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Short communication

Synthesis, crystal structure and anti-inflammatory properties of curcumin analogues

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Abstract

Curcuminoids have been reported to possess multifunctional bioactivities, especially the ability to inhibit proinflammatory induction. Since it has been suggested that the seven-carbon β-diketone linker in curcumin is responsible for its instability, nine mono-carbonyl five-carbon linker containing analogues were designed and synthesized. Their bioactivity against lipopolysaccharide-induced TNF-α amd IL-6 secretion was evaluated by using mouse J774.1 macrophages. The results showed that the 3′-methoxyl plays an important role in bioactivity and cyclohexanone containing analogues exhibited stronger inflammatory inhibition than acetone and cyclopentanone analogues. Subsequently the most active analogue **3c** was determined using single-crystal X-ray diffraction. X-ray analysis and comparison with curcumin reveals that the presence of cyclohexanone in **3c**, which remotely resembles the 6-membered ring in the enol tautomer in curcumin, may play an important role in the bioactivity. It is suggested that five-carbon linker analogues containing a cyclohexane ring which are synthetically assessable may be pharmacologically important.

Keywords: Curcumin analogue; Anti-inflammation; X-ray diffraction; Cyclohexanone

1. Introduction

Proinflammatory cytokines are involved in the pathogenesis of a variety of diseases such as immunological disease and inflammation. For example, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are two multifunctional proinflammatory cytokines involved in the pathogenesis of cardiovascular diseases, cancer and neurodegenerational disease through a series of cytokine signaling pathways [1,2]. The inhibition of release of cytokines becomes a major focus of current drug development and an important method for evaluating the bioactivity of drugs [3].

Curcumin (Fig. 1), a yellow compound isolated from turmeric, possesses multifunctional pharmacological applications in a variety of diseases such as liver fibrosis, inflammation, cardiovascular disease and cancer [4–6]. Curcumin has been reported to alter the cytokine profiles [7,8]. Curcumin potently inhibited the production of TNF-α and IL-12 in a dose-dependent manner from mouse macrophages stimulated by lipopolysaccharide (LPS) and reduced the LPS-induced activation of NF-kB [9,10]. However, pre-clinical and clinical studies showed that curcumin possessed several disadvantages such as instability, poor bioavailability and fast metabolism [11]. It is suggested that the presence of the methylene group and β-diketone moiety contributes to the instability of curcumin under physiological conditions [12,13]. Therefore, the deletion of the β-diketone moiety may contribute to the enhancement of stability of curcumin compounds. We present here the synthesis of nine mono-carbonyl analogues of curcumin, as well

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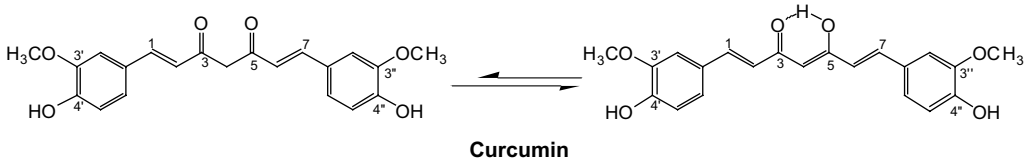


Fig. 1. Chemical structure of curcumin, where the α,β-unsaturated β-diketone (heptadiene–dione) moiety undergoes keto–enol tautomerism and forms a hydrogen bond containing 6-membered ring [14].

as their pharmacological screening in inhibition of LPS-induced TNF-α and IL-6 secretion in macrophages. The crystal structure of (2*E*,6*E*)-2,6-bis(4-fluorobenzylidene) cyclohexanone (**3c**), which has the highest bioactivity among analogues, is reported by a single-crystal X-ray diffraction. The difference in crystal structures of curcumin and **3c** is analyzed.

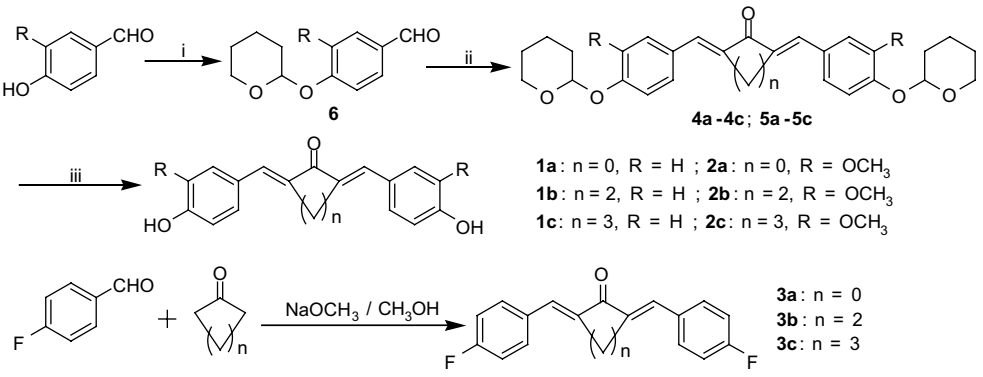
2. Results and discussion

Compounds **3a**–**3c** were synthesized (Scheme 1) by direct coupling of *p*-fluorobenzaldhyde with the three ketones, acetone, cyclopentanone and cyclohexanone in alkaline media. The synthesis of compounds **1** and **2**, shown in Scheme 1, begins by the protection of 4-hydroxybenzaldehyde with tetrahydropyran-2-yl to afford compound **6**. Aldol condensation of **6** with acetone, cyclopentanone and cyclohexanone gave compounds **4a**–**4c** and **5a**–**5c**, respectively. Compounds **1** (or **2**) were subsequently obtained by hydrolysis of **4** (or **5**) using a catalytic amount of *p*-toluenesulfonic acid. A diaryl structure is confirmed by the absence of methyl protons in the ^{1}H NMR spectra of **a** class compounds and the absence of two methylene protons in the spectra of **b** and **c** class compounds.

These nine analogues containing five-carbon linker with mono-carbonyl were evaluated their anti-inflammatory properties through inhibiting the LPS-induced TNF-α and IL-6 release in macrophages [14]. As shown in Fig. 2, the mono-carbonyl compounds **2a**–**2c** exhibited enhanced inhibition on both TNF-α and IL-6 than curcumin. In the structure of curcumin, the hydrogen bonds are formed between 4′-hydroxyl and 3′-methoxyl, reducing the molecular polarity of curcumin. The 3′-methoxyl-deleting compound **1a**, however, exhibited an opposing activity to enhance the release of inflammatory cytokines induced by LPS. Although it was reduced when the cyclopentanone and cyclohexanone were introduced as rigid linker chains in structures of **1b** and **1c**, only **1c** reached a comparable activity to curcumin. This result indicates that (1) the 3′-methoxyl plays an important role in bioactivity, and (2) the six-member ring containing linker of mono-canbonyl analogues may be favorable to the inflammatory inhibition. With the same electron-withdrawing fluoride displacing hydroxyl group, compounds **3a**–**3c** were synthesized and all of them showed elevated activity against LPS-induced inflammatory response. Especially, from **3a** to **3c**, an increasing trend similar to hydroxyl analogues **1** appeared and **3c** exhibited the highest ability in analogues **3**, reaching 65.3% (TNF-α) and 54.8% (IL-6).

Our data given above showed that the cyclohexanone containing linker may play an important role in the bioactivity of analogues. To demonstrate the structure of cyclohexanone linker, an X-ray crystal structure of **3c**, (2*E*,5*E*)-2,5-bis(4-fluorobenzylidene) cyclohexanone was determined and the atom labeling scheme is presented in Fig. 3, as well as that of curcumin for comparison according to Parimita et al. [15]. The selected bond lengths and bond angles are given in Table 1.

Single-crystal X-ray structure analysis reveals that there are two **3c** molecules in one unit cell with different bond lengths and angles. No intermolecular hydrogen bond and interaction appear. All H atoms were located in difference Fourier maps and were refined freely in idealized positions, with C–H = 0.93 Å for aromatic H and vinyl H or 0.96 Å for methylene H. In **3c**, the dihedral angle between the two least-square planes passing through benzene rings is 34.2(6)°, while that of curcumin is 19.1(3)°. Compound **3c** possesses more rigid structure than curcumin due to the introduction of cyclohexanone. The lengths of C–F bonds including C1–F1, C18–F2, C21–F3 and C38–F4 are similar to that of C–O bonds of curcumin in the corresponding position (data showed in Table 1). In one of **3c** molecule in unit cell, the C12 is symmetrically placed between atoms C11 and C13 and the 6-membered



Scheme 1. Synthesis of presented compounds. Reagents and conditions: (i) 3,4-dihydro-α-pyran, pyridine-PTSA, CH_2Cl_2, rt; (ii) acetone, cyclopentanone or cyclohexanone, NaOH/EtOH, rt; (iii) PTSA, MeOH, rt.

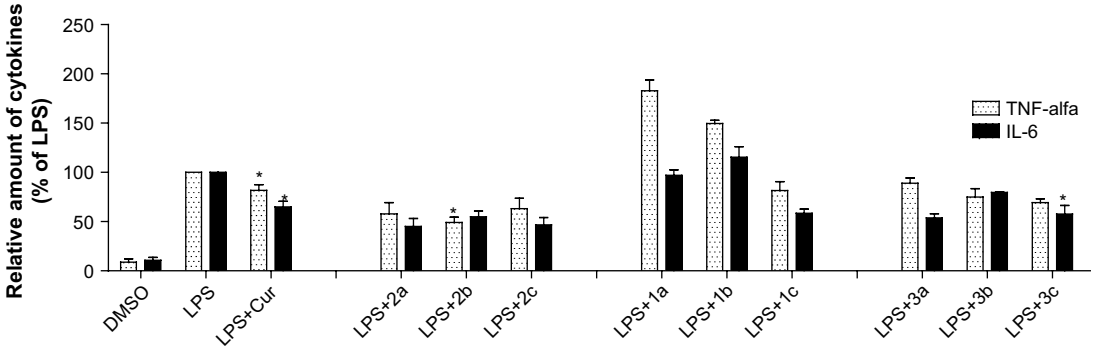


Fig. 2. Inhibition of LPS-induced TNF-α and IL-6 by curcumin and its analogues in J774A.1 macrophages. Cells were pre-treated with curcumin or its analogues (10 μM) for 3 h, then treated with LPS (0.5 μg/ml) for 24 h. TNF-α (A) and IL-6 (B) levels in the culture media were measured by ELISA. The results were expressed as percent of LPS control. Each bar represents mean ± S.E. of three independent experiments ($^{*}n > 5$ and $p < 0.01$).

ring of cyclohenxanone was positioned with C11–C13 distance being 2.483 Å, similar to the 6-membered ring in curcumin formed by hydrogen bonding among H23, O2 and O3, in which the O2–O3 distance is 2.455 Å. In the other molecule of the cell, the C31–C32 length is 1.506(7) Å while the C32–C33 length is 1.522(7) Å. This result reveals that the structure of cyclohexanone in **3c** is similar to 6-membered ring formed by hydrogen bonding in curcumin. Although the conformation of drugs in micro-environment is decisive to activity, close structure may easily result in close conformation. So, the structural similarity may play an important role in the bioactivity.

3. Conclusion

In conclusion, our data show that the 3′-methoxyl group is important for activity and cyclohexanone containing analogues exhibited higher anti-inflammatory activity than acetone and cyclopentanone analogues. The structure of cyclohexanone moiety is similar to 6-membered ring formed by β-diketone tautomer in curcumin, which may render **1c** and **3c** stronger ability on inhibition of TNF-α and IL-6. Recent reports indicated that β-diketone moiety may lead to instability of curcumin and affect the metabolic profile. Therefore, it is suggested that five-carbon linker analogues containing a cyclohexane ring may be important for the development of anti-inflammatory curcumin drugs.

4. Experimental

^{1}H NMR spectra were recorded on a Varian INOVA-400 spectrometer. Electron-spray ionization mass spectra in positive mode (ESI-MS) data were recorded on a Bruker Esquire 3000^{+} spectrometer. Melting points were determined on a Fisher–Johns melting apparatus and are corrected. Column chromatography purifications were carried out on Silica Gel 60 (E. Merck, 70–230 mesh).

4.1. Synthesis

A solution of 3,4-dihydro-α-pyran (0.2 mol) in dichloromethane (10 mL) was added dropwise to a well-stirred suspension of *p*-hydroxylbenzaldehyde (0.1 mol) and pyridinium

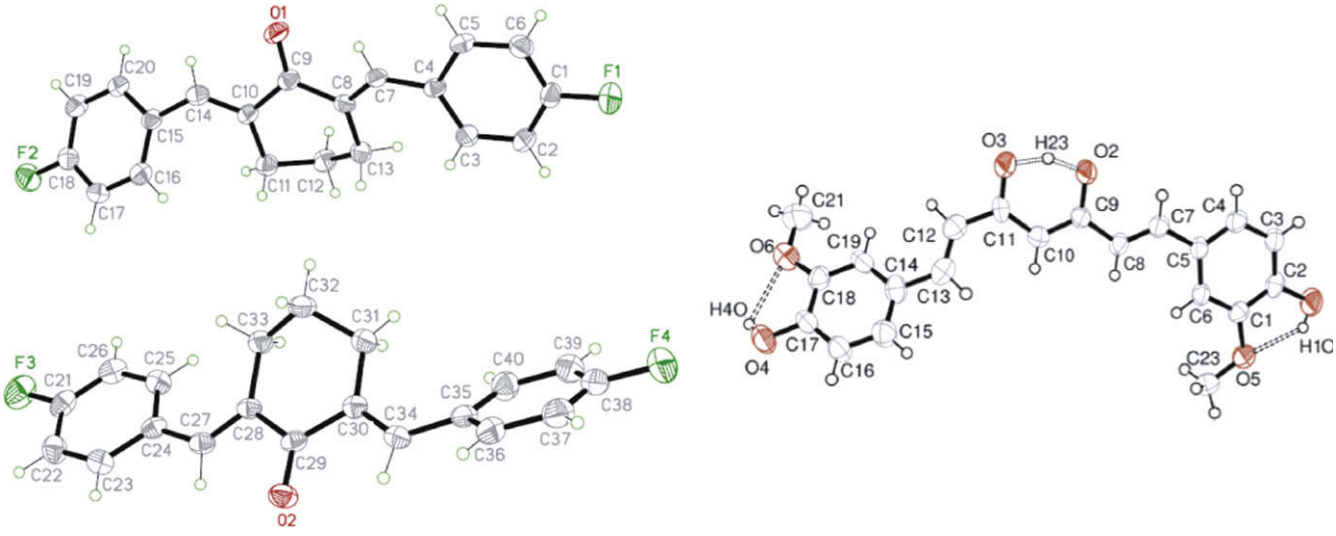


Fig. 3. The crystal structure of **2c** (left) in a unit cell and curcumin (right) [15] with labeled non-hydrogen atom, showing displacement ellipsoids at the 50% probability level. Dashed lines indicate intramolecular hydrogen bonding. (To see curcumin in Lit. [15].)

Table 1
Selected bond lengths (Å) and bond angles (°)

Bond	Distance	Bond	Distance	Bond	Distance
F1–C1	1.364 (6)	C8–C9	1.487 (7)	C28–C33	1.497 (7)
F2–C18	1.353 (6)	C8–C13	1.500 (7)	C28–C29	1.498 (7)
F3–C21	1.356 (6)	C9–C10	1.495 (7)	C29–C30	1.504 (7)
F4–C38	1.362 (6)	C10–C11	1.497 (7)	C30–C31	1.499 (7)
O1–C9	1.224 (6)	C11–C12	1.511 (7)	C31–C32	1.506 (7)
O2–C29	1.218 (5)	C12–C13	1.511 (7)	C32–C33	1.522 (7)

Bond	Angle	Bond	Angle	Bond	Angle
C7–C8–C9	116.0 (4)	C11–C12–C13	110.5 (4)	C34–C30–C29	117.1 (4)
C7–C8–C13	125.7 (5)	C8–C13–C12	113.3 (4)	C30–C31–C32	110.7 (4)
C14–C10–C9	117.8 (4)	C27–C28–C33	124.6 (5)	C31–C32–C33	109.5 (4)
C14–C10–C11	124.2 (5)	C27–C28–C29	115.7 (4)	C28–C33–C32	112.0 (4)
C10–C11–C12	111.0 (4)	C34–C30–C31	124.6 (5)		

p-toluenesulfonate (catalytic) in dichloromethane (40 mL). The mixture was stirred at room temperature for 3 h and monitored by TLC, and then washed with saturated $NaHCO_3$ solution (50 mL × 3) and brine (50 mL × 3) and dried over Na_2SO_4. Evaporation of the solvent affords crude product which was purified by column chromatography on silica gel (hexane/ethyl acetate, 8:2, as eluent) to give the protective compound **6** (0.09 mol). A mixture of **6** (10 mmol) and acetone, cyclopentanone or cyclohexanone (5 mmol) in ethanol (10 mL) was stirred at rt for 10 min and 18% (w/v) $NaOCH_3$ in methanol (1.5 mL, 5 mmol) was added dropwise. After stirring for 2 h, the resulting precipitate was filtered. The protected derivative **4** (10 mmol) was suspended in ethanol (20 mL) and treated with a catalytic amount of *p*-toluenesulfonic acid. After stirring at room temperature for 10 h, 100 ml cold water was added and the mixture was filtered. The residue was washed with cold water and then recrystallized from C_2H_5OH/H_2O (9:1) to obtain pure **1a**, **1b** or **1c**. Compounds **2** were obtained by the same way.

*4.1.1. 1,5-Bis(4-hydroxyphenyl)penta-1,4-dien-3-one (**1a**)*

Orange crystal, 92% yield, mp 246–248 °C [Lit. [16] 243–245 °C].

*4.1.2. 2,5-Bis(4-hydroxybenzylidene)cyclopentanone (**1b**)*

Yellow crystal, 89.3% yield, mp >300 °C. ^{1}H NMR (DMSO-d_6) δ: 2.98 (4H, s, CH_2–CH_2), 6.88 (4H, d, $J = 8.4$ Hz, Ar–$H^{2,6}$ × 2), 7.33 (2H, s, Ar–CH=C × 2), 7.53 (4H, d, $J = 8.4$ Hz, Ar–$H^{3,5}$ × 2), 10.01 (2H, br, Ar–OH × 2). ESI-MS *m/z*: 291.13 $(M-1)^+$, calcd for $C_{19}H_{16}O_3$: 292.33.

*4.1.3. 2,6-Bis(4-hydroxybenzylidene)cyclohexanone (**1c**)*

Yellow crystal, 59% yield, mp >300 °C. ^{1}H NMR (DMSO-d_6) δ: 1.71 (2H, m), 2.84 (4H, s, C–CH_2 × 2), 6.83 (4H, d, $J = 6.0$ Hz, Ar–$H^{2,6}$ × 2), 7.40 (4H, d, $J = 6.0$ Hz, Ar-$H^{3,5}$ × 2), 7.53 (2H, s, Ar–CH=C × 2), 9.50 (2H, br, Ar–OH × 2). ESI-MS *m/z*: 305.18 $(M-1)^+$, calcd for $C_{20}H_{18}O_3$: 306.36.

*4.1.4. 1,5-Bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (**2a**)*

Yellow powder, 85% yield, mp 87–89 °C [Lit. [17] 84–86 °C].

*4.1.5. 2,5-Bis(4-hydroxy-3-methoxybenzylidene)cyclopentanone (**2b**)*

Yellow powder, 76% yield, mp 214 °C [Lit. [16] 212–214 °C].

*4.1.6. 2,6-Bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone (**2c**)*

Yellow powder, 67% yield, mp 174–176 °C [Lit. [16] 178–179 °C].

To a solution of 10 mmol 4-fluorobenzaldehyde in dried EtOH (10 ml) was added 5 mmol acetone, cyclopentanone or cyclohexanone. The solution was stirred at room temperature for 10 min, followed by dropwise addition of 18% (w/v) $NaOCH_3$ in methanol (1.5 ml, 5 mmol). The mixture was stirred at rt for 2 h. The resulting precipitate was filtered and washed with water, cold ethanol, and then cold acetone and dried in vacuum. The solid was purified by chromatography over silica gel using CH_2Cl_2/CH_3OH as the eluent affording compounds **3a**–**3c**.

*4.1.7. 1,5-Bis(4-fluorophenyl)penta-1,4-dien-3-one (**3a**)*

Yellow crystal, 67% yield, mp 152 °C [Lit. [18] 150–152 °C].

*4.1.8. 2,5-Bis(4-fluorobenzylidene)cyclopentanone (**3b**)*

Yellow crystal, 88% yield, mp 212–214 °C [Lit. [19] 210 °C].

*4.1.9. 2,6-Bis(4-fluorobenzylidene)cyclohexanone (**3c**)*

The product was recrystallized from ethanol/water (9:1), yellow crystal, 69% yield, mp 156 °C. ^{1}H NMR ($CDCl_3$) δ: 1.81 (2H, q, $J = 12$ Hz, CH_2), 2.90 (4H, t, $J = 5.4$ Hz, CH_2 × 2), 7.12 (4H, m, Ar–$H^{2,6}$ × 2), 7.45 (4H, m, Ar–$H^{3,5}$ × 2), 7.76 (2H, s, Ar–CH=C × 2). ESI-MS *m/z*: 311.11 $(M+1)^+$, calcd for $C_{20}H_{16}F_2O$: 310.34.

Table 2
Crystal data of analogue **6**

Crystal data		
$C_{20}H_{16}F_2O$	$\alpha = 78.479\ (2)^\circ$	$\lambda = 0.71073$ Å
FW = 310.33	$\beta = 74.498\ (2)^\circ$	Cell parameters
	$\gamma = 86.226\ (2)^\circ$	from 1584 reflections
Triclinic, *P*-1	$V = 1554.9\ (3)$ Å^3	
Hall symbol: -*P*1	$Z = 4$	$\theta = 2.51-24.26^\circ$
$a = 9.6947\ (11)$ Å	$F_{000} = 648$	$\mu = 0.097$ mm^{-1}
$b = 11.8447\ (12)$ Å	$D_x = 1.326$ Mg m^{-3}	$T = 298\ (2)$ K
$c = 14.3422\ (15)$ Å	Mo Kα radiation	$0.25 \times 0.16 \times 0.14$ mm
Data collection [20,21]		
$T_{min} = 0.976$	8202 Independent	$h = -9 \rightarrow 11$;
$T_{mix} = 0.991$	reflections, 3778	$k = -13 \rightarrow 14$;
	reflections	$l = -12 \rightarrow 17$
	with $I > 2\sigma(I)$	
$\theta_{max} = 25.02^\circ$	$\theta_{min} = 1.75^\circ$	$R_{int} = 0.0295$
Refinement [21]		
$R[F^2 > 2\sigma(F^2)] = 0.1024$	5414 Reflections	$(\Delta/\sigma)_{max} = 0.000$
$wR(F^2) = 0.3438$	415 Parameters	$\Delta\rho_{max} = 0.176$ e Å^{-3}
$S = 1.021$	$w = 1/[\sigma^2(F_o^2) + (0.2253P)^2 + 0.4116P]$, where $P = (F_o^2 + 2F_c^2)/3$	$\Delta\rho_{min} = -0.214$ e Å^{-3}

4.2. Crystal data and structure determination

The crystal and refinement data of the analogue **3c**, (2*E*,6*E*)-2,6-bis(4-fluorobenzylidene)-cyclohexanone are presented in Table 2. A yellow rhombus crystal of $C_{20}H_{16}F_2O$ from CH_3OH/CH_2Cl_2 (9:1) having approximate dimensions of $0.25 \times 0.16 \times 0.14$ mm was mounted on a glass fiber. All measurements were made on a Bruker X8 APEX diffractometer with graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). Of the 8202 reflections that were collected in the range of $2.51 < \theta < 24.26^\circ$, 3778 were unique ($R_{int} = 0.0295$). Data were collected and integrated using the Bruker SAINT [20] software package. All non-hydrogen atoms were refined anisotropically. All other hydrogen atoms were included in calculated positions but not refined. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.176 and −0.214 e Å^{-3}, respectively. All refinements were performed using the SHELXTL-97 crystallographic software package [21].

4.3. Anti-inflammatory test by ELISA

The mouse J774A.1 cells in our experiments belongs to macrophage-like cell line and is widely used as a kind of cell model in vitro for inflammatory research. Cells were plated in DMEM medium (including 10% FBS, 100 μg/ml penicillin and 100 μg/ml streptomycin) with a density of 1.2×10^6/plate for overnight in 37 °C and 5% CO_2. Cells then were pre-treated with 10 μM of curcumin, each analogue or vehicle control for 3 h, followed by treatment with LPS (0.5 μg/ml) for 21 h. At the end of treatment, the culture media and cells were collected, respectively. The levels of tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) in the media were determined by ELISA using mouse TNF-α and mouse IL-6 ELISA Max™ Set Deluxe Kits (Biolegend, USA). The tests were performed according to the manufacturer's instruction. The cells collected were added into the total lysis buffer (Tris–HCl, 20 mM; NP40, 1%; NaCl, 150 mM; EDTA, 2 mM; SDS, 0.1%; NaF, 20 mM; Na_3VO_4, 20 mM; H_2O) and votaxed, and then centrifuged at 8000 rpm for 5 min to extract the total proteins. The total protein concentrations of the viable cell pellets were determined using Bio-Rad protein assay reagents. Total amounts of the TNF-α and IL-6 in the media were normalized to the total protein amount of the viable cell pellets. Each compound was repeatedly tested in three independent experiments.

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