Niloticin binds to MD-2 to promote anti-inflammatory pathway activation in macrophage cells

Guirong Chen¹,², Chang Liu², Mingbo Zhang², Xiaobo Wang¹ and Yubin Xu³

Abstract

Objectives: Niloticin is an active compound isolated from Cortex phellodendri with uncharacterized anti-inflammatory activity. We assessed the drug potential of niloticin and examined its ability to target myeloid differentiation protein 2 (MD-2) to ascertain the mechanism for its anti-inflammatory activity.

Methods: The Traditional Chinese Medicine Systems Pharmacology Database was used to evaluate niloticin. Bio-layer interferometry and molecular docking technologies were used to explore how niloticin targets MD-2, which mediates a series of toll-like receptor 4 (TLR4)-dependent inflammatory responses. The cytokines involved in the lipopolysaccharide (LPS)-TLR4/MD-2-NF-κB pathway were evaluated using ELISA, RT-qPCR, and western blotting.

Results: Niloticin could bind to MD-2 and had no evident effects on cell viability. Niloticin treatment significantly decreased the levels of NO, IL-6, TNF-α, and IL-1β induced by LPS (p < 0.01). IL-1β, IL-6, iNOS, TNF-α, and COX-2 mRNA expression levels were decreased by niloticin (all p < 0.01). Compared with that in the control group, the increase in TLR4, p65, MyD88, p-p65, and iNOS expression levels induced by LPS were suppressed by niloticin (all p < 0.01).

Conclusion: Our results suggest that niloticin has therapeutic potential and binds to MD-2. Niloticin binding to MD-2 antagonized the effects of LPS binding to the TLR4/MD-2 complex, resulting in the inhibition of the LPS-TLR4/MD-2-NF-κB signaling pathway.

Keywords
niloticin, anti-inflammatory activity, myeloid differentiation protein 2, mechanism

Date received: 19 October 2021; revised: 28 September 2022; accepted: 24 August 2022

Introduction

Bacterial infections constitute a major threat to human health. Bacteria can enter the body through damaged skin and mucous membranes, triggering specific defense responses by the immune system essential to ensure human survival. However, severe systemic infections such as sepsis induce an excessive or uncontrolled immune response that can lead to systemic inflammatory response syndrome (SIRS), which is a life-threatening condition that
promotes the development of multiple organ dysfunction syndrome (MODS).\textsuperscript{1,2}

Lipopolysaccharide (LPS) recognition by the host is crucial for defense response to bacterial infections.\textsuperscript{3} LPS action on the membrane CD14 receptors of monocytes and macrophages requires the LPS-binding protein (LBP), toll-like receptor (TLR)\textsubscript{4}, and myeloid differentiation protein 2 (MD-2) for signal transduction.\textsuperscript{4-6} A previous study found that the immune cells have an absent or weak response to LPS when TLR4 is present, indicating that TLR4 recognizes LPS only when associated with MD-2.\textsuperscript{7} In addition, the LPS response is blocked more efficiently by the inhibition of MD-2 than TLR4/MD-2.\textsuperscript{8} Therefore, drugs that intervene with the binding of LPS to MD-2 or suppress the interaction between MD-2 and TLR4 may constitute effective treatment strategies to control the inflammatory and excessive immune responses caused by LPS.

Natural products used in traditional Chinese medicine (TCM) are structurally and chemically diverse and constitute a valuable source of active compounds for drug discovery.\textsuperscript{9,10} Cortex phellodendri is a plant used in TCM that exhibits a broad range of pharmacological activities including anti-inflammatory, anti-apoptotic, anti-tumor, and anti-oxidative properties.\textsuperscript{11-14} Niloticin (Figure 1(a)), one of the active compounds found in Cortex phellodendri, has good drug potential, high oral bioavailability (OB), and drug-likeness (DL).\textsuperscript{15} Several studies have reported the anti-dengue, antioxidant, and anti-respiratory syncytial virus activity properties of niloticin, but its anti-inflammatory activity has not been fully explored.\textsuperscript{15-17} Thus, the aim of this study was to evaluate the drug potential of niloticin and investigate the MD-2-targeting mechanism for its anti-inflammatory activity. Our results suggest that niloticin has therapeutic potential and lay the foundation for the development of niloticin as a new drug against SIRS.

**Materials and methods**

**Drug potential assessment**

The Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (http://lsp.nwu.edu.cn/tcmsp.php) was used to obtain the information on absorption, distribution,
metabolism, and excretion (ADME), OB, DL, and Lipinski’s rule of five of niloticin, allowing its screening and evaluation as a drug candidate with potential bioeffects.  

**Bio-layer interferometry assay**

The bio-layer interferometry assay was conducted using the Octet K2 system (Pall ForteBio Corp., Menlo Park, CA, USA). To assess the affinity of niloticin and MD-2, MD-2 (10 µg/mL) was immobilized on NTA biosensors, and a set of sensors without protein was used as a control. Niloticin at 3000, 1500, 750, 375, and 187.5 µM was prepared in a buffer containing 10 mM phosphate-buffered saline, 0.5% Tween-20, and 0.5% dimethyl sulfoxide (DMSO) at pH 7.5. A baseline step of 60 s, an association step of 45 s, and a dissociation step of 60 s were acquired using protein-loaded and control sensors for each point of the concentration series. Data were processed to remove drift and well-to-well artifacts using ForteBio Data Analysis 10.0 (Molecular Devices, LLC, San Jose, CA, USA).

**Docking of niloticin to the MD-2 protein**

Molecular docking of niloticin to MD-2 was performed to assay the binding of niloticin and MD-2. Niloticin was constructed in ChemBio3D Ultra 13.0, and the structure was minimized using an MMFF94x force field. The crystal structure of MD-2 (PDB code: 2Z65.pdb) was obtained from a protein databank (www.rcsb.org) and preprocessed using Autodock Tool (ADT), including the addition of polar hydrogen atoms and the assignment of Kollman united partial charge. The docking calculation was performed with Autodock Vina using default parameters unless otherwise stated. The center of the grid box (30 × 30 × 30 Å) was set to 23.36 Å, −56.98 Å, and −3.97 Å by referring to the position of the ligand co-crystallized with MD-2. The lowest binding energy conformation was chosen as the final result. The binding mode was analyzed and visualized with ADT.

**Cells**

Macrophages are the first line of defense for host immunity and epidemic prevention. The balance between pro-inflammatory and anti-inflammatory cytokines released after injury affects the degree of inflammatory response. Therefore, the macrophage RAW264.7 cell line was selected to evaluate anti-inflammatory activity. Macrophage RAW264.7 cells (#CL-0190, Procell Life Science & Technology Co., Ltd., Wuhan, China) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 100 mg/L penicillin-streptomycin (GIBCO, Invitrogen Inc., Carlsbad, CA, USA) in an incubator with 5% CO₂ at 37°C. Cells were subcultured when they reached 80–90% confluence. For experimental measurements, cells (5 × 10⁵ cells/well) were plated in 96-well plates and cultured overnight before being subjected to any treatment.

**Methylthiazolyterazolium (MTT) assay for cell viability analysis**

The MTT assay was used to evaluate cell viability. The experimental samples were divided into an untreated control group, a DMSO group (treated with 0.5% DMSO), and a niloticin-treated group. Cells were treated with 8, 40, or 100 µg/mL niloticin (six replicates per concentration) for 24 h, and then MTT was added in the medium at 1:10 (v/v; Sigma, St Louis, MO, USA), and the cells were cultured for 4 h. The optical density (OD) was measured using a microplate spectrophotometer at 570 nm (Tecan Group Ltd., Mannedorf, Switzerland).

**Cytokine measurement**

To measure the effect of niloticin on cytokine release, RAW264.7 cells were divided into three experimental groups. The niloticin group was treated with 8, 40, or 100 µg/mL niloticin before incubation with 100 ng/mL LPS (six replicates per concentration). The model group was treated only with 100 ng/mL LPS, and the control group received no treatment. Then, the cells were incubated for 24 h, and the levels of nitric oxide (NO), tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β were analyzed in the cell supernatants using ELISA following the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA).

**Quantitative reverse-transcription PCR (RT-qPCR) analysis**

TRizol reagent and Turbo DNase® (Invitrogen Inc., Carlsbad, CA, USA) were used to extract total RNA and inactive DNase, respectively. The Superscript First-Strand cDNA Synthesis Kit (Vazyme Biotech, Co., Ltd., Nanjing, China) was used to generate cDNA following the manufacturer’s instructions. The primer sequences for IL-1β, IL-6, TNF-α, inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), and β-actin are presented in Table 1. RT-qPCR analysis was performed in triplicate as described previously, and all reagents were purchased from Tiangen Biotech (Beijing) Co., Ltd., China. The levels of expression of each gene were determined with a standard curve, and the values were normalized to β-actin.
Western blot assay

Cells were lysed with RIPA buffer, and the nuclear protein isolation-translocation assay kit was used to isolate nuclear and cytosolic protein fractions following the manufacturer’s instructions. Protein concentration was measured using the BCA protein assay kit according to the manufacturer’s instructions. The RIPA lysis buffer and BCA protein assay kit were obtained from Beyotime Institute of Biotechnology (Haimen, China). Protein extracts were separated by electrophoresis using 10% sodium dodecyl sulfate polyacrylamide gels and electroblotted onto polyvinylidene difluoride membranes. The membranes were incubated with blocking solution for 2 h at room temperature, followed by incubation overnight at 4°C with the primary antibodies as described previously. Then, the blots were washed six times with TBST and incubated for 2 h with HRP-labeled goat anti-mouse or anti-rabbit secondary antibodies at 37°C. The membranes were washed with TBST, and the bands were developed using a substrate for enhanced chemiluminescence (Pierce Chemical, Dallas, TX, USA). Band intensities were quantified using Image-Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA).

Statistical analysis

All data are presented as mean ± standard deviation. Significant differences between groups were evaluated using the one-way analysis of variance (ANOVA) followed by the LSD post-hoc test. All statistical analyses were carried out using SPSS 20.0 (IBM, Armonk, NY, USA). Two-sided p-values were considered to indicate significance at p < 0.05.

Results

Drug potential assessment

The ADME features of niloticin were obtained from the TCMSP database. Notably, the OB of niloticin was 41.4%, and the DL was 0.82, suggesting that it had good drug potential. In addition, the molecular weight (MW), partition coefficient between octanol and water (AlogP), H-bond donors (Hdon), H-bond acceptors (Hacc), and rotatable bond number (RBN) of niloticin were 456.78 Da, 5.62, 1, 3, and 5.19, respectively, consistent with Lipinski’s rule of five.

Bio-layer interferometry assay

The global-fitting analysis of a 1:1 binding interaction model conducted in the Octet system was used to assay the binding affinities of various niloticin concentrations to MD-2. The concentration of niloticin varied from 0.0313, 0.0625, 0.125, 0.25, and 0.5 mM/mL, and the KD value of niloticin was 7.16E-04 (Figure 1(d)), which indicated that niloticin could bind to MD-2 and block the interaction between LPS and TLR4/MD-2.

Molecular docking

To investigate the interaction between niloticin and MD-2 at the molecular level, niloticin was docked to the LPS-binding pocket in MD-2 (Figure 1(b)). The docking results showed that niloticin bound to MD-2 through hydrophobic interactions with a binding energy of −10.0 kcal/mol. A total of 12 residues of MD-2 were involved in the interaction with niloticin, namely, ILE80, VAL135, TYR131, PHE151, ILE135, ILE32, ILE124, LE054, PHE121, LEU61, ILE63, and VAL135. No hydrogen-bonding interactions were observed between niloticin and MD-2.

MTT assay for viability effects of niloticin

The MTT assay was performed to determine the cell viability of macrophages treated with niloticin. These results showed a dose-dependent decrease in OD value with increasing concentrations of niloticin (Figure 1(c)). Compared with that in the control group, no significant differences were found in the cell viability of the DMSO (p = 1), niloticin-8 (p = 1), and niloticin-40 (p = 0.067) groups, whereas a significant decrease was detected in that of the niloticin-100 group (p = 0.000). However, the cell viability of the niloticin-100 group was close to 90%, indicating that niloticin had no significant effect on cell viability.
**Effect of niloticin treatment on cytokine expression in RAW264.7 cells**

ELISA was used to measure NO, TNF-α, IL-6, and IL-1β levels in RAW264.7 cells treated with niloticin (Figure 2(a)). The results showed that the levels of all inflammatory proteins analyzed were significantly higher in the LPS group than in the control group ($p = 0.000$, all $p < 0.01$). Conversely, a significant decrease in the expression levels of all markers was observed in the cells treated with LPS and niloticin compared with that in the LPS group ($p$-value of each group was $0.000$, all $p < 0.01$), although they were still significantly higher than those in the control group (IL-1β, $p = 0.041$; IL-6, $p = 0.035$ in cells treated with 100 μg/mL niloticin; all other $p$ values <0.01). Altogether, these results indicated that niloticin inhibited the LPS-induced release of NO, TNF-α, IL-6, and IL-1β in RAW264.7 cells.

**Effect of niloticin on cytokine mRNA expression**

RT-qPCR was used to analyze the mRNA expression levels of the cytokines IL-1β, TNF-α, iNOS, IL-6, and COX-2 (Figure 2(b)). Compared with the control group, the LPS group showed high levels of iNOS, IL-1β, IL-6, TNF-α, and COX-2 mRNA expression (each group had a $p = 0.000$, with all $p < 0.01$). Cells treated with LPS and niloticin showed significantly decreased iNOS, IL-1β, IL-6, TNF-α, and COX-2 mRNA expression compared with the LPS alone group ($p = 0.000$ for each group, and all $p < 0.01$), but the levels were still higher than those in control cells, where $p = 0.05$ for iNOS and <0.01 for all other groups. These data indicate that niloticin significantly suppressed the mRNA expression of IL-1β, TNF-α, iNOS, IL-6, and COX-2 induced by LPS in RAW264.7 cells.

---

**Figure 2.** Effects of niloticin treatment on interleukin expression in LPS-stimulated RAW264.7 cells. RAW264.7 cells were treated or untreated with LPS and different concentrations of niloticin and the expression of interleukins was measured by ELISA (a), RT-qPCR (b), and western blotting (c). Data are presented as mean ± standard deviation of three replicates obtained from three independent experiments. One-way ANOVA followed by LSD post-hoc test; *$p < 0.05$, **$p < 0.01$ vs. the control group. #$p < 0.05$, ###$p < 0.01$ vs. the LPS group.
Effect of niloticin on protein expression levels of the LPS-TLR4/MD-2-NF-κB signaling pathway

Protein expression levels of the LPS-TLR4/MD-2-NF-κB signaling pathway were analyzed using western blotting (Figure 2(c)). This analysis revealed that iNOS, TLR4, MyD88, p65, and p-p65 expression levels were up-regulated in the cells treated with LPS compared with that of the control group (p = 0.006 for p65 and 0.000 for all other groups, p < 0.01). This increase in the expression levels induced by the LPS treatment was significantly downregulated by niloticin treatment (p = 0.000 for each group; p < 0.01), although these levels were still higher than those in the control group, with p values of 0.008 and 0.007 for TLR4 and p65 groups, respectively, and 0.000 for all other groups. In conclusion, niloticin treatment markedly reduced the expression levels of iNOS, TLR4, MyD88, NF-κB p65, NF-κB, and p-p65 in RAW264.7 cells.

Discussion

LPS is recognized as the most potent pathogenic factor implicated in endotoxemia and sepsis, which can lead to SIRS and MODS. The pathogenicity of LPS relies on its ability to cause a cascade reaction of uncontrolled systemic inflammation through the activation of the TLR4 transmembrane signaling pathway, where TNF-α and IL-6 also play significant roles. Several signal proteins and receptors are involved in the regulation of the TLR4 signaling pathway. MD-2 is the gatekeeper of TLR4 in the LPS-induced inflammation signaling cascade and is considered as an indispensable receptor for the activation of the TLR4 signaling pathway. Therefore, the inflammatory response could be regulated by antagonizing MD-2 activation.

Niloticin is an active compound produced by Cortex phellodendri, and its anti-inflammatory activity has not been explored. The aim of this study was to assess not only the drug potential of niloticin but also the MD-2-mediated mechanism as a possible cause for its anti-inflammatory activity. The pharmacokinetics properties of niloticin, including OB, DL, and Lipinski’s rules, were investigated because they are fundamental factors for assessing the efficacy of drug distribution in systemic circulation. We found that these properties indicated good drug potential for niloticin. Further analysis using bio-layer interferometry and molecular docking technology indicated that niloticin could bind to MD-2 within 5 s with a stable bonding curve (Figure 1(d)) and that there was high affinity between these two molecules. Generally, docking scores with binding energy ≤ 5.0 kcal/mol indicate good target-compound interaction. The binding energy between niloticin and MD-2 was −10.0 kcal/mol, while the binding energy between the LPS antagonist eritoran to MD-2 was only −7.9 kcal/mol, suggesting that MD-2 interaction with niloticin is stronger than that with LPS, probably due to the strong hydrophobic bonding between niloticin and MD-2.

Importantly, the MTT assay results showed that niloticin had no evident effect on cell viability, suggesting that it can be developed as a new drug to target MD-2. Subsequently, the molecular mechanism of niloticin was explored. MD-2 and TLR4 on the cell surfaces of macrophages form the TLR4-MD-2 complex that recognizes and binds LPS to activate the NF-κB signal transduction pathway, promoting the release of several inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, which further induces an excessive inflammatory reaction. In the present study, niloticin was shown to reduce the increased levels of TNF-α, IL-6, and IL-1β induced by LPS treatment. Conversely, NO is released from LPS-stimulated macrophage cells to induce an inflammation reaction. In a pathological state, iNOS is the main source of NO production; consequently, reduction in the levels of NO or iNOS might be important for the treatment of SIRS.

Interestingly, we found that the release of NO and iNOS expression was suppressed by niloticin. In addition, niloticin downregulated the expression of COX-2, which is proportional to the degree of inflammation. A previous study showed that COX-2-deficient rats presented delayed leukocyte infiltration into organs and were resistant to LPS-mediated infection. Therefore, molecules that decrease the expression of COX-2 could be potent drug candidates to modulate inflammation.

LPS can induce NF-κB activity through a signaling cascade that requires MyD88 and TLR4. NF-κB activity is also stimulated by pro-inflammatory cytokines, bacteria, and viruses and promotes inflammation, immunomodulation, metastasis, and radiotherapy and chemotherapy resistance. The analysis of the effects of niloticin treatment on the LPS-TLR4/MD-2-NF-κB signaling pathway suggested that niloticin exhibits anti-inflammatory properties through inhibiting the expression of P65, p-P65, TLR4, MyD88, and iNOS. In summary, niloticin interacted with MD-2 through the rapid and stable formation of hydrophobic bonds, which helped to antagonize the binding of LPS to the TLR4/MD-2 complex. Therefore, niloticin may inhibit the release of pro-inflammatory factors (e.g., TNF-α, IL-6, and IL-1β) and NO by suppressing the activation of the LPS-TLR4/MD-2-NF-κB signaling pathway (Figure 3).

In this study, we aimed to characterize the therapeutic potential of niloticin as an anti-inflammatory drug. We found that niloticin can interact with MD-2, a receptor involved in the TLR4-mediated LPS responses. Importantly, niloticin treatment did not evidently affect the
cell viability and lead to a significant decrease in the LPS-induced expression of pro-inflammatory molecules. Altogether, these results suggest that niloticin is a drug candidate for the treatment of SIRS. However, there are some limitations in the current study. We conducted the experiments of the anti-inflammatory activity and mechanism of niloticin in vitro. Thus, further in vivo studies are required to clarify the anti-inflammatory activity and mechanism of niloticin. Furthermore, we only carried out the experiment 24 h after niloticin treatment, so the follow-up effects of niloticin remain uncertain. Therefore, the long-term anti-inflammatory effects of niloticin also need to be studied.

**Conclusion**

Niloticin is a potential MD-2 antagonist that binds to MD-2 through stable hydrophobic bonds and promotes an anti-inflammatory effect by suppressing the LPS-TLR4/MD-2-NF-κB signaling pathway.

**Acknowledgments**

We would like to thank Editage (www.editage.com) for English language editing.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Natural Science Foundation of China (No. 81303205 and 81803681) and the intercollegiate joint training program of colleges and universities in Liaoning Province and Scientific research project of Liaoning Provincial Department of Education (LJKZ0897). The recipients of these funds were Guirong Chen and Yubin Xu.

**ORCID iDs**

Guirong Chen @ https://orcid.org/0000-0001-6562-2204
Yubin Xu @ https://orcid.org/0000-0003-0340-4425

**References**

1. Gao YL, Zhai JH and Chai YF (2018) Recent advances in the molecular mechanisms underlying pyroptosis in sepsis. *Mediators Inflamm:* 5823823.
2. Kaner Z, Ochayon DE, Shahaf G, et al. (2015) Acute phase protein alpha1-antitrypsin reduces the bacterial burden in mice by selective modulation of innate cell responses. *J Infect Dis* 211(9): 1489–1498.
3. Nakamura N, Yoshida M, Umeda M, et al. (2006) Extended exposure of lipopolysaccharide fraction from *Porphyromonas gingivalis* facilitates mononuclear cell adhesion to vascular endothelium via Toll-like receptor-2 dependent mechanism. *Atherosclerosis* 196: 59–67.
4. Li S, Guo Q, Li S, et al. (2019) Glutamine protects against LPS-induced inflammation via adjusted NODs signaling and enhanced immunoglobulins secretion in rainbow trout leukocytes. *Dev Comp Immunol* 98: 148–156.
5. Wang X, Han C, Qin J, et al. (2019) Pretreatment with *Salvia miltiorrhiza* polysaccharides protects from lipopolysaccharides/d-galactosamine-induced liver injury in mice through inhibiting TLR4/MyD88 signaling pathway. *J Interferon Cytokine Res* 39: 495–505.
6. Qu RN and Qu W (2019) Metformin inhibits LPS-induced inflammatory response in VSMCs by regulating TLR4 and PPAR-gamma. *Eur Rev Med Pharmacol Sci* 23: 4988–4995.
7. Kawasaki K, Akashi S, Shimazu R, et al. (2001) Involvement of TLR4/MD-2 complex in species-specific
lipopolysaccharide-mimetic signal transduction by Taxol. *J Endotoxin Res* 7: 232–236.
8. Park BS, Song DH, Kim HM, et al. (2009) The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature* 458: 1191–1195.
9. Koparde AA, Doijad RC and Magdum CS (2019) Natural Products in Drug Discovery. In: Perveen S (ed), *Pharmacognosy-Medicinal Plants*. London: IntechOpen. DOI: 10.5772/intechopen.82860.
10. Li B, Ma C, Zhao X, et al. (2018) YaTCM: yet another traditional Chinese medicine database for drug discovery. *Comput Struct Biotechnol J* 16: 600–610.
11. Xian YF, Mao QQ, Ip SP, et al. (2011) Comparison on the anti-inflammatory effect of *Cortex phellodendri* Chinensis and *Cortex phellodendri* Amurensis in 12-O-tetradecanoylphorbol-13-acetate-induced ear edema in mice. *J Ethnopharmacol* 137: 1425–1430.
12. Jung HW, Jin GZ, Kim SY, et al. (2009) Neuroprotective effect of methanol extract of Phellodendri Cortex against 1-methyl-4-phenylpyridinium (MPP+)-induced apoptosis in PC-12 cells. *Cell Biol Int* 33: 957–963.
13. Park SD, Lai YS and Kim CH (2004) Immunopotentiating and antitumor activities of the purified polysaccharides from Phellodendron chinese SCHNEID. *Life Sci* 75: 2621–2632.
14. Kong LD, Yang C, Qiu X, et al. (2001) Effects of different processing products of *Cortex phellodendri* against scavenging oxygen free radicals and anti-lipid peroxidation. *Zhongguo Zhong Yao Za Zhi* 26: 245–248.
15. Reegan AD, Stalain A, Paulraj MG, et al. (2016) Silico molecular docking of niloticin with acetylcholinesterase 1 (AChE1) of *Aedes aegypti* L. (Diptera: Culicidae): a promising molecular target. *Med Chem Res* 25: 1411–1419.
16. Kiplimo JJ, Shahidul Islam M and Koobanally NA (2012) Ring A-seco limonoids and flavonoids from the Kenyan *Vepris uguenensis* Engl. and their antioxidant activity. *Phytochemistry* 83: 136–143.
17. Esimone CO, Eck G, Duong TN, et al. (2008) Potential antirespiratory syncytial virus lead compounds from Aglaia species. *Pharmazie* 63: 768–773.
18. Ru J, Li P, Wang J, et al. (2014) TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform* 6: 13.
19. Zhang YF, Huang Y, Ni YH, et al. (2019) Systematic elucidation of the mechanism of geraniol via network pharmacology. *Drug Des Devel Ther* 13: 1069–1075.
20. Pei T, Zheng C, Huang C, et al. (2016) Systematic understanding of the mechanisms of vitiligo pathogenesis and its treatment by Qubaibabuqi formula. *J Ethnopharmacol* 190: 272–287.
21. Morris GM, Huey R, Lindstrom W, et al. (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem* 30: 2785–2791.
acute pancreatitis and pancreatitis-associated lung injury. *Gastroenterology* 123: 1311–1322.

37. Zarghi A and Arfaei S (2011) Selective COX-2 inhibitors: a review of their structure-activity relationships. *Iran J Pharm Res* 10: 655–683.

38. Biswas SK, Bist P, Dhillon MK, et al. (2007) Role for MyD88-independent, TRIF pathway in lipid A/TLR4-induced endotoxin tolerance. *J Immunol* 179: 4083–4092.

39. Tu C, Huang X, Xiao Y, et al. (2019) Schisandrin A inhibits the IL-1β-induced inflammation and cartilage degradation via suppression of MAPK and NF-kappaB signal pathways in rat chondrocytes. *Front Pharmacol* 10: 41.

40. Naugler WE and Karin M (2008) NF-kappaB and cancer—identifying targets and mechanisms. *Curr Opin Genet Dev* 18: 19–26.