

Progress in the structural modification and pharmacological activity of isosteviol

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ABSTRACT

Isosteviol and its derivatives, diterpene compounds with a wide range of biological activities, play important antiviral, antibacterial, antioxidant, antitumor, analgesic, anti-inflammatory, antipyretic, antifungal, and anticardiovascular disease roles. Because of its unique diterpene skeleton, isosteviol is frequently used as an active framework in drug synthesis, thus helping medicinal chemists design highly selective, potentially active, and multifunctional isosteviol analogs for the treatment of various diseases. In recent years, rapid developments in the design and synthesis of isosteviol derivatives have proven effective and important in the field of medicinal chemistry research. This article briefly reviews the novel derivatives obtained through structural modification of isosteviol, including their pharmacological activities, to provide a reference for the development of new synthetic strategies and the construction of new isosteviol derivatives, and to guide preclinical studies and development of new drugs with greater pharmacological efficacy.

Keywords: Isosteviol, Diterpene, Skeleton construction, Structural modification, Pharmacological activity

1. INTRODUCTION

Natural products are secondary metabolites that have been retained by organisms over millions of years of evolution and have diverse structural types [1]. Currently, approximately 40% of the basic skeleton types in the discovered natural products are unavailable in organic synthetic compound libraries; moreover, natural products have diverse bioactivities [2]. The natural structures of these novel skeletons have provided new ideas for drug research and development. After the discovery of morphine from opium in 1803 [3], many chemical drugs used clinically worldwide were initially discovered to have medicinal value after their extraction and isolation from natural medicines, structural determination, and screening for biological activity. These compounds were subsequently developed into new drugs after extensive research and clinical trials. The antimalarial drug quinine (of plant origin), the antispasmodic drug atropine, antitumor drug comedones, the lipid-lowering drug lovastatin, and the antimicrobial drug penicillin (of microbial origin) are typical representatives. Terpenoids are widely

present in many naturally occurring active substances, and are characterized by their complex structures, variety, and broad biological activities. These compounds serve as useful resources in the search for natural medicines [4]. Frequently used clinical drugs, such as artemisinin [5], paclitaxel [6], and andrographolide [7], are all terpenoids isolated from plants (Figure 1).

Stevia rebaudiana Bertonii, an herbaceous perennial plant of the *Stevia* genus in the Asteraceae family, grows naturally in South America and is widely cultivated in many parts of the world [8]. This plant contains high levels of diterpene glycosides, including stevioside and rebaudiosides (A, B, C, D, E, and F), which are used as sweeteners worldwide, owing to their sweetening properties 450 times higher than those of sucrose [9]. *Stevia* has potential medical applications. For example, stevia extract has antiviral properties, and contains functional compounds for the treatment of neuralgia, lumbago, anemia, eczema, rheumatism and dermatitis [10]. Stevioside, a favorable lead compound for diterpenoid discovery in medicinal chemistry, has one glycosidic element (steviol) and three glucose molecules.

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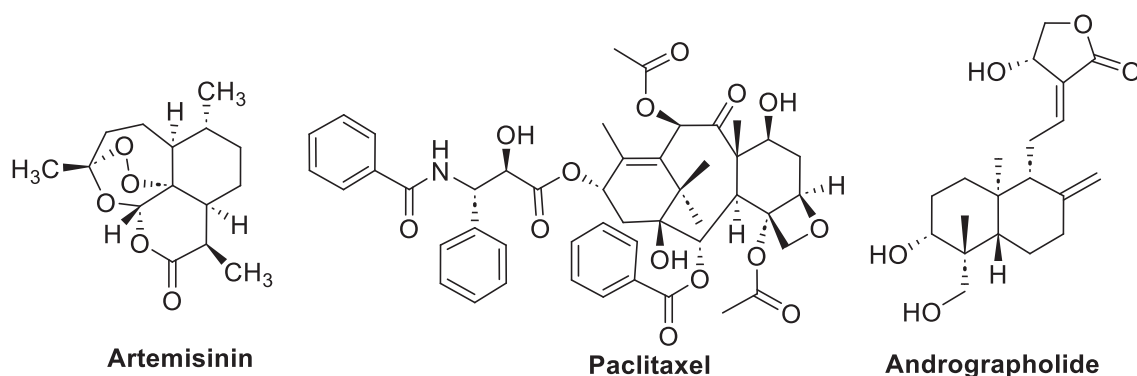


Figure 1 | Clinical terpenoid drugs.

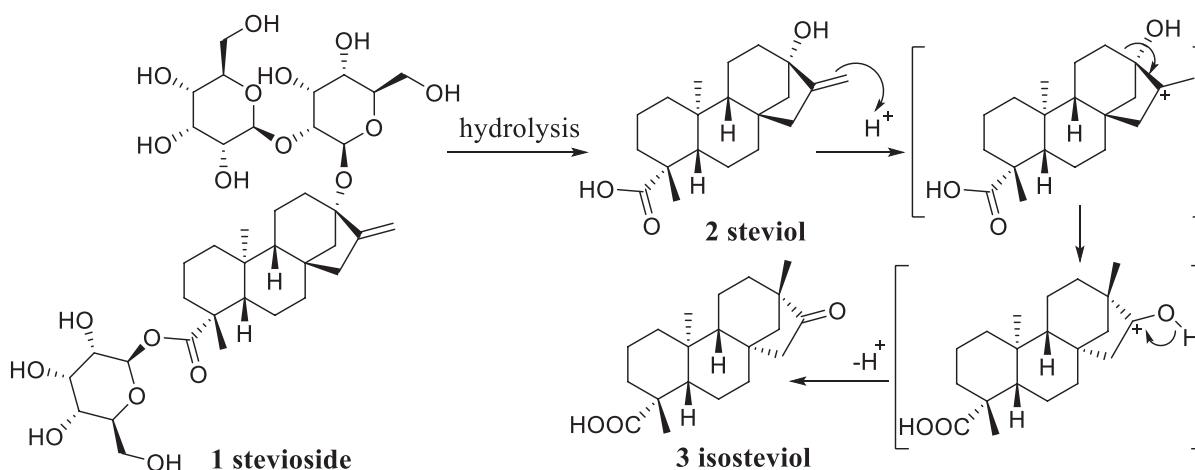


Figure 2 | Preparation of isosteviol.

Oxidative hydrolysis of steviol glycosides by sodium periodate yields steviol, and Wagner-Meerwein rearrangement [11] of steviol glycosides or steviol under acidic conditions yields isosteviol (Figure 2). Isosteviol and its derivatives also exhibit a variety of pharmacological activities, such as anti-tuberculosis [12], DNA polymerase and DNA topoisomerase inhibitory [13], hypoglycemic [14], neuroprotective [15], and plant growth regulatory [16] functions. Isosteviol is a diterpenoid that provides a unique diterpenoid core skeleton for drug development. Therefore this article reviews the pharmacological activity, structural modification, and chemical synthesis of isosteviol and its derivatives.

2. PHARMACOLOGICAL ACTIVITY OF ISOSTEVIOL

2.1 Amelioration of cardiovascular diseases

Isosteviol has been found to have salience prognostic value for numerous cardiovascular diseases, such as myocardial ischemia, diabetic heart disease, stroke, and cardiac hypertrophy. Liu established a rat model

of permanent coronary artery occlusion and administered intraperitoneal injection of isosteviol (4 mg/kg twice daily for 4 weeks), to investigate the protective effects and pro-angiogenic activity of isosteviol after myocardial infarction. The isosteviol group showed an ejection fraction 32% higher and a systolic index 25% higher than observed in the model group. *In vitro*, various concentrations of isosteviol increased the cell viability, branch length, and number of human umbilical vein endothelial cells. Moreover, isosteviol was found to promote angiogenesis in zebrafish and to up-regulate the expression of vascular endothelial growth factor five-fold. These findings provide potential new ideas for the treatment of myocardial ischemia [17]. Sodium isosteviol (STVNa), the sodium salt of isosteviol, has greater water solubility and bioavailability than isosteviol. The nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathway plays a crucial role in ischemic stroke. Zhang investigated the relationship between STVNa and miR-181 (microRNA-181) in the NF- κ B signaling pathway in a brain ischemia model

in mice administered 30 mg/kg STVNa intravenously. STVNa was found to up-regulate the expression of CYLD (cylindromatosis), inhibit the NF- κ B signaling pathway, and down-regulate the expression of miRNA-181b, thus playing a role in the treatment of ischemic stroke in the mice. Moreover, this treatment protected mouse N2A neuroblastoma cells against 300 μ M CoCl₂ induced hypoxia injury. Regulation of the miR181b-CYLD-NF- κ B signaling pathway by STVNa may provide a new treatment method for ischemic stroke [18]. Tang studied the therapeutic effect of STVNa on diabetic cardiomyopathy. Male Wistar rats were injected with streptozotocin (55 mg/kg) to induce diabetes. The STVNa group was orally administered 8 mg/kg STVNa, whereas the control group was administered solvent. The diabetic group showed problems related to heart function, structure, oxidative stress, and inflammation. STVNa treatment significantly inhibited cardiac hypertrophy, fibrosis, and inflammation, without altering blood glucose and insulin levels. Moreover, this treatment inhibited extracellular regulated protein kinases and NF- κ B. The finding that this signaling pathway ameliorates diabetic cardiomyopathy in rats suggests that STVNa might potentially be used to treat diabetic cardiomyopathy [19].

2.2 Anti-inflammatory effects

Isosteviol has been reported to have anti-inflammatory effects; however, the mechanism underlying its anti-inflammatory action is unclear. Yao has shown that isosteviol, when administered orally at a concentration of 10 μ M, protects against dextran sulfate sodium-induced colitis, by decreasing colonic inflammation and inflammatory cytokine levels and improving intestinal barrier function. The colon length increased; the inflammation score decreased; and the expression of the tight junction proteins Zona Occludens 1, occludin, and claudin-1 was up-regulated. The underlying mechanism involves inhibition of tumor necrosis factor- α (TNF- α)-induced cell barrier dysfunction by regulating the PDK1/AKT/NF- κ B signaling pathway. Therefore, isosteviol might potentially be used for treating inflammatory bowel disease, by maintaining the intestinal barrier and decreasing inflammation [20]. Matrix metalloproteinase 9 (MMP9) has important roles in various models of inflammation and wound healing [21]. This protein is closely associated with the development of inflammation, and it mediates shedding of the pro-inflammatory cytokine TNF and recruitment of pro-inflammatory cells in inflammatory regions, thereby promoting inflammation [22]. Qiao, in a study of burn inflammation, screened CCL3, CCL4, MMP9, and other macrophage-associated inflammatory factors as potential targets through bioinformatics. Subsequently, male mice were subjected to burn treatment. The treatment group was intraperitoneally injected with 20 mg/kg isosteviol after the burn, then fed normally. The expression of factors was detected with immunohistochemistry. Isosteviol treatment decreased the expression of interleukin-6

and TNF- α and increased the expression of MMP9 in burn wound tissue during acute inflammation, thereby decreasing inflammation after burn injury [23].

2.3 Hypoglycemic effects

Diabetes mellitus is a chronic multifactorial disease characterized by persistently high blood glucose levels resulting from impaired insulin secretion, and consequent defects in carbohydrate, lipid, and protein metabolism [24]. Isosteviol has been found to have anti-hyperglycemic properties *in vivo* in diabetic animal models [25]. Foxa2, Pax6, Nkx2.2, and Nkx6-1 are islet-enriched transcription factors that can act as both insulin sensitizers and inhibitors of insulin secretion by pancreatic β cells [26, 27]. Nordentoft randomly divided diabetic KKAY mice into two groups: a control group fed a regular diet and a treatment group receiving 20 mg/kg isosteviol for 6 weeks. Normal C57BL mice were fed a standard diet and served as a non-diabetic control group in a study of the effect of isosteviol on diabetes prognosis. Compared with the control group, the isosteviol treatment group of diabetic KKAY mice showed improved glucose homeostasis, higher insulin sensitivity, and lower plasma triglycerides and body weight. Simultaneously, the expression of the insulin essential regulatory genes *Ins 1*, *Ins 2*, and *Pax6*, and the β -cell transcription factors *Nkx2.2*, *Nkx6-1*, and *FoxA2* in isolated islets of mice were significantly altered, thereby providing a basis for the use of isosteviol as a potential anti-diabetic drug [28]. Xu established hyperglycemia and hyperlipidemia models through intragastric administration of a high-fat emulsion for 14 days, then orally administered isosteviol at 0.2, 1.0, or 5.0 mg/kg/day for 7 days. Rosiglitazone maleate (5.0 mg/kg/day) served as a positive control to study the effects of isosteviol on hyperglycemia and hyperlipidemia. Isosteviol decreased serum glucose levels, and all doses of isosteviol decreased fasting blood glucose, fasting serum insulin, total cholesterol, and low-density lipoprotein levels, but increased high-density lipoprotein levels. The mechanism of action might be associated with isosteviol's ability to partially decrease damage to islet cells and enhance the hepatic expression of PPAR α mRNA. Isosteviol has been shown to regulate blood glucose and lipids, and therefore might have potential value in treating hyperglycemia and hyperlipidemia [29].

2.4 Antitumor effects

Cancer, the second leading cause of human death after cardiovascular disease, poses a severe threat to human health. Anti-cancer research has consistently garnered global attention [30]. Sui observed enhanced inhibitory effects of isosteviol on various human gastric cancer cells, human colorectal cancer cells, and human liver cancer cells, in a concentration- and time-dependent manner (with tested concentrations of 50, 100, 150, 200, or 250 μ g/mL, and times of 24, 48, or 72 h). Moreover, the drug action was highest at 48 h. The IC₅₀ values

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of isosteviol in HepG2 cells were 181.1 $\mu\text{g/mL}$ (24 h), 119.0 $\mu\text{g/mL}$ (48 h), and 85.7 $\mu\text{g/mL}$ (72 h). The inhibition rate of isosteviol showed the most rapid increase at 48 h, and the IC_{50} value showed the smallest decrease. Subsequently, the effects of isosteviol on the morphology of liver cancer cells and the cell cycle of cancer cells were studied. Isosteviol decreased the density of cancer cells, thus distinguishing them from the surrounding cells, and caused loss of the translucent morphology of cells in the normal growth state. Isosteviol showed different blocking periods for cancer cells in G1, S, and G2 phases. Isosteviol might potentially interfere with the transcription or replication of DNA by blocking different cell cycle stages and inhibiting the synthesis of proteins required for specific cell cycle stages, thereby exerting anti-tumor activity [31].

Although isosteviol has many biological activities, its low solubility limits its application. Chemical conversion of isosteviol to increase bioavailability and optimize pharmacological activity is crucial to enhance its medicinal value. Isosteviol derivatives belong to a class of drugs with critical pharmacological activities. To date, thousands of new isosteviol derivatives have been obtained through organic synthesis, and have shown more favorable pharmacological activities than isosteviol, including antibacterial, anti-inflammatory, antiviral, anti-tumor, plant growth regulatory, and enzyme inhibitory pharmacological effects. The many compounds are discussed herein, categorized according to the location of the structural modification within the ring and the year of publication.

3. STRUCTURAL MODIFICATION AND PHARMACOLOGICAL ACTIVITY OF ISOSTEVIOL

The molecular structure of isosteviol (Figure 3) has two main active groups: the carboxyl group (COOH) at position C-19 on the A-ring and the carbonyl group ($\text{C}=\text{O}$)

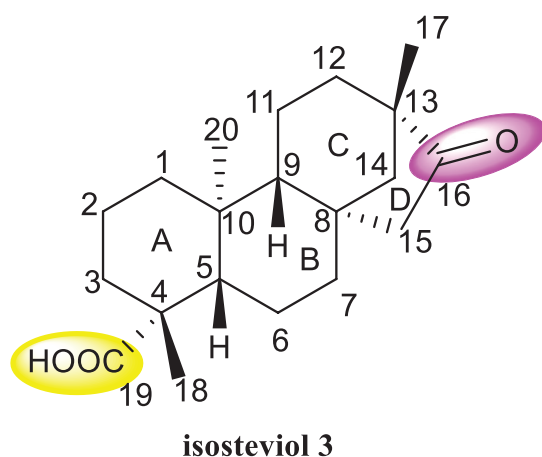


Figure 3 | Structure of isosteviol.

at position C-16 on the D-ring. Structural modification of isosteviol has focused on these two rings, and a variety of derivatives with differing pharmacological activities have been obtained. We have summarized the synthetic scheme (Scheme S1–S33) of isosteviol derivatives and compiled tables (Table S1) summarizing the pharmacological effects of isosteviol derivatives as shown in the supporting material.

3.1 Modification of the isosteviol A-ring

Modification of the A-ring of isosteviol has focused primarily on the carboxyl group (COOH) at the C-19 position. In 2024, Denner designed and synthesized various isosteviol derivatives (Scheme S1) containing aminosulfonic acid groups with carbonic anhydrase II (CA II) as the target. Screening of the CA II inhibitory activity of the compounds revealed that derivatives **7**, **9**, and **11** were the optimal inhibitors of the enzyme, with inhibition rates exceeding 94%. Additional kinetic measurements revealed that these compounds were competitive inhibitors of CA II, and derivative **11** had the highest activity, with an inhibition rate of 99.9% for CA II and a K_i of 2.59 μM [32]. Chen systematically synthesized 24 isosteviol derivatives (Scheme S2) from isosteviol through a three-step reaction and evaluated the cardioprotective activity of the derivatives in a doxorubicin (DOX)-induced zebrafish model. The activity of **14a–14c** was highest. The zebrafish survival rate after exposure to compound **14b** was 95%, that for **14a** was 92%, and that for **14c** was 88%, at concentrations of 5 μM . Compound **14b** showed the clearest activity, but its limited solubility introduced further research and application challenges. Therefore, **14b** was converted into the corresponding sodium salt **15** to increase its solubility. The two compounds showed nearly identical activity in decreasing cardiac edema, restoring normal heart shape, and ameliorating DOX-induced cardiac dysfunction. Moreover, compound **15** was not toxic. Further studies indicated that derivative **15** protected H9c2 cardiomyocytes against DOX-induced oxidative damage and ameliorated DOX-induced cardiomyopathy in mice by activating the SIRT3/SOD2 and OPA1 pathways [33].

In 2021, Voloshina prepared derivative **18** (Scheme S3) through a three-step reaction. This compound showed favorable potential in antibacterial and anti-cancer aspects. Antibacterial activity studies indicated that the inhibitory activity of derivative **18** against MRSA-1 strain ($\text{MIC} = 2.80 \pm 0.22 \mu\text{M}$) was significantly higher than that of the reference drugs amoxicillin and ciprofloxacin. The activity against MRSA-2 strain ($\text{MIC} = 1.40 \pm 0.11 \mu\text{M}$) was comparable to that of ciprofloxacin but 15–20 times higher than that of amoxicillin. Derivative **18** also had high bactericidal activity against *Staphylococcus aureus* and may serve as a scaffold for designing efficient new antibacterial drugs. Antitumor activity studies revealed that the derivatives had strong cytotoxic effects on the tumor cell lines MCF-7, M-HeLa, A-549, PC3, HepG2, and T98G. Derivative **18** had the

highest activity against MCF-7 human breast cancer cells ($IC_{50} = 5.0 \mu M$), but differed from the control drug DOX in its selective effects on cancer cells and lower toxicity to normal cells. Although compound **18** might have the potential to treat cancer, further studies are required to verify its safety and efficacy [34, 35]. Garifullin prepared a series of conjugates of diterpene isosteviol and N-acetyl-D-glucosamine (Scheme S4). Activity assays indicated that most conjugates were cytotoxic to several human cancer cell lines (M-HeLa, MCF-7, Hep G2, PANC-1, and PC-3). Conjugates **25a** and **25b** showed selective cytotoxicity toward M-HeLa cells ($IC_{50} = 13 \mu M$ and $14 \mu M$), to a level twice that of the control anticancer drug tamoxifen ($IC_{50} = 28 \mu M$). The antiproliferative activity against M-HeLa cells was due to the induction of apoptosis [36]. Cutaneous leishmaniasis (Kala-Azar) is an endemic infectious disease in tropical and subtropical countries. This disease is transmitted by phlebotomines infected with protozoa of the genus *Leishmania*; the currently available drug treatment options are limited. Murillo used the Grignard, Mitsunobu, and Williamson reactions, and other classical reactions, to modify the structures of the A-ring and D-ring; synthesized various types of isosteviol derivatives; and evaluated the anti-*Leishmania* activity and cytotoxicity (Scheme S5). Activity screening revealed that derivative **31** had low cytotoxicity ($LC_{50} = 279.7 \mu M$) and high anti-*Leishmania* activity ($EC_{50} = 33.5 \mu M$) [37].

In 2020, Andreeva synthesized a series of glycosylated 1,2,3-triazole derivatives of isosteviol (Scheme S6) through a two-step click reaction, and evaluated their cytotoxicity against human cancer cells and normal cell lines. The derivatives **36**, **37**, and **38**, showed highly selective cytotoxic activity against M-HeLa cells ($IC_{50} = 1.7\text{--}1.9 \mu M$), which was comparable to that of the anticancer drug DOX ($IC_{50} = 3.0 \mu M$). The compounds showed no cytotoxicity against normal human liver cell lines ($IC_{50} > 100 \mu M$). The isosteviol glycoconjugate containing a 1,2,3-triazolyl ring is therefore a promising scaffold for designing new anticancer drugs [38].

In 2019, Sharipova synthesized a variety of glycosides and glycoconjugates of isosteviol, and determined their antiproliferative activity against tumor cells (Scheme S7). Most derivatives showed moderate to significant cytotoxicity against M-HeLa and MCF-7 cancer cells. Derivatives **40** ($IC_{50} = 15.1 \pm 1.2 \mu M$), **43a** ($IC_{50} = 10.0 \pm 0.7 \mu M$), and **44a** ($IC_{50} = 11.0 \pm 0.9 \mu M$) showed a selective cytotoxicity against M-HeLa cells three times that of the anticancer drug tamoxifen ($IC_{50} = 28.0 \mu M$). Most derivatives showed no cytotoxicity toward normal human hepatocytes ($IC_{50} > 100 \mu M$), whereas tamoxifen inhibited normal human hepatocytes (IC_{50} of $46.0 \mu M$). Further studies revealed that the cytotoxicity of the derivatives was due to apoptosis induction through the mitochondrial pathway, and structure-activity relationship studies indicated the anticancer activity of the derivatives was largely dependent on whether the monosaccharide residues

were directly attached to the isosteviol backbone or linked through polymethylene [39].

In 2018, Sharipova synthesized isosteviol and conjugates linked by various glycosides, then evaluated their antibacterial and anti-tuberculosis activity. Compound **48** had high antibacterial activity against *S. aureus* ATCC 209P, with an MIC of $31.3 \mu g/mL$, twice that of the antibiotic chloramphenicol. Compound **48** inhibited the growth of *Mycobacterium tuberculosis* H37Rv, and had similar activity to the anti-tuberculosis drug pyrazinamide (Scheme S8) [40].

In 2017, Liu synthesized acyl thiosemicarbazide derivatives **51** through a three-step reaction. The oxadiazole isosteviol derivative **52** (Scheme S9) was finally cyclized after $Hg(OAc)_2$ treatment. The structure-activity relationship and *in vitro* activity studies indicated that derivatives **51a–51e** exhibited extremely low IC_{50} values ($0.95\text{--}5.33 \mu M$), whereas compounds with strong electron-withdrawing substituents exhibited favorable cytotoxic activity. After introduction of acyl thiosemicarbazides into the framework of isosteviol, the inhibitory activity of three cancer cell lines (colon cancer HCT-116, gastric cancer HGC-127, and lymphoma JEKO-1) significantly increased, and the antitumor activity of acyl thiosemicarbazide substituted isosteviol derivatives was significantly higher than that of oxadiazole derivatives. The anti-proliferative activity of derivative **51e** ($IC_{50} = 0.95 \mu M$) against the HCT-116 cell line was higher than that of cisplatin ($IC_{50} = 3.91 \mu M$); The activity of derivative **51b** ($IC_{50} = 2.31 \mu M$) on the HGC-127 cell line was equivalent to that of cisplatin ($IC_{50} = 2.03 \mu M$). For the JAKO-1 cell line, only **52f** ($IC_{50} = 2.50 \mu M$) of most oxadiazole derivatives had higher inhibitory activity than cisplatin ($IC_{50} = 2.74 \mu M$). These findings provide an important reference for the development of new drugs for tumor treatment [41].

In 2015, Huang synthesized derivative **55** (Scheme S10), using isosteviol as the starting material through a three-step reaction, then tested its *in vitro* anti-hepatitis B virus (HBV) activity. Derivative **55** showed better inhibition of the antigen of HBsAg and HBeAg in HepG2 2.2.15 cells and inhibition of the replication activity of HBV DNA [42].

In 2014, Wang synthesized a series of fusion compounds of a nitric oxide donor and isosteviol (Scheme S11). Cytotoxicity tests showed that the derivatives had different degrees of anti-proliferative activity against B16-F10 melanoma cells and HepG-2 cells. The C-19-coupled derivative **63** showed the strongest anti-proliferative effect against B16-F10 melanoma cells ($IC_{50} = 0.02 \mu M$), whereas the anti-proliferative activity of compound **58** against HepG-2 cells ($IC_{50} = 4.24 \mu M$) was better than that of the positive control CDDO-Me ($IC_{50} = 4.99 \mu M$) [43]. Through click chemistry, Khaybullin designed and synthesized a series of 1,2,3-triazole and isosteviol conjugates (Scheme S12). Anti-tumor activity tests indicated that the derivative **65a** had the most significant anti-tumor activity among all compounds and showed good

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activity against all tested cancer cell lines. The IC_{50} values in lung cancer A549, pancreatic cancer ASPC-1, and colon cancer HCT-116 cells were lowest, at 9.95, 4.79, and 6.60 μM , respectively. In addition, conjugate **65b**, without an adaptor, lost anti-tumor activity against lung, pancreatic, breast, and prostate cancer cells, yet still specifically inhibited the growth of cervical cancer cells, with an IC_{50} value of 5.83 μM . A structure-activity relationship study demonstrated that the increased polarity of the triazole moiety significantly decreased the anticancer activity of the isosteviol derivatives, and the hydrophobicity of the triazole moiety played a key role in the anti-proliferative activity of the isosteviol conjugates [44].

In 2013, Ukiya synthesized isosteviol derivatives **68a–68e** and **69a–69e** through three steps. The toxic activity of derivatives **68a**, **68d**, and **68e** against lung cancer (A549) and gastric cancer (AZ521) cells was greater than that of the positive control drug cisplatin, and the toxic activity of derivatives **68a**, **68c**, and **68d** against breast cancer (SK-BR-3) cells was greater than that of cisplatin (Scheme S13) [45].

In 2004, Lin synthesized isosteviolamide dimer derivative **71** (Scheme S14) by using alkyl diamine **70** to form an alkylamine linkage between the C-19 carboxyl groups. The derivative showed favorable anti-proliferative effects against HL-60 human leukemia cells, HepG-2 human hepatoma cells, and H460 lung cancer cells, with IC_{50} values of 4.8 mM, 17.4 mM, and 14.0 mM, respectively [46].

Several A-ring modified derivatives exhibited alterations in antibacterial and anti-inflammatory activities. The modified molecules augmented the inhibitory effects against particular bacterial strains and decreased the release of inflammatory factors. The underlying mechanism involved modification of the mode of interaction with the target or regulation of the inflammatory signaling pathway. Nevertheless, current A-ring modification efforts predominantly focus on specific sites and a limited number of reaction types. This constraint restricts the expansion of structural diversity and reflects an inadequate understanding of the molecular mechanism of action and structure-activity relationships. Collectively, these limitations impede further progress in A-ring modification research and must be overcome.

3.2 Modification of the isosteviol D-ring

The D-ring modification accompanies A-ring structural modification. In most cases, the carboxyl group of the A-ring is esterified and protected first; some columns of chemical reactions are performed through the carbonyl group of the D-ring; and a variety of biologically active isosteviol derivatives are obtained.

In 2024, Qi modified the C-15 and C-16 positions of the D-ring of isosteviol, and designed and synthesized isosteviol derivatives of different structural types (Scheme S15). The inhibitory effect of compound **74** ($IC_{50} = 16.1 \pm 3.18 \mu M$) against HeLa cells was comparable to that of the positive control drug gefitinib ($IC_{50} = 15.4 \pm$

4.65 μM). The inhibitory effects of compound **74** ($IC_{50} = 7.84 \pm 0.84 \mu M$) and compound **78** ($IC_{50} = 6.89 \pm 0.33 \mu M$) against A549 cells were significantly better than that of gefitinib ($IC_{50} = 11.0 \pm 3.27 \mu M$) [47]. Ozsvár synthesized free carboxylic acids, and benzyl, propargyl, and acryloyl ester derivatives from isosteviol (Scheme S16). The anti-proliferative activities of the derivatives against human tumor cell lines (A2780, HeLa, MCF-7, and MDA-MB-231) were studied. Compound **82** was the most active compound, with low IC_{50} values of 1.99 μM , 1.78 μM , and 1.37 μM for HeLa, MDA-MB-231, and MCF-7 cancer cells, respectively. In addition, the Boc-protected amino alcohol derivative **89** showed a selective cell growth inhibition effect against A2780 cells ($IC_{50} = 4.78 \mu M$), thereby suggesting that the structure might potentially serve as a candidate drug for ovarian cancer [48].

In 2023, Li treated isosteviol with bromoethane and KOH at room temperature to obtain ethyl ester derivative **72**, which was subjected to stereoselective reduction with $NaBH_4$ to obtain hydroxyl-containing derivative **73**, then treated with benzoic acid or p-hydroxybenzoic acid. Intermediate **73** was converted into aromatic ester derivatives **90a** and **90b**. Subsequently, through a base-catalyzed nucleophilic substitution reaction of isosteviol with organic halides in DMF, derivatives **91a** and **91b** were obtained. Ethyl ester derivative **72** was treated with excess ethyl bromide and dimethyl sulfoxide at 80°C to obtain the D-ring brominated derivative **92** (Scheme S17). Derivatives **90b**, **91a**, **91b**, and **92** significantly inhibited tyrosine phosphatase 1B (PTP1B), with IC_{50} values of 0.24–0.40 μM . Derivative **92** had the strongest inhibitory activity against PTP1B ($IC_{50} = 0.24 \mu M$) and had potential anti-diabetic activity [49]. Chen reacted isosteviol with excess paraformaldehyde to obtain hydroxylated derivative **93**, which was then subjected to a condensation reaction with benzo-1,3-dioxacyclo-5-acetic acid to obtain derivative **95**; subsequently, the hydroxyl group of the D-ring was oxidized to a carbonyl group with PDC reagent to obtain derivative **96** (Scheme S18). Biological activity studies indicated that derivatives **95** and **96** have cardioprotective effects, can effectively maintain normal heart shape, decrease pericardial edema, and improve DOX-damaged heart performance. Mechanistic studies have demonstrated that derivative **95** protects cardiomyocytes against oxidative stress by inhibiting excessive accumulation of reactive oxygen species, activating superoxide dismutase 2 expression, enhancing the endogenous antioxidant defense system, and maintaining a normal cardiac phenotype. Its cardioprotective effects exceed those of its parent compound, isosteviol, and positive drug levosimendan. These findings have provided a pharmacological basis for developing potential clinical candidates for treating cardiovascular diseases [50].

In 2021, Zhang prepared intermediate **93**, whose carboxyl group was converted to $-NH_2$ through Curtius rearrangement. Derivatives **99a–99e** were then synthesized through a four-step reaction of esterification

and oxidation (Scheme S19). Finally, the cardiovascular protective activity of the derivatives *in vivo* was evaluated in a zebrafish model. The new derivative **99d** effectively decreased cardiac edema in DOX-induced zebrafish cardiomyopathy; maintained normal heart shape; decreased levels of the cardiomyopathy biomarkers cTnT and ANP mRNA; and prevented cardiomyocyte death and injury by inhibiting the excessive accumulation of cellular reactive oxygen species [51]. Consequently, mitochondrial membrane potential was restored, and mitochondrial morphology was maintained. Covalently bound drugs exert corresponding biological activities by forming covalent bonds with targets; the advantages of these drugs include high efficacy, high ligand efficiency, and long-lasting efficacy. Two covalent warheads, α , β -unsaturated ketones [52], and five-membered unsaturated lactones [53], are present in many natural products and synthetic molecules, and have strong anti-tumor effects. Covalent drugs react with protein sulfhydryl groups and subsequently covalently bind proteins, thus resulting in protein structure changes and cell death; however, the destruction of functional groups leads to loss of biological activity [54]. Liu constructed two covalent warheads in the skeleton of the A-ring of isosteviol and chemically modified them to synthesize various isosteviol derivatives (Scheme S20). These new molecules showed effective anti-proliferative effects against various human cancer cells. Compounds **106** and **107a–107c** showed low IC_{50} values (0.29–7.6 μ M) against various cancer cells. In particular, compound **107a** had an IC_{50} value of 0.29 μ M for the A549 cell line, and showed low toxicity toward normal human mammary epithelial cells and normal human lung cells (Beas-2b), with IC_{50} values of 2.9 μ M and 14 μ M, respectively. The mechanism of action of compound **107a** was found to involve lysosome targeting and induction of cell death. This compound also showed a significant anti-proliferative effect in a zebrafish xenotransplantation model; therefore, the *in vivo* activity of compound **107a** suggests potential for the development of this compound as a new anticancer agent [55].

In 2020, Shi et al. [56] used isosteviol ethyl ester **72** as an intermediate, and reacted it with hydrazine hydrate to form imine derivative **108**, which in turn was reacted with various isothiocyanates to obtain a series of derivatives containing thiourea structure in the D-ring (Scheme S21). The anticoagulant factor X (FXa) inhibitory activity of the derivatives was evaluated. The inhibitory activity of compounds **109a** (IC_{50} = 153 nM), **109b** (IC_{50} = 196 nM), and **109c** (IC_{50} = 209) toward FXa was better than that of isosteviol (IC_{50} = 642 nM).

In 2019, Chen prepared oxime ether derivatives **111a** and **111b** through a three-step reaction (Scheme S22). Antithrombotic activity studies indicated that the efficacy of intermediate **110** was lower than that of clinical drugs, but oxime ether derivatives containing thiophene group (**111a**, K_i = 15 μ M) or 3-methyloxetane group (**111b**, K_i = 28 μ M) showed excellent inhibition of FXa activity, which

was more than 95 times better than that of derivative **110** (K_i = 2.70 mM). Compound **111a** had greater effects than compound **111b** and showed favorable anticoagulant activity, by significantly prolonging thrombosis time in rats, decreasing platelet aggregation, and decreasing thrombus wet and dry weights [57]. Jayachandra modified the structure of isosteviol at C-15 and C-16 positions and obtained derivatives **112** and **113** through Canizzaro/aldol addition and oxidation (Scheme S23). The cardioprotective activity of the compounds was evaluated in a DOX-induced zebrafish cardiomyopathy model. Compounds **93**, **112**, and **113** showed significant cardioprotective effects, and compound **113** showed the greatest protective effect, with an LC_{50} of 92.6 μ M [58].

In 2016, Liu designed and synthesized a series of 1,2,3-triazole-linked isosteviol derivatives through a Huisgen click reaction (Scheme S24). The inhibitory activity of the derivatives toward HCT-116 and JEKO-1 cancer cell lines was studied *in vitro*, with cisplatin as a positive control. Most tested compounds showed significant anticancer properties. Compound **117b** (IC_{50} = 2.99 ± 0.098 μ M) had significantly greater anticancer activity against HCT-116 cells than the positive control cisplatin (IC_{50} = 3.91 ± 0.261 μ M) [59]. Liu synthesized a series of novel isosteviol derivatives containing amino alcohol or thiourea fragments in the D-ring and screened the anti-proliferative activity toward three human cancer cells (HCT-116, HGC-27, and JEKO-1) *in vitro* (Scheme S25). Derivatives **121a** and **121b**, **122**, and **124** had low IC_{50} values (1.45–2.38 μ M). Among them, compound **121b** had the most potent inhibitory activity against HCT-116 cells and HGC-27 cells, with IC_{50} values of 1.45 μ M and 2.36 μ M, respectively. Compound **124** had the most potent anti-proliferative activity against JEKO-1 cells, with an IC_{50} value of 2.16 μ M [60]. Macrocyclic terpenoid glycosides have a variety of biological activities. For example, monoterpenoid glycosides have significant analgesic, anti-inflammatory, hepatoprotective, and hypoglycemic activities; diterpenoid glycosides inhibit apoptosis; and macrocyclic triterpenoid saponins inhibit tumor cell growth. Garifullin reduced the D-ring carbonyl group of isosteviol to a hydroxyl group, after five reaction steps, macrocyclic glycoterpenoids **129** and **130** containing glucosamine and isosteviol skeleton were synthesized (Scheme S26), and their inhibitory activity against *M. tuberculosis* H37Rv was evaluated. All tested compounds showed moderate anti-tuberculosis activity, and the MIC against *M. tuberculosis* H37Rv *in vitro* was 6.40–17.4 μ M. Compound **130** had favorable activity, with an MIC of 6.40 μ M [61].

In 2013, Zhu stereoselectively synthesized isosteviol derivatives containing pyrazole and isoxazole heterocyclic fragments in high yield, and studied the anti-proliferative activity of derivatives against gastric cancer (SGC7901), lung cancer (A549), lymphatic cancer (Raji), and cervical cancer (HeLa) cells (Scheme S27). The introduction of thioformamide-substituted pyrazole and isoxazole heterocyclic fragments in isosteviol increased the

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antitumor activity, and the pyrazole derivatives **133a–133u** showed favorable cell activity. The derivatives **133a**, **133d**, **133j**, and **133p** showed favorable anti-proliferative activity against A549 (IC_{50} = 17.6 μ M), HeLa (IC_{50} = 13.3 μ M), SGC 7901 (IC_{50} = 7.06 μ M), and Raji (IC_{50} = 6.51 μ M) cells [62]. In the same year, Zhu's group [63] further modified the structure of intermediate **131** and synthesized a new isosteviol derivative **134** containing a pyrazole heterocyclic fragment in one step (Scheme S27). The IC_{50} values against SGC7901, A549, Raji, and HeLa cells were 2.71, 3.18, 1.09, and 13.5 μ M, respectively—values superior to those of the drug cisplatin (IC_{50} values of 7.56, 17.8, 17.3, and 14.3 μ M, respectively). These compounds have strong cytotoxicity and are expected to be used as lead compounds to develop antitumor drugs.

In 2012, Zhang converted the carboxyl group of isosteviol into ethyl ester, then synthesized derivative **137** through five steps of reduction, oxidation, and oximation. The antiproliferative activity against four human cancer cell lines was studied *in vitro* (Scheme S28). The antiproliferative activity against **137** pairs of EC9706 (IC_{50} = 4.01 μ M), PC-36 (IC_{50} = 15.3 μ M), and HCT-116 (IC_{50} = 12.3 μ M) cell lines was significantly better than that of the positive control cisplatin, and the activity against the Eca109 cell line was comparable to that of the control drug cisplatin. A structure-activity relationship study indicated that introducing hydroxyl and amino alcohol fragments was beneficial to the anti-cancer activity of isosteviol, whereas the D-ring ring-opening derivatives were ineffective in promoting the antiproliferative effect of isosteviol [64].

In 2009, Wu et al. [65] used isosteviol as a starting material to synthesize various types of isosteviol derivatives through multi-step reactions. Derivatives **73** and **114** were synthesized by a two-step reaction, and a three-step reaction was used to synthesize the derivatives **138** and **139**. Four compounds showed varying anti-proliferative activity against B16-F10 melanoma cell lines, among which compound **138** (IC_{50} = 22.0 μ M) had the lowest IC_{50} value. Similarly, a four-step reaction was used to prepare the intermediate **141**, and the derivative **142** (IC_{50} = 24.0 μ M) was obtained through functional group conversion of the aldehyde group of **141**, which had specific anti-melanoma activity. Through the 1,3-dipolar cycloaddition reaction of intermediate **141**, isosteviol derivatives **144** (IC_{50} = 15.0 μ M), **146** (IC_{50} = 21.0 μ M), and **147** (IC_{50} = 19.0 μ M) containing a pyrazole or isoxazole ring were synthesized, and showed higher anti-melanoma activity. A structure-activity relationship study indicated that the isosteviol derivatives containing carboxyl groups were inactive, and the activity was improved after introduction of hydroxyl and hydroxymethyl groups into the isosteviol skeleton. The ring-opening and fused heterocyclic derivatives containing hydroxyl, amine, and oxime groups were more active than the ring-opening product **141** (Scheme S29). In the same year, Wu modified the structure with isosteviol as the lead compound and synthesized a series

of derivatives with inhibitory activity against α -glycosidase (Scheme S30). Indole-isosteviol derivatives **148a** and **148b** were obtained through a Fischer reaction between isosteviol **3** and ethyl ester intermediate **72** under acidic conditions. Subsequently, in ethanol and the presence of sodium bicarbonate, compounds **3** and **72** were oximated with hydroxylamine hydrochloride to produce derivatives **149** and **110**, which were then combined with TsCl or H_2SO_4 or BF_3 to produce lactam derivatives **150a** and **150b**. Compound **148b** (IC_{50} = 68.2 μ M) and **150b** (IC_{50} = 72.4 μ M) demonstrated higher α -glucosidase inhibitory activity compared to other related compounds [66].

Research on the chemical modification of isosteviol has demonstrated that the outcomes of D-ring modification are highly significant and have substantial potential in broadening the diversity of the chemical structure of isosteviol and providing abundant materials for the further exploration of structure-activity relationships. Nevertheless, several limitations exist. The elusive mechanisms of action of most compounds pose an obstacle to the optimization of drug design. Pharmacokinetic studies have been relatively scarce, thereby precluding accurate assessment of pharmacokinetic properties. Because the verification of *in vivo* activity has been restricted, determining the actual medicinal value is an arduous process. Additionally, the intricate multistep synthesis methods for some derivatives are disadvantageous for large-scale production. Overall, despite the numerous breakthroughs achieved in D-ring modification, overcoming these drawbacks will be essential to fully exploit its value in drug development.

3.3 Other modifications

Some isosteviol derivatives have been used to catalyze the Michael addition reaction and the asymmetric aldol condensation reaction. Ma et al. [67] converted the A-ring carboxyl group of isosteviol to the amino group to obtain isosteviolamine derivative **54**, then reacted it with dimethyl squamate to obtain derivative **151**. Derivative **151** was reacted with various configurations of 1,2-cyclohexane diamine to obtain the novel chiral bifunctional primary amine squaramide-type organic catalysts **152** and **153**. Similarly, Song et al. [68] obtained isosteviol isothiocyanate **154** through reaction of CS_2 with derivative **54**, then reacted derivative **154** with diaminocyclohexane with various configurations, to prepare isosteviol thiourea bifunctional catalysts **155** and **156** (Scheme S31). Ma's group also designed and synthesized catalysts for the carbonyl group of the D-ring. After conversion of isosteviol into intermediate **122** through a three-step reaction, compound **122** was treated with CS_2 , Boc_2O , DMAP, and TEA in EtOH to obtain isosteviol C-16 isothiocyanate derivative **157**. The addition of derivative **157** with cyclohexane-1,2-diamine yielded the chiral amphiphilic bifunctional primary amine thiourea catalyst **158** [69]. Through a similar method, intermediate **122** underwent a two-step

reaction for preparation of the novel catalysts **160** [70] and **161** [71] (Scheme S32). These catalysts have been successfully applied in the catalysis of Michael addition reactions, with yields as high as 98% and enantioselectivity as high as 99% ee. These catalysts can maintain the same high yield and enantioselectivity levels as in small-scale reactions and are applicable to large-scale catalytic synthesis. In addition, isosteviol and L-proline have been used as raw materials to synthesize two new amphiphilic catalysts, **162** and **163** (Scheme S33) with the one-pot method [72]. Catalyst **162** showed varying activity (as high as 99% yield) and stereoselectivity (as high as 99:1 Dr, >99% ee) for the direct aldol condensation of cyclohexanone with substituted benzaldehydes in the presence of water.

These derivatives present novel opportunities in organic synthetic chemistry, extend the application horizons of isosteviol, augment the repertoire of catalyst resources, conform to the developmental trends of green chemistry and asymmetric synthesis, and might potentially have enhanced environmental compatibility and atomic economy. However, the synthesis method requires optimization to decrease costs, and enhance production feasibility and practicality, thereby enabling extensive application in organic synthesis.

4. SUMMARY AND OUTLOOK

Given the increasingly severe population ageing problem, health awareness is continually improving. Many traditional drugs for treating cancer, cerebral infarction, thrombosis, heart disease, skin disease, inflammation, influenza, and other diseases have serious adverse effects and low patient acceptability, and therefore no longer meet current needs. Isosteviol, a hydrolysis product of stevia glycoside, has captured the attention of numerous researchers, because of its distinctive molecular architecture and diverse pharmacological activities. Research on chemical structure modification of isosteviol has focused on derivatives modified at the C-19 carboxyl group, C-16 carbonyl group, C-15 methylene group, and D-ring. At the C-19 site, a variety of substituents have been introduced, and many of the resultant derivatives exhibit favorable activities including anti-PTP1B, antibacterial, and anticancer functions. The functional alteration of the C-16 carbonyl group has led to the generation of compounds with antibacterial, antitumor, and anticoagulant activities. Although relatively fewer modification reactions at C-15 have been reported, the associated derivatives have also demonstrated biological activity. D-ring modified derivatives show remarkable cytotoxicity, cardiac protection, and enzyme inhibition capabilities, and some have played a major role in organic catalytic reactions. However, the development of these derivatives also faces many challenges, such as a lack of information regarding the exact molecular action mode of most tested isosteviol derivatives, and the need to clarify the pharmacokinetics of isosteviol and

its derivatives, including *in vivo* absorption, distribution, metabolism, and excretion [73]. In conclusion, derivatization and further isosteviol research is an important field of medicinal chemistry, in which existing molecules can be adjusted and refined to serve as references for future searches for compounds with better biological activities and easier synthesis, in the development of more effective and selective drug molecules.

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CONFLICTS OF INTEREST

No potential conflicts of interest are reported by the authors.

SUPPLEMENTARY DATA

Supplementary data for this article can be found online at: https://amm-journal.org/wp-content/uploads/2025/02/amm20240051_Suppl.pdf.

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