

Evodiamine: A Extremely Potential Drug Development Candidate of Alkaloids from *Evodia rutaecarpa*

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Abstract: Evodiamine (EVO) is a tryptamine indole alkaloid and the main active ingredient in *Evodia rutaecarpa*. In recent years, the antitumor, cardioprotective, anti-inflammatory, and anti-Alzheimer's disease effects of EVO have been reported. EVO exerts antitumor effects by inhibiting tumor cell activity and proliferation, blocking the cell cycle, promoting apoptosis and autophagy, and inhibiting the formation of the tumor microvasculature. However, EVO has poor solubility and low bioavailability. Several derivatives with high antitumor activity have been discovered through the structural optimization of EVO, and new drug delivery systems have been developed to improve the solubility and bioavailability of EVO. Current research found that EVO could have toxic effects, such as hepatotoxicity, nephrotoxicity, and cardiac toxicity. This article reviews the pharmacological activity, derivatives, drug delivery systems, toxicity, and pharmacokinetics of EVO and provides research ideas and references for its further in-depth development and clinical applications.

Keywords: evodiamine, pharmacology, derivatives, drug delivery systems

Introduction

Evodia rutaecarpa is the dried, nearly ripe fruit of *Evodia rutaecarpa* (Juss). Benth., *E. rutaecarpa* (Juss). Benth. var. *officinalis* (Dode) Huang, or *E. rutaecarpa* (Juss). Benth. var. *bodinieri* (Dode) Huang, which are plants belonging to the Rutaceae family. Fructus Evodiae is mainly distributed in Shanxi, Hebei, Shandong, Jiangsu, Anhui, Sichuan, Yunnan, northern Guizhou, and Hanzhong in the Shaanxi Province and other places in China. It was first recorded in "Sheng Nong's herbal classic" and is described in the *Compendium of Materia Medica* as follows: "Eating it too much will cause eye irritation and hair loss". "It has small poison, stimulates spleen fire, and should be avoided by those with eye diseases". It has anti-emetic, anti-ulcer, analgesic, and other effects, and is often used to treat nausea with acid regurgitation, Jueyin headache, and dawn diarrhea.

The chemical components of Fructus Evodiae are alkaloids, terpenoids (including bitter principles), flavonoids, phenylpropanoids, anthraquinones, sterols, and volatile oils, with alkaloids being the main active ingredients. Numerous alkaloids have been isolated, which can be divided into indole quinoline alkaloids, quinolone alkaloids, and other alkaloids according to their chemical structures. Among them, evodiamine (EVO) and rutaecarpine are the main active components. Modern pharmacological research showed that they have antitumor, cardioprotective, anti-gastric ulcer, antibacterial, anti-inflammatory, and analgesic effects. Dehydroevodiamine, which is the symbolic component of Fructus Evodiae, has a vasodilatory effect.

With the widespread use of *Evodia rutaecarpa* in clinical practice, its toxicity has gradually been exposed. The 2020 edition of the *Chinese Pharmacopoeia* reported that *Evodia rutaecarpa* has little toxicity and stipulates that the amount of 2–5 g, and the appropriate amount is for external use. In clinical practice, excessive doses of *Evodia rutaecarpa* often lead to toxicity, which is mainly manifested as liver toxicity, indicated by significant increases in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Adverse reactions caused by the improper use of *Evodia rutaecarpa* occur in clinical practice. However, the main toxic components and their mechanism of action are still unclear.

EVO is a tryptamine indole alkaloid appearing as yellow, flaky crystals and having a relative molecular weight of 303.36, molecular formula of $C_{19}H_{17}N_3O$, and melting point of 278 °C. It is insoluble in water but soluble in acetone and other organic solvents. The structure of EVO is unstable when the temperature exceeds 60 °C or when the pH is < 5 or > 9. Under such conditions, EVO is prone to chemical changes. It also contains several reactive groups with the potential for chemical modification. In recent years, EVO has received extensive attention due to its low cytotoxicity, wide bioactivity, and excellent physicochemical properties. Although some reviews on EVO have been published, most are older reviews and focused on its pharmacological effects. Limited current studies have addressed the synthesis, derivatives, and formulation of EVO, which are key information for the application of EVO. This is the first review to comprehensively summarize the pharmacological activity, toxicity, absorption, and metabolism of ECO in vivo; the synthesis, species, and activity of its derivatives; and the preparation, development, and other aspects of EVO to provide ideas and references for its further in-depth research, development, and utilization.

Pharmacological Effects

Studies have shown that EVO has many pharmacological activities, such as anticancer, cardioprotective, anti-atherosclerosis, anti-inflammatory, anti-Alzheimer's disease (AD), and antibacterial activity (Figure 1), indicating high medicinal value.

Anticancer Activity

In recent years, studies have found that EVO has potent anticancer activity, can significantly inhibit the proliferation of various cancer cells, and has a curative effect in liver cancer, lung cancer, gastric cancer, and colorectal cancer. Its mechanism of action is mainly related to inducing cancer-cell apoptosis, inhibiting cancer-cell proliferation, and affecting the cancer cell cycle and cell migration (Table 1).

Liver Cancer

Liver cancer is a common malignant tumor characterized by high malignancy, rapid development, and high mortality. Its incidence rate ranks fifth among all cancers, and the mortality rate in males ranks second.¹ Clinical data show that the 5-year survival rate of patients with liver cancer is less than 10%. Surgery in the early stages is the usual treatment; however, it is associated with poor postoperative prognosis and a high recurrence rate.² Studies in an immunocompetent mouse model of orthotopic intrahepatic xenotransplantation confirmed that EVO could significantly inhibit the growth of cancer cells in model mice.³ Other studies showed that EVO could significantly induce the cell-cycle arrest of hepatocellular carcinoma (HCC) cells in the G2/M phase, upregulate P53 and BAX, and decrease the expression of NOD1, p-P65, p-ERK, p-p28, and p-JNK. The mechanism may be due to the induction of apoptosis in HCC cells by inhibiting the NOD1 signaling pathway in vitro and in vivo.⁴ Treatment with EVO was reported to increase WWOX expression in a dose-dependent manner in HepG2 and Hepa1-6 tumor-bearing mice. As a new inducer of oxidative stress, EVO exerts its anti-HCC effect by activating the WWOX-dependent pathway.⁵ EVO can also reduce phospho-protein kinase B (p-Akt) level that is activated by SC79, decrease the activity of tumor-specific growth factors and alpha-fetoprotein, leading to the cleavage of caspase-3 and an increase in Bax/Bcl2, and anti-liver cancer effects.⁶ Yes-associated protein (YAP)/transcriptional co-activator with PDZ-binding motif (TAZ) is associated with metastasis and cancer formation and progression in various cancers. YAP is a key target in the treatment of HCC. Studies have shown that EVO significantly reduced YAP levels and HCC growth in a dose-dependent manner. It was also shown to exert anti-

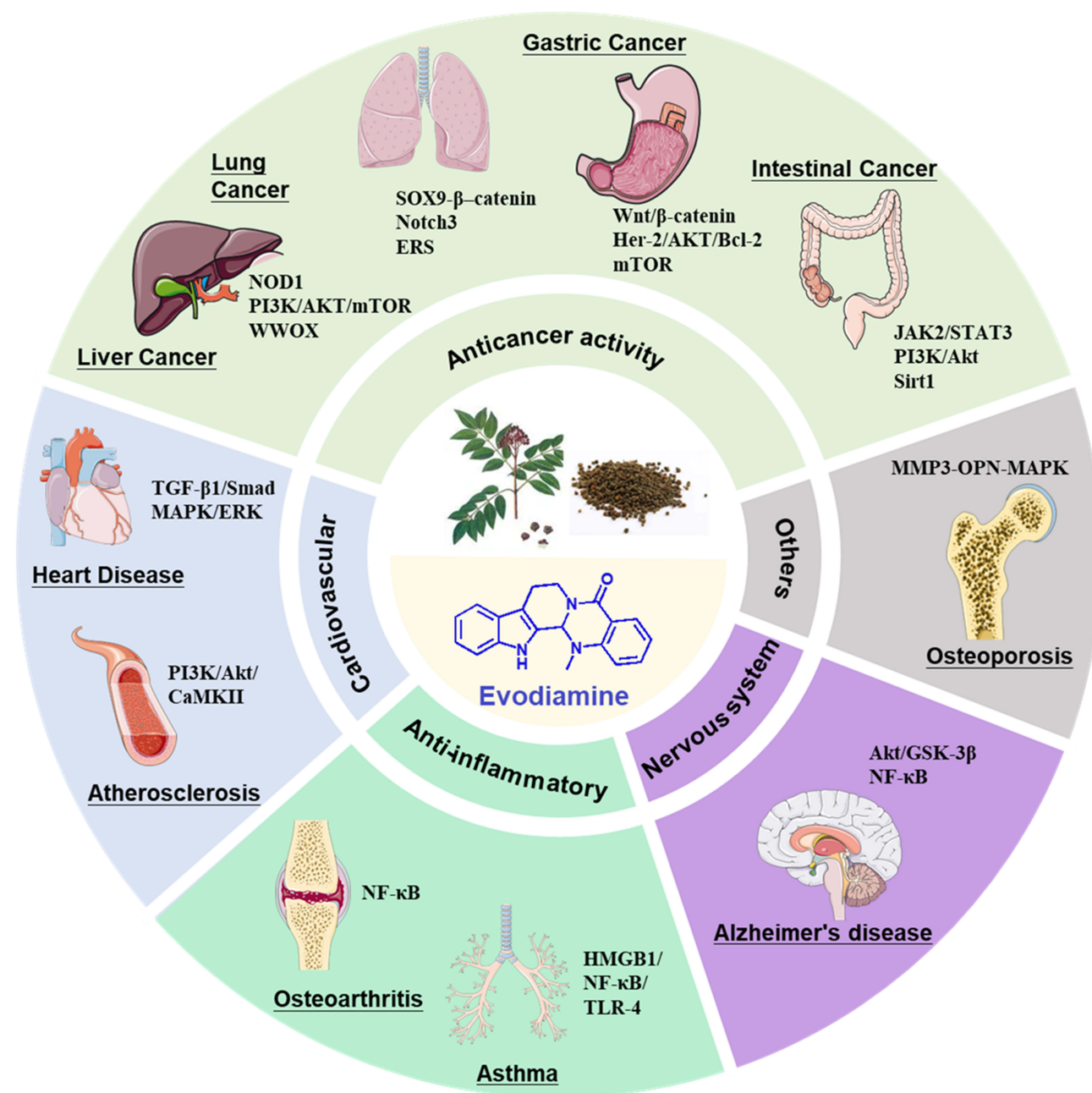


Figure 1 The pharmacological activity and mechanism of evodiamine.

metastatic effects on Hep3B and Huh-7 cells^{7,8}. EVO combined with vorinostat synergistically inhibited the proliferation of liver cancer cells under hypoxia by downregulating HIF-1 α .⁹ Thus, overall, it can be used as an anti-liver cancer drug.

Lung Cancer

Currently, lung cancer is one of the most malignant and rapidly growing tumors and is associated with high morbidity and mortality. More than 2.2 million new cases of lung cancer and nearly 1.8 million deaths worldwide due to lung cancer were reported in 2020.

EVO decreased the cell viability of A549 and NCI-H522 cells in a dose- and time-dependent manner, induced DNA damage, and arrested the cell cycle in the G2/M phase, significantly inhibiting cell cycle-related proteins.¹⁰ It also reduced the expression of pro-caspase-3 protein, indicating that EVO may induce the apoptosis of lung cancer cells via

Table I The Summary of Anticancer Effect and Mechanism of Evodiamine on Different Cancers

Tumor Type	Cell or Animal Model	Mechanism	Pathway
Liver cancer	HepG2 cells; A human hepatoma xenograft model	c-myc was the key molecule	/
	SMMC-7721 and HepG2 Cells	Inhibiting NOD1 to suppress NF- κ B and MAPK activation	NOD1 signal pathway
	HCC, Hepa1-6 and HepG2 cell; Hepa1-6 hepatoma-bearing mice	Inhibits both Mus musculus and Homo sapiens HCC, Hepa1-6 and HepG2 cell proliferation	WVVOX-dependent pathway
	HepG2, SMMC-7721 and H22 cell; H22 xenograft mouse model	Decreased p-Akt levels activated by SC79, which led to the increase of bax/bcl-2 and cleaved-caspase3.	PI3K/Akt pathway
	Hep3B and Huh-7 cells	Reduced both the Yes-associated protein (YAP) level and cell growth of HCC; mitochondria dysfunction-mediated apoptosis	/
	HCC cell lines	Inhibit cell proliferation and induce apoptosis in HCC cells under hypoxia, and inhibit HIF-1 α induced by hypoxia in HCC cells	PI3K/AKT/mTOR pathway
Lung cancer	A549 human lung cancer cells	Induction of G2/M phase cell cycle arrest and apoptosis, and down-regulation of ERK in human lung cancer cells.	Intrinsic apoptotic pathway
	Human SCLC cell lines NCI-H446 and NCI-H1688	Induced cell cycle arrest at G2/M phase, induced apoptosis by up-regulating the expression of caspase-12 and cytochrome C protein, and induced the expression of Bax mRNA and by down-regulating of the expression of Bcl-2 mRNA	Intrinsic caspase-dependent pathways
	A549 and NCI-H522 lung cancer cells	Induced DNA damage and arrested the cell cycle at the G2/M phase; inhibit cell invasion was mediated by altering the expression of E-cadherin, ZO-1, N-cadherin, and Vimentin	SOX9- β -catenin signaling pathway
	A549 and H1299 cells	Involving higher expression of DR5 and cytochrome-c release with dissipation of mitochondrial membrane potential	Both extrinsic and intrinsic pathways
	Lewis lung carcinoma	Suppressed HGF-stimulated invasion and migration of tumor cells through inhibition of cell spreadin	/
	NSCLC A549 and LLC cells; LLC tumor-bearing mouse model	Up-regulated the expression of apoptosis-related genes and proteins and increased activation of the endoplasmic reticulum stress (ERS) pathway	ERS pathway
	A549 and H1299 cells	Induced G2/M cell cycle arrest; prevented the γ -secretase cleavage of Notch3 at the cell surface and hence inhibited Notch3 activation	Notch3 signaling pathway
	H1650, H1975 and NSCLC cells	Elevating of CD8 ⁺ T cells and downregulating of the MUC1-C/PD-L1 axis	/
	CLI cells and human umbilical vein endothelial cells (HUVECs)	Inhibited the expression of vascular endothelial growth factor (VEGF) and the p44/p42 mitogen-activated protein kinase (MAPK, ERK) that correlated with endothelial cells angiogenesis	p38MAPK, MAPK, ERK

(Continued)

Table 1 (Continued).

Tumor Type	Cell or Animal Model	Mechanism	Pathway
Gastric cancer	Gastric cancer stem cells	Alterations in the sphere formation ability, the expression of induced-pluripotent stem cell factors, expression of epithelial-to-mesenchymal transition (EMT) factors	Wnt/ β -catenin signaling pathway
	SGC-7901 cells	Downregulation of survivin and upregulation of caspase-3 mRNA.	/
	SGC-7901 cells	Inhibiting the cell cycle at G2/M phase, activating caspase-3, -8 and -9, and altering the expression of caspase-3, Bax and Bcl-2.	
	SGC-7901 cells	Mitotic arrest and subsequent mitotic slippage	
	BGC-823 cells; BGC-823 cell-derived xenograft models	Induced cell cycle arrest in G2/M phase; decreased the expression of Bcl-2, phosphorylated Akt and Her-2, increased the expression of Bax	Her-2/AKT/Bcl-2 signaling pathway
	SGC-7901 cells	Inhibit the protein expressions of mTOR and p-mTOR	mTOR signal pathway
	Freshly-removed gastric tumor tissues	Reducing the mRNA expression levels of excision repair cross-complementing 1 (ERCC1) and thymidylate synthase(TS)	DNA repair pathway
Intestinal cancer	Bulk cultured cancer cells (BCC) of colon cancers	Induce apoptosis of the cells captured in G2/M phase; decreased the CD133 and SCD1 gene expression	WNT and NOTCH signaling pathway
	HCT-116 Human Colorectal Cancer Cells	Accumulation of cells in S and G2/M phases; downregulate MMP3 and PGI expression	JAK2/STAT3 pathway; p53 signaling pathway
	HT-29 and HCT-116 CRC cells; HCT-116 cell xenograft models	Suppresses the migration and invasion of CRC cells by inhibiting the acetyl-NF- κ B p65 by Sirt1, and suppression of metalloproteinase-9 expression	Sirt1 signaling pathway
	SW480 colon cancer cells	Upregulation of caspase-3 and Bax and suppression of Bcl-2; enhancing the expression of LC3 II and Beclin I	/
	LoVo cells; LoVo cells xenograft models	Downregulating HIF-1 α expression	PI3K/Akt signaling pathway
	SW480 colon cancer cells; C57BL/6 colorectal cancer mice	Caused G2/M-phase arrest and suppressed the translocation of phosphorylated-NF- κ B from the cytoplasm into the nucleus; Reduce the levels of inflammatory factors	NF- κ B signaling pathway
Pancreatic cancer	PANC-1 and SW1990 PC cell; orthotopic pancreatic tumor model	Inhibiting phosphoinositide 3-kinase/AKT and mitogen-activated protein kinase/ERK, and inhibiting the phosphorylation of signal transducer and activator of transcription activator 3	PI3K/Akt and MAPK/ERK signaling pathways
Thyroid carcinoma	TPC-1 and SW1736 human thyroid carcinoma cells	Increased the protein levels of γ H2AX and cleaved PARP, and ROS production	PI3K/Akt signaling pathway
Kidney cancer	786-O, Caki-1 and HK-2 cells	Arrest cells in the G2/M phase, and regulate the expression of apoptosis- and cell cycle-related genes	MAPK and NF- κ B signalling pathway.
Human urothelial cell carcinoma	5637 and HT1197 cells	Caused G2/M cell-cycle arrest and induced caspase-dependent apoptosis in UCC cells.	MAPK signaling pathways

(Continued)

Table 1 (Continued).

Tumor Type	Cell or Animal Model	Mechanism	Pathway
Bladder cancer	253J and T24 cells	Induces apoptosis and enhances TRAIL-induced apoptosis possibly through mTOR/S6K1-mediated downregulation of Mcl-1	mTOR/S6K1 pathway
Ovarian cancer	HO-8910PM cells	Inhibit the growth of ovarian cancer cells by G2/M arrest and intrinsic and extrinsic apoptosis	PI3K/Akt, ERK1/2 MAPK, and p38 MAPK signaling pathway
	A2780, A2780CP, ES-2 and SKOV-3 cells	Increases in the percentage of apoptotic cells, DNA ladders, and cleavage of caspase 3 and poly(ADP ribose) polymerase (PARP) proteins	PERK pathway
Cervical carcinoma	HeLa cell	G2/M transition by inductions of ROS/NO generations	Ras-Raf-JNK and PTK pathway
Oral cancer	MC3 and HSC4 cells	Cleavages of PARP (poly (ADP-ribose) polymerase) and caspase-3; increased the level of Bax protein	AKT pathway
	A-253, HSC-4, CAL-27, SCC-4 and HUVEC cell lines; HSC-4 cell xenograft model	Reduce the activity of RAGE pathway by affecting the binding between HMBG1 and RAGE	HMGB1/RAGE signaling pathway
Nasopharyngeal carcinoma	HONE1 and CNE1 cells	Decreased mRNA and protein levels of MMP-2 and its activity; inhibited the translocation of NF- κ B p65	MAKP and Akt signaling pathways
Tongue cancer	CAL-27 cell	Upregulated the expression levels of SOCS2, DUSP1, and NRAS, downregulated STAT2, MMP1, and FGFR3	Toll-like receptor (TLR), IL-6 signaling pathway, et al
Melanoma	A375-S2 cell	Led to I κ B phosphorylation and degradation that reflect translocation of NF- κ B	PI3K/Akt/caspase and Fas-L/NF- κ B signaling pathways
Cholangiocarcinoma	HuCC-T1 and TFK-1 cells; TFK-1 cell xenograft models	Upregulation of phosphatase shatterproof 2 (SHP-2), a negative feedback regulator of IL-6/STAT3	IL-6/STAT3 signaling pathway
Breast cancer	MCF7 and MDAMB 231	Selectively activated p53 and p21 and decreased inactive Rb, the master molecules in G1/S checkpoint	p53-p21-Rb pathway
Prostate cancer	LNCaP and 22Rv1 cell;	Targeted Src and reduced DHT-induced Src activation	/
Esophageal cancer	EC1, Eca109 and Kyse150 cells; Eca109 and Kyse150 cells xenograft model	Induced M-phase cell-cycle arrest by inactivation of CUL4A E3 ligase, which mediates degradation blockage of p53 and transcriptional activation of p21	p53/p21 signaling pathway

the endogenous apoptosis pathway.¹¹ It significantly reduced the protein levels of epithelial-mesenchymal transition (EMT)-related molecules (N-cadherin, vimentin, and E-cadherin) in cells and increased the expression of E-cadherin (epithelial marker) protein to mediate the inhibition of cell invasion.^{12,13} Another study found that EVO could induce the release of cytochrome C, activate the cascade reactions of caspase-3 and caspase-9 endogenous pathways and the exogenous apoptosis pathway induced by death receptor 5 and caspase-8, suggesting that EVO may induce apoptosis in lung cancer cells via both endogenous and exogenous induction pathways.¹⁴ Besides upregulating the expression of apoptosis-related genes and proteins in tumors, EVO was also shown to activate the endoplasmic reticulum stress pathway and inhibit the invasion and migration of lung cancer cells stimulated by hepatocyte growth factor¹⁵ to inhibit proliferation and increase the apoptosis rate of A549 and LLC cells.¹⁶ In addition, EVO was reported to activate DNA

methyltransferase-induced Notch3 methylation, prevent Notch3 from splitting on the cell surface by γ -exosome complex, and inhibit Notch3 signal pathway conduction, thereby inhibiting cancer cell proliferation and enhancing the anticancer activity of EVO.^{17,18} EVO significantly inhibited the growth of non-small cell lung cancer (NSCLC) cells, induced apoptosis, and arrested the cell cycle in the G2 phase. The mechanism for these effects may be through increasing CD8+ T cells and downregulating the MUC1-C/PD-L1 axis to inhibit NSCLC.¹⁹ Regarding the inhibition of tumor angiogenesis, EVO pretreatment significantly decreased the formation of human umbilical vein endothelial cells (HUVECs) and may regulate the formation of capillaries by downregulating the expression of phosphorylated ERK (extracellularly regulated protein kinase) and vascular endothelial growth factor.²⁰

Gastric Cancer

Gastric cancer is a common malignant tumor of the digestive system and was the fifth most common cancer and the fourth leading cause of cancer deaths worldwide in 2020. In the same year, the total number of new cases of gastric cancer worldwide was approximately 1.1 million, and the death toll was approximately 770,000, accounting for 8% of all cancer-related deaths. Gastric cancer is associated with a higher mortality rate due to its lower rate of early detection.^{21,22}

Studies have shown that EVO can inhibit the proliferation of gastric cancer stem cells (GCSCs), change their activity, and reduce the spheroid-formation ability of gastric cancer cells. EVO downregulated the Wnt/ β -catenin pathway by reducing the expression of β -catenin, c-myc, and cyclin D1, thus inhibiting the proliferation and stem-cell self-renewal characteristics of GCSCs and reducing the expression of EMT factors.²³ The expression of Survivin decreased in a dose-dependent manner, the expression of caspase-3/8/9 and Bax increased significantly, and the expression of Bcl2 decreased significantly in the EVO-treated SGC7901 gastric cancer cell line, suggesting that EVO induces apoptosis in these cells by downregulating the expression of the anti-apoptosis gene Survivin and upregulating the expression of the pro-apoptosis gene caspase-3, as well as inhibiting the G2/M phase of the cell cycle in these cells. Additionally, EVO inhibits the proliferation of gastric cancer cells by mitotic arrest and subsequent mitotic slippage.^{24–26} EVO increased the sensitivity of the BGC-823 gastric cancer cell line to radiotherapy and inhibited the growth of xenograft tumors.²⁷ The expression of *mTOR*, *4E-BP1*, and *p70S6K* genes was significantly inhibited after treatment of SGC7901 cells or human peripheral blood monocyte models with EVO or EVO combined with z-VADfmk, respectively.²⁸ In addition, the inhibitory efficiency of EVO was higher when combined with other cancer-treatment approaches.²⁹

Intestinal Cancer

Intestinal cancer can be mainly divided into colon cancer and rectal cancer. It is a malignant tumor with a high worldwide incidence, and its morbidity and mortality have always been at the forefront of malignant tumors. Tumor metastasis and inflammatory infiltration are the main characteristics of intestinal cancer. Studies have reported the effects of 75 natural compounds on the migration and proliferation of intestinal cancer cells. EVO has been reported to have the most obvious effect in affecting the migration ability of cells with the lowest migration/proliferation value; 10 mg/mL EVO was reported to inhibit cancer cell movement activity by 70% to exert anticancer effects by regulating the Notch and WNT signaling pathways.^{30,31} EVO can significantly reduce the protein levels of autocrine motility factor in cell supernatants and inhibit PGI to reduce cancer cell migration. It can also induce cell apoptosis by inhibiting the JAK2/STAT3 pathway, regulating the activity of the p53 signaling pathway, and downregulating the expression of recombinant matrix metalloproteinase (MMP)-3.³² EVO significantly inhibited the migration and invasion of HT-116 and HT-29 cells, the sirtuin1-mediated NF- κ B pathway, and the migration and invasion of CRC cells.³³

EVO inhibited Bcl2 in SW480 colon cancer cells; induced the expression of caspase-3, Bax, LC3II, and Beclin1; and induced the synergistic effect of cell autophagy and apoptosis on cell death.³⁴ EVO prevented the proliferation of human colorectal cancer cells (HCT-116) and colon cancer (LoVo) cells, wherein the expression of IGF-1 was inhibited, PI3K/Akt signal transduction was reduced, and HIF-1 α expression was downregulated, thus exerting an inhibitory effect on colon cancer.³⁵ EVO was also reported to reduce the levels of inflammatory factors, such as interleukin (IL)-1 β , IL-2, IL-6, IL-15, IL-17, IL-22, tumor necrosis factor (TNF)- α , NF- κ B, IKK α + β , and I κ B α , in colon cancer models and exert its antitumor effect.³⁶

Other Tumors

In addition to its efficacy in the above cancers, EVO was shown to exert significant pharmacological activity in cancers of the endocrine system (pancreatic cancer,³⁷ thyroid carcinoma,³⁸ renal carcinoma³⁹), urinary system (urothelial cell carcinoma,⁴⁰ bladder cancer⁴¹), reproductive system (ovarian cancer,⁴² cervical carcinoma,⁴³ breast carcinoma), respiratory system (oral cancer,⁴⁴ nasopharyngeal carcinoma,⁴⁵ tongue squamous cell carcinoma⁴⁶), nervous system (melanoma⁴⁷), and digestive system (cholangiocarcinoma⁴⁸).

The anticancer activity of EVO against these cancers involves multiple pathways. EVO inhibits the activation of IL-6-induced STAT3 signaling, upregulates the expression of SHP-2, and plays a role in inhibiting the proliferation of cholangiocarcinoma tumors. In several cancer models, apoptotic bodies were significantly increased after treatment with EVO. At the same time, cell arrest in the G0/G1 phase and the abnormal expression of caspase-related proteins⁴⁹ were shown to also target the p53-p21-Rb pathway to inhibit the proliferation of breast cancer MCF-7 cells and MDA-MB-231 cells.⁵⁰ EVO activated Janus kinase and PERK in human ovarian cancer cells to destroy the mitochondrial membrane potential and induce apoptosis in these cells.⁵¹ The mechanism of the anti-prostate cancer action of EVO is related to the direct targeting of Src, the reduction of dihydrotestosterone-induced Src activation, and the blockage of the transcriptional activity of androgen receptors (ARs).⁵² EVO induced M-phase cell-cycle arrest by inactivating CUL4A E3 ligase, thereby blocking the degradation of p53 and the transcriptional activation of p21 and inhibiting the growth of esophageal squamous cell carcinoma (ESCC).⁵³ EVO inhibited the proliferation, invasion, and angiogenesis of oral squamous cell carcinoma (OSCC) by targeting the receptor for advanced glycation end products (RAGE) and affecting the downstream signal transduction system of advanced glycation end products/RAGE.⁵⁴ In addition, EVO disrupted the heat shock protein (HSP) system by binding to the N-terminal ATP-binding pocket of HSP70 and causing ubiquitin-mediated degradation, thereby acting as an effective HSP70-targeted anticancer drug.⁵⁵

Disorders of the Cardiovascular and Cerebrovascular Systems

Cardioprotection

EVO can protect the heart by preventing cardiac fibrosis, inhibiting the proliferation of vascular smooth muscle cells, and alleviating myocardial ischemic injury. EVO can block the TGF- β 1/Smad signaling pathway, decrease the expression of fibroblast-specific protein α -smooth muscle actin and vimentin, and increase the expression of the interstitial system markers CD31 and CD34, thereby inhibiting endothelial cell migration and cardiac EMT. EVO was shown to significantly prevent the activation of Smad2, Smad3, ERK1/2, and Akt, and the nuclear translocation of Smad4 in cardiac fibroblasts and HUVECs, thus preventing cardiac fibrosis, suggesting the potential therapeutic effect of EVO in myocardial fibrosis.⁵⁶ EVO also prevented isoproterenol-induced myocardial fibrosis by regulating the interstitial transformation of endothelial cells, and its mechanism was related to blocking the TGF- β 1/Smad signal pathway.⁵⁷ EVO caused cell-cycle arrest in the G0/G1 phase by reducing the expression of proliferating cell nuclear antigen, cyclin-dependent kinase (CDK2/4/6), and cyclin D, which inhibited the proliferation of platelet-derived growth factor-BB (PDGF-BB)-induced vascular smooth muscle cells (VSMCs) by inhibiting the MAPK/ERK signaling pathway and decreasing the production of reactive oxygen species (ROS) in cells.⁵⁸ β 1-AR antagonists are mainly used to treat ischemic heart disease and chronic heart failure. Studies have found that EVO is a potential β 1-AR antagonist, and in vivo experiments showed that it can alleviate myocardial ischemic injury by regulating β 1-AR.^{59,60} Additionally, EVO significantly reduced symptoms, such as decreased cardiac blood flow due to cardiac allergy, and protected rats from cardiac allergic injury and myocardial ischemic injury by regulating CGRP release.^{61,62}

Anti-Atherosclerosis Effects

Atherosclerosis is the leading cause of coronary heart disease, cerebral infarction, and peripheral vascular disease, and lipid metabolism disorders are the pathological basis of atherosclerosis. Elevated cholesterol levels are one of the reasons for the development of atherosclerosis. Studies have found that EVO can significantly regulate cholesterol absorption. When combined with berberine, serum cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and liver total cholesterol levels were decreased in hyperlipidemic rats, and the combination significantly promoted serum

cholesterol decreases in high-fat diet-induced rats.⁶³ Activation of the capsaicin receptor (TRPV1) improved high-fat diet-induced atherosclerosis.⁶⁴ The activation of TRPV1 was shown to regulate lipid metabolism, reduce foam cell formation, protect endothelial cells, inhibit smooth muscle cell proliferation, and inhibit inflammation and oxidation.⁶⁵ After 4 weeks of EVO gavage (10 mg/kg), the atherosclerotic plaques in the aortic sinuses of ApoE^{-/-}TRPV1^{-/-} mice became larger, serum cholesterol levels increased, HDL levels decreased, and TNF- α , MCP-1, IL-6, and MIP-2 levels increased compared to ApoE^{-/-} mice, indicating that EVO reduced serum cholesterol, alleviated hyperlipidemia, and played an anti-atherosclerosis role by mediating the TRPV1 receptor.⁶⁶ EVO also activated the Ca²⁺-dependent PI3K/Akt/CaMKII signaling pathway via the TRPV1 receptor, phosphorylated eNOS protein in endothelial cells, increased the synthesis and release of nitric oxide (NO), and exerted an anti-atherosclerosis effects.⁶⁷

The uncontrolled migration of VSMCs into the intima is a key process in the development of atherosclerosis. EVO (0.1 μ mol/L and 0.5 μ mol/L) inhibited the PDGF-BB-induced migration of VSMCs by activating peroxisome proliferator-activated receptor- γ and reducing the expression of MMPs, cell adhesion molecules, and osteopontin, thereby exerting an anti-atherosclerosis effect.⁶⁸

Anti-Inflammatory Activity

At the cellular level, EVO inhibits the secretion of various cell-related inflammatory factors by the following mechanisms. (1) Inhibition of the secretion of macrophage (M ϕ) inflammatory factors: EVO can inhibit the secretion of lipopolysaccharide (LPS)-induced prostaglandin (PG)E₂ and affect macrophage migration by inhibiting the activities of NF- κ B and its mediated cyclooxygenase-2 (COX-2).⁶⁹ In an LPS-induced macrophage model, EVO enhanced the activation of the NLRP3 inflammasome in a dose-dependent manner, induced an increase in IL-1 β and caspase-1 cleavage, and enhanced the activity of inflammasomes by inducing α -tubulin acetylation, thus reducing the inflammatory response.⁷⁰ (2) Inhibition of the secretion of monocyte inflammatory factors: EVO can inhibit the secretion of inflammatory factors, the expression of numerous chemokine receptors such as CCR1 and CCR2, and the migration of human MOs induced by the lymphotoxin analog (LIGHT).⁷¹ (3) Inhibition of ROS production in neutrophilic granulocytes: EVO can reduce the production of ROS and superoxide anions by inhibiting the activity of reduced coenzyme II (NADPH) oxidase in mouse NE.⁷² (4) Inhibition of the production of proinflammatory factors in microglial cells (MCs): After pretreatment of mouse BV2 cells with LPS, EVO significantly inhibited the synthesis of inducible nitric oxide synthase (iNOS), which, in turn, significantly inhibited the production of proinflammatory factors in MCs.⁷³

In vivo studies found that EVO could significantly reduce the expression of inflammatory cytokines such as TNF- α and IL-4 in mouse models of allergies, regulate the synthesis of cytokines related to allergic reactions in mast cells and basophils, and further alleviate immunoglobulin (Ig)E-induced allergic diseases.⁷⁴ EVO was shown to reduce intracellular LPS content, rebalance the abundance of *Escherichia coli* and lactic acid bacteria, alleviate dextran sodium sulfate-induced inflammatory reactions, and maintain the integrity of the intestinal tract and homeostasis of the intestinal flora, thereby showing benefits in treating ulcerative colitis.⁷⁵ EVO is also effective in treating osteoarthritis. It was shown to decrease the production of NO, IL-6, TNF- α , and PGE₂ in mice with osteoarthritis and inhibit the phosphorylation of the NF- κ B signaling pathway stimulated by IL-1 β , thus exerting an anti-inflammatory effect and inhibiting cartilage degeneration.⁷⁶ EVO reduced IgE and interferon- γ levels and inflammatory cell infiltration in the lung tissues of asthmatic rats, reduced toll-like receptor (TLR-4), MyD88, NF- κ B, and HMGB1 mRNA levels in lung tissues, and decreased airway inflammation and lung tissue remodeling through the HMGB1/NF- κ B/TLR-4 pathway.⁷⁷

EVO has been reported to inhibit HIF-1 α by dephosphorylating Akt and p70S6K in a hypoxia model. It also inhibited the expression of COX-2 and iNOS and the release of PGE₂, indicating the potential of EVO in treating hypoxic inflammatory diseases.⁷⁸ The P2X4 receptor (P2X4R) is a subtype of ATP receptors that plays an important role in pain, inflammatory, and immune responses. EVO administration to HUVECs stimulated with a high concentration of glucose in vitro inhibited the ATP receptor P2X4R signaling pathway to downregulate the expression of inflammatory factors NF- κ B and TNF- α , reduce ROS production, promote NO synthesis, exert anti-inflammatory effects, and protect HUVECs from glucose toxicity.⁷⁹

Alzheimer's Disease

With the acceleration of population aging in recent years, the incidence of neurodegenerative diseases, such as AD, has increased significantly, and 15 million people are expected to be affected by dementia by 2050.⁸⁰ Studies have shown the efficacy of EVO in treating AD. A Morris water-maze experiment of AD mice found that EVO could significantly reduce the average latency, significantly improve the spatial cognition and learning ability, significantly reduce the deposition of amyloid plaques in the brain, increase the levels of serum acetylcholine and choline acetyltransferase, and reduce acetylcholinesterase levels in the serum, hypothalamus, and brain.⁸¹ EVO reduced the expression of inflammatory cytokines in the brains of mice with AD, increased glucose uptake in the brain tissues, significantly increased glutathione activity and decreased superoxide dismutase activity in the hippocampus, increased Akt/GSK-3 β signaling pathway and inhibited NF- κ B activity, and significantly inhibited the activation of glial cells and the levels of inflammatory factors (TNF- α , IL-1 β , and IL-6) in the hippocampus, suggesting that anti-inflammatory activity was involved in the anti-AD effect of EVO.^{82,83} In addition, EVO can passively diffuse through the single-cell blood-brain barrier. It showed a concentration-dependent neuroprotective effect on PC12 cells injured by MPP+ or H₂O₂, indicating its potential as a neuroprotective drug.⁸⁴

Antibacterial Effects

EVO was shown to enhance NLRP3-mediated IL-1 β production and neutrophil recruitment by promoting the LPS-induced acetylation of α -tubulin around microtubule tissue centers in macrophages, which enhanced innate immunity to bacterial infections and resistance to pathogenic infections in the host.⁷⁰ EVO was reported to interact with arginine 161 and aspartic acid 551 residues of *E. coli* topoisomerase (Topo)1, inhibit bacterial Topo1, and show a significantly lower minimal inhibitory concentration value of 128 μ g/mL compared to antibiotics (> 512 μ g/mL) against a clinical isolate of *Klebsiella pneumoniae*.⁸⁵ The growth of *Helicobacter pylori* reference strains and clinical isolates was inhibited by EVO. Its inhibitory mechanisms were attributed to the downregulation of the gene expression of both the replication and transcription machinery of *H. pylori*.⁸⁶

Other Activities

In addition to the pharmacological activities described above, research studies are continuously confirming other pharmacological activities of EVO. EVO can upregulate the expression of brain-derived neurotrophic factor and phosphorylated myoprotein-related kinase B and reduce the levels of 5-HT and NA, thus exerting an antidepressant effect.⁸⁷ EVO restored the MMP3-OPN-MAPK pathway in a zebrafish model of osteoporosis, significantly increasing the calcium and phosphorus content and increasing bone formation, indicating its significant anti-osteoporosis effect.⁸⁸

Toxicology

China Pharmacopoeia (2020 Edition) reports that *E. rutaecarpa* has little toxicity, and adverse reactions caused by its improper use may sometimes occur in a clinical setting. Six main alkaloids were isolated from *E. rutaecarpa*, among which EVO had the most acute toxicity, with an LD₅₀ of 77.8 mg/kg.⁸⁹ Recent studies found that EVO is toxic to several organs: (1) Hepatotoxicity: EVO can induce an increase in the release of AST, ALT, lactate dehydrogenase (LDH), and alkaline phosphatase; enhance p-p38/p38 expression; and induce mitochondrial swelling, vacuolation, MPT pore opening, and a significant decrease in the mitochondrial potential, leading to ATP depletion and cytochrome C release and ultimately triggering cell death.⁹⁰ The mechanism of EVO-induced liver injury is likely related to the MAPK signaling pathway (p38).⁹¹ The hepatotoxicity of EVO has also been suggested to be related to lipid-peroxidation injury, cell apoptosis, and cholestasis.⁹² (2) Cardiotoxicity: using a cardiomyocyte model in in vitro studies, the IC₅₀ of cells treated with EVO for 24 h was reported to be 28.44 μ g/mL. LDH release and malondialdehyde (MDA) levels of the treated cardiomyocytes increased significantly, whereas the activity of superoxide dismutase decreased. The LC₁₀ of EVO was reported to be 354 ng/mL in a zebrafish heart model. After treatment with EVO, the heart rate and blood circulation in zebrafish changed significantly; the pericardium appeared malformed, and heart failure was induced.⁹³ (3) Nephrotoxicity: Screening of traditional Chinese medicine drugs for nephrotoxicity identified EVO as potentially

nephrotoxic. In vitro experiments with different concentrations of EVO showed that EVO could significantly inhibit the activity of HEK293 cells. LDH release increased in treated cells, the morphology of renal cells changed significantly, and the expression of autophagy-related proteins increased, indicating that the renal cytotoxicity of EVO may be related to autophagy-related pathways.⁹⁴

Pharmacokinetic Studies of EVO

In recent years, scholars have conducted pharmacokinetic studies of EVO isolated from different species and have a good understanding of its mechanism in vivo. EVO was administered to rats via intragastric administration at a dose of 100 mg/kg, and blood concentrations were determined using LC-MS/MS.⁹⁵ The maximum drug concentration in the blood (C_{\max}) was determined to be 5.3 ng/mL. It was also administered intravenously to rats at a dose of 2 mg/kg, and the area under the curve (AUC) was determined to be 21 $\mu\text{g}\cdot\text{mL}\cdot\text{min}^{-1}$.⁹⁶ Beagle dogs are commonly used to study the metabolism of drugs in vivo. Some researchers orally administered 10 mg/kg EVO to beagle dogs and established a method for determining blood drug concentrations using LC-MS/MS, where the C_{\max} was determined to be 30.9 ng/mL.⁹⁷ In vivo studies of EVO were conducted in rabbits after they were injected via the ear vein.⁹⁸ EVO had poor absorption and poor bioavailability after oral administration. In addition, many studies have reported the pharmacokinetics of Fructus Evodiae extract and prescriptions containing Fructus Evodiae and analyzed the absorption and metabolism of EVO.^{99–102} One study analyzed the tissue distribution of EVO. After injecting EVO into the tail veins of mice, the C_{\max} was attained within 15 min, and the tissue distribution concentration from highest to lowest was in the order of the lungs, kidneys, brain, heart, and liver, indicating lung targeting.¹⁰³ An MDCK pHMDR cell monolayer model was used to confirm that EVO could pass through the blood-brain barrier via passive diffusion,⁸⁴ which is consistent with its tissue distribution.

Metabolism of EVO in vivo: After the oral administration of EVO in rats, 6 metabolites (2 Phase I metabolites and 4 Phase II metabolites) were identified using UPLC-LTQ-Orbitrap, namely, 10-hydroxyevodiamine (M1), 3-hydroxyevodiamine (M2), 10-hydroxyevodiamine sulfate (M3), 3-hydroxyevodiamine sulfate (M4), 10-hydroxyevodiamine glucuronide (M5), and 3-hydroxyevodiamine glucuronide (M6).¹⁰⁴

In vitro studies have been conducted on the metabolic characteristics of EVO-conditioned human liver microsomes and liver cells.¹⁰⁵ A total of 12 phase I metabolites were detected in human liver microsomes, and 19 metabolites were detected in human liver cells, including 7 phase II metabolites. Among them, 12 metabolites were identified for the first time.¹⁰⁶ EVO was orally administered to rats at a dose of 30 mg/kg, and the levels of 4 metabolites, namely, 10-hydroxyevodiamine, 3-hydroxyevodiamine, 10-hydroxyevodiamine glucuronide, and 3-hydroxyevodiamine glucuronide, were determined. The content of 18-hydroxyevodiamine was the highest, with a C_{\max} of 302.09 ng/mL and an AUC_{0-1} of 3279.39 ng.h/mL.¹⁰⁷ Oxidation, N-demethylation, dehydrogenation, glucuronidation, and glutathione (GSH) binding were shown to be the main metabolic pathways of EVO. A study found that EVO inhibited the activity of cytochrome P450 (CYP)1A2, CYP2C9, and CYP2D6, suggesting that its disposition by the body depends on these metabolic pathways.¹⁰⁸ The GSH conjugate of EVO is dominated by benzylsulfide adducts at position C-8, and its formation is mainly catalyzed by heterologously expressed recombinant CYP3A4, CYP1A2, and CYP2D6.¹⁰⁹ The metabolic pathway of EVO is shown in Figure 2.

The study also found that EVO could affect the composition of the intestinal flora, increase the abundance of *Lactobacillus acidophilus* and levels of acetic acid, and exert therapeutic effects in ulcerative colitis.¹¹⁰

Derivatives of EVO

The structural modification of the active ingredients isolated from plants is an effective approach to discovering new drugs.^{111,112} EVO shows outstanding pharmacological activity because of its special chemical structure, and molecular-targeting studies have found that EVO can serve as a basic skeleton of multitargeted antitumor drugs. In recent years, numerous derivatives have been developed by chemically modifying the structure of EVO to significantly enhance its antitumor activity. The effectiveness of EVO has been gradually improved by structure-activity relationship studies and continuous in-depth research to yield novel EVO derivatives with higher antitumor activity. The structural modification of EVO mainly involves modifications of the A-E rings. The main derivatives of EVO are shown in Figure 3.

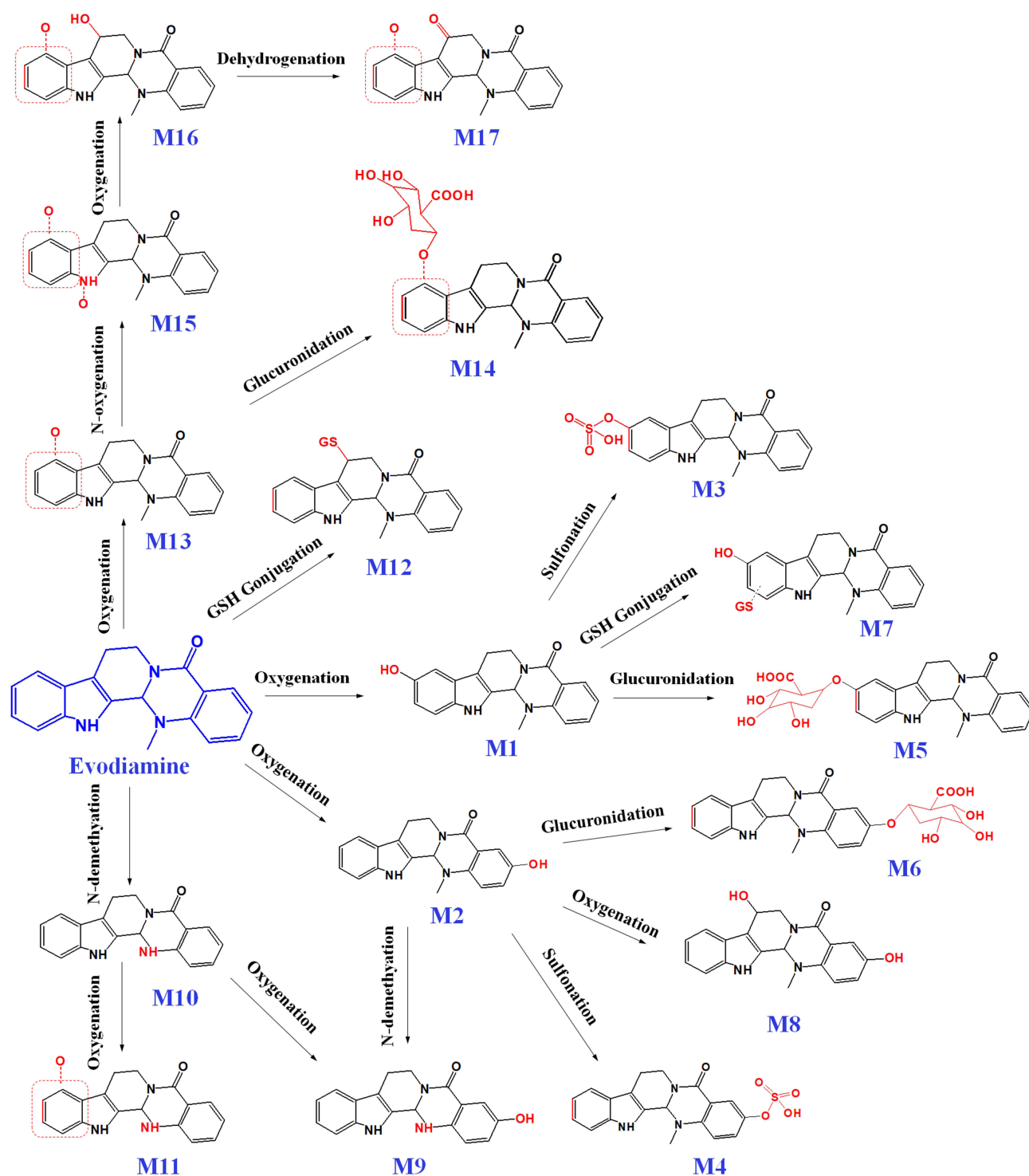


Figure 2 Proposed major metabolic pathway of evodiamine in vivo.

Synthesis of EVO

Currently, 5 synthesis methods are commonly used for EVO, of which 4 are based on N-methylo-aminobenzoic acid(1) as a raw material, and the other is synthesized using a one-pot method. The synthesis route is shown in Figure 4. Method 1: N-methylo-aminobenzoic acid first undergoes acylation and masonry and then undergoes intermediate states (2) and (3) to generate the key intermediate ketone (4), which is then reacted with 3,4-dihydrocarboline to obtain EVO. Method

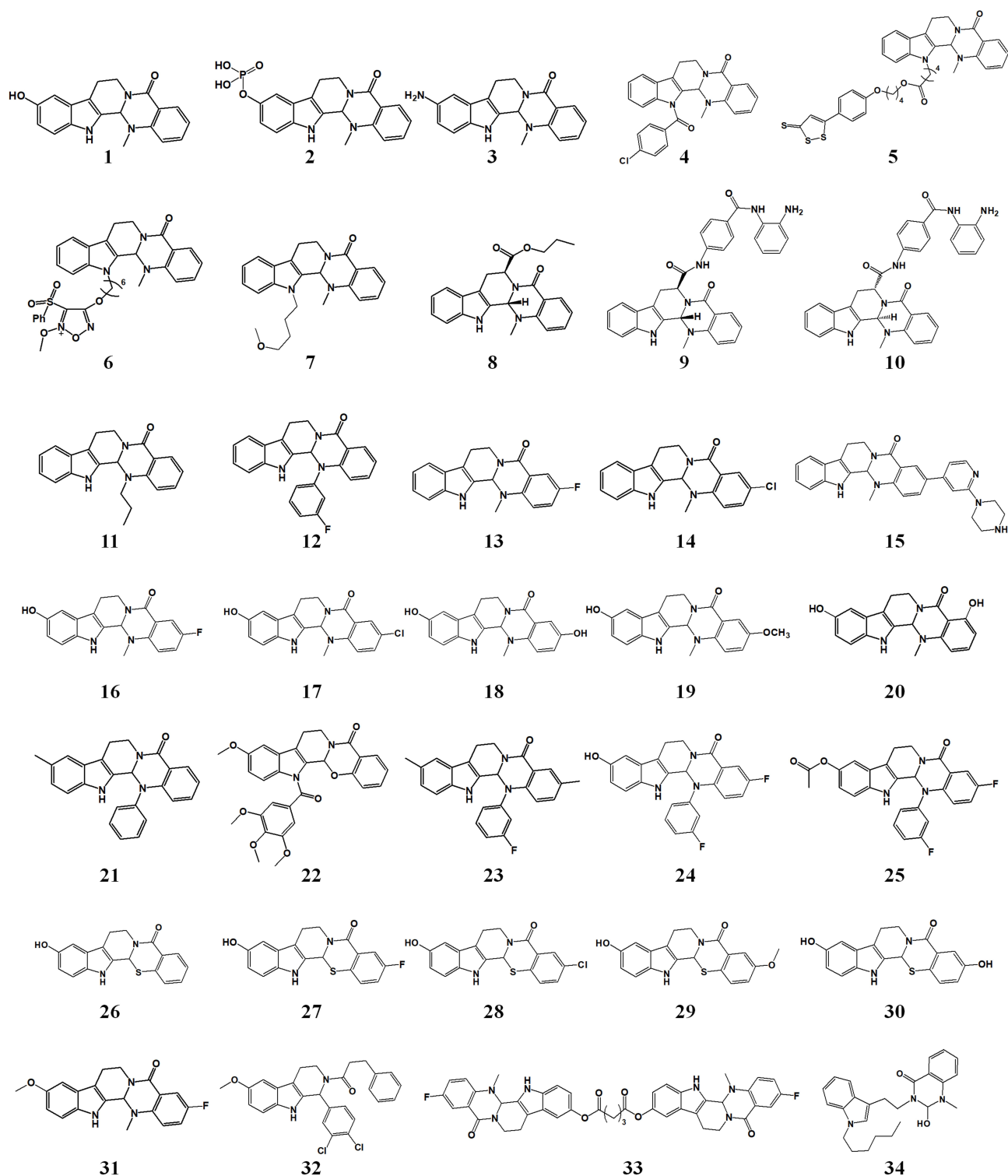


Figure 3 Structure of the main derivatives of evodiamine.

2: N-methyl-aminobenzoic acid is also used as the raw material and is first reacted with phosgene to generate intermediate (5) and then reacted with 3,4-dihydrocarboline to obtain EVO. Method 3: In this method, N-methyl-aminobenzoic acid is reacted with phosgene (5) to form an amide, which is then substituted with 3-indoleethylamine to obtain the product (7). After cyclization, EVO can be obtained. Method 4: The product is obtained by reacting

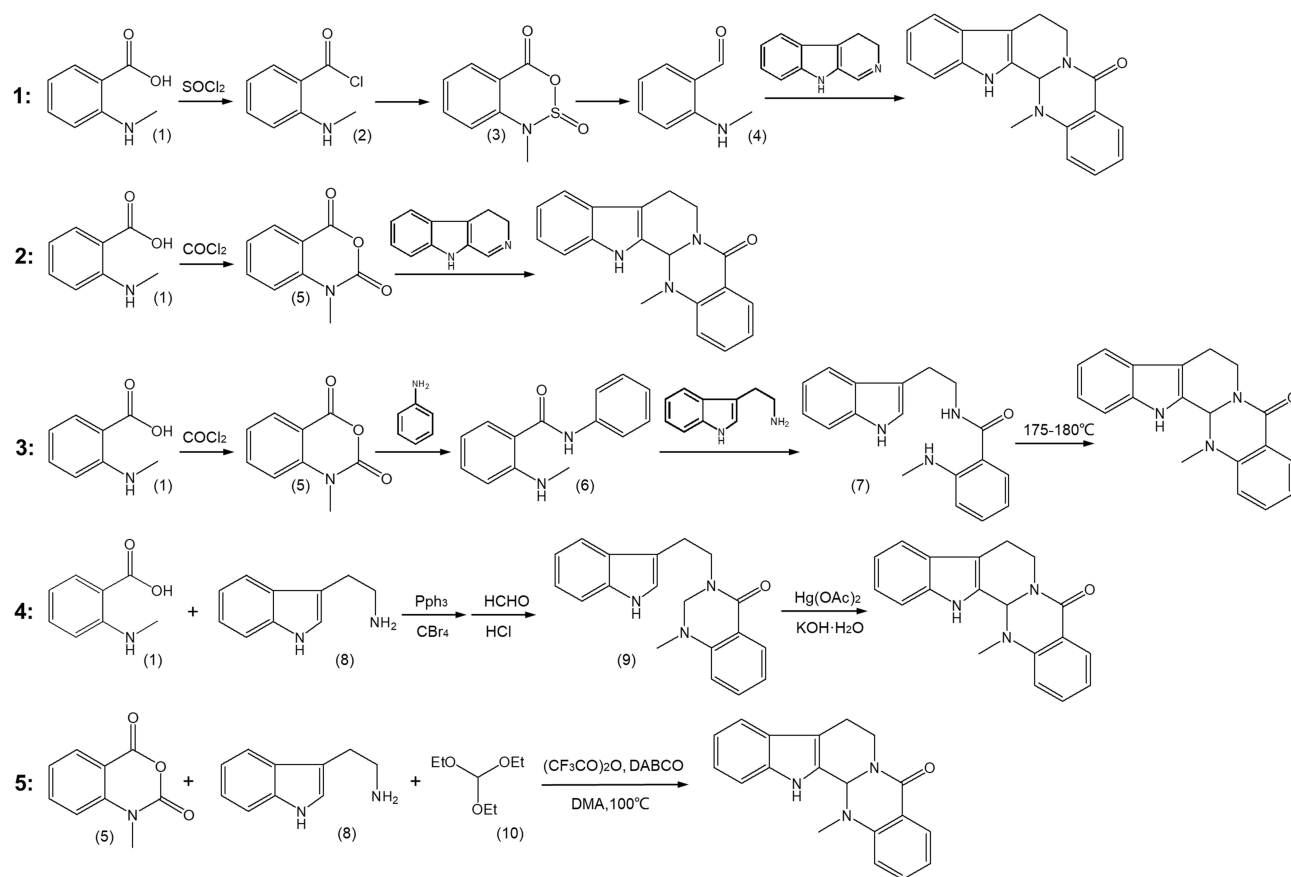


Figure 4 The main synthesis routes of evodiamine.

N-methyl-L-tryptophan and 3-indole-ethylamine (8) with triphenyl phosphite to form an amide, which reacts with formaldehyde to cyclize under the action of mercury acetate. Method 5: Tryptophan, N-methylindocyanine anhydride, triethylenediamine, trifluoroacetic anhydride, triethyl orthoformate, and N, N-dimethylacetamide are used as raw materials and are sequentially integrated into a reaction bottle through acylation, decarboxylation, condensation, and Pictet-Spengler reactions to achieve a one-pot synthesis of EVO.^{113,114}

Modification of the A Ring of EVO

The A ring of EVO can be modified mainly at the C-10 and C-12 positions. The introduction of different substituents at the C-10 position showed the following increasing order of antitumor activity strength: hydroxyl > iodine > fluorine > methoxy > bromine. The hydroxyl-substituted compound 1 obtained by substitution at the C-10 position showed better anticancer activity than camptothecin, topotecan, or irinotecan. The IC₅₀s of A549, MDAMB-435, and HCT116 were < 3 nM,¹¹⁵ and they exerted a good inhibitory effect on the proliferation of certain drug-resistant cells. The mechanism of action was by inhibiting HSP70 function by directly binding and destabilizing HSP70 protein.¹¹⁶ The C-13b position has an asymmetric center with enantiomers, and the (S)-isomer is more active than the (R)-isomer.¹¹⁷

10-Hydroxy EVO has good activity in vitro, but its in vivo antitumor effect is not very potent. A phosphate derivative (compound 2) was designed and synthesized to overcome this obstacle, which was orally active and showed improved antitumor efficacy in vivo in an HCT116 xenograft model (TGI value of 59%).¹¹⁸ Compound 3 obtained by amino substitution at the C-10 position showed enhanced antitumor effects. Its IC₅₀ for H1688 and H446 cells was 1.14 and 5.40 μM, respectively. A significant improvement in bioavailability was noted, and its AUC and C_{max} was 7.02 and 4.62 times that of EVO, respectively.¹¹⁹

Modification of the B Ring of EVO

N-13 on the B ring of EVO is the site that is most easily modified. In 2010, Dong et al first discovered that EVO was an effective inhibitor of the antitumor target Topo using molecular docking and reported that it could be used as a lead compound to develop antitumor drugs. They subsequently introduced different substituents at the N-13 position of EVO and found that the introduction of a 4-chlorophenyl moiety led to compound 4 with the highest activity and IC_{50} values ranging from 0.049 to 2.60 $\mu\text{mol/L}$.¹²⁰

The substitution of N-13 with a sulfur-containing group yielded compound 5 with good antitumor effects. Its IC_{50} values in human leukemia HL-60 and colon epithelial adenocarcinoma Caco-2 cells were 0.58 and 2.02 μM , respectively. Compound 5 could inhibit the cell apoptosis cycle in the G2/M phase and lead to mitochondrial dysfunction in HL-60 cells.¹²¹ An NO donor (compound 6) that was synthesized based on the N-13 site modification showed good cytotoxicity and selectivity between tumor and normal liver cells, with an IC_{50} in BGC-823 cells of 0.07 μM . The mechanism at the molecular level revealed that 13c caused cell-cycle arrest in the S phase and induced apoptosis in Bel-7402 cells through mitochondria-related caspase-dependent pathways.¹²² Replacing N-13 with ether-containing groups not only improved the antitumor effect but also enhanced the water solubility of EVO. For example, the IC_{50} of compound 7 for various tumors was in the range of 1–2 μM , indicating its potential to be developed as an antitumor drug.¹²³

Modification of the C Ring of EVO

Enantiomers of EVO can be obtained based on the spatial characteristics of the C ring.¹²⁴ De Petrocellis et al synthesized a series of enantiomeric EVO derivatives and found that S-EVO derivatives were more effective than R-EVO derivatives in human and rat transient receptor potential TRPV1 in transfected HEK-293 cells. The EC_{50} of compound 8 was 2 nM.¹²⁵

A series of derivatives with dual effects of inhibiting histone deacetylase 1 (HDAC1) and Topo2 were synthesized by substituting the C-7 position of the C ring. Compounds 9 and 10 showed good antitumor activities both in vitro and in vivo. In a mouse HCT116 xenograft model, the oral administration of compound 9 at a dose of 150 mg/kg for 15 days led to a tumor inhibition rate of 75.2% without obvious toxicity.¹²⁶

Modification of the D Ring of EVO

Modification of the D ring of EVO has been performed mainly at the C-5 and N-methyl (N14) sites, with the antitumor activity of carbonylthio substitutions at the C-5 site being stronger than that of carbonyl and methylene ($C=S > C=O, CH_2$). The increasing order of activity of substituents at the N14 site of the D ring of EVO was $S > O > N\text{-Me} > NH > CN$.

A series of N-14 alkyl-substituted EVO derivatives were designed and synthesized. Among them, the N(14)-propyl-substituted derivative (compound 11) showed good inhibitory activity on MGC-803, RKO, and SGC-7901 cells and could significantly inhibit the polymerization of Topo1 and tubulin.¹²⁷ Substituting the N-14 position of the D ring with 3'-fluorophenyl afforded compound 12 with good antitumor activity, which could inhibit Topo1 at 200 μM and arrest the cell cycle in both HGC-27 and HT-29 cells at the G2/M phase.¹²⁸

Modification of the E Ring of EVO

The C-3 position on the E ring is the optimal active site. When electron-donating substituents (hydroxyl, amino, and alkyl groups) or weak electron-withdrawing substituents (halogens) are introduced at the C-3 position, the antitumor activity increases. For example, when C-3 was replaced by F or Cl (compounds 13 and 14), their IC_{50} s against A549, MDAMB-435, and HCT116 were all < 3 nM. However, introducing strong electron-withdrawing groups (trifluoromethyl or nitro groups) at the C-3 position did not increase antitumor activity. Compound 15, with substitution at the C-3 position, showed good antitumor activity with a broad spectrum of activity and was an inhibitor of Topo1/Topo2. Molecular docking studies further confirmed that the derivatives acted by the dual inhibition of Topo1 and Topo2.¹²⁹

Synergistic Modification of EVO

Synergistic Modification of the A and E Rings

Hydroxyl and halogen groups can be substituted at the third position of the E ring on the basis of hydroxyl substitution at the tenth position of the A ring to further optimize the structure of EVO, which can not only retain the antitumor effect but also significantly improve its water solubility, which is very helpful in enhancing the *in vivo* antitumor effect (compounds 16–20).

Synergistic Modification of the A and D Rings

A series of compounds were synthesized by the synergistic modification of the C-10 site of the A ring and the N-14 site of the D ring. N-phenyl and sulfur atom substitution at the N14 site yielded compounds that were more active than those with oxygen atom substitution. Among them, compound 21 had a methyl substitution at the C-10 position and phenyl substitution at the N-14 position and showed good antitumor effects both *in vitro* and *in vivo*.¹³⁰

Synergistic Modification of the A, B, and E Rings

Researchers have synthesized a series of compounds based on 3,4,5-trimethoxyphenyl substitution at the N-13 position of the B ring, as well as the substitution of functional groups, such as methyl and halogen, on the A and E rings. All synthesized derivatives showed good antitumor activity. Among them, the IC₅₀ of compound 22 (only for A and B) against HGC-27 cells was $3.3 \pm 1.5 \mu\text{M}$.¹³¹

Synergistic Modification of the A, D, and E Rings

N-14 phenyl-substituted EVO derivatives show potential as antitumor drugs, but the preparation of these derivatives usually involves the modification of other rings. Compound 23 prepared by the substitution of N14-3'-fluorophenyl in the D ring combined with methyl substitution at the C-10 position of the A ring and the C-3 position of the E ring, showed good anti-gastric cancer effects *in vitro* and *in vivo* with a TGI of 70.12% in a MGC-803 xenograft model. Further studies on the antitumor mechanism showed that compound 23 inhibited Topo1.¹³² Further modification by N14-3'-fluorophenyl substitution led to derivatives 24 and 25,¹³³ with high antitumor activity and good inhibition against Topo1 and Topo2. Compound 24 inhibited Topo1 and Topo2 by 55.15% and 55.50% at 25 μM , respectively, whereas compound 25 had a tumor inhibition rate of 36.35%.¹³⁴

Wang et al designed 11 EVO-inspired novel scaffolds and their derivatives, among which compounds 26–30 were based on C-10 hydroxyl substitutions. All synthesized compounds showed excellent *in vitro* and *in vivo* antitumor efficacy with good tolerability and low toxicity. 10-Hydroxyl thio-EVO (26) was the most potent antitumor compound, with a tumor growth inhibition rate of 48% *in vivo*. 3-Chloro-10-hydroxyl thio EVO (28) is the first-in-class triple Topo1/Topo2/tubulin inhibitor.¹³⁵

Further optimization studies were carried out on this basis, and the synthesized compound 31 showed inhibitory activity in the HCT116 cell line in the low nanomolar range. Further mechanistic studies on the antitumor effect indicated that compound 31 acted by the dual inhibition of Topo1 and tubulin and induced apoptosis by G2 cell-cycle arrest. The quaternary ammonium salt of compound 31 exhibited excellent *in vivo* antitumor activity (TGI = 66.6%) and low toxicity in an HCT116 xenograft model.¹³⁶

Other EVO Modifications

Some researchers have made other modifications to EVO and synthesized a series of tetrahydro- β -carboline derivatives by eliminating the D ring of EVO. Among them, compound 32 showed good antitumor effects, with an IC₅₀ < 1 μM against HCT116, A549, and MDA-MB-231 cells.¹³⁷

The design and *in vitro* antitumor effects of a series of bis-EVO derivatives have been reported, wherein compound 33 effectively inhibited the proliferation and migration of HCT116 cells. The mechanism of action in HCT116 cells was attributed to the induction of apoptosis and arresting the cell cycle at the G2/M phase.¹³⁸

In another study, a series of C ring-opened EVO derivatives were synthesized by treatment with TFA, among which, compound 34 showed strong antitumor activity with IC₅₀ values against BGC803 and SW480 cells of 2.53 and 2.84 μM , respectively, and it exhibited improved solubility by nearly 20 times.¹³⁹

The main structural modification paths and characteristics of EVO are shown in Figure 5, and the antitumor activity of the modified derivatives is shown in Supporting Data. In addition to studying the antitumor activity, modified and transformed EVO derivatives have been evaluated for the treatment of AD^{140,141} and pulmonary hypertension¹⁴² and were also found to exert bacteriostatic¹⁴³ and insecticidal¹⁴⁴ effects.

EVO Preparations

EVO has many pharmacological activities, and many new preparations have been developed to improve the solubility and bioavailability of EVO (Figure 6) and also to realize some special functions, such as targeted enrichment, to better improve efficacy or reduce toxic side effects.^{145–147}

Particulate Preparation

Particulate drug delivery systems refer to solid, liquid, semi-solid, or gaseous drug preparations composed of particles with a certain size (in the micron or nanometer range) prepared using a drug or a suitable carrier and a certain dispersion embedding technology. These systems can improve the solubility of insoluble drugs, the bioavailability of drugs, and drug stability, as well as reduce adverse drug reactions, delay drug release, and improve drug targeting.

Liposomes

Liposomes are microcystic carrier preparations with lipid bilayer encapsulation. They can wrap water-soluble or fat-soluble drugs in the vesicles or bilayer membranes formed by the hydrophilic head and have good biocompatibility in vivo. Liposomes have become a hotspot in drug research because of their advantages in increasing drug solubility, delaying drug release, improving the stability of encapsulated drugs, improving drug bioavailability, and reducing the toxicity of encapsulated drugs. A study used soybean lecithin and cholesterol as starting materials to prepare EVO and berberine hydrochloride co-encapsulated liposomes using the film-dispersion method. The particle size was 126 nm, and the average encapsulation efficiency of rutaecarpine liposomes was 99%. Its inhibitory effect on the proliferation of B16 melanoma cells was significantly better than that of the original drug.¹⁴⁸ Using phosphatidylcholine and DSPE-mPEG₂₀₀₀ as carrier materials and the film-dispersion method, EVO and the photosensitizer indocyanine green were integrated into a liposomal nanoplatfor for noninvasive diagnostic imaging and combinatorial therapy against OSCC. The particle size of the liposomes was about 120 nm, and the drug loading was $87.49\% \pm 2.79\%$. The IC₅₀ against CAL

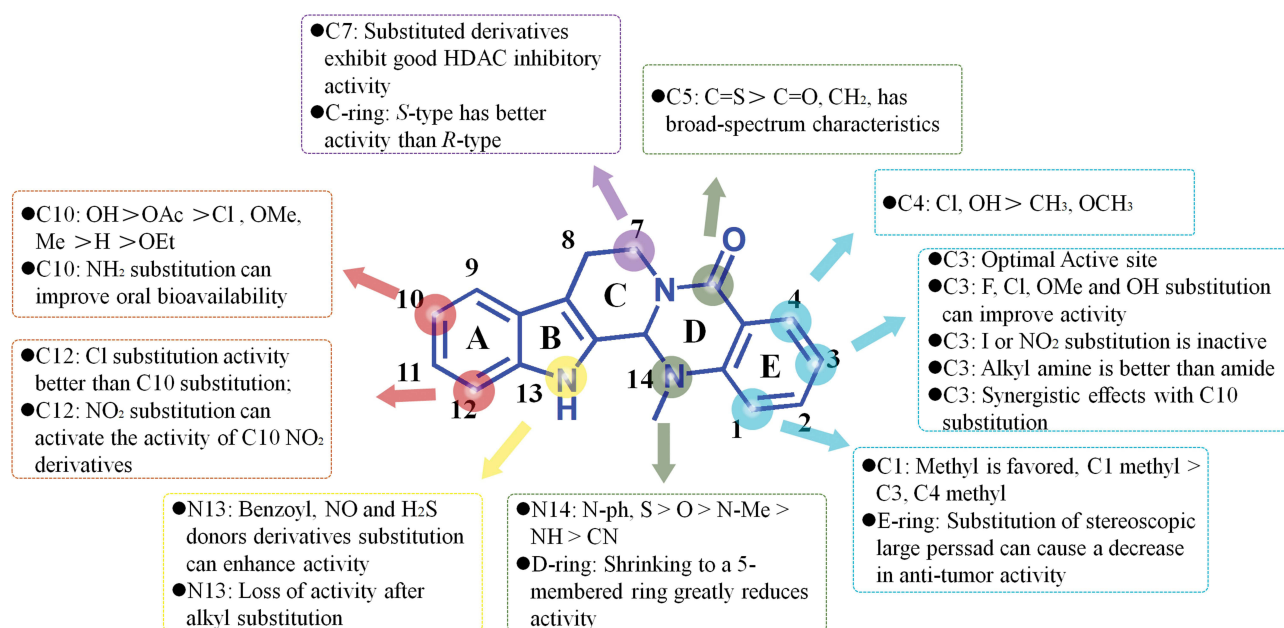


Figure 5 Modified derivatives of evodiamine A-E rings and their main characteristics.

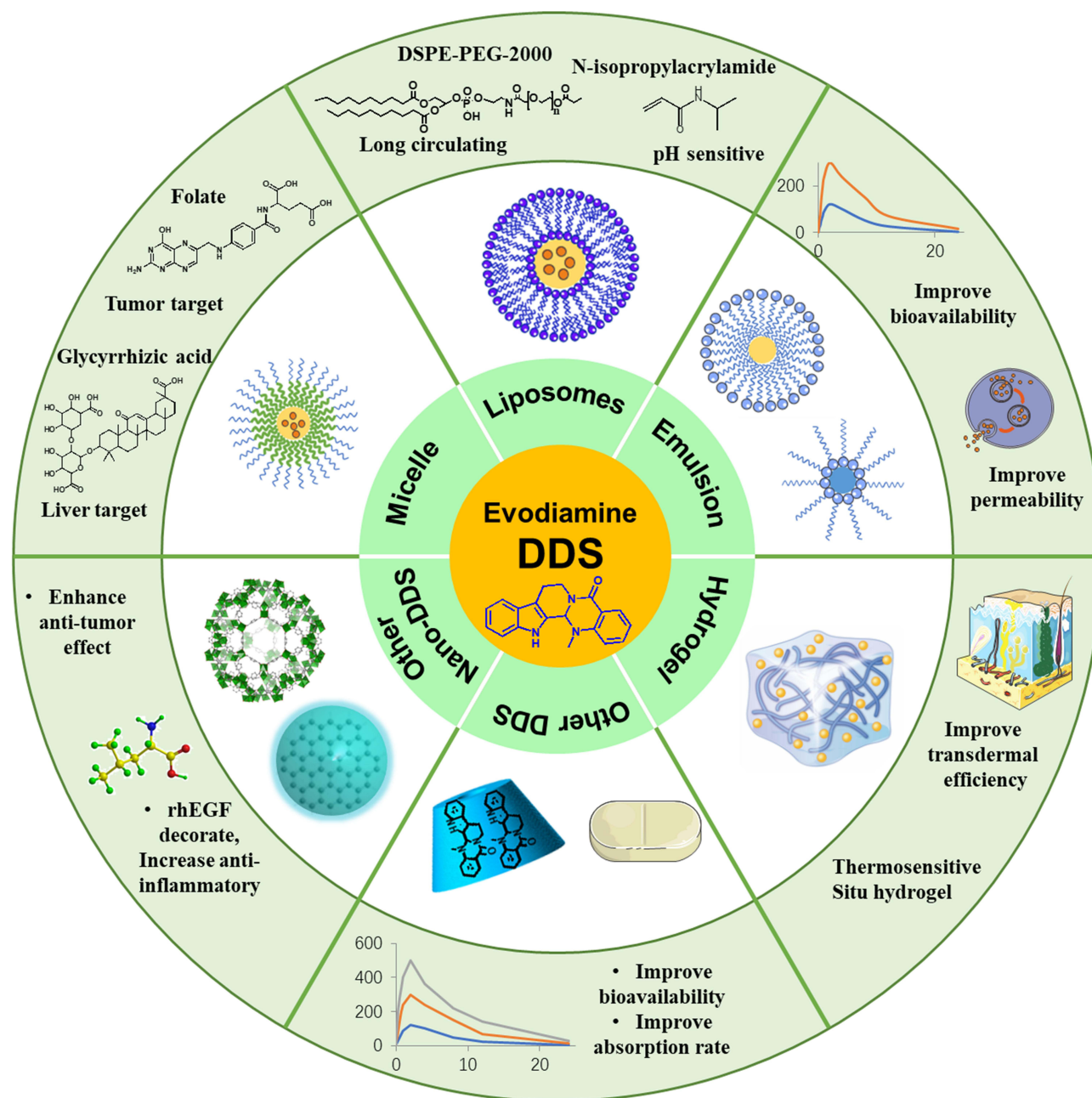


Figure 6 Evodiamine preparations.

27 and Fadu cells was decreased, and the liposomes had a significant inhibitory effect on in situ tongue tumors compared to the original drug.¹⁴⁹

Mesoporous silica nanoparticles (MSNs)@p(NIPAM-co-MA) were constructed for the co-delivery of EVO and BBR to further enrich the functions of the liposomes. The liposomes were temperature-sensitive and pH-sensitive due to N-isopropylacrylamide (NIPAM) and methacrylic acid (MA) modifications and had a sustained release effect due to the DSPE-PEG-2000 coating on the MSNs. The particle size of the nanoparticles was 230 nm, with an encapsulation efficiency of 98% and a drug-loading efficiency of 56%. The IC_{50} in HUVECs, HepG-2, HCT-8, and HeLa cells was in the range of 7.40 to 16.75 $\mu\text{g/mL}$, which was much higher than that of the free drug.¹⁵⁰

Micelles

Polymer micelles are self-assembled nanoparticles with hydrophobic cores and hydrophilic shells. They have the characteristics of a wide drug-loading range, stable structure, good tissue permeability, and long in vivo retention time, and they can effectively carry a drug to the target. A study used poly(lactic co-glycolic acid) (PLGA) as a polymer material and prepared EVO-PLGA nanoparticles using the solvent evaporation method. The drug loading was 5.8%, the encapsulation efficiency was 63.8%, and the particle size was 157.3 nm. In vitro studies showed that the proliferation of MCF-7 breast cancer cells decreased by > 50% after 48 h of incubation with 16 mM EVO-PLGA nanoparticles, which was obviously better than that of the free drug.¹⁵¹ EVO-glycyrrhizic acid micelles were prepared with glycyrrhizic acid as a functional carrier using the film dispersion method to treat liver fibrosis. The average size of the micelles was 130.8 nm, the encapsulation efficiency was 91.23%, and the drug loading was 8.42%. In the treatment of carbon tetrachloride-induced liver fibrosis in rats, the experimental results showed that its effects in improving liver function and liver fibrosis indices were superior to those of free EVO.¹⁵²

Targeting is an important characteristic of micelles. Pluronic F108 nanoparticles loaded with folic acid were prepared using solvent evaporation to achieve tumor targeting. The particle size of the micelle was 50.33 ± 3.09 nm, and the entrapment efficiency was $71.30 \pm 3.76\%$. In vitro studies showed that the IC_{50} for the original EVO drug and nanomicelles in HeLa cells was 45.41 ± 6.50 and 27.86 ± 1.63 $\mu\text{g/mL}$, respectively.¹⁵³ Other studies constructed nanomicelles loaded with EVO and the EGF-targeting ligand GE11 and PEG-polyamino acids using fluorescent labeling, which could effectively target colorectal cancer cells. The average particle size was 255 nm, the encapsulation efficiency was 94.1%, and drug loading was 10%. The IC_{50} of the nanoparticles and free drugs in LoVo cells was 9.732 and 19.51 $\mu\text{g/mL}$, respectively. Fluorescence imaging studies showed that the nanomicelles aggregated at tumor sites in nude mice, and the antitumor effects in mouse colon cancer models in situ showed that the nanoparticles could inhibit tumor growth efficiently, achieving an inhibitory effect analogous to that of cetuximab.¹⁵⁴ Some studies developed mitochondrial-targeted reduction-sensitive micelles that can co-deliver doxorubicin and EVO. The micelles first target mitochondria through triphenylphosphorus ions and then release the drugs by cleaving the disulfide bonds of the micelles by GSH. The particle size of the micelles was about 85 nm, and the drug loading and encapsulation efficiency of EVO were 5.6% and 62%, respectively. The IC_{50} value of the micelles against 4T1 cells was 1.45 $\mu\text{g/mL}$, and the tumor inhibition rate in 4T1 tumor model mice was up to 90%, both of which were superior to those of the free drugs.¹⁵⁵

Other Nanoparticles

Some EVO nanopreparations have been synthesized using inorganic materials, magnetic materials, and cyclodextrins to achieve targeting and magnetic responses, as well as to improve bioavailability and other functions. A study used graphene quantum dots (GQDs) as a carrier to deliver EVO, functionalized it with folic acid (FA), and synthesized a GQDs-FA-EVO nanocomposite system using a self-assembly method. The particle size was 85–130 nm, and the drug loading was 10%.

In vitro experimental results showed that the nanoparticles inhibited the growth of OSCC cells by > 50%. In tumor-bearing nude mice, GQDs-FA-EVO significantly reduced the tumor volume by 19% after 18 days compared with the original drug.¹⁵⁶ Polymer nanoparticles loaded with superparamagnetic iron oxide nanoparticles and EVO were synthesized using Fe_3O_4 and an MPEG-PLGA copolymer. The hydrodynamic diameter was approximately 261 nm, and the drug loading and encapsulation efficiency were $8.61 \pm 0.73\%$ and $40.36 \pm 3.42\%$, respectively. The iron content was about 9.34%, the sustained drug release lasted more than 70 h, and the IC_{50} of HeLa cell nanocarriers and free EVO was 13.8 and 9.84 g/mL , respectively. Using the H22 tumor ascites mouse model, the tumor inhibition rate of the nanoparticles reached 70% at a dose of 10 mg/kg .¹⁵⁷ EVO nanoparticles modified using recombinant human epidermal growth factor were prepared using the solvent evaporation method and carbodiimide chemistry. These nanoparticles can be used to treat gastric mucosal injury by activating the EGFR signaling pathway and the anti-inflammatory effect of EVO. The nanoparticle size was 179.07 nm, and the encapsulation efficiency was 94%. The degree of gastric mucosal injury was significantly reduced in male Sprague–Dawley rats with IND-associated gastric mucosal injury, the serum levels TNF- α and IL-6 were decreased, COX-2 levels in gastric tissue were decreased, and NO levels in gastric tissue were increased after the oral administration of the nanoparticle preparation.¹⁵⁸ Phospholipid and hydroxypropyl- β -

cyclodextrin were used to construct EVO-loaded nanoparticles using the solvent evaporation method. The synthesized nanoparticles had a size of 183.23 nm and showed better absorption than free EVO in the entire gastrointestinal tract. The relative bioavailability was 4.58 times that of free EVO. The IC_{50} of nanoparticles (EA) in H446 (human small cell lung cancer) cells was 17.20 μ M, 0.78 μ M, and 0.91 μ M at 24 h, 48 h, and 72 h, respectively, and the inhibition rates were better than those of free EVO (> 30.00 μ M, 29.54 μ M, and 3.97 μ M, respectively). In vivo experiments showed that the nanoparticles inhibited 76% of the H446 cell-transplanted tumors in nude mice and were better than that of free EVO (46%).¹⁵⁹

Microemulsions and Nanoemulsions

Microemulsions are composed of a water phase, a surfactant, a cosurfactant, and an oil phase. They have low viscosity, isotropic and stable thermodynamic properties, and are translucent, transparent, or slightly milky. These features of nanoemulsions can improve the bioavailability and permeability of EVO. Studies have used Cremophor® EL and PEG400 as surfactants or co-surfactants to prepare EVO microemulsions with a particle size of 10.7–48.1 nm. In an in vitro transdermal experiment, the skin throughput of the EVO microemulsion was 2.55–11.36 times that of the aqueous suspension. In microemulsion application with in vivo microdialysis, the maximum concentrations of EVO from the microemulsion were 1.52 and 3.06 times that from ointment and tincture, respectively,¹⁶⁰ and its transport mechanism was mainly attributed to the intercellular space.¹⁶¹ Water-in-oil EVO nanoemulsions were constructed using soybean phospholipid, Cremophor EL 35, and PEG400. The relative bioavailability of the nanoemulsion was 6.3 times higher, and its effective permeability in the colon was 8.64 times that of free EVO.¹⁶² A study used Fructus bruce oil to prepare emulsified nanosystems containing EVO. Fructus bruce oil has 2 excipient properties (oil phase and stabilizer) and a drug-like property (antitumor effect). The particle size of the nanoemulsion was 31.2 nm, and the IC_{50} against A549 cells was 6.79 μ M, 1.95 μ M, and 1.55 μ M for 12 h, 24 h, and 48 h, respectively. In vivo studies showed that its absorption constant and bioavailability were 8.38 times and 4.6 times higher than those of free EVO, respectively, and its tumor inhibition rate in the subcutaneous tumorigenesis model of the human A549 NSCLC cell line in nude mice was obviously better than that of free EVO.¹⁶³ The EVO nanoemulsion was prepared using ethyl oleate, Cremophor EL 35, and PEG400 as the oil phase, surfactant, and co-surfactant, respectively. The particle size was about 29 nm, and the IC_{50} for A549 cells was 31.53 μ M, 17.62 μ M, and 7.24 μ M for 12 h, 24 h, and 48 h, respectively, which were better than those of free EVO. Pharmacokinetic studies showed that the bioavailability (4.7 times that of the original drug) and in situ absorption characteristics (2–14 fold) of the nanoemulsions were significantly better than those of free EVO.¹⁶⁴ An EVO-phospholipid complex was prepared using solvent evaporation, and *Brucea javanica* oil, Cremophor EL 35, and polyethylene glycol 400 were added to prepare water-in-oil emulsions with a particle size of 613.3 nm. The preparation exhibited improved oral absorption and bioavailability of EVO. The absorption rate constant and permeability in different intestinal segments were 3.65–6.76 times that of free EVO and the relative bioavailability was 8.47 times that of free EVO.¹⁶⁵

Hydrogels

A hydrogel is a 3-dimensional network structure formed by the cross-linking of hydrophilic polymer chains. Hydrogels have good biocompatibility and can regulate the amount and rate of drug release by swelling and responding to specific stimuli (pH and temperature) to achieve sustained drug release. Using Cremophor EL as a surfactant and ethyl oleate as an emulsifier, a microemulsion was prepared and loaded into a hydrogel with a hyaluronic acid structure to increase the transdermal penetration of EVO by 2.60 times and enhance the analgesic effect in a mouse model of pain.¹⁶⁶ Studies have also prepared a self-assembled thermosensitive in situ hydrogel by constructing a berberine hydrochloride/HP- β -CD inclusion complex and EVO/HP- β -CD inclusion complex and then adding PEG8000, p188, and p407 and stirring to achieve the continuous co-delivery of berberine hydrochloride and EVO. After intranasal administration of this hydrogel, the bioavailability of berberine and EVO increased by approximately 135 and 112 times, respectively, compared with the free drugs after intragastric administration.¹⁶⁷

Others

In addition to the above preparations, some studies have reported the preparation of EVO in a phospholipid complex as a cyclodextrin-inclusion compound and as a dispersible tablet to improve the solubility and dissolution rate of fat-soluble drugs and further improve their bioavailability. Some studies designed a new type of rutaecarpine-phospholipid complex by the noncovalent complexation of phospholipid and rutaecarpine, with an average particle size of 246.1 nm. Its complexation rate reached 96.5%. Pharmacokinetic studies showed that its relative bioavailability increased significantly to 218.82% compared with free EVO.¹⁶⁸ An EVO inclusion complex was prepared using hydroxypropyl- β -cyclodextrin, and its inclusion rate reached 96%, obviously improving drug pharmacokinetics. The relative bioavailability was 256.73% that of free EVO,¹⁶⁹ and the inclusion complex significantly enhanced the antitumor effects. The IC_{50} of EVO and EVO/HP- β -CD in HepG2 cells was 8.516 and 0.977 μ M, respectively.¹⁷⁰ Solid dispersions of different concentrations of EVO and PVP-K30 were prepared using the solvent method, and pharmacokinetic studies in beagle dogs showed that the C_{max} and $AUC_{0-24 h}$ of EVO solid dispersion were 2.66 and 1.50 times higher than those of the physical mixture of samples, respectively, significantly improving the absorption and bioavailability rates.¹⁷¹

Summary

EVO is the main alkaloid in *E. rutaecarpa*, and it has great potential for development as an anticancer, anti-inflammatory, cardioprotective, and anti-AD agent. Its antitumor effects, especially, appear to be of the most interest, and numerous studies have confirmed the antitumor effects of EVO.^{172–174} Its mechanism of action is by acting on Topo1, blocking the cell cycle, inhibiting cell proliferation, inducing apoptosis, and inhibiting the invasion and metastasis of tumor cells. The pharmacological effects of this natural and novel anticancer drug are worthy of further exploration in basic research and clinical trials and may bring new options for treating patients with tumors. EVO also exerts anti-inflammatory, cardioprotective, anti-atherosclerosis, and other effects, but its exact curative effect and mechanism of action need elucidation. With the gradual deepening of the research on the main components of *Evodia rutaecarpa*, the multiple targets and mechanisms of the pharmacological activity of EVO have gradually emerged. However, studies reported that EVO is associated with potential liver, heart, and kidney toxicity, and there are potential risks in its clinical use, which seriously limit its clinical application. Previous studies revealed that the effective-toxic effects were mainly related to administration time and concentration. Long-term, high-dose administration may induce toxicity; therefore, when using EVO or drugs containing EVO to treat diseases, attention should be paid to the administration time and dosage to reduce toxicity and side effects. Further in-depth research is also needed on the toxicological mechanisms, and new clinical strategies need to be sought from the perspectives of toxicity efficacy conversion, efficacy enhancement, and toxicity reduction. In addition, comprehensive systematic exposure and liver exposure studies should be carried out based on the absorption and metabolism of EVO to identify a range for the safe clinical use of EVO. The supervision of the clinical use of *Evodia rutaecarpa* must be strengthened to control its dosage strictly and reduce the occurrence of adverse reactions.

Prospects

The poor water solubility of EVO limits its wide use in clinical settings. The following 2 pathways are currently used to further develop and utilize EVO: (1) Structural modifications of EVO: Studies on the structural modification of EVO and synthesis of novel EVO derivatives to improve the in vivo biological activity of EVO, increase the in vivo absorption of EVO, improve the bioavailability of EVO, and enhance the curative effect of EVO are useful approaches. Numerous potent EVO derivatives have been synthesized by modifying the A–E rings of EVO. However, further studies and verification are warranted before using these compounds in clinical settings. (2) Studies on EVO nanopreparations: Nanopreparations of EVO have been synthesized to improve the solubility, bioavailability, and therapeutic efficacy of EVO. Microparticle preparations, hydrogel, cyclodextrin-inclusion compounds, and solid dispersions have led to an increase in EVO absorption, bioavailability, and efficacy. Nanopreparations, such as liposomes and micelles, can improve the sustained-release and targeting effects, which can further improve the curative effect of EVO and reduce its toxicity and side effects.

However, current studies on EVO need further corroboration. Future research should focus on the following aspects. (1) Although many studies have reported the pharmacological activities of EVO, they are generally limited to cell or animal (preclinical) studies, and no clinical studies have been conducted. Thus, its efficacy needs to be further confirmed, and its complex mechanism of action needs to be elucidated. (2) Tumor multidrug resistance reversal agents are a current worldwide hot research topic, and subsequent research should focus on whether EVO can reverse drug resistance in tumor cells. (3) Several studies have shown that EVO has a certain toxicity risk. Thus, further studies on the affected sites, mechanism of toxicity, and its “dose-effect toxicity” relationship are necessary. Additional studies on the toxicological mechanisms and detoxification strategies are also warranted to provide guidance for subsequent clinical research. (4) At present, most of the pharmacokinetic studies on EVO have focused on absorption and metabolism, but there are few studies on its distribution in tissues and metabolism in vivo. In addition, most of the previous studies were pharmacokinetic studies conducted in animals. Therefore, an in-depth pharmacokinetic study of EVO in humans is needed to provide a basis for its later clinical translation. (5) EVO derivatives can improve the physicochemical properties and bioavailability of EVO; however, the stability, efficacy, and safety of several derivatives need to be further confirmed. (6) At present, research findings related to EVO preparations indicate that the derivatives have better bioavailability than the parent drug. However, these compounds are mostly at the basic laboratory research stage. A means to further their industrial production is an aspect that should be focused on.

Limitations: This article has many limitations. (1) It is only a summary of the current research on EVO and does not discuss the heterogeneity among studies or variations in experimental methodologies. There may be one-sidedness in some pharmacological effects or therapeutic effects. (2) At present, there is limited research on the toxicity of EVO, and this article cannot fully reveal the potential toxic effects of EVO or the adverse reactions that may occur in clinical practice. (3) Since EVO and its formulations have not yet reached the clinical trial stage, the current study is only based on the summary of preclinical studies.

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Disclosure

The authors declare that they have no competing financial interests.

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