Current Topics

Recent Research in Bioactive Natural Products from Traditional Medicinal Plants

Review

Natural Inhibitors on Over-Activation of Microglia from Herbals

Zisong Bai,[a,b] Gang Chen,[a] Wei Li,[c] Yue Hou,*[,b] and Ning Li*[,a]

[a] *School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University; Shenyang 110016, P. R. China:* [b] *College of Life and Health Sciences, Northeastern University; Shenyang 110169, P. R. China: and* [c] *Faculty of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan.*

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Neuroinflammation manifested by over-activation of microglial cells plays an essential role in neurodegenerative diseases. Short-term activation of microglia can be beneficial, but chronically activated microglia can aggravate neuronal dysfunction possibly by secreting potentially cytotoxic substances such as tumor necrosis factor-alpha (TNF-*α*) and nitric oxide (NO), which can result in dysfunction and death of neurons. Therefore inhibiting over-activation of microglia and the production of cytotoxic intermediates may become an effective therapeutic approach for neuroinflammation. In this paper, we review our continuous research on natural inhibitors of over-activated microglia from traditional herbals, including flavonoids, lignans, sesquiterpene coumarins, and stilbenes.

Key words microglia; neuroinflammation; neurodegenerative disease; natural neuroinflammatory inhibitor; flavonoid; sesquiterpene coumarin

1. Introduction

Neuroinflammation, manifested by over-activation of microglia, is associated with the progression of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD).[1–4] Microglial cells, as the resident macrophage-like immune cells in the brain, are important contributors to neuroinflammation.[5–8] However, under inflammatory conditions, microglia are over-activated and therefore release various pro-inflammatory cytokines, including interleukin (IL)-1*β*, IL-6, tumor necrosis factor-alpha (TNF-*α*), interferon-*γ* (IFN-*γ*), and free radical mediators including reactive oxygen species (ROS) and nitric oxide (NO).[9–11] In turn, the redundant pro-inflammatory mediators will activate microglia and aggravate the pathogenesis of neurodegenerative diseases.[12–14] Thus over-activated microglial cells have become a therapeutic target for neuroinflammation-mediated neurodegenerative diseases. However, there remains a lack of effective drugs for the treatment of neuroinflammation.

Currently available drugs (memantine, acetylcholinesterase inhibitors (AChEIs), levodopa, and rotigotine) for AD and PD, unfortunately, mainly aim at mitigating symptoms, but not curing these diseases.[15,16] The high costs, side effects, and other shortcomings for new synthetic drugs make them second choices for both prevention and treatment of neurodegeneration.[17] Extensive scientific research has revealed that herbal medicine and many identified natural products could reduce over-activation of microglia both *in vitro* and *in vivo*. Thus novel bioactive products from natural sources have attracted enormous research interest from chemists and pharmacologists.[18]

In our previous research, we elicited lipopolysaccharide (LPS)-induced NO production in microglia as a systematic screening tool to identify neuroinflammatory inhibitors from medicinal plants, and further obtained effective extracts and bioactive components that can significantly inhibit hyperactive microglia. We obtained ten herbals as potential anti-neuroinflammatory agents, including Traditional Chinese Medicine *Tamarix hohenackeri* Bunge, *Ferula sinkiangensis*, and Chinese Dragon's Blood, medicine plants *Alhagi sparsifolia* Shap, *Pongamia pinnata* (L.) Pierre, *Xanthoceras sorbifolia* Bunge, and *Coreopsis tinctoria*, and functional food *Humulus lupulus* (Hop). Activity-guided phytochemical research revealed 76 highly effective neuroinflammation inhibitors, structurally including 44 flavonoids, 18 sesquiterpene coumarins, 9 lignans, 5 stilbenes, and other compounds. These active ingredients may be promising resources for the development of new anti-neuroinflammation drugs.[19–28]

A large number of natural products have shown strong anti-neuroinflammatory activities *in vitro*, and some of them have been demonstrated effective in animal models. For example, we reported that pterostilbene, the major component of Chinese Dragon's Blood, could reduce LPS-induced learning and memory impairment evaluated by Morris water maze and Y-maze tests. Such protective effects may be related to protective effects on neuronal damage and inhibition of microglia over-activation in mice. Neurodegenerative diseases such as AD are accompanied by neuroinflammation and cognitive deficits. Pterostilbene seems to be a recognized neuroprotective substance for neurodegenerative diseases.[29]

In this review, we focus on natural flavonoids, lignans, sesquiterpene coumarins, and stilbenes from herbals with anti-neuroinflammatory effects. Furthermore, their structure–

* To whom correspondence should be addressed. e-mail: houyue@mail.neu.edu.cn; liningsypharm@163.com

activity relationships (SARs) and related mechanisms of action are summarized based on our previous research.

2. Flavonoids

Flavonoids, widely distributed in the plant kingdom, have demonstrated a wide range of biological activities. Both *in vitro* and *in vivo* studies of the anti-neuroinflammatory actions of flavonoids have been explored. In our study, we characterized more than 100 flavonoids from the bioactive extract of traditional herbals.[19,20,23–27] Some of these showed significant inhibitory effects on microglia over-activation both *in vitro* and *in vivo*. For example, flavonoids (isorhamnetin and quercetin), isoflavone (3′,7-dihydroxyl-4′-methoxylisoflavone and 3′,7-dihydroxyl-4′,6-dimethoxy isoflavone), and flavans (5,4′-dihydroxy-7-methoxy-6-methylflavane and (2*S*)-5,7,3′,5′-tetrahydroxy flavanone) showed significant anti-neuroinflammatory effects in our assay. Furthermore, many researchers have identified more than 70 flavonoids as potential inhibitors of neuroinflammation.[30–35] Herein, the SARs between flavonoids and anti-neuroinflammatory activities were studied in detail.

2.1. SARs of Flavonoids for Inhibition Over-Activation of Microglia

We summarized the relations between the structure and anti-neuroinflammatory activity of the 30 flavonoids, and outlined the main SARs. Based on the molecular structures and bioassay activities, the following SARs of flavonoids were suggested (Fig. 1): 1) With regard to [2″,3″:7,8]-furanoflavone, the presence of 5-OCH_3 group might play a role in enhancing anti-neuroinflammatory effect, such as compounds **3** and **4**. 2) While comparing compounds **4** to **6**, it was revealed that the 3-OCH_3 group might decrease the inhibitory activity. 3) Furthermore, comparing between compounds **4**, **1** and **2**, **7**, we can reveal that flavonoids with 2′,5′-OCH_3, 3′,5′-OCH_3, and 3′,4′-methylenedioxy groups at B ring showed lower activity. 4) In addition, compounds **4** and **5**, which have a different substitution at C-3′, also exhibited different IC_{50} values. Thus for [2″,3″:7,8]-furanoflavones, methoxy groups at C-3′ might enhance the effect. 5) The substitution of methylenedioxy group might improve the anti-neuroinflammatory effect in the 3,7-dimethoxy flavone such as **17** *vs.* **18**.[24] 6) Flavonoid aglycones were favorable to anti-inflammatory effects, whereas glycosylation decreased the activity by comparing compounds **10** *vs.* **9**, **13** and **16** *vs* **14**. 7) As for 3,4,7-trihydroxy-4′-methoxyflavonoid, the substitu-

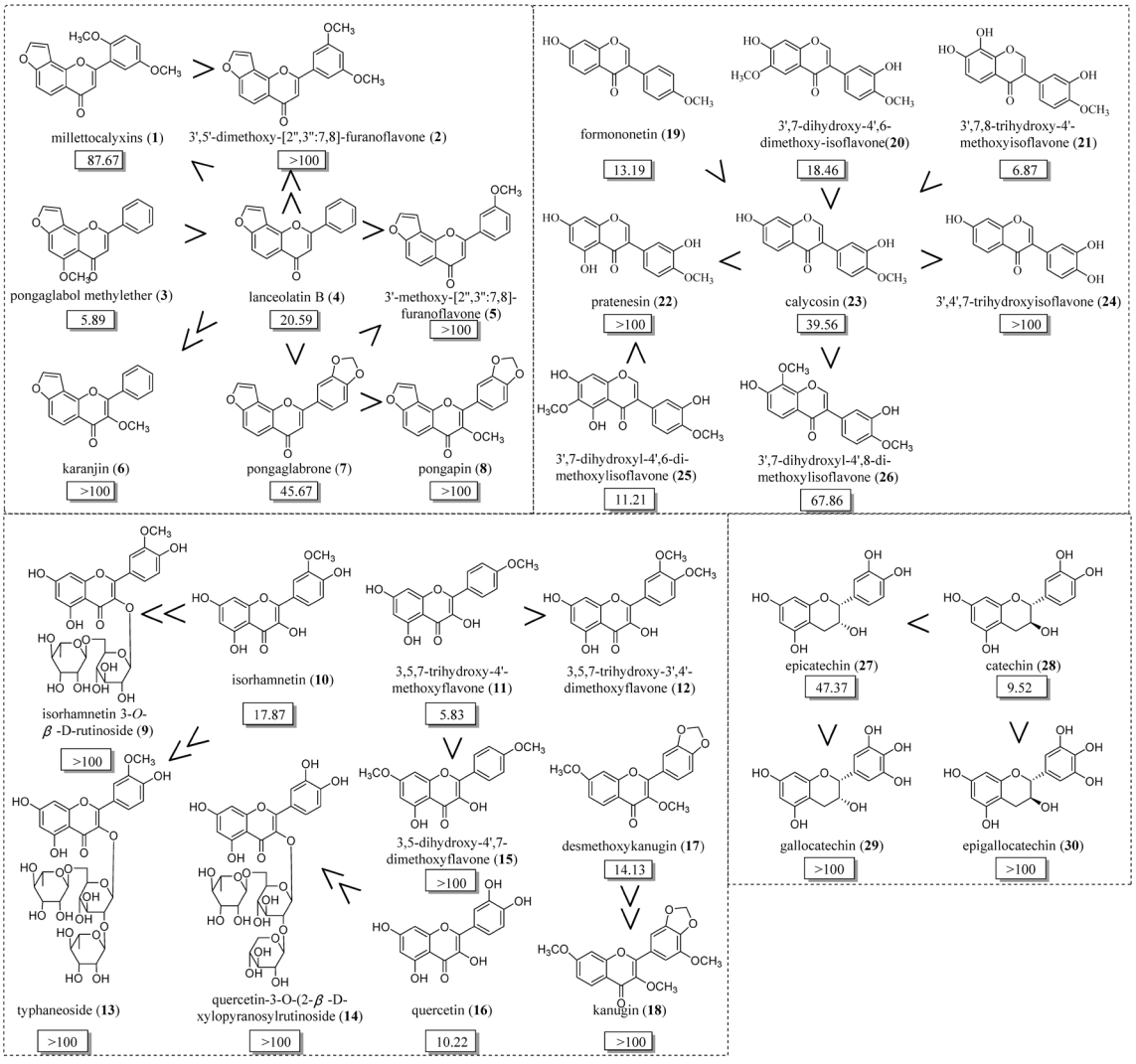

Fig. 1. Effects of Flavonoids on NO Release Induced by LPS in Microglial Cells[a)]

a) Data taken from refs. 22, 24–26; IC_{50} values in μM.

tion of methoxy group at C-7 might significantly reduce the bioactivities.[22] 8) We concluded that the presence of 5-OH was unfavorable to the anti-neuroinflammatory activity in the 3′,7-dihydroxyl-4′-methoxylisoflavone by comparing **22** to **23**. Furthermore, the presence of 8-OH, 6-OCH_3, and 4′-OCH_3 groups improved the activity, such as **21** *vs.* **23**, **20** *vs.* **23** and **23** *vs.* **24**, whereas the presence of 8-OCH_3 reduced the anti-neuroinflammatory effect. 9) With regard to 3′,5,7-trihydroxy-4′-methoxy isoflavone, the presence of 6-OCH_3 significantly improved the inhibitory action.[25] 10) We also revealed the configuration could play a major role in increasing anti-neuroinflammatory effect in the 3,5,7,3′,4′-pentahydroxyflavane, such as **27** and **28**.[26]

2.2. Possible Target Molecules of Flavonoids Considerable research has revealed that the nuclear factor *κ*B (NF-*κ*B) signaling pathway is a vital activator in inflammatory processes. Expression levels of chemokines and inflammatory cytokines are modulated by NF-*κ*B in many different cells including microglia.[36–38] NF-*κ*B consists of Rel A p65 and NF-*κ*B p50 subunits, and is combined to inhibitory protein inhibitor of NF-*κ*B (I*κ*B) in an inactive form in cytoplasm.[39–41] LPS and pro-inflammatory cytokines such as TNF-*α* can induce phosphorylation and proteasomal degradation of I*κ*B, and result in the release of NF-*κ*B. Then, NF-*κ*B heterodimer p50–65 rapidly translocates into the nucleus and combines with response elements of DNA to regulate the transcription of target genes, such as inducible nitric oxide synthase (iNOS), IL-1*β*, IL-6, TNF-*α*, and cyclooxygenase-2 (COX-2).[42] The mitogen-activated protein kinases (MAPKs) also play important roles in the process of cellular biological reactions including inflammatory processes. To date, the following three MAPKs signaling pathways have been found in mammalian cells, including c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 MAPK.[43] Production of pro-inflammatory cytokines and expression of iNOS and COX-2 are promoted by LPS in the JNK, ERK, and p38 signaling pathways[44,45] (Fig. 2).

Several flavonoids such as tectorigenin,[46] luteolin, sophoraflavanone G,[47] cudraflavanone D,[48] nobiletin,[49] and fisetin[50] may attenuate LPS-induced pro-inflammatory responses and activation of MAPK/NF-*κ*B signaling pathway in BV-2 cells (Fig. S1). In our study, quercetin (**16**) significantly inhibited LPS-induced mRNA expression of pro-inflammatory mediators, including TNF-*α*, iNOS, IL-1*β*, and IL-6, and phosphorylation of I*κ*B*α*. Furthermore, quercetin antagonized translocation of NF-*κ*B p65 to the nucleus induced by LPS and suppressed phosphorylation of ERK and MEK.[17]

Yuan *et al.* reported isoorientin decreased LPS-induced COX-2, iNOS, TNF-*α* and IL-1*β* production in a dose- and time-dependent manner (Fig. S1). Isoorientin suppressed LPS-induced translocation of NF-*κ*B from cytosol to nucleus through inhibiting protein expression in the nucleus. Obvious inhibition of phosphorylation of JNK, ERK1/2, and p38MAPK was also observed.[51]

In our study, we have shown that LPS-induced over-activation of microglia was attenuated by ethyl acetate (EtOAc) extract of *C. tinctoria* Nutt both *in vitro* and *in vivo*. The major constituent of the EtOAc extract could be okanin, which significantly inhibited production of IL-6 and TNF-*α* and mRNA expression, suppressing iNOS expression in BV-2 cells after LPS treatment (Fig. S1). Western blot showed that okanin downregulated levels of nuclear NF-*κ*B p65 and suppressed phosphorylation of I*κ*B*α*, indicating that it could inhibit LPS-induced activation of the NF-*κ*B signaling pathway.[52]

3. Lignans

A. sparsifolia is an ethnodrug in Xinjiang. The aerial parts are used for the treatment of cancer and rheumatism in China.[53,54] Its secretion from aerial parts is called “alhagi sugar,” which has been used to treat neurogenic headache.[54]

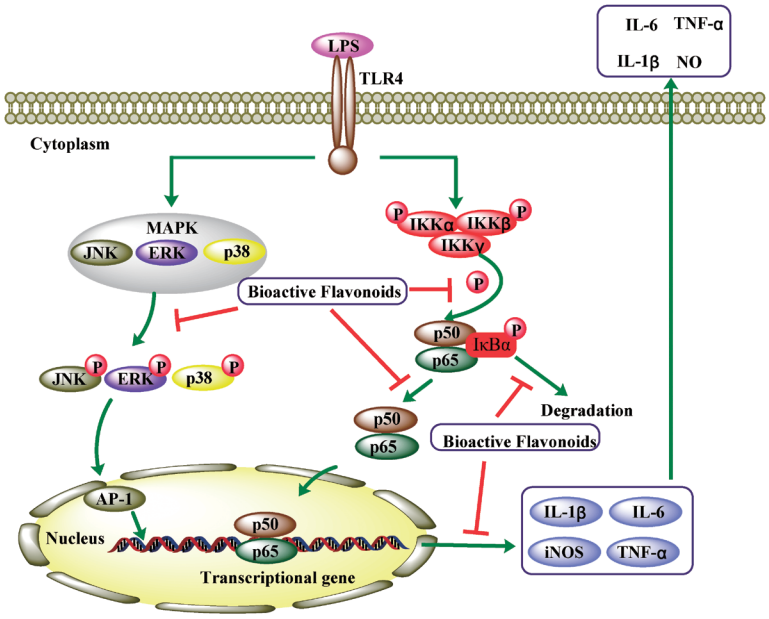

Fig. 2. NF-*κ*B and MAPK Signaling Pathway and Possible Target Molecules of Flavonoids

AP-1: activator protein 1, ERK: extracellular signal-regulated kinase, JNK: c-Jun N-terminal kinase, IL-1*β*: interleukin-1*β*, IL-6: interleukin-6, iNOS: inducible nitric oxide synthase, IKK: I*κ*B kinase, I*κ*B*α*: inhibitory protein, LPS: lipopolysaccharide, MAPK: mitogen-activated protein kinases, p38: p38 mitogen-activated protein kinase, TLR4: Toll-like receptors 4, TNF-*α*: tumor necrosis factor-alpha.

In our study, EtOAc extract of dried aerial parts of *A. sparsifolia* was found to suppress the release of NO stimulated by LPS in N9 cells ($IC_{50} = 4.16\,\mu g/mL$). Seven lignans and 15 flavonoids were isolated from the active fraction accordingly. Then, the effects of lignans and their derivatives on NO release stimulated by LPS were examined in N9 cells.

3.1. SARs of Lignans for Inhibition of Microglia Over-Activation Syringgaresinol (**31**) showed potent inhibitory activities with IC_{50} values of $2.68\,\mu M$, being stronger than that of the positive control minocycline ($IC_{50} = 19.89\,\mu M$) (Fig. 3). The inhibitory effect of pinoresinol-4-*O*-*β*-D-glucopyranoside (**33**) and (+) tortoside A (**36**) was decreased ($IC_{50} > 100\,\mu M$) by glycosylation. Our results suggest that lignans may be favorable to anti-neuroinflammation activity, whereas the effects were decreased by glycosylation such as compounds **31**/**34** and **32**/**33**. As for pinoresinol, the presence of 3-OCH_3

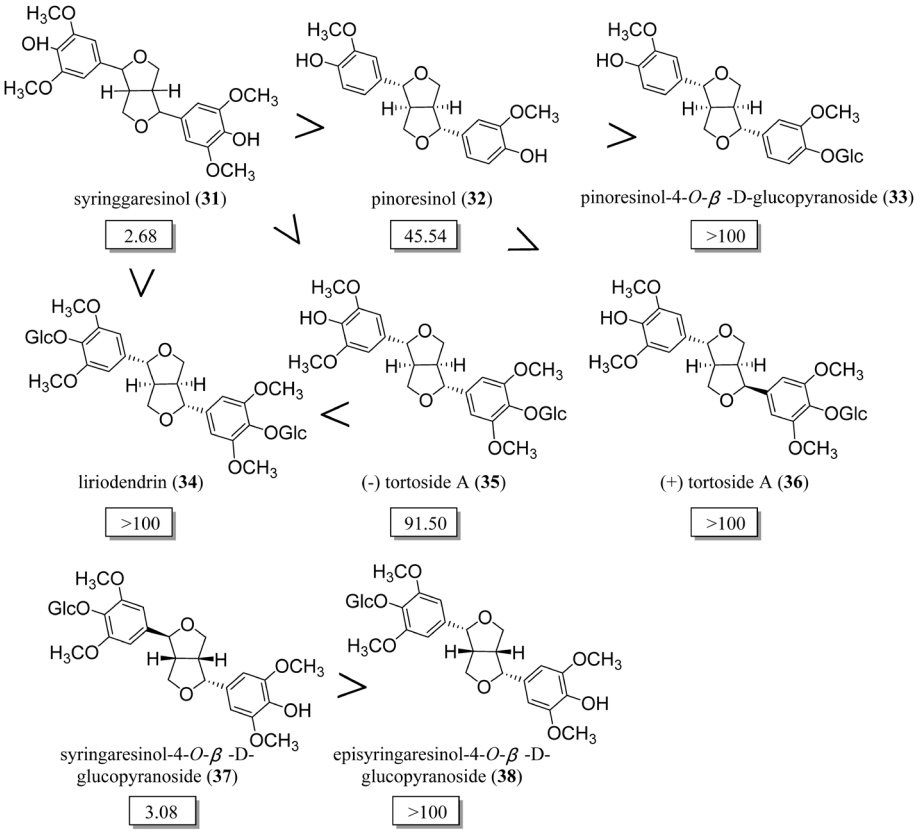

Fig. 3. Effects of Lignans from *A. sparsifolia* on Release of NO Induced by LPS in Microglia[a)]

a) Data taken from ref. 20 and 26; IC_{50} values in μM.

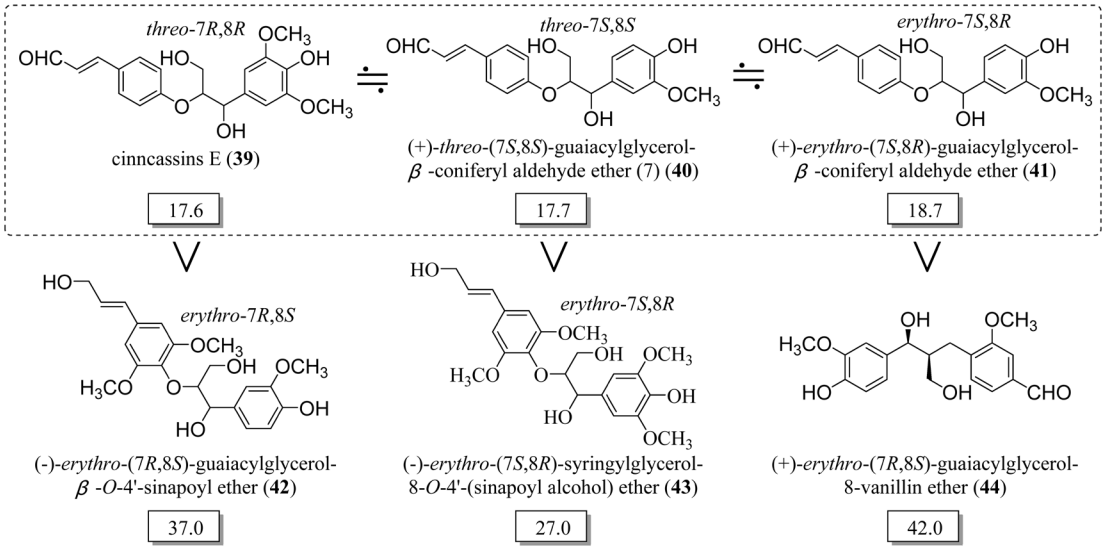

Fig. 4. Effects of Lignans from *Clematis armandii* on Release of NO Induced by LPS in Microglia[a)]

a) Data taken from ref. 55; IC_{50} values in μM.

and 3′-OCH_3 could obviously increase anti-neuroinflammatory effect by comparing between compounds **31** and **32**.[20] As for syringaresinol-4-*O*-*β*-D-glucopyranoside, the configuration of 7 and 7′ dominated the anti-neuroinflammatory effects, such as **37** (7*S*,7′*R*) with IC_{50} 3.08 μM and **38** (7*R*,7′*R*) with IC_{50} >100 μM.[26]

He *et al.* reported 16 lignans from the barks of *Cinnamomum cassia* and their anti-neuroinflammatory activities. Their SARs are summarized as follows: (Fig. 4) 1) As for 8-*O*-4′-lignans, it was shown that the acrylaldehyde group could increase anti-inflammatory activities, such as compounds **39**, **40**, and **41**; however, aldehyde group showed weaker effect than acrylaldehyde group, such as compound **44**. 2) When the acrylaldehyde group was reduced to allyl alcohol group, the inhibitory effect became much weaker (**39**, **40**, **41** > **42**, **43**). 3) The methoxyl group in the phenyl of the compounds did not affect the anti-inflammatory activities; for example, the effect of **39** was similar to those of **40** and **41**.[55]

3.2. Possible Target Molecules of Lignans Lim *et al.* reported 19 bioactive lignan derivatives from the stems of *Firmiana simplex*. Among them, balanophonin (IC_{50} = 7.07 μM), a new neolignan derivative, showed a stronger inhibitory effect on NO production than the positive control (N^G-Monomethyl-L-arginine mono-acetate salt (L-NMMA); IC_{50} = 16.27 μM) in LPS-activated BV-2 cells (Fig. S1). Because of its potent effect, the mechanism was further investigated, and it was suggested that balanophonin could significantly inhibit production of inflammatory mediators, including prostaglandin (PG)E_2, NO, IL-1*β*, and TNF-*α* in over-activated BV-2 cells. Moreover, balanophonin attenuated protein expression levels of COX2 and iNOS, and decreased phosphorylation of MAPKs such as p-ERK, p-JNK, and p-p38.[57]

Several lignans from *Magnoliae* plants downregulated inflammatory gene products through inhibition of phosphorylation and nuclear translocation of p65 subunit of NF-*κ*B and the inactivation of inhibitory *κ*B kinase (IKK)/MAPK.[58,59] Ji *et al.* identified three lignans (kobusin, aschantin, and fargesin) from the flower buds of *Magnolia fargesii*. Fargesin

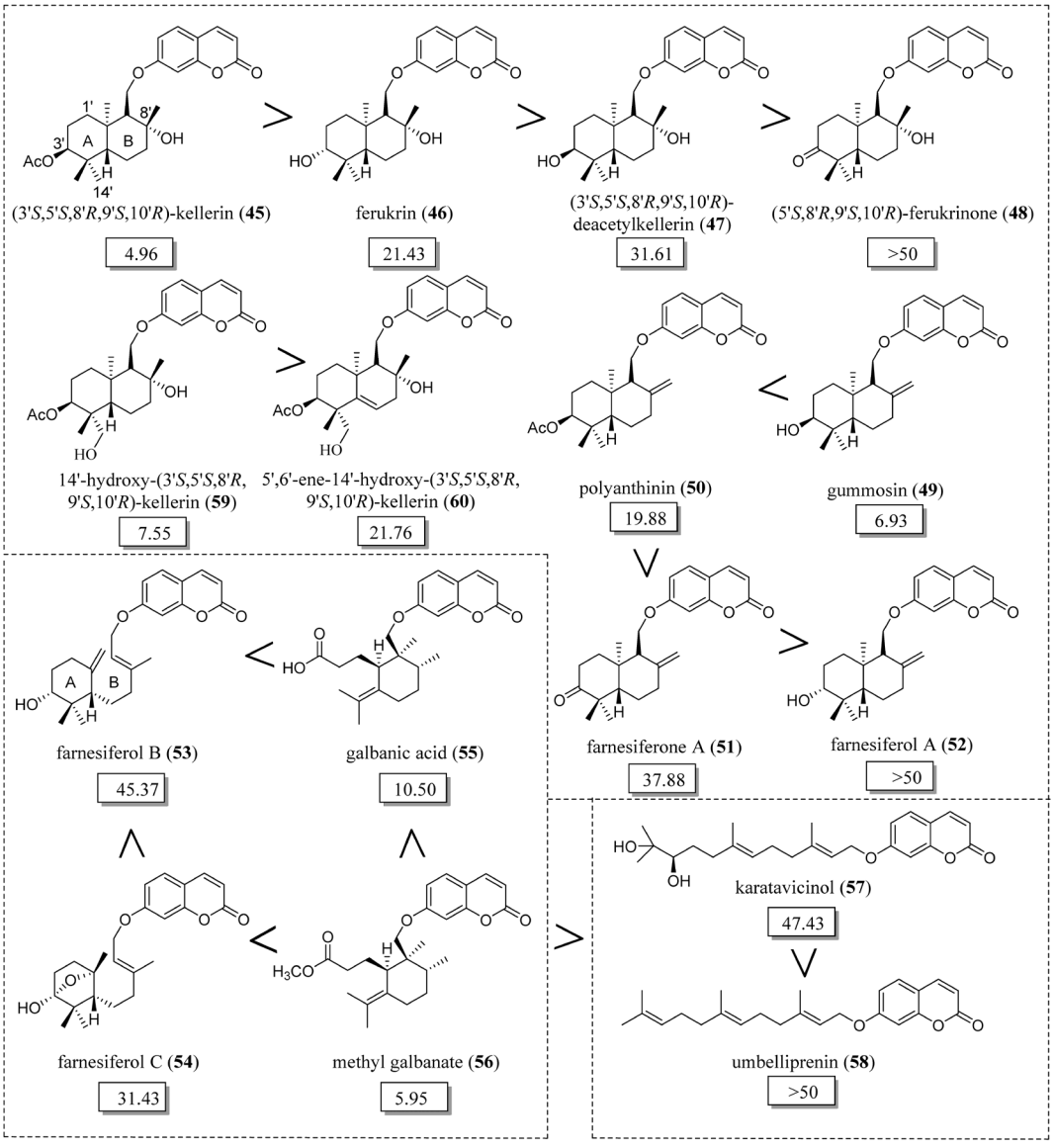

Fig. 5. Effects of Sesquiterpene Coumarins from Gum of *Ferula sinkiangensis* on NO Release Induced by LPS in Microglia[a]

a) Data taken from ref. 56; IC_{50} values in μM.

(IC_{50} = 10.4 μg/mL) showed more potent inhibition of LPS-induced NO production than kobusin and aschantin (IC_{50} = 21.8 and 14.8 μg/mL, respectively) (Fig. S1). All tested lignans decreased expression of iNOS protein in LPS-induced microglia. Aschantin and fargesin significantly inhibited expression of iNOS mRNA. Kobusin, aschantin, and fargesin inhibited LPS-induced NF-κB activation in microglia by luciferase reporter assay.[60]

4. Sesquiterpene Coumarin

The gum resin of the *Ferula* genus is usually called "Awei" in China. The gum Awei from *F. sinkiangensis* and *Ferula fukanensis* is used as Traditional Chinese Medicines. Sesquiterpene coumarins from the *Ferula* genus exert diverse bioactivities, including anti-inflammatory,[61,62] antitumor,[63] and antibacterial activities.[64,65] In our previous study, several bioactive sesquiterpene coumarins with inhibitory effects on over-activation of microglia were identified from Awei, having potency to serve as therapeutic agents against AD.

4.1. SARs of Sesquiterpene Coumarin for Inhibition of Over-Activation of Microglia We previously identified 16 sesquiterpene coumarins including two new compounds, from the effective extract of Awei gum of *F. sinkiangensis*. We showed that substitution at C-3′ in bicyclic sesquiterpene coumarins, which held the substitution of hydroxyl and methyl at C-8′(*R*), may play a significant role in regulating anti-neuroinflammatory activity (Fig. 5). Substituted groups of acetoxyl group, α-hydroxy group, β-hydroxy group, and carbonyl group at C-3′enhance the activity with successively decreasing IC_{50} values, such as **45**–**48**. However, the sequential order was different in bicyclic sesquiterpene coumarins with a terminal double bond substituted at C-8′. The order for those was β-hydroxy group, acetoxyl group, carbonyl group, and α-hydroxy group. Furthermore, the chain sesquiterpene coumarins exhibited weak activities, such as compounds **57** and **58**. We therefore concluded that the ring of sesquiterpene is necessary for the anti-neuroinflammatory effect in a sesquiterpene moiety. Finally, the crack position of the ring in the monocyclic sesquiterpene moiety could obviously affect the activities. Compounds **53** and **54**, with the B ring cracked, exhibited weaker anti-neuroinflammatory activities than **55** and **56** with the A ring cracked. Furthermore, comparing **55** and **56**, we confirmed that the presence of an oxygen bridge in the A ring could enhance the activity.[19]

(3′*S*,5′*S*,8′*R*,9′*S*,1″*R*)-Kellerin has been identified as a major constituent from *F. sinkiangensis* as a potential natural therapeutic agent for neuroinflammation. To afford more novel derivatives, modification of kellerin was carried out by biotransformation using the callus of *Angelica sinensi* (Oliv.) Diels. All the natural and modified sesquiterpene coumarins were evaluated for their anti-neuroinflammatory effects. As a result, two main conclusions were made: 1) The presence of hydroxyl group at C-14′ was propitious to the anti-inflammatory effect, although hydroxylation of C-14′ reduced the activity, such as **59** (IC_{50} = 7.55 μM) and **45** (IC_{50} = 4.96 μM). 2) Compounds with a double bond present at $\Delta^{5',6'}$ in B-ring revealed a remarkable decrease of activity, which showed that the saturated B-ring was critical for bioactivity in the bicyclic skeleton of sesquiterpene coumarins.[66]

4.2. Possible Target Molecules of Sesquiterpene Coumarins Our previous results revealed that (3′*S*,5′*S*,8′*R*,9′*S*,1″*R*)-kellerin (1.5%, w/w) may be the critical effective constituent of Awei. The primary mechanism of kellerin was involved in inhibiting the production and mRNA expression of inflammatory factors, such as NO, TNF-α, IL-1β, IL-6, and COX-2 in over-activated BV-2 cells.[19]

5. Stilbenes

Stilbenes originate from two isomers of 1, 2-diphenylethene with substitution of methoxyl, hydroxyl, and other groups. Several herbals rich in stilbenes have been reported to exert anti-neuroinflammatory effects, such as *Dracaena cochinchinensis*,[18] *Caragana turfanensis*,[23] *Polygonum multiflorum*,[67] *Milicia excelsa*, *Morus alba*, *Gnetum africanum*, *Vitis vinifera*,[68] and *Vaccinium ashei* cultivar Tifblue.[69] Among the reported effective stilbenes, *E*-resveratrol (**62**), *E*-piceatannol (**65**),[68] cochinchinenene E, cochinchinenene F, and pterostilbene[18] (Fig. S1) exhibited significant inhibitory activities with IC_{50} values ranging from 3.33 to 34.02 μM.

5.1. SARs of Stilbenes for Inhibition of Over-Activa-

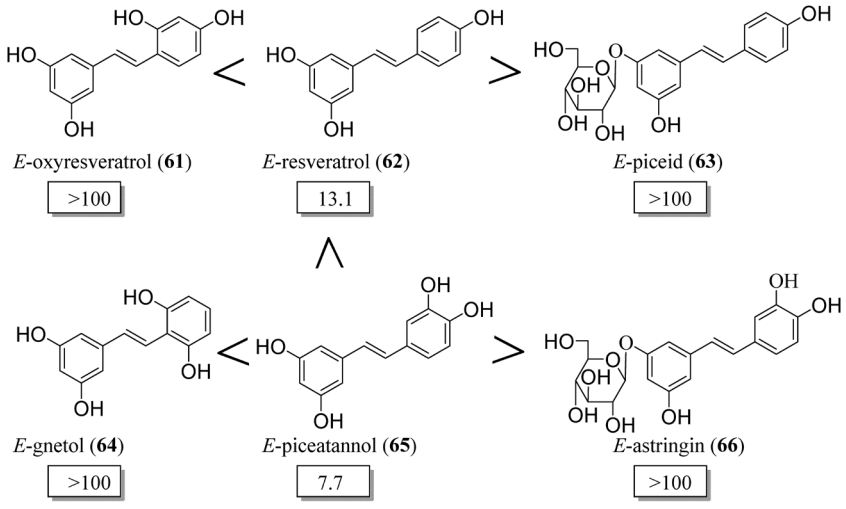

Fig. 6. Effects of Stilbenes on Release of NO Induced by LPS in Microglia[a]

a) Data taken from ref. 68; IC_{50} values in μM.

tion of Microglia Nassra *et al.* reported twenty-five stilbenes from the active fraction and evaluated the inhibitory effect on the release of NO in BV-2 microglial cells stimulated by LPS. Their findings suggest that glycosylation might reduce the activity, such as **61** and **62** *vs.* **64** and **65** (Fig. 6). Moreover, it was indicated that the location of hydroxyl groups is related to pharmacological activity. Indeed, **60** and **63** with hydroxyl groups substituted at the *meta* position were found to exhibit weaker effects than **64** with hydroxyl groups located at *ortho* position. Our previous study also showed that **61** ($IC_{50} = 18.66\,\mu M$) with methoxyl groups at C-3, C-5, and C-4′ exhibited 4-fold-higher inhibitory activity than **64** (IC_{50} $80.51\,\mu M$) with methoxy groups at the C-3, C-5, C-3′, and C-4′.[68]

5.2. Possible Target Molecules of Stilbenes Pterostilbene (Fig. S1), which was characterized from Chinese Dragon's Blood, significantly inhibited LPS-induced production of IL-6, TNF-α, and NO, and obviously decreased mRNA expression of IL-6, TNF-α, and iNOS in LPS-stimulated BV-2 microglia. Furthermore, pterostilbene significantly inhibited phosphorylation of p38, ERK, and JNK, indicating that pterostilbene might exhibit its anti-neuroinammatory activities through suppressing activation of MAPK signaling pathway. We also confirmed the effect of pterostilbene *in vivo* using a rat neuroinflammation model. Immunohistochemical test showed that pterostilbene could alleviate LPS-induced microglial activation in rat dentate gyrus (DG) and hippocampal CA1. Moreover, pterostilbene obviously inhibited mRNA expression of IL-6 and TNF-α in hippocampus and lowered corresponding contents in serum induced by LPS in rat.[70]

Han *et al*. reported that resveratrol (**62**) can inhibit phosphorylation of p38, translocation of NF-κB, and mRNA expression of proinflammatory factors such as IL-1β, IL-6, and TNF-α in overactive BV-2 cells induced by morphine. Systemic or spinal administration with resveratrol remarkably blocked morphine-induced microglial activation in the spinal cord of both male and female mice.[71]

Zhang *et al.* reported that resveratrol significantly suppressed hypoxia-induced release of NO, and inhibited hypoxia-increased iNOS expression in LPS-induced BV-2 cells. They demonstrated that resveratrol reduced NO release through downregulating expression of iNOS in hypoxia-stimulated group. Their results also showed that treatment with resveratrol could inhibit hypoxia-induced degradation of IκBα and phosphorylation of JNK, ERK1/2, and p65 NF-κB protein. The anti-neuroinflammatory effect of resveratrol was mediated by JNK/p38, MAPKs, and NF-κB pathways.[72]

6. Conclusion

Neuroinflammation is an unavoidable and vital pathological process involved in all types of brain damage. Over-activated microglial cells and ensuing neuroinflammation lead to multiple neurodegenerative diseases. In our continuous studies, we have found a variety of natural structures with anti-neuroinflammatory effects from medicinal plants. These active constituents may be promising resources for the development of new anti-neuroinflammation drugs. Furthermore, we also summarized the relations between the structure and anti-neuroinflammatory activity of flavonoids, lignans, sesquiterpene coumarins, and stilbenes providing guidance for us to find new neuroinflammatory inhibitors. In future studies, we will endeavor to discover further novel bioactive constituents with anti-neuroinflammatory effects. On the other hand, most of the active compounds we found in *in vitro* experiments have not been evaluated in animal experiments, and therefore *in vivo* confirmation for the anti-neuroinflammatory activities of these potential neuroinflammatory inhibitors remains to be performed.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

References

1) Gertig U., Hanisch U. K., *Front. Cell. Neurosci.*, **8**, 101–110 (2014).
2) Liu B., Hong J. S., *J. Pharmacol. Exp. Ther.*, **304**, 1–7 (2003).
3) Prinz M., Mildner A., *Glia*, **59**, 177–187 (2011).
4) Groh J., Martini R., *Glia*, **65**, 1407–1422 (2017).
5) Dong H., Zhang X., Qian Y., *Med. Sci. Monit. Basic. Res.*, **20**, 200–206 (2014).
6) Lull M. E., Block M. L., *Neurotherapeutics*, **7**, 354–365 (2010).
7) Block M. L., Hong J. S., *Prog. Neurobiol.*, **76**, 77–98 (2005).
8) Galve-Roperh I., Aguado T., Palazuelos J., Guzman M., *Curr. Pharm. Des.*, **14**, 2279–2288 (2008).
9) Chen J. C., Liu K. S., Yang T. J., Hwang J. H., Chan Y. C., Lee I. T., *Nutr. Neurosci.*, **15**, 252–256 (2012).
10) Rajan T. S., Giacoppo S., Iori R., De Nicola G. R., Grassi G., Pollastro F., Bramanti P., Mazzon E., *Fitoterapia*, **112**, 104–115 (2016).
11) Xia Q., Hu Q., Wang H., Yang H., Gao F., Ren H., Chen D., Fu C., Zheng L., Zhen X., Ying Z., Wang G., *Cell Death Dis.*, **6**, e1702–e1713 (2015).
12) Jeohn G. H., Kong L. Y., Wilson B., Hudson P., Hong J.-S., *J. Neuroimmunol.*, **85**, 1–10 (1998).
13) Song G. J., Kim J., Kim J. H., Song S., Park H., Zhang Z. Y., Suk K., *Exp. Neurobiol.*, **25**, 252–261 (2016).
14) Suk K., *Neurosignals*, **14**, 23–33 (2005).
15) Zhuo C., Zhu X., Jiang R., Ji F., Su Z., Xue R., Zhou Y., *Sci. Rep.*, **8**, 45865 (2017).
16) Kumar A., Singh A., Ekavali, *Pharmacol. Rep.*, **67**, 195–203 (2015).
17) Lim S. Y., Subedi L., Shin D., Kim C. S., Lee K. R., Kim S. Y., *Biomol. Ther.*, **25**, 519–527 (2017).
18) Luo X. L., Liu S. Y., Wang L. J., Zhang Q. Y., Xu P., Pan L. L., Hu J. F., *Eur. J. Pharmacol.*, **774**, 135–143 (2016).
19) Chen R., Yang Y., Xu J., Pan Y., Zhang W., Xing Y., Ni H., Sun Y., Hou Y., Li N., *Phytomedicine*, **40**, 10–19 (2018).
20) Li N., Ma Z., Li M., Xing Y., Hou Y., *J. Ethnopharmacol.*, **152**, 508–521 (2014).
21) Xing Y., Li N., Zhou D., Chen G., Jiao K., Wang W., Si Y., Hou Y., *Planta Med.*, **83**, 135–142 (2017).
22) Zhou D., Wei H., Jiang Z., Li X., Jiao K., Jia X., Hou Y., Li N., *Bioorg. Med. Chem. Lett.*, **27**, 973–978 (2017).
23) Li J., Li N., Li X., Chen G., Wang C., Lin B., Hou Y., *J. Nat. Prod.*, **80**, 3081–3092 (2017).
24) Li J., Jiang Z., Li X., Hou Y., Liu F., Li N., Liu X., Yang L., Chen G., *Bioorg. Med. Chem. Lett.*, **25**, 53–58 (2015).
25) Song Y., Pan L., Li W., Si Y., Zhou D., Zheng C., Hao X., Jia X., Jia Y., Shi M., Jia X., Li N., Hou Y., *Bioorg. Med. Chem. Lett.*, **27**, 4765–4769 (2017).
26) Li N., Wang Y., Li X., Zhang H., Zhou D., Wang W., Li W., Zhang X., Li X., Hou Y., Meng D., *Bioorg. Med. Chem. Lett.*, **26**, 5018–

5023 (2016).
27) Li N., Meng D., Pan Y., Cui Q., Li G., Ni H., Sun Y., Qing D., Jia X., Pan Y., Hou Y., *J. Func. Foods.*, **17**, 837–846 (2015).
28) Zhao W. H., Li N., Chu Y., Sun T., Wang J., Wang W. L., Li J. Y., Lin B., Chen R., Hou Y., *Sci. Rep.*, **7**, 13608 (2017).
29) Hou Y., Xie G., Miao F., Ding L., Mou Y., Wang L., Su G., Chen G., Yang J., Wu C., *Prog. Neuro-psychoph.*, **54**, 92–102 (2014).
30) Kim D. H., Li H., Han Y. E., Jeong J. H., Lee H. J., Ryu J. H., *Molecules*, **23**, 109–117 (2018).
31) Wu X. Y., Xiong J., Liu X. H., Hu J. F., *Chem. Biodivers.*, **13**, 1030–1037 (2016).
32) Woo K. W., Moon E., Park S. Y., Kim S. Y., Lee K. R., *Bioorg. Med. Chem. Lett.*, **22**, 7465–7470 (2012).
33) Guo H., Bai Z., Xu Y., Wu X., Li N., Zhu Y., Wang X., Zhang P., *Arch. Pharm. Res.*, **40**, 818–824 (2017).
34) Wen R., Lv H.-N., Jiang Y., Tu P.-F., *Phytochemistry*, **149**, 56–63 (2018).
35) Wen R., Lv H., Jiang Y., Tu P., *Bioorg. Med. Chem. Lett.*, **28**, 1050–1055 (2018).
36) DiDonato J. A., Mercurio F., Karin M., *Immunol. Rev.*, **246**, 379–400 (2012).
37) Xu L. Y., Qi J. N., Liu X., Ma H. X., Yuan W., Zhao P. Q., Liang X. H., Xu Y., Wang H. X., Xu X. Y., Wang W., Ma C. H., Gao L. F., *PLOS ONE*, **10**, e0124771 (2015).
38) Benedetti F., Davinelli S., Krishnan S., Gallo R. C., Scapagnini G., Zella D., Curreli S., *J. Transl. Med.*, **12**, 145 (2014).
39) Chai E. Z., Siveen K. S., Shanmugam M. K., Arfuso F., Sethi G., *Biochem. J.*, **468**, 1–15 (2015).
40) Li F., Zhang J., Arfuso F., Chinnathambi A., Zayed M. E., Alharbi S. A., Kumar A. P., Ahn K. S., Sethi G., *Arch. Toxicol.*, **89**, 711–731 (2015).
41) Loercher A., Lee T. L., Ricker J. L., Howard A., Geoghegen J., Chen Z., Sunwoo J. B., Sitcheran R., Chuang E. Y., Mitchell J. B., Baldwin A. S., Waes C. V., *Cancer Res.*, **64**, 6511–6523 (2004).
42) Wan F., Anderson D. E., Barnitz R. A., Snow A., Bidere N., Zheng L., Hegde V., Lam L. T., Staudt L. M., Levens D., Deutsch W. A., Lenardo M. J., *Cell*, **131**, 927–939 (2007).
43) Liu Y., Shepherd E. G., Nelin L. D., *Nat. Rev. Immunol.*, **7**, 202–212 (2007).
44) Rao K. M. K., *J. Leukoc. Biol.*, **69**, 3–10 (2001).
45) Sun L. D., Wang F., Dai F., Wang Y. H., Lin D., Zhou B., *Biochem. Pharmacol.*, **95**, 156–169 (2015).
46) Lim H. S., Kim Y. J., Kim B. Y., Park G., Jeong S. J., *Front. Pharmacol.*, **9**, 462 (2018).
47) Guo C., Yang L., Wan C. X., Xia Y. Z., Zhang C., Chen M. H., Wang Z. D., Li Z. R., Li X. M., Geng Y. D., Kong L. Y., *Phytomedicine*, **23**, 1629–1637 (2016).
48) Kim D. C., Yoon C. S., Quang T. H., Ko W., Kim J. S., Oh H., Kim Y. C., *Int. J. Mol. Sci.*, **17**, 255 (2016).
49) Cui Y., Wu J., Jung S. C., Park D. P., Maeng Y. H., Hong S. J., Kim S. J., Lee S. R., Kim S. J., Kim S. J., Eun S. Y., *Biol. Pharm. Bull.*, **33**, 1814–1821 (2010).
50) Zheng L. T., Ock J., Kwon B. M., Suk K., *Int. Immunopharmacol.*, **8**, 484–494 (2008).
51) Yuan L., Wu Y., Ren X., Liu Q., Wang J., Liu X., *Mol. Cell. Biochem.*, **386**, 153–165 (2014).
52) Hou Y., Li G., Wang J., Pan Y., Jiao K., Du J., Chen R., Wang B., Li N., *Sci. Rep.*, **7**, 45705 (2017).
53) Dawut M., Abliz A., Ghopur W., Behti P., Obulkhasim B., Mijit G., *J. Plant. Pathol. Microbiol.*, **7**, 11 (2016).
54) Li N., Zhang G., Xiong Y., Makhabel B., Li X., Jia X., *Fitoterapia*, **81**, 1058–1061 (2010).
55) He S., Zeng K. W., Jiang Y., Tu P. F., *Fitoterapia*, **112**, 153–160 (2016).
56) Xiong J., Bui V. B., Liu X. H., Hong Z. L., Yang G. X., Hu J. F., *J. Ethnopharmacol.*, **153**, 737–743 (2014).
57) Lim S. Y., Subedi L., Shin D., Kim C. S., Lee K. R., Kim S. Y., *Biomol. Ther.*, **25**, 519–527 (2017).
58) Choi M. S., Lee S. H., Cho H. S., Kim Y., Yun Y. P., Jung H. Y., Jung J. K., Lee B. C., Pyo H. B., Hong J. T., *Eur. J. Pharmacol.*, **556**, 181–189 (2007).
59) Tse A. K., Wan C. K., Zhu G. Y., Shen X. L., Cheung H. Y., Yang M., Fong W. F., *Mol. Immunol.*, **44**, 2647–2658 (2007).
60) Kim J. S., Kim J. Y., Lee H. J., Lim H. J., Lee D. Y., Kim D. H., Ryu J. H., *Phytother. Res.*, **24**, 748–753 (2010).
61) Motai T., Kitanaka S., *J. Nat. Prod.*, **68**, 365–368 (2005).
62) Motai T., Daikonya A., Kitanaka S., *Chem. Pharm. Bull.*, **61**, 618–623 (2013).
63) Kasaian J., Iranshahy M., Masullo M., Piacente S., Ebrahimi F., Iranshahi M., *J. Asian Nat. Prod. Res.*, **16**, 248–253 (2014).
64) Liu T., Osman K., Kaatz G. W., Gibbons S., Mu Q., *Planta Med.*, **79**, 701–706 (2013).
65) Appendino G., Mercalli E., Fuzzati N., Arnoldi L., Stavri M., Gibbons S., Ballero M., Maxia A., *J. Nat. Prod.*, **67**, 2108–2110 (2004).
66) Zhou D., Li N., Zhang Y., Yan C., Jiao K., Sun Y., Ni H., Lin B., Hou Y., *Rsc. Adv.*, **6**, 97302–97312 (2016).
67) Chang M. J., Xiao J. H., Wang Y., Yan Y. L., Yang J., Wang J. L., *PLOS ONE*, **7**, e50291 (2012).
68) Nassra M., Krisa S., Papastamoulis Y., Kapche G. D., Bisson J., André C., Konsman J. P., Schmitter J. M., Mérillon J. M., Téguo P. W., *Planta Med.*, **79**, 966–970 (2013).
69) Carey A. N., Fisher D. R., Rimando A. M., Gomes S. M., Bielinski D. F., Shukitt-Hale B., *J. Agric. Food Chem.*, **61**, 5979–5986 (2013).
70) Hou Y., Li N., Xie G., Wang J., Yuan Q., Jia C., Liu X., Li G., Tang Y., Wang B., *J. Func. Foods*, **19**, 676–687 (2015).
71) Han Y., Jiang C., Tang J., Wang C., Wu P., Zhang G., Liu W., Jamangulova N., Wu X., Song X., *Eur. J. Pain*, **18**, 1458–1470 (2014).
72) Zhang Q., Yuan L., Zhang Q., Gao Y., Liu G., Xiu M., Wei X., Wang Z., Liu D., *Int. Immunopharmacol.*, **28**, 578–587 (2015).