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Research progress on the pharmacological activities of senkyunolides

Qingquan Li[1], Jian-Bo Wan[2,*], Lu Zhao[1,*]

Abstract

Senkyunolides are a group of phthalide molecules that exist in Umbelliferae plants, with the highest abundance in *Ligusticum chuanxiong* Hort, a medicinal herb with therapeutic applications in various diseases such as coronary heart disease, migraine, and rheumatism. Modern research has highlighted the anti-inflammatory, analgesic, and cardiovascular-protective properties of senkyunolides. Recently, some novel effects, such as antibacterial, anti-osteoporosis, and anti-fibrosis, have been reported for senkyunolides, indicating their diverse biological activities. In addition, because of its high bioavailability and ability to cross the blood-brain barrier (BBB), particularly exemplified by senkyunolide I (SEI), holds promise for the treatment of brain diseases. Multiple signaling pathways have been related to the pharmacological activities of senkyunolides, such as the toll-like receptor 4/nuclear factor-kappa B (NF-κB) signaling, extracellular signal–regulated kinase (ERK) pathway, p38 mitogen-activated protein kinase, and c-Jun N-terminal kinase (JNK) pathways. Here, we review the research progress on the pharmacokinetics and pharmacological effects of senkyunolides, which are promising candidates for future drug development and have significant clinical value in the treatment of various diseases.

Keywords: *Ligusticum chuanxiong* Hort, Pharmacokinetics, Pharmacological activities, Senkyunolides

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Introduction

Rhizomes of *Ligusticum chuanxiong* Hort. (*Ligusticum striatum DG*, *L. striatum*) have a long history of medicinal use. According to traditional Chinese medicine (TCM) theory, *L. striatum* has beneficial functions of facilitating blood circulation and dispersing blood stasis, expressing wind dump, and relieving pain, and is a major component in multiple TCM formulae, such as Xuefu Zhuyu decoction, Siwu decoction, Naodesheng tablets, and Shunaoxin dropping pills, for the treatment of diverse diseases, including coronary heart disease, migraine, rheumatism, and irregular menstruation[1]. Modern pharmacological studies have shown that *L. striatum* exhibits anti-oxidative, neuroprotective, anti-inflammatory, and antibacterial activities[2]. The bioactive ingredients of *L. striatum* can be divided into four categories: phenols and organic acids (such as ferulic acid), phthalides (such as ligustilide and senkyunolide), alkaloids (such as ligustrazine), and polysaccharides[2]. Recent pharmacological studies have suggested that phthalide molecules are among the most important active substances in *L. striatum*[3–5]. Among these small molecules, senkyunolides may play an essential pharmacodynamic role *in vivo* because of their high oral bioavailability (BA) and have received extensive attention[6]. Here, we reviewed the research progresses of the pharmacokinetics and pharmacological effects of senkyunolides. A literature review was conducted to find all published works on senkyunolides' pharmacokinetics and pharmacological activities. A comprehensive systematic literature has conducted in the PubMed, Web of Science, and CNKI electronic databases. The literature search (systematic and hand-search) covered the period from February 2007 to March 2023.

Pharmacokinetics

Senkyunolides are a class of molecules with an O-hydroxymethylbenzoic acid lactone nuclear structure distinguished by substitutions at the C-3 and benzene ring positions. Most senkyunolides are phthalide monomers, with a few dimeric phthalides such as senkyunolide O (SEO)[7–8]. A study examined the plasma of rats after oral administration of *L. striatum* decoction, the prototypes of most senkyunolide were detected, such as senkyunolide A (SEA), senkyunolide D (SED), senkyunolide F (SEF), senkyunolide G (SEG), senkyunolide H (SEH), senkyunolide I (SEI), senkyunolide J (SEJ), senkyunolide M (SEM), and senkyunolide Q (SEQ)[9]. In another study, through dynamic and continuous monitoring of the absorption and metabolic processes of *L. striatum* decoction after oral administration, the metabolic status of senkyunolides at various absorption stages was

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obtained through digestive juices, digestive enzymes, gut microbiota, intestinal wall cells, and the liver[10]. Among them, SEF, SEG, SEI, and SEJ are absorbed into the blood as prototype drugs. SEH is mostly metabolized by the liver, whereas SEM is mainly metabolized by the intestinal flora after entering the intestine. However, SEA and SEQ were not detected in blood samples during the absorption process[10]. A possible reason for the different spectra of senkyunolides detected in the two studies is the difference in the doses and administration routes of *L. striatum*. Further studies are required to the *in vivo* concentrations of different senkyunolides in *L. striatum* treatment.

SEI is the metabolite with the highest content after ligustilide is metabolized by rat and human hepatocytes[11]. In rats, SEI was rapidly cleared in the plasma after intravenous administration and rapidly absorbed from the gastrointestinal tract after oral administration, with an oral BA of approximately 37.25%[12]. The pharmacokinetic parameters were not related to dosage in either administration approach[12]. The BA of SEI was 81.17% when administered *via* the intrahepatic portal vein, with an 18.83% first-pass effect on the liver[12]. When administered intraduodenally, the BA of the SEI was 36.91%, which is similar to oral administration and indicates that the first-pass effect in the stomach was negligible[12]. SEI are widely distributed throughout the body after oral administration, especially in the kidney, liver, lung, and brain, suggesting that the SEI prototype drug may be excreted through the kidney and can penetrate the blood-brain barrier (BBB)[12]. Xiong et al.[13] analyzed the parent molecule and possible metabolites in the plasma, urine, and bile of rats following intravenous administration of SEI. Methylation, hydration, epoxidation, glucuronidation, and glutathione (GSH) binding are the main metabolic pathways of the SEI *in vivo*, among which the GSH-binding complex is the main metabolite[13]. Another *in vivo* study in rats reported that SEI is excreted mainly through bile, in which the four major SEI adducts were characterized to be SEI-6S-O-β-D-glucuronide, SEI-7S-O-β-D-glucuronide, SEI-7S-S-GSH, and SEI-7R-S-GSH[14]. The SEI showed a high degree of glucuronidation, which may suggest hepatic-intestinal circulation of the molecule. In rats that underwent bile drainage surgery after gavage administration of *L. striatum* decoction, bile drainage enhanced SEI absorption[15]. The blocked hepatic-intestinal circulation may result in more SEI being transported from the liver into the plasma and less into the bile[15]. Moreover, SEI exhibits similar pharmacokinetic and BA profiles in rats and dogs, that is, rapid absorption, rapid clearance, moderate BA, and a dose-independent pharmacokinetic profile, suggesting that this metabolic profile may also be conserved in humans[16].

SEA is rapidly absorbed after oral administration, exhibits broad distribution and rapid elimination by different routes of administration, and has dose-independent pharmacokinetic parameters[17]. Unlike the high BA of SEI, the oral availability of SEA is only 8%[17]. During the absorption process, the largest SEA loss occurs in the gastrointestinal tract, accounting for 67%[17]. The first-pass effect of SEA in the liver is approximately 25%, which may be related to its nicotinamide adenine dinucleotide phosphate-dependent oxidation in the liver and binding to GSH[17]. Five metabolites were found in the rat plasma after oral, intravenous, or intraperitoneal administration of SEA, namely, 3-butylphthalide, 11-hydroxysenkyunolide A, 11-hydroxy-3-butylphthalide, the GSH conjugate of 7-hydroxysenkyunolide A, and the cysteine conjugate of 7-hydroxysenkyunolide A[17]. Liver metabolism is the primary mechanism underlying SEA elimination *in vivo*. The metabolic spectrum of SEA in mice, rats, dogs, monkeys, and human hepatocytes shows that the depletion of the parent molecule follows a first-order reaction, with rapid metabolism and elimination[18]. However, there are large differences in the metabolic rate among species, and metabolism is the slowest in human hepatocytes[18]. By analyzing the structure of metabolites in liver cells, the major metabolic pathways of SEA in the liver have been suggested as follows: 1) hydroxylation to form 10- and 11-hydroxysenkyunolide A, followed by epoxidation and binding with GSH; 2) direct epoxidation, followed by hydrolyzation or conjugation with GSH; and 3) aromatization to generate 3-butylphthalide, followed by hydroxylation to form hydroxy derivatives[18].

Notably, *L. striatum* was often processed together with yellow rice wine in ancient China to improve its efficacy. Several studies have tested the effects of wine processing on the pharmacokinetics of *L. striatum*. Ning et al.[19] compared the pharmacokinetic parameters of representative molecules in the plasma of healthy rats administered *L. striatum* extracts processed with and without wine. They found that compared to raw *L. striatum*-administered mice, the maximum plasma concentrations and area under the concentration-time curve (AUC) of butylidenephthalide, ligustilide, SEA, and ferulic acid decreased in the plasma of rats receiving wine-processed *L. striatum* extract, but the apparent volume of distribution increased significantly[19]. They speculated that wine processing expands the body distribution of the components in *L. striatum* extract and enhances its biological efficacy[19]. However, in another study, the maximum plasma concentrations and AUC of SEA and ferulic acid were significantly higher in middle cerebral artery occlusion rats treated with wine-processed *L. striatum* than in those treated with raw *L. striatum*[20]. Further studies are necessary to answer this question, and a better understanding of the pharmacokinetic characteristics of senkyunolides is important.

Pharmacological activity

Nervous system

Neuroprotective effect

Neuroprotective effects of senkyunolides have been demonstrated in several murine models. In a mouse model of intracerebral hemorrhage, SEH alleviated brain edema, neuronal damage, glial cell activation, and leukocyte infiltration[21]. Another study reported that in rats with cerebral ischemia (CI), SEH-loaded nanoparticle treatment reduced the neurological deficit score, cell apoptosis, and neuronal autophagy[22]. SEI also showed significant protective effects in ischemia-reperfusion (I/R) rats, including improved neural function, reduced infarct volume and brain edema, reversed brain morphological damage, and increased brain tissue superoxide

dismutase activity[23]. The beneficial effects of SEI treatment have also been reported in septic mice with diffuse brain dysfunction. SEI administration reduced neural inflammation and cell apoptosis in the hippocampus and improved memory loss[24]. The neuroprotective effects of senkyunolides have also been observed in cellular models of microglia, which are intrinsic immune effector cells within the central nervous system that play a central role in neuroinflammation[25]. SEH treatment attenuated the lipopolysaccharide (LPS)-mediated activation of BV2 microglia in a dose-dependent manner, and promoted the conversion of BV2 microglia from the M1 (pro-inflammatory) to the M2 (anti-inflammatory) phenotype[26]. In an oxygen-glucose deprivation/reoxygenation-treated microglia stroke model, SEI suppressed neuroinflammation by downregulating the transcription and expression of inflammation-related molecules[27].

At the molecular level, SEH and SEI ameliorated nerve injury through multiple biological pathways. The expression levels of pro-inflammatory factors, such as interleukin-6 (IL-6) and interleukin-1β (IL-1β), and the expression of peroxiredoxins 1, a kind of damage-associated molecular pattern involved in ischemic/hemorrhagic stroke, were found to be decreased with SEH treatment. SEI can directly inhibit nuclear factor-kappa B (NF-κB) activation and nuclear translocation, and indirectly inhibit NF-κB *via* the toll-like receptor 4/myeloid differentiation primary response gene 88 pathway[27]. In contrast, the expression of anti-oxidative factors such as GSH, catalase, and superoxide dismutase increased with SEH or SEI administration[21,26]. High-dose SEI (100 μM) can activate the nuclear factor erythroid 2–related factor 2 (Nrf2)/anti-oxidant response element pathway by upregulating extracellular signal–regulated kinase (ERK)1/2 phosphorylation and inducing Nrf2 nuclear translocation, thereby playing an anti-oxidant role[23]. In addition, both molecules showed anti-apoptotic activity and downregulated the expression of pro-apoptotic factor B-cell lymphoma-2–associated X protein, cytochrome C, and caspase-3[23,28]. SEH may also inhibit neuronal autophagy through the phosphoinositide 3-kinase/protein kinase B/mammalian target of the rapamycin signaling pathway[22], whereas SEI may regulate glutamate-induced calcium-dependent cell death signals by inhibiting c-Jun N-terminal kinase (JNK)/caspase-3 signaling pathway[29–30]. Nevertheless, the molecular mechanism mediating the therapeutic potential of senkyunolides in neural damage is rather complicated, and a causal relationship between the neuroprotective effects of these molecules and the above signaling pathways remains to be established.

BBB function regulation

The clinical application of some poorly liposoluble molecules is limited because it is difficult for them to reach the target tissue through the BBB. *L. striatum* is often used as a messenger drug in the TCM prescriptions for glioma and stroke, such as Jiuwei Tongqiao decoction, to facilitate the distribution of effective ingredients to the target site[31–32]. This suggests that certain components of *L. striatum* promote drug absorption through the BBB. Ligustilide, SEA, and SEI were reported to increase the apparent permeability coefficients of paeoniflorin in a concentration-dependent manner in human MDR1 gene-transfected Madin Darby canine kidney (MDCK-MDR1) cells, which are often used as models to study the BBB *in vitro*[31,33].

Further mechanistic research showed that these small molecules could reduce the expression of the cell efflux protein p-glycoprotein in a dose-dependent manner and open tight junctions between cells to promote BBB transport[31]. BBB function is closely related to the expression of tight junction proteins claudin-5 and zonula occludens-1 (ZO-1)[34–35]. In the oxygen-glucose deprivation/reoxygenation-induced MDCK-MDR1 model, SEA downregulated the expression levels of tight junction proteins ZO-1, occluding, and claudin-5 at the dose of both 40 and 80 μg/mL, whereas SEI upregulated the expression levels of all three tight junction proteins at 40 μg/mL, but downregulate the expression of ZO-1 at 80 μg/mL[36]. Nevertheless, in another study on MDCK-MDR1 cells, ligustilide, SEA, and SEI were found to downregulate the tight junction proteins claudin-5 and ZO-1 at 120 μg/mL and promote the uptake of the drug by the paracellular pathway[37]. These interesting findings suggest that SEI may have a two-way regulatory effect on BBB function; low doses of SEI led to a decrease in BBB permeability, whereas high doses resulted in an increase. However, whether this effect is specific to certain diseases and dependent on the duration of exposure requires further investigation. Further studies are necessary to clarify the specific mechanism of action of senkyunolides in BBB regulation, provide a reference for appropriate drug use, and determine the optimal timing for interventions.

Promoting neuronal growth

Neuronal axonal regeneration is regulated by multiple factors that play important roles in the recovery of neurological function[38–39]. Interestingly, a study using rat models of septic-associated encephalopathy reported that SEI can increase the expression of neuroglobin, a neuronal marker, in brain tissue, suggesting that SEI may play a role in promoting the growth of neuronal processes[40]. One possible mechanism is related to positive regulation of the p38 mitogen-activated protein kinase signaling pathway[40].

Cardiovascular system

Anti-coagulation and anti-thrombotic effect

L. striatum has long been used in China to treat thrombosis (remove blood stasis) especially in combination with other medicinal herbs such as *Salvia miltiorrhiza* Bunge. We previously reported the anti-thrombotic activity of *L. striatum* and SEI *in vivo* using erythrocyte-labeled *Tg*(*LCR: eGFP*) and platelet-labeled *Tg*(*CD41:eGFP*) transgenic zebrafish lines with phenylhydrazine-induced thrombosis[41]. Zhu et al.[42] compared the activities of Siwu decoction with or without SEI and paeoniflorin, and demonstrated that SEI is required for SWD to exert inhibitory effects on adenosine diphosphate (ADP)–induced platelet aggregation. The half maximal inhibitory concentration (IC_{50}) value

of SEI was approximately 140 μg/mL in the assay of ADP-induced platelet aggregation, indicating a strong anti-coagulant activity[43]. Mechanistically, SEI may play an anti-thrombotic role by regulating the coagulation cascade by downregulating the expression of coagulation factor VII. Moreover, the SEI was suggested to bind to thrombin and effectively inhibit its enzymatic activity with an IC_{50} value of 197.23 μM[44]. It was detected by molecular docking that SEI may interact with ASP189, ALA190, CYS191, CYS220, GLY216, GLY219, GLY226, PHE227, TRP215 on thrombin, and insert into its S1 catalytic active pocket, with a binding energy of –6.99 kcal/mol[45]. It would be interesting to further examine the therapeutic potential of SEI in mammalian thrombotic disease models.

Vasorelaxation effect

As the key category of molecules in *L. striatum*, whose primary role is to circulate *qi* according to the TCM theory, senkyunolides have long been suspected to have vasodilatory effects. A study on an isolated rat aorta contraction model stimulated with multiple vasoconstrictors, including the prostanoid TP receptor agonist U46619, α-adrenoceptor agonist phenylephrine, 5-hydroxytryptamine (5-HT), and potassium chloride, found consistent vasorelaxation effects of SEA and ligustilide, which were independent of the type of vasoconstrictor agent or the removal of the endothelium[46]. Using a luciferase reporter system, Lei et al.[47] found that SEA showed calcium-antagonistic activity in HEK 293 cells, possibly by blocking ryanodine receptors and voltage-operated Ca^{2+} channels, which could explain the vasodilatory activity. Based on the potential pharmacological activities of senkyunolides on vasodilation and increasing BBB permeability, it is tempting to speculate that these groups of compounds may indeed possess the biological effects implied in the TCM theory, such as facilitating chemical movement and diffusion.

Pro-angiogenesis effects and anti-proliferation of smooth muscle cells

Interestingly, several studies have suggested that senkyunolides play contrasting roles in the proliferation of vascular endothelial cells and smooth muscle cells of the aorta. Wang et al.[48] evaluated the effect of SEI on promoting the formation of the lumen structure *in vitro* using human microvascular endothelial cells and found that SEI may promote the angiogenesis of human microvascular endothelial cells by upregulating placental growth factor. Kobayashi et al.[49] studied the effect of the rhizome of *Cnidium officinale* on the proliferation of smooth muscle cells (SMC) in the primary SMC of the mouse aorta. SEH was suggested to be the major active compound responsible for the anti-SMC proliferative effect, with an IC_{50} value of less than 0.1 μg/mL[50]. Due to the limited research available, we cannot infer whether senkyunolides have contrasting regulatory effects on cell proliferation in the venous and arterial systems. However, the cell-specific regulatory effects of senkyunolides require further investigation.

Anti-inflammatory and analgesia

Anti-oxidant activity

Heme oxygenase-1 (HO-1) is an anti-oxidant protein regulated by the transcription factor Nrf2 that plays an important role in the cellular response to oxidative stress[51]. In mouse models of liver I/R injury, SEI treatment was found to protect liver function, which was also related to the regulation of HO-1 expression and anti-oxidative signaling[52]. In another study using a mouse renal I/R injury model[53], the anti-oxidant effects of SEI were also observed. Upregulated expression of Nrf2, HO-1, and nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase-1 was detected in SEI-treated mice, along with reduced production of reactive oxygen species (ROS), which was suggested to be related to alleviated inflammation and reduced cell apoptosis in the kidneys[53]. In hydrogen peroxide–stimulated HepG2 cells, the SEI was reported to induce the expression of HO-1 in a dose-dependent manner and inhibit the production of ROS and malondialdehyde[54]. In addition, SEI may downregulate the expression of endoplasmic reticulum stress–related proteins GRP78 and CHOP, thereby inhibiting endoplasmic reticulum stress-induced cell death[53]. These findings suggest that SEI holds promise as a potential therapeutic intervention for oxidative stress–related liver and kidney injuries; however, future research is needed to further explore its mechanisms of action and evaluate its efficacy in clinical settings.

Inhibition of inflammatory reaction

Multiple senkyunolides have been suggested as the major active substances that drive the anti-inflammatory and immunomodulatory roles of *L. striatum*–containing TCM formulae. For example, a study comparing the therapeutic effects of the Suxiao Jiuxin pill (SX, a TCM for cardiovascular diseases) and atorvastatin on atherosclerosis showed that SX plays a regulatory role in atherosclerosis-related immune responses[55]. In the SX mixture, SEA was identified as a key molecule that significantly downregulated the expression of the tumor necrosis factor (TNF)-α–induced CD137, which is a member of the TNF receptor superfamily associated with increased plaque necrosis, macrophage infiltration, and decreased collagen content in the progression of unstable atherosclerotic plaques[55–56]. In a study using an osteoarthritis mouse model, SEA treatment was found to alleviate osteoarthritis progression by inhibiting the expression of multiple inflammatory cytokines and the NLRP3 signaling pathway[57]. Several studies have reported the anti-inflammatory effects of SEA in different cell models, *via* blocking the release of IL-1β–induced pro-inflammatory mediator nitric oxide (NO), inhibiting the expression of nod-like receptor family pyrin domain-containing 3, and downregulating the expression of inflammatory cytokines (IL-1β, IL-6, TNF-α) and chemokines (C-C motif chemokine ligand 20)[57–58].

The SEI also exhibits anti-inflammatory activity. Xuebijing injection is a TCM formula with *L. striatum* as the key herbal component. Multiple clinical trials have demonstrated that Xuebijing improves 28-day mortality, Acute Physiology and Chronic Health Evaluation (APACHE) II score, and white blood cell (WBC) count in

patients with sepsis[59]. Jiang et al.[60] used a cecal ligation and puncture (CLP)–induced sepsis model to screen active ingredients with anti-inflammatory effects in Xuebijing injection, and found that SEI inhibited TNF-α–induced NF-κB activation, and reversed the increase of IL-6 and IL-8 in LPS-stimulated human monocyte THP-1[60]. SEI effectively inhibited the infiltration of neutrophils and the formation of neutrophil extracellular traps in the lungs of CLP-treated mice and reduced the levels of inflammatory factors and oxidative stress in the lung homogenate[61]. In addition, the SEI directly inhibited the formation of neutrophil extracellular traps in neutrophils treated with 12-myristate 13-acetate or CLP mouse platelets *in vitro*[61]. Although multiple botanical compounds have been reported to exhibit anti-inflammatory activities, the unique *in vivo* diffusion properties of senkyunolides may enhance their potential value in anti-inflammatory treatments at specific sites, such as the central nervous system. Further exploration of their applications in targeted anti-inflammatory therapies, particularly in the context of the central nervous system, holds promise.

Anti-pyretic-analgesia effect

Non-steroidal anti-inflammatory drugs act on cyclooxygenase (COX) and the production of prostaglandins to relieve the symptoms of fever and pain at the inflammatory site[62]. SEO was reported to have selective inhibitory activity against COX-2, which was more than five times that against COX-1[63]. Bae et al.[64] showed that SEH downregulates COX-2 mRNA levels in LPS-stimulated macrophages RAW 264.7 in a dose-dependent manner, which may play a role in COX-2 inhibition and anti-inflammatory. The inhibitory activity of the senkyunolides on COX may be responsible for the analgesic effect of *L. striatum*, providing a basis for the development of new analgesic/anti-pyretic drugs.

Other effects

Anti-migraine activity

The exact pathophysiology of migraines is not fully understood[65]. According to the neurovascular hypothesis, migraine is triggered by the activation of the trigeminovascular system, resulting in neurogenic inflammation and pain impulses, which are transmitted to higher cortical pain centers of the brain through the trigeminovascular system[65]. A pharmacokinetic study reported significant differences in SEI between migraine rats and normal rats, suggesting that SEI may be a key effective component of *L. striatum* in the treatment of migraine[66]. In migraine rats, the clearance rate of SEI decreased by 68%, the apparent volume of distribution increased by 342%, and the half-life time and AUC also increased significantly[67]. The change in 5-HT levels is considered a key factor in a migraine attack. SEI can improve the metabolism of monoamine neurotransmitters in the plasma and brain tissue of nitroglycerin-induced migraine rats, and maintain a stable level of 5-HT[68]. Cortical spreading depression is an important pathophysiological mechanism that explains the migraine aura. In a potassium chloride–treated rat model of cortical spreading depression, it was observed that high-dose SEI significantly inhibited the change in brain potential amplitude and reduced the frequency of brain potential waves[69]. Moreover, the SEI reduced the content of calcitonin gene–related peptides and NO, two important substances that cause vasodilation and are also related to migraine[69]. In another study, chemometric analysis was used to evaluate the anti-migraine activity of 18 small molecules from *L. striatum* and *Cyperus rotundus* L., SEI was not identified as an anti-migraine active ingredient[70]. Combined with its pharmacokinetic characteristics, one speculation is that SEI is rapidly removed from the plasma after oral administration, resulting in difficulty in detection. Whether SEI is a promising candidate for migraine treatment requires further study.

Insecticidal and bactericidal activity

Kim et al.[71] found that *Cnidium officinale* hexane extract showed antibacterial activity against *Propionibacterium acnes* at a concentration of 50 mg/mL, in which SEA was the major active substance. SEA also showed significant antibacterial effects against *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Pythium aphanidermatum*, *Alternaria alternata*, and *Didymella glomerata*, with minimum inhibitory concentrations of 7.81, 250, 250, and 250 mg/L, respectively[72]. Thus, senkyunolides are expected to become novel botanical fungicides. However, a significant amount of research is still needed to investigate the mechanisms of action of these compounds and evaluate their *in vivo* effects. Further studies are required to fully understand the potential of these compounds and their implications for clinical applications.

Anti-tumor activity

SEA displayed significant anti-mutagenic activity against the *S. Typhimurium* strain TA100 mutation induced by 2-nitrofluorone, and strongly inhibited mouse epidermal JB6 P^+ cell transformation and colony formation induced by epidermal growth factor in a dose-dependent manner with an IC_{50} value of approximately 0.2 μg/mL[73]. SEA may block the transmission of carcinogenic signals by inhibiting the expression of activator protein-1, the main transcription factor involved in epidermal growth factor-induced cell transformation[73]. Nevertheless, research on the anti-tumor activity of senkyunolides remains limited and *in vivo* studies are needed to confirm this effect.

Anti-osteoporosis activity

Excessive osteoclast production leads to osteoporosis, which is common in postmenopausal women[74]. SEH has been reported to alleviate osteoporosis by increasing bone mineral density and trabecular thickness/number, and reducing trabecular space and the number of tartrate-resistant acid phosphatase–positive osteoclasts in ovariectomized mice[75]. Moreover, SEH dose-dependently inhibited the differentiation of bone marrow–derived macrophages and RAW264.7 cells to osteoclasts under the stimulation of the receptor activator of nuclear factor-κB ligand and reduced the bone absorption function of osteoclasts[75]. SEH can also inhibit NF-κB, JNK,

and ERK signal pathways and downregulate the gene expression of cathepsin K, recombinant nuclear factor of activated T-cells cytoplasmic 1, TNF receptor–associated factor 6, c-fos, and dendritic cell–specific transmembrane protein, thus inhibiting osteoclast formation[75].

Anti-fibrotic activity

The therapeutic effect of SEA on renal interstitial fibrosis (RIF) has been reported in a rat model with unilateral ureteral obstruction[76]. Moreover, SEA was found to effectively alleviate renal interstitial injury, inflammatory cell infiltration, and fibrous tissue proliferation, which could be related to the potential inhibitory role of SEA on the Wnt/β-catenin signaling[76]. In addition, after SEA treatment, reduced expression of the mesenchymal phenotypic characteristic markers vimentin and α-smooth muscle actin was detected, together with elevated expression of the epithelial phenotypic characteristic marker E-cadherin, and reduced Collagen I in the renal tissue[76]. Thus, SEA may exert anti-RIF activity by inhibiting the epithelial-mesenchymal transformation. The potential efficacy of SEA in fibrosis goes beyond the traditional pharmacological activity of *L. striatum* and is an inviting new field for future investigation.

Toxicity analysis

Although toxicity studies on senkyunolides are not yet available, several groups have evaluated the toxicity of *L. striatum* using different model systems. In pregnant mice, *L. striatum* of 2 or 8 g/kg/day showed no impact on maternal or embryonic development, whereas an increased number of absorbed fetuses was observed in pregnant mice treated with 32 g/kg/day *L. striatum* decoction[77]. In another study, using the essential oil extracted from *L. striatum* (contains mostly ligustilide and senkyunolides), the oral and intraperitoneal median lethal dose in mice was 7.23 g/kg (approximately 14,606 times the clinical dose) and 2.25 g/kg (approximately 5,091 times the clinical dose)[78]. On rabbit skin, doses of 0.115 and 0.23 g/kg *L. striatum* essential oil (approximately 232.5 and 465 times of the respective clinical doses used) revealed slight irritation effects, but 1 g/kg treatment (approximately 2020 the clinical dose used) showed no observable effect on guinea pig skin in the skin sensitization test[78]. Although the aforementioned experiments suggest that *L. striatum* is safe in most experimental settings, further confirmation is needed regarding the *in vivo* toxicity of senkyunolides.

Summary and future scope

In summary, based on recent pharmacological studies, senkyunolides have diverse biological effects, including anti-inflammation, anti-oxidative stress, anti-coagulation/thrombosis, vasodilatory, neuroprotective, anti-migraine, and analgesic effects (Figure 1). In addition, some studies have suggested novel roles for

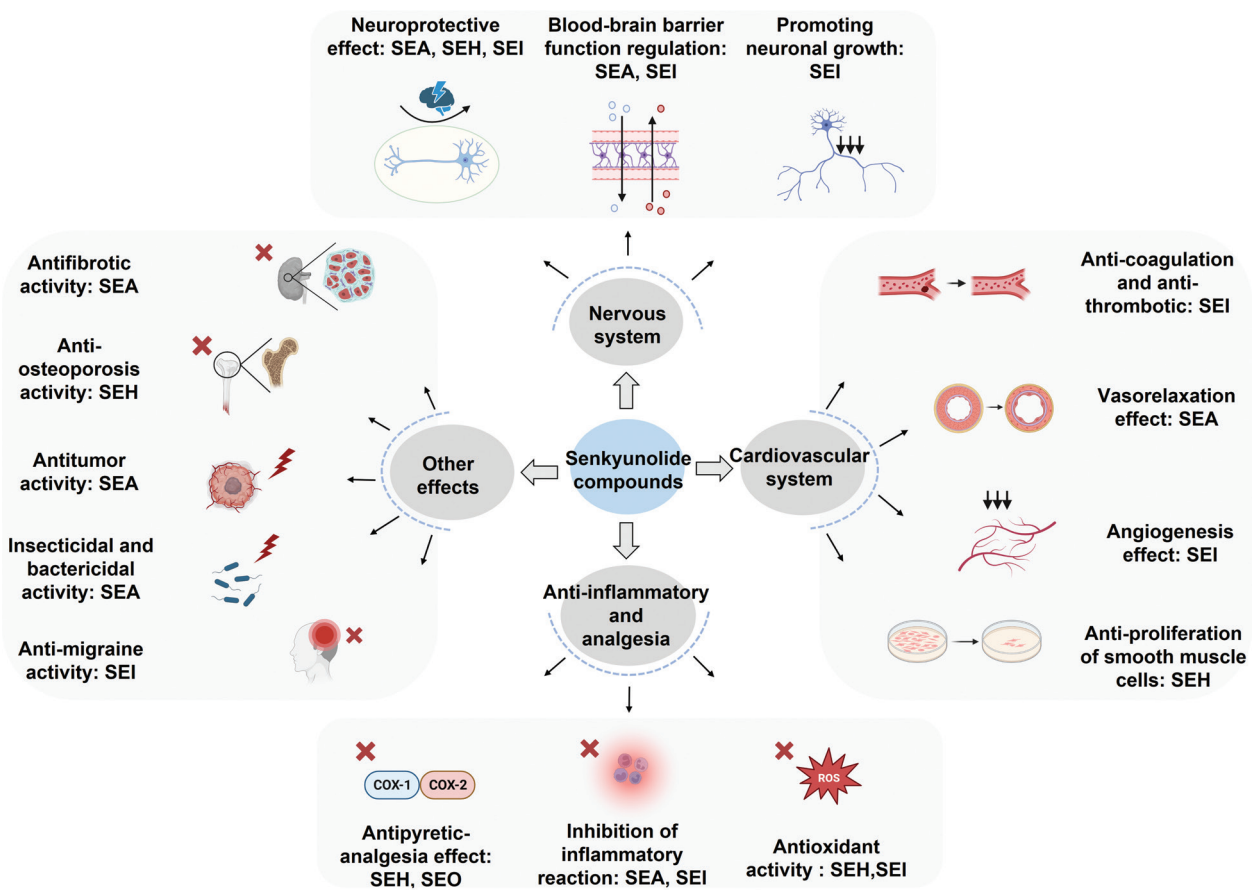

Figure 1. Overview of the pharmacological activities of senkyunolides. SEA: Senkyunolide A; SEH: Senkyunolide H; SEI: Senkyunolide I; SEO: Senkyunolide O.

senkyunolides in regulating the BBB, preventing the onset of cancer, anti-RIF, treating osteoporosis, and bactericidal effects (Figure 1). Several years of clinical experience have shown that TCM has high safety and significant biological activity, and many natural products screened from herbal medicines have become potential drug candidates. Given the numerous pharmacological effects of senkyunolides, it is valuable to further develop and characterize this special class of natural molecules.

Finally, the structure of senkyunolides is unstable, which may limit their clinical use. This problem can be solved by designing and synthesizing structural analogs with both stability and pharmacological activity. For example, the structure of SEA is similar to that of racemic 3-n-butylphthalide, which has been approved to treat ischemic stroke[79]. Fang et al.[80] designed 12 novel senkyunolide analogs and evaluated their biological activity. Among them, derivatives containing the NO-donated group 5-hydroxy benzofuran-3-one showed significant protective effects in the HT-22 cell oxygen-glucose deprivation model, providing a new idea for the structural modification of natural products with neuroprotective activity[80]. In addition to monomeric phthalide derivatives, many new phthalide dimers and polymers have been discovered. Chuanxiongdiolides R4-R6 identified from the aboveground part of *L. striatum* belong to three new types of phthalide dimers, of which Chuanxiongdiolides R4 and R6 have significant vasodilative effects, which may depend on their effects on the L-type calcium channel $Ca_V1.2$ activation blockade[81]. This study provides a good example of the discovery of small molecules with potential biological activities from undeveloped medicinal plants.

Conclusions

Multifaceted pharmacological effects of senkyunolides have been reported in recent years, including neuroprotection, cardiovascular protection, anti-inflammation, anti-oxidative stress, and multiple novel applications, highlighting the potential of this natural compound family as a valuable pool of drug candidates for a broad range of diseases. However, the detailed molecular mechanisms underlying the pharmacological activities remain unclear. Future studies combining multi-disciplinary approaches in pharmaceutical analysis, pharmacology, and medicinal chemistry of senkyunolides are warranted to unravel the underlying molecular mechanisms of action, explore the *in vivo* efficacy in diverse models, and ultimately translate these findings into clinical applications.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

Lu Zhao conceived the study and revised the manuscript. Qingquan Li collected the published data, prepared the manuscript, and prepared the figure. Jian-Bo Wan revised the manuscript. All the authors have read and approved the final manuscript.

Ethical approval of studies and informed consent

Not applicable.

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None.

Data availability statement

All data generated or analyzed during this study are included in this published article.

References

[1] Chinese Pharmacopoeia Commission. *Chinese Pharmacopoeia 1*. Beijing: China Medical Science Press; 2020.
[2] Li H, Hu S, Zhou Y, et al. Pharmacology progress on the main bioactive components of Ligusticum chuanxiong hort. *Chin J Clin Pharmacol Ther* 2018;23(11):1302–1308.
[3] Wei X, Zeng Y, Sun C, et al. Recent advances in natural phthalides: distribution, chemistry, and biological activities. *Fitoterapia* 2022;160:105223.
[4] Pannek J, Gach J, Boratyński F, et al. Antimicrobial activity of extracts and phthalides occurring in Apiaceae plants. *Phytother Res* 2018;32(8):1459–1487.
[5] Chen WR, Chou CC, Wang CC. Phthalides serve as potent modulators to boost fetal hemoglobin induction therapy for β-hemoglobinopathies. *Blood Adv* 2019;3(9):1493–1498.
[6] Zhang LJ, Liu JY, Yao C, et al. Research progress in pharmacological activities of Senkyunolides. *Chin Pharm J* 2015;50(13):1081–1084.
[7] Lyu J-L, Duan J-A, Tang Y-P, et al. Phthalide mono- and dimers from the radix of Angelica sinensis. *Biochem Syst Ecol* 2009;37(4):405–411.
[8] Yang Y, Feng Y, Chen H, et al. Research advance in phthalides and their biological activities. *Nat Prod Res Dev* 2022;34(8):1439–1453.
[9] Zuo A, Wang L, Xiao H, et al. Identification of the absorbed components and metabolites in rat plasma after oral administration of Rhizoma Chuanxiong decoction by HPLC-ESI-MS/MS. *J Pharm Biomed Anal* 2011;56(5):1046–1056.
[10] Liu Y, Luo Z-Q, Lyu B-R, et al. Absorption and metabolism of Chuanxiong Rhizoma decoction with multi-component sequential metabolism method. *Chin J Chin Mat Med* 2016;41(7):1178–1182.
[11] Tao S, Li H, Liu J. Metabolic profiling of ligustilide and identification of the metabolite in rat and human hepatocytes by liquid chromatography combined with high-resolution mass spectrometry. *J Sep Sci* 2020;43(24):4405–4413.
[12] He CY, Wang S, Feng Y, et al. Pharmacokinetics, tissue distribution and metabolism of senkyunolide I, a major bioactive component in Ligusticum chuanxiong Hort. (Umbelliferae). *J Ethnopharmacol* 2012;142(3):706–713.
[13] Xiong YK, Lin X, Liang S, et al. Identification of senkyunolide I metabolites in rats using ultra performance liquid chromatography/quadrupole-time-of-flight tandem mass spectrometry. *J Pharm Biomed Anal* 2013;81-82:178–186.
[14] Ma Q, Ma C, Wu F, et al. Preparation and structural determination of four metabolites of senkyunolide I in rats using ultra performance liquid chromatography/quadrupole-time-of-flight tandem mass and nuclear magnetic resonance spectra. *BMC Complement Altern Med* 2016;16(1):504.
[15] Li H, Jiang Y, Wang Y, et al. The effects of Warfarin on the pharmacokinetics of senkyunolide I in a rat model of biliary drainage after administration of Chuanxiong. *Front Pharmacol* 2018;9:1461.

[16] Li JQ, Wang JF, Li J, et al. A validated LC-MS/MS method for the determination of senkyunolide I in dog plasma and its application to a pharmacokinetic and bioavailability studies. *Biomed Chromatogr* 2018;32(5):e4182.
[17] Yan R, Lin G, Ko NL, et al. Low oral bioavailability and pharmacokinetics of senkyunolide a, a major bioactive component in Rhizoma Chuanxiong, in the rat. *Ther Drug Monit* 2007;29(1):49–56.
[18] Zhang H, Liu C, Wang M, et al. Metabolic profiling of senkyunolide A and identification of its metabolites in hepatocytes by ultra-high-performance liquid chromatography combined with diode-array detector and high-resolution mass spectrometry. *Rapid Commun Mass Spectrom* 2020;34(21):e8894.
[19] Ning Y, Pei K, Cao G, et al. Comparative study on pharmacokinetics of four active compounds in rat plasma after oral administration of raw and wine processed Chuanxiong Rhizoma. *Molecules* 2019;25(1):93.
[20] Pei K, Cao L, Cao G, et al. A reasonable evaluation of Chuanxiong Rhizoma processing with wine through comparative pharmacokinetic study of bioactive components: dominant effect on middle cerebral artery occlusion model rats. *J Anal Methods Chem* 2022;2022:8252038.
[21] Han L, Liu DL, Zeng QK, et al. The neuroprotective effects and probable mechanisms of Ligustilide and its degradative products on intracerebral hemorrhage in mice. *Int Immunopharmacol* 2018;63:43–57.
[22] Zhao B, Tang KC, Zhao Y, et al. Senkyunolide H affects cerebral ischemic injury through regulation on autophagy of neuronal cells *via* P13K/AKT/mTOR signaling pathway. *Dis Markers* 2022;2022:9211621.
[23] Hu Y, Duan M, Liang S, et al. Senkyunolide I protects rat brain against focal cerebral ischemia-reperfusion injury by up-regulating p-Erk1/2, Nrf2/HO-1 and inhibiting caspase 3. *Brain Res* 2015;1605:39–48.
[24] Xie J, Zhao ZZ, Li P, et al. Senkyunolide I protects against sepsis-associated encephalopathy by attenuating sleep deprivation in a murine model of cecal ligation and puncture. *Oxid Med Cell Longevity* 2021;2021:6647258.
[25] Shabab T, Khanabdali R, Moghadamtousi SZ, et al. Neuroinflammation pathways: a general review. *Int J Neurosci* 2017;127(7):624–633.
[26] Tan J, Li W, Teng Z, et al. Senkyunolide H inhibits activation of microglia and attenuates lipopolysaccharide-mediated neuroinflammation and oxidative stress in BV2 microglia cells *via* regulating ERK and NF-κB pathway. *Kaohsiung J Med Sci* 2022;38(4):378–384.
[27] Hu YY, Wang Y, Liang S, et al. Senkyunolide I attenuates oxygen-glucose deprivation/reoxygenation-induced inflammation in microglial cells. *Brain Res* 2016;1649(Pt A):123–131.
[28] Luo Y, Li X, Liu T, et al. Senkyunolide H protects against MPP(+)-induced apoptosis *via* the ROS-mediated mitogen-activated protein kinase pathway in PC12 cells. *Environ Toxicol Pharmacol* 2019;65:73–81.
[29] Gu Y, Huang P, Cheng T, et al. A multiomics and network pharmacological study reveals the neuroprotective efficacy of Fu-Fang-Dan-Zhi tablets against glutamate-induced oxidative cell death. *Comput Biol Med* 2022;148:105873.
[30] Wang M, Hayashi H, Horinokita I, et al. Neuroprotective effects of senkyunolide I against glutamate-induced cells death by attenuating JNK/caspase-3 activation and apoptosis. *Biomed Pharmacother* 2021;140:111696.
[31] Hu PY, Liu D, Zheng Q, et al. Elucidation of transport mechanism of paeoniflorin and the influence of ligustilide, senkyunolide I and senkyunolide A on paeoniflorin transport through Mdck-Mdr1 cells as blood-brain barrier *in vitro* model. *Molecules* 2016;21(3):300.
[32] Shuai S-Y, Liu S-S, Liu X-J, et al. Essential oil of Ligusticum chuanxiong Hort. Regulated P-gp protein and tight junction protein to change pharmacokinetic parameters of temozolomide in blood, brain and tumor. *J Ethnopharmacol* 2022;298:115646.
[33] Hellinger E, Veszelka S, Tóth AE, et al. Comparison of brain capillary endothelial cell-based and epithelial (MDCK-MDR1, Caco-2, and VB-Caco-2) cell-based surrogate blood-brain barrier penetration models. *Eur J Pharm Biopharm* 2012;82(2):340–351.
[34] Hashimoto Y, Greene C, Munnich A, et al. The CLDN5 gene at the blood-brain barrier in health and disease. *Fluids Barriers CNS* 2023;20(1):22.
[35] Wang Y, Peng F, Xie G, et al. Rhubarb attenuates blood-brain barrier disruption *via* increased zonula occludens-1 expression in a rat model of intracerebral hemorrhage. *Exp Ther Med* 2016;12(1):250–256.
[36] Liu S-S, Zheng Q, Yue P-F, et al. Effect and mechanism of phthalides from Ligusticum chuanxiong on oxygen-glucose deprivation/reperfusion-induced MDCK-MDR1 cell injury. *Chin Tradit Herbal Drugs* 2021;52(10):2958–2966.
[37] Zheng Q, Tang Y, Hu PY, et al. The influence and mechanism of ligustilide, senkyunolide I, and senkyunolide A on echinacoside transport through MDCK-MDR1 cells as blood-brain barrier *in vitro* model. *Phytother Res* 2018;32(3):426–435.
[38] Mahar M, Cavalli V. Intrinsic mechanisms of neuronal axon regeneration. *Nat Rev Neurosci* 2018;19(6):323–337.
[39] Doron-Mandel E, Fainzilber M, Terenzio M. Growth control mechanisms in neuronal regeneration. *FEBS Lett* 2015;589(14):1669–1677.
[40] Zheng S, L X, Yang L, et al. Study on cerebral protective effect of Senkyunolide trol mechanisms in neuronal regeneration. ion p38 MAPK signaling pathway. *Tradit Chin Drug Res Clin Pharmacol* 2019;30(9):1083–1087.
[41] Li J, Liu H, Yang Z, et al. Synergistic effects of cryptotanshinone and ssenkyunolide I in Guanxinning tablet against endogenous thrombus formation in Zebrafish. *Front Pharmacol* 2020;11:622787.
[42] Zhu M, Tang Y, Duan JA, et al. Roles of paeoniflorin and senkyunolide I in SiWu decoction on antiplatelet and anticoagulation activities. *J Sep Sci* 2010;33(21):3335–3340.
[43] Zhang K, Shen X, Yang L, et al. Exploring the Q-markers of Angelica sinensis (Oliv.) Diels of anti-platelet aggregation activity based on spectrum-effect relationships. *Biomed Chromatogr* 2022;36(9):e5422.
[44] Zhang Q, Yang YX, Li SY, et al. An ultrafiltration and high performance liquid chromatography coupled with diode array detector and mass spectrometry approach for screening and characterizing thrombin inhibitors from Rhizoma Chuanxiong. *J Chromatogr B Analyt Technol Biomed Life Sci* 2017;1061-1062:421–429.
[45] Yang YY, Wu ZY, Xia FB, et al. Characterization of thrombin/factor Xa inhibitors in Rhizoma Chuanxiong through UPLC-MS-based multivariate statistical analysis. *Chin Med* 2020;15:93.
[46] Chan SS, Cheng TY, Lin G. Relaxation effects of ligustilide and senkyunolide A, two main constituents of Ligusticum chuanxiong, in rat isolated aorta. *J Ethnopharmacol* 2007;111(3):677–680.
[47] Lei W, Ni J, Xia X, et al. Searching for synergistic calcium antagonists and novel therapeutic regimens for coronary heart disease therapy from a Traditional Chinese Medicine, Suxiao Jiuxin Pill. *J Chromatogr B Analyt Technol Biomed Life Sci* 2018;1092:220–227.
[48] Wang S, Feng Y, Wang Y, et al. Senkyunolide I promotes endothelial cells angiogenesis through PIGF signaling pathway. *Pharm Biotechnol* 2011;18(3):211–214.
[49] Kobayashi S, Mimura Y, Naitoh T, et al. Chemical structure-activity of cnidium rhizome-derived phthalides for the competence inhibition of proliferation in primary cultures of mouse aorta smooth muscle cells. *Jpn J Pharmacol* 1993;63(3):353–359.
[50] Kobayashi S, Mimura Y, Notoya K, et al. Antiproliferative effects of the traditional Chinese medicine shimotsu-to, its component cnidium rhizome and derived compounds on primary cultures of mouse aorta smooth muscle cells. *Jpn J Pharmacol* 1992;60(4):397–401.
[51] Araujo J, Zhang M, Yin F. Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. *Front Pharmacol* 2012;3:119.
[52] Yang Q, Zhao ZZ, Xie J, et al. Senkyunolide I attenuates hepatic ischemia/reperfusion injury in mice *via* anti-oxidative, anti-inflammatory and anti-apoptotic pathways. *Int Immunopharmacol* 2021;97:107717.
[53] Zhu YL, Huang J, Chen XY, et al. Senkyunolide I alleviates renal ischemia-reperfusion injury by inhibiting oxidative stress, endoplasmic reticulum stress and apoptosis. *Int Immunopharmacol* 2022;102:108393.
[54] Qi H, Siu SO, Chen Y, et al. Senkyunolides reduce hydrogen peroxide-induced oxidative damage in human liver HepG2 cells *via* induction of heme oxygenase-1. *Chem Biol Interact* 2010;183(3):380–389.
[55] Lei W, Deng YF, Hu XY, et al. Phthalides, senkyunolide A and ligustilide, show immunomodulatory effect in improving atherosclerosis, through inhibiting AP-1 and NF-κB expression. *Biomed Pharmacother* 2019;117:109074.
[56] Jung IH, Choi JH, Jin J, et al. CD137-inducing factors from T cells and macrophages accelerate the destabilization of atherosclerotic plaques in hyperlipidemic mice. *FASEB J* 2014;28(11):4779–4791.

[57] Shao M, Lyu D, Zhou K, et al. Senkyunolide A inhibits the progression of osteoarthritis by inhibiting the NLRP3 signalling pathway. *Pharm Biol* 2022;60(1):535–542.
[58] Ningsih FN, Okuyama T, To S, et al. Comparative analysis of anti-inflammatory activity of the constituents of the rhizome of Cnidium officinale using rat hepatocytes. *Biol Pharm Bull* 2020;43(12):1867–1875.
[59] Li C, Wang P, Li M, et al. The current evidence for the treatment of sepsis with Xuebijing injection: bioactive constituents, findings of clinical studies and potential mechanisms. *J Ethnopharmacol* 2021;265:113301.
[60] Jiang M, Zhou M, Han Y, et al. Identification of NF-κB inhibitors in Xuebijing injection for sepsis treatment based on bioactivity-integrated UPLC-Q/TOF. *J Ethnopharmacol* 2013;147(2):426–433.
[61] Zha YF, Xie J, Ding P, et al. Senkyunolide I protect against lung injury *via* inhibiting formation of neutrophil extracellular trap in a murine model of cecal ligation and puncture. *Int Immunopharmacol* 2021;99:107922.
[62] Stiller CO, Hjemdahl P. Lessons from 20 years with COX-2 inhibitors: importance of dose-response considerations and fair play in comparative trials. *J Intern Med* 2022;292(4):557–574.
[63] Cao H, Yu R, Choi Y, et al. Discovery of cyclooxygenase inhibitors from medicinal plants used to treat inflammation. *Pharmacol Res* 2010;61(6):519–524.
[64] Bae KE, Choi YW, Kim ST, et al. Components of rhizome extract of Cnidium officinale Makino and their *in vitro* biological effects. *Molecules* 2011;16(10):8833–8847.
[65] Peters GL. Migraine overview and summary of current and emerging treatment options. *Am J Manag Care* 2019;25(2 Suppl):S23–S34.
[66] Zhao X, Ma T, Zhang C, et al. Simultaneous determination of senkyunolide I and senkyunolide H in rat plasma by LC-MS: application to a comparative pharmacokinetic study in normal and migrainous rats after oral administration of Chuanxiong Rhizoma extract. *Biomed Chromatogr* 2015;29(9):1297–1303.
[67] Wang Y-H, Hong Y-L, Feng Y, et al. Comparative pharmacokinetics of senkyunolide I in a rat model of migraine versus normal controls. *Eur J Drug Metab Pharmacokinet* 2012;37(2):91–97.
[68] Mittal M, Siddiqui MR, Tran K, et al. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 2014;20(7):1126–1167.
[69] DuanMu Y, Gu CC, Dong X, et al. Pharmacodynamic action of Senkyunolide I on cortical spreading depression in migraine rats. *Tradit Chin Drug Res Clin Pharmacol* 2013;24(3):217–221.
[70] Guo L, Gong M, Wu S, et al. Identification and quantification of the quality markers and anti-migraine active components in Chuanxiong Rhizoma and Cyperi Rhizoma herbal pair based on chemometric analysis between chemical constituents and pharmacological effects. *J Ethnopharmacol* 2020;246:112228.
[71] Kim TY, Kwon HC, Lee SY, et al. Antibacterial activity of senkyunolide A isolated from Cnidium Officinale extract. *J Cosmet Sci* 2020;71(6):377–383.
[72] Chen H, Zhao Y, Qin G, et al. Antifungal effects and active components of Ligusticum chuanxiong. *Molecules* 2022;27(14):4589.
[73] Liu MM, Huang KM, Qian L, et al. Effects of bioactive constituents in the Traditional Chinese Medicinal formula Si-Wu-Tang on Nrf2 signaling and neoplastic cellular transformation. *Phytomedicine* 2018;40:1–9.
[74] Wang L-T, Chen L-R, Chen K-H. Hormone-related and drug-induced osteoporosis: a cellular and molecular overview. *Int J Mol Sci* 2023;24(6):5814.
[75] Yang D, Liu T, Jiang G, et al. Senkyunolide H attenuates osteoclastogenesis and postmenopausal osteoporosis by regulating the NF-κB, JNK and ERK signaling pathways. *Biochem Biophys Res Commun* 2020;533(3):510–518.
[76] Zheng L, C X, Yin B, et al. Effect and mechanism of senkyunolide A on renal interstitial fibrosis in unilateral ureteral obstruction rats. *Chin J Surg Integr Tradit West Med* 2020;26(2):237–242.
[77] Wang H, Bao Q, Yi H, et al. The evaluation of embryotoxicity of Ligusticum chuanxiong on mice and embryonic stem cells. *J Ethnopharmacol* 2019;239:111895.
[78] Zhang H, Han T, Yu CH, et al. Analysis of the chemical composition, acute toxicity and skin sensitivity of essential oil from rhizomes of Ligusticum chuanxiong. *J Ethnopharmacol* 2012;144(3):791–796.
[79] Wang S, Ma F, Huang L, et al. Dl-3-n-butylphthalide (NBP): a promising therapeutic agent for ischemic stroke. *CNS Neurol Disord Drug Targets* 2018;17(5):338–347.
[80] Fang Y, Wang R, Wang Q, et al. Design and synthesis of novel senkyunolide analogues as neuroprotective agents. *Bioorg Med Chem Lett* 2018;28(4):668–672.
[81] Tang F, Yan YM, Yan HL, et al. Chuanxiongdiolides R4 and R5, phthalide dimers with a complex polycyclic skeleton from the aerial parts of Ligusticum chuanxiong and their vasodilator activity. *Bioorg Chem* 2021;107:104523.

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