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Article

Design and Synthesis of L-1′-Homologated Adenosine Derivatives as Potential Anti-inflammatory Agents

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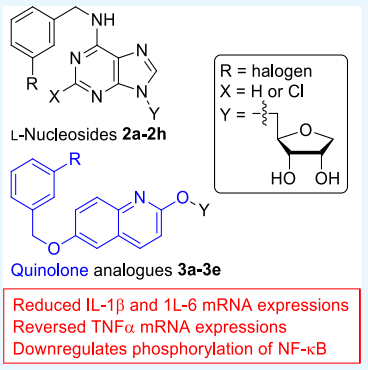
ABSTRACT: Inflammatory responses are fundamental protective warning mechanisms. However, in certain instances, they contribute significantly to the development of several chronic diseases such as cancer. Based on previous studies of truncated 1′-homologated adenosine derivatives, L-nucleosides and their nucleobase-modified quinolone analogues were designed, synthesized, and evaluated for anti-inflammatory activities. The target molecules were synthesized via the key intramolecular cyclization of monotosylate and Mitsunobu condensation from the natural product, D-ribose. All compounds tested and showed potent anti-inflammatory activities, as indicated by their inhibition of LPS-induced IL-1β secretion from the RAW 264.7 macrophages. Gene expressions of pro-inflammatory cytokines showed that all compounds, except **3a** and **3b**, significantly reduced LPS-induced IL-1β and IL-6 mRNA expressions. The half-maximal inhibitory concentrations (IC_{50}) of **2g** and **2h** against IL-1β were 1.08 and 2.28 μM, respectively. In contrast, only **2d**, **2g**, and **3d** effectively reversed LPS-induced TNFα mRNA expression. Our mechanistic study revealed that LPS-induced phosphorylation of NF-κB was significantly downregulated by all compounds tested, providing evidence that the NF-κB signaling pathway is involved in their anti-inflammatory activities. Among the compounds tested, **2g** and **2h** had the most potent anti-inflammatory effects, as shown by the extent of decrease in pro-inflammatory gene expression, protein secretion, and NF-κB phosphorylation. These findings suggest that the L-truncated 1′-homologated adenosine skeleton and its nucleobase-modified analogues have therapeutic potential as treatments for various human diseases by mediating inflammatory processes.



INTRODUCTION

Inflammation is the body's first warning signal of microbial invaders and pathogens. This process involves the release of various signaling molecules, including cytokines, that play vital roles in coordinating the immune response. In particular, inflammation-related cytokines, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor alpha (TNFα), are a group of signaling molecules involved in regulating inflammation. These cytokines can be produced by various immune cells, such as macrophages, T cells, and B cells.[1,2] These cytokines can either promote or inhibit the inflammatory response depending on the specific circumstances. While acute inflammation is a necessary process for tissue repair and pathogen clearance, chronic inflammation can contribute to the development and growth of tumors by promoting genetic mutations, enhancing cell survival and proliferation, and suppressing the immune system.[3−7]

The search for new anti-inflammatory agents that can target the nuclear factor kappa B (NF-κB) signaling pathway has been intensely researched in recent years.[8] NF-κB is a transcription factor that is critical for regulating inflammation, immune response, and cell survival. Aberrant activation of NF-κB is associated with various diseases, including cancer, autoimmune disorders, and chronic inflammatory diseases.[9,10] Therefore, developing NF-κB inhibitors is key for the development of therapeutic agents.

Nucleoside analogues are effective therapeutic agents because they can mimic and therefore interfere with natural nucleosides. Since modification of the sugar ring led to clinically relevant results for some nucleoside analogues, one class of nucleoside analogues that has also gained considerable attention is nucleobase-modified nucleosides. It refers to the addition, removal, or relocation of various atoms in the purine ring, resulting in altered physicochemical properties and unique modes of action.[11] These changes can alter the effects of hydrogen bonding interactions in enzyme binding sites. Expanding the size of the purine nucleobase is one of the strategies for structural modifications. Such modifications can alter $\pi-\pi$ stacking, aromaticity, and polarizability of the heterocyclic base, all of which are important elements of recognition in enzyme binding sites. In this regards, aza/deaza-

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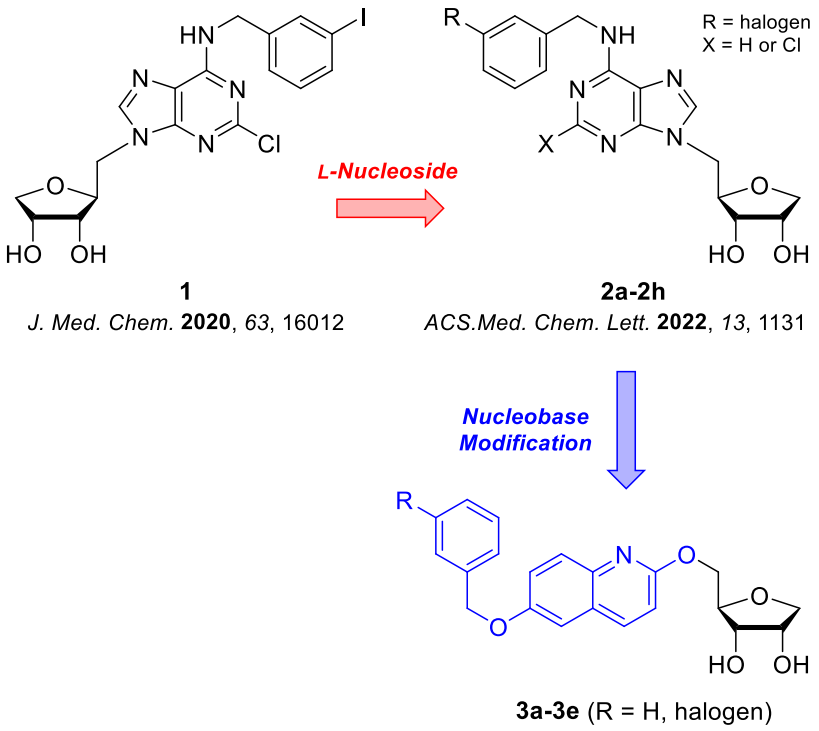

Figure 1. Rationale for the synthesis of truncated L-1′-homologated adenosine derivatives and their quinolone-analogues.

modified purines have shown promising results in the treatment of viral infections, including HIV, hepatitis B and C, and herpes viruses.[12]

Previously, we reported that truncated D and L-1′-homologated adenosine derivatives exhibited polypharmacological profiles, such as modulation of peroxisome proliferator-activated receptors (PPARs) and adiponectin production.[13−15] In this work, we investigated anti-inflammatory effects of truncated D and L-1′-homologated adenosine derivatives and the synthesis of novel nucleobase-modified derivatives with introduced quinolone and truncated L-1′-homologated adenosine analogues (Figure 1).

RESULTS AND DISCUSSION

Chemistry. To synthesize the final nucleoside analogues, a glycosyl donor **7** was synthesized in five steps from D-ribose and then condensed with a purine base. As shown in Scheme 1, D-ribose was converted to the 2,3-acetonide **4** by using acetone and a catalytic amount of sulfuric acid. The primary hydroxyl group of **4** was protected with the trityl (Tr) group and reduced with $NaBH_4$ to produce diol **5**. Regioselective tosylation of the primary alcohol of **5** was followed by tandem intramolecular cyclization to produce the 4-oxo sugar **6**.[16] Removal of the Tr group of **6** was achieved by treatment with diethylaluminum chloride to produce the key glycosyl donor **7**.[17] The process of synthesizing the base-condensed intermediates was carried out by treating the glycosyl donor **7** with 2,6-dichloropurine and 6-chloropurine under the Mitsunobu conditions.[14,18] The final truncated 1′-homologated adenosine derivatives **2a−2h** were obtained by treating **8** or **9** with 3-halobenzylamines, which was followed by acetonide deprotection.

As illustrated in Scheme 2, we synthesized the nucleobase-modified quinolone analogues in which the purine base is replaced by quinolone for comparison of their biological activities (compounds **3a−3e**). The halobenzyloxy quinoline **11a−11e** was obtained from 2,6-quinolinediol by chlorination and Williamson ether synthesis. Treatment of **11a−11e** with glycosyl donor **7** and *t*-BuOK in THF at 80 °C gave the base-condensed products, which was treated with 1 N HCl in MeOH at 60 °C to yield the final **3a−3e**

Evaluation of Pharmacological Profiles of 1, 2a−2h, and 3a−3e. Cell viability was assessed by treating RAW 264.7 cells with 5, 10, 20, 50, and 100 μM of all compounds for 24 h. While some compounds do not show any effects on cell viability in the range of concentrations tested, compounds **1**, **2d**, **2g**, **3a**, **3b**, and **3e** induced cytotoxicity at 100 μM concentration (Figure 2). **3e** was the only compound that induced cytotoxicity at 50 μM concentration. Based on these results, a concentration of 20 μM was selected for all subsequent biological analyses.

Next, we measured IL-1β secretion in cell culture media as an indication of inflammation. As shown in Figure 3, lipopolysaccharide (LPS)-induced IL-1β secretion was significantly reduced by pretreatment with all of the compounds tested. The lowest concentration of IL-1β was observed by **2b** treatment, followed by **2h**. The anti-inflammatory potential of the compounds was confirmed by measuring the gene expression of pro-inflammatory cytokines, including IL-1β, IL-6, and TNFα (Figure 4). Results of IL-1β and IL-6 mRNA expression showed that pretreatment with all compounds, except compounds **3a** and **3b**, significantly reduced LPS-induced gene expressions. Compounds **2f** and **2g** showed the most potent reduction in IL-1β and IL-6 gene expression. The half-maximal inhibitory concentrations (IC_{50}) of **2g** and **2h** against IL-1β were 1.08 and 2.28 μM, respectively. In contrast, only compounds **2d**, **2g**, **3d**, and **3e** significantly inhibited the LPS-induced TNFα expression.

Finally, a mechanistic study was conducted by the evaluation of the effect of the compounds on the NF-κB signaling pathway. Activation of the signaling pathway, as shown by

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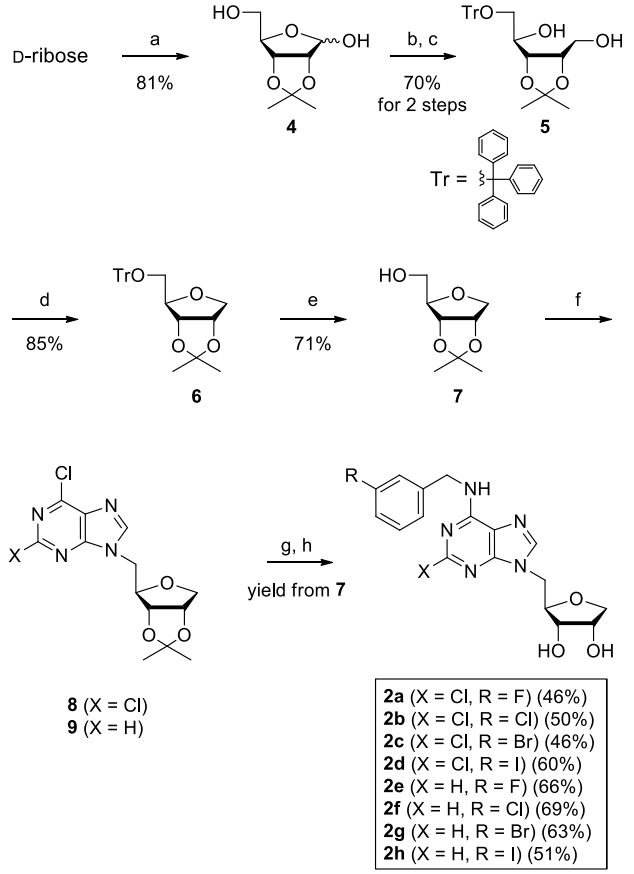
Scheme 1. Synthesis of L-Truncated Homologated 4′-Oxoadenosine Derivatives, 2a−2h[a]



[a]Reagents and conditions: (a) cat. H_2SO_4, acetone, rt, 3 h; (b) trityl chloride, DMAP, pyridine/CH_2Cl_2 (1:1), 40 °C, 12 h; (c) $NaBH_4$, EtOH, rt, 2 h; (d) TsCl, DMAP, pyridine, CH_2Cl_2, 50 °C, 5 h; (e) Et_2AlCl, CH_2Cl_2, −40 °C, 2 h; (f) 2,6-dichloropurine or 6-chloropurine, PPh_3, DIAD, THF, 60 °C, 24 h; (g) 3-halobenzylamines, Et_3N, THF, rt, 20 h; and (h) 1 N HCl, MeOH, 60 °C, 28 h.

increased phosphorylation of NF-κB after 5 min LPS treatment, was significantly reduced by 2 h pretreatment with all the compounds tested (Figure 5). Consistent with the results of IL-1β secretion and expression, **2g** and **2h** inhibited the NF-κB signaling pathway most.

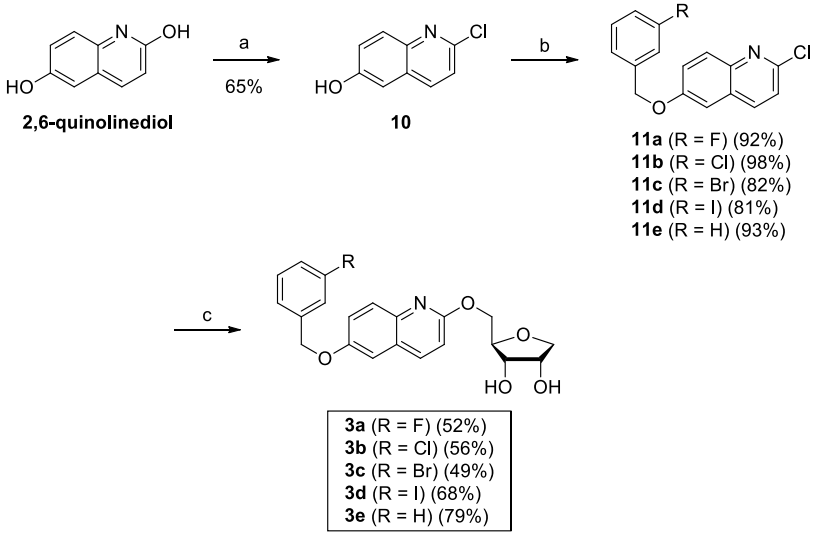
These results indicate that no substituent at the C2 position on the adenine nucleobase improved biological activity, which means a less sterically hindered and neutral substituent is favored. Notably, in comparison with D-nucleoside **1** and L-nucleoside **2d**, L-enantiomer exhibited improved pharmacological activities. Among 3-halobenzyl quinolone analogues, iodine-containing analogue **3d** showed potent anti-inflammatory activities, which indicates that electropositivity is an important factor for biological activities.

Activated PPARs regulate the inflammatory response by controlling the expression of several inflammation-related genes.[19] Therefore, we investigated the anti-inflammation activities associated with PPARs. In a previous study, compound **2d** acted as a PPARγ partial agonist (K_i = 4.3 μM) and a PPARδ antagonist (K_i = 1.0 μM). However, these compounds showed less IL-1β inhibitory effects than compounds **2g** and **2h**, which have weak PPAR modulating activities. Compounds **3a−3e**, possessing moderate anti-inflammatory activities, did not show any PPAR-modulating activities (data not shown). These results suggest a lack of clear correlation between PPARs and anti-inflammation activities of these compounds. The anti-inflammatory effect could be attributed to polypharmacology, wherein these compounds modulate multiple drug targets.[20]

CONCLUSIONS

We have synthesized truncated L-1′-homologated adenosine derivatives and their nucleobase-modified quinolone analogues and evaluated them for anti-inflammatory activities. The target molecules were synthesized via the key intramolecular cyclization of monotosylate and Mitsunobu condensation from the natural product, D-ribose. The nucleobase-modified quinolone analogues were synthesized from 2,6-quinolinediol by chlorination and Williamson ether synthesis. All compounds

Scheme 2. Synthesis of Nucleobase-Modified Quinolone Analogues 3a−3e[a]



[a]Reagents and conditions: (a) $POCl_3$, MeCN, 80 °C, 23 h; (b) 3-halobenzyl chloride, K_2CO_3, DMF, 80 °C, 7 h; and (c) (i) **7**, *t*-BuOK, THF, 80 °C, 2 h and (ii) 1 N HCl, MeOH, 60 °C, 14 h.

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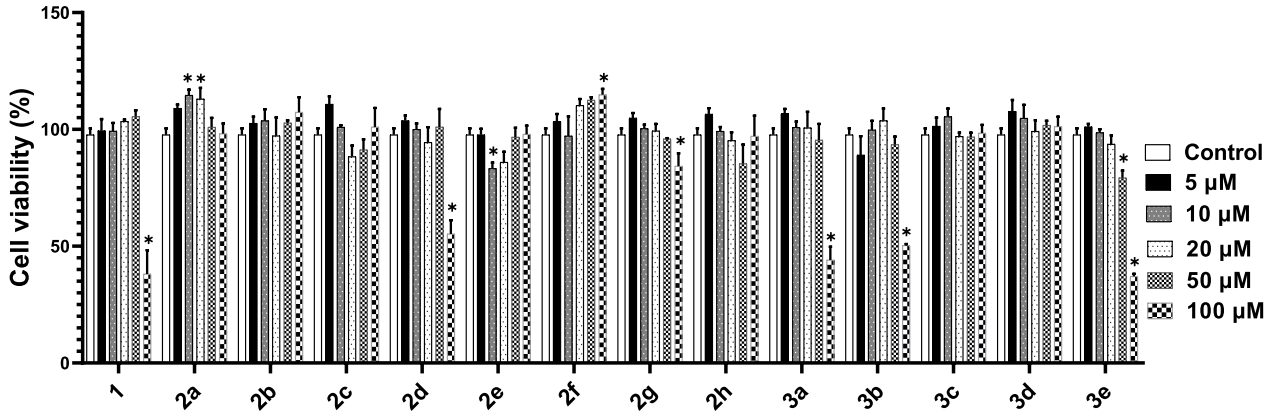

Figure 2. MTT colorimetric assay of the active compounds. RAW 264.7 cells were pretreated with 5, 10, 20, 50, and 100 μM active compounds for 24 h, and the data represent the cell viability percentages of RAW 264.7 cells. Values are presented as means ± standard errors. *p < 0.05 compared with the control.

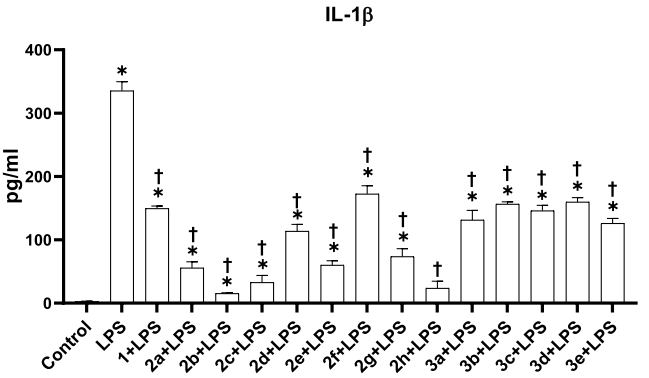

Figure 3. Inhibitory effect of active compounds on IL-1β production. RAW 264.7 cells were pretreated with 20 μM active compounds for 2 h, followed by 100 ng/mL LPS for 24 h. An IL-1β ELISA kit was used to check IL-1β production in the media of the RAW 264.7 cells. Values are presented as means ± standard errors. *p < 0.05 compared with the control, $^{\dagger}p$ < 0.05 compared with LPS alone.

tested exhibited potent anti-inflammatory activities, indicated by inhibition of LPS-induced IL-1β secretion from RAW 264.7 macrophages. Gene expressions of pro-inflammatory cytokines showed that all compounds except **3a** and **3b** significantly reduced LPS-induced IL-1β and IL-6 mRNA expressions. The half-maximal inhibitory concentrations (IC_{50}) of **2g** and **2h** against IL-1β were 1.08 and 2.28 μM, respectively. In contrast, only **2d**, **2g**, and **3d** effectively reversed LPS-induced TNFα mRNA expression. Our mechanistic study revealed that LPS-induced phosphorylation of NF-κB was significantly down-regulated by all compounds tested, providing evidence for the involvement of this signaling pathway involved in their anti-inflammatory activity. Among the compounds tested, **2g** and **2h** had the most potent anti-inflammatory effects, as shown by the extent to which they decreased in pro-inflammatory gene expression, protein secretion, and NF-κB phosphorylation. These findings suggest that the L-truncated 1′-homologated adenosine skeleton and its nucleobase-modified analogues have therapeutic insights for the treatment of inflammation-related diseases. Given the potent anti-inflammatory activities of the truncated L-1′-homologated adenosine skeleton and its nucleobase-modified analogues, the therapeutic effect of these compounds on metabolic diseases could be tested in animal models such as high-fat-diet-induced obese mice or genetically obese db/db type 2 diabetic mice, and the results will be reported elsewhere.

EXPERIMENTAL SECTION

Chemical Synthesis. *General Methods.* Proton (^{1}H) and carbon (^{13}C) NMR spectra were obtained on a Bruker AVANCE III HD 400 (400/100 MHz) spectrometer (Bruker, Billerica, MA, USA). Chemical shifts are reported as parts per million (δ) relative to the solvent peak. Coupling constants (J) are reported in hertz (Hz). Mass spectra were recorded on a JMS-T200GC instrument. Optical rotations were determined on a Jasco P-1030 in an appropriate solvent. UV spectra were recorded in methanol or water on a U-3000 instrument (Hitachi High-Technologies Corp., Tokyo, Japan). Melting points were determined on a 6427-H10 Thomas-Hoover melting point apparatus made by Thomas Scientific and were uncorrected. The crude compounds were purified by column chromatography on silica gel (Kieselgel 60, 70-230 mesh, Merck). Elemental analyses (C, H, and N) were used to determine the purity of all synthesized compounds, and the results were within ±0.4% of the calculated values, confirming ≥95% purity.

*(3aR,6R,6aR)-6-(Hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-ol (**4**).* To a stirred suspension of D-ribose (10.0 g, 66.6 mmol) in acetone (333.0 mL, 0.2 M) was added dropwise conc. sulfuric acid (0.3 mL) at room temperature, and the reaction mixture was stirred at the same temperature for 3 h. The mixture was neutralized with solid $NaHCO_3$, filtered, and evaporated under reduced pressure to give a colorless syrup. The residue was purified by silica gel column chromatography (*n*-hexanes/EtOAc = 1:2) to give **4** as a colorless syrup (10.2 g, 81%): $[\alpha]_D^{25} = -14.0$ (*c* 0.46, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 5.26 (s, 1H), 4.77 (d, J = 6.0 Hz, 1H), 4.52 (d, J = 6.0 Hz, 1H), 4.19 (irregular t, J = 4.4, 5.2 Hz, 1H), 3.63 (dd, J = 4.8, 12.0 Hz, 1H), 3.59 (dd, J = 5.6, 12.0, 1H), 1.44 (s, 3H), 1.31 (s, 3H); Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.48; H, 7.36.

*(3aR,6R,6aR)-2,2-Dimethyl-6-((trityloxy)methyl)-tetrahydrofuro[3,4-d][1,3]dioxol-4-ol (**5**).* To a stirred solution of **4** (9.7 g, 50.9 mmol) in anhydrous CH_2Cl_2 (254.5 mL, 0.2 M) was added trityl chloride (28.4 g, 101.8 mmol), 4-dimethylaminopyridine (1.8 g, 15.3 mmol), and pyridine (12.3 mL, 152.7 mmol) at 0 °C, and the mixture was stirred with

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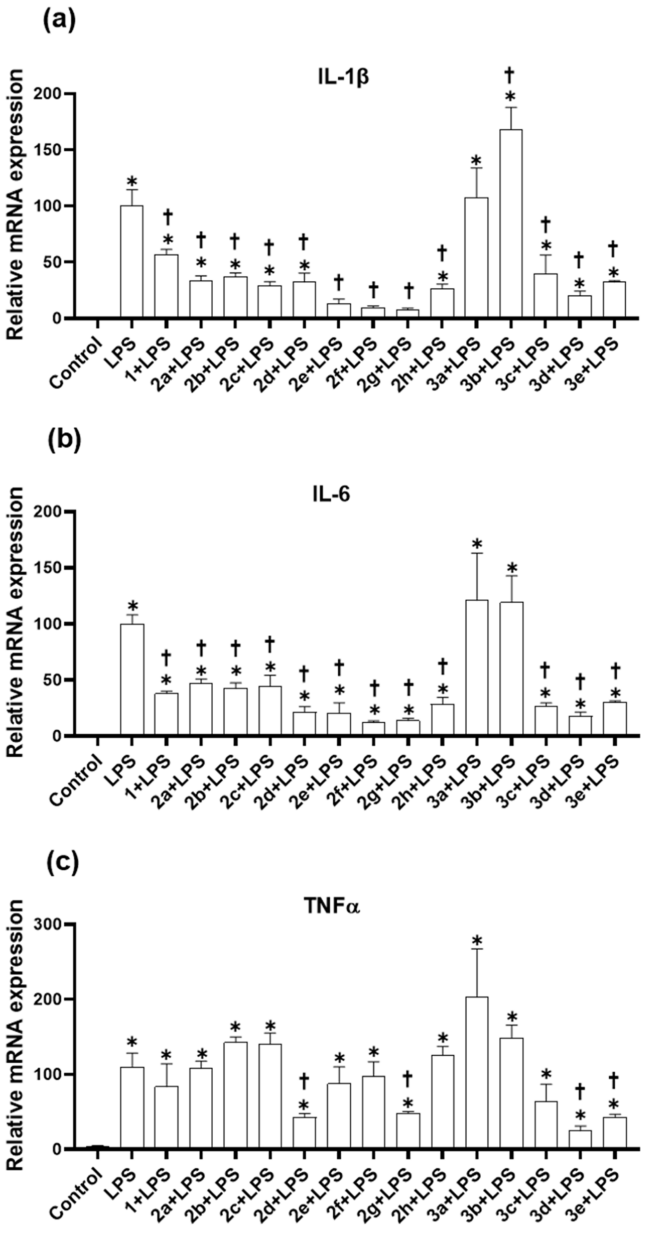

Figure 4. Anti-inflammatory potential of active compounds. RAW 264.7 cells were pretreated with 20 μM active compounds for 2 h, followed by 100 ng/mL LPS for 6 h. Real-time PCR was used to measure the gene expression of (a) IL-1β, (b) IL-6, and (c) TNFα, and quantification was normalized to 18S rRNA levels. Values are presented as the means ± standard errors. *p < 0.05 compared with the control and $^{\dagger}p$ < 0.05 compared with LPS alone.

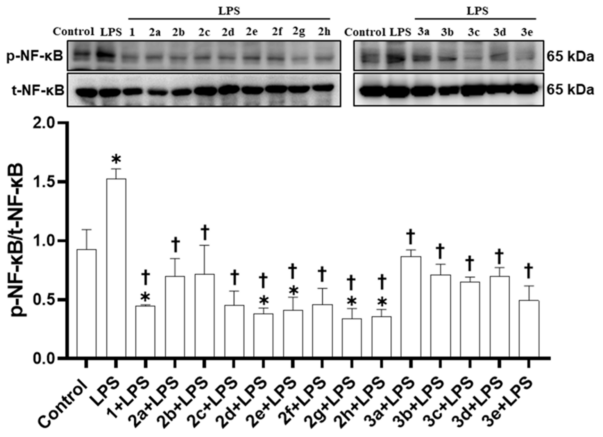

Figure 5. Inhibitory effect of active compounds on NF-κB pathway activation. RAW 264.7 cells were pretreated with 20 μM active compounds for 2 h, followed by 100 ng/mL LPS treatment for 5 min. Phosphorylated and total NF-κB expression were determined by western blot analysis. Values are presented as the means ± standard errors. *p < 0.05 compared with the control and $^{\dagger}p$ < 0.05 compared with LPS alone.

heating at 40 °C under a N_2 atmosphere. After being stirred for 12 h, the mixture was cooled, diluted with EtOAc, washed with saturated NH_4Cl solution and brine, and evaporated. To a stirred solution of the crude intermediate in methanol (170.0 mL, 0.2 M) was added portionwise sodium borohydride (4.0 g, 105.7 mmol) at 0 °C under a N_2 atmosphere. After being stirred for 2 h, the mixture was evaporated, and the resulting white suspension was diluted with EtOAc and washed with saturated NH_4Cl solution and brine. The separated organic layer was evaporated, and the residue was purified by silica gel column chromatography (n-hexanes/EtOAc = 3:1) to give diol **5** (15.4 g, 70% for two steps) as a syrup: $[\alpha]_D^{25}$ = −38.0 (c 0.044, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.42−7.36 (m, 6H), 7.35−7.23 (m, 9H), 5.74 (dd, J = 4.0, 11.3 Hz, 0.4H), 5.33 (d, J = 9.2 Hz, 0.6H), 4.78 (d, J = 5.88 Hz, 0.6H), 4.74 (dd, J = 4.0, 6.2 Hz, 0.4H), 4.66 (d, J = 5.9 Hz, 0.6H), 4.58 (d, J = 6.2 Hz, 0.4H), 4.35 (t, J = 3.3 Hz, 0.6H), 4.19 (s, 0.4H), 3.99 (d, J = 11.3 Hz, 0.4H), 3.89 (d, J = 9.2 Hz, 0.6H), 3.43 (ddd, J = 3.3, 10.3, 12.7 Hz, 1H), 3.34 (dd, J = 3.7, 10.3 Hz, 0.6H), 3.01 (dd, J = 2.8, 10.2 Hz, 0.4H), 1.55 (s, 1H), 1.48 (s, 2H), 1.36 (d, J = 9.1 Hz, 3H). Anal. Calcd for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53. Found: C, 74.68; H, 6.26.

*(3aR,4R,6aS)-2,2-Dimethyl-4-((trityloxy)methyl)-tetrahydrofuro[3,4-d][1,3]dioxole (**6**).* To a cooled (0 °C) solution of **5** (12.8 g, 29.6 mmol) in anhydrous pyridine/CH_2Cl_2 (1:1, total 99 mL, 0.3 M) was added 4-toluenesulfonyl chloride (71.3 g, 59.1 mmol, 2 equiv) and 4-dimethylaminopyridine (0.36 g, 3.0 mmol, 0.1 equiv) under a N_2 atmosphere. After the reaction mixture was stirred with heating (50 °C) for 5 h, the reaction mixture was cooled and quenched with a saturated ammonium chloride solution. The organic layer was washed with brine, dried with anhydrous $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (n-hexanes/EtOAc = 30:1) to give **6** as a colorless syrup (10.5 g, 85%): $[\alpha]_D^{25}$ = −84.5 (c 0.1, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.40 (d, J = 7.5 Hz, 6H), 7.29 (dd, J = 7.3, 15.2 Hz, 6H), 7.20−7.25 (m, 3H), 4.87 (t, J = 5.1 Hz, 1H), 4.64 (d, J = 6.1 Hz, 1H), 4.20 (dd, J = 3.8, 7.7 Hz, 1H), 4.15 (dd, J = 4.3, 10.2 Hz, 1H), 4.04 (d, J = 10.2 Hz, 1H), 3.25 (dd, J = 4.0, 10.0 Hz, 1H), 3.09 (dd, J = 4.3, 10.0 Hz, 1H), 1.50 (s, 3H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 143.6, 128.6, 127.8, 127.0, 112.3, 87.0, 84.0, 83.0, 81.6, 74.3, 64.5, 26.6, 25.0; Anal. Calcd for $C_{27}H_{28}O_4$: C, 77.86; H, 6.78. Found: C, 77.65; H, 6.59.

*((3aR,4R,6aS)-2,2-Dimethyltetrahydrofuro[3,4-d][1,3]-dioxol-4-yl)methanol (**7**).* To a cooled (−40 °C) solution of **6** (10.5 g, 25.2 mmol) in anhydrous CH_2Cl_2 (126 mL, 0.2 M) was added dropwise diethylaluminum chloride 1.0 M solution in hexanes (75.6 mL, 75.6 mmol, 3 equiv) under a N_2 atmosphere. After the reaction mixture was stirred at the same temperature for 2 h, the mixture was quenched with saturated potassium sodium tartrate solution and stirred for an additional 1 h. The organic layer was washed with brine, dried

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with anhydrous $MgSO_4$, and evaporated at 5 °C. The residue was purified by silica gel column chromatography (*n*-hexanes/EtOAc = 4:1) to give **7** as a colorless syrup (3.1 g, 71%): $[\alpha]_D^{25}$ = −50.0 (*c* 0.1, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.79 (m, 1H), 4.58 (dd, *J* = 1.9, 6.3 Hz, 1H), 4.11 (ddd, *J* = 2.1, 3.9, 6.2 Hz, 1H), 3.95 (m, 2H), 3.65 (dd, *J* = 3.7, 11.6 Hz, 1H), 3.58 (dd, *J* = 6.7, 11.5 Hz, 1H), 1.50 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 112.9, 84.8, 81.8, 81.0, 72.8, 61.7, 26.6, 24.9. Anal. Calcd for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 55.36; H, 8.30.

General Procedure for the Synthesis of ***2a*****−****2h***. To a stirred solution of 6-chloropurine or 2,6-dichloropurine (2.0 equiv) and triphenylphosphine (Ph_3P) (2.0 equiv) in anhydrous THF (0.15 M) was added diisopropyl azodicarboxylate (DIAD) (4.0 equiv) in anhydrous THF (0.3 M) at 0 °C under a N_2 atmosphere, and the mixture was stirred at the same temperature for 15 min. To this solution was added a solution of compound **7** (1.0 equiv) in anhydrous THF (0.3 M) at 0 °C, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure, and the crude residue was purified by flash silica gel column chromatography to give **8** and **9** combined with triphenylphosphine oxide, respectively. To a stirred solution of **8** or **9** and an appropriate amine hydrochloride salts or free amines (3.0 equiv) in anhydrous THF (0.3 M) was added Et_3N (3.0 equiv), and the solution was stirred overnight at room temperature. After the solvent was removed under reduced pressure, the residue was purified by flash silica gel column chromatography to give the triphenylphosphine oxide-contaminated N^6-substituted amine intermediates. To a stirred solution of N^6-substituted amine intermediates in methanol (0.1 M) was added dropwise 1 N HCl (0.1 equiv) at room temperature. After being stirred at room temperature, at which time TLC indicated the absence of the starting material, the reaction mixture was evaporated under reduced pressure. The residue was purified by flash column chromatography (reversed-phase silica gel, H_2O/MeOH, 10:1) to give **2a**−**2h**.

2,6-Dichloro-9-(((3aR,4R,6aS)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-9H-purine (***8***). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.51 (s, 1H), 4.83−4.90 (m, 1H), 4.31−4.39 (m, 3H), 4.06 (dd, *J* = 3.6, 10.8 Hz, 1H), 3.90 (d, *J* = 10.8 Hz, 1H), 1.41 (s, 3H), 1.30 (s, 3H).

6-Chloro-9-(((3aR,4R,6aS)-2,2-dimethyltetrahydrofuro-[3,4-d][1,3]dioxol-4-yl)methyl)-9H-purine (***9***). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.77 (s, 1H), 8.22 (s, 1H), 4.78−4.81 (m, 1H), 4.55 (dd, *J* = 2.5, 6.2 Hz, 1H), 4.47 (dd, *J* = 2.4, 12.8 Hz, 1H), 4.28−4.37 (m, 2H), 3.97−4.05 (m, 2H), 1.50 (s, 3H), 1.33 (s, 3H).

(2R,3S,4S)-2-((2-Chloro-6-((3-fluorobenzyl)amino)-9H-purin-9-yl)methyl)tetrahydrofuran-3,4-diol (***2a***). Yield = 46%; white solid; mp 185−186 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.93 (s, 1H), 7.20−7.26 (m, 1H), 7.11 (d, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 10.0 Hz, 1H), 6.88 (ddd, *J* = 1.9, 8.4, 16.9 Hz, 1H), 4.66 (s, 2H), 4.34 (dd, *J* = 3.5, 14.4 Hz, 1H), 4.19 (dd, *J* = 6.7, 14.4 Hz, 1H), 3.87−3.97 (m, 3H), 3.71 (dd, *J* = 4.8, 7.2 Hz, 1H), 3.60 (dd, *J* = 2.2, 9.2 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ 165.7, 164.5, 157.1, 156.3, 152.4, 144.1, 143.8, 132.0 (d, *J* = 8.1 Hz), 125.3, 119.7, 116.2 (d, *J* = 21.7 Hz), 115.7 (d, *J* = 21.2 Hz), 81.8, 75.6, 74.8, 73.1, 47.3; $[\alpha]_D^{25}$ = −102.0 (*c* 0.015, MeOH); ESI-MS *m*/*z*: 394.1065 (M + H^+). Anal. Calcd for $C_{17}H_{17}ClFN_5O_3$: C, 51.85; H, 4.35; N, 17.78. Found: C, 51.75; H, 4.75; N, 17.68.

(2R,3S,4S)-2-((2-Chloro-6-((3-chlorobenzyl)amino)-9H-purin-9-yl)methyl)tetrahydrofuran-3,4-diol (***2b***). Yield = 50%; white solid; mp 139−144 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.00 (s, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 7.22−7.31 (m, 3H), 4.72 (s, 2H), 4.42 (dd, *J* = 3.4, 14.4 Hz, 1H), 4.27 (dd, *J* = 6.7, 14.4 Hz, 1H), 3.94−4.05 (m, 3H), 3.79 (dd, *J* = 4.2, 7.6 Hz, 1H), 3.68 (dd, *J* = 2.2, 9.3 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ 157.1, 156.3, 152.4, 144.1, 143.5, 138.7, 138.2, 132.1, 129.0, 119.7, 95.7, 81.7, 75.6, 74.8, 73.1, 47.3, 45.2; $[\alpha]_D^{25}$ = +30.0 (*c* 0.03, MeOH); FAB-MS *m*/*z*: 410.0729 (M + H^+). Anal. Calcd for $C_{17}H_{17}Cl_2N_5O_3$: C, 49.77; H, 4.18; N, 17.07. Found: C, 49.97; H, 4.38; N, 17.37.

(2R,3S,4S)-2-((6-((3-Bromobenzyl)amino)-2-chloro-9H-purin-9-yl)methyl)tetrahydrofuran-3,4-diol (***2c***). Yield = 46%; white solid; mp 130−132 °C; ^{1}H NMR (CD_3OD, 800 MHz): δ 8.01 (s, 1H), 7.55 (s, 1H), 7.36 (dd, *J* = 7.9, 25.9 Hz, 2H), 7.21 (t, *J* = 7.8 Hz, 1H), 4.71 (br s, 2H), 4.42 (dd, *J* = 3.5, 14.4 Hz, 1H), 4.27 (dd, *J* = 6.8, 14.4 Hz, 1H), 4.03 (ddd, *J* = 3.4, 6.9, 10.4 Hz, 1H), 3.97 (dd, *J* = 4.5, 9.6 Hz, 1H), 3.80 (dd, *J* = 5.0, 7.2 Hz, 1H), 3.69 (dd, *J* = 2.8, 9.6 Hz, 1H); ^{13}C NMR (CD_3OD, 200 MHz): δ 157.0, 156.3, 152.4, 144.1, 143.5, 142.7, 132.5, 132.1, 128.4, 124.1, 119.6, 81.7, 75.6, 74.8, 73.1, 47.3, 45.2; $[\alpha]_D^{25}$ = +147.0 (*c* 0.015, MeOH); FAB-MS *m*/*z*: 454.0287 (M + H^+). Anal. Calcd for $C_{17}H_{17}BrClN_5O_3$: C, 44.90; H, 3.77; N, 15.40. Found: C, 44.90; H, 3.87; N, 15.60.

(2R,3S,4S)-2-((2-Chloro-6-((3-iodobenzyl)amino)-9H-purin-9-yl)methyl)tetrahydrofuran-3,4-diol (***2d***). Yield = 60%; white solid; mp 146−148 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.00 (s, 1H), 7.76 (s, 1H), 7.58 (d, *J* = 7.2 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.07 (t, *J* = 8.4 Hz, 1H), 4.68 (br s, 2H), 4.41 (dd, *J* = 3.6, 14.4 Hz, 1H), 4.27 (dd, *J* = 7.2, 14.8 Hz, 1H), 3.94−4.03 (m, 3H), 3.78 (dd, *J* = 5.2, 7.2 Hz, 1H), 3.68 (dd, *J* = 2.4, 9.2 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ 163.8, 157.1, 144.2, 143.5, 138.7, 138.2, 132.1, 130.3, 129.1, 129.0, 119.7, 108.8, 81.8, 75.6, 74.8, 73.1, 47.3; $[\alpha]_D^{25}$ = −90.0 (*c* 0.01, MeOH); FAB-MS *m*/*z*: 502.0151 (M + H^+). Anal. Calcd for $C_{17}H_{17}ClIN_5O_3$: C, 40.70; H, 3.42; N, 13.96. Found: C, 40.90; H, 3.62; N, 13.96.

(2R,3S,4S)-2-((6-((3-Fluorobenzyl)amino)-9H-purin-9-yl)-methyl)tetrahydrofuran-3,4-diol (***2e***). Yield = 66%; white solid; mp 140−141 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.29 (s, 1H), 8.10 (s, 1H), 7.31−7.37 (m, 1H), 7.22 (d, *J* = 7.7 Hz, 1H), 7.14 (d, *J* = 10.0 Hz, 1H), 6.99 (ddd, *J* = 2.2, 8.6, 16.9 Hz, 1H), 4.85 (s, 2H), 4.51 (dd, *J* = 3.5, 14.5 Hz, 1H), 4.38 (dd, *J* = 6.5, 14.4 Hz, 1H), 4.09 (ddd, *J* = 3.5, 6.9, 13.7 Hz, 1H), 3.98−4.04 (m, 2H), 3.82 (dd, *J* = 4.8, 7.2 Hz, 1H), 3.73 (dd, *J* = 2.2, 9.2 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ 156.2, 153.9, 143.6, 143.3, 135.2, 131.4, 131.3, 124.6, 115.3, 115.1, 115.0, 114.8, 81.2, 74.9, 74.2, 72.4, 46.5; $[\alpha]_D^{25}$ = +125.7 (*c* 0.02, MeOH); ESI-MS *m*/*z*: 360.1461 (M + H^+). Anal. Calcd for $C_{17}H_{18}FN_5O_3$: C, 56.82; H, 5.05; N, 19.49. Found: C, 56.92; H, 5.15; N, 19.59.

(2R,3S,4S)-2-((6-((3-Chlorobenzyl)amino)-9H-purin-9-yl)-methyl)tetrahydrofuran-3,4-diol (***2f***). Yield = 69%; white solid; mp 150−153 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.25 (s, 1H), 8.06 (s, 1H), 7.37 (s, 1H), 7.20−7.30 (m, 3H), 4.79 (s, 2H), 4.47 (dd, *J* = 3.5, 14.4 Hz, 1H), 4.33 (dd, *J* = 6.5, 14.4 Hz, 1H), 4.05 (ddd, *J* = 3.5, 6.9, 13.8 Hz, 1H), 3.93−4.00 (m, 2H), 3.78 (dd, *J* = 4.8, 7.2 Hz, 1H), 3.68 (dd, *J* = 2.3, 9.3 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ 156.7, 154.6, 151.1, 143.9, 138.2, 135.2, 131.3, 130.9, 130.5, 128.9, 120.8, 108.8, 81.8, 75.6, 74.8, 73.1, 47.2; $[\alpha]_D^{25}$ = +138.2 (*c* 0.02, MeOH); ESI-MS *m*/*z*: 376.1182 (M + H^+). Anal. Calcd for

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$C_{17}H_{18}ClN_5O_3$: C, 54.33; H, 4.83; N, 18.64. Found: C, 54.53; H, 4.93; N, 18.84.

*(2R,3S,4S)-2-((6-((3-Bromobenzyl)amino)-9H-purin-9-yl)-methyl)tetrahydrofuran-3,4-diol (**2g**).* Yield = 63%; white solid; mp 148−151 °C; ^{1}H NMR (CD_3OD, 500 MHz): δ 8.25 (s, 1H), 8.07 (s, 1H), 7.53 (s, 1H), 7.35 (dd, J = 7.8, 16.8 Hz, 2H), 7.20 (t, J = 7.8 Hz, 1H), 4.78 (br s, 2H), 4.47 (dd, J = 3.4, 14.4 Hz, 1H), 4.33 (dd, J = 6.6, 14.4 Hz, 1H), 4.05 (ddd, J = 3.4, 6.9, 10.4 Hz, 1H), 3.98−4.02 (m, 1H), 3.94−3.99 (m, 1H), 3.79 (dd, J = 5.0, 7.2 Hz, 1H), 3.69 (dd, J = 2.5, 9.6 Hz, 1H), 3.29−3.30 (m, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 156.1, 153.9, 143.39, 143.33, 131.5 (d, J = 11.4 Hz), 131.3, 127.4, 123.5, 120.1, 108.5, 81.2, 74.9, 74.2, 72.4, 46.5; $[\alpha]_D^{25}$ = +166.8 (c 0.02, MeOH); ESI-MS m/z: 422.0642 (M + H$^+$). Anal. Calcd for $C_{17}H_{18}BrN_5O_3$: C, 48.58; H, 4.32; N, 16.66. Found: C, 48.78; H, 4.52; N, 16.86.

*(2R,3S,4S)-2-((6-((3-Iodobenzyl)amino)-9H-purin-9-yl)-methyl)tetrahydrofuran-3,4-diol (**2h**).* Yield = 51%; white solid; mp 177−179 °C; ^{1}H NMR (DMSO-d_6, 800 MHz): δ 8.35 (br s, 1H), 8.20 (br s, 1H), 8.07 (s, 1H), 7.72 (s, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 7.6 Hz, 1H), 7.10 (t, J = 7.7 Hz, 1H), 4.96 (d, J = 6.2 Hz, 1H), 4.83 (d, J = 4.6 Hz, 1H), 4.64 (br s, 2H), 4.34 (dd, J = 3.8, 14.2 Hz, 1H), 4.20 (dd, J = 7.3, 14.2 Hz, 1H), 3.95 (ddd, J = 3.9, 6.9, 10.7 Hz, 1H), 3.88−3.92 (m, 1H), 3.86 (dd, J = 4.8, 9.2 Hz, 1H), 3.71 (dd, J = 6.4, 11.5 Hz, 1H), 3.50 (dd, J = 3.3, 9.2 Hz, 1H), 3.29 (br s, 1H); ^{13}C NMR (DMSO-d_6, 200 MHz): δ 154.1, 152.2, 149.0, 142.9, 141.3, 135.7, 135.2, 130.4, 126.6, 94.6, 79.6, 73.2, 72.4, 70.2, 64.8, 45.2; $[\alpha]_D^{25}$ = +138.8 (c 0.0125, MeOH); FAB-MS m/z: 468.0529 (M + H$^+$). Anal. Calcd for $C_{17}H_{18}IN_5O_3$: C, 43.70; H, 3.88; N, 14.99. Found: C, 43.90; H, 3.98; 14.99.

*2-Chloroquinolin-6-ol (**10**).* To a stirred suspension of quinoline-2,6-diol (1.0 g, 6.2 mmol) in acetonitrile (20.7 mL, 0.3 M) was added dropwise phosphorus oxychloride (0.9 mL, 1.5 equiv) at room temperature, and the reaction mixture was stirred at 80 °C for 23 h. The reaction mixture was cooled and neutralized with solid $NaHCO_3$, filtered, and evaporated under reduced pressure to give a colorless syrup. The residue was purified by silica gel column chromatography (n-hexanes/EtOAc = 6:1) to give **10** as a white solid (723.6 mg, 65%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.95 (dd, J = 11.8, 8.9 Hz, 2H), 7.34 (dd, J = 8.8, 2.2 Hz, 2H), 7.13 (d, J = 2.8 Hz, 1H), 5.36 (s, 1H).

*General Procedure for the Synthesis of **11a−11e**.* To a stirred solution of **10** (1.0 equiv) and K_2CO_3 (1.5 equiv) in DMF (0.3 M) was added 3-halobenzyl chloride or benzyl chloride (1.5 equiv) at room temperature, and the reaction mixture was stirred at 80 °C for 7 h. The reaction mixture was cooled and quenched with saturated ammonium chloride solution. The organic layer was washed with brine, dried with anhydrous $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (n-hexanes/EtOAc = 20:1) to give **11a−11e**.

*2-Chloro-6-((3-fluorobenzyl)oxy)quinoline (**11a**).* Yield = 92%; white solid; mp 99−100 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.97 (dd, J = 13.5, 8.9 Hz, 2H), 7.47 (dd, J = 9.2, 2.7 Hz, 1H), 7.31−7.41 (m, 2H), 7.17−7.25 (m, 2H), 7.13 (d, J = 2.6 Hz, 1H), 7.05 (t, J = 7.3 Hz, 1H), 5.18 (s, 2H); ^{13}C NMR (CD_3OD, 100 MHz): δ 156.88, 148.37, 143.96, 138.82 (d, J = 7.2 Hz), 137.74, 130.33 (d, J = 8.2 Hz), 130.20, 127.83, 123.26, 122.80, 122.66, 115.16 (d, J = 21.2 Hz), 114.31 (d, J = 22.1 Hz), 106.71, 69.54; HRMS (FD$^+$) m/z: [M$^+$] calcd for $C_{16}H_{11}ClFNO$, 287.0508; found, 287.0507.

*2-Chloro-6-((3-chlorobenzyl)oxy)quinoline (**11b**).* Yield = 98%; white solid; mp 105−106 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.96 (dd, J = 14.1, 8.9 Hz, 2H), 7.46 (dd, J = 9.4, 3.0 Hz, 2H), 7.35 (d, J = 4.5 Hz, 4H), 7.12 (d, J = 2.7 Hz, 1H), 5.15 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.88, 148.39, 143.98, 138.32, 137.72, 134.69, 130.12 (d, J = 19.0 Hz), 128.40, 127.82, 127.48, 125.40, 123.22, 122.65, 106.73, 69.51; HRMS (FD$^+$) m/z: [M$^+$] calcd for $C_{16}H_{11}Cl_2NO$, 303.0212; found, 303.0213.

*2-Chloro-6-((3-bromobenzyl)oxy)quinoline (**11c**).* Yield = 82%; white solid; mp 123−124 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.92 (dd, J = 13.9, 8.9 Hz, 2H), 7.60 (s, 1H), 7.43 (s, 2H), 7.35 (d, J = 7.7 Hz, 1H), 7.30 (d, J = 8.6 Hz, 1H), 7.21−7.27 (m, 1H), 7.08 (d, J = 2.7 Hz, 1H), 5.10 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.87, 148.39, 143.98, 138.57, 137.72, 131.34, 130.40, 130.26 (d, J = 8.8 Hz), 127.82, 125.89, 123.22, 122.74 (d, J = 17.9 Hz), 106.73, 69.44; HRMS (FD$^+$) m/z: [M$^+$] calcd for $C_{16}H_{11}BrClNO$, 346.9707; found, 346.9712.

*2-Chloro-6-((3-iodobenzyl)oxy)quinoline (**11d**).* Yield = 81%; white solid; mp 128−129 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.97 (dd, J = 18.2, 8.9 Hz, 2H), 7.84 (s, 1H), 7.70 (d, J = 7.9 Hz, 1H), 7.40−7.51(m, 2H), 7.35 (d, J = 8.6 Hz, 1H), 7.14 (dd, J = 13.5, 5.4 Hz, 2H), 5.12 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.88, 148.38, 143.97, 138.59, 137.72, 137.31, 136.33, 130.32 (d, J = 22.6 Hz), 127.82, 126.58, 123.22, 122.65, 106.72, 94.55, 69.33; HRMS (FD$^+$) m/z: [M$^+$] calcd for $C_{16}H_{11}ClINO$, 394.9568; found, 394.9577.

*6-(Benzyloxy)-2-chloroquinoline (**11e**).* Yield = 93%; white solid; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.97 (dd, J = 21.6, 8.9 Hz, 2H), 7.46 (ddd, J = 19.2, 10.5, 5.3 Hz, 5H), 7.33−7.39 (m, 2H), 7.16 (d, J = 2.7 Hz, 1H), 5.19 (s, 2H).

*General Procedure for the Synthesis of **3a−3e**.* To a stirred solution of **7** (2.0 equiv) in THF (0.2 M) was added t-BuOK (3.0 equiv), followed by compounds **11a−11e** (1.0 equiv) at room temperature, and the mixture was stirred at 80 °C for 2 h. The reaction mixture was cooled and quenched with saturated ammonium chloride solution. The organic layer was washed with brine, dried with anhydrous $MgSO_4$, and evaporated to give crude compounds. To the solution of crude intermediates in methanol (0.1 M) was added dropwise 1 N HCl (1 equiv) at room temperature. After being stirred at 60 °C for 14 h, the reaction mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane/MeOH = 50:1) to give **3a−3e**.

*(2R,3S,4S)-2-(((6-((3-Fluorobenzyl)oxy)quinolin-2-yl)oxy)-methyl)tetrahydrofuran-3,4-diol (**3a**).* Yield = 52%; white solid; mp 118−119 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.06 (d, J = 8.9 Hz, 1H), 7.76 (d, J = 9.1 Hz, 1H), 7.36−7.48 (m, 2H), 7.22−7.35 (m, 3H), 7.07 (td, J = 8.5, 2.3 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H), 5.22 (s, 2H), 4.65 (dd, J = 11.6, 2.6 Hz, 1H), 4.47 (dd, J = 11.6, 5.3 Hz, 1H), 4.21−4.30 (m, 1H), 4.07−4.19 (m, 3H), 3.80 (dd, J = 9.6, 3.2 Hz, 1H); ^{13}C NMR (CD_3OD, 150 MHz): δ 165.36, 163.74, 162.37, 156.73, 143.29, 141.63 (d, J = 7.4 Hz), 139.76, 131.48 (d, J = 8.2 Hz), 129.46, 127.39, 124.27, 122.75, 115.67 (d, J = 21.4 Hz), 115.24 (d, J = 22.3 Hz), 114.21, 109.19, 81.88, 74.16 (d, J = 5.6 Hz), 72.52, 70.56, 67.51; $[\alpha]_D^{25}$ = +412.0 (c 0.01, MeOH); HRMS (FD$^+$) m/z: [M$^+$] calcd for $C_{21}H_{20}FNO_5$, 385.1320; found, 385.1323.

*(2R,3S,4S)-2-(((6-((3-Chlorobenzyl)oxy)quinolin-2-yl)oxy)-methyl)tetrahydrofuran-3,4-diol (**3b**).* Yield = 56%; white

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solid; mp 103−104 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.07 (d, J = 8.9 Hz, 1H), 7.76 (d, J = 9.1 Hz, 1H), 7.54 (s, 1H), 7.25−7.49 (m, 5H), 6.97 (d, J = 8.8 Hz, 1H), 5.20 (s, 2H), 4.65 (dd, J = 11.6, 2.5 Hz, 1H), 4.47 (dd, J = 11.6, 5.2 Hz, 1H), 4.20−4.29 (m, 1H), 4.07−4.19 (m, 3H), 3.81 (dd, J = 9.6, 3.1 Hz, 1H); ^{13}C NMR (CD_3OD, 150 MHz): δ 162.38, 156.71, 143.31, 141.14, 139.77, 135.60, 131.25, 129.48, 129.09, 128.57, 127.39, 126.93, 122.74, 114.22, 109.20, 81.89, 74.17 (d, J = 5.5 Hz), 72.52, 70.52, 67.51; $[\alpha]_D^{25}$ = +212.3 (c 0.02, MeOH); HRMS (FD$^+$) m/z: [M$^+$] calcd for $C_{21}H_{20}ClNO_5$, 401.1025; found, 401.1025.

*(2R,3S,4S)-2-(((6-((3-Bromobenzyl)oxy)quinolin-2-yl)oxy)-methyl)tetrahydrofuran-3,4-diol (**3c**).* Yield = 49%; white solid; mp 170−171 °C; ^{1}H NMR (DMSO, 400 MHz): δ 8.14 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 9.2 Hz, 2H), 7.48−7.57 (m, 2H), 7.34−7.45 (m, 3H), 6.99 (d, J = 8.8 Hz, 1H), 5.21 (s, 2H), 4.98 (d, J = 6.1 Hz, 1H), 4.85 (d, J = 4.4 Hz, 1H), 4.52 (d, J = 2.8 Hz, 1H), 4.32 (s, 1H), 4.03−4.09 (m, 1H), 3.95 (dd, J = 8.1, 4.0 Hz, 2H), 3.89 (dd, J = 11.9, 6.1 Hz, 1H), 3.57 (dd, J = 9.3, 3.3 Hz, 1H); ^{13}C NMR (DMSO, 100 MHz): δ 160.79, 154.95, 141.70, 140.21, 138.97, 131.20 (d, J = 3.9 Hz), 130.82, 128.58, 127.22, 125.95, 122.02 (d, J = 36.6 Hz), 113.64, 108.68, 80.12, 72.89 (d, J = 18.9 Hz), 70.72, 69.02, 66.82; $[\alpha]_D^{25}$ = +172.0 (c 0.025, MeOH); HRMS (FD$^+$) m/z: [M$^+$] calcd for $C_{21}H_{20}BrNO_5$, 445.0519; found, 445.0516.

*(2R,3S,4S)-2-(((6-((3-Iodobenzyl)oxy)quinolin-2-yl)oxy)-methyl)tetrahydrofuran-3,4-diol (**3d**).* Yield = 68%; white solid; mp 124−125 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.11 (d, J = 8.9 Hz, 1H), 7.85 (s, 1H), 7.67 (t, J = 8.3 Hz, 2H), 7.49 (d, J = 7.5 Hz, 1H), 7.40 (d, J = 2.6 Hz, 1H), 7.36 (dd, J = 9.0, 2.8 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 6.97 (d, J = 8.8 Hz, 1H), 5.14 (s, 2H), 4.99 (d, J = 6.3 Hz, 1H), 4.86 (d, J = 4.5 Hz, 1H), 4.50 (dd, J = 11.6, 2.6 Hz, 1H), 4.28 (dd, J = 11.6, 6.4 Hz, 1H), 4.00−4.08(m, 1H), 3.80−3.96(m, 3H), 3.54 (dd, J = 9.3, 3.2 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ 165.54, 159.74, 146.44, 144.80, 143.74, 141.82, 141.36, 135.90, 133.31, 132.35, 130.69, 126.58, 118.38, 113.47, 100.08, 84.91, 77.64 (d, J = 18.8 Hz), 75.46, 73.74, 71.55; $[\alpha]_D^{25}$ = +438.7 (c 0.01, MeOH); HRMS (FD$^+$) m/z: [M$^+$] calcd for $C_{21}H_{20}INO_5$, 493.0381; found, 493.0380.

*(2R,3S,4S)-2-(((6-(Benzyloxy)quinolin-2-yl)oxy)methyl)-tetrahydrofuran-3,4-diol (**3e**).* Yield = 79%; white solid; mp 139−140 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.05 (d, J = 8.9 Hz, 1H), 7.75 (d, J = 9.1 Hz, 1H), 7.51 (d, J = 7.3 Hz, 2H), 7.27−7.46 (m, 5H), 6.96 (d, J = 8.9 Hz, 1H), 5.20 (s, 2H), 4.65 (dd, J = 11.6, 2.5 Hz, 1H), 4.46 (dd, J = 11.6, 5.2 Hz, 1H), 4.25 (dd, J = 7.7, 4.4 Hz, 1H), 4.08−4.22 (m, 3H), 3.81 (dd, J = 9.6, 3.2 Hz, 1H); ^{13}C NMR (CD_3OD, 150 MHz): δ 162.30, 156.99, 143.20, 139.75, 138.69, 129.67, 129.36, 129.09, 128.81, 127.41, 122.85, 114.14, 109.13, 81.89, 74.16 (d, J = 5.4 Hz), 72.52, 71.48, 67.49; $[\alpha]_D^{25}$ = +388.0 (c 0.01, MeOH); HRMS (FD$^+$) m/z: [M$^+$] calcd for $C_{21}H_{21}NO_5$, 367.1414; found, 367.1424.

BIOLOGY (IN VITRO ANTI-INFLAMMATION TEST)

Statistical Analysis. The data were presented as the mean ± standard error of the mean (SEM) of three independent readings. Statistical differences were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison analysis, using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). The statistical significance was set at $P < 0.05$.

Cell Culture. The mouse macrophage cell line RAW 264.7 (ATCC, Manassas, VA, USA) was cultured in DMEM containing 10% FBS (HyClone; Logan, UT, USA) and 1% penicillin/streptomycin. Cells were incubated at 37 °C in an incubator with 5% CO_2. To confirm cell viability, RAW 264.7 cells at 50% confluency were treated with various concentrations of the compounds for 24 h. Then, the cells were treated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) assay reagent (Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of 0.5 mg/mL for 2 h until purple crystals formed. Then, DMSO was added for 10 min to dissolve the crystals, and the absorbance was measured at 540 nm.

Gene Expression Analysis. Total RNA from RAW 264.7 cells was extracted using TRI Reagent (MRC, Cincinnati, OH, USA). cDNA was synthesized using TOPscriptTM RT DryMIX (Enzynomics; Daejeon, South Korea). mRNA levels were measured by real-time PCR using a Rotor-Gene Q (QIAGEN; Hilden, Germany) in a 10 μL reaction volume consisting of cDNA transcripts, primer pairs, and the TOPreal SYBR Green PCR Kit (Enzynomics). Gene expression levels were normalized to the 18S rRNA levels. Primer pairs for IL-6, IL-1β, and TNFα were used, and their forward and reverse sequences are as follows. IL-1β forward: 5′-TGCCACCTTTTGACAGTGATG-3′; IL-1β reverse: 5′-TTCTTGTGACCCTGAGCGAC-3′, TNFα forward: 5′-CCCTCACACTCAGATCATCTTCT-3′; TNFα reverse: 5′-GCTACGACGTGGGCTACAG-3′, IL-6 forward: 5′-AGTTGCCTTCTTGGGACTGA-3′; IL-6 reverse: 5′-TCCACGATTTCCCAGAGAAC-3′; 18S forward; 5′-CGAAAGCATTTGCCAAGAAT-3′; 18S reverse: 5′-AGTCGGCATCGTTTATGGTC-3′.

Enzyme-Linked Immunosorbent Assay. RAW 264.7 cells were pretreated for 2 h with active compounds, followed by treatment with 100 ng/mL LPS for 24 h (Sigma-Aldrich). IL-1β levels were then determined in the supernatants using the mouse IL-1 beta Quantitative ELISA Kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Western Blotting. RAW 264.7 cells were lysed with RIPA buffer (Thermo Fisher Scientific; Rockford, IL, USA), and the BCA kit (Thermo Fisher Scientific) was used for protein concentration quantification. 30 μg of protein samples was separated by SDS-PAGE and then transferred to PVDF membranes (Cytiva, Amersham, Germany), which were incubated overnight with primary antibodies (4 °C). After the membranes were washed, secondary antibodies were added, and the membranes were incubated at room temperature for 2 h. Finally, proteins were visualized with a LAS-4000 (Fuji Photo Film, Tokyo, Japan). Mouse antirabbit IgG-HRP (#sc-2357, 1:2000) was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA), while phospho NF-κB (#3033, 1:2000) and total NF-κB (#8242, 1:2000) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05029.

Copies of ^{1}H and ^{13}C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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