

# Research progress on the pharmacological activities of senkyunolides

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## 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- $\kappa$ B) 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

**Graphical abstract:** <http://links.lww.com/AHM/A66>.

## 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<sup>[1]</sup>. Modern pharmacological studies have shown that *L. striatum* exhibits anti-oxidative, neuroprotective, anti-inflammatory, and antibacterial activities<sup>[2]</sup>. 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<sup>[2]</sup>. Recent pharmacological studies have suggested that phthalide molecules are among the most important active substances in *L. striatum*<sup>[3–5]</sup>. 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<sup>[6]</sup>. 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)<sup>[7–8]</sup>. 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)<sup>[9]</sup>. 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<sup>[10]</sup>. 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<sup>[10]</sup>. 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<sup>[11]</sup>. 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%<sup>[12]</sup>. The pharmacokinetic parameters were not related to dosage in either administration approach<sup>[12]</sup>. The BA of SEI was 81.17% when administered *via* the intrahepatic portal vein, with an 18.83% first-pass effect on the liver<sup>[12]</sup>. 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<sup>[12]</sup>. 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)<sup>[12]</sup>. Xiong et al.<sup>[13]</sup> 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<sup>[13]</sup>. 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<sup>[14]</sup>. 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<sup>[15]</sup>. The blocked hepatic-intestinal circulation may result in more SEI being transported from the liver into the plasma and less into the bile<sup>[15]</sup>. 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<sup>[16]</sup>.

SEA is rapidly absorbed after oral administration, exhibits broad distribution and rapid elimination by different routes of administration, and has dose-independent pharmacokinetic parameters<sup>[17]</sup>. Unlike the high BA of SEI, the oral availability of SEA is only 8%<sup>[17]</sup>. During the absorption process, the largest SEA loss occurs in the gastrointestinal tract, accounting for 67%<sup>[17]</sup>. 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<sup>[17]</sup>. 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<sup>[17]</sup>. 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<sup>[18]</sup>. However, there are large differences in the metabolic rate among species, and metabolism is the slowest in human hepatocytes<sup>[18]</sup>. 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<sup>[18]</sup>.

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.<sup>[19]</sup> 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<sup>[19]</sup>. They speculated that wine processing expands the body distribution of the components in *L. striatum* extract and enhances its biological efficacy<sup>[19]</sup>. 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*<sup>[20]</sup>. 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<sup>[21]</sup>. Another study reported that in rats with cerebral ischemia (CI), SEH-loaded nanoparticle treatment reduced the neurological deficit score, cell apoptosis, and neuronal autophagy<sup>[22]</sup>. 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<sup>[23]</sup>. 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<sup>[24]</sup>. 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<sup>[25]</sup>. 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<sup>[26]</sup>. In an oxygen-glucose deprivation/reoxygenation-treated microglia stroke model, SEI suppressed neuroinflammation by downregulating the transcription and expression of inflammation-related molecules<sup>[27]</sup>.

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-1 $\beta$  (IL-1 $\beta$ ), 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- $\kappa$ B) activation and nuclear translocation, and indirectly inhibit NF- $\kappa$ B *via* the toll-like receptor 4/myeloid differentiation primary response gene 88 pathway<sup>[27]</sup>. In contrast, the expression of anti-oxidative factors such as GSH, catalase, and superoxide dismutase increased with SEH or SEI administration<sup>[21,26]</sup>. High-dose SEI (100  $\mu$ M) 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<sup>[23]</sup>. 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<sup>[23,28]</sup>. SEH may also inhibit neuronal autophagy through the phosphoinositide 3-kinase/protein kinase B/mammalian target of the rapamycin signaling pathway<sup>[22]</sup>, whereas SEI may regulate glutamate-induced calcium-dependent cell death signals by inhibiting c-Jun N-terminal kinase (JNK)/caspase-3 signaling pathway<sup>[29-30]</sup>. 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<sup>[31-32]</sup>. 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*<sup>[31,33]</sup>.

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<sup>[31]</sup>. BBB function is closely related to the expression of tight junction proteins claudin-5 and zonula occludens-1 (ZO-1)<sup>[34-35]</sup>. 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  $\mu$ g/mL, whereas SEI upregulated the expression levels of all three tight junction proteins at 40  $\mu$ g/mL, but downregulate the expression of ZO-1 at 80  $\mu$ g/mL<sup>[36]</sup>. 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  $\mu$ g/mL and promote the uptake of the drug by the paracellular pathway<sup>[37]</sup>. 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<sup>[38-39]</sup>. 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<sup>[40]</sup>. One possible mechanism is related to positive regulation of the p38 mitogen-activated protein kinase signaling pathway<sup>[40]</sup>.

#### 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<sup>[41]</sup>. Zhu et al.<sup>[42]</sup> 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<sub>50</sub>) value

of SEI was approximately 140 µg/mL in the assay of ADP-induced platelet aggregation, indicating a strong anti-coagulant activity<sup>[43]</sup>. 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<sub>50</sub> value of 197.23 µM<sup>[44]</sup>. 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<sup>[45]</sup>. 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<sup>[46]</sup>. Using a luciferase reporter system, Lei et al.<sup>[47]</sup> found that SEA showed calcium-antagonistic activity in HEK 293 cells, possibly by blocking ryanodine receptors and voltage-operated Ca<sup>2+</sup> 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.<sup>[48]</sup> 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.<sup>[49]</sup> 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<sub>50</sub> value of less than 0.1 µg/mL<sup>[50]</sup>. 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<sup>[51]</sup>. 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<sup>[52]</sup>. In another study using a mouse renal I/R injury model<sup>[53]</sup>, 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<sup>[53]</sup>. 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<sup>[54]</sup>. In addition, SEI may downregulate the expression of endoplasmic reticulum stress-related proteins GRP78 and CHOP, thereby inhibiting endoplasmic reticulum stress-induced cell death<sup>[53]</sup>. 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<sup>[55]</sup>. 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<sup>[55-56]</sup>. 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<sup>[57]</sup>. 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)<sup>[57-58]</sup>.

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



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<sup>[75]</sup>.

**Anti-fibrotic activity**

The therapeutic effect of SEA on renal interstitial fibrosis (RIF) has been reported in a rat model with unilateral ureteral obstruction<sup>[76]</sup>. 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/ $\beta$ -catenin signaling<sup>[76]</sup>. In addition, after SEA treatment, reduced expression of the mesenchymal phenotypic characteristic markers vimentin and  $\alpha$ -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<sup>[76]</sup>. 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.

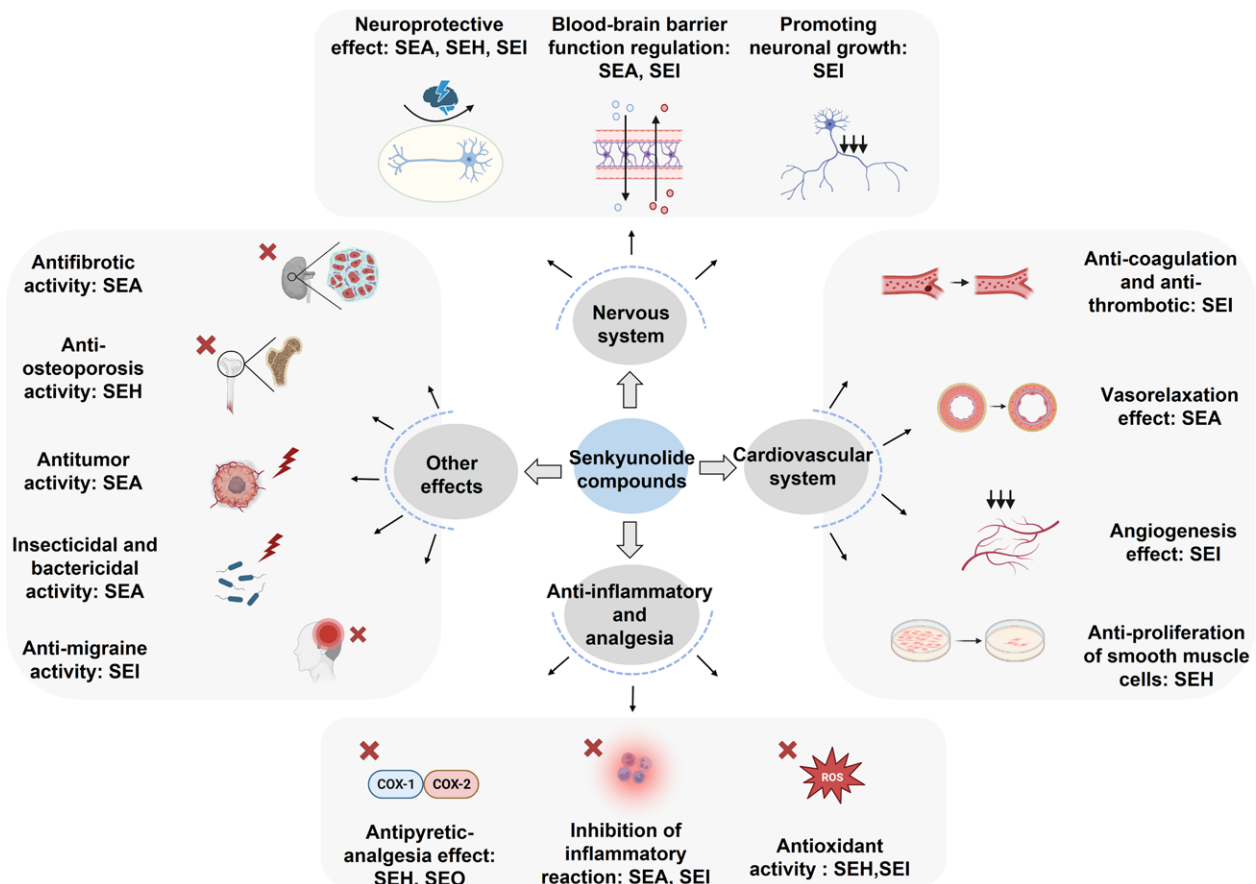
**Toxicity analysis**

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<sup>[77]</sup>. 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)<sup>[78]</sup>. 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<sup>[78]</sup>. 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.

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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<sup>[79]</sup>. Fang et al.<sup>[80]</sup> 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<sup>[80]</sup>. 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<sub>v</sub>1.2 activation blockade<sup>[81]</sup>. 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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## Data availability statement

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

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