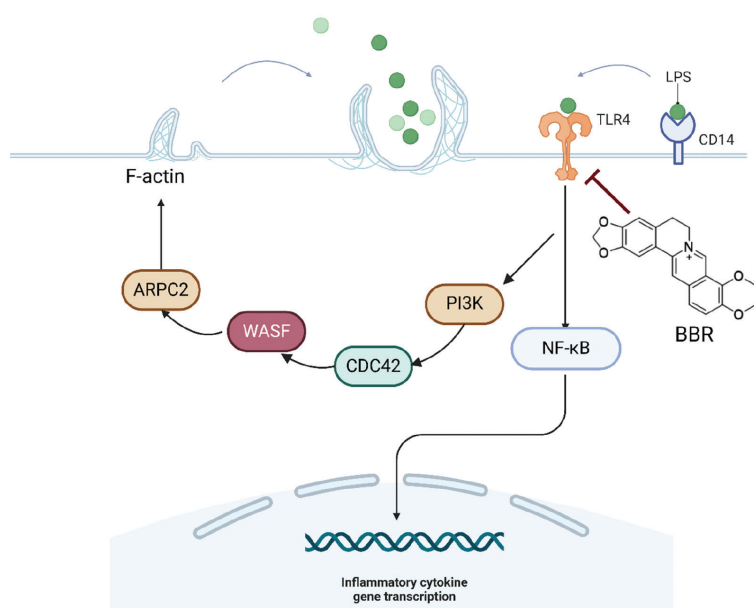


Berberine inhibits phagocytosis through the TLR4-PI3K-CDC42 pathway

Graphical abstract



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In brief

This study identified that BBR significantly inhibits macrophage phagocytosis through the TLR4-PI3K-CDC42 pathway, beyond its anti-inflammatory effects.

Highlights

- Among a library of natural products with a variety of structures, berberine significantly inhibited macrophage phagocytosis.
- Berberine effectively restrained macrophage phagocytosis via the TLR4-PI3K-CDC42 signaling pathway.

Short Communication

Berberine inhibits phagocytosis through the TLR4-PI3K-CDC42 pathway

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ABSTRACT

Phagocytosis is a fundamental mechanism used by the body to resist pathogens and restore physiological homeostasis. Herein, to identify small molecules with anti-inflammatory properties via phagocytosis inhibition, we constructed a library of natural products and evaluated their ability to modulate phagocytosis in RAW264.7 macrophages. Berberine (BBR) is the major constituent of traditional Chinese medicine *Coptidis Rhizoma* that is recorded in Chinese Pharmacopoeia with the effect of clearing heat-toxin, and is used in the therapeutic management of various inflammatory diseases. BBR was found to inhibit phagocytosis and significantly alleviate inflammation via suppressing interleukin-1 α (IL-1 α), interleukin-1 β (IL-1 β), inducible nitric oxide synthase (iNOS), and tumor necrosis factor- α (TNF- α), according to real-time quantitative polymerase chain reaction (RT-qPCR) analyses, and phosphorylated-p65 (p-p65), iNOS, and cyclooxygenase-2 (COX-2), according to western blot analyses. BBR inhibited the expression of F-actin, a key protein in phagosome formation. Notably, BBR exerted its phagocytosis effects through targeting phosphoinositide 3-kinase (PI3K), thereby activating the small GTPase-Cdc42 (CDC42), Wiskott-Aldrich syndrome protein (WASP), and actin-related protein 2/3 complex subunit 2 (Arp2/3). BBR attenuated LPS-mediated inflammation through promoting macrophage phagocytosis. We determined that BBR targets the toll-like receptor 4 (TLR4)-PI3K-CDC42 pathway, thereby inhibiting the nuclear factor-kappa B (NF- κ B) pathway, and consequently regulating phagocytosis and the inflammatory response. Our findings suggest that BBR might serve as a candidate for the development of phagocytic inhibitors.

Keywords: Berberine, Phagocytosis, TLR4-PI3K-CDC42

1. INTRODUCTION

A recent health study in 22 million people has indicated that approximately 1 in 10 people (10%) have an autoimmune disease [1]. Macrophages, the primary components of heterologous phagocytes, are integral to the innate immune system. These cells play crucial roles in the phagocytic clearance of apoptotic cells and cells damaged by inflammation [2]. In mammals, resident macrophages have dual functions in promoting tissue regeneration and organogenesis after damage, and defending against infectious disease [2-4]. The physiological functions of phagocytosis involve recognizing, internalizing, and digesting large particles, including bacteria, apoptotic cells, and cell debris [5, 6]. Phagocytosis is critical in various diseases, such as neurodegeneration, infectious inflammatory, and

auto-immune disorders [7]. Polysaccharides, polyphenols, and flavonoids have been shown to regulate phagocytosis [8-13]. Notable studies have demonstrated that resveratrol alleviates inflammation by inhibiting the phagocytic function of macrophages and the NF- κ B pathway [8, 14]. Furthermore, *Ganoderma lucidum* has been found to suppress both phagocytosis and microglial migration in mice with A β -induced neuroinflammation [9]. Consequently, regulating phagocytosis is a promising therapeutic strategy for various diseases.

Macrophage phagocytosis is closely associated with the aggregation of actin, a critical component in establishing the phagocytic cup and generating the necessary driving force for plasma membrane deformation. Recent advancements have indicated that F-actin is essential in phagocytosis [15, 16]. During microbial invasion, TLR4 initiates a signaling cascade within cells,

down-regulating the PI3K signaling pathway; subsequently leads to actin polymerization and formation through the stimulation of GTP-Rho family proteins; and ultimately facilitates phagosome formation [17]. Onyishi et al. have reported that TLR4 signals through myeloid differentiation primary response 88 (MyD88), which also drives actin remodeling and pathogen uptake [18]. The Rho family of small GTPases, a subset of mammalian small-GTP-binding proteins, encompasses several members including Rho family small GTPase (RHO), RAC, CDC42, Rho family small GTPase D (RHOD), Rho family small GTPase G (RHOG), TC10, RND, and TTF [19, 20]. These proteins play distinct roles in cellular processes: RAC induces the formation of lamellar pseudopodia through peripheral actin polymerization, whereas Rho mediates the assembly of actin and myosin filaments into stress fibers. F-actin, as a phagocytic marker, targets the downstream factor CDC42 to jointly activate the inflammatory response [21, 22]. CDC42, in conjunction with WASP and the verprolin homologous protein WASP subfamily (WASF, also known as WAVE), is essential in macrophage polarization as a critical regulator of cytoskeletal rearrangements during phagocytosis [23, 24]. WASF2, a downstream gene of CDC42, interacts with the Arp2/3 complex, providing the necessary cytoskeletal basis for the formation of pseudopodia and the formation of phagosomes during phagocytosis by regulating the assembly of actin. The Arp 2/3 complex is also crucial in cytoskeletal remodeling and the formation of the dense dendritic network in the pseudopodia and phagosomes [25]. Studies have highlighted the potential of certain traditional Chinese medicine preparations in modulating phagocytosis via the Rho family small GTPase A (RHOA), CDC42, and actin-related protein 2/3 complex subunit 2 (ARPC2). For instance, Houshiheisan suppresses RHOA while enhancing CDC42 expression, thereby promoting axon regeneration after ischemic brain injury [26]. You-Gui pills have shown efficacy in promoting nerve regeneration, specifically in the brain and spinal cord in experimental mouse models of autoimmune encephalomyelitis, through modulating the expression of RHOA, RAC1, and CDC42 [27]. In addition, Liu et al. have reported that modified *danzhi xiaoyao* powder effectively regulates CDC42, ARPC2, and actin-associated protein 3 (ACTR3), thereby attenuating inflammation and decreasing phagocytosis in a mouse model of dry eye disease [28]. Collectively, these insights underscore the major role of the TLR4-PI3K-CDC42 signaling pathway in regulating phagocytic processes and highlight the potential of traditional Chinese medicine formulations to modulate this pathway and achieve therapeutic benefits.

Berberine (BBR), a bioactive alkaloid compound naturally found in *Coptis chinensis*, has an extensive history of use in traditional Chinese medicine. BBR exerts

significant inhibitory effects on inflammation in various diseases, such as colitis, pancreatitis, and adipose inflammation [29, 30]. Herein, we constructed a library of natural products and used neutral red assays to screen for compounds modulating phagocytosis. Our investigation revealed that BBR notably inhibited macrophage phagocytosis and mitigated inflammation in lipopolysaccharide (LPS)-activated macrophages. Our research was aimed at identifying the mechanism through which BBR modulates phagocytosis, as well as providing potential drugs for the modulation of phagocytosis activity.

2. MATERIALS AND METHODS

2.1 Materials

The 49 compounds methyl 3,5-dicaffeoylquininate (1), 6,8-dihydroxycoumarin (2), 1-*O*-caffeoylglycerol (3), 4'-*O*-methyl dihydroquercetin (4), 3,5,4'-trihydroxy-6,7,3'-trimethoxy-flavone (5), britanicaflavin E (6), 1,5-*O*-dicaffeoylquinic acid (7), spinacetin (8), quercetin (9), luteolin (10), temifoliuslignan A (11), 5,6,4'-trihydroxy-3,7-dimethoxyflavone (12), 6-dodecyloxy coumarin (13), britanicaflavin C (14), britanicaflavin D (15), (2*R*,3*R*)-taxifolin (16), wedelolactone (17), epitaxifolin (18), (2*S*,3*S*)-britanicaflavin A (19), (2*R*,3*R*)-britanicaflavin A (20), (2*R*,3*S*)-britanicaflavin B (21), (2*S*,3*R*)-britanicaflavin B (22), BBR (23), uncarialin D (24), uncarialin H (25), isocorynoxene (26), villocarin A (27), hirsuteine *N*-oxide (28), cadambine (29), akuammigine (30), dihydrocorynantheine (31), 3 α -dihydrocadambine (32), corynantheine (33), uncarialin C (34), geissoschizine methyl ether *N*-oxide (35), tetrahydroalstonine (36), uncarialin G (37), strictosamide (38), isorhynchophylline (39), hirsutine *N*-oxide (40), vincosamide (41), hirsutine (42), uncarialin B (43), uncarialin E (44), uncarialin F (45), uncarialin A (46), *Z*-geissoschizine (47), corynoxene (48), and uncarialin I (49) (Figure 1) were previously isolated from traditional Chinese medicines by Cheng-Peng Sun, and their structures were determined through NMR and MS spectroscopy. HPLC assays confirmed that the compounds' purity exceeded 98%.

2.2 Cell culture and treatment

RAW264.7 cells were incubated under a 5% CO₂ atmosphere at 37°C, and the culture medium consisted of high-glucose Dulbecco's modified Eagle's medium (Pricella Biotechnology, PM150210) supplemented with 10% v/v fetal bovine serum, penicillin, and streptomycin. Cells were plated at optimal confluency, pre-treated with compounds for 1 h, and then exposed to LPS at a final concentration of 1 µg/mL for 24 h.

2.3 Neutral red screening

For the neutral red phagocytosis experiments, cells were cultured in 96-well plates and treated with the

Short Communication

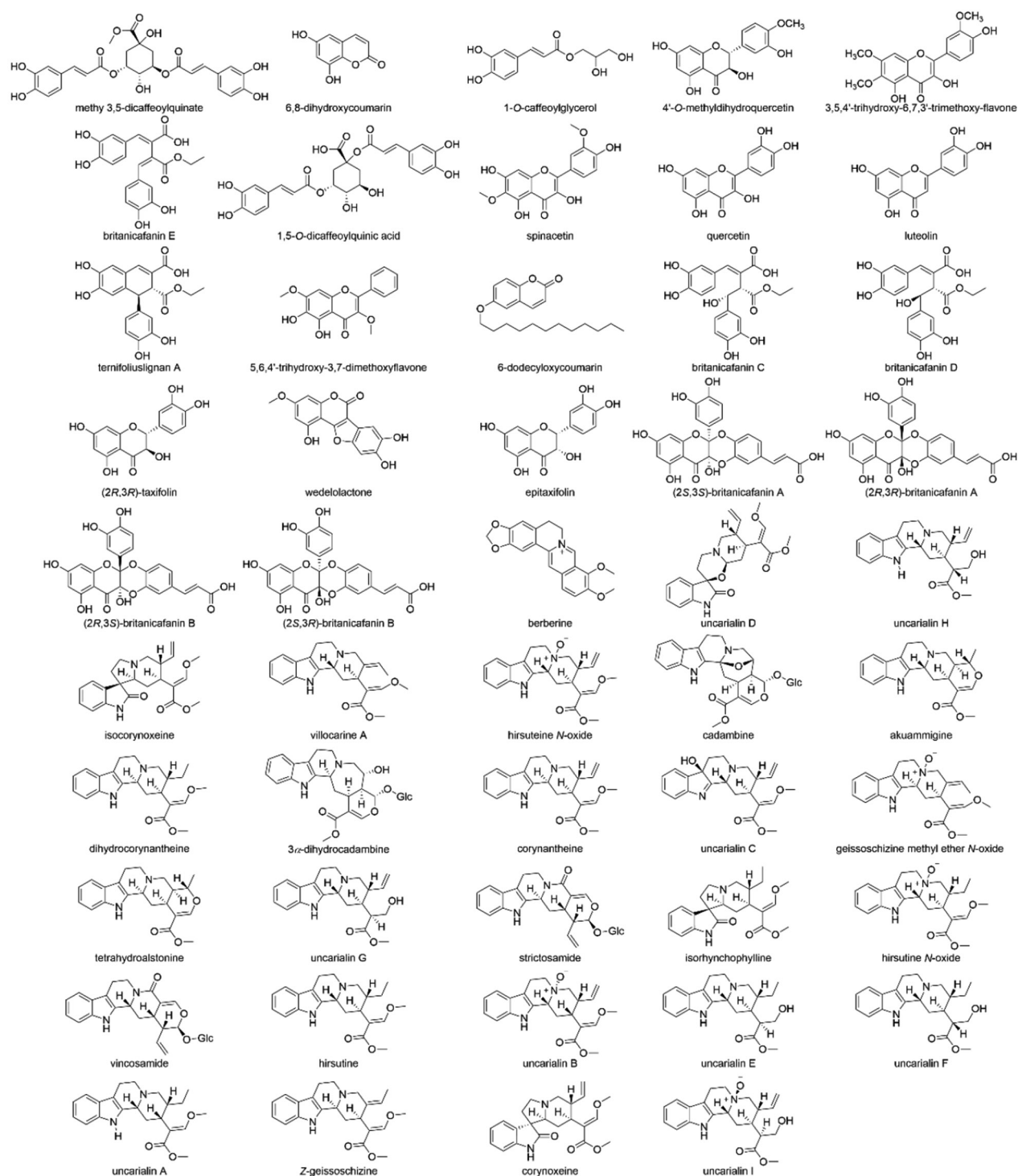


Figure 1 | Structures of 49 natural products.

49 compounds (10 μ M) for 24 h [31]. The medium in each well was discarded, and neutral red solution (0.1%, w/v) was added and incubated for 30

min. Each well was rinsed with PBS three times. The absorbance was recorded at 540 nm with a microplate spectrophotometer.

2.4 Real-time quantitative PCR

RAW264.7 cells were plated into six-well dishes and cultured. The cells were pre-treated with BBR for 1 h (2.5, 5, or 10 μ M), then treated with LPS at 1 μ g/mL for an additional 24 h. Total RNA was extracted from cells with TRIzol reagent (Accurate Biology, AG21101), and reverse-transcribed into cDNA with a cDNA synthesis kit (AB clonal, RK20433). Diluted cDNA was then mixed with primers and 2 $\times$ NovoStan SYBR qPCR SuperMix Plus (Novoprotein, E096-01B), and real-time qPCR was performed on a PCR System (Thermo Fisher Scientific, Quant Studio 1). The relative mRNA expression was determined with the $2^{-\Delta\Delta C_t}$ method [32]. The primers used for RT-PCR were as follows (Table 1).

2.5 Western blotting

Cells were collected, and total protein was extracted with lysis buffer and protease inhibitor cocktail. Protein concentration was quantified with a BCA kit (Genesand, PA101). Equal amounts of proteins were separated with 10% SDS-PAGE, and the target proteins were transferred to polyvinylidene difluoride membranes. After blocking of non-specific proteins with 5% skim milk at room temperature for 2 h, the membranes were incubated with primary antibodies overnight at 4°C. The membranes were incubated with secondary antibodies for 2 h and detected with a chemiluminescence system [33].

2.6 Rhodamine-phalloidin staining

Cells were seeded in 24-well plates (6×10^4 cells/well) and treated with BBR for 24 h, and the supernatant was subsequently discarded. Cells were fixed with 4% para-formaldehyde and then treated with 0.1% Triton X-100 to increase membrane permeability. The cells were washed with PBS and incubated with Actin-tracker-red (Beyotime, C2207S, 1:100) at room temperature, in the dark for 40 min. Subsequently, the cells were washed with PBS and analyzed with a DM6B thunder system [34].

2.7 Flow cytometry

Cells were seeded in six-well plates and divided into a control group, LPS group, and BBR group. Collected cells were stained for CD14 (Biolegend, 123309) and TLR4 (Biolegend, 145403), and CD14-positive cells or TLR4-positive cells were recorded through CytoFlex flow cytometry (Beckman, USA) [32].

2.8 Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (SEM) and were analyzed with one-way ANOVA in GraphPad Prism 9. Probability (p) values <0.05 were considered to indicate significant differences.

3. RESULTS

3.1 BBR inhibits RAW264.7 cell phagocytosis

First, we constructed a natural product library comprising 49 compounds (Figure 1), and assessed their effects

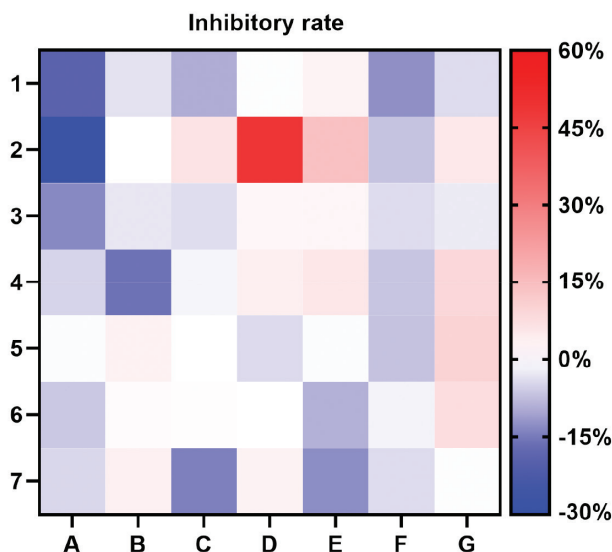


Figure 2 | Inhibitory rates of 49 compounds on phagocytic activity.

on RAW264.7 cell phagocytosis. The inhibitory ratios of 49 compounds are displayed in Figure 2 and Table 2. Macrophages maintain normal physiological function through participating in immune regulation, which plays a crucial role in defense mechanisms. However, excess phagocytosis can damage to normal cells and lead to metabolic disorders. Thus, phagocytosis inhibition plays a pivotal role in inflammatory diseases [35]. Methyl 3,5-dicaffeoylquininate, 6,8-dihydroxy coumarin, 1-O-caffeoyglycerol, ternifoliuslignan, geissoschizine methyl ether *N*-oxide, and tetrahydroalstonine were found to promote phagocytosis by more than 10%, whereas BBR, akuammigine, and Z-geissoschizine inhibited the phagocytic effect by more than 10% (Figure 2). BBR had the most significant inhibitory effect on phagocytosis, achieving an inhibition rate of 48.3% at 10 μ M (Figure 2). Therefore, we focused on BBR in further investigations.

3.2 BBR alleviates inflammation in LPS-induced macrophages

Macrophage phagocytosis, a form of non-specific immunomodulation, is a defense mechanism against potentially pathogenic substances. LPS, the primary molecular component in the outer cell wall in gram-negative bacteria, activates monocyte release of proinflammatory cytokines, which in turn initiate a systemic inflammatory response during the activation of phagocytosis [36–38]. Macrophage phagocytosis blocks timely termination of the inflammatory response and prevents tissue necrosis [39, 40]. To assess the modulatory effects of BBR on macrophage inflammation, we detected levels of various inflammatory factors. As shown in Figure 3, LPS treatment increased the mRNA levels of *IL-1 α* , *IL-1 β* , *TNF- α* ,

Short Communication

Table 1 | Primer sequences.

Primer	Forward	Reverse
Mouse-IL-1 α	ACGTCAAGCAACGGGAAGAT	AAGGTGCTGATCTGGGTTGG
Mouse-IL-1 β	GAAATGCCACCTTTTGACAGTGA	GTCCTCATCCTGGAAGGTCC
Mouse-TNF- α	ATGGCCTCCCTCTCATCAGT	TTTGCTACGACGTGGGCTAC
Mouse-iNOS	GTTTGACCAGAGGCCAGA	GTGAGCTGGTAGGTTCTGT
Mouse-CDC42	AGAAAAGTGGGTGCCTGAGA	GGGCAAGAGCACTCCACATAC
Mouse-ARPC2	TGTGGGTGACAACATTGGCT	GATAGTCCCGGAACGTGTGG
Mouse-WASF	TGCCTAGCGATACAAGCGAG	CCGCTCAGCAAGGGAGTTTA
Mouse-GAPDH	TCAAGATCATCAGCAATGCC	CGATACCAAAGTTGTCATGGA

Table 2 | Names of 49 compounds in the heat map.

Number	Name	Number	Name
A1	Methyl 3,5-dicaffeoylquinic acid	D5	Isocorynoxine
A2	6,8-Dihydroxycoumarin	D6	Villocarin A
A3	1-O-caffeoylglycerol	D7	Hirsutine <i>n</i> -oxide
A4	4'-O methyl dihydroquercetin	E1	Cadambine
A5	3,5,4'-Trihydroxy-6,7,3'-trimethoxy-flavone	E2	Akuammigine
A6	Britanicafanin E	E3	Dihydrocorynantheine
A7	1,5-O-dicaffeoylquinic acid	E4	3 α -Dihydrocadambine
B1	Spinacetin	E5	Corynantheine
B2	Quercetin	E6	Uncaralin C
B3	Luteolin	E7	Geissoschizine methyl ether <i>n</i> -oxide
B4	Ternifoliuslignan A	F1	Tetrahydroalstonine
B5	5,6,4'-Trihydroxy-3,7-dimethoxyflavone	F2	Uncaralin G
B6	6-Dodecyloxy coumarin	F3	Strictosamide
B7	Britanicafanin C	F4	Isorhynchophylline
C1	Britanicafanin D	F5	Hirsutine <i>n</i> -oxide
C2	(2 <i>R</i> ,3 <i>R</i>)-taxifolin	F6	Vincosamide
C3	Wedelolactone	F7	Hirsutine
C4	Epitaxifolin	G1	Uncaralin B
C5	(2 <i>S</i> ,3 <i>S</i>)-britanicafanin A	G2	Uncaralin E
C6	(2 <i>R</i> ,3 <i>R</i>)-britanicafanin A	G3	Uncaralin F
C7	(2 <i>R</i> ,3 <i>S</i>)-britanicafanin B	G4	Uncaralin A
D1	(2 <i>S</i> ,3 <i>R</i>)-britanicafanin B	G5	Z-geissoschizine
D2	Berberine	G6	Corynoxine
D3	Uncaralin D	G7	Uncaralin I
D4	Uncaralin H		

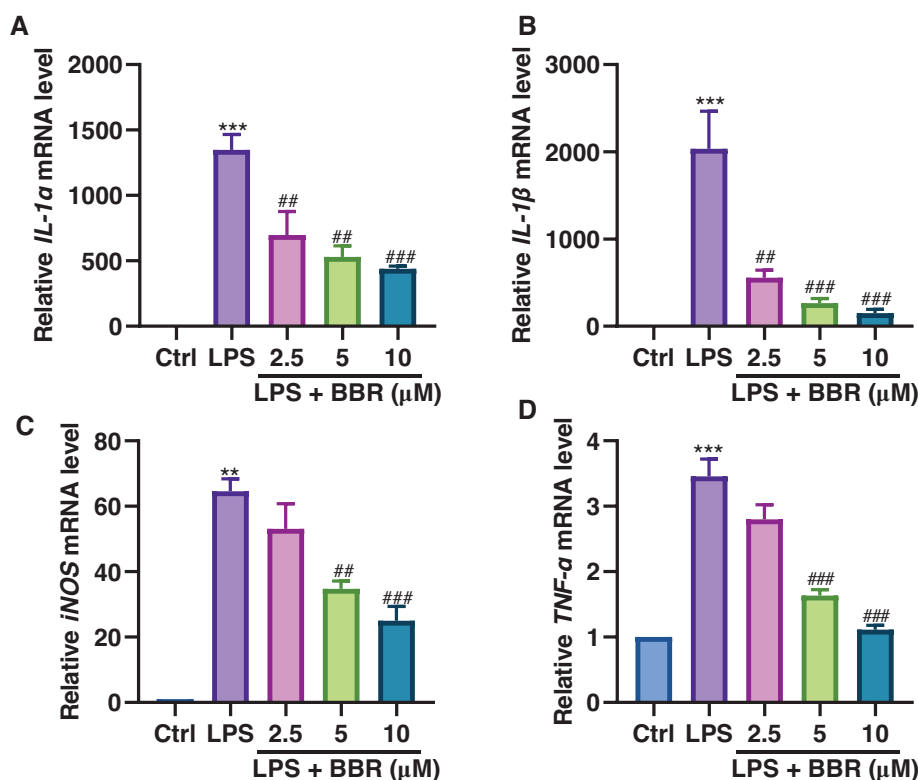


Figure 3 | BBR attenuates inflammation in LPS-induced macrophages.

(A-D) Effects of BBR on mRNA expression of inflammatory *iNOS*, *IL-6*, *IL-1β*, and *TNF-α*. Data are presented as mean ± SEM (n = 3), **p* < 0.05, ***p* < 0.01, and ****p* < 0.001, vs control group; #*p* < 0.05, ##*p* < 0.01, and ###*p* < 0.001, vs LPS group.

and *iNOS* above those in the control group, whereas administration of BBR (2.5, 5, or 10 μM) reversed these changes.

NF-κB signaling is considered a prototypical pathway enhancing the release of IL-1β and TNF-α, and the expression of *iNOS* and COX-2, all of which are important contributors to the inflammatory response [33, 41–44]. Therefore, we investigated the effects of BBR on the expression of proteins associated with the NF-κB pathway after LPS treatment. Western blotting suggested that BBR treatment suppressed the increased expression of COX-2 and *iNOS* as well as the LPS-mediated phosphorylation of p65 (Figure 4A–D). Moreover, confocal microscopy findings supported the observation that LPS stimulation led to translocation of the transcription factor p65 to the nucleus, whereas BBR administration inhibited this effect. The above results demonstrated the anti-inflammatory effects of BBR in vitro.

3.3 BBR inhibits F-actin expression

Phagocytosis, a crucial process for the clearance of pathogens, involves dynamic rearrangement of various cytoskeletal proteins [45]. F-actin, a major cytoskeletal protein with crucial roles in phagocytosis, induces rearrangement and pitting of the cell membrane, and further formation of phagosomes that engulf pathogens.

The synergistic actions of phagocyte receptors and actin polymerization regulators are responsible for the assembly and closure of the phagocytic cup [46, 47].

To determine whether BBR might affect the phagocytic function of macrophages, we detected F-actin by staining with rhodamine-labeled phalloidin. As expected, the fluorescence intensity significantly increased in the LPS group, where more pseudopodia were observed. Administration of BBR markedly decreased the fluorescence intensity and pseudopodia (Figure 5). BBR upregulated F-actin expression, thereby enhancing cell dedifferentiation ability and strengthening membrane-actin cortical adhesion [48]. These results supported that the inhibitory effect of BBR on phagocytosis is mediated by F-actin.

3.4 BBR inhibits phagocytosis through the PI3K-CDC42 pathway

CDC42 and PI3K have been demonstrated to be critical regulators of F-actin, through the modulation of F-actin expression and pseudopodia formation by WASF and ARPC2 [49, 50]. Consequently, we measured proteins in the PI3K signaling pathway and CDC42-related proteins with RT-qPCR and western blotting. The expression of p-PI3K in RAW264.7 cells was significantly greater after LPS stimulation than observed in the control group, and

Short Communication

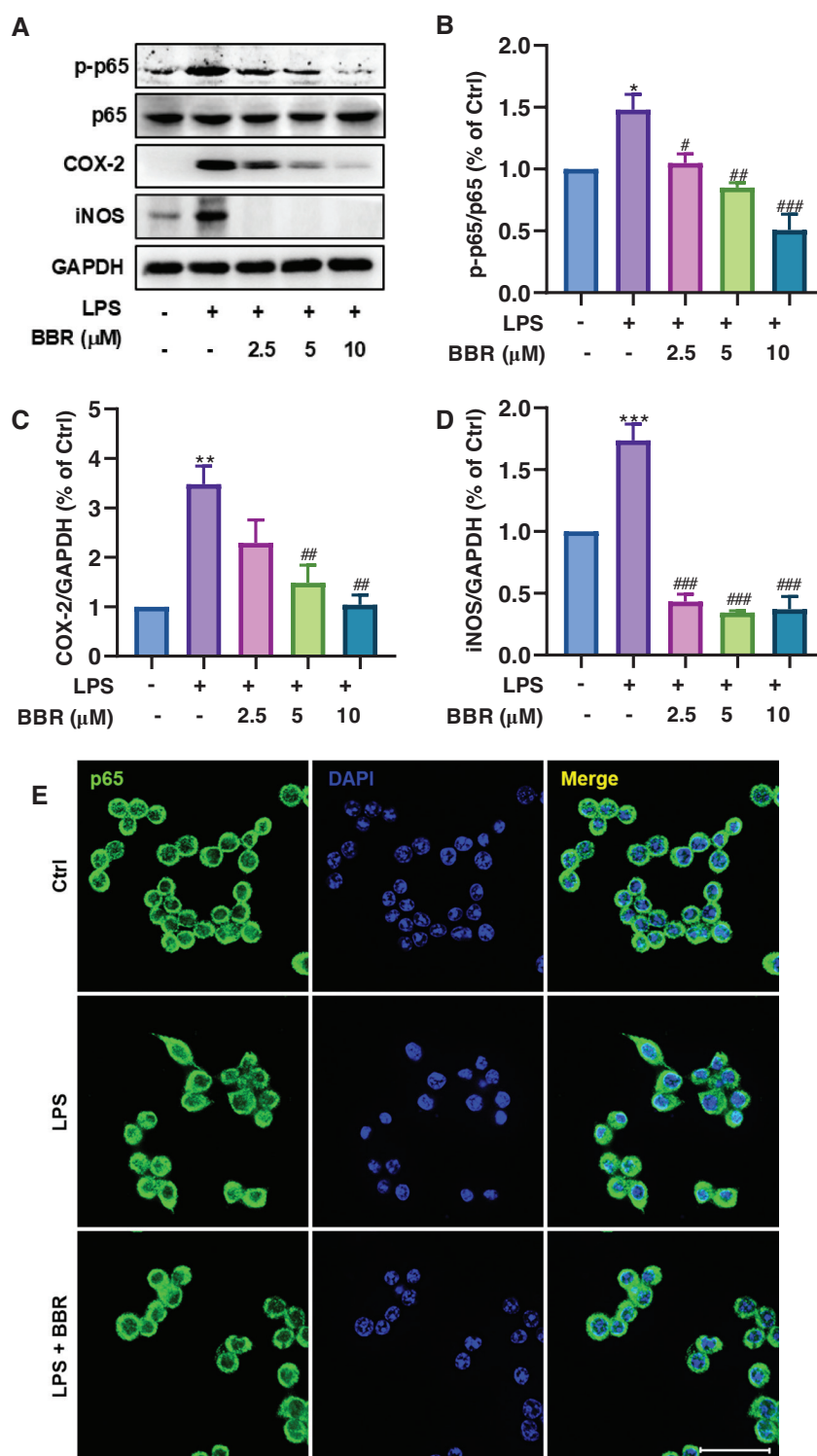


Figure 4 | BBR alleviates the inflammatory response through the NF-κB pathway.

(A) Effects of BBR on the expression of p-p65, p65, COX-2, and iNOS. (B-D) Quantification of p-p65/p65, COX-2, and iNOS. (E) BBR suppresses the nuclear translocation of p65, as indicated by confocal microscopy. Data are presented as mean $\pm$ SEM (n = 3), * p <0.05, ** p <0.01, and *** p <0.001, vs control group; # p <0.05, ## p <0.01, and ### p <0.001, vs LPS group.

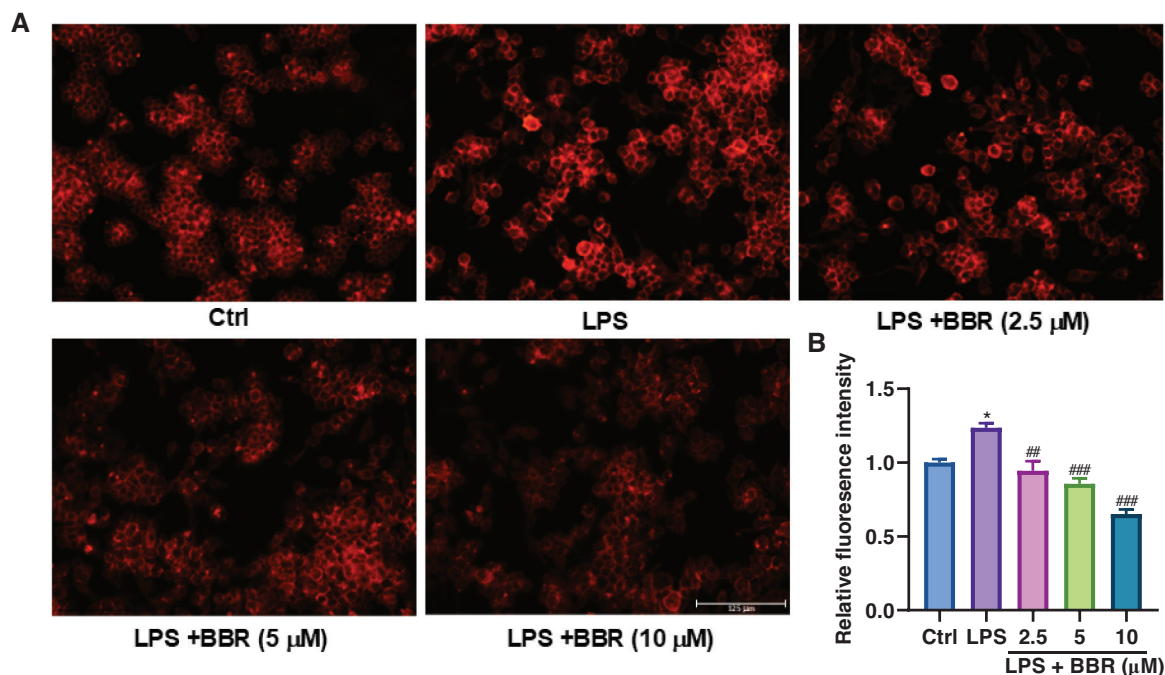


Figure 5 | BBR inhibits expression of the phagocytosis protein F-actin.

(A) F-actin in the indicated groups of cells was stained with rhodamine-phalloidin. (B) Quantification of rhodamine-phalloidin. Data are presented as mean $\pm$ SEM ($n = 3$), * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, vs control group; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$, vs LPS group.

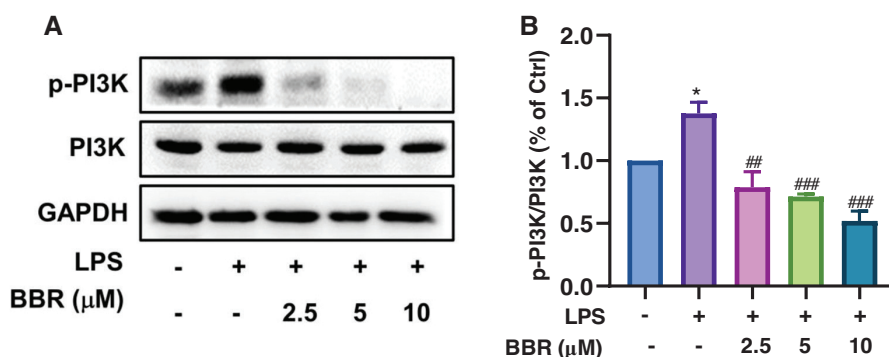


Figure 6 | BBR inhibits PI3K activation in RAW 264.7 cells.

(A) Effects of BBR on the expression of p-PI3K and PI3K in LPS-induced RAW264.7 cells. (B) Quantification of p-PI3K/PI3K. Data are presented as mean $\pm$ SEM ($n = 3$), * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, vs control group; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$, vs LPS group.

BBR decreased p-PI3K expression (Figure 6). In addition, pretreatment with BBR (2.5, 5, or 10 μ M) upregulated the mRNA levels of *CDC42*, *WASF*, and *ARPC2* in LPS-induced macrophages (Figure 7). These results suggested that BBR inhibits the expression of *CDC42*, *WASF*, and *ARPC2*, thereby inhibiting actin expression and pseudopodia production, and ultimately inhibiting phagocytosis. The inhibitory effect of BBR on macrophage phagocytosis is therefore associated with PI3K and *CDC42*.

3.5 BBR inhibits the TLR4 receptor rather than CD14

TLR4 and CD14 activate the PI3K signaling pathway, thereby promoting cell division and proliferation and regulating the production and release of inflammatory cytokines [51, 52]. To identify whether BBR might affect the PI3K signaling pathway via TLR4 or CD14, we conducted flow cytometry experiments. LPS exposure significantly increased the expression of TLR4, whereas BBR (10 μ M) administration suppressed this effect (Figure 8).

Short Communication

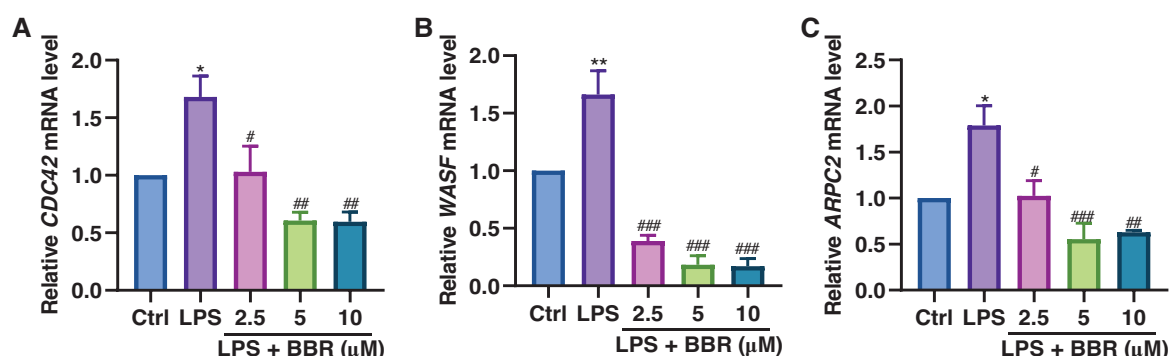


Figure 7 | Effects of BBR on CDC42-related genes.

(A-C) Detection of mRNA levels of CDC42, WASF, and ARPC2 genes by qPCR. Data are presented as mean $\pm$ SEM ($n = 3$), * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, vs control group; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$, vs LPS group.

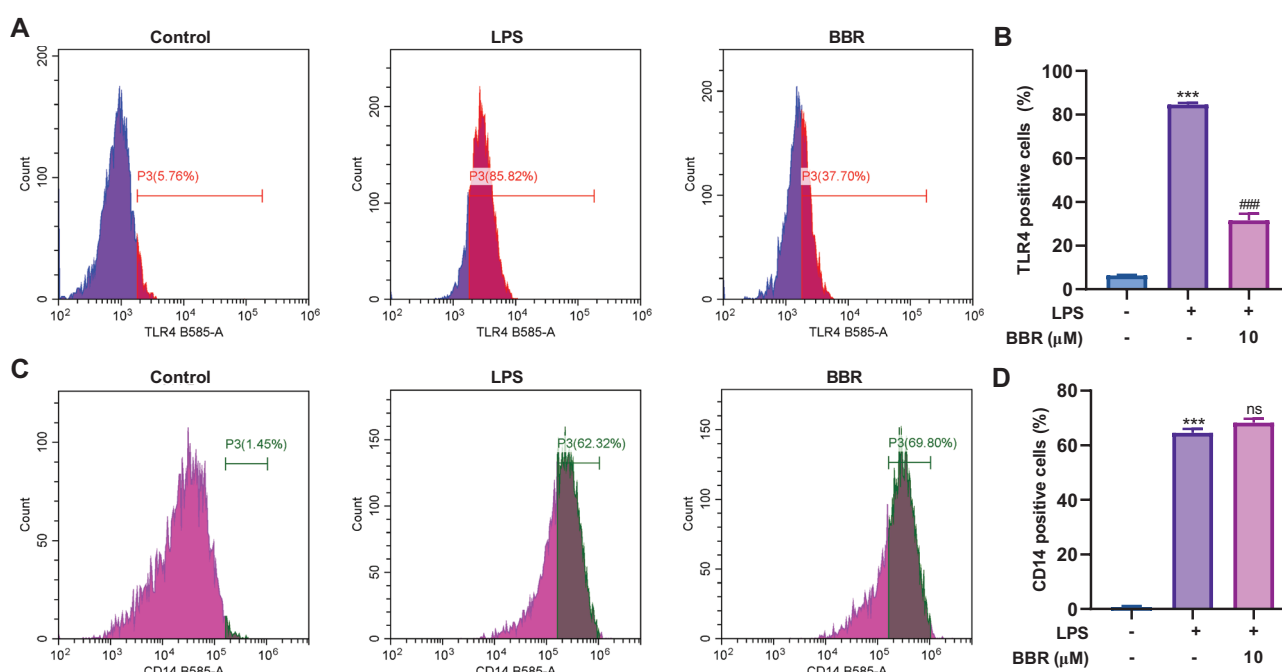


Figure 8 | Effects of BBR on TLR4 and CD14.

(A and B) BBR significantly inhibits TLR4 in LPS-induced macrophages. (C and D) BBR has no significant effect on CD14. All data are presented as mean $\pm$ SEM ($n = 3$), * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, vs control group; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$, vs LPS group; ns = no significance.

After TLR4 activation, endocytosis, mediated by CD14, subsequently led to the activation of transcription regulators such as NF- κ B and IRF3 [53-55]. BBR had no significant effect on the LPS-induced CD14 elevation. Therefore, we hypothesized that BBR inhibits endocytosis through TLR4 rather than CD14.

4. DISCUSSION

Phagocytosis, a fundamental mechanism in both innate and adaptive immunity, plays a critical role in defending

against bacteria, viruses, and fungi. Phagocytosis is a crucial process in mitigating inflammation and eliminating pathogens. Because excessive phagocytosis can disrupt cellular function and metabolism, modulating excessive phagocytosis is a promising therapeutic for maintaining cellular homeostasis [56]. This finding might provide a molecular basis for the use of small molecules in the treatment of autoimmune diseases and inflammatory diseases. Overactivity of microglia in phagocytosis has been reported to lead to loss of synapses within the brain, and to contribute to the onset and exacerbation of

neurodegenerative diseases such as Alzheimer's disease [57]. Loriani et al. have discovered that FcγR-mediated phagocytosis is a key factor leading to immune cytopenias [58]. Therefore, developing small-molecule drugs that block phagocytosis might potentially ameliorate disease progression. In this study, we built a library of 49 compounds, which we screened them for the ability to modulate phagocytosis. Among these compounds, BBR significantly inhibited phagocytosis.

BBR, a bioactive alkaloid with a protoberberine skeleton, is isolated from *Coptis chinensis*. Recently, many studies have reported BBR's remarkable pharmacological effects, including antimicrobial, antidiabetic, anti-inflammatory, anticancer, and anti-oxidant activities [59–64]. Dong and co-workers have reported that BBR controls DSS-induced colon inflammation and maintains the structure and function of the intestinal mucosal barrier through the Wnt/β-catenin pathway [65]. Moreover, BBR effectively resists metabolic disorder and inflammation by decreasing p65^{Lys310} acetylation in peritoneal macrophages and in obese mice [66]. Herein, we found that BBR is an anti-inflammatory compound that effectively inhibits macrophage phagocytosis, by acting through the NF-κB pathway. Although BBR was found to significantly inhibit phagocytosis, with an inhibition rate of 48.3% at 10 μM, its inhibition rates at other concentrations were not measured. BBR plays a crucial role in the remission of inflammatory diseases, yet the mechanism underlying its effects on phagocytosis remains unclear. Phagocytosis is responsible for bacterial invasion, abnormal apoptosis under pathological conditions, and abnormal expression of surface markers. Our neutral red experiments indicated that BBR strongly inhibits phagocytosis. The increased research on phagocytosis is focused on cell surface signal transduction and intracellular activation of signaling pathways [67]. F-actin is involved in the internalization of phagocytic particles and is essential for the movement of phagosomes [68]. Bacterial infection or LPS stimulation triggers activates TLR4, thereby augmenting macrophage phagocytosis; regulating the downstream genes PI3K, CDC42, WASF, and ARPC2; causing F-actin remodeling [52, 69]; and ultimately inhibiting phagocytosis. In this study, BBR reversed the formation of pseudopodia and the aggregation of F-actin induced by LPS, and downregulated the expression of CDC42, WASF, and the PI3K pathway, in agreement with previously reported results [70, 71].

5. CONCLUSION

In summary, BBR was found to block macrophage-mediated phagocytosis and alleviate inflammation through regulating the TLR4-PI3K-CDC42 pathway. These results highlight the therapeutic potential of BBR in the management of inflammatory disorders, such as autoimmune and chronic inflammatory diseases. Consequently, additional studies on its clinical efficacy are warranted.

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CONFLICTS OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

CONSENT TO PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data are available on reasonable request.

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Short Communication

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