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Construction, structural modification, and bioactivity evaluation of pentacyclic triterpenoid privileged scaffolds in active natural products

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Pentacyclic triterpenoids, as important representatives of natural products, have garnered widespread attention due to their diverse biological activities, including anti-inflammatory, antiviral, and antitumor effects. Oleanolic acid (**OA**), betulinic acid (**BA**), ursolic acid (**UA**), triptolide, and glycyrrhetinic acid (**GA**) are typical examples of pentacyclic triterpenoids. Despite their significant biological activities, their poor water solubility and low bioavailability have limited further development and application. In recent years, researchers have developed a series of derivatives with enhanced biological activities and improved drug properties through structural modifications of these compounds, particularly achieving notable progress in the field of antitumor therapy. This review summarizes recent advances in the structural modification of pentacyclic triterpenoids and explores their promising applications in the development of antitumor, antiviral, and other therapeutic agents.

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Terpenoids are a class of compounds and their derivatives with a molecular scaffold based on isoprene units, which are widely distributed in nature. They are major components of secondary metabolites such as plant essential oils, resins, and pigments. Terpenoids are classified into monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, tetraterpenes, and polyterpenes based on the number of isoprene units in their molecular structure. Triterpenes, composed of a basic skeleton of 30 carbon atoms, are important plant secondary metabolites found in nature either in free form or as glycosides or esters bound to sugars. They exhibit various pharmacological activities, including anti-cancer, antiviral, and cholesterol-lowering effects. Triterpenoids can be further classified into monocyclic, bicyclic, tricyclic, tetracyclic, and pentacyclic triterpenes, based on the number of rings in their structure, with tetracyclic and pentacyclic triterpenes being the most common.

Pentacyclic triterpenoids possess various modification types in their carbon skeletons, resulting in complex and diverse structures, which have garnered significant attention due to their wide range of functions. Based on their aglycone types, pentacyclic triterpenoids are divided into four main categories: oleanane-type [*e.g.*, **OA**, **GA**, and hawthorn acid (HA)], lupane-type (*e.g.*, **BA**, lupeol, and betulin), ursane-type [*e.g.*, **UA** and asiatic acid (AA)], and friedelane-type (*e.g.*, *Tripterygium wilfordii* polyglycosides). Oleanane-type triterpenoids exhibit extensive pharmacological activity; **OA** is commonly used clinically for its hepatoprotective effects,[1–3] while glycyrrhizin-based formulations have been marketed for the treatment of hepatitis.[4] The oleanane-type, also known as the β-amyrane-type, includes compounds such as β-amyrin, germanicol, **OA**, and **GA**.

Lupane-type compounds mainly include lupeol, betulin, **BA**, and their derivatives. Lupeol is widely distributed in nature and serves as the core skeleton for lupane-type triterpenoids, showing significant anti-inflammatory[5] and anti-cancer[6] activities. Betulinic acid, formed through three consecutive oxidation steps at the C-28 position of lupeol, not only demonstrates potent anti-HIV activity,[7] but also selectively inhibits melanoma cell proliferation,[8] making it a promising candidate for anti-HIV and anti-melanoma drug development.[9,10] **BA** is predominantly found in the bark of birch trees.

The ursane-type, also known as the α-amyrane-type, includes compounds such as α-amyrin, **UA**, and taraxasterol. α-Amyrin is a precursor for ursane-type triterpenoids, whose structural diversity can be enhanced through glycosylation, acylation, and redox reactions. **UA**, also known as ursane acid or **UA**, is an oxidation product of α-amyrin at the C-28 position and exhibits anti-cancer, anti-diabetic, anti-ulcer, and lipid-lowering effects.[11–13] **UA** is found in various plants, including fruit trees, aromatic herbs, and traditional Chinese medicinal herbs.

1 Construction of pentacyclic triterpenoid privileged scaffolds

The synthesis of pentacyclic triterpenoid scaffolds primarily occurs through two pathways:[14] the methylerythritol phosphate (MEP) pathway in plastids and the mevalonate (MVA) pathway.

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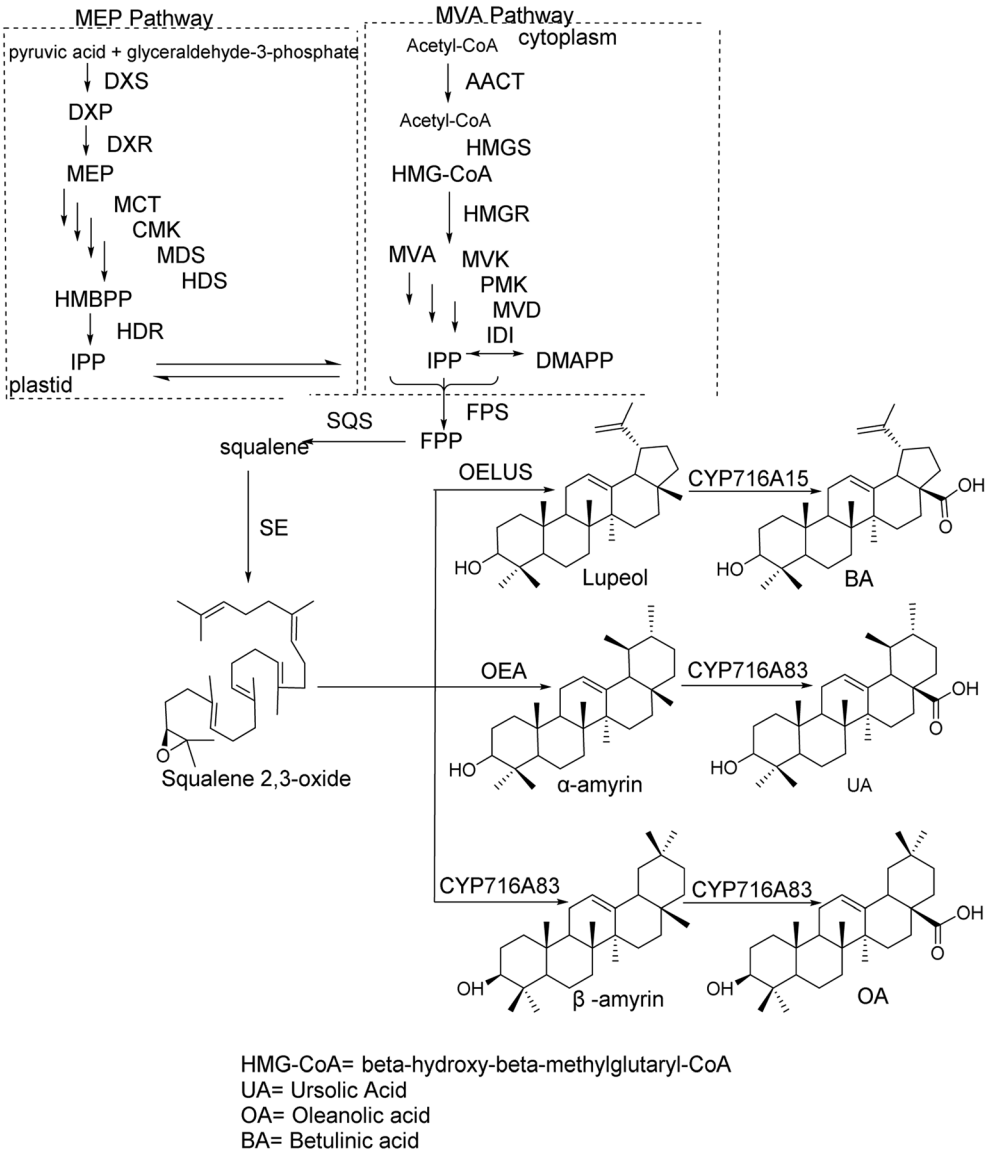


Fig. 1 Synthesis of the pentacyclic triterpenoid frameworks **OA**, **BA**, and **UA**.

In plastids, isopentenyl diphosphate (IPP) is synthesized from pyruvate and glyceraldehyde-3-phosphate *via* seven enzymatic reactions, after which IPP is transported into the cytoplasm to participate in terpenoid synthesis. Under the action of IPP isomerase, IPP and dimethylallyl pyrophosphate (DMAPP) interconvert. In the cytoplasmic MVA pathway, acetyl-CoA acts as the initial donor to produce IPP. IPP and DMAPP are then converted into farnesyl diphosphate under the action of farnesyl diphosphate synthase, which is subsequently converted into squalene by squalene synthase. Squalene is further oxidized into 2,3-oxidosqualene *via* squalene epoxidase (SE).[15] The cyclization of 2,3-oxidosqualene, catalyzed by oxidosqualene cyclase (OSC), produces various tetracyclic and pentacyclic triterpenoid scaffolds. These scaffolds undergo chemical modifications, such as oxidation, substitution, and glycosylation, through the action of cytochrome P450 monooxygenases and glycosyltransferases, with uridine diphosphate (UDP) as the glycosyl donor, ultimately forming various pentacyclic triterpenoid compounds. The synthesis pathways of **OA**, **BA**, and **UA** scaffolds are shown in Fig. 1. OSC, a key enzyme in this process, catalyzes the cyclization of 2,3-oxidosqualene to produce sterol and triterpenoid precursors, representing the critical step in generating the diversity of triterpenoid products.

2 Structural modification of pentacyclic triterpenoid compounds

In the structure of pentacyclic triterpenoids, the modifications of **OA**, **BA**, and **UA** primarily occur at the hydroxyl group on the A-ring, the carboxyl group at the C-28 position, and the double bond on the C-ring. Rational structural modifications of these active groups can significantly enhance the antitumor activity of these compounds.

2.1 Salt formation modification

Based on the structural characteristics of pentacyclic triterpenoid compounds, there are three direct methods of salt formation: carboxyl groups forming salts with alkali metal ions; introduction of hydrophilic groups followed by salt formation with alkali metal ions; and the introduction of quaternary ammonium salts. Zhao *et al.*[16] and Zhai *et al.*[17] demonstrated that converting **OA** into its sodium salt significantly increased its solubility in water compared to **OA**. After administering sodium oleanolate to rats, Li *et al.*[18] observed a marked improvement in subacute liver injury, with the hepatoprotective effect being significantly stronger than that of **OA**. This indicates that salt formation significantly enhances the water solubility, bioavailability, and bioactivity of **OA**.

2.2 Amino acid conjugation

Amino acids, as essential nutrients for the human body, are commonly used in the treatment of liver diseases, neurological disorders, and psychiatric conditions. Due to the presence of both carboxyl and hydroxyl polar groups in their structure, coupling amino acids with pentacyclic triterpenoid compounds can enhance the water solubility of the drug and improve its bioavailability. Zhang *et al.*[19] introduced amino acids at the C-28 position of **OA**, and the resulting **OA** derivatives exhibited significantly higher antitumor activity compared to the parent compound.

2.3 Glycosylation

Glycosides are polyhydroxy compounds widely distributed in the roots, stems, and leaves of plants, characterized by high solubility and strong polarity. Currently, numerous studies have focused on using **OA** as a starting material to design and synthesize **OA** glycoside derivatives with enhanced biological activities. These **OA** derivatives have shown significantly improved pharmacological activities. Meng *et al.*[20] synthesized 10 **OA** derivatives through a series of reactions, using natural **OA** as the lead compound. Pharmacological activity analysis of two glycoside derivatives revealed that one of the derivatives exhibited significantly better antitumor activity than **OA**, while the other showed even stronger antitumor activity, comparable to the antitumor drug gefitinib.

2.4 Introduction of ester and amide bonds

Based on the structural characteristics of **UA**, **OA**, and **BA**, the hydroxyl group at the C-3 position can undergo esterification reactions with compounds containing carboxyl groups to form ester bonds. Additionally, the carboxyl group at the C-28 position can react with amino or hydroxyl groups through dehydration to form either ester or amide bonds, thereby improving their water solubility and enhancing bioavailability. Zhang *et al.*[21] applied the principle of molecular hybridization in drug synthesis, coupling oxo-oleanolic acid with aminophosphonic acid aryl esters through amide bonds. The resulting novel **OA** derivatives exhibited better water solubility and higher bioactivity compared to the parent compound.

3 Structural modification and bioactivity of oleanolic acid

OA, also known as Cenoside, is a pentacyclic triterpenoid compound of the oleanane type. It is widely distributed in nature and can be found in various plants such as the fruit of *Ligustrum lucidum* and the whole herb of *Rabdosia rubescens*, existing either in its free form or as glycosides. **OA** exhibits a wide range of biological activities, common to pentacyclic triterpenoids, including antitumor activity,[22–24] antiviral properties,[25] antihyperlipidemic effects,[26,27] anti-inflammatory actions,[28,29] and hypoglycemic effects. **OA** is considered an ideal drug for the treatment of chronic viral hepatitis and acute jaundice hepatitis[30] due to its low toxicity and side effects. The anticancer activity of **OA** and its derivatives is particularly broad, showing inhibitory effects on various human tumor cells. Yang *et al.*[31] reported that **OA** and its derivatives are well-known for their potent antitumor activity, including inhibiting tumor cell proliferation, inducing cell cycle arrest, apoptosis, and differentiation. These effects have been observed in various cancer cell lines, such as A549, SGC7901, MCF-7, HT1080, and MKN-45 cells. Jannus *et al.*[32] further highlighted that the anticancer properties of **OA** and its derivatives have been demonstrated in numerous *in vitro* and *in vivo* models, showing significant anticancer effects. In cancer, the homeostatic balance between cell proliferation and death is disrupted, and apoptosis, a physiological process of programmed cell death, is one of the key mechanisms for controlling this balance.

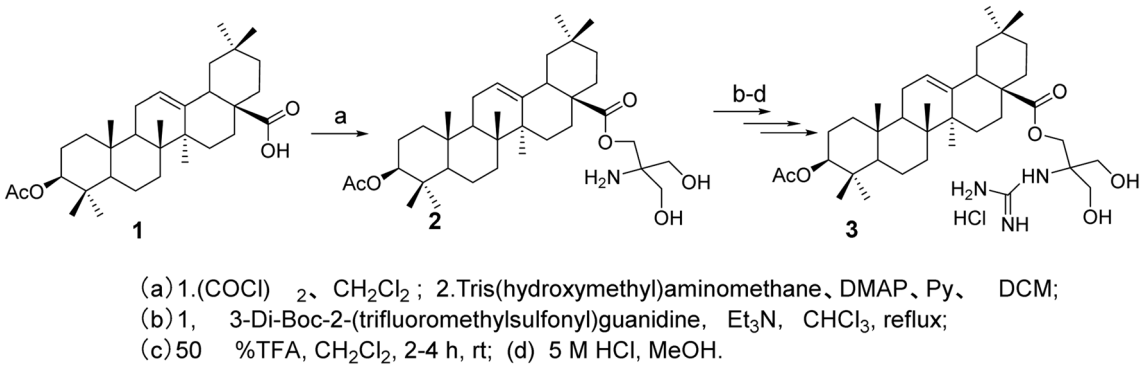
However, the major limitation of **OA** and its derivatives as anticancer agents is their poor water solubility, which leads to low bioavailability *in vivo*. Therefore, it is necessary to introduce polar groups and perform rational structural modifications to enhance solubility and bioavailability, thus improving the clinical applicability of these compounds.

3.1 Structural modifications of oleanolic acid and antitumor activity

3.1.1 Modification of hydroxyl group at C-3 or carboxyl group at C-28 of oleanolic acid. Spivak *et al.*[33] designed and synthesized derivatives of **OA** with a C-28 carboxyl group connected to a guanidine group through various linkers to investigate the influence of the guanidine moiety on the anticancer properties of **OA**. The synthesized guanidine-containing **OA** derivatives (compound **3**, Fig. 2) exhibited significantly higher cytotoxicity in Jurkat cells compared to the original **OA**. Most of the guanidine derivatives tested demonstrated higher IC_{50} values than the amine counterparts, while showing lower toxicity towards human fibroblasts.

Chouaïb *et al.*[34] synthesized two series of **OA** 1,2,3-triazole derivatives (compounds **4** and **5**, Fig. 3) using CuAAC or RuAAC as catalysts under microwave conditions. The introduction of the 1,2,3-triazole moiety enhanced their activity against mouse mammary cancer (EMT-6) and human colon cancer (SW480) cells.

Khusnutdinova *et al.*[35] synthesized alkynyl derivatives **6** and **7** (Fig. 4) through amidation and the Mannich reaction. These



(a) 1.$(COCl)_2$、CH_2Cl_2; 2.Tris(hydroxymethyl)aminomethane、DMAP、Py、 DCM;
(b) 1, 3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine, Et_3N, $CHCl_3$, reflux;
(c) 50 %TFA, CH_2Cl_2, 2-4 h, rt; (d) 5 M HCl, MeOH.

Fig. 2 Synthesis of OA derivative 3.

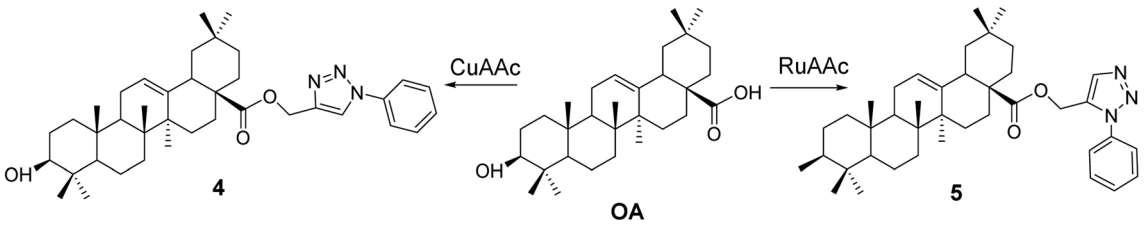
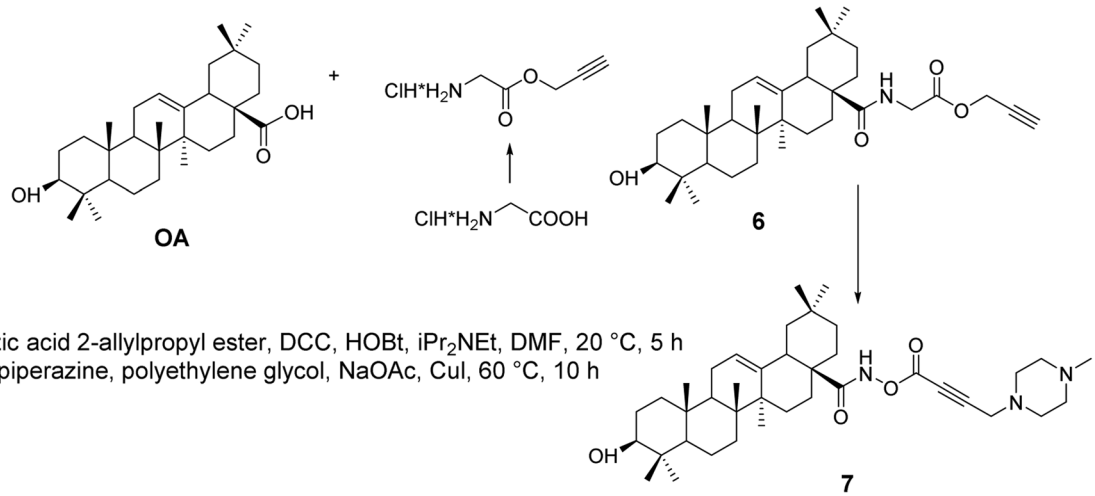


Fig. 3 Synthesis of OA derivatives 4 and 5.



i)Glycyrrhizic acid 2-allylpropyl ester, DCC, HOBt, iPr_2NEt, DMF, 20 °C, 5 h
ii)N-methylpiperazine, polyethylene glycol, NaOAc, CuI, 60 °C, 10 h

Fig. 4 Synthesis of OA derivatives 6 and 7.

derivatives exhibited *in vitro* anticancer activity against leukemia cells and human colon cancer, acting through their pro-apoptotic activity, stimulating the activity of apoptosis-related caspases 3 and 8, enhancing the activity of NF-κB transcription factors, influencing BAX protein expression, and inhibiting DNA polymerase.

3.1.2 Modification of C-2, ring A, and other positions of oleanolic acid. Liu *et al.*[36] synthesized a variety of **OA** derivatives (compounds **8** and **9**, Fig. 5) involving the condensation of formaldehyde at the C-2 position, oxidation of the C-3 hydroxyl group to a carbonyl, and the attachment of ethylenediamine at C-28. Compounds **8** and **9** demonstrated inhibitory effects on various types of cancer stem cells (CSCs) involved in tumorigenesis, tumor recurrence, invasion, metastasis, and resistance.

Meng *et al.*[37] modified the A ring and C-28 position of **OA** to synthesize ten **OA** derivatives (compounds **12–21**, Fig. 6). The inhibitory activities of these derivatives were evaluated against the SGC7901 and A549 cell lines, confirmed through the MTT assay. The experimental results indicated that all compounds exhibited certain antitumor activities against the SGC-7901 and A-549 cell lines. Among them, the **OA** derivatives **15** and **21** demonstrated superior antitumor activity against both SGC7901 and A549 cells compared to the positive drug gefitinib.

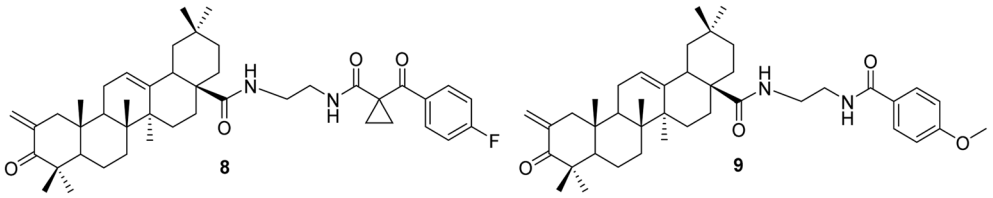
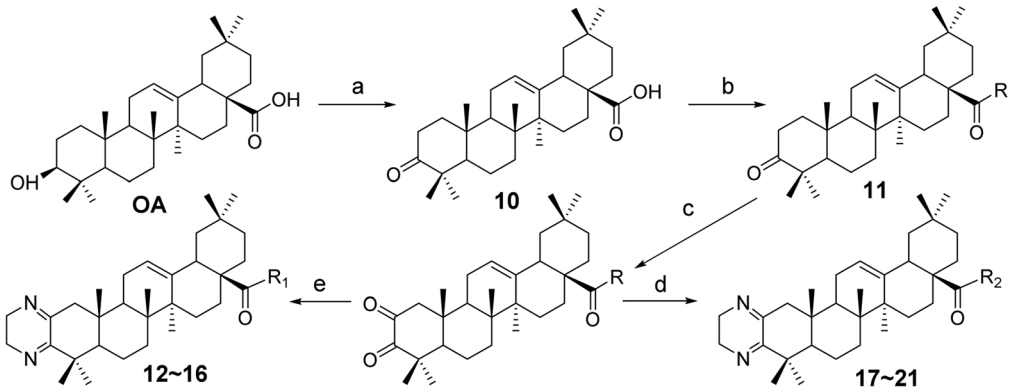


Fig. 5 The structures of OA derivatives 8 and 9.



12. $R_1=CH_2C_6H_5$; 13. $R_1=CH_2CH_3$; 14. $R_1=CH(CH_3)_2$; 15. $R_1=CH_2CH(CH_3)_2$; 16. $R_1=(CH_2)_5CH_3$;
17. $R_2=CH_2CH_3$; 18. $R_2=CH(CH_3)_2$; 19. $R_2=(CH_2)_3CH_3$; 20. $R_2=C_2H_4CH(CH_3)_2$; 21. $R_2=(CH_2)_5CH_3$

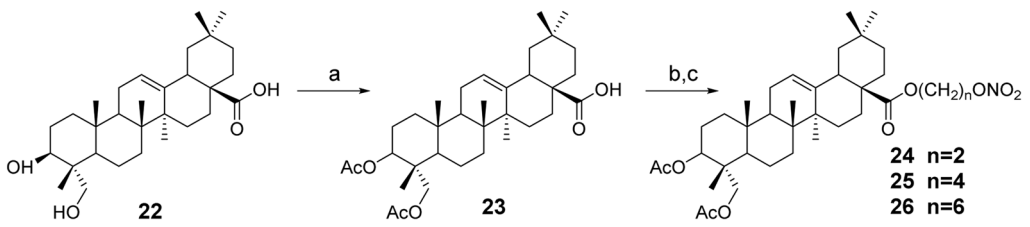
Reagents and conditions: (a) acetone, Jones Reagent, rt; (b) DMF, anhydrous K_2CO_3, RBr, rt; (c) t-BuOH, t-BuOK, 50 °C; (d) anhydrous ethanol, $C_2H_8N_2$, Magnesium sulfate anhydrous, 80 °C; (e) 1, 2-Benzenediamine、anhydrous ethanol, reflux, 8 h。

Fig. 6 Synthesis of OA derivatives 12 to 21.

Nitric oxide (NO) is associated with several carcinogenic signaling pathways. Ling's group synthesized several hybrid compounds of **OA** and ivy saponin NO donors[38] (compounds **24–26**, Fig. 7) and evaluated their cytotoxicity against various cancer cell lines. The most effective derivative, compound **25**, significantly inhibited the proliferation of five cancer cell lines (IC_{50} = 4.6–5.2 μmol L^{-1}). Furthermore, compound **25** exhibited a stronger inhibitory effect on EGFR-LTC kinase activity (IC_{50} = 0.01 μmol L^{-1}) compared to ivy saponin (IC_{50} > 20 μmol L^{-1}) and inhibited the proliferation of gefitinib-resistant H1975 (IC_{50} = 8.1 μmol L^{-1}) and osimertinib-resistant H1975-LTC (IC_{50} = 7.6 μmol L^{-1}) non-small cell lung cancer (NSCLC) cells. This suggests that NO may synergistically act with compound **15** (Fig. 6) to induce the maximum production of NO in H1975 cells.

In 2018, Raghuvanshi *et al.*[39] synthesized a series of **OA**-based chromene derivatives and evaluated their anticancer activity. Among the derivatives with promising activity (compounds **27** and **28**, Fig. 8), they further analyzed their ability to trigger apoptosis in A549 and MDA-MB-231 cells and found that these compounds caused cell cycle arrest in the G2/M phase. The most effective compound, **27** (IC_{50} = 3.6 μmol L^{-1}), exhibited anticancer activity through microtubule destabilization, and it was found to be non-toxic to human red blood cells.

Froelich *et al.*[40] introduced a lactam system into the pentacyclic triterpenoid structure of **OA**, facilitating the Beckmann



(a) Ac_2O, pyridine, rt, 12 h; (b) $Br(CH_2)nBr$, K_2CO_3, DMF, rt, 8 h; (c) $AgNO_3$, CH_3CN, light-protected, reflux 3 h.

Fig. 7 Synthesis of OA derivatives 24 to 26.

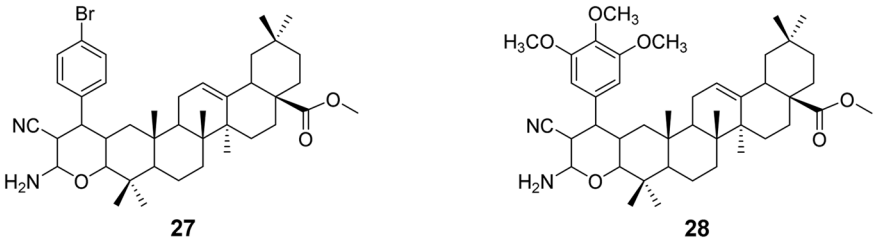


Fig. 8 The structures of OA derivatives 27 and 28.

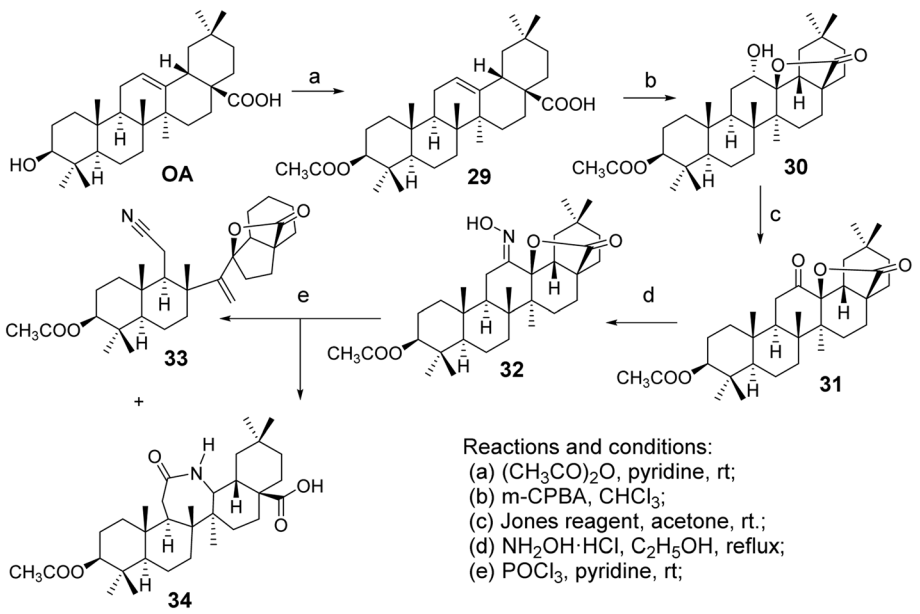


Fig. 9 Synthesis of OA derivatives 32 to 34.

rearrangement of the C-ring lactone to form **OA** derivatives (oxime **32**, nitrile **33**, and lactam **34**, Fig. 9). Zaprutko *et al.*[41] conducted tests on these derivatives as percutaneous permeation enhancers, finding that their activity was comparable to that of the positive control drug Azone.

3.1.3 Modifications of C-3, C-4 of the A ring and C-17 of oleanolic acid. Xu *et al.*[42] discovered that methoxy substitutions in triterpene structures exhibit better anticancer activity compared to hydroxyl substitutions. Based on this finding, they modified the pentacyclic triterpene acid through esterification, using compound **35** as the ester moiety to synthesize a series of triterpene derivatives (compounds **36** and **37**, Fig. 10), with modifications at the C-3 and C-4 positions of the A ring and the C-17 position of the five-membered ring. Activity assays indicated that most derivatives showed enhanced antifibrotic and antiproliferative effects. The increased lipophilicity improved the ability of the drugs to penetrate cell membranes, demonstrating that the introduction of ester moieties can enhance the anticancer activity of triterpenes. Furthermore, studies have shown that introducing amide bonds at the C-28 position and carbonyl groups at the C-11 position can also enhance the antitumor activity of triterpene acids.[43] Heise *et al.*[44] modified **OA** derivatives to obtain two structurally unique lactone triterpenes (derivatives **38–40**, Fig. 10) and a tri-hydroxy **OA** derivative (compound **40**). Among these, derivative **38** exhibited no cytotoxicity against all tested cell lines, while derivative **39** showed a degree of selectivity against MCF-7 and FaDu cancer cells. The tri-hydroxy compound **40** demonstrated the strongest cytotoxic effect on MCF-7 cells, with toxicity approximately 5.5 times greater than that observed in non-malignant fibroblasts. This unique structural modification provides new avenues for enhancing the anticancer activity and selectivity of triterpenes.

3.1.4 Improvements and antitumor activity of CDDO based on oleanolic acid derivatives. Honda *et al.*[45] synthesized a derivative of **OA** over two decades ago, known as 2-cyano-3,12-dioxo-olean-1,9(11)-dien-28-oic acid (**CDDO**, Fig. 11). Their research revealed that **CDDO** not only prevents somatic cancers but also shows increasing evidence of efficacy against gender-specific cancers. **CDDO** and its derivatives are relatively non-toxic and serve as promising candidates for testing therapies for various diseases, demonstrating effectiveness against multiple types of cancer. The **CDDO** compound provides protection against ROS-induced cell death in normal cells by upregulating Nrf2, while simultaneously inducing death in cancerous or abnormal cells. In ovarian cancer models, **CDDO** directly targets and inhibits hsp90, reducing cell proliferation;

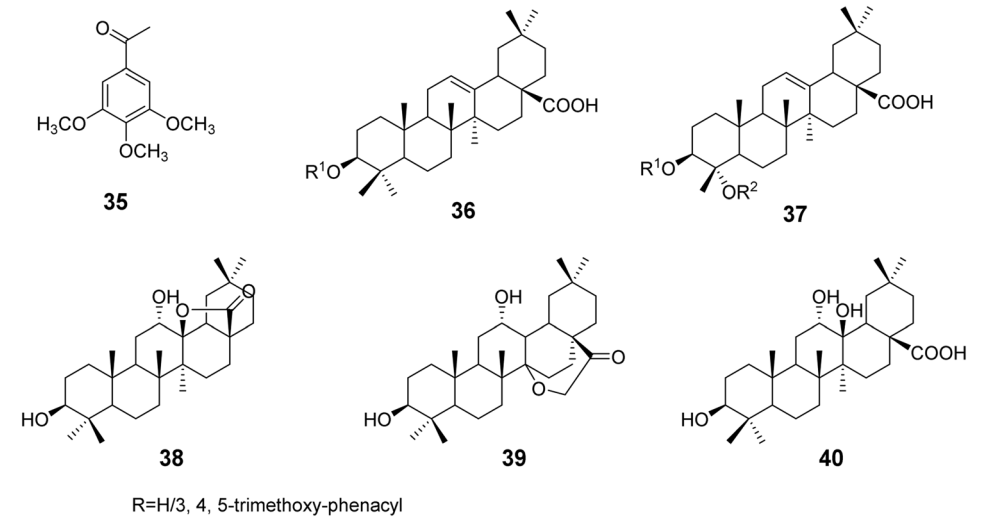


Fig. 10 The structures of triterpenic acid derivatives **35** to **40**.

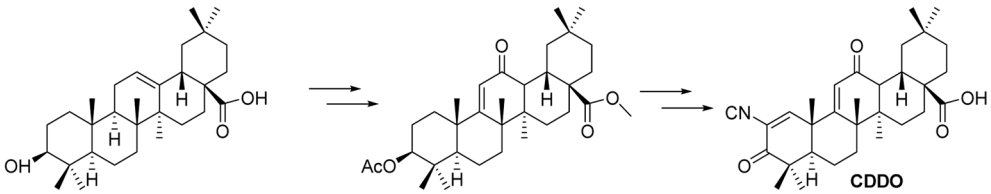


Fig. 11 Synthesis of **OA** derivative **CDDO**.

it also upregulates NQO1, which contributes to PARP1-mediated programmed necrosis in breast cancer, offering a potential therapeutic approach for this malignancy.

The most common modification site for **CDDO** is the C-28 carboxyl group. For instance, the introduction of a methyl ester at C-28 yields **CDDO-Me** (Fig. 12), which exhibits potent anti-inflammatory and anticancer activity.[46] **CDDO-Me** has shown inhibitory effects on prostate cancer cells, ovarian cancer cells, acute myeloid leukemia, pancreatic cancer cells, esophageal cancer cell lines, and triple-negative breast cancer cells. In ovarian cancer, **CDDO-Me** induces apoptosis by inhibiting AKT, NF-κB, and mTOR signaling pathways, without affecting PDK1 kinase or PP2A activity. It induces prostate cancer cell death by inhibiting Akt, and apoptosis in acute myeloid leukemia by inhibiting ERK 1/2 phosphorylation through activation of p38/MAPK. **CDDO-Me** can also downregulate telomerase activity (hTERT) and induce cell death in pancreatic cancer cell lines.[47] Wong *et al.*[48] synthesized a novel **CDDO-Me** analog (compound **41**, Fig. 12), which can irreversibly react with model thiols, aiding in the identification of pharmacologically relevant target interaction sites. Compound **41** exhibited cytotoxicity in rat liver cancer cells and comparable efficacy to **CDDO-Me** in activating Nrf2. Molecular modeling supported the stable modification of specific cysteine residues within Keap1 by compound **41**. Gao *et al.*[49] explored whether **CDDO-NFM** (Fig. 12) has potential antitumor effects for cancer treatment, and they found that **CDDO-NFM** effectively inhibits OS cell growth, and this inhibitory effect is independent of apoptosis-related and cell cycle-related proteins.

Zhang *et al.*[50] designed and synthesized **CDDO** derivatives (compounds **42–44**, Fig. 13). Their anticancer activity was further evaluated by measuring IC_{50} values using the MTT assay. These compounds demonstrated potent inhibitory activity against both A549/Taxol (IC_{50} = 0.349–1.087 μmol L^{-1}) and A549 (IC_{50} = 1.066–1.438 μmol L^{-1}) cells, showing greater antiproliferative efficacy than **CDDO-Me** (IC_{50} = 1.703 and 2.313

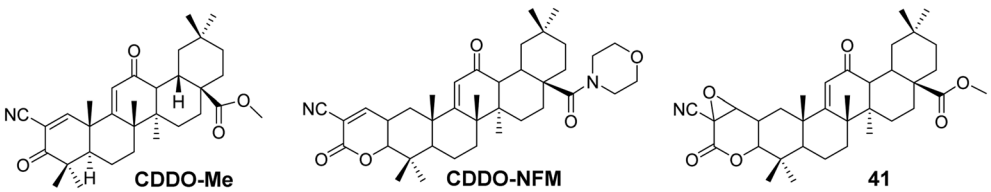


Fig. 12 The structures of **OA** derivatives **CDDO-Me**, **CDDO-NFM**, and **41**.

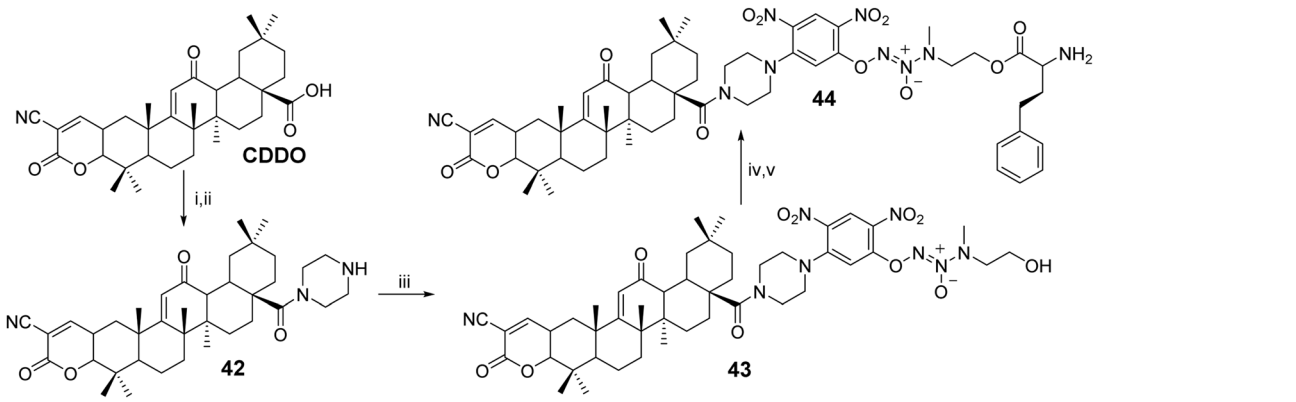


Fig. 13 Synthesis of OA derivatives 42 to 44.

μmol L^{-1}) and JS-K (IC_{50} = 1.987 μmol L^{-1} and 2.313 μmol L^{-1}). The most active compound, **44**, induced higher levels of NO and reactive oxygen species (ROS) in paclitaxel-resistant A549/Taxol cells with overexpression of glutathione *S*-transferase π (GSTπ), significantly inhibiting cell proliferation (IC_{50} = 0.349 ± 0.051 μmol L^{-1}). Moreover, compound **44** showed stronger inhibition of Lon protease expression, as well as more pronounced induction of apoptosis and cell cycle arrest in A549/Taxol cells, compared to **CDDO-Me**.

3.2 Structural modifications of oleanolic acid and antiviral activity

Yan *et al.*[51] synthesized a series of **OA** derivatives by introducing conjugated dienes and utilizing photosensitized oxidation to form epoxides. The cytotoxicity of these derivatives was evaluated in HepG 2.2.15 cells using the standard MTS assay. Most of the derivatives exhibited activity in inhibiting HBV antigen secretion in HepG 2.2.15 cells. Among the compounds tested, the most active derivative (compound **46**, Fig. 14) demonstrated significant activity in inhibiting the secretion of HBsAg, HBeAg, and the replication of HBV DNA. It was more effective than lamivudine in preventing the rebound of viral replication.

Li *et al.*[52] introduced amino conjugates at the C-28 position of **OA** (compounds **48–52**, Fig. 15) and evaluated their antiviral activity against influenza A virus A/WSN/33 (H1N1) in Madin–Darby Canine Kidney (MDCK) cells. Based on the biological results, the structure–activity relationship of the compounds was discussed. Compound **52**, which contains a six-carbon chain and a terminal hydroxyl group, exhibited the strongest anti-influenza activity, with an IC_{50} of 2.98 μmol L^{-1}. Hemagglutination

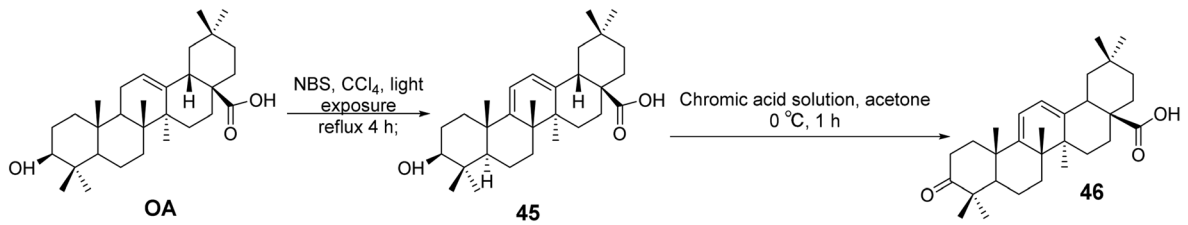


Fig. 14 Synthesis of OA derivative 46.

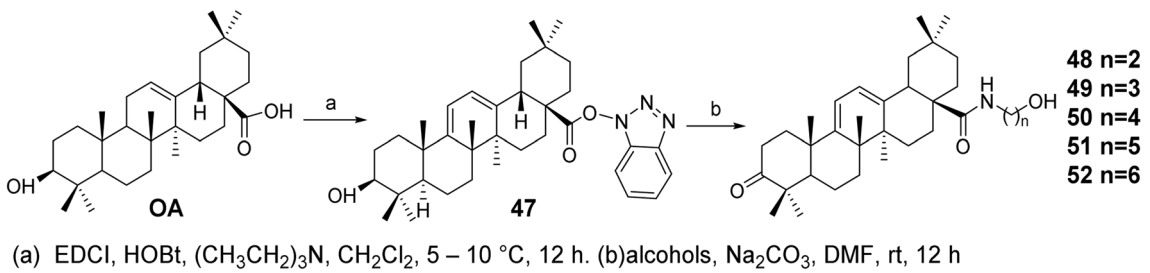


Fig. 15 Synthesis of OA derivatives 48 to 52.

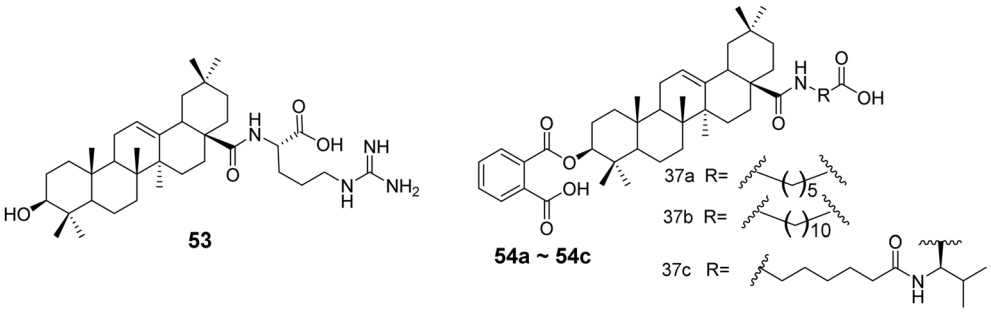


Fig. 16 Synthesis of OA derivative 53 and derivatives 54a to 54c.

inhibition and surface plasmon resonance (SPR) analysis suggested that compound **52** may interfere with viral invasion by interacting with the HA protein of the influenza virus.

In 2019, Yang's team[53] designed and synthesized **32** derivatives by coupling various amino acids with the 28-COOH of **OA**. The most active derivative (compound **53**, Fig. 16) demonstrated robust efficacy and a broad antiviral spectrum against four different influenza strains. Hemagglutination inhibition (HI) assays and docking studies suggested that these derivatives likely share the same mechanism as their parent compound, blocking viral entry by binding to hemagglutinin (HA) and preventing the interaction between the viral HA protein and the sialic acid receptors on host cells. In the same year, Medina-O'Donnell *et al.*[54] esterified one or two amino acids at the C-28 position and substituted phthaloyl groups at the C-3 position to obtain three 3-phthaloyl derivatives (compounds **54a–54c**, Fig. 16), which showed enhanced inhibition of HIV-1 protease activity.

4 Structural modifications and biological activities of glycyrrhetinic acid

Glycyrrhiza, derived from the dried roots and rhizomes of *Glycyrrhiza uralensis*, *Glycyrrhiza inflate*, or *Glycyrrhiza glabra*, is a valuable traditional Chinese medicine. The active constituents found in its dried roots and rhizomes primarily include flavonoids such as glycyrrhizin, **GA**, liquiritin, and isoliquiritin. Current research on the structural modifications of **GA** mainly focuses on the hydroxyl group at C-3, the carboxyl group at C-30, and the carbonyl group at C-11. Through modifications of the **GA** skeleton, a series of derivatives with physiological activities have been developed.

For instance, after replacing the hydroxyl group at the C-3 position of **GA**, the anti-inflammatory and anti-ulcer activities were significantly enhanced. When acyl groups, glycosides, or alkoxy and glycosyl groups were introduced at the C-3 hydroxyl or C-30 carboxyl positions, the anti-ulcer and anti-inflammatory activities were improved. Introducing substituents at both the C-3 hydroxyl and C-30 carboxyl positions further increased anti-inflammatory and antiviral activities. Additionally, the removal of the hydroxyl group at the C-11 position of **GA** has been shown to reduce or eliminate side effects and enhance pharmacological activity.

4.1 Structural modifications of the C-30 carboxyl group of glycyrrhetinic acid

Wang *et al.*[55,56] utilized addition and elimination reactions to modify substituted benzaldehyde, yielding substituted methylamine intermediates. These intermediates were then reacted with **GA** and deoxyglycyrrhetinic acid (DGA), respectively, to produce **GA** and DGA derivatives at the C-30 position with amide bonds, forming heterocyclic derivatives (compound **55**, Fig. 17).

The activity testing results for these compounds indicated that they exhibited high analgesic, anti-inflammatory, and antitussive activities, along with increased safety. The findings also demonstrated that 1-(3-methoxyphenyl)-5-aminomethyl-2-isoxazole DGA possesses certain hepatoprotective effects, effectively reducing the mortality rate of mice infected with the H1N1 influenza virus at a dose of 100 mg kg^{-1}. Lee *et al.*[57] synthesized 11 **GA** derivatives by introducing dehydrogingerol at the C-30 position. The activity tests revealed that these **GA** derivatives significantly inhibited tumor cell replication and exhibited anticancer effects. Some derivatives displayed strong cytotoxic activity; however, substituting the methoxy groups in compounds **56** and **57** with ethoxy groups resulted in a reduction in the cytotoxic activity of compounds **60** and **61** (Fig. 18).

Zhang Yan *et al.*[58] synthesized **GA** methyl ester and **GA** ethyl ester through esterification reactions between **GA** and methanol or ethanol. **GA** and its derivatives reacted with peracetic acid, resulting in the oxidation of the C-3 hydroxyl group to a carbonyl group, yielding oxidized **GA** and its methyl and ethyl ester derivatives. The catalytic asymmetric epoxidation activity of these compounds was tested, revealing the following activity order: oxidized glycyrrhetinic acid (OGA) > oxidized

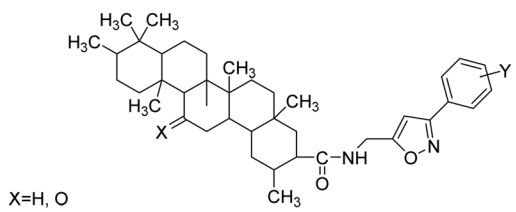
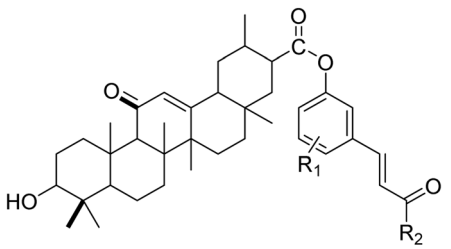


Fig. 17 The structure of isoxazole heterocyclic derivatives **55** of glycyrrhizic acid and dehydroglycyrrhizic acid.



56. 4-OGA R_1=3-OCH_3 R_2=CH_3
57. 2-OGA R_1=3-OCH_3 R_2=CH_3
58. 3-OGA R_1=4-OCH_3 R_2=CH_3
59. 2-OGA R_1=3-OCH_3 R_2=CH_3
60. 4-OGA R_1=3-OC_2H_5 R_2=CH_3
61. 2-OGA R_1=3-OC_2H_5 R_2=CH_3
62. 2-OGA R_1=3-F R_2=CH_3
63. 4-OGA R_1=3-OCH_3 R_2=Ph
64. 2-OGA R_1=3-OCH_3 R_2=Ph
65. 3-OGA R_1=4-OCH_3 R_2=Ph
66. 2-OGA R_1=4-OCH_3 R_2=Ph

Fig. 18 The structures of dehydrogingdiolide derivatives of glycyrrhizic acid **56** to **66**.

glycyrrhetinic acid derivatives > **GA** derivatives. Furthermore, **GA** derivatives were found to be ineffective in catalyzing the reactions of sterically hindered alkenes.

4.2 Structural modifications of C-3 hydroxyl and C-30 carboxyl groups of glycyrrhetinic acid

Zhang Na *et al.*[59] introduced an acetyl group at the C-3 position through an acetylation reaction to synthesize compound **67**. Subsequently, under the reaction conditions of $(COCl)_2$, compounds **56** and **57** (Fig. 18) were reacted to obtain compounds **68** and **69**. Using berberine, 18α-**GA**, and methotrexate as controls, it was found that compounds a and b exhibited significant inhibitory effects on the liver cancer cell line SMMC-7721, with IC_{50} values of 85.7 μmol L^{-1} and 178.2 μmol L^{-1}, respectively. Animal experiments indicated that these compounds were almost non-toxic to normal liver cells (Fig. 19).

Csuk *et al.*[60] modified **GA** methyl ester (**GA-Me**) by reacting it with chloroacetyl chloride, followed by a condensation reaction with various diamines to introduce straight-chain amino groups at the C-3 hydroxyl position, resulting in a series of **GA** derivatives **70**. The results of antitumor activity testing demonstrated that the introduction of straight-chain amino groups at the C-3 position enhanced the activity of **GA**, with the highest activity observed when the carbon chain length $n = 6$, yielding an IC_{50} range of 0.6–3.0 μmol L^{-1} (Fig. 20).

Schwarz *et al.*[61] synthesized **GA** derivatives by introducing methoxy, ethoxy, and benzyloxy groups at the C-30 position through esterification reactions. They also introduced different configurations of alanine at the C-3 position, resulting in a series of C-3 and C-30 **GA** derivatives **71**. The activity and selectivity of these derivatives against tumor cells were found to be superior to those of the control group, which consisted of **GA** (Fig. 21).

Gao *et al.*[62] simultaneously modified the C-30 carboxyl and C-3 hydroxyl groups of **GA** through oxidation, esterification, substitution, and hydrolysis reactions to obtain compound **72**.

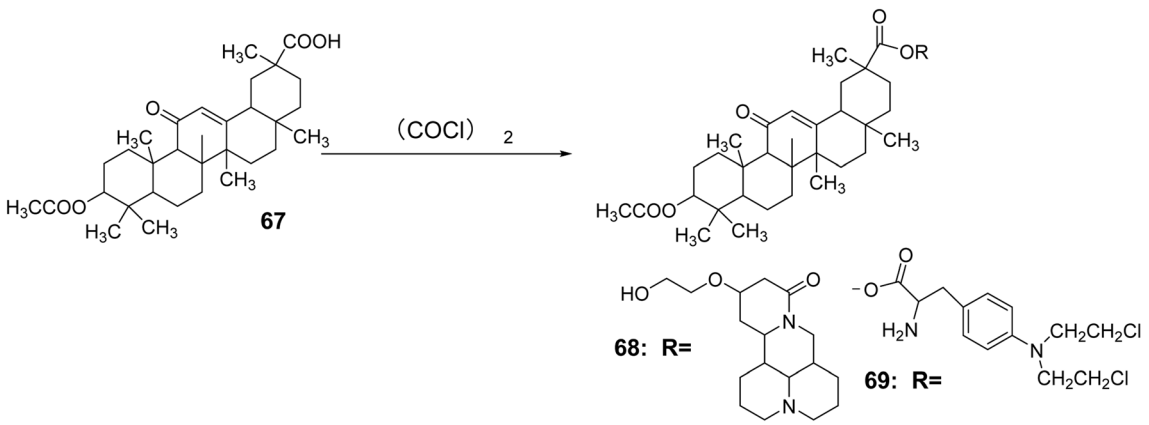


Fig. 19 Synthesis of 3-acetyl glycyrrhizic acid lipid derivatives **68** to **69**.

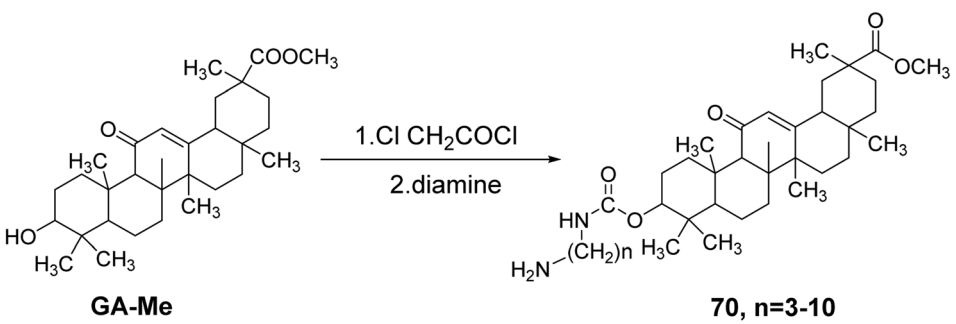


Fig. 20 Synthesis of 3-*O*-(linear aminocarbonyl) glycyrrhizic acid lipid derivative **70**.

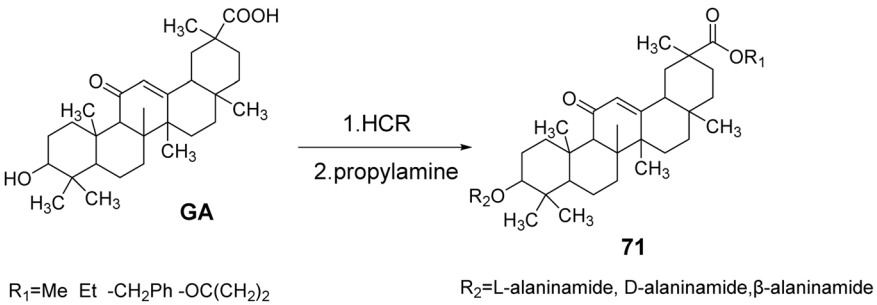


Fig. 21 Synthesis of 3-alanine type glycyrrhizic acid lipid derivative **71**.

Compound **72** underwent nucleophilic substitution to introduce various *N*-substituents at the carboxyl group, yielding amide derivatives **73a–73c.** Compound **2** was synthesized by forming a ring between C-3 and C-2, followed by nucleophilic substitution to obtain compounds **74a–74d.** Subsequently, ring-opening elimination and reduction led to various structural derivatives of oxidized **GA** amides, including **75a–75d** and **76a–76d.** Activity studies indicated that these compounds exhibited strong inhibitory effects on the growth of HL-60 leukemic cells (Fig. 22).

4.3 Structural modification of the C-11 carbonyl group of glycyrrhetinic acid

Meng Yanqiu *et al.*[63] reduced the C-11 carbonyl group of **GA** to obtain DGA (compound **77**) using zinc–mercury amalgam. Subsequently, they introduced an ester bond at the C-30 position through an esterification reaction. The reaction with *p*-toluenesulfonyl chloride in a dichloromethane and triethylamine medium at low temperatures facilitated the introduction of a *p*-toluenesulfonyl group at the C-3 hydroxyl. Finally, refluxing with sodium azide in tetrahydrofuran yielded compounds **80a–80g.**

In vitro antitumor activity tests, using paclitaxel and gefitinib as controls, revealed that compounds **80a**, **80c**, and **80e** exhibited significant inhibitory effects on human breast cancer cells (MCF-7) and lung cancer cells (A549). At a concentration of 10 μmol L^{-1}, the inhibition rates against MCF-7 were 71.57%, 14.53%, and 22.02%, respectively. Notably, compound **80a** demonstrated a significantly enhanced inhibitory capacity against breast cancer cells compared to glycyrrhetinic acid, with

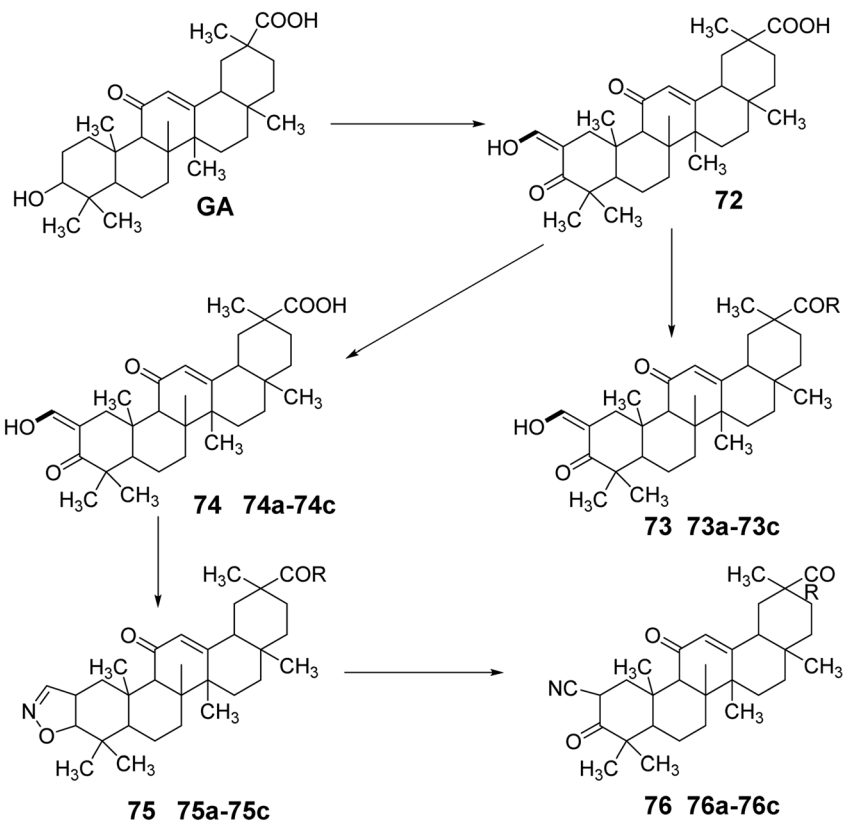


Fig. 22 Synthetic route of oxidized glycyrrhizic acid amide derivatives.

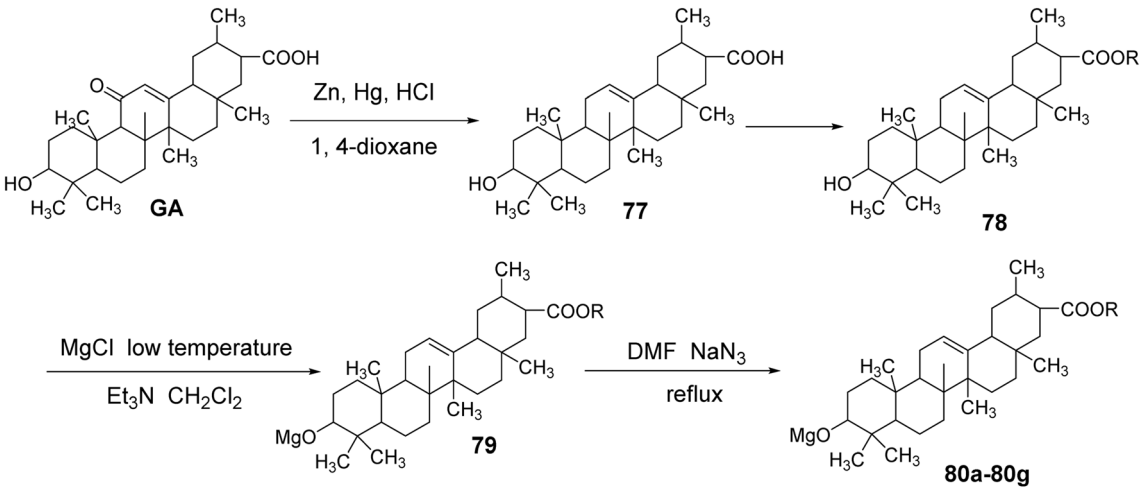


Fig. 23 Synthesis route of 3-azido glycyrrhizic acid derivatives.

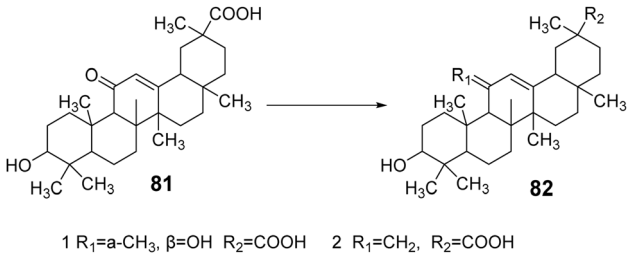


Fig. 24 The synthesis route of 11-position modified glycyrrhizic acid derivatives **81** and **82**.

an IC_{50} of 4.29 ± 0.07 μmol L^{-1}. The inhibition rates against A549 cells at 10 μmol L^{-1} were 4.91%, 26.60%, and 19.97%, all exceeding that of glycyrrhetinic acid. The activity testing results of compounds **80a**, **80c**, and **80e** further indicated that the different ester bonds at the C-30 position significantly influenced the inhibition of MCF-7 cells (Fig. 23).

Su *et al.*[64] modified the carbonyl group at the C-11 position and the carboxyl group at the C-30 position of **GA**, yielding two **GA** derivatives, **81** and **82**. These derivatives exhibited strong inhibitory activity against 11β-HSD1, with IC_{50} values of 0.4 μmol L^{-1} and 1.1 μmol L^{-1}, respectively. Notably, the presence of the carboxyl group at the C-30 position contributed to the inhibition of 11β-HSD1 activity (Fig. 24).

5 Betulinic acid: structural modifications and biological activity

5.1 Structural modifications of betulinic acid and antitumor activity

5.1.1 Structural modifications at C-3 and C-28 of betulinic acid. Research has indicated that derivatives with modifications at the C-3 and C-28 positions show promising potential. The introduction of amino acid conjugates at the C-28 position enhances solubility and cytotoxicity, while the incorporation of

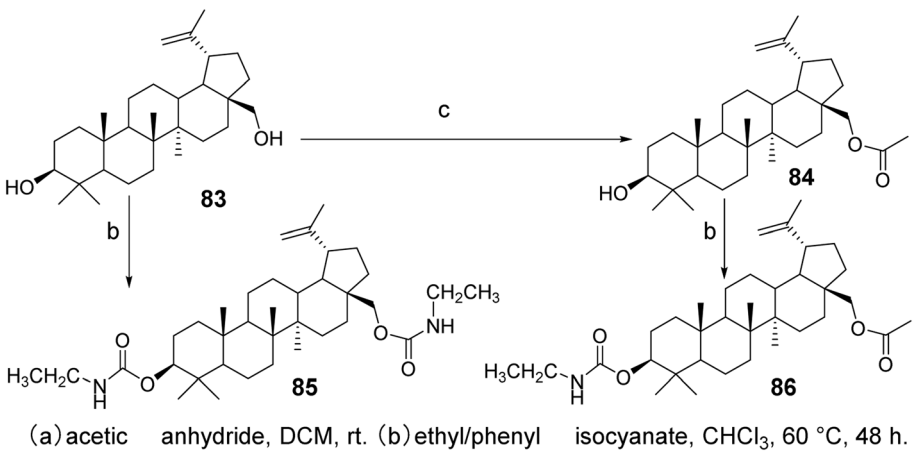
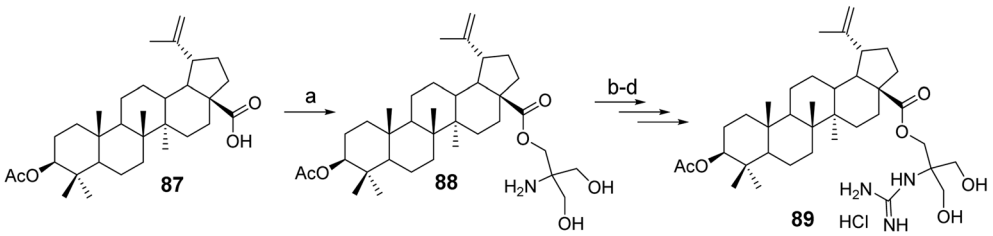


Fig. 25 Synthesis of BA derivatives **85** and **86**.



(a) 1.$(COCl)_2$、CH_2Cl_2; 2.Tris(hydroxymethyl)aminomethane、DMAP、Py、 DCM;
(b) 1, 3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine, Et_3N, $CHCl_3$, reflux;
(c) 50 %TFA, CH_2Cl_2, 2-4 h, rt; (d) 5 M HCl, MeOH.

Fig. 26 Synthesis of BA derivative 89.

polar groups such as acids, amines, or hydroxyls increases biological activity.[65] Hydroxylation at the C-3 position has yielded encouraging results against murine melanoma cells. Additionally, another chemical modification at C-3, namely dimethylsuccinoyl **BA**, has transformed **BA** from a proteasome activator to a proteasome inhibitor. **BA** derivatives substituted with new carbamate derivatives at C-3 and C-28 (compounds **85** and **86**, Fig. 25) exhibit strong cytotoxicity and can induce apoptosis in lung tumor cell lines. Furthermore, compounds **85** and **86** demonstrate higher selectivity for tumor cells compared to normal cells.[66]

Spivak *et al.*[33] designed and synthesized guanidine-containing **BA** derivatives (compound **89**, Fig. 26), which exhibited higher cytotoxicity in Jurkat cells compared to the original triterpenic acid. Most of the tested guanidine derivatives showed higher IC_{50} values than amines, while demonstrating lower toxicity to human fibroblasts.

5.1.2 Structural modifications of betulinic acid in the A ring. Chatterjee *et al.*[67] investigated the metabolic conversion of **BA** using *Bacillus subtilis* to biosynthesize derivative **90** (Fig. 27). Compared to the activity of **BA** (ED_{50} ranging from 2.1 μmol L^{-1} to 7.0 μmol L^{-1}), compound **90** exhibited cytotoxic activity against human melanoma cell lines (Mel-1 and Mel-2) in the micromolar range (ED_{50} < 40 μmol L^{-1}).

In the experiment by Mukherjee *et al.*,[68] **BA** was oxidized with Jones reagent to form its 3-oxo derivative, **BA** ketone (compound

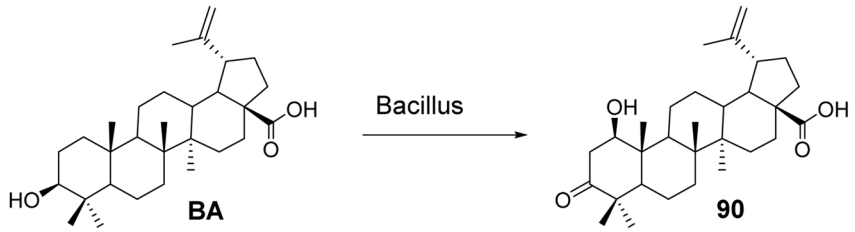
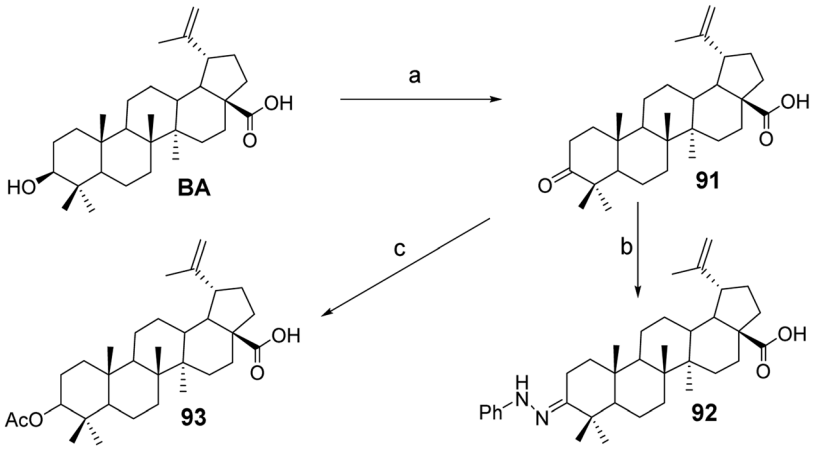


Fig. 27 Synthesis of BA derivative 90.



(a) CrO_3, H_2SO_4; (b) $PhNHNH_2$, NaOAc, MeOH; (c) Isopropenyl acetate, TsCl,

Fig. 28 Synthesis of BA derivatives 91 to 93.

91, Fig. 28), which then reacted with phenylhydrazine and sodium acetate to yield the 3-phenylhydrazone (compound **92**, Fig. 28). The results from anti-angiogenesis screening indicated that derivative **91** exhibited lower cytotoxicity compared to **BA** (with an IC_{50} of 4.4 μmol L^{-1} against the ECV304 endothelial cell line) and comparable low endothelial cell specificity (with an EC_{50} of 1.9–4.2 μmol L^{-1} across four cancer cell lines). Compound **91** was then reacted with isopropenyl acetate and *p*-toluenesulfonyl chloride under reflux for 9 hours, resulting in the enol acetate (compound **93**, Fig. 28). Compound **93** was screened against a panel of five cancer cell lines and demonstrated low cytotoxic activity (IC_{50} ranging from 40 μmol L^{-1} to 100 μmol L^{-1}).

Li *et al.*[69] synthesized difluorinated derivatives (compounds **96** and **97**, Fig. 29) starting from dihydrobetulinic acid (DHBA) through a five-step or six-step reaction pathway. They investigated the cytotoxic activity of all compounds on the CCRF-CEM cell line. Compared to DHBA, the difluorinated derivatives **96** and **97** exhibited higher cytotoxicity, with IC_{50} values of 2.2 μmol L^{-1} and 4.0 μmol L^{-1}, respectively.

5.1.3 Structural modifications of betulinic acid at the C-3 and C-28 positions with phosphorus. Tsepaeva *et al.*[70] used **BA** as the starting material and, in the presence of K_2CO_3, reacted it with phosphoric acid esters in DMF solvent to obtain derivative **98** (Fig. 30). The compounds containing triphenylphosphonium cations are promising ionic molecules for the targeted delivery of neutral bioactive compounds to the mitochondria of cancer cells, including anticancer agents that possess pro-oxidant properties and destabilize the mitochondrial membrane.

In the study by Spivak *et al.*,[71] **BA** derivatives (compounds **99–104**, Fig. 31) containing triphenylphosphonium cations featured various functional groups at the C-3 and C-28 positions (3β-OAc, 3α-OAc, 3β-OH, 3α-OH, 3β-*O*-phthalate, 28-COOMe, 28-COOH). Despite the differences in their chemical structures, the cytotoxic effects exhibited against EAC cells and mastocytoma P-815 cells were not significantly different, with IC_{50} values in Ehrlich cells ranging from 1.2 to 1.9 μmol L^{-1} and in P-815 cells from 0.9 to 1.1 μmol L^{-1}. However, the benzyl ester of DHBA (compound **99**) demonstrated reduced antitumor activity against both cell types, with IC_{50} values of 4.80 μmol L^{-1} for Ehrlich cells and 4.89 μmol L^{-1} for P-815 cells.

5.2 Structural modifications of betulinic acid and their antiviral activity against HIV-1

Chen *et al.*[72] designed and synthesized a series of C-28 amine derivatives of **BA** (**107**) based on C-3 benzoyl-substituted **BA** derivatives (**105**, **106**) as HIV-1 inhibitors. Compared to the C-28 amide series, the C-28 amine derivatives (**107**) showed enhanced inhibitory activity against HIV-1, specifically targeting Gag polyprotein polymorphisms. Their activity was further improved in the presence of human serum, even though basic amines generally exhibit lower plasma exposure after oral administration in rats. Among the C-28 amine series, the

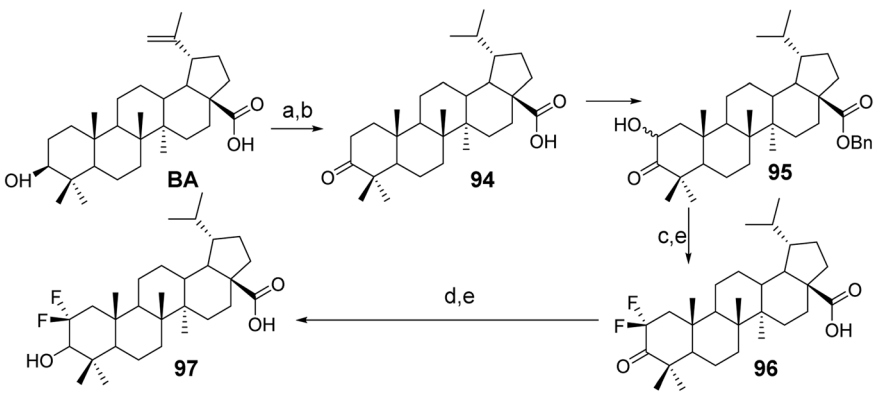


Fig. 29 Synthesis of BA derivatives 96 and 97.

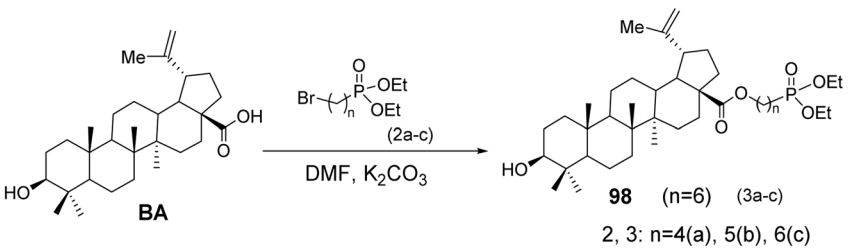


Fig. 30 Synthesis of BA derivative 98.

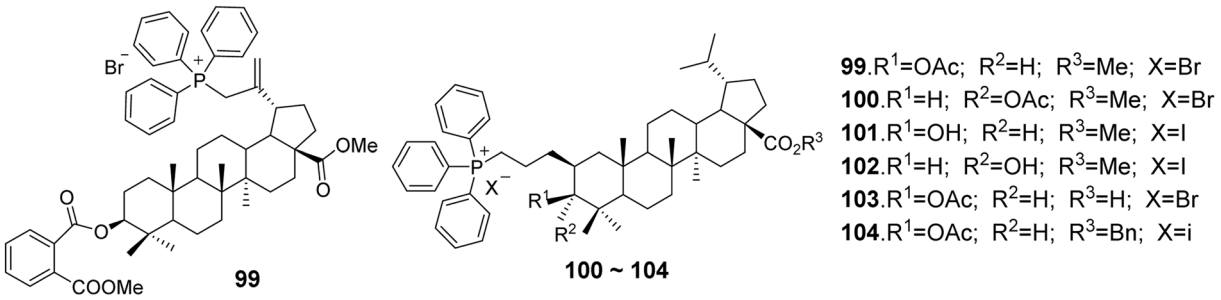


Fig. 31 The structures of BA derivatives 99 to 104.

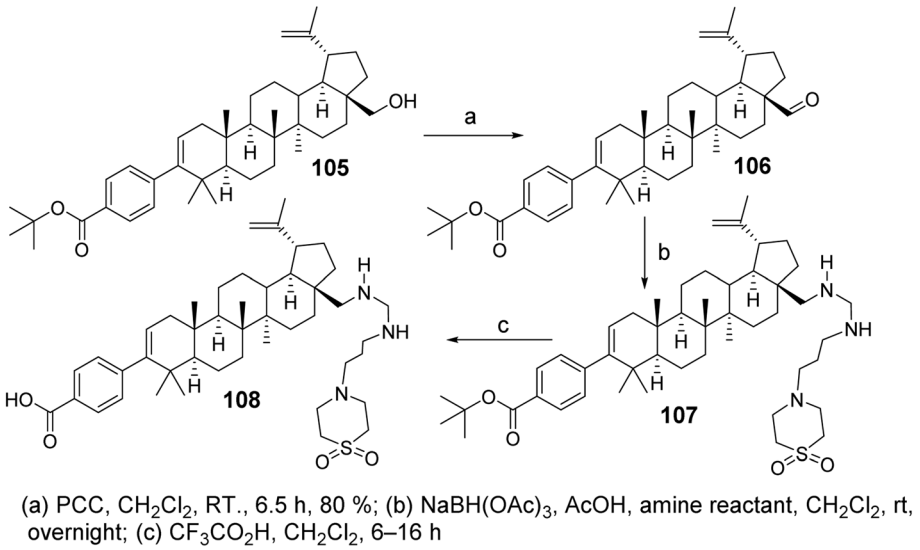


Fig. 32 Synthesis of BA derivative 108.

derivative with the best antiviral activity and acceptable pharmacokinetic properties (compound **108**, Fig. 32) demonstrated a 2- to 4-fold improvement in antiviral potency compared to the C-28 amide. It exhibited low EC_{50} shifts in the presence of human serum or human serum albumin against the V370A and DV370 viral strains and showed promising efficacy against polymorphic variants T371A and V362I.

Reutrakul *et al.*[73] isolated two partially acetylated 2α-hydroxy **BA** derivatives (compounds **109** and **110**, Fig. 33) from the leaves, branches, and resin of *Garcinia* species. Both acetylated derivatives, **109** and **110**, exhibited anti-HIV-1 activity in HIV-1 reverse transcriptase and syncytium formation assays. In the syncytium formation assay, compounds **109** and **110** demonstrated efficacy with IC_{50} values of 30.9 μmol L^{-1} and 38.6 μmol L^{-1}, respectively, which is 2–3 times more potent than the activity of **BA**.

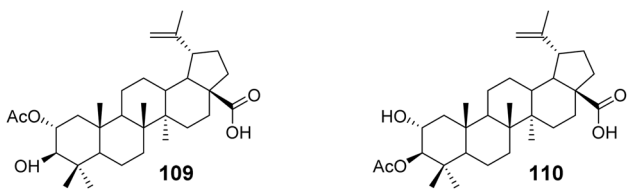


Fig. 33 Structure of BA derivatives 109 and 110.

6 Structural modifications of ursolic acid and its biological activities

Ursolic acid (**UA**), also known as **UA** or ursolic acid, is a representative compound of the ursane-type pentacyclic triterpenoids. It is widely distributed in many plants, including medicinal herbs and edible plants such as the leaves of Rosaceae plants like loquat and species from the Ericaceae family. Significant progress has been made in recent years in studying the antitumor activities of **UA**. Li *et al.*[74] found that **UA** can exert anticancer effects by inhibiting cancer initiation and progression as well as DNA mutations. Young *et al.*[75] reported that **UA** can induce the differentiation of teratoma stem cells, demonstrating its anti-carcinogenic properties, and has been recognized as one of the most promising natural compounds for cancer prevention. Additionally, **UA** shows potential therapeutic applications for prostate cancer by controlling the proliferation and apoptosis of PC-3 and LNCaP cells.[76] **UA** exerts its

anticancer activity by inhibiting kinases involved in cancer cell signaling, showing antitumor effects in various cell types.

6.1 Structural modifications of ursolic acid and antitumor activity

6.1.1 Structural modifications of ursolic acid at the C-3 and C-28 positions. Zhang Dajun *et al.*[77] synthesized benzyl phosphonic acid diethyl ester acetamide derivatives of **UA** (compounds **111–115**, Fig. 34) through a substitution reaction between **UA** and various substituted diethyl benzyl phosphonic acids. The *in vitro* anti-hepatocellular activity of the target compounds was evaluated using the MTT assay. All target compounds exhibited low cytotoxicity against normal human liver cells (L02), while compound **115** demonstrated significant antiproliferative activity against HepG2 cells, with an IC_{50} value of 2.69 μmol L^{-1}, showing good selectivity between tumor cells and normal cells.

Spivak *et al.*[33] designed and synthesized guanidine-containing **UA** derivatives (compound **118**, Fig. 35). Compared to the original **UA**, these guanidine-containing derivatives exhibited lower toxicity toward human fibroblasts while demonstrating higher cytotoxicity in Jurkat cells. Most of the guanidine derivatives tested showed higher IC_{50} values compared to their amine counterparts.

Tian *et al.*[78] designed and synthesized a series of novel nitrogen-containing **UA** derivatives by introducing diamine fragments at the C-3 and C-28 positions. The derivatives with the highest activity (compounds **120** and **122**, Fig. 36) were tested for their cytotoxicity against MCF-7, HeLa, and A549 cell lines. **UA**-4a and **UA**-8a showed significant inhibitory activity against all three human cancer cell lines, with IC_{50} values of less than 10 μmol L^{-1}. Analyzing the antitumor activity of the synthesized compounds revealed no significant difference in activity between the introduction of diamine compounds at the C-3 and C-28 positions, both displaying strong antitumor properties.

Furthermore, analysis of the synthesized series suggested that the carbon chain length of the diamine compounds had little impact on antitumor activity, and no linear correlation was observed. However, a slight decrease in activity was noted when the carbon chain length reached six carbons. Considering factors such as ease of isolation, purification, yield, and activity during synthesis, the diamine compounds with a two-carbon chain were deemed the most valuable for further research. These compounds were characterized by low synthesis cost, easy purification, simple synthetic steps, and high cytotoxicity against cancer cell lines, making them promising candidates for further study as novel antitumor drugs. Additionally, they provide the potential for further salt formation modifications.

Bai *et al.*[79–81] conducted comprehensive studies on modifications at the C-3 and C-28 positions of **UA**. The introduction of hydrophilic polyethylene glycol (PEG) significantly enhanced the solubility of the drug. Conjugating PEG at the C-28 position of the **UA** scaffold notably improved the *in vitro* antitumor activity. For example, compound **123** (Fig. 37) exhibited a solubility between 0.01 g mL^{-1} and 0.033 g mL^{-1} in water and demonstrated potent anticancer activity with IC_{50} values below 10 μmol L^{-1} against five tumor cell lines: HepG2, BGC-823, AGS, HT-29, and PC-3.

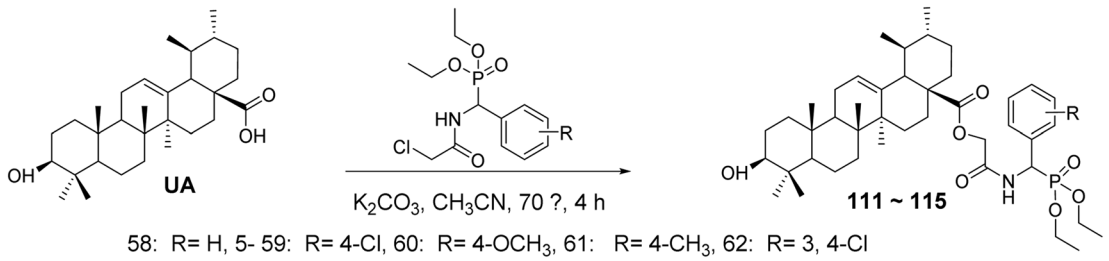


Fig. 34 Synthesis of UA derivatives 111–115.

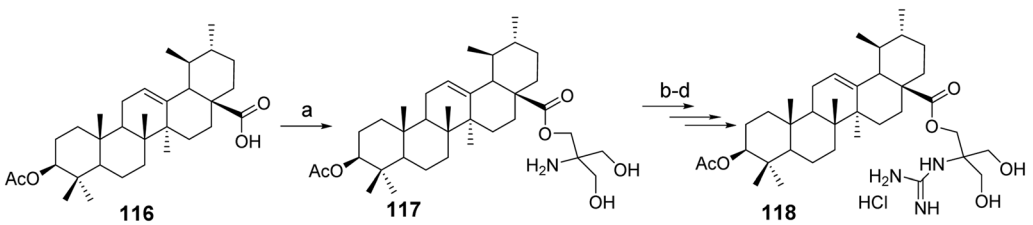


Fig. 35 Synthesis of UA derivative 118.

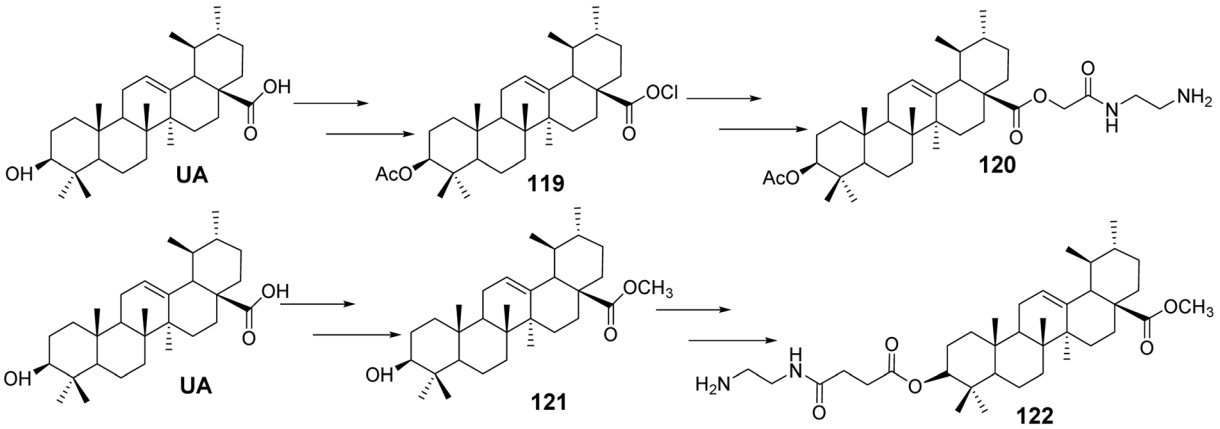


Fig. 36 Synthesis of UA derivatives 120 and 122.

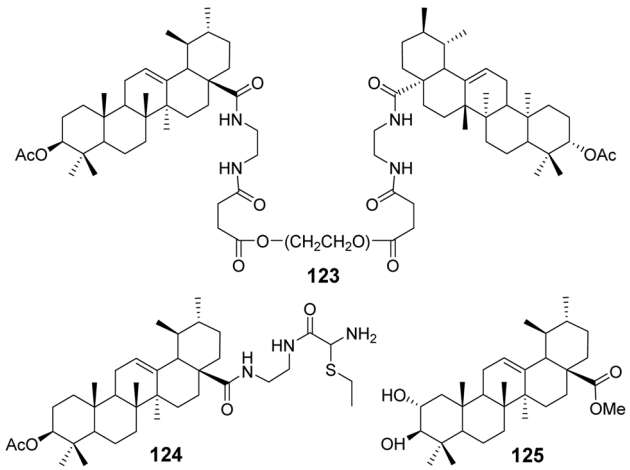


Fig. 37 Structure of UA derivatives 123 to 125.

Furthermore, the attachment of groups with varying electronegativities to **UA** effectively improved its water solubility, particularly the positively charged **UA** derivatives, which exhibited significantly enhanced anticancer activity. Compound **124** (Fig. 37) displayed a 70-fold increase in water solubility and an IC_{50} value below 10 μmol L^{-1} against PC-3 cancer cells. Additionally, it was found that introducing amino acid derivatives with primary amino groups at the C-28 position of **UA** substantially enhanced its anticancer activity compared to **UA** alone, with the mechanism of action involving the induction of apoptosis.

Zhao *et al.*[82] carried out structural modifications on the A and C rings of **UA**, as exemplified by compound **125** (Fig. 37). These modifications involved multiple oxygen substitutions on the A ring and oxidation at the 11th position of the C ring. The resulting compounds exhibited cytotoxicity against cancer cells such as KB and PC-3, comparable to cisplatin, and demonstrated an inhibitory effect on α-glucosidase that was an order of magnitude stronger than that of acarbose.

6.1.2 Structural modifications of ursolic acid at the C-2 and C-3 positions of the A ring. Research on the structural modification of **UA** at the C-2 and C-3 positions of the A ring has provided insights into enhancing its biological activity. Modifications in these positions have been shown to impact the compound's pharmacological properties, particularly its anticancer effects. Wu *et al.*[83] designed and synthesized a series of **UA** derivatives containing an aminoguanidine structure and evaluated their activity as HIF-1α inhibitors and anticancer agents. Using a luciferase reporter gene assay based on Hep3B cells, most compounds exhibited good HIF-1α inhibitory activity. Among them, the most active derivative (compound **128**, Fig. 38) demonstrated the strongest HIF-1α inhibition under hypoxic conditions, with an IC_{50} value of 4.0 μmol L^{-1}, and showed no significant cytotoxicity in the tested cells. The mechanism of action may involve the downregulation of HIF-1α protein expression through inhibition of HIF-1α protein synthesis, as indicated by western blotting, RT-PCR assays, and cell aggregation experiments. This reduction in HIF-1α expression subsequently leads to decreased production of vascular endothelial growth factor (VEGF), thereby inhibiting cancer cell proliferation.

Wang *et al.*[84] synthesized derivatives of **UA** with a furan moiety at the C-2 and C-3 positions based on the method of Khan (compound **130**, Fig. 31). These derivatives were further modified by conjugation with ethyl-piperazine to obtain piperidine-containing derivatives (compound **131**, Fig. 39). The antiproliferative activities of the target compounds against the HeLa and MKN45 cancer cell lines were evaluated using the MTT assay, with cisplatin and **UA** used as positive controls. Derivative **130** exhibited significantly higher activity than **UA**, with an IC_{50} value below 10 μmol L^{-1}, while derivative **131** displayed moderate to low activity against both cancer cell lines. Structure–activity relationship studies indicated that the introduction of thiazole on the A ring and the incorporation of triazole or tetrazole structures at C-28 had minimal impact on enhancing antitumor activity. However, the inclusion of piperazine or higher piperazine derivatives significantly improved the antitumor activity.

6.2 Structural modifications of ursolic acid and antiviral activity

The structural modifications of **UA** have been investigated to enhance its antiviral activity. Various derivatives have been

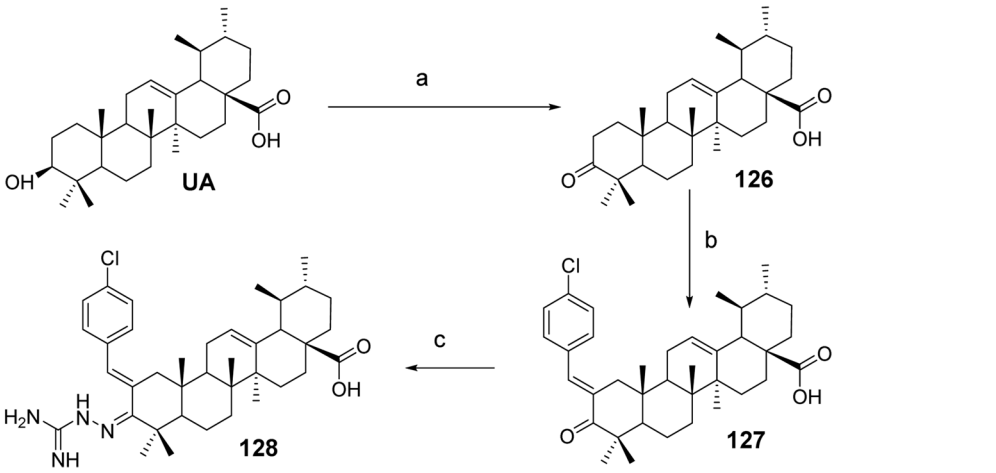


Fig. 38 Synthesis of UA derivative 128.

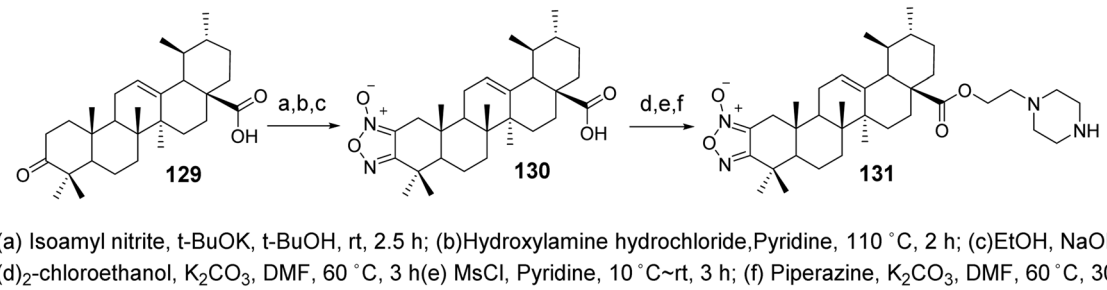


Fig. 39 Synthesis of UA derivatives 130 and 131.

synthesized to explore their potential effects against different viral strains. Min *et al.*[85] reported that the 3-*O*-acyl derivatives of ursolic acid (compounds **132–142**, Fig. 40) exhibited significant inhibitory effects on HIV replication in human T lymphocytes (H9 cells), with IC_{50} values of 0.31 and 2.1 μmol L^{-1}, respectively. Compared to the lead compound ursolic acid (IC_{50} value of 4.4 μmol L^{-1}), these two compounds demonstrated improved anti-HIV activity and safety profiles. They further reported that a series of anhydrides were introduced at the C-3 position of **UA** to form corresponding dicarboxylic acid monoesters, evaluating the impact of the length of the anhydride chain on anti-HIV activity. Analysis of the IC_{50} values revealed that, when introducing anhydride chains containing up to six carbons, the anti-HIV activity of the compounds increased with the number of carbon atoms. However, when two methylene units were added, a slight decrease in activity was observed. Additionally, when the carboxyl groups of the anhydride formed carboxylic acid methyl esters, the anti-HIV activity of the compounds decreased, and in some cases, was completely lost.

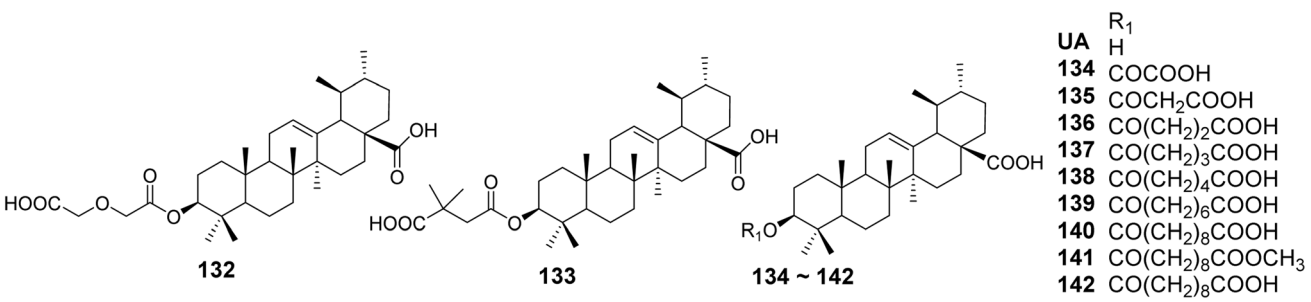


Fig. 40 Structure of UA derivatives 132 to 142.

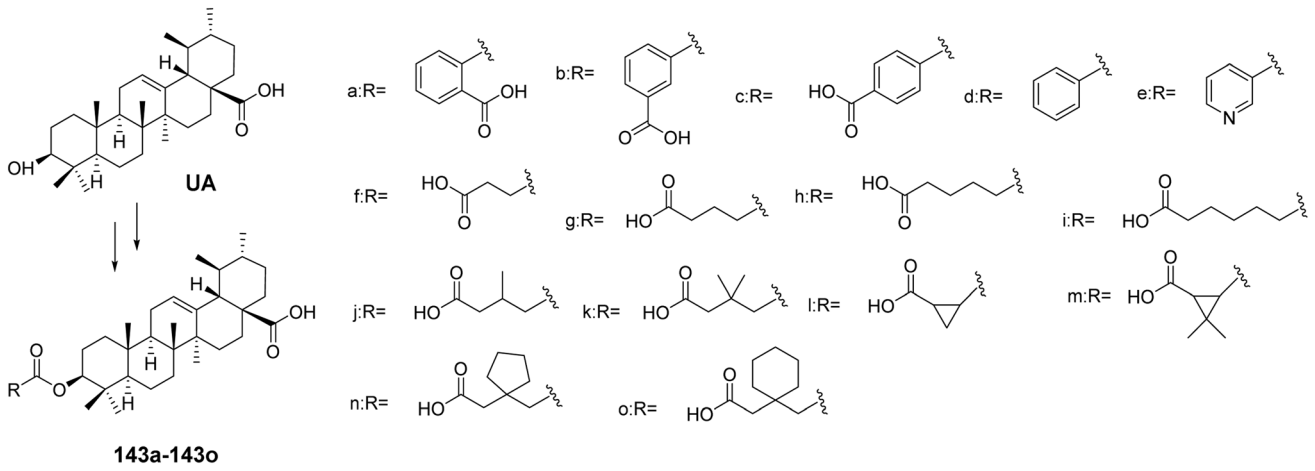


Fig. 41 Synthesis of ursolic acid 3-β ester derivatives (**143a–143o**).

6.3 Ursolic acid 3-β ester derivatives and corresponding activity evaluation

UA has garnered attention for its versatile pharmacological properties, and the development of 3-β ester derivatives is a promising approach to enhance its biological activity. Various studies have explored the synthesis of these ester derivatives and evaluated their potential in different therapeutic areas.

For instance, Chen *et al.*[86] synthesized a series of **UA** 3-β ester derivatives (**143a–143o**, Fig. 41). Activity assays indicated that compounds **143a** and **143b**, which contain phthalate esters or isophthalate esters, exhibited moderate inhibitory effects on cholesterol ester transfer protein (CETP), while the inhibitory effect of derivative **143c**, containing terephthalate esters, was completely lost. Further comparison of derivatives **143b**, **143d**, and **143e** revealed that the carboxyl group on the backbone chain is essential for enhancing inhibitory activity. In contrast to derivative **143g**, the derivatives containing succinate esters (**143j** and **143k**) lost their inhibitory effects on CETP, possibly due to steric hindrance from the methyl group. Derivative **143l**, which contains 1,2-cyclopropanedicarboxylate esters, exhibited the strongest CETP inhibitory activity, with an IC_{50} of 2.4 μmol L^{-1}. The presence of a carboxyl group at the end of the backbone chain and the length of the backbone chain are critical factors for maintaining or enhancing CETP inhibitory activity. These types of 3-β ester triterpenoid derivatives (*e.g.*, derivative **143l**) hold promise as lead compounds with potent CETP enzyme inhibitory activity. Xu *et al.*[87] found that glycosylated corosolic acid derivatives possess certain α-glucosidase inhibitory activity and improved water solubility, which is associated with the presence of hydroxyl groups at the C-2 and C-3 positions. This activity is also closely related to the substituents at the C-28 position.[88,89]

7 Structural modifications and pharmacological activities of celastrol

Celastrol, also known as tripterine, is a triterpenoid compound belonging to the oleanane-type pentacyclic triterpenoids. It was first extracted from the traditional Chinese medicinal herb *Tripterygium wilfordii* by the renowned organic chemist Zhao Chenggu in 1936.[90] As the first active compound isolated from the root bark of *Tripterygium wilfordii*, celastrol is the most abundant bioactive component in this plant and has been demonstrated to exhibit potent anticancer activity.[91] Compared to some conventional chemotherapeutic agents, celastrol shows similar or even superior anticancer effects, particularly against multidrug-resistant cancer cells. In 2007, it was listed by *Cell* magazine as one of the five natural products most likely to be developed into modern pharmaceuticals.[92] Despite extensive research into its potential anticancer properties, its further application remains limited due to challenges such as poor water solubility, low bioavailability, and high toxicity.[93]

7.1 Structural modifications of celastrol at the C-29 position and its anticancer activity

Feng *et al.*[94] modified the hydroxyl group at C-3 and the carboxyl group at C-29 of celastrol to synthesize a series of 1,2,3-triazole derivatives of celastrol (compound **145**, Fig. 42). They evaluated the anti-glioma activity of these derivatives *in vitro* against four human glioma cell lines (A172, LN229, U87, and U251). Structure–activity relationship studies indicated that the modifications at C-3 and C-29 significantly enhanced the anti-proliferative activity compared to celastrol, with the C-29 carboxyl group derivatives exhibiting stronger anti-proliferative activity than the C-3 hydroxyl group derivatives across the four human glioma cell lines. Among these, compound **145** demonstrated the highest anti-proliferative activity in the U251 cell line, with an IC_{50} of 0.94 μmol L^{-1}. Further studies revealed that its effective anti-glioma activity is associated with the activation of the receptor–interacting protein **1**/receptor–interacting protein **3**/mixed lineage kinase domain-like protein (RIPI/RIP3/MLKL) pathway.

Ferulic acid (FA) is a type of phenolic acid that has been demonstrated to possess various pharmacological activities, including antioxidant, anti-diabetic, anti-inflammatory, and neuroprotective effects. FA and its derivatives have also shown

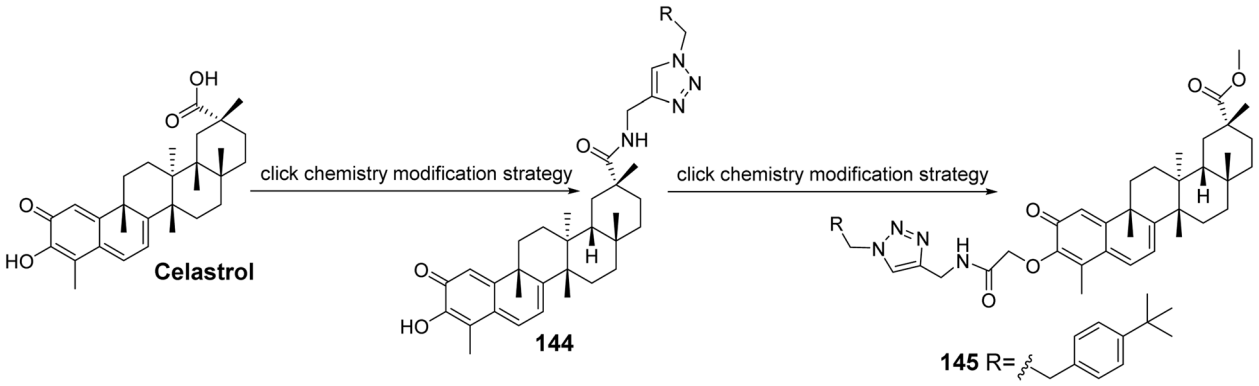


Fig. 42 Synthesis of celastrol derivative **145**.

significant anticancer efficacy both *in vitro* and *in vivo*.[95] Coghi *et al.*[96] designed and synthesized a series of novel hybrids of celastrol and ferulic acid, evaluating their antitumor activity. Structure–activity relationship (SAR) studies indicated that different linkers influenced the pharmacological effects, with a two-carbon linker exhibiting stronger anticancer capabilities compared to a three-carbon linker. Moreover, when a methoxy group was substituted on the FA moiety, the hybrids displayed improved efficacy. Among these, the most active derivative (compound **146**, Fig. 43) exhibited the strongest inhibitory capacity. Mechanistic studies revealed that compound **146** could disrupt the heat shock protein Hsp90–Cdc37 complex while also inducing abnormal regulation of heat shock protein (Hsp90) receptors (*p*-Akt and Cdk4), leading to concentration-dependent cell cycle arrest in the G0/G1 phase.

Disrupting the interaction between Hsp90 and Cdc37 has emerged as a promising strategy for investigating the antitumor activity of celastrol.[97] To further explore the effects of celastrol C-29 derivatives on the Hsp90–Cdc37 complex, Li *et al.*[98] introduced several lipophilic fragments into celastrol C-29, synthesizing 48 new celastrol derivatives and testing their effects on the Hsp90–Cdc37 complex and antitumor cell proliferation activity. SAR studies revealed that the length of the linker significantly influenced the activity of the compounds. Using two-carbon and three-carbon linkers resulted in much higher inhibition rates on tumor cells compared to four-carbon linkers, with 8-cyanide groups exhibiting better efficacy than α-hydroxyl groups. Among the three-amine substitutions, introducing electron-donating groups onto the phenyl ring enhanced the antiproliferative activity of the compounds. One particularly active derivative (compound **147**, Fig. 44) demonstrated excellent antiproliferative activity against relevant cancer cells (IC_{50}: 0.41–0.94 μmol L^{-1}). Further mechanistic studies indicated that compound **147** could reduce the expression of the Hsp90–Cdc37 complex in A549 cells and form a complex with Cdc37 in tumor cells, thereby disrupting the Hsp90–Cdc37 interaction.

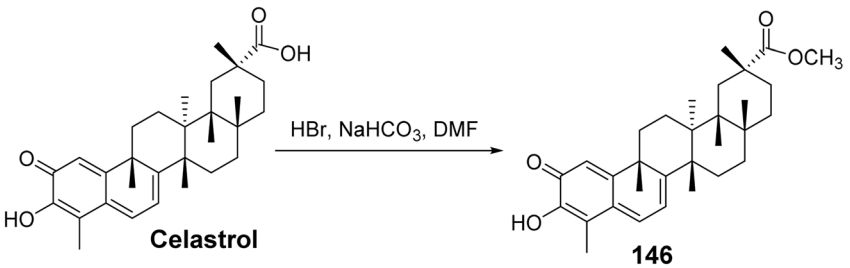


Fig. 43 Synthesis of celastrol derivative **146**.

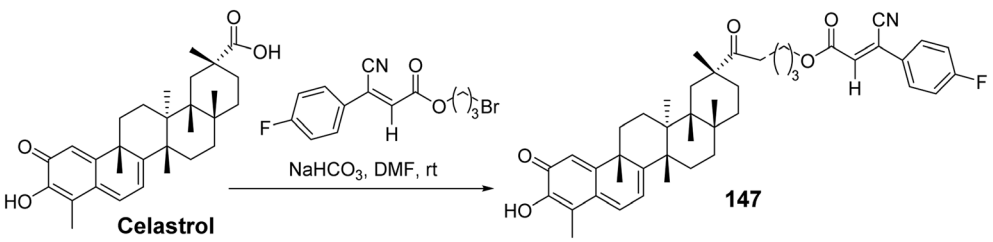


Fig. 44 Synthesis of celastrol derivative **147**.

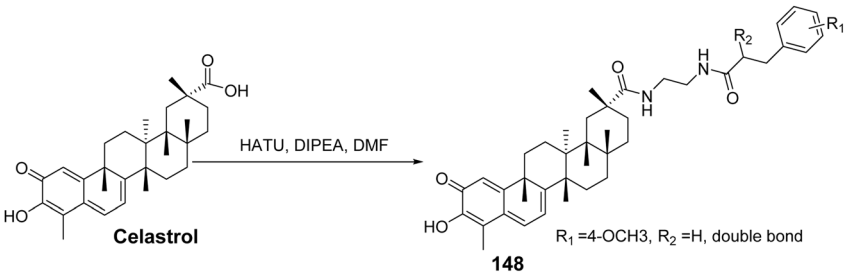


Fig. 45 Synthesis of celastrol derivative **148**.

CSCs are associated with cancer progression, tumor recurrence, metastasis, and chemotherapy resistance. To develop effective and selective anti-CSC agents, Li *et al.*[99] synthesized two series of celastrol derivatives with cinnamoylamine linkers, utilizing ethylenediamine and piperazine as connecting chains. Their antitumor activity against ovarian cancer was evaluated. SAR analysis revealed that most compounds exhibited stronger antiproliferative activity compared to celastrol. Additionally, no significant differences in activity were observed between the two series of compounds with different linker chains. However, the celastrol derivative bearing a 3,4,5-trimethoxy cinnamoylamine side chain (compound **148**, Fig. 45) demonstrated the strongest antiproliferative effect against ovarian cancer cells (IC_{50} = 0.6 μmol L^{-1}). Further pharmacological tests indicated that compound **148** significantly inhibited the colony formation ability of tumor cells, reduced the number of tumor spheres, and decreased the percentage of ovarian CSCs.

Nitric oxide (NO) is a key signaling and effector molecule in tumor development. Generally, high concentrations of NO can induce apoptosis in tumor cells, while low concentrations typically offer cellular protection. Based on this, Tang *et al.*[100] synthesized a series of celastrol nitric oxide donor drugs and evaluated their NO release capabilities and antiproliferative activities. The results showed that the most active derivative (compound **149**, Fig. 46) released the highest levels of NO *in vitro* and exhibited greater antiproliferative activity compared to other compounds (A549: IC_{50} = 0.48 μmol L^{-1}). SAR analysis indicated that the C-29 amide bond was beneficial for enhancing antiproliferative activity. Furthermore, furoxan nitrogen oxides demonstrated stronger antiproliferative activity as NO-releasing components than nitrate esters. Additional pharmacological studies revealed that compound **149** could induce dysregulation of the Hsp90 receptor, apoptosis, and cell cycle arrest at the G0/G1 phase. These findings suggest that the inhibition of Hsp90 and NO release have a synergistic effect in tumor cells, providing new directions for the discovery of antitumor drugs.

7.2 A/B ring-modified celastrol derivatives and their anticancer activity

In the exploration of the anticancer activity of celastrol, the modification of its A/B rings has become an important strategy. By chemically modifying the A/B rings of celastrol, it is possible to enhance its biological activity and improve its drug properties. Tang *et al.*[101] synthesized a series of derivatives of celastrol with urea moieties as linkers at the C-29 position and modifications at the A/B rings, followed by testing their antitumor cell proliferation activity. SAR analysis revealed that the C-29 carboxy urea derivatives exhibited enhanced cytotoxicity against tumor cell lines compared to celastrol. However, modifications to the A/B rings resulted in diminished antiproliferative activity of the derivatives. This suggests that the intact quinone methide structure of the A/B rings and the reactive Michael addition site at position 6 may be essential groups for the anticancer effects of celastrol. Among these, the most active derivative (compound **150**, Fig. 47) demonstrated superior antiproliferative activity with greater selectivity. Preliminary studies on its mechanism of action indicated that compound **150** induces apoptosis through the activation of cysteine protease-8 (caspase-8) and cysteine protease-3 (caspase-3), as well as the cleavage of poly (ADP-ribose) polymerase (PARP) *via* an extrinsic pathway. Additionally, it induced downregulation of p53 in the SKOV-3 cell line, which is a p53-mutant cell line.

7.3 C-6 modified celastrol derivatives and anticancer activity

To explore the impact of A-ring and C-6 modifications on the antitumor activity of celastrol, Tang *et al.*[102] synthesized a series

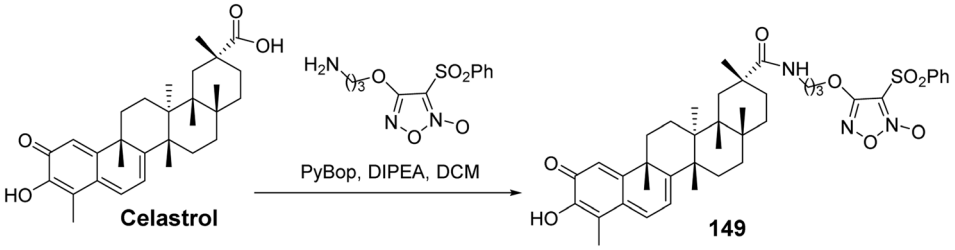


Fig. 46 Synthesis of celastrol derivative **149**.

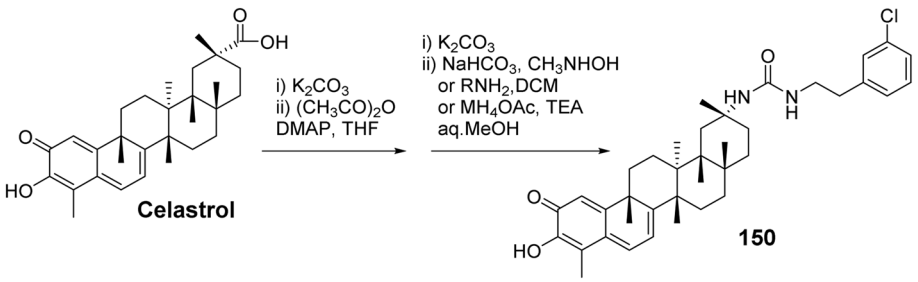


Fig. 47 Synthesis of celastrol derivative **150**.

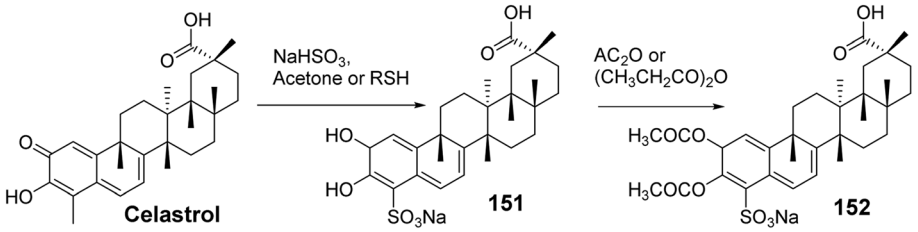


Fig. 48 Synthesis of celastrol derivative **152**.

of C-6 sulfonated, thiolated, and carbon-modified celastrol derivatives *via* a Michael addition reaction. The cytotoxicity of these derivatives was evaluated in human cancer cell lines (BGC-823, H4, Bel7402, H522, Colo205, HepG2, and MDA-MB468). SAR analysis showed that C-6 sulfonated and thiolated derivatives exhibited stronger cytotoxicity compared to carbon-modified derivatives at the C-6 position, with 3-hydroxy acetylation proving more effective than propionylation. Among these derivatives, compound **152** (Fig. 48) demonstrated the most potent bioactivity in the Colo205 cell line (IC_{50} = 0.06 μmol L^{-1}). Further experimental results revealed that, in nude mice bearing human colorectal cancer xenografts, compound **152** effectively inhibited the growth of Colo205 xenografts *in vivo* and exhibited better safety profiles compared to celastrol.

8 Conclusion

Natural products, as an important source of lead compounds for drug development, play a crucial role in pharmaceutical research. However, their inherent limitations, such as poor water solubility, low bioavailability, and significant toxicity, have led researchers to focus increasingly on structural modifications to obtain more efficient and less toxic new drugs. Among these, pentacyclic triterpenes have garnered growing attention due to their diverse biological activities, with research on these compounds becoming progressively more in-depth in recent years. Despite the structural diversity of pentacyclic triterpenes, their limited content in plants, complex extraction processes, and our insufficient understanding of their biosynthetic pathways, effective chemical modifications offer promising opportunities for their development. Structural modifications, particularly aimed at their hydrophobic framework, are essential for further exploration.

OA, **BA** and **UA** are pentacyclic triterpenes widely distributed in nature, known for their anti-inflammatory, antiviral, and anticancer properties, showcasing significant potential for therapeutic applications. However, due to their poor water solubility and low bioavailability, structural modifications can effectively improve these properties by enhancing water solubility, increasing bioactivity, and reducing toxic side effects. Although numerous bioactive derivatives have been developed, few have advanced to clinical application. Research into the structural modification of **OA** and **BA** has yielded significant progress in the field of anticancer therapy, identifying key targets and mechanisms of action. SAR studies have provided insights that will guide the design of more potent pentacyclic triterpene derivatives in the future.[103–106] Additionally, as research continues, **UA** has also become a focal point for anticancer drug development through structural modification. The continued exploration of **OA**, **BA**, and **UA** derivatives for the development of highly effective, low-toxicity anticancer drugs remains a promising area of research.

The structural modification of celastrol has mainly focused on the C-29 position, with relatively little research on the 3-OH group and A/B rings. However, the high reactivity of the A/B rings may be crucial to celastrol's pharmacological effects. Therefore, further studies on the modification of the A/B rings are essential, and even ring-opening reactions could be explored to generate celastrol derivatives with reduced toxicity and enhanced efficacy. This could also lead to a more comprehensive understanding of the SAR of celastrol. Moreover, biotransformation could provide novel approaches for the structural modification of celastrol, potentially yielding derivatives that are difficult to obtain through chemical methods, thus offering new directions for celastrol's modification.

GA is another pentacyclic triterpene with broad biological activities, particularly in the fields of anti-inflammatory and anticancer research, showcasing immense potential for therapeutic applications. Similar to **OA**, **BA**, and **UA**, **GA**'s poor water solubility and high toxicity have limited its clinical application. Recent studies on **GA**'s structural modifications have led to the discovery of derivatives with promising anticancer activities, along with initial insights into their mechanisms of action, laying the groundwork for the development of **GA** as an anticancer agent. Further optimization of **GA**'s structural modifications to enhance bioactivity and reduce toxicity will facilitate the clinical translation of **GA** and its derivatives.

In conclusion, structural modifications of natural products hold great potential for the development of novel anticancer drugs. The modification studies on pentacyclic triterpenes, such as **OA**, **BA**, **UA**, and celastrol, not only enrich the theoretical foundation of chemistry and pharmacology but also provide new avenues for drug discovery.

Data availability

No new data were created or analyzed in this study. This article is a review of existing literature, and all data supporting the findings are derived from previously published sources, which are appropriately cited in the manuscript. Should any further clarification on data sources be needed, the corresponding author can provide additional information upon request.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgements

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