Regular Article

Benzoylaconitine Inhibits Production of IL-6 and IL-8 via MAPK, Akt, NF- κ B Signaling in IL-1 β -Induced Human Synovial Cells

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Benzoylaconitine (BAC), the main hydrolysate of aconitine, is a lower toxic monoester type alkaloid considered as the pharmacodynamic constituent in *Aconitum* species. In this study, the effects and mechanisms of BAC on production of inflammatory cytokines interleukin (IL)-6 and IL-8 were investigated in IL-1 β -stimulated human synovial SW982 cells. The SW982 cells were incubated with BAC (0, 5 and 10 μ M) before stimulating with IL-1 β (10 ng/mL). The results revealed that BAC suppressed gene and protein expression of IL-6 and IL-8 induced by IL-1 β . BAC decreased activation of mitogen-activated protein kinase (MAPK) and phosphorylation of Akt. BAC also inhibited degradation of inhibitor of kappaB (I κ B)- α , phosphorylation and nuclear transposition of p65 protein. The results demonstrate that BAC exerts an anti-inflammatory effect dependent on MAPK, Akt and nuclear factor- κ B (NF- κ B) pathways in human synovial cells stimulated with IL-1 β , suggesting that BAC may be exploited as a potential therapeutic agent for rheumatoid arthritis (RA) treatment.

Key words benzoylaconitine; cytokine; signaling pathway; synovial cell

INTRODUCTION

Aconitum species are widely used for the treatment of syncope, rheumatoid fever, painful joints, bronchial asthma, gastroenteritis and other diseases in China, Japan and a great deal of Asian countries for thousands of years.^{1,2} *Aconitum* medicines are also decidedly noxious, and diester-diterpene alkaloids, such as aconitine, are the main noxious components. The roots of *Aconitum* herbs are commonly applied after processing that diester alkaloids are mainly hydrolyzed to lower toxic monoester alkaloids.^{3,4} Benzoylaconitine (BAC; C₃₂H₄₅NO₁₀, molecular weight = 603.705) is a monoester alkaloid (Fig. 1A) and is the main hydrolysate of aconitine. Its toxicity is much lower than aconitine, which is considered as one of the pharmacodynamic constituents in *Aconitum* species.^{5,6}

Rheumatoid arthritis (RA) is a long-lasting, systemic, inflammatory disorder that influences synovial joints in a symmetric pattern leading to joint destruction and deformity. RA usually does not directly lead to death but critically affects the life expectancy and quality of patients if untreated.⁷ The traditional *Aconitum* Chinese medicines including *Aconitum kusnezoffii* Reichb. and *Aconitum carmichaeli* Debx. are frequently used for RA in China. However, the effects and mechanisms of BAC on RA are still largely unknown.

The pathological characterization of RA is hyperplasia of synovial fibroblasts (SFs), soakage of interstitial inflammatory cells, formation of pannus and destruction of skeleton.^{8,9} SFs produce a great deal of pro-inflammatory factors, such as interleukin (IL)-1 β , IL-6, IL-8, which induce articular chondrocytes apoptosis and cartilage degeneration leading to joint destruction.^{10–12} Mitogen-activated protein kinase

(MAPK), phosphatidylinositol 3-kinase (PI3K)-Akt and nuclear factor- κ B (NF- κ B) are crucial signaling cascades that modulate the biosynthesis and activation of inflammatory cytokines and other mediators involved in RA.^{13–15} Reducing SFs inflammation is an important strategy for treatment of RA. Nevertheless, the anti-inflammatory effects of BAC on RA synovial inflammation remains unclear.

The human synovial sarcoma cell SW982 is a frequently-used tool to investigate SFs inflammation of RA.¹⁶ In this present research, we evaluated the role of BAC in IL-1 β -mediated inflammatory response in SW982 cells. In addition, we investigated the potential anti-inflammatory mechanisms of BAC.

MATERIALS AND METHODS

Materials BAC (HPLC $\geq$ 98%, CAS No. 466-24-0) was obtained from Chengdu Must Bio-Technology Co., Ltd. (Chengdu, China). Interleukin-1 β was purchased from PeproTech (Rocky Hill, NJ, U.S.A.). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), streptomycin and penicillin were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Fetal bovine serum (FBS) was purchased from Gibco (Grand Island, NY, U.S.A.). Other chemicals were purchased from Sigma-Aldrich unless otherwise stated.

Cell Cultures The human synovial sarcoma cell line SW982, originally purchased from American Type Culture Collection (Rockville, MD, U.S.A.), was maintained in incubator with 5% CO₂ at 37°C and was cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Thermo Fisher Scientific Inc., Logan, UT, U.S.A.) supplemented with 10% FBS,

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Fig. 1. Chemical Structure of BAC (A) and Effects of BAC on Cell Vitality in SW982 Cells (B)

Cells were treated with different doses of BAC for 48h, the cell vitality was measured by MTT. The data are presented as mean $\pm$ standard deviation (S.D.) of three independent experiments.

Table 1. Antibodies Used in Western Blot Assays

Antibody	Host	Dilution	Source
p-ERK	Rabbit	1:1000	Cell Signaling Technology
p-JNK	Mouse	1:1000	Cell Signaling Technology
p38	Rabbit	1:1000	Cell Signaling Technology
p-p38	Rabbit	1:1000	Cell Signaling Technology
Akt	Rabbit	1:1000	Cell Signaling Technology
p-Akt	Mouse	1:1000	Cell Signaling Technology
I κ B- α	Mouse	1:1000	Cell Signaling Technology
p-p65	Rabbit	1:1000	Cell Signaling Technology
NF- κ B (p65)	Rabbit	1:1000	Cell Signaling Technology
β -Actin	Mouse	1:2000	Cell Signaling Technology
Lamin B	Mouse	1:2000	Cell Signaling Technology
JNK2	Rabbit	1:1000	Santa Cruz Biotechnology
ERK2	Rabbit	1:1000	Proteintech
Anti-mouse		1:5000	Cell Signaling Technology
Anti-rabbit		1:5000	Cell Signaling Technology

Table 2. Primer Sequences Used for Real-Time PCR

Gene	Primer	Sequence (5'→3')
IL-6	Forward	TACATCCTCGACGGCATCTCA
	Reverse	CACCAGGCAAGTCTCCTCATTG
IL-8	Forward	TCTTGGCAGCCTTCCTGATT
	Reverse	TGGTCCACTCTCAATCACTCTCAGT
GAPDH	Forward	TGCCCTCAACGACCACTTTG
	Reverse	TACTCCTTGGAGGCCATGTG

ern blot assay was performed as previously described.¹⁷⁾ The antibodies for Western blot were used as follows Table 1. Photographs were quantified by measuring the immunoreactive protein band density with software ImageJ.

Real-Time PCR After incubation, total RNA was isolated by TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer's instructions, and then reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit. Real-time quantitative PCR was performed using SYBR Green real-time PCR analysis (KAPA Biosystems, MA, U.S.A.) on ABI Prism 7500 Sequence detection system with the specific primers (Table 2). PCR results, recorded as cycle threshold (Ct), were normalized against an internal control (glyceraldehyde-3-phosphate dehydrogenase (GAPDH)).

Statistical Analysis All data were analyzed with GraphPad Prism 5 software using one-way ANOVA to analyze the multiple groups and $p < 0.05$ was considered to be statistical significance. Data were presented as mean $\pm$ standard deviation (S.D.).

RESULTS

BAC Is Nontoxic to SW982 Cells at Concentrations Up to 50 μ M Before examining the effects of BAC, its potential cytotoxicity on SW982 cells was detected with MTT assay. The cells were disposed with different concentrations of BAC (0, 0.1, 1, 2, 5, 10, 20, 50 μ M) for 48h, then the cell viability was examined by MTT assay. The results showed that BAC had no cytotoxic effects at the concentrations up to 50 μ M (Fig. 1B).

BAC Inhibits the Production of IL-6 and IL-8 SW982 cells were treated with various concentrations (0, 5 and 10 μ M)

100 μ g/mL streptomycin and 100 U/mL penicillin.

BAC was dissolved with DMSO for *in vitro* experiments, the final concentration of DMSO added to cells was $<0.1\%$.

Cell Vitality Assay The cell viability was assessed with MTT assay. 1×10^4 SW982 cells were seeded into 96-well plates and incubated overnight. After washing with fresh medium thrice, SW982 cells were treated with BAC for 48h in absence of FBS. At the indicated time, 1 mg/mL MTT was added and incubated for 4h at 37°C, then the medium was removed and the cells were dissolved in DMSO. The absorbance at 570nm was measured using a microplate reader (Molecular Devices).

Enzyme-Linked Immunosorbent Assay (ELISA) The SW982 cells were pretreated with various doses (0, 5 and 10 μ M) of BAC for 30min, followed by IL-1 β stimulation (10ng/mL) for 12h. The levels of IL-6 and IL-8 in supernatants of cell cultures were determined by ELISA kits (Pierce Endogen, Rockford, IL, U.S.A.) according to the manufacturer's recommendations.

Western Blot SW982 cells were seeded into 6-well plates. After incubation with various doses (0, 5 and 10 μ M) of BAC and IL-1 β , total cells were washed twice with ice-cold PBS and then lysed in ice-cold lysis buffer (Sigma-Aldrich). Lysates were centrifuged at $15000 \times g$ for 15min at 4°C. West-

Fig. 2. Effects of BAC on IL-1 β -Induced Production of IL-6 and IL-8 in SW982 Cells

Cells were pretreated with various concentrations (0, 5 and 10 μ M) of BAC for 30 min and then stimulated with IL-1 β (10 ng/mL) for 12 h. The supernatants were collected and the levels of IL-6 (A), IL-8 (B) were measured by ELISA. The data are presented as mean $\pm$ S.D. of three independent experiments. * p < 0.05, ** p < 0.01, *** p < 0.001 vs. IL-1 β without BAC.

Fig. 3. Effects of BAC on IL-1 β -Induced mRNA Expression of IL-6 and IL-8

SW982 cells were pretreated with various concentrations (0, 5 and 10 μ M) of BAC for 30 min and then stimulated with IL-1 β (10 ng/mL) for 6 h. The total RNA was collected and the mRNA levels of IL-6 (A), IL-8 (B) were determined by real-time PCR. Relative gene-expression levels are expressed with GAPDH as an internal reference. The results are expressed as the mean $\pm$ S.D. of three independent experiments. * p < 0.05, ** p < 0.01, *** p < 0.001 vs. IL-1 β without BAC.

of BAC for 30 min before incubation with 10 ng/mL IL-1 β for 12 h. The supernates of cell culture were collected and the levels of IL-6 and IL-8 were measured by ELISA. After IL-1 β stimulation, the levels of IL-6 and IL-8 were significantly increased. However, BAC treatment decreased the production of IL-6 and IL-8 in IL-1 β -induced SW982 cells (Figs. 2A, B).

BAC Suppresses the Gene Expression of IL-6 and IL-8 After pre-treated with different doses (0, 5 and 10 μ M) of BAC for 30 min, SW982 cells were stimulated with IL-1 β (10 ng/mL) for 6 h. Total RNA was collected and the mRNA levels were detected by quantitative real-time PCR. As shown in Fig. 3, IL-1 β obviously increased the mRNA expression of IL-6 and IL-8. BAC treatment suppressed the levels of IL-6 and IL-8 expression in IL-1 β -stimulated SW982 cells.

BAC Inhibits Activation of MAPK Signaling Pathways MAPK signaling pathways, including extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK) and p38, play a crucial role in regulation of inflammation mediators.¹⁸⁾ Pre-treating with IL-1 β triggered MAPKs signaling activation in SW982 cells. However, BAC significantly inhibited the IL-1 β -induced phosphorylation of ERK, JNK and p38 (Figs. 4A–D). These results indicate that BAC may suppress the expression of IL-6 and IL-8 by inhibiting MAPK signaling pathways in IL-1 β -stimulated SW982 cells.

BAC Inhibits the Activation in IL-1 β -Mediated Akt

Signaling Pathway The PI3K–Akt signaling pathway is a crucial intracellular transduction pathway and has previously been demonstrated to be connected with the deterioration of RA.¹⁹⁾ We investigated the effects of BAC on PI3K–Akt signaling by measuring the phosphorylation of the key molecule Akt using Western blotting. As shown in Figs. 5A and B, an increased phosphorylation of Akt was observed in IL-1 β -induced SW982 cells. In contrast, the level of phospho-Akt was decreased in BAC-treated cells. The results suggest that BAC may inhibit IL-1 β -stimulated secretion of IL-6 and IL-8 by blocking Akt signaling pathway.

BAC Inhibits IL-1 β -Mediated NF- κ B Activation The NF- κ B pathway is a crucial pathway in the regulation of the inflammatory cytokine in RA.²⁰⁾ To explore the anti-inflammatory mechanisms of BAC in IL-1 β -mediated SW982 cells, we examined the effects of BAC on the activation of NF- κ B. As a result of BAC treatment, the degradation of inhibitor of kappaB (I κ B)- α (Figs. 6A, C) and phosphorylation of p65 (Figs. 6A, D) were significantly decreased as compared to that in IL-1 β -stimulated cells. Furthermore, the level of intranuclear NF- κ B p65 was detected in nuclear extracts using Western blotting. As shown in Figs. 6B and E, IL-1 β -induced nucleus translocation of p65 was largely blocked by BAC. These results indicate that NF- κ B signaling is involved in the anti-inflammatory effects of BAC in IL-1 β -stimulated SW982

Fig. 4. Effects of BAC on Phosphorylation of ERK, JNK and p38 in IL-1 β -Induced SW982 Cells

(A) The cells were pretreated with different doses (0, 5 and 10 μ M) of BAC for 30 min before IL-1 β (10 ng/mL) stimulation for 30 min. The level of phosphorylated MAPK was analysed by Western blot, and β -actin was used as loading control. Histogram analysis of the levels of p-ERK (B), p-p38 (C) and p-JNK (D). $n=3$, $x \pm s$. ** $p < 0.01$, *** $p < 0.001$ vs. IL-1 β without BAC.

Fig. 5. Effects of BAC on IL-1 β -Induced Activation of Akt in SW982 Cells

(A) The cells were incubated with BAC for 30 min, followed by IL-1 β (10 ng/mL) stimulation for 30 min. Western blot was performed using specific phospho-Akt and Akt antibodies. (B) Histogram analysis for the expression levels of phospho-Akt. Actin was used as an internal control. The data are presented as mean $\pm$ S.D. of three independent experiments. ** $p < 0.01$, *** $p < 0.001$ vs. IL-1 β without BAC.

cells.

DISCUSSION

Some of Traditional Chinese medicines (TCM) prescriptions have been utilizing for treatment of RA for thousands of years in China. As is recording in Treatise on Febrile and Miscellaneous Diseases, Aconite Decoction is a typical prescription to cure RA, consisting of the root of *Aconitum* species.²¹⁾ BAC is one of effective constituents of *Aconitum* herbs and has pharmacological anti-inflammatory effects, but the potential function of BAC on RA remains unclear. In this study, we demonstrated that BAC inhibited IL-1 β -induced production of IL-6 and IL-8 dependent on MAPK, Akt and

NF- κ B signaling pathways in human synovial cells.

IL-1 β is a significant molecule in sensitization of synovio-cyte and release of inflammatory mediators, such as IL-6 and IL-8, in the pathogenesis of RA.²²⁾ The pro-inflammatory cytokine IL-6 is discovered in the blood and SFs of RA patients relates with chronic inflammation and joint destruction owing to the neutrophil secretion of reactive oxygen intermediates and proteolytic enzymes. IL-6 is persistently generated by immunocyte, SFs, and many other cells in the pathogenesis of RA.^{23,24)} With the chemoattractant properties of neutrophils, monocytes, macrophages, IL-8 is one of critical chemokines in the deteriorating procedure of RA.^{25,26)} In this study, our data report the first experimental evidence for inhibitory effects of BAC on production of pro-inflammatory cytokines

Fig. 6. Effects of BAC on IL-1 β -Induced Activation of NF- κ B in SW982 Cells

The cells were treated with various doses (0, 5, and 10 μ M) of BAC for 30 min before IL-1 β (10 ng/mL) stimulation for 5 min. Western blot was performed using specific cytoplasmic I κ B- α , phospho-p65, total p65 (A) and intranuclear p65 (B). Histogram analysis of the levels of I κ B- α (C), phospho-p65 (D) and intranuclear p65 (E). Actin and lamin B were used as internal controls. The data are presented as mean $\pm$ S.D. of three independent experiments. ** p < 0.01, *** p < 0.001 vs. IL-1 β without BAC.

IL-6 and IL-8 in IL-1 β -induced human synovial cells, suggesting that BAC plays a protective role against RA.

MAPKs are relative to the expression of IL-1 β -induced inflammatory mediators and the deterioration of joints in RA.²⁷⁾ When irritated with relevant pro-inflammatory mediators, MAPKs in turn activate various genes encoding inflammatory factors.²⁸⁾ Three main MAPK pathways were found in mammals: ERK, JNK and p38 MAPK. The present results showed that BAC suppressed the phosphorylation of ERK, JNK and p38 irritated by IL-1 β , which suggests that BAC may inhibit pro-inflammatory cytokines expression through suppression of MAPK pathways.

The PI3K-Akt is a significant pathway involved in the expression of inflammatory mediators and the destruction of joints in the pathogenesis of RA.^{29,30)} Akt signaling pathway is reported to be relevant with the aberrant multiplication of synovial fibroblasts and the regulation of IL-6 in RA.¹³⁾ NF- κ B is a crucially transcriptional factor regulating the production of cytokines leading to joint inflammation in SFs of RA.^{31,32)} In this study, we demonstrated that BAC obviously reduced Akt phosphorylation and degradation, blocked p65 migration into the nucleus in IL-1 β -mediated SW982 cells. These results indicate that Akt and NF- κ B pathways may be involved in the anti-inflammatory effects of BAC on IL-6 and IL-8 release in SW982 cells induced by IL-1 β .

CONCLUSION

In summary, we demonstrated that BAC suppressed IL-1 β -induced expression of IL-6 and IL-8 *via* inhibition the activation of MAPK (ERK, JNK, and p38), Akt, and NF- κ B pathways in SW982 human synovial sarcoma cells. These findings suggested that BAC may be potentially utilized as a

novel therapeutic agent for treatment of synovial inflammation in RA. Further fundamental and clinical studies are needed to verify the anti-inflammatory role of BAC *in vivo* and evaluate its potential toxicity.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

- 1) Singhuber J, Zhu M, Prinz S, Kopp B. Aconitum in traditional Chinese medicine: a valuable drug or an unpredictable risk? *J. Ethnopharmacol.*, **126**, 18–30 (2009).
- 2) Zhang P, Kong D, Du Q, Zhao J, Li Q, Zhang J, Li T, Ren L. A conscious rat model involving bradycardia and hypotension after oral administration: a toxicokinetic study of aconitine. *Xenobiotica*, **47**, 515–525 (2017).
- 3) Chen JH, Lee CY, Liao BC, Lee MR, Jong TT, Chiang ST. Determination of aconitine-type alkaloids as markers in fuzi (*Aconitum carmichaeli*) by LC/ (+) ESI/MS (3). *J. Pharm. Biomed. Anal.*, **48**, 1105–1111 (2008).
- 4) Wang Y, Shi L, Song F, Liu Z, Liu S. Exploring the ester-exchange reactions of diester-diterpenoid alkaloids in the aconite decoction process by electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.*, **17**, 279–284 (2003).
- 5) Liu CC, Xu YH, Yuan S, Xu Y, Hua ML. An enzyme-linked immunosorbent assay for monoester-type aconitic alkaloids and its

- application in the pharmacokinetic study of benzoylhyaconine in rats. *J. Asian Nat. Prod. Res.*, **20**, 352–360 (2018).
- 6) Wada K, Nihira M, Hayakawa H, Tomita Y, Hayashida M, Ohno Y. Effects of long-term administrations of aconitine on electrocardiogram and tissue concentrations of aconitine and its metabolites in mice. *Forensic Sci. Int.*, **148**, 21–29 (2005).
 - 7) Yap HY, Tee SZ, Wong MM, Chow SK, Peh SC, Teow SY. Pathogenic role of immune cells in rheumatoid arthritis: implications in clinical treatment and biomarker development. *Cells*, **7**, 161 (2018).
 - 8) Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet*, **376**, 1094–1108 (2010).
 - 9) Zhao J, Liu T, Xu F, You S, Xu F, Li C, Gu Z. Anti-arthritis effects of total flavonoids from *Juniperus sabina* on complete freund's adjuvant induced arthritis in rats. *Pharmacogn. Mag.*, **12**, 178–183 (2016).
 - 10) Cooles FA, Isaacs JD. Pathophysiology of rheumatoid arthritis. *Curr. Opin. Rheumatol.*, **23**, 233–240 (2011).
 - 11) Kloesch B, Becker T, Dietersdorfer E, Kiener H, Steiner G. Anti-inflammatory and apoptotic effects of the polyphenol curcumin on human fibroblast-like synoviocytes. *Int. Immunopharmacol.*, **15**, 400–405 (2013).
 - 12) Mateen S, Zafar A, Moin S, Khan AQ, Zubair S. Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. *Clin. Chim. Acta*, **455**, 161–171 (2016).
 - 13) Malemud CJ. The PI3K/Akt/PTEN/mTOR pathway: a fruitful target for inducing cell death in rheumatoid arthritis? *Future Med. Chem.*, **7**, 1137–1147 (2015).
 - 14) Simmonds RE, Foxwell BM. Signalling, inflammation and arthritis: NF-kappaB and its relevance to arthritis and inflammation. *Rheumatology*, **47**, 584–590 (2008).
 - 15) Thalhamer T, McGrath MA, Harnett MM. MAPKs and their relevance to arthritis and inflammation. *Rheumatology*, **47**, 409–414 (2008).
 - 16) Yamazaki T, Yokoyama T, Akatsu H, Tukiya T, Tokiwa T. Phenotypic characterization of a human synovial sarcoma cell line, SW982, and its response to dexamethasone. *In Vitro Cell. Dev. Biol. Anim.*, **39**, 337–339 (2003).
 - 17) Cheng BF, Gao YX, Lian JJ, Guo DD, Wang L, Wang M, Yang HJ, Feng ZW. Hydroxysafflor yellow A inhibits IL-1beta-induced release of IL-6, IL-8, and MMP-1 via suppression of ERK, NF-kappaB and AP-1 signaling in SW982 human synovial cells. *Food Funct.*, **7**, 4516–4522 (2016).
 - 18) Kaminska B. MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits. *Biochim. Biophys. Acta*, **175**, 253–262 (2005).
 - 19) Yuan H, Yang P, Zhou D, Gao W, Qiu Z, Fang F, Ding S, Xiao W. Knockdown of sphingosine kinase 1 inhibits the migration and invasion of human rheumatoid arthritis fibroblast-like synoviocytes by down-regulating the PI3K/AKT activation and MMP-2/9 production *in vitro*. *Mol. Biol. Rep.*, **41**, 5157–5165 (2014).
 - 20) Xu H, He Y, Yang X, Liang L, Zhan Z, Ye Y, Yang X, Lian F, Sun L. Anti-malarial agent artesunate inhibits TNF-alpha-induced production of proinflammatory cytokines via inhibition of NF-kappaB and PI3 kinase/Akt signal pathway in human rheumatoid arthritis fibroblast-like synoviocytes. *Rheumatology*, **46**, 920–926 (2007).
 - 21) Lü S, Wang Q, Li G, Sun S, Guo Y, Kuang H. The treatment of rheumatoid arthritis using Chinese medicinal plants: from pharmacology to potential molecular mechanisms. *J. Ethnopharmacol.*, **176**, 177–206 (2015).
 - 22) Alam J, Jantan I, Bukhari SNA. Rheumatoid arthritis: recent advances on its etiology, role of cytokines and pharmacotherapy. *Biomed. Pharmacother.*, **92**, 615–633 (2017).
 - 23) Yoshida Y, Tanaka T. Interleukin 6 and rheumatoid arthritis. *Biomed. Res. Int.*, **2014**, 698313 (2014).
 - 24) Dayer JM, Choy E. Therapeutic targets in rheumatoid arthritis: the interleukin-6 receptor. *Rheumatology*, **49**, 15–24 (2010).
 - 25) Szekecz Z, Koch AE. Successes and failures of chemokine-pathway targeting in rheumatoid arthritis. *Nat. Rev. Rheumatol.*, **12**, 5–13 (2016).
 - 26) Vergunst CE, Tak PP. Chemokines: their role in rheumatoid arthritis. *Curr. Rheumatol. Rep.*, **7**, 382–388 (2005).
 - 27) Barchowsky A, Frelto D, Vincenti MP. Integration of the NF-kappaB and mitogen-activated protein kinase/AP-1 pathways at the collagenase-1 promoter: divergence of IL-1 and TNF-dependent signal transduction in rabbit primary synovial fibroblasts. *Cytokine*, **12**, 1469–1479 (2000).
 - 28) Guha M, Mackman N. LPS induction of gene expression in human monocytes. *Cell. Signal.*, **13**, 85–94 (2001).
 - 29) Wu T, Mohan C. The AKT axis as a therapeutic target in autoimmune diseases. *Endocr. Metab. Immune Disord. Drug Targets*, **9**, 145–150 (2009).
 - 30) Cohen S, Fleischmann R. Kinase inhibitors: a new approach to rheumatoid arthritis treatment. *Curr. Opin. Rheumatol.*, **22**, 330–335 (2010).
 - 31) Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu. Rev. Immunol.*, **27**, 693–733 (2009).
 - 32) Hah YS, Lee YR, Jun JS, Lim HS, Kim HO, Jeong YG, Hur GM, Lee SY, Chung MJ, Park JW, Lee SI, Park BH. A20 suppresses inflammatory responses and bone destruction in human fibroblast-like synoviocytes and in mice with collagen-induced arthritis. *Arthritis Rheum.*, **62**, 2313–2321 (2010).