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The Precision Design, Synthesis, and Application of Novel Bioactive Small Molecules to Power the Aquaculture Green Revolution

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ABSTRACT

Environmentally friendly bioactive small molecules are structurally defined, low-molecular-weight organic compounds that exert biological effects while posing relatively low risk to human health and the environment. Their chemical synthesis and their application as green inputs in aquaculture provide a key pathway to reduce and replace antibiotics and improve quality and efficiency, thereby supporting the sustainable development of the aquaculture industry. This study systematically reviews research advances in the green synthesis and application of five key bioactive small molecules: short-chain fatty acids (SCFAs), tryptophan metabolites, tricarboxylic acid (TCA) cycle regulators, bile acid derivatives, and the quorum sensing inhibitor bromofuranone C-30. Chemical and physical modifications (e.g., salinization, esterification, and encapsulation) significantly enhance SCFA stability and targeted delivery. Efficient and green synthetic processes, such as Michael addition and Friedel–Crafts alkylation, enable the high-yield, low-cost preparation of indole-3-propionic acid and indole-3-lactic acid (ILA). Furthermore, innovative catalytic oxidation and lactonization routes provide new synthetic strategies for α-ketoglutaric acid and its derivatives. For bile acid derivatives (e.g., ursodeoxycholic acid (UDCA), sodium taurocholate, and 5α/5β-cyprinol sulfate), novel synthetic and extraction processes featuring streamlined steps, mild conditions, and high atom economy have been developed. A novel two-step synthesis of bromofuranone C-30 enables its scalable production, and a nano-encapsulated sustained-release formulation effectively resolves issues of thermal instability and rapid gastric absorption. These advances overcome the traditional extraction bottlenecks of limited raw materials, cumbersome procedures, and low yields. This review offers theoretical and technical perspectives to advance the targeted design, efficient synthesis, and industrial application of green aquaculture inputs, ultimately contributing to a more sustainable industry marked by greater safety and efficiency.

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1. Introduction

Bioactive small molecules are low-molecular-weight organic compounds with well-defined structures that interact with specific biological targets (e.g., proteins, enzymes, or receptors) in

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organisms, thereby regulating key biological processes. With favorable cell membrane permeability and oral bioavailability, these molecules serve as the material basis for the research and development of drugs and functional products. In aquaculture, antibiotic overuse has led to resistance, residues, and environmental pollution. As the world's largest producer (~60% of global output) of aquaculture, China has implemented strict antibiotic-restriction policies, promoting the development of green alternatives, and the market demand continues to grow.

The chemical synthesis and application of bioactive small molecules as green inputs in aquaculture comprise an emerging interdisciplinary field that integrates aquaculture science, chemistry, chemical engineering, biology, and materials science. In response to the specific needs of the aquaculture industry, this field leverages small molecules with potential functions as molecular templates. The strategy involves selecting appropriate enhancement methods based on molecular characteristics and general principles for functional improvement to guide targeted product design. Nontoxic, environmentally friendly, and benign substances should be prioritized in raw material selection. Simple, efficient, high-conversion-rate, and environmentally friendly techniques are applied to synthesize or process the active template during its production. The primary technical approaches involve green chemical engineering methods, including physical processing, chemical modification, and chemical synthesis. The aim is to develop processing, modification, and synthesis techniques that are highly functional, cost-effective, and environmentally friendly. To provide a unified evaluative framework, we define "green synthesis" based on four core criteria: atom economy, high yield, environmental friendliness (i.e., avoiding toxic reagents and minimizing waste), and industrial feasibility (i.e., mild conditions, simple operation, and scalability). These benchmarks guide this review's assessment of synthetic routes and modification technologies.

Once process development is complete, the resulting products must be applied in a practical aquaculture setting to verify their actual performance, evaluate their physiological efficacy and biological value, and establish usage guidelines suitable for feed and aquaculture enterprises. Furthermore, by integrating the results of product performance evaluation, pharmacokinetic characteristics, absorption and metabolic mechanisms, and structural property analysis, the efficiency of the product and its mechanism of action can be deeply analyzed, providing a scientific basis for the iterative upgrading of the product. Thus, the chemical synthesis and application of green inputs in aquaculture are characterized by targeted design, green efficiency, economic viability, clear mechanisms, and sustainable innovation, offering a key technological pathway toward the industry's green transition and high-quality development.

The sustainable cultivation of aquatic animals is directly linked to food safety and product quality. By applying green biochemical technologies to design and develop safe, efficient, and environmentally friendly aquaculture inputs, the sustainable development of China's aquaculture industry can be fostered. The research and application of such green inputs contribute to reducing disease incidence in aquatic animals, lowering reliance on antibiotics, improving overall aquaculture productivity, ensuring the quality and safety of aquatic products, and alleviating environmental impacts. These advances provide key technical support for achieving the industry goals of reducing and replacing antibiotics and improving quality and efficiency. This article systematically reviews recent progress in the green chemical synthesis and application of several key bioactive small molecules, including short-chain fatty acids (SCFAs), tryptophan metabolites (e.g., indole-3-propionic acid and indole-3-lactic acid (ILA)), tricarboxylic acid (TCA) cycle regulators (e.g., α-ketoglutaric acid and its derivatives), bile acids and their derivatives, and the quorum sensing inhibitor bromofuranone C-30 (Table 1). It aims to serve as a reference for the further development and utilization of green inputs in aquaculture.

2. SCFAs

SCFAs are a class of organic fatty acids containing fewer than six carbon atoms; common SCFAs include formic acid, acetic acid, propionic acid, and butyric acid. Due to their volatile nature, SCFAs are also known as volatile fatty acids. These compounds are largely generated through the microbial fermentation of dietary fiber in the intestine and exhibit physicochemical properties such as water solubility and weak acidity ($pKa \approx 4.8$) [1,2]. Research indicates that SCFAs not only act as a rapid energy source for the host [3,4] but also promote animal growth [5,6], improve intestinal health [7,8], modulate immune and inflammatory responses [9,10], enhance antioxidant capacity [5,8], and support bone health [11,12]. Based on these characteristics, SCFAs are widely considered as potential alternatives to antibiotics in animal husbandry.

Despite their promising applications, the inherent physicochemical properties of SCFAs significantly restrict their direct use. SCFAs exhibit strong volatility, degradability, and hygroscopicity, as well as a pungent odor and high acidity, which complicate feed processing and storage. In addition, due to their high polarity and water solubility, SCFAs are readily and rapidly absorbed in the foregut of aquatic animals, leading to a short duration of action and low bioavailability, which limit their effective distribution to the hindgut and systemic circulation. Therefore, current research mainly focuses on improving the stability and targeted utilization efficiency of SCFAs through dosage form optimization and delivery system design. Current technological advances can generally be categorized as physical, chemical, or physico–chemical hybrid methods. Considerable progress has been achieved in each direction, resulting in product systems at varying levels of technological sophistication.

The chemical modification of SCFAs primarily involves molecular alteration through two principal methods: salination and esterification. Salination technology improves product stability and processing tolerance by producing salts such as sodium acetate or sodium butyrate. Incorporating these salts into feed effectively improves intestinal health, growth performance, and disease resistance in fish [7,13–16]. In contrast, esterification technology enables precise intestinal targeting through the synthesis of esters such as tributyrin. This approach not only significantly increases bioavailability but also removes the limitations imposed by gastric acid conditions on efficacy, representing a more advanced technological direction [17,18]. Tributyrin is an ester compound formed by the reaction of butyric acid with glycerol (Fig. 1). It effectively addresses the inherent drawbacks of butyric acid, such as volatility, pungent odor, and difficulty incorporating in feed uniformly. Upon ingestion, tributyrin is hydrolyzed by endogenous lipase in the small intestine, releasing butyric acid in a targeted manner. This provides energy for intestinal epithelial cells, promotes mucosa development, optimizes the balance of the digestive tract microbiota, and enhances immune function, thereby contributing to disease prevention [19–22]. However, tributyrin's inherent bitter and astringent taste and its instability during processing and storage still restrict its widespread application to a certain extent. Zhou et al. [23] chemically modified glucose with butyric acid (Fig. 1), successfully synthesizing glucose pentabutyrate (PGE) with a butyric acid content of 67% while achieving co-production with butyric acid. This product exhibits high lipid solubility and excellent

Table 1
Summary of bioactive small-molecule products, their production characteristics, and upgrade methods.

	Main dosage forms	Biological activity	Product or production features	Product upgrade methods
SCFAs	Sodium acetate, sodium butyrate, triglycerides, etc.	Maintaining the intestinal barrier, regulating immunity, anti-inflammation, and affecting energy metabolism	Volatile, degradable, hygroscopic, pungent odor, bitter taste, low bioavailability	Dosage form innovations (TVCE, PGE), or processing technology innovation
Tryptophan metabolites	Indole-3-propionic acid, ILA	Antioxidant and neuroprotective activities, maintaining the intestinal barrier, and anti-inflammation	Multiple steps, low yield (< 50%), poor safety, high cost, and low environmental compatibility	Dosage form innovation (chiral isomers) and synthetic process innovation (87% overall yield)
TCA cycle regulators	α-Ketoglutaric acid, sodium α-ketoglutarate	Regulating the TCA cycle and metabolism of carbohydrates, fats, and amino acids	Complicated process, low reaction efficiency (< 50%), high energy consumption, difficult product separation, and relatively high comprehensive cost	Dosage form innovation (α-ketoglutarate lactone) and synthetic process (80% overall yield) innovation
Bile acids and their derivatives	UDCA, sodium taurocholate, 5α-cyprinol sulfate, 5α-cyprinol	Promoting the digestion and absorption of lipids and fat-soluble vitamins and regulating liver metabolism, energy homeostasis, intestinal barrier, and inflammatory response	Long synthetic route, low catalytic efficiency, poor stereoselectivity, numerous byproducts, harsh reaction conditions, exorbitant cost, large amounts of three wastes	Dosage form (chiral isomers) and synthetic process innovation
Quorum sensing inhibitor (bromofuranone C-30)	Bromofuranone C-30 and bromofuranone C-30 sustained-release agent	Anti-virulence, anti-biofilm, quorum sensing inhibition	Unstable and absorbed quickly, low yield, dangerous operation, not environmentally friendly	Dosage form (slow release) and synthetic process innovation (increase yield by 11 times)

UDCA: ursodeoxycholic acid; TVCE: tetraacetyl vitamin C ester; PGE: glucose pentabutyrate.

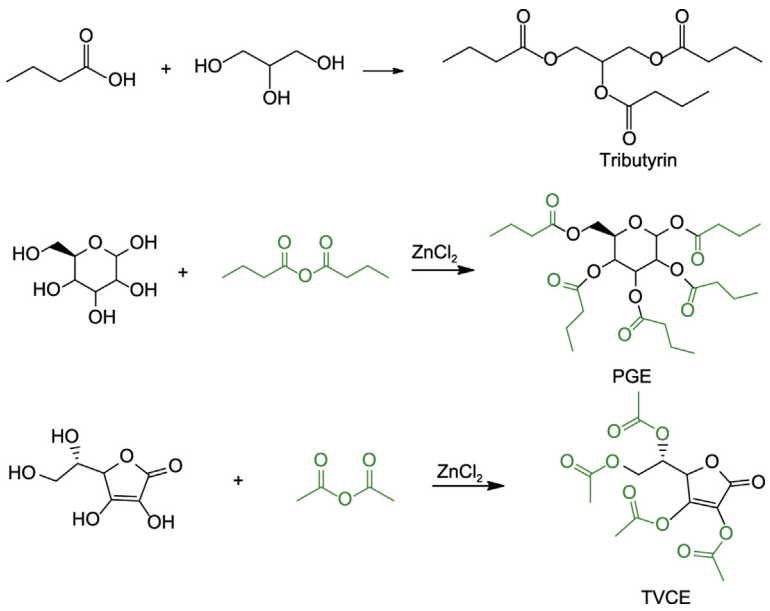


Fig. 1. Synthetic routes for tributyrin, PGE, and TVCE [23–25].

thermal stability (withstanding temperatures up to 210 °C) and has a pleasant fatty aroma, effectively masking the off-flavor and bitterness of raw butyric acid. It combines the physiological functions of butyric acid and glucose, demonstrating a synergistic enhancement of physiological activity. A study showed that, at equivalent effective butyric acid content, PGE promoted zebrafish growth more effectively than tributyrin or sodium butyrate [24]. Concurrently, the researchers applied full acetylation technology to vitamin C for the first time, producing tetraacetyl vitamin C ester (TVCE) (Fig. 1 [23–25]). This compound contains 50% acetic acid and has favorable physical properties, including lipid solubility, high stability, non-volatility, and non-hygroscopicity, with a heat resistance up to 160 °C. It also features an improved flavor profile. Functionally, TVCE integrates the physiological activities of acetic acid and vitamin C, and their synergy significantly increases efficacy, such that a 50% dosage reduction achieves equivalent effects (Table 1). Research indicates that TVCE improves fish health via the microbiota–gut–liver axis, thereby promoting growth. Its unique value lies in combining the physiological roles of vitamin C and acetic acid, enabling it to replace sodium acetate and vitamin C phosphate. Importantly, the two components exhibit a synergistic interaction in this ester form, producing an effect greater than the sum of the individual effects. Experiments confirm that TVCE is more effective than sodium acetate and vitamin C phosphate in activating the physiological functions of various bodily components [25,26].

Physical barrier technology is an effective strategy for improving the stability and targetability of SCFAs. There are two main

technical methods: lipid coating and adsorption carriers. Lipid coating technology uses high-melting-point lipid materials (e.g., palm oil stearin, natural waxes) to form core-shell structured particles. This creates a physical barrier that mitigates volatility and degradation, while enabling targeted intestinal release. By employing composite coating materials with precise process control, this method strengthens resistance to high temperature and pressure during feed processing, increases active ingredient retention by over 30%, and achieves controlled release in the posterior intestine (e.g., triggered by local temperature and enzymatic activity). Consequently, the intestinal delivery rate of active components exceeds 85%, maximizing the functional benefits of SCFAs in regulating gut flora, promoting nutrient absorption, and inhibiting pathogens [27–31]. Adsorption carrier technology employs porous materials such as silica, natural zeolite powder, montmorillonite, and attapulgite to immobilize SCFAs through vacuum adsorption or modification treatments. This approach mainly addresses issues related to volatility, caking, and handling convenience [32–34]. However, because the loading mechanism primarily relies on physical adsorption, improvements in controlled release and targeting remain limited. Consequently, this method is less common in SCFA-modification applications that require specific release functionalities.

The integrated physical–chemical approach has been used to successfully overcome SCFA application bottlenecks by combining the advantages of physical embedding and chemical modification, producing a synergistic effect greater than the sum of the individual effects. Among such strategies, salt-coating technology utilizes lipids or enteric-coated polymers to form a core-coating structure, providing a versatile stability solution for aquatic animals [35]. Coated sodium butyrate is one of the most successful and classical applications of coating technology. Uncoated sodium butyrate tends to absorb moisture, emits an unpleasant odor, and exhibits poor flowability, complicating storage and processing. Following coating treatment, product flowability is improved and issues related to palatability and controlled release are effectively resolved [30]. Studies indicate that coated sodium butyrate outperforms its uncoated counterpart in maintaining intestinal health and reducing hepatic lipid accumulation in fish [36,37]. Based on this premise, compound acidifier synergy technology integrates acidification + antibacterial + antioxidant functions through scientifically formulated blends of SCFAs, organic acids, and plant essential oils with varying pKa values. This formulation can be tailored to the physiological traits of aquatic animals, such as digestive tract length [38,39]. A composite system enables gradient dissociation across different pH environments. Organic acids with low pKa values mainly regulate acidity and exert bactericidal effects in the stomach, whereas SCFAs with higher pKa values extend their action to the intestinal tract, continuously inhibiting pathogenic bacteria proliferation and promoting intestinal mucosa repair. The incorporation of plant essential oils not only broadens the antibacterial spectrum but also enhances the bioavailability of the organic acids by increasing cell membrane permeability. Research shows that such precisely proportioned acidifiers can effectively modulate intestinal flora structure, improve nutrient digestion and absorption, enhance intestinal morphology, and significantly reduce infection risks in aquatic animals caused by common pathogens such as *Aeromonas hydrophila* [40–42]. pH-sensitive polymer coating technology represents the pinnacle of delivery systems, enabling precise triggered release via polymers that dissolve in the higher-pH environment of the intestine. Although this technology is currently applied mainly in high-value aquatic seedling production due to its cost, its advanced targeting capability outlines a direction for future technological development [43]. Together, the three technical pathways of physical, chemical, or physico–chemical hybrid methods form a comprehensive system encompassing stability enhancement, functional integration, and precise delivery (Fig. 2). Research in these areas is collectively advancing the industrial application of SCFAs in aquaculture and providing sustained momentum for high-quality industry development.

Despite these advances, several limitations remain. The high cost of advanced delivery systems limits widespread adoption, and the potential environmental impact of residual coating materials remains poorly understood. Finally, other SCFAs such as propionate and acetate remain underexplored compared with butyrate. Future research should focus on cost-effective formulations and environmental risk assessment.

3. Tryptophan metabolites

The microbial indole pathway is a key process through which gut microbiota convert dietary tryptophan into a series of bioactive indole derivatives. This pathway is mainly catalyzed by tryptophanase produced by specific bacteria such as *Escherichia coli* and *Clostridium*, yielding core products including indole, indole-3-propionic acid, ILA, and indole-3-acrylic acid [44]. These metabolites share an indole ring as their core structure, and differences in their biological activities mainly stem from variations in the functional groups attached to the third carbon of the indole ring. Among them, indole-3-acetic acid, indole-3-propionic acid, and ILA have emerged as research hotspots in recent years and have been demonstrated to possess potent antioxidant and neuroprotective activities. They not only help maintain intestinal barrier integrity but also positively regulate overall host health through mechanisms such as anti-inflammatory effects [45–47].

3.1. Indole-3-propionic acid

Indole-3-propionic acid, a promising green feed additive, has attracted growing research interest for its use in livestock and poultry breeding. Its application in aquatic animal farming, however, remains relatively underexplored. Physiologically, indole-3-propionic acid demonstrates notable antioxidant and anti-stress properties. It effectively scavenges free radicals and reduces oxidative damage to key tissues such as the intestines and liver, thereby improving animals' overall health [48]. It contributes to maintaining the structural and functional integrity of the intestinal mucosal barrier and may further modulate host physiology systemically via pathways such as the gut–liver axis and gut–brain axis [49,50].

Diverse methods are used to chemically synthesize indole-3-propionic acid (Table 2 [51–57]), including the Grignard reagent/-cyanation method [51], Mannich reaction method [52], reduction method [53], Darzens condensation of 3-indolealdehyde [54], side-chain functional-group transformation [55] and Fischer indole synthesis [56]. Each method has distinct characteristics and is suited to different synthetic requirements. Although the Grignard reagent/cyanation method using indole as starting material is well-established, it involves lengthy procedures, complex operations, and highly toxic reagents, which limit its broad application [51]. The Mannich reaction is a common laboratory method, thanks to its accessible starting materials, straightforward procedure, and relatively high yield. However, the potential for side reactions requires attention [52]. Reduction methods offer mild reaction conditions, good selectivity, and high product purity, presenting distinct advantages, but their reliance on precious metal catalysts leads to higher costs [53]. Synthetic pathways employing other indole derivatives also have specific features. The Darzens condensation route starting from 3-indolealdehyde is versatile and yields multifunctional intermediates, yet it often requires noble metal catalysts and toxic reagents such as pyridine [54]. The side-chain functional-group transformation approach ingeniously utilizes

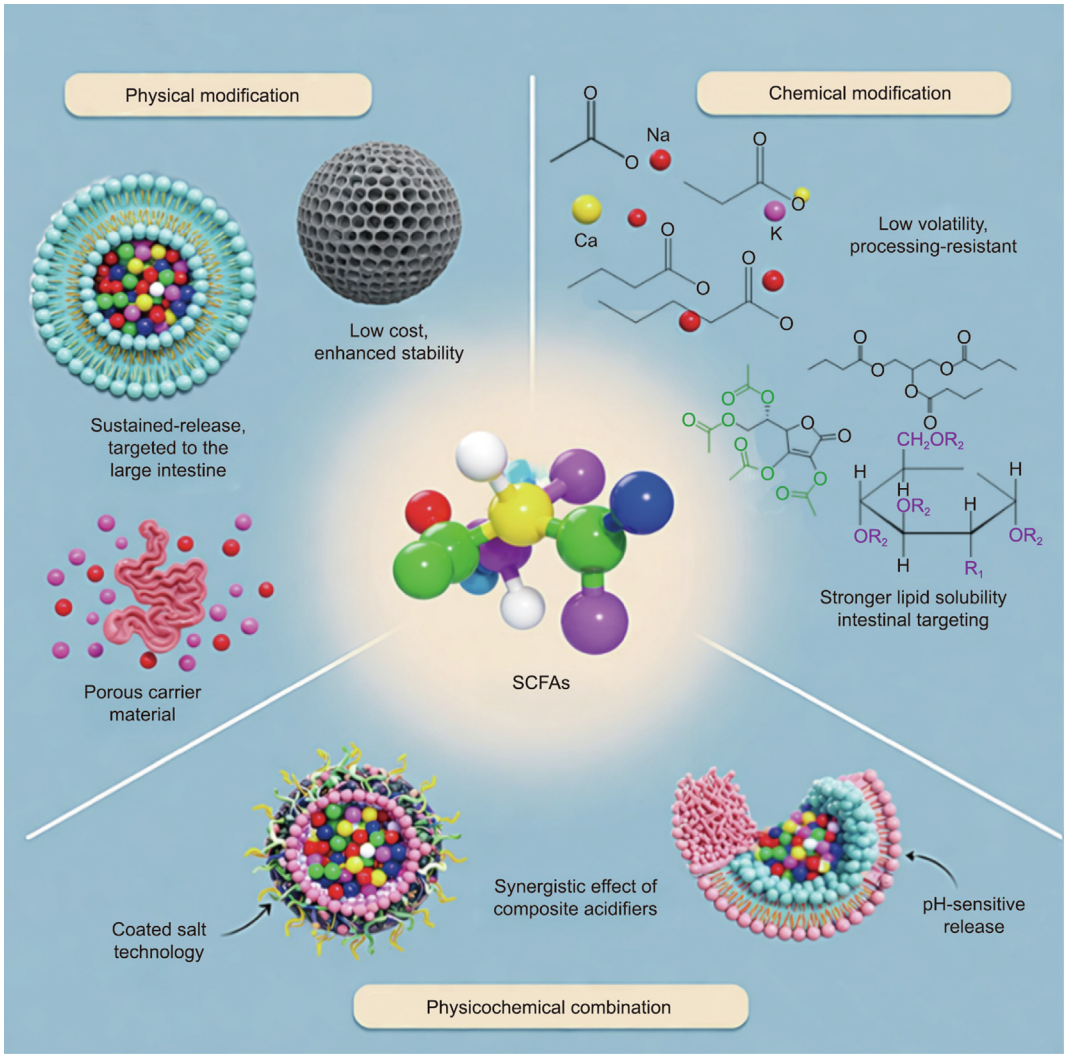


Fig. 2. Three product upgrade strategies for SCFAs.

Table 2
Chemical synthesis methods and characteristics of indole-3-propionic acid.

Synthetic method	Reaction steps	Advantages	Disadvantages
Grignard reagent/cyanidation method [51]	3	Classic	Low yield, long route, complex operation, and highly toxic reagents
Mannich reaction method [52]	2	Raw materials are readily available, operation is simple, and yield is relatively high	Byproducts such as through dialkylation are produced, making separation and purification difficult
Reduction method [53]	2	Reaction conditions are mild, good selectivity, few byproducts, and high product purity	Involves the use of precious metal catalysts, which are costly
Darzens contraction method [54]	2	The synthetic route is flexible	Involves the use of toxic reagents such as pyridine and precious metal catalysts, which are costly and not environmentally friendly
Side-chain functional-group transformation method [55]	3	Strong selectivity and high yield	Involves the use of strong oxidants, heavy metal catalysts, or highly toxic reagents
Fischer indole synthesis method [56]	2	High regional selectivity	Low yield, numerous side reactions, and difficult purification
Michael addition reaction [57]	2	Green economy, simple, and efficient	

existing derivative structures to construct indole-3-propionic acid. However, this process frequently involves strong oxidants, heavy metal catalysts, or highly toxic reagents, posing safety and environmental concerns [55]. Additionally, the Fischer indole synthesis, which builds the indole ring system, is particularly effective for preparing derivatives with specific substituents and serves as a powerful tool in drug development. Nevertheless, its drawbacks, which include low yield, numerous side reactions, and challenging purification processes, necessitate further improvement [56]. Zhou et al. [57] developed an alternative route using a Michael addition

reaction between indole and ethyl acrylate catalyzed by a Lewis acid to synthesize ethyl indole-3-propionate. This intermediate was subsequently hydrolyzed to produce indole-3-propionic acid. This two-step route achieves a total yield of 87% and is characterized by its simplicity, minimal side reactions, high yield, and excellent product purity. Moreover, the reagents used are low in toxicity, cost-effective, and require modest equipment, demonstrating strong potential for industrial scale-up. Indole-3-propionic acid is an important pharmaceutical intermediate and plant growth regulator, and it benefits greatly from this green, economical, and efficient synthesis process, whose successful development not only ensures a reliable supply via the large-scale production of indole-3-propionic acid but also provides a valuable reference for the synthesis of other indole derivatives.

3.2. ILA

ILA is a significant natural active molecule. It is a reduced hydroxylated derivative of the plant hormone indole-3-acetic acid and one of the main metabolites derived from tryptophan metabolism by intestinal microorganisms in humans and mammals. ILA is widely present in human serum, urine, and fermented foods [58]. It plays a central role in regulating intestinal health: Not only does it act as a key ligand for the aryl hydrocarbon receptor, helping to maintain intestinal barrier integrity, reduce inflammation, and regulate immune balance through pathway activation, but its levels also correlate positively with the abundance of beneficial bacteria such as *Bifidobacterium*, indicating potential prebiotic properties. This molecule also exhibits notable antioxidant capacity [59].

Thus far, research on the application of ILA as a feed additive in aquaculture remains relatively limited. In a zebrafish model fed a high-fat diet, ILA demonstrated multi-target efficacy against metabolic syndrome. Studies have shown that ILA alleviates hepatic steatosis by modulating lipid metabolism genes, reduces endotoxin levels by enhancing intestinal barrier function, and improves inflammatory status by suppressing pro-inflammatory cytokines while promoting anti-inflammatory cytokine expression. The underlying mechanisms involve activation of the AhR2-Cyp1a1 signaling axis, remodeling of the gut microbiota, and multi-omics network regulation, confirming that ILA can ameliorate metabolic disorders through synergistic multi-pathway interactions [60].

Research on the chemical synthesis of ILA remains relatively limited, with current strategies mainly focusing on the efficient construction of its characteristic side chain. Among reported methods, the most representative route involves selective reduction using indole-3-pyruvate as a key intermediate. This approach typically begins with the preparation of indole-3-pyruvate via Friedel–Crafts acylation of indole with reagents such as oxalyl chloride, followed by selective reduction of the ketone carbonyl group through catalytic hydrogenation or metal hydride reduction to yield the target product. Another synthetic pathway starts from readily available indole-3-propionic acid. Through steps including activation, diazotization, and Wolfe rearrangement, the terminal carboxyl group is converted into a hydroxyl group [61]. However, such alternative methods generally suffer from operation complexity, low reaction yields (typically below 50%), and the frequent use of explosive or highly toxic reagents, rendering them suitable mainly for laboratory-scale studies. In contrast, Zhou et al. [62,63] developed a more efficient synthetic route (Fig. 3). Using indole, methyl 2,3-epoxypropionate, and tin tetrachloride as raw materials, racemic ILA (*DL*-ILA) can be obtained in just two steps, through a Friedel–Crafts alkylation followed by alkaline hydrolysis, achieving an overall yield of 70% and a purity of 96%. This method offers several advantages, including straightforward operation, high yield and purity, low equipment requirements, minimal reagent toxicity, and low cost, demonstrating strong potential for industrial scale-up [62].

The same team has also expanded the chiral synthesis pathway (Fig. 3). Using indole, chiral methyl 2,3-epoxypropionate, and ytterbium trifluoromethanesulfonate as starting materials, the researchers successfully prepared chiral (*R*/*S*) ILA (*R*/*S*-ILA) with a total yield of 80%, a purity of up to 99%, and an enantiomeric excess (ee) of 99%. This chiral synthesis method offers several advantages, including simple operation, high yield, excellent stereoselectivity, and low equipment and cost requirements, making it well-suited for industrial production [63]. In applied research, this research team systematically evaluated the effects of racemic ILA and its *R*- and *S*-enantiomers on the growth performance and stress resistance of zebrafish. Their experimental results demonstrated that, within the concentration range of 25–100 mg·kg $^{-1}$, both racemic ILA and the *S*-enantiomer effectively promoted zebrafish growth and significantly increased resistance to high temperature, saline-alkali, and ammonia nitrogen stress (Fig. 3). In contrast, the *R*-enantiomer inhibited zebrafish growth and reduced resistance to the same three stressors. The specific mechanism underlying this inhibitory effect warrants further investigation [63].

Several limitations to the synthesis and application of ILA persist. The high cost of chiral synthesis catalysts hinders industrial-scale production, and the finding that the *R*-enantiomer inhibits zebrafish growth raises safety concerns that require urgent investigation. Additionally, the stability of indole derivatives during feed storage and processing has not been systematically evaluated. Future research should focus on elucidating the toxicity mechanism of the *R*-enantiomer and developing cost-effective scalable synthesis.

4. TCA cycle regulators: α-Ketoglutaric acid and its derivatives

The TCA cycle, also known as the Krebs cycle, is the central hub of cellular aerobic respiration and is tightly regulated by various metabolites [64]. Among these, α-ketoglutaric acid transcends its conventional role as an intermediate metabolite to function as a key metabolic regulatory node [65]. Through multiple mechanisms, including substrate supply, feedback inhibition, and cellular signal transduction, α-ketoglutaric acid not only precisely modulates the flux of the cycle itself but also bridges the three major metabolic networks of carbohydrates, lipids, and amino acids, thereby playing a pivotal role in maintaining cellular energy and material homeostasis [66]. As a feed additive in aquaculture, α-ketoglutaric acid or its disodium salt exhibits multifaceted benefits. Studies have demonstrated that dietary supplementation with appropriate amounts of α-ketoglutaric acid significantly promotes growth performance, increases weight gain, and improves feed utilization efficiency in freshwater finfish (e.g., grass carp and rainbow trout) [67,68] and crustaceans (e.g., *Eriocheir sinensis*) [69]. Furthermore, α-ketoglutaric acid effectively improves the organism's antioxidant capacity and immune function, alleviating the oxidative damage and tissue inflammation induced by environmental stressors such as ammonia nitrogen and thereby improving overall health status and reducing mortality [70,71]. These findings collectively highlight α-ketoglutaric acid as a promising green feed additive that supports the healthy and sustainable development of aquaculture.

The chemical synthesis of α-ketoglutaric acid has evolved significantly from traditional pathways to modern green processes. Early industrial production primarily relied on the itaconic acid oxidation method and the succinic anhydride oxidation method. The itaconic acid oxidation method uses itaconic acid as the raw material, undergoing oxidative ring-opening with oxidants such as hydrogen peroxide to yield α-ketoglutaric acid [72,73]. Although

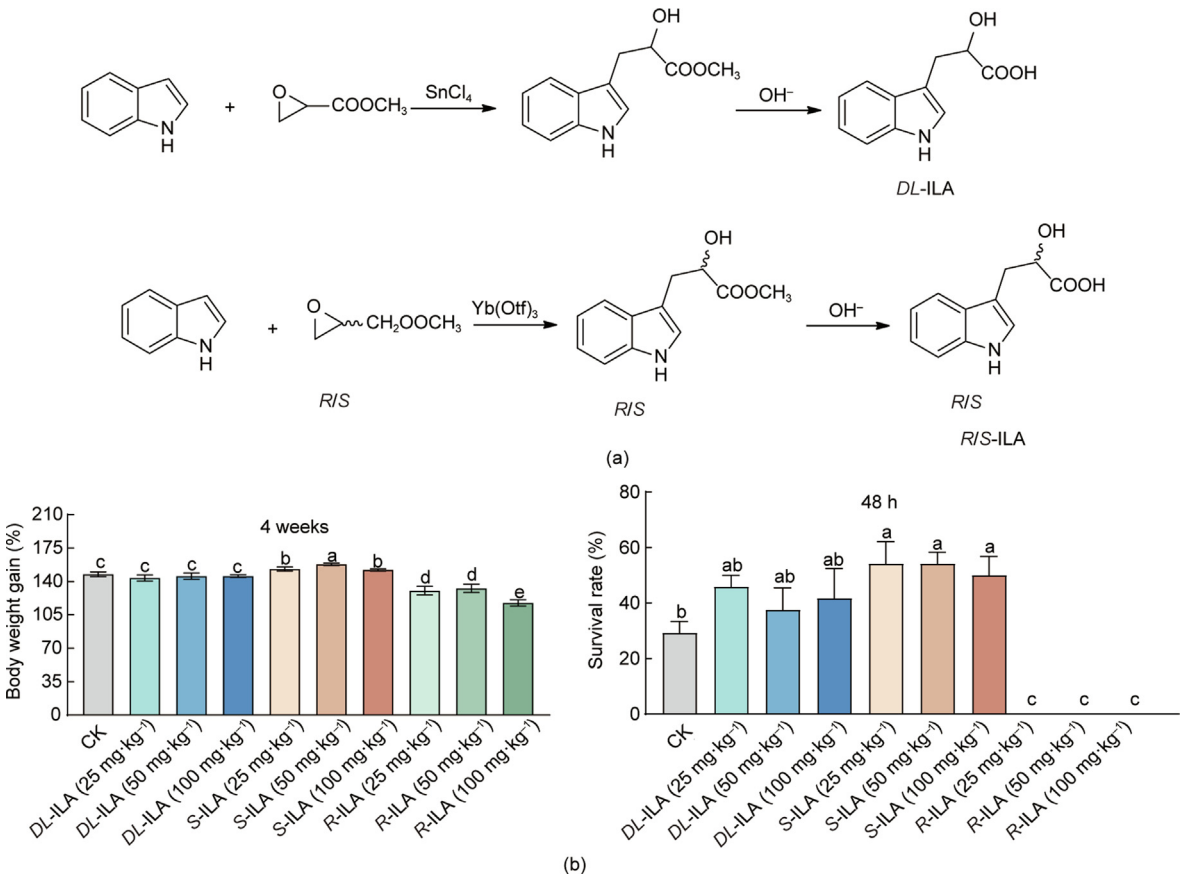


Fig. 3. (a) Synthetic routes for racemic ILA (*DL*-ILA) and (*R*/*S*) ILA (*R*/*S*-ILA), (b) the influence of these compounds on the growth and survival rate of zebrafish under high-temperature stress. Different letters above the bars indicate significant differences ($P < 0.05$ [62,63].

mature, this process involves lengthy steps and high costs. The succinic anhydride route starts with succinic anhydride and synthesizes α-ketoglutaric acid through multi-step reactions including chlorination, cyanidation, and hydrolysis. While classic, this method employs highly toxic cyanides, posing considerable environmental concerns [74]. In recent years, to overcome the limitations of these approaches, research has shifted toward green and efficient catalytic oxidation routes [75]. One particularly promising direction is the selective catalytic oxidation of glutaric acid or its derivatives, such as dimethyl glutarate. The core of this route lies in the development of highly selective catalysts, including metal oxide and complex catalysts, to achieve directed oxidation of the methyl groups at specific sites (C1 or C5) into ketone groups, thereby directly producing α-ketoglutaric acid. In terms of derivative production, the industrial preparation of disodium α-ketoglutarate typically uses α-ketoglutaric acid as the starting material. This undergoes a neutralization reaction with sodium hydroxide or sodium carbonate in aqueous solution, followed by purification steps such as concentration, crystallization, and drying to obtain a high-purity product [76].

As described above, the existing synthetic routes for α-ketoglutaric acid generally still present challenges, including complex processes, low reaction efficiency, high energy consumption, difficult product separation, and relatively high overall costs, which constrain the large-scale production and economical application of α-ketoglutaric acid and its sodium salts. Therefore, developing a new process characterized by concise steps, mild conditions, environmental friendliness, and cost controllability has become crucial in promoting the broader industrial application of α-ketoglutaric acid in fields such as aquaculture. Notably, the research team led by Zhou et al. [77] recently developed a novel chemical synthesis route distinguished by high atom economy, green efficiency, short process flow, and simple operation. This route (Fig. 4 [77]) uses methyl dichloroacetate and methyl acrylate as starting materials. Under sodium methoxide catalysis, these compounds react to form the intermediate dimethyl 2,2-dichloropentanedioate. Subsequent hydrolysis under alkaline conditions, while precisely regulating temperature and pH, enables the selective production of a mixture of 2-oxopentanedioic acid and α-ketoglutarolactone (65:35), disodium α-ketoglutarate (sodium 2-oxopentanedioate), or α-ketoglutarolactone (2-hydroxy-5-oxote trahydrofuran-2-carboxylic acid). Structural analysis of commercial α-ketoglutaric acid revealed that its composition matched that of the mixture obtained from the aforementioned synthetic route, both consisting of approximately 65% 2-oxopentanedioic acid and 35% α-ketoglutarolactone. This finding further validates the accuracy and practicality of the synthetic strategy. This strategy not only avoids highly toxic reagents and achieves flexible regulation of product structures but also provides a new concept for the green and economic synthesis of α-ketoglutaric acid and its derivatives, offering significant reference value for understanding the actual compositions of and synthetic pathways for these compounds.

Through a systematic comparison of the physiological regulatory effects of α-ketoglutarolactone and α-ketoglutaric acid under saline-alkaline stress, the research team led by Zhou et al. [77] revealed for the first time the unique role of α-ketoglutarolactone in improving the saline-alkaline tolerance of fish. Studies demonstrated that adding feed with 0.5%–1.0% α-ketoglutarolactone effectively alleviated growth inhibition and improved the feed utilization efficiency of gibel carp (*Carassius*

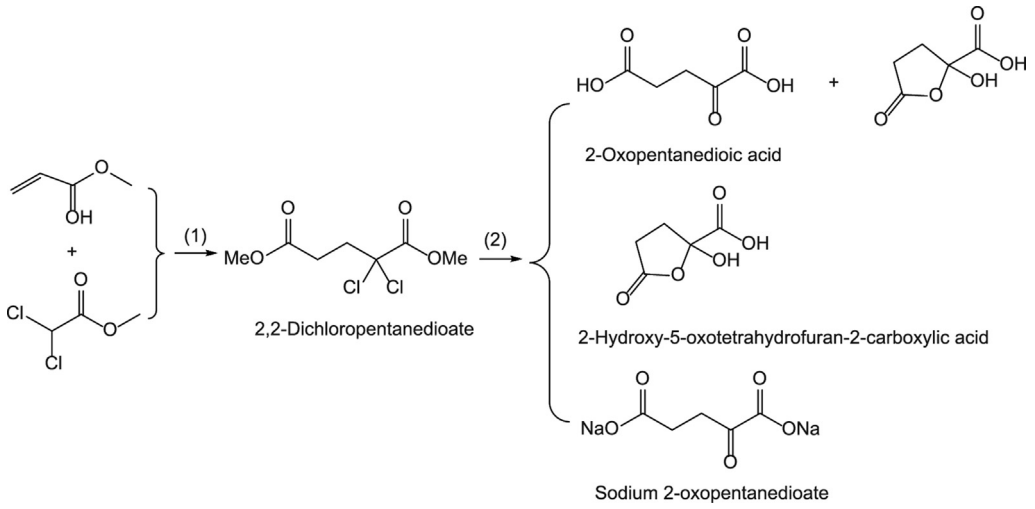


Fig. 4. Synthetic routes for α-ketoglutaric acid and its derivatives [77].

gibelio) under carbonate–induced saline–alkaline stress. It also promoted ammonia-nitrogen metabolism and excretion while maintaining osmotic homeostasis in the gills and kidneys. Compared with commercially available α-ketoglutaric acid, α-ketoglutarolactone exhibited a more pronounced effect in comprehensively alleviating saline-alkaline stress. It is speculated that the mechanism may involve a multi–pathway synergistic enhancement of physiological functions, such as improved bioavailability, regulated energy metabolism, and balanced nitrogen metabolism, providing a clear direction for further exploration of α–ketoglutar olactone's mode of action (Fig. 5 [77]). This work not only offers a scientific basis for the application of α-ketoglutarolactone as a novel feed additive to enhance saline-alkaline tolerance but also offers a new technical approach for the sustainable development of fishery resources in saline-alkaline waters in China (Table 1).

Despite these advances, several limitations to the commercial use of α-ketoglutarolactone and α-ketoglutaric acid in aquaculture remain. The equilibrium mixture of α-ketoglutaric acid and its lactone form (~65:35) complicates product standardization, and the superior efficacy of the lactone form warrants further mechanistic investigation. Additionally, the metabolic fate of these compounds in aquatic species has not been systematically characterized. Future research should focus on establishing product specifications and elucidating the differential bioactivities of the two forms.

5. Bile acids and their derivatives

The molecular structure of bile acids is characterized by a unique rigid steroid skeleton, formed by the fusion of one five-membered ring (ring D) and three six-membered rings (rings A, B, and C). Structural variations among different bile acids mainly depend on the number and positions of hydroxyl groups attached to the steroid nucleus, which are typically located on carbon atoms at positions C-3, C-7, and C-12 (Fig. 6). Based on their origin, bile acids can be classified into two categories: primary bile acids, such as cholic acid and chenodeoxycholic acid (CDCA), which are synthesized directly in the liver; and secondary bile acids, including deoxycholic acid, lithocholic acid, and the medicinally used ursodeoxycholic acid (UDCA), which are produced through bacterial modification in the intestines [78–81]. Structurally, bile acids can also be categorized into free and conjugated forms, with sodium taurocholate being an example of the latter. Physiologically, bile acids play a crucial role in lipid digestion and absorption. As effective bioemulsifiers, they emulsify dietary fats into fine droplets, greatly increasing the surface area accessible to lipase, and subsequently form mixed micelles to facilitate the transport of breakdown products, such as fatty acids and fat-soluble vitamins (A, D, E, K), across the intestinal mucosal barrier. Bile acids also serve as a major pathway for cholesterol excretion, promote bile

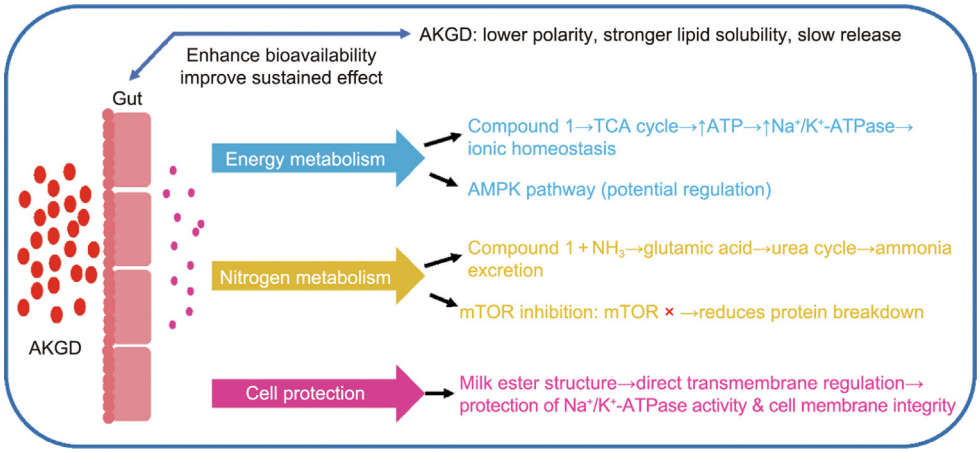


Fig. 5. Diagram of the possible molecular mechanism by which α-ketoglutarolactone (AKGD) alleviates saline-alkali stress in crucian carp [77].

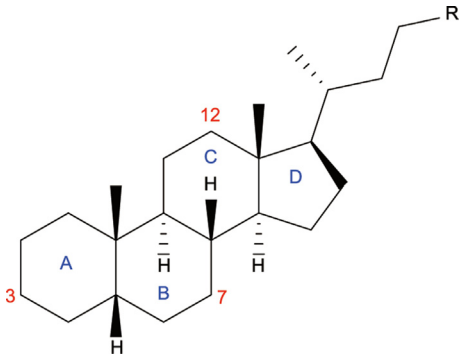


Fig. 6. The rigid steroid skeleton structure in bile acid molecules.

secretion, and act as important signaling molecules involved in metabolic regulation and the maintenance of intestinal microenvironment homeostasis [82,83].

5.1. UDCA

UDCA is a hydrophilic secondary bile acid that was originally isolated from bear bile. Its core value lies in its excellent hepatobiliary protective effects. It can replace endogenous hydrophobic bile acids, stabilize hepatocyte membranes, and alleviate cholestasis. As a result, UDCA is widely used as a first-line therapeutic agent for primary biliary cholangitis and cholesterol gallstones. With ongoing research, the application of UDCA has extended into animal husbandry and aquaculture. In aquaculture, as a high-efficiency feed additive, USDCA significantly promotes the absorption of fats and fat-soluble vitamins in crustaceans such as *Penaeus vannamei*, thereby improving growth performance [84,85]. Additionally, leveraging its notable hepatoprotective properties, UDCA effectively alleviates issues such as high-fat-diet-induced fatty liver in fish and increases overall stress resistance [86]. Recent studies have further explored UDCA's potential in alleviating oxidative stress, modulating intestinal flora, and serving as a pharmaceutical excipient to improve the bioavailability of lipid-based drugs [87,88]. This reflects a shift from traditional nutrition and health applications toward more refined health-regulation strategies. While UDCA is traditionally extracted from bear bile, this method suffers from low extraction efficiency and ethical concerns related to animal welfare, making it unable to meet the growing demand for UDCA in clinical and aquaculture fields. Therefore, developing efficient and scalable chemical synthesis processes holds great practical and economic importance for ensuring a stable UDCA supply and reducing its costs as a pharmaceutical and feed additive. At present, UDCA is chemically synthesized mainly from cholic acid, hyodeoxycholic acid, or CDCA derived from the bile of livestock such as cattle, sheep, pigs, chickens, and ducks. Synthetic routes starting from cholic acid or hyodeoxycholic acid are generally lengthy, typically requiring more than seven reaction steps, and result in low overall yields. Moreover, they often involve highly toxic reagents, such as selenium dioxide, or harsh conditions such as high temperature and pressure, failing to meet industrial standards in terms of safety, environmental friendliness, and cost-effectiveness [89–92]. In contrast, the route using CDCA as the starting material offers advantages due to structural similarity (as C-7 epimers). Nevertheless, this route still presents key challenges, particularly in achieving efficient and precise inversion of the hydroxyl group configuration at the C-7 position. Existing methods, which typically employ oxidation-reduction or isomerization strategies, often suffer from extended synthetic routes, low catalytic efficiency, poor stereoselectivity, and considerable byproduct formation. Additionally, the use of noble metal catalysts or strong bases leads to high costs and operational risks. Consequently, the yield and purity often fall short of industrial requirements, limiting the large-scale application of this pathway [93,94]. To address these technical bottlenecks, the research team led by Zhou et al. [95] developed an efficient four-step synthesis of UDCA from CDCA (Fig. 7). This process involves sequential key steps including methanol esterification, selective acetylation protection with acetic anhydride, mild oxidation using *N*-bromosuccinimide (NBS), and reduction in an n-butanol/sodium metal system. The entire process is conducted under mild conditions (reaction temperature 10–35 °C) and is operationally safe and straightforward. It avoids the highly toxic reagents and harsh conditions (e.g., high temperature and pressure) associated with traditional methods, while achieving regioselective oxidation of the 7-position hydroxyl group and efficient stereoinversion through precise process control. Compared with conventional synthetic approaches, this route reduces the typical five to seven steps to only four, significantly improving reaction efficiency and atom economy. The experimental results show a total yield of up to 71%, with high product purity. This technology features readily available raw materials, controllable costs, and low emission of waste gas, wastewater, and solid waste. It effectively overcomes the limitations of traditional processes, such as lengthy synthesis, low total yields (reported in the literature as only 26%–57%), poor safety, and challenges in scale-up. Thus, it provides a reliable and green solution for the low-cost industrial production of UDCA [95].

5.2. Sodium taurocholate

Sodium taurocholate, a key bioactive component of animal bile acids, is a natural amphiphilic compound synthesized in the liver through amide bond formation between cholic acid and taurine. Its unique molecular structure, featuring a hydrophilic sulfonic acid group at one end and a hydrophobic steroid skeleton at the other, confers exceptional surface activity, emulsifying properties, and biofilm permeability [80]. Owing to these favorable physicochemical properties and its biocompatibility, sodium taurocholate has become an essential functional ingredient in pharmaceutical formulations, the food industry, and functional feed additives. As a conjugated bile acid, sodium taurocholate exhibits promising potential as a functional additive, primarily due to its excellent emulsifying ability and biocompatibility. It improves nutrient utilization by promoting the digestion and absorption of lipids and fat-soluble compounds, while also alleviating the hepatobiliary burden induced by high-fat diets. Studies on rainbow trout (*Oncorhynchus mykiss*) and yellowtail amberjack (*Seriola lalandi*) have shown that sodium taurocholate can restore intestinal bile acid levels, improve bile acid homeostasis, and expand the endogenous bile acid pool, thereby increasing fat digestibility and mitigating digestive disorders caused by high non-starch polysaccharide (NSP) content or plant-based protein replacement of fishmeal [96,97]. Further studies on gilthead seabream (*Sparus aurata*) indicated that sodium taurocholate increases transepithelial electrical resistance (TEER) in foregut tissues and acts synergistically with other bile salts to promote ion absorption in the hindgut, with dose-dependent effects [98]. Collectively, these findings underscore the value of sodium taurocholate in optimizing intestinal function and addressing diet-related digestive challenges in aquatic species.

Traditionally, sodium tauroursodeoxycholate has been extracted from animal bile—a method limited by unstable supply, high cost, and inconsistent product quality. Thus, developing efficient chemical synthesis routes has become a major research focus. Conventional synthetic approaches, such as the ethyl chloroformate method [99] and the N-ethoxycarbonyl-2-ethoxy-1,2-dihy droquinoline (EEDQ) method [100–102], rely on ethyl chlorofor-

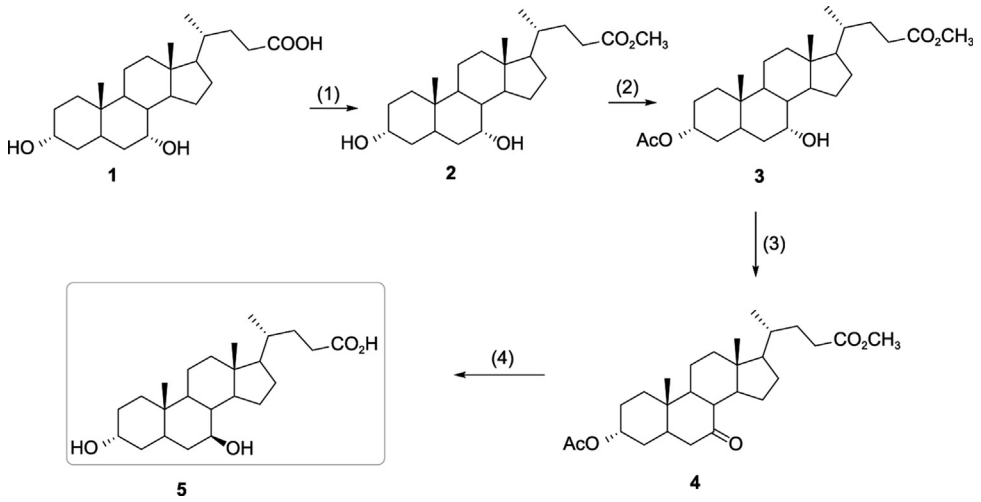


Fig. 7. Synthetic route for preparing UDCA using CDCA as the starting material [95].

mate, a highly toxic and strictly regulated chemical that poses operational, environmental, and residual safety concerns, leading to significant compliance and pollution-control costs. To overcome these drawbacks, the Zhigang Zhou et al. [103] proposed an innovative approach, replacing highly toxic, regulated reagents such as ethyl chloroformate with low-toxic, non-regulated alternatives such as propyl chloroformate or butyl chloroformate. Under mild conditions, these were reacted with quinoline and its derivatives to successfully prepare four novel EEDQ-analogous catalysts (C1–C4 in Fig. 8). This catalyst series efficiently promotes amination between cholic acid and taurine under mild conditions, establishing a safer and more environmentally friendly synthetic route for sodium taurocholate (Fig. 8). The process offers notable advantages, including readily available raw materials, low cost, mild reaction conditions, and straightforward post-processing. Under optimal conditions, the yield of sodium taurocholate is as high as 92%, with a product purity consistently above 95%. The entire procedure is simple to operate and easily scalable, effectively addressing the safety hazards, environmental impact, and regulatory constraints associated with traditional methods. This approach provides an efficient, economical, and sustainable technical solution for the industrial production of sodium taurocholate [103].

5.3. 5α-Cyprinol sulfate

5α-Cyprinol sulfate is a naturally occurring sulfated sterol derivative found in the bile of cyprinid fish such as common carp, grass carp, and other related species. It consists of a cholesterol backbone linked to a sulfate group via an ester bond and is thus classified as a sulfated sterol. This compound exhibits high polarity and water solubility and plays important roles in physiological processes including biological metabolism, signal transduction, and bile emulsification [104,105]. Research indicates that 5α-cyprinol sulfate possesses potential biological activity such as anti-inflammatory and antibacterial effects, as well as the ability to regulate lipid metabolism. These properties suggest its functional significance in fish physiology and adaptive evolution. In recent years, due to its unique structure and bioactivity, this compound has attracted increasing attention in the fields of natural product chemistry, pharmacology, and aquaculture. It is regarded as a promising candidate for novel biomarkers or functional ingredients, with significant potential for use in health-product development and aquaculture disease prevention [106].

Currently, the production of 5α-cyprinol sulfate relies primarily on natural extraction from fish bile. However, existing methods

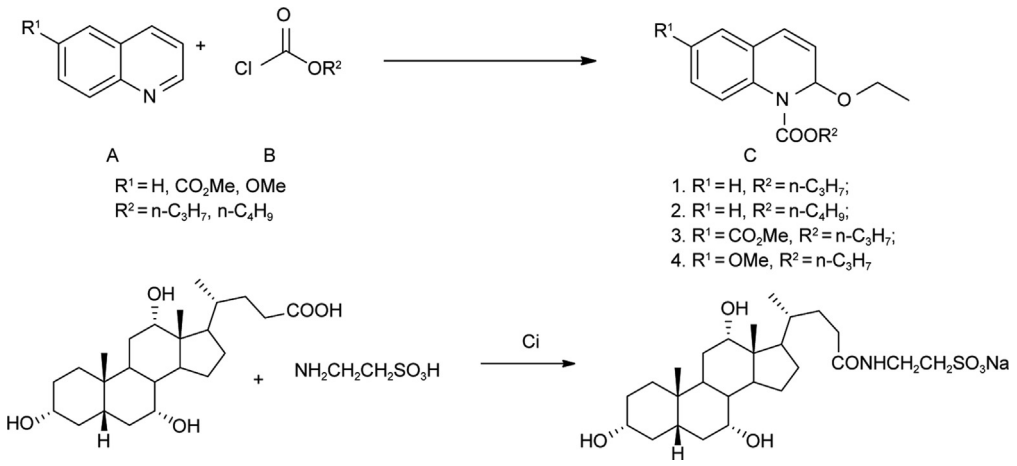


Fig. 8. Synthetic route for preparing EEDQ-analogous catalysts and sodium taurocholate [103].

have encountered several technical bottlenecks. First, the compound is present in relatively low concentrations in bile and coexists with various impurities such as bile acids, proteins, and lipids, making purification difficult and separation efficiency low. Second, traditional purification typically involves multi-step procedures including organic solvent extraction, column chromatography, and high-performance liquid chromatography (HPLC). These processes are operationally cumbersome, require large volumes of solvents, and result in relatively low overall recovery rates. Third, the extensive use of toxic organic solvents such as *N,N*-dimethylformamide and chloroform not only raises production costs but also creates issues related to waste treatment and environmental pollution, failing to align with green chemistry and sustainability principles [107,108]. Furthermore, chemical synthesis routes for this compound remain underdeveloped, lacking efficient and economical alternatives. Therefore, developing a novel extraction and purification process that is high-yielding, cost-effective, and environmentally friendly is of great importance for advancing basic research on 5α-cyprinol sulfate and its applications in pharmaceuticals and animal husbandry.

Zhou et al. [109] proposed an innovative method for extracting 5α-cyprinol sulfate from the bile of common carp and grass carp. The key innovation lies in a simplified purification process termed "thermal dissolution–gradient precipitation." After obtaining the crude product via freeze-drying or rotary evaporation concentration, hot methanol is used to selectively dissolve the target compound. By controlling the methanol–ethyl acetate ratio (1:1 to 1:2), high-purity products can be precipitated under mild static conditions. This approach consolidates the multi-step purification of traditional methods into two key operations, significantly improving both efficiency and selectivity. This extraction method exhibits prominent technical advantages: First, only low-toxicity solvents such as methanol and ethyl acetate are used, avoiding harmful reagents such as chloroform and *N,N*-dimethylformamide. This aligns with green chemistry principles and reduces waste treatment costs by over 30%. Second, without the need for complex equipment such as chromatography columns, a single extraction achieves a purity greater than 95% with a consistent yield of 79%–82%, greatly increasing production efficiency and lowering costs. This method is simple, mild, and easily scalable, providing a reliable, economical, and environmentally friendly route for the large-scale preparation of 5α-cyprinol sulfate and supporting its applications in pharmaceuticals and aquaculture.

5.4. 5α-Cyprinol

Like its sulfinated form, 5α-cyprinol sulfate, 5α-cyprinol is a unique polyhydroxylated bile alcohol isolated from the bile of cyprinid fish. Its core structure differs from 5α-cyprinol sulfate by the absence of just one sulfate group—a structural difference that determines their respective native states and physiological roles. In fish, 5α-cyprinol is synthesized and stored almost exclusively in its sulfated form within the bile, as this form exhibits high water solubility and stability. As the direct functional molecule responsible for emulsifying fats and regulating cholesterol metabolism, 5α-cyprinol sulfate accounts for the overwhelmingly dominant fraction. In contrast, free 5α-cyprinol is present only in trace amounts, resembling a biosynthetic precursor or intermediate. Studies indicate that both free 5α-cyprinol and 5α-cyprinol sulfate have specific physiological activities, such as serving as bile acid precursors, participating in lipid digestion regulation, and demonstrating potential anti-inflammatory, antibacterial, and lipid metabolism-modulating effects. This makes them valuable resources for exploring natural-product lead compounds. Given the difficulty of obtaining substantial quantities of free 5α-cyprinol directly from natural bile, Zhou et al. (the work has not yet been published) developed a key process to extract abundant 5α-cyprinol sulfate from fish bile, followed by its efficient and controllable conversion into high-purity 5α-cyprinol via a specific chemical hydrolysis reaction (Fig. 9). This process enables the large-scale preparation of the free sterol, provides a reliable material basis and technical pathway for comparative studies on the activity differences and structure–activity relationships between the two forms, and opens up a route for subsequent drug development.

5.5. 5β-cyprinol and its sulfate

The stereochemical properties of a compound are a decisive factor governing its physiological activity. Subtle chiral differences often lead to significant variations in efficacy, metabolism, and toxicity, and may even produce opposite biological effects. This principle is particularly evident in the field of steroidal drugs [110,111]. Although research on the naturally abundant 5α-configured cyprinol and its sulfate has laid a certain foundation, its key stereoisomers, the 5β-cyprinol series of compounds, have long presented a bottleneck in sourcing due to extremely low natural abundance and challenging purification, severely restricting systematic exploration of their biological functions. Therefore, establishing an efficient and stereoselective synthetic route for 5β-cyprinol and its sulfate is both practically urgent and scientifically valuable.

This research would hold value in three areas: First, it would overcome the limitations of natural extraction, providing sufficient high-purity reference substances for in-depth activity evaluation, mechanism studies, and safety assessments. Second, it would enable rigorous structure–activity relationships (SARs) analysis. It is only by synthesizing both the 5α and 5β stereochemically pure compounds that we can clarify how the core stereochemical difference—that is, the *cis* (5β-) versus *trans* (5α-) fusion of the A/B rings—regulates interactions with biological targets and influences anti-inflammation and metabolic activities, including potency, selectivity, and *in vivo* metabolic fate. Third, it would lay a chemical foundation for drug development. A robust synthetic route is essential for subsequent structural optimization and derivatization to improve druggability by optimizing solubility, stability, and bioavailability, thereby providing a versatile platform for novel

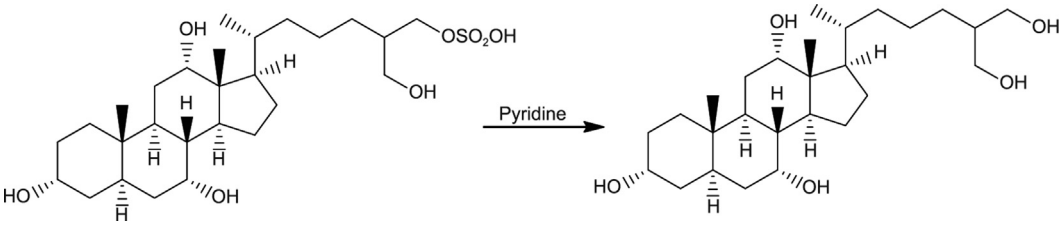


Fig. 9. Synthetic route for preparing 5α-cyprinol from 5α-cyprinol sulfate.

lead compound discovery. The successful establishment of this synthetic method would thus hold significance beyond the preparation of a single class of compounds. Scientifically, it would fill a gap in the field of the selective synthesis of complex polyhydroxylated bile sterols, offering strategic insights for synthesizing other challenging natural products with similar structures. In practical terms, synthetic access to the 5β-cyprinol series may provide novel functional additives to prevent prevalent metabolic diseases, such as fatty liver and hepatobiliary syndrome, in intensively farmed fish. Furthermore, SAR studies based on these compounds could guide the optimization of hepatobiliary-regulating molecules with higher activity and stability than their natural 5α counterparts. This would deliver original material and key technical support for developing green and efficient aquatic feeds.

Based on this background, the research group led by Zhou et al. [112] innovatively designed and validated a novel synthetic route for the efficient preparation of high-purity 5β-cyprinol sulfate and its free sterol via a total chemical synthesis approach, using cheap and readily available cholic acid as the starting material (Fig. 10 [109]). Through seven key steps, including intramolecular lactonization, simultaneous protection of multiple hydroxyl groups, selective reduction, sulfonylation activation, malonate alkylation and extension, double reduction, and regioselective sulfation, this route achieves the highly stereoselective construction of the 5β–steroidal core skeleton (*cis*–fused A/B rings) and complete side–chain assembly. This systematic synthetic strategy not only overcomes the inherent limitations of traditional fish bile extraction methods in terms of raw material scarcity, separation efficiency, and scalability but also accomplishes, for the first time, gram-scale controllable synthesis of this otherwise scarce compound. This method exhibits comprehensive advantages in multiple areas. In terms of raw material economy, cholic acid—a bulk

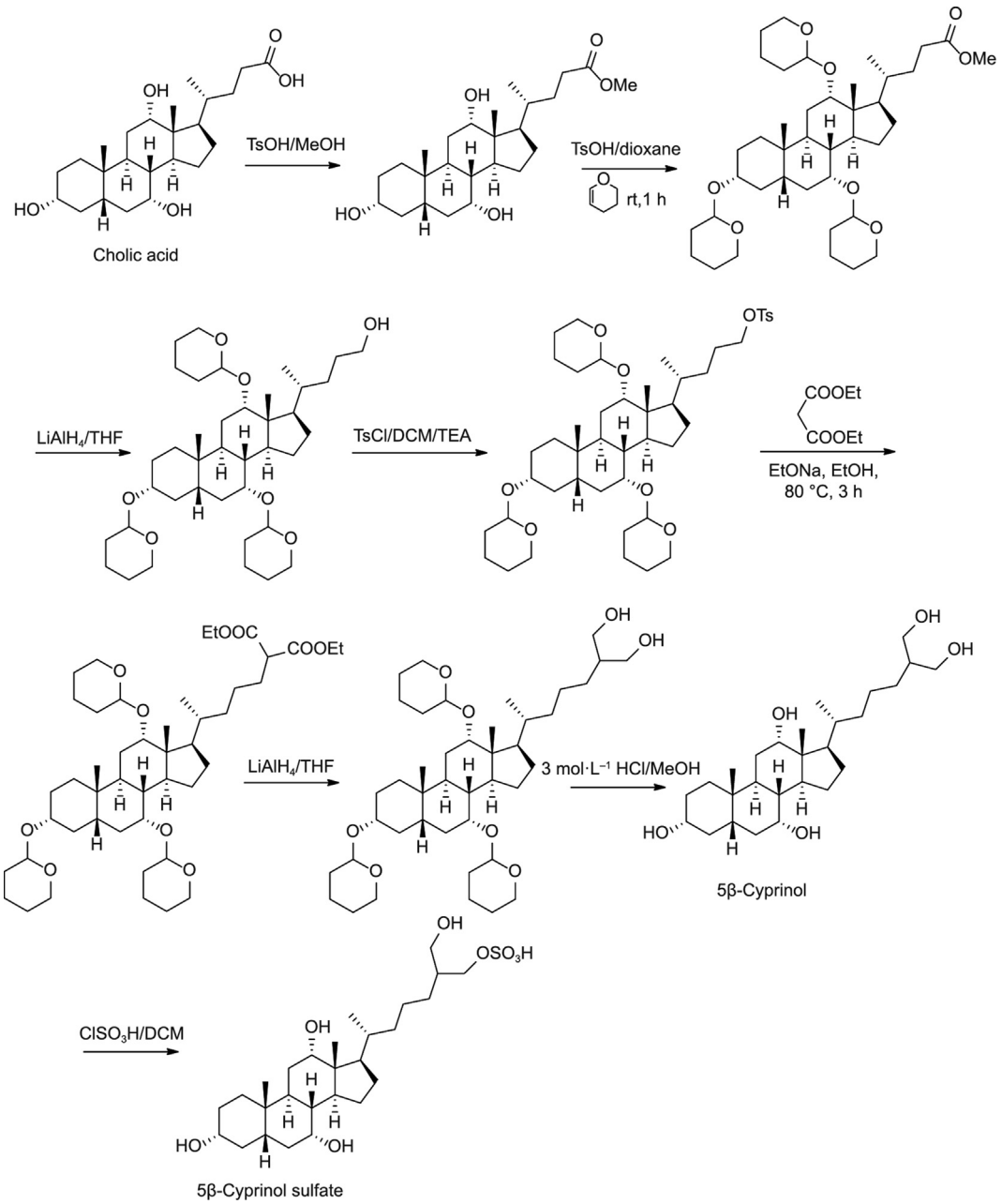


Fig. 10. Synthetic route for preparing 5β-cyprinol and 5β-cyprinol sulfate from cholic acid [109]. DCM: dichloromethane; TEA: triethylamine; THF: tetrahydrofuran; rt: room temperature.

industrial chemical with wide availability—serves as the starting material, eliminating dependence on limited biological resources and offering significant cost advantages for large–scale production. In terms of process feasibility, the reaction conditions are mild and controllable, the key steps have high yields (overall yield >15%, in linear synthesis, an overall yield of 15%–30% over eight steps is generally regarded as relatively high), and purification relies on conventional column chromatography, making the route concise, reproducible, and readily scalable in terms of quality control. The process stably delivers the target products with high chemical purity and stereoselectivity (> 97%). All intermediates and final products have been unequivocally characterized by nuclear magnetic resonance (NMR) spectroscopy, providing a reliable material foundation for subsequent studies. The establishment of this synthetic platform not only offers a key tool for systematic SAR studies of 5α-/5β-series compounds but also lays a solid technical foundation for the development of novel feed additives and drug candidates based on this scaffold (Table 1).

Despite substantial progress in the preparation of bile acids and their derivatives, several critical limitations persist. The industrial scalability of UDCA synthesis requires further validation, and the long-term biocompatibility of synthetic catalysts for sodium taurocholate remains unassessed. For 5β-cyprinol sulfate, the multi-step synthesis and use of column chromatography limit cost-effective production, and its *in vivo* functions await validation. Future research should focus on simplifying the synthetic routes and validating 5β-series functions *in vivo*.

6. Quorum sensing inhibitors (bromofuranone C-30)

Halogenated furanones are a novel class of antibacterial agents that are both found in nature and created synthetically. As important members of the furanone family, with a group of heterocyclic compounds containing a furan ring and a ketone group, halogenated furanones occur naturally in fruits such as strawberries and pineapples, contributing to their unique fruity aromas. Through structural modifications such as halogenation, these compounds acquire pronounced biological activity. Studies have confirmed that both naturally derived and synthetic halogenated furanones, such as bromofuranone C-30, can specifically disrupt bacterial quorum-sensing systems and interfere with associated signal transduction pathways [113,114]. In this way, they effectively inhibit biofilm formation in various Gram-positive and Gram-negative bacteria, reducing bacterial virulence. Rather than acting through direct bactericidal activity, the mechanism relies on anti-virulence strategies, which lower the risk of inducing bacterial resistance while maintaining high biological safety. These properties underscore the strong potential halogenated furanones hold for the development of novel antibacterial approaches.

Bromofuranone C-30 is a well-characterized quorum sensing inhibitor whose mechanism and application value have been confirmed by multiple studies. It specifically disrupts the acyl-homoserine lactone (AHL)-mediated signaling system in bacteria, thereby suppressing the expression of virulence factors and the formation of biofilm. This compound exhibits particularly clear effects against *Pseudomonas aeruginosa*, significantly inhibiting pyocyanin production, swarming motility, and biofilm development [115,116], and has been shown to improve pulmonary bacterial clearance in murine infection models [117]. Furthermore, studies demonstrate that it can protect the larvae of the giant freshwater prawn *Macrobrachium rosenbergii* from *Vibrio harveyi* infection [113].

The key strategy in the chemical synthesis of bromofuranone C-30 lies in the efficient and highly selective construction of its 3,4-dibromo-5-hydroxyfuran-2(5*H*)-one skeleton [118]. The synthetic route is generally designed following the principle of first constructing the furanone core, followed by selective bromination, with the aim of achieving the target structure through the most straightforward steps. Mainstream synthetic approaches typically start from inexpensive and readily available furoic acid or its derivatives. First, the starting material undergoes cyclization and hydroxylation under mild alkaline conditions to form the key intermediate, 5-hydroxyfuran-2(5*H*)-one. Subsequently, in the most critical step of the synthesis, this intermediate is subjected to electrophilic bromination using reagents such as bromine (Br_2) or NBS at low temperatures (e.g., 0 °C) under an inert atmosphere. This allows the precise introduction of two Br_2 atoms at the 3- and 4-positions of the furan ring, and the pure product is finally obtained via crystallization or similar methods [119–121].

However, this route has notable drawbacks. The key bromination step requires stringent conditions (i.e., precise temperature control and an anhydrous, oxygen-free environment) and involves hazardous reagents (e.g., highly corrosive liquid Br_2). Moreover, the labile furanone lactone ring is prone to side reactions such as ring-opening and over-bromination. These issues lead to cumbersome operations, low overall yield, and poor reproducibility, which severely hinder large-scale preparation and further research on this molecule.

To overcome this bottleneck, Zhou et al. [122] developed a novel synthetic route for bromofuranone C-30 using levulinic acid as the starting material, with a two-step bromination–cyclization sequence (Fig. 11). This approach exhibits significantly improved synthetic efficiency: By optimizing the catalyst (employing a hydrobromic acid–acetic acid solution) and reaction conditions (i.e., elevating the temperature of the first-step bromination to 50 °C), the yield of the first intermediate (3,5-dibromo-4-oxopentanoic acid) was sharply increased from 48% using the conventional method to 80%–85%, and the yield of the second-step cyclization reaction was raised from 11.2% to 70%–80%. This new synthetic route fundamentally addresses the major bottleneck of low yield in existing synthetic routes. Furthermore, this process eliminates the need for stringent low-temperature, anhydrous, and oxygen-free operations and reduces reliance on highly toxic brominating reagents (e.g., liquid Br_2), making the synthesis simpler and safer. It thereby lays a solid material and technical foundation for the large-scale preparation of bromofuranone C-30 and its applications in fields such as feed additives and pharmaceuticals [122].

Zhou et al. [122] have thus provided a clear paradigm for translating quorum sensing inhibitors from laboratory-scale development to industrialization. A key insight is that excellent *in vitro* activity alone is far from sufficient to ensure successful practical application. Effective translation must systematically address two challenges: ① establishing an efficient, safe, and scalable synthetic route to overcome the chemical bottlenecks that constrain supply, such as low yield and hazardous operations; and ② developing precise formulation engineering tailored to target application scenarios in order to mitigate inherent molecular limitations, including instability and pharmacokinetic shortcomings. Clearly, the development of future quorum sensing inhibitors should adopt a tripartite, systematic strategy that integrates bioactive molecules, efficient synthesis, and application-adapted formulations from the outset, with well-defined end-use requirements, such as serving as antibiotic alternatives in aquaculture.

Based on this framework, the future development of bromofuranone-based products can proceed along two main avenues. On the one hand, molecular structure optimization can be pursued. Leveraging the core pharmacophore of C-30, a series of derivatives with varied halogen substitution positions and functional groups can be synthesized using computer-aided drug

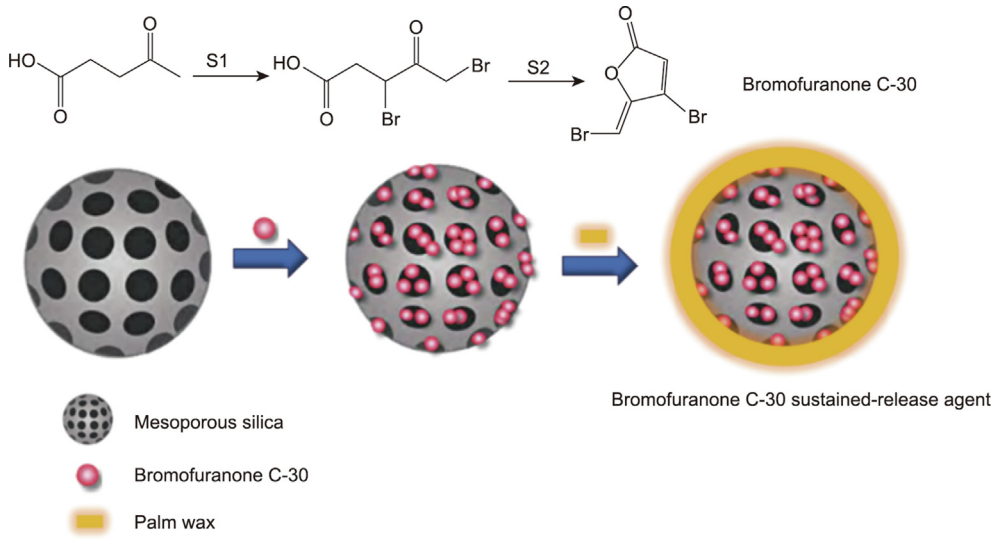


Fig. 11. Synthetic route for preparing bromofuranone C-30 and a bromofuranone C-30 sustained-release agent [122].

design, with the aim of screening next-generation lead compounds that exhibit enhanced activity, improved stability, or broader antimicrobial spectra. On the other hand, formulation diversification and application expansion should be explored. Novel delivery systems, such as nanoparticles, hydrogels, or sprays, can be developed for specific infection sites (e.g., the intestines of aquatic animals, skin wounds, or the lungs) and different administration routes (i.e., injection, immersion, or spraying). Concurrently, in-depth investigations into the synergistic mechanisms between bromofuranones and existing antibiotics are warranted in order to develop anti-virulence and bactericidal combination formulations capable of addressing more complex clinical infections. Through these efforts, bromofuranones can be evolved from a successful proof-of-concept case into a sustainable product platform with enduring innovation.

Several important limitations in the application of bromofuranone C-30 sustained-release agent in aquaculture remain. The safety profile of bromofuranone C-30 in aquatic animals has not been characterized, and the potential for resistance development to quorum sensing inhibitors remains unexplored. Additionally, the nano-encapsulated formulation introduces concerns regarding nanoparticle safety and environmental release. Future research should focus on comprehensive toxicological assessment and environmental risk evaluation.

7. Conclusions

The chemical synthesis and application of green aquaculture inputs have progressed from traditional extraction and simple compounding toward a systematic, function-driven innovation paradigm underpinned by green synthesis technologies. Based on a comprehensive review of four key categories—namely, SCFAs, tryptophan metabolites, TCA cycle regulators, bile acid derivatives, and quorum sensing inhibitors (bromofuranone C-30)—this study arrived at the following core conclusions:

Green synthesis and targeted modification are fundamental in improving product performance. Practices such as stabilizing and achieving targeted delivery of SCFAs through esterification and encapsulation, or preparing high-purity indole metabolites via efficient catalysis and chiral synthesis, highlight how chemical and process innovations are crucial for advancing product functionality. Moreover, synthetic processes characterized by atom economy and streamlined steps are central in enabling industrialization. Breakthroughs made by the research group led by Zhigang Zhou in synthesizing complex molecules, such as UDCA, sodium taurocholate, and 5β-cyprinol sulfate, demonstrate that, through strategic route design and condition optimization, it is possible to significantly improve yield and purity while lowering production costs and environmental impact. In addition, in-depth analyses of SARs and mechanisms of action provide a scientific basis for product iteration and upgrading. Comparative studies on the activity and mechanisms of compounds with different stereoconfigurations (e.g., 5α- vs 5β-cyprinol) and forms (e.g., α-ketoglutaric acid vs its lactone) offer clear guidance for the targeted optimization of product performance. Finally, interdisciplinary integration and collaboration among industry, academia, and research institutions are essential for advancing the field. From molecular design and green synthesis to efficacy verification and the establishment of application standards, progress in this area depends on the close integration of chemistry, biology, aquaculture science, and engineering technologies.

Looking forward, several emerging technologies hold great promise for further advancing the development of green aquaculture inputs. In particular, the integration of artificial intelligence (AI) and intelligent delivery systems will drive the field toward greater activity, enhanced specificity, and improved environmental compatibility.

In the realm of AI-assisted molecular design, future research can leverage machine learning and deep learning algorithms to predict the biological activity, toxicity, pharmacokinetic properties, and synthetic accessibility of candidate small molecules prior to laboratory synthesis. For example, generative models (e.g., variational autoencoders or generative adversarial networks) can be trained on existing bioactive small-molecule databases to propose novel molecular structures with optimized properties. Quantitative structure–activity relationship (QSAR) models can accelerate the screening of derivatives with improved stability, target affinity, or reduced off-target effects. AI can also facilitate retrosynthetic analysis, recommending the shortest, most atom-economical, and environmentally friendly synthetic routes. These approaches will significantly reduce the trial-and-error cycle in molecular discovery and enable the rational design of next-generation green inputs.

Regarding smart delivery systems, future efforts should focus on developing nano-enabled platforms that respond to specific physiological or environmental triggers. For instance, pH-sensitive nanoparticles or nanocoatings can be engineered to

release their payloads only upon reaching the neutral-to-slightly-alkaline environment of the fish intestine, thereby protecting acid-labile compounds (e.g., SCFAs) from premature degradation in the stomach. Thermo-responsive hydrogels or lipid nanoparticles could be designed to release bioactive molecules at specific temperature thresholds associated with febrile responses or seasonal variations. Enzyme-triggered release systems, utilizing proteases or lipases enriched in the gut of aquatic species, can achieve site-specific delivery of therapeutics or growth promoters. Additionally, targeted nano-delivery vehicles functionalized with ligands (e.g., lectins or antibodies) that recognize specific receptors on intestinal epithelial cells or immune cells could increase cellular uptake and therapeutic efficacy while minimizing systemic exposure. These smart delivery systems could not only improve bioavailability and reduce required dosages but also mitigate the potential environmental release of active compounds, aligning with the principles of green and sustainable aquaculture.

The convergence of AI-driven molecular design with intelligent nano-delivery systems will underpin the development of an aquaculture system that is resource-efficient, environmentally sound, and product-safe, accelerating the transition from empirical formulation to precision aquaculture.

CRediT authorship contribution statement

Lijuan Yu: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Zhiyong Xue:** Supervision, Project administration, Methodology. **Yanqing Li:** Investigation, Data curation. **Min Fu:** Investigation, Formal analysis, Data curation. **Jian Zhang:** Investigation, Formal analysis, Data curation. **Lanhui Huang:** Data curation. **Zhen Zhang:** Supervision. **Yuanyuan Yao:** Supervision. **Yalin Yang:** Visualization, Supervision, Resources. **Chao Ran:** Supervision. **Kangsen Mai:** Conceptualization. **Shouqi Xie:** Conceptualization. **Einar Ringø:** Writing – review & editing. **Rolf Erik Olsen:** Writing – review & editing. **Jihong Liu Clarke:** Writing – review & editing. **Qianwen Ding:** Writing – review & editing, Methodology, Investigation. **Zhigang Zhou:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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