany) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C with 5% CO_2.

Determination of TNF-α and IL-6. The TNF-α and IL-6 levels in the medium and serum were determined by ELISA analysis as previously described.[13b] RAW 264.7 macrophages were seeded into six-well plates at a density of 400 000 cells per well in DMEM medium. Cells were incubated at 37 °C in 5% CO_2 for 24 h.

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Macrophages were pretreated with compounds for 30 min, which was followed by treatment with 0.5 μg/mL LPS. After treatment, the cells were incubated for 24 h. ELISA was used to screen the inhibition of all synthetic compounds for LPS-induced TNF-α and IL-6 release in RAW 264.7 mouse macrophages.

Real-Time Quantitative PCR. Cells were homogenized in a TRIZOL kit (Invitrogen, Carlsbad, CA, USA) for extraction of RNA according to the manufacturer's protocol. Both reverse transcription and quantitative PCR were carried out using a two-step M-MLV Platinum SYBR Green qPCR SuperMix-UDG kit (Invitrogen). An Eppendorf Mastercycler ep Realplex detection system (Eppendorf, Hamburg, Germany) was used for qPCR analysis. The primers of genes including TNF-α, IL-6, IL-1β, ICAM-1, MCP-1, COX-2, and β-actin were synthesized by Invitrogen. Details have been described previously.[2] The primer sequences of mouse genes used are shown as follows: mouse TNF-α sense primer, 5′-TGGAACTGGCAGAAGAGG-3′; mouse TNF-α antisense primer, 5′-AGACAGAAGAGCGTGGTG-3′; mouse IL-6 sense primer, 5′-GAGGATACCACTCCCAACAGACC-3′; mouse IL-6 antisense primer, 5′-AAGTGCATCATCGTTGTTCATACA-3′; mouse IL-1β sense primer, 5′-ACTCCTTAGTCCTCGGCCA-3′; mouse IL-1β antisense primer, 5′-CCATCAGAGGCAAGGAGGAA-3′; mouse ICAM sense primer, 5′-GCCTTGGTAGAGGTGACTGAG-3′; mouse ICAM antisense primer, 5′-CTGGCGGCTCAGTATCTCCT-3′; mouse MCP-1 sense primer, 5′-TCAGCCAGATGCAATCAATGCCC-3′; mouse MCP-1 antisense primer, 5′-TGGGTTTGCTTGTCCAGGTGGT-3′; mouse COX-2 sense primer, 5′-TGGTGCCTGGTCTGATGATG-3′; mouse COX-2 antisense primer, 5′-GTGGTAACCGCTCAGGTGTTG-3′; mouse β-actin sense primer, 5′-TGGAATCCTGTGGCATCCATGAAAC-3′; mouse β-actin antisense primer, 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′. The amount of each gene was determined and normalized by the amount of β-actin.

Statistical Analysis. Data are expressed as the mean ± standard error of the mean (SEM). Student's *t* test was employed to analyze the differences between sets of data. Statistics were performed using GraphPad Pro (GraphPad, San Diego, CA, USA). *P* values less than 0.05 ($p < 0.05$) were considered indicative of significance. All experiments were repeated at least three times.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00596.

Copies of ^{1}H and ^{13}C NMR spectra, HPLC data, and HR-MS data (PDF)

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Notes

The authors declare no competing financial interest.

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