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STRUCTURE-DIRECTED URSOLIC ACID MODIFICATIONS AND THEIR IMPACT ON BIOLOGICAL ACTIVITY

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Abstract. Ursolic acid is a natural pentacyclic triterpene found in many medicinal plants. Due to its wide biological activity, including antitumor, anti-inflammatory, antioxidant, hepatoprotective and antimicrobial effects, it is considered a promising structure for developing new broad-spectrum therapeutic agents. However, its clinical application is limited by low water solubility, weak bioavailability, and limited selectivity. Therefore, considerable attention is given to the structural modification of ursolic acid to obtain derivatives with improved pharmacological and physicochemical properties and high therapeutic value. The purpose of the study was to demonstrate a way to increase the biological activity and improve the pharmacokinetic properties of ursolic acid by means of chemical modifications. The following research methods were used: synthetic pathways, analysis of the influence of structural changes on biological activity, and comparison of experimental data obtained on various biological models. Modern methods of chemical synthesis of ursolic acid derivatives, grouped by key reactive positions of the molecule C-3, C-28, as well as other parts of the skeleton, such as ring A, double bond between C-12 and C-13, are considered in the review article. Studies have shown that targeted modification in positions C-3 and C-28 is most effective for enhancing antitumor, anti-inflammatory, antibacterial and cytotoxic activity. The

practical significance of the study is that it shows the high potential of modified ursolic acid in the development of new pharmaceuticals and substantiates synthesis strategies aimed at targeted enhancement of biological activity.

Keywords: ursolic acid, chemical modification, reaction centers, triterpenoid derivatives, biological activity

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УРСОЛ ҚЫШҚЫЛЫНЫҢ ҚҰРЫЛЫМДЫҚ-БАҒЫТТАЛҒАН МОДИФИКАЦИЯЛАРЫ ЖӘНЕ ОЛАРДЫҢ БИОЛОГИЯЛЫҚ БЕЛСЕНДІЛІККЕ ӘСЕРІ

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Аннотация. Урсол қышқылы көптеген дәрілік өсімдіктерде кездесетін табиғи пентациклды тритерпен. Ісікке қарсы, қабынуға қарсы, антиоксиданттық, гепатопротекторлық және микробқа қарсы әсерді қоса алғанда, биологиялық белсенділіктің кең спектріне байланысты ол жаңа кең спектрлі емдік агенттерді жасау үшін перспективалы құрылым ретінде қарастырылады. Дегенмен, урсол қышқылының клиникалық қолданылуы оның суда төмен ерігіштігімен, нашар биожетімділігімен және әсер етудің шектеулі селективтілігімен шектеледі. Осыған байланысты жоғары емдік маңызы бар фармакологиялық және физика-химиялық сипаттамалары жақсартылған туындыларды алу үшін урсол қышқылының құрылымдық модификациясына көп көңіл бөлінеді. Зерттеудің мақсаты – химиялық модификациялар арқылы урсол қышқылының биологиялық белсенділігін арттыру және фармакокинетикалық қасиеттерін жақсарту жолдарын көрсету. Зерттеу әдістері ретінде синтез жолдары, құрылымдық өзгерістердің биологиялық белсенділікке әсерін талдау, сондай-ақ әртүрлі биологиялық модельдерде алынған эксперименттік деректерді салыстыру қолданылды. Шолу мақалада молекуланың негізгі реакциялық орталықтары - С-3, С-28, сондай-ақ қаңқаның басқа бөліктері, мысалы, А сақинасы, С-12 және С-13 арасындағы қос байланыс бойынша топтастырылған урсол қышқылы туындыларының химиялық синтезінің заманауи әдістері қарастырылады. Зерттеулер нәтижесінде С-3 және

С-28 позицияларындағы мақсатты модификация ісікке қарсы, қабынуға қарсы, бактерияға қарсы және цитоуыттылық белсенділікті арттыруда ең тиімді екенін көрсетеді. Шолуда ұсынылған нәтижелер онкологиялық, қабыну, жұқпалы ауруларды емдеуге бағытталған жаңа препараттарды әзірлеуде пайдаланылуы мүмкін, сондай-ақ дәрілік химия, фармакология және биомедициналық технологиялар саласындағы одан әрі зерттеулерге негіз болады. Зерттеулердің практикалық маңыздылығы – модификацияланған урсол қышқылының жаңа фармацевтикалық агенттерді әзірлеуде жоғары потенциалы бар екенін айқындауы және биологиялық белсенділікті мақсатты түрде арттыруға бағытталған синтез стратегияларын негіздеуі болып табылады.

Түйін сөздер: урсол қышқылы, химиялық модификация, реакциялық орталықтар, тритерпеноидтардың туындылары, биологиялық белсенділік

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СТРУКТУРНО-НАПРАВЛЕННЫЕ МОДИФИКАЦИИ УРСОЛОВОЙ КИСЛОТЫ И ИХ ВЛИЯНИЕ НА БИОЛОГИЧЕСКУЮ АКТИВНОСТЬ

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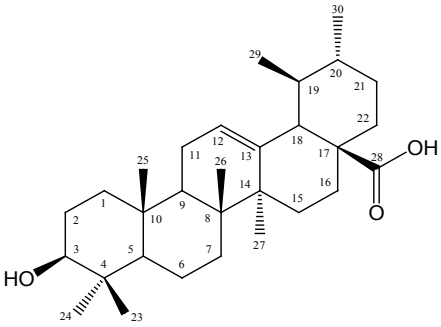
Аннотация. Урсоловая кислота — природный пентациклический тритерпен, содержащийся во многих лекарственных растениях. Благодаря широкому спектру биологической активности, включающему противоопухолевое, противовоспалительное, антиоксидантное, гепатопротекторное и антимикробное действие, она рассматривается как перспективная структура для разработки новых терапевтических агентов. Однако её клиническое применение ограничено низкой растворимостью в воде, слабой биодоступностью и умеренной селективностью, что существенно снижает фармакологическую эффективность. В связи с этим значительное внимание уделяется структурной модификации урсоловой кислоты с целью получения производных с улучшенными фармакологическими и физико-химическими характеристиками. Целью исследования было проанализировать

способы повышения биологической активности и улучшения фармакокинетических свойств урсоловой кислоты посредством различных химических модификаций. В качестве методов исследования использовались анализ современных химических подходов к модификации структуры, изучение влияния функциональных превращений на биологическую активность и сопоставление данных, полученных на различных биомоделях. В обзорной статье рассмотрены актуальные методы синтеза производных урсоловой кислоты, классифицированные по ключевым реакционноспособным позициям молекулы — C-3, C-28, а также по модификациям кольца А и области двойной связи C-12=C-13. Показано, что целенаправленные преобразования в позициях C-3 и C-28 являются наиболее эффективными для усиления противоопухолевой, противовоспалительной, антибактериальной и цитотоксической активности в отношении опухолевых клеток. Представленные в обзоре результаты могут быть использованы при создании новых лекарственных препаратов для лечения онкологических, воспалительных и инфекционных заболеваний, а также служить основой для дальнейших исследований в области медицинской химии, фармакологии и биомедицинских технологий. Практическая значимость исследования заключается в демонстрации высокого потенциала модифицированной урсоловой кислоты в разработке новых фармацевтических средств и обосновании стратегий синтеза, направленных на целевое усиление биологической активности.

Ключевые слова: урсоловая кислота, химическая модификация, реакционные центры, производные тритерпеноидов, биологическая активность

Introduction. Among natural compounds, triterpenoids are among the most abundant and structurally diverse biologically active secondary metabolites in plants. They play protective, signaling, and physiological regulatory roles in plants. In addition, triterpenoids have attracted special attention in the medical and pharmaceutical industries due to their anti-inflammatory, antitumor, antiviral, antioxidant, and hepatoprotective effects (Malik et al, 2024; Wei et al, 2024; Hu et al, 2025). One of the most widely distributed compounds among these triterpenoids is ursolic acid (Cargnin et al, 2017; Szakiel et al, 2012). Ursolic acid (3β-hydroxy-urs-12-en-28-oylic acid) (**1**) is a five-membered triterpenoid.



(**1**)

Various studies on the synthesis and biological properties of ursolic acid obtained from plants have revealed its importance in the fields of industry, agriculture and medicine.

Among oncological diseases, hormone-dependent types - in particular, breast and endometrial cancer in women, as well as prostate cancer in men - remain one of the medical and biological problems that have not lost their relevance today. In the treatment of such pathologies, antihormonal drugs aimed at regulating hormonal balance and inhibitors of enzymes involved in the biosynthesis of hormones (for example, aromatase) are widely used. Studies have shown that ursolic acid is also able to inhibit the activity of the aromatase enzyme (Jeong et al, 2000), which makes it a promising agent in the therapy of hormone-dependent malignant tumors.

Ursolic acid exhibits pronounced antitumor activity by suppressing key signaling pathways involved in tumor development. It also significantly reduces proliferation and enhances apoptosis of cancer cells by blocking their pathological activation (Mbaveng et al, 2014).

Ursolic acid exhibits a wide range of biological activities, however, its practical application is limited by its low solubility, poor bioavailability, and limited selectivity of action. Therefore, it is important to chemically modify ursolic acid to improve its pharmacokinetic properties, enhance its therapeutic activity, and obtain new derivatives with high selectivity and safety.

This work examines the modifications of the triterpenoid ursolic acid, which has a wide spectrum of biological activity, at its reactive centers, and the pharmacological activities of the synthesized derivatives.

Materials and methodology. The scientific information and data that formed the basis of this review were systematically selected from international scientific databases. In particular, information on chemical modifications performed on the reactive centers in the molecular structure of ursolic acid, their synthesis routes and biological activity were searched and analyzed from leading databases such as ScienceDirect, SpringerLink, and PubChem.

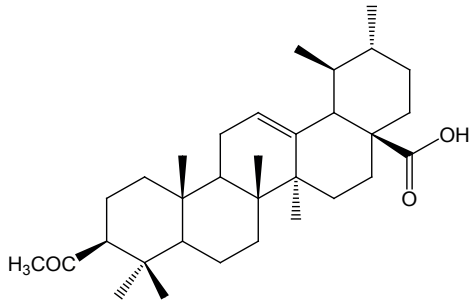
The following keywords were used during the information search: ursolic acid, chemical modification, reactive centers, biological activity, triterpenoids, derivatives, etc.

The collected literature data were systematized according to the directions of chemical modification (for example, C3-hydroxyl group, C28-carboxyl group and C12–C13 double bond), and the synthetic methods used for each reactive center and their effect on pharmacological activity were comparatively analyzed.

Results and discussion. Modifications at the C-3 position. The C-3 hydroxyl group is one of the most frequently modified moieties in the structure of ursolic acid. Various chemical modifications at this position, including esterification, oxidation, glycosylation, oximation, and the formation of carbamate or amide derivatives, are widely used to enhance anticancer activity.

Thien and colleagues synthesized a number of new derivatives of ursolic acid by chemical modification. In the study, the hydroxyl group at the C-3 position was

mainly modified with various functional groups. The purpose of these modifications was to increase the anticancer activity of the molecule. During the synthesis, the C-3 hydroxyl group of ursolic acid was first subjected to various acetylation and ester and amide introduction reactions. As a result, compounds (2–4) were obtained. In addition, oxidation, oximation, and further acetylation were carried out to obtain the acetoximino group compound (**5**) (Thien et al, 2013). The results of the study showed that the presence of an acetoximino group in the molecule significantly increased its anti-lung cancer activity compared to the original ursolic acid. These structural modifications demonstrate the possibility of creating effective anti-cancer drugs based on ursolic acid, and the reactions are aimed at improving lipophilicity and biological activity by modifying the C-3 position.



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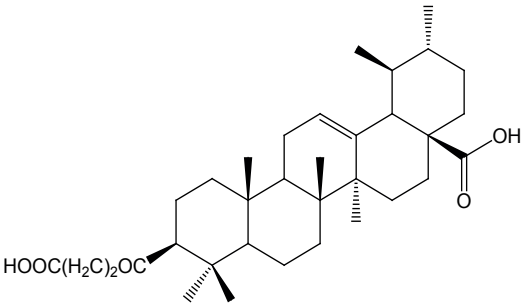

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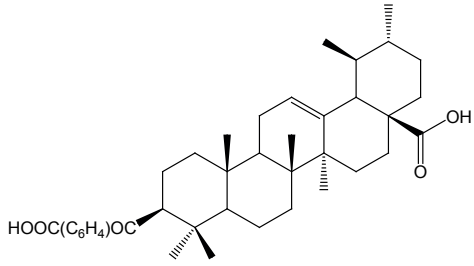

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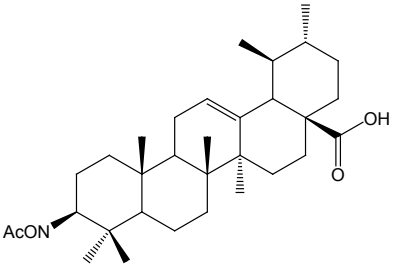
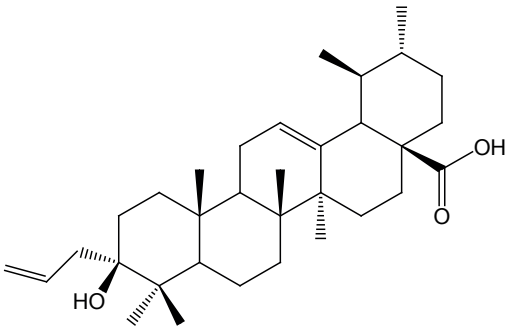
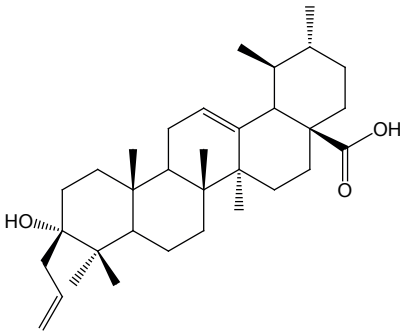


(**5**)

Fontana and colleagues conducted a series of modifications and studied the effect of the synthesized compounds on malignant liver cancer cell lines (HepG2, HA22T/VGH and Hep3B) [Fontana et al, 2019]. Among the ursolic acid derivatives, compounds (**6**) and (**7**), in which a three-carbon side chain was introduced at the C-3 position, were obtained as stereoisomers by the Barbier–Grignard method and showed high activity against all three cell lines.

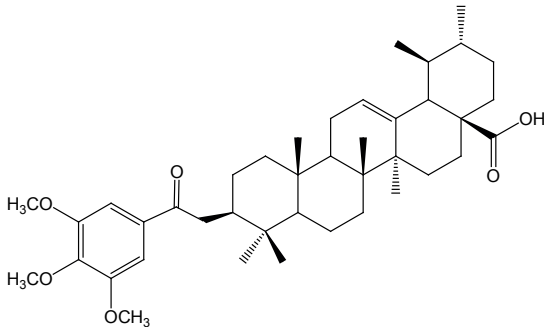


(**6**)



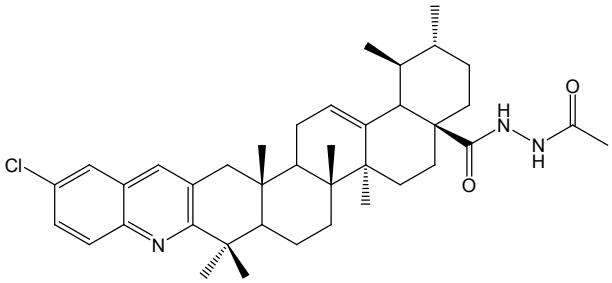
(**7**)

Xu and his research group reacted ursolic acid and a number of other triterpenoids with 3,4,5-methoxy benzoic acid and tested the antitumor effects of the resulting compounds on A549, MCF-7, H1975, and BGC-823 tumor cells. In particular, compound (**8**) with a 3,4,5-trimethoxyphenacyl group introduced at the C-3 position had a specific antiproliferative effect, with an IC_{50} value ranging from 5.32–15.23 μM (Xu et al, 2020).



(**8**)

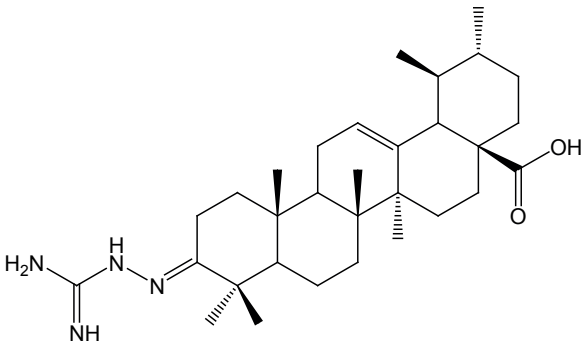
Jin and colleagues synthesized novel derivatives based on ursolic acid containing a quinoline structure and containing thiadiazole, hydrazide, and oxadiazole moieties. The antitumor activity of these compounds was evaluated *in vitro* against cancer cell lines such as SMMC-7721, MDA-MB-231, and HeLa. As a result of the studies, a number of compounds showed significant activity against at least one cell line. Among them, compound (**9**) showed the strongest activity against all cancer cell lines, and this activity was higher than that of etoposide. In particular, compound (**9**) can induce apoptosis of HeLa cells, arrest the cell cycle at the G0/G1 phase, increase the intracellular level of reactive oxygen species, and reduce the mitochondrial membrane potential (Jin et al, 2019).



(**9**)

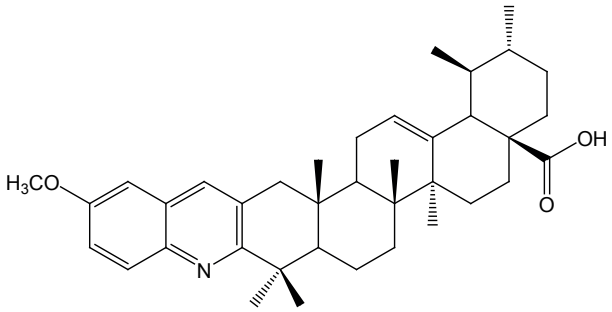
In addition, compound (**9**) can significantly inhibit MEK1 kinase activity and interfere with the Ras/Raf/MEK/ERK transduction pathway. Thus, compound (**9**) has been identified as a potential anticancer agent and a promising compound worthy of further investigation.

Wu et al. developed a series of derivatives by modifying the structure of ursolic acid with an aminoguanidine fragment and evaluated their anti-inflammatory and antibacterial properties (Wu et al, 2019). Among these derivatives, compound (**10**) showed the highest antibacterial activity against various bacteria tested with MIC values in the range of 2–16 μg/mL, as well as the highest anti-inflammatory activity with 81.61% inhibition. Structural analysis revealed that the aminoguanidine group is the main factor influencing the activity. It was also observed that the retention of the carboxyl group at C-28 in the molecule enhances the pharmacological efficacy of the compound.



(**10**)

Gu and his research group synthesized new quinoline derivatives based on ursolic acid. The antitumor effects of these compounds were tested in vitro on a series of human cancer cells, such as MDA-MB-231 (breast cancer), HeLa (cervical cancer), and SMMC-7721 (liver cancer) (Gu et al, 2017). In particular, compound (**11**) was found to be the most potent derivative, with IC_{50} values of 0.61 ± 0.07, 0.36 ± 0.05, and 12.49 ± 0.08 μM, respectively, against MDA-MB-231, HeLa, and SMMC-7721 cells, respectively, compared with the control.

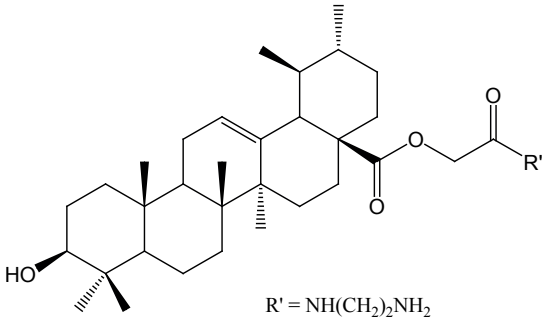


(**11**)

Modifications at the C-28 position

The carboxyl group at the C-28 position is one of the structural elements that is frequently subjected to chemical modification. Functional groups such as piperazine ring and nitrogen chains are introduced at this position through esterification, amidation. Such modifications are often carried out to enhance anti-lung cancer activity.

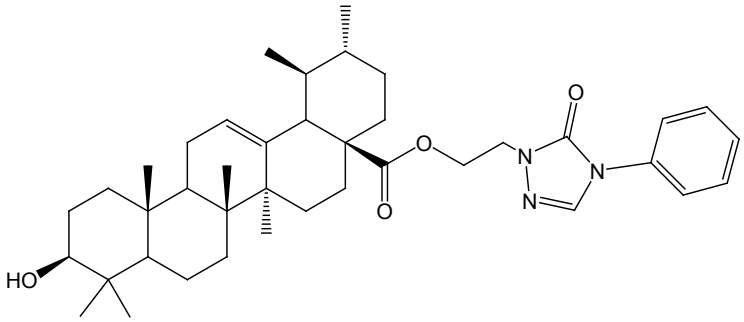
Tian and colleagues synthesized new derivatives by introducing various diamine groups at the C-28 position of ursolic acid and studied their anti-cancer activity against MCF-7, HeLa and A549 cancer cells (Tian et al, 2017). During the synthesis, the C-3 hydroxyl group was first protected with an acetyl group, and then the C-28 position was modified with 2-hydroxyacetate and subjected to amidation reaction with various diamines. The amines used included piperazine, N-methylpiperazine and various alkane diamines. The results of the study showed that some of these derivatives, especially compound (**12**), had higher antiproliferative activity compared to gefitinib. In particular, compounds with primary amine groups were significantly more effective than secondary and tertiary amine derivatives. In addition, secondary amine derivatives showed higher activity compared to tertiary amines.



(**12**)

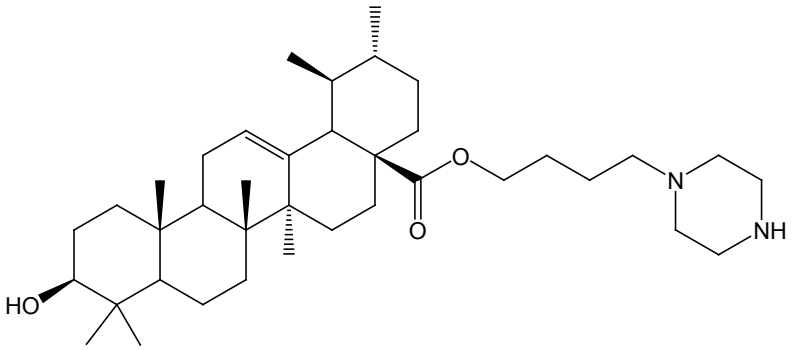
Chi et al. synthesized a series of novel derivatives containing oxadiazole, piperazine, and triazolone groups based on ursolic acid and evaluated their antitumor activity (Chi et al, 2017). The antihypoxic effects of these derivatives, in particular, their ability to inhibit the expression of hypoxia-induced factor-1α (HIF-1α), were investigated. In vitro

experiments showed that these novel compounds significantly enhanced their antitumor activity by inhibiting the expression of HIF-1α. Among them, derivative (**13**) inhibited HIF-1α activity the most, but did not show cytotoxic effects on cancer cells. These results indicated that simple esterification of the carboxyl group of ursolic acid could significantly enhance its ability to inhibit HIF-1α activity and reduce its toxicity to cells. In addition, the study revealed that derivative (**13**) could slow down cell proliferation and induce cell cycle arrest in the G1 phase.



(**13**)

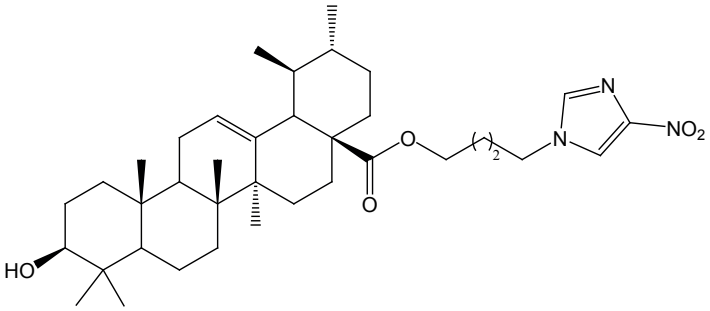
Gou et al. obtained several compounds by structural modification of ursolic acid. Among them, compound (**14**) with a piperazine moiety in particular showed a more pronounced inhibitory effect on lung cancer cell proliferation (IC_{50} = 5.4–6.1 μM for A549 cells and IC50 = 3.9–5.7 μM for H460 cells) compared to the original ursolic acid. Compound (**14**) was shown to not only induce cell cycle arrest at the G0/G1 phase but also induce apoptosis in A549 and H460 cells. Thus, compound (**14**) is a drug with anti-lung cancer properties (Gou et al, 2020).



(**14**)

Li and his research group synthesized a series of novel derivatives based on ursolic acid and investigated their ability to inhibit hypoxia-inducible factor 1α (HIF-1α) activity and anti-inflammatory properties. The single compound (**15**) inhibited HIF-1α activity more effectively than ursolic acid and showed anti-inflammatory effects comparable to

celecoxib in in vivo studies. In addition, this compound moderately inhibited the COX-2 enzyme, which also confirms its anti-inflammatory potential (Li et al, 2022).

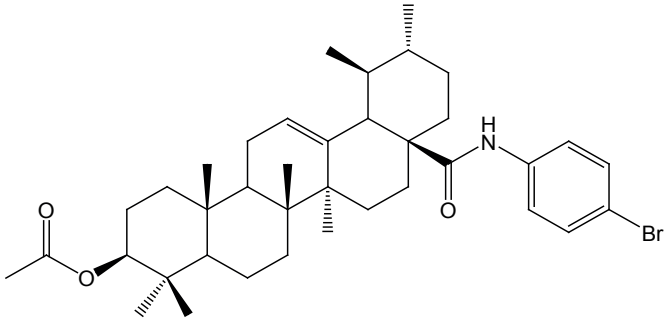


(**15**)

Based on these results, compound (**15**) is considered a promising molecule and can be proposed as a starting structure for the development of new HIF-1α inhibitors and anti-inflammatory agents.

Modifications at positions C-3 and C-28

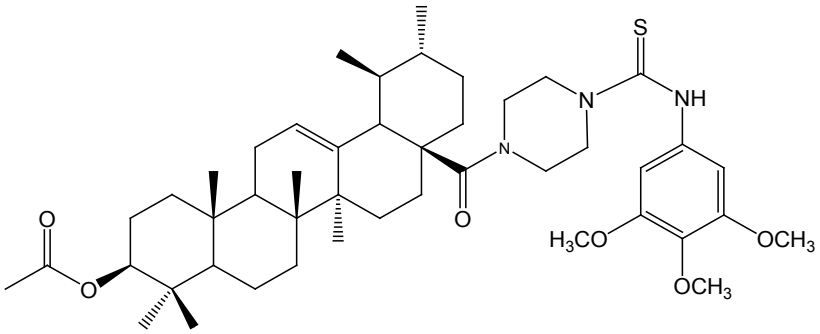
Kalani and colleagues modified the molecular structure of ursolic acid and studied its characteristics using quantitative structure-activity relationship (QSAR) models (Kalani et al, 2012). They applied these models to human lung cancer cell lines (A549). The antitumor activity of the compounds obtained in the study was in good agreement with experimental data. Among them, the 4-bromoanilamidoursolic acid analogue (**16**) was the most active of all and had higher cytotoxic activity than the control drug Adriamycin.



(**16**)

Based on previous studies, Hua and his research group developed a series of novel biologically active derivatives by purposefully modifying the structure of ursolic acid. In their study, a series of novel ursolic acid derivatives modified at positions C-3 and C-28 were designed and synthesized with the aim of developing potential anticancer agents (Hua et al, 2015). They were evaluated against five cancer cell lines (MGC-803, HCT-116, T24, HepG2 and A549) and a normal cell (HL-7702). The screening results showed that some of these targeted compounds exhibited moderate to high levels of

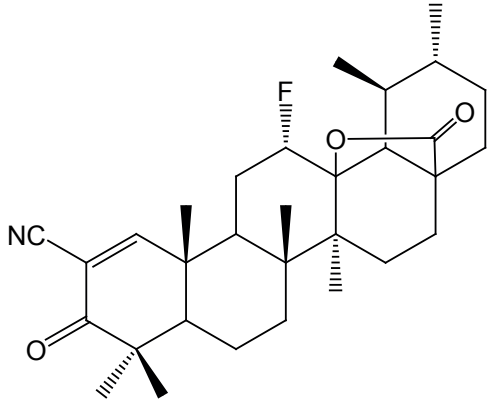
antiproliferative activity. The results indicated that the anticancer activity of compound (**17**) could be achieved by inducing cell apoptosis by arresting the cell cycle at the G1 phase, and could induce apoptosis through both intrinsic and extrinsic apoptotic pathways.



(**17**)

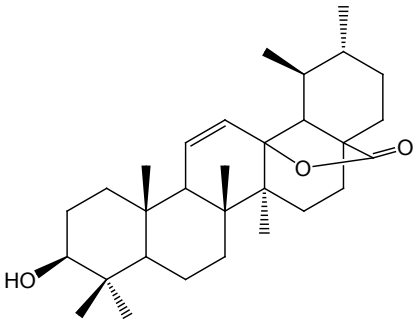
Other modifications

Leal and colleagues synthesized a series of ursolic acid derivatives using an electrophilic fluorinating reagent. The antiproliferative activity of these novel compounds was evaluated in AsPC-1 pancreatic cancer cells and their structure-activity relationships (SARs) were investigated. Among the synthesized compounds, ursolic acid derivatives with heterocyclic rings such as imidazole or methylimidazole and cyanones were among the most potent inhibitors of AsPC-1 pancreatic cancer cell growth. 2-cyano-3-oxo-12α-fluoro-urs-1-ene-13,28β-olide, compound (**18**), was the most potent inhibitor with IC50 values of 0.7, 0.9 and 1.8 μM in AsPC-1, MIA PaCa-2 and PANC-1 cancer cells, respectively. This compound also showed good antiproliferative activity against breast (MCF7), prostate (PC-3), hepatocellular (Hep G2), and lung (A549) tumor cells with IC50 values below 1 μM (Leal et al, 2012).

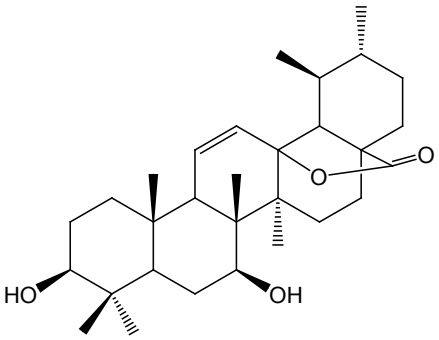


(**18**)

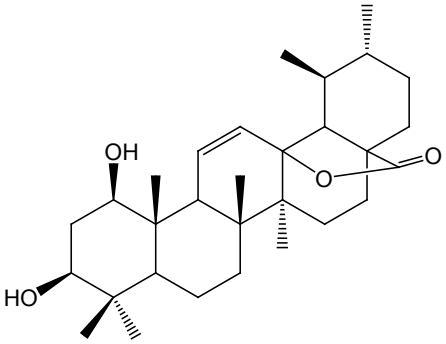
Fu et al. reported that the biotransformation of ursolic acid by the endophytic fungus *Ustilago isabellina* introduced a number of important structural changes. During the study, it was found that the double bond of ursolic acid was shifted from the original C-12–C-13 position to the C-11–C-12 position, and a hydroxyl group was introduced at the C-13 carbon atom. As a result, new compounds (**19-21**) were obtained (Fu et al, 2011). In addition, a lactone ring was formed between the C-28 carboxyl group and the C-13 hydroxyl group. These structural changes are an important step in increasing the biological activity of ursane-type triterpenes and obtaining new natural compounds. The authors showed that endophytic fungi can be used not only as metabolite producers, but also as effective biocatalysts in complex structural transformations of natural compounds. This study will increase the possibility of obtaining new bioactive compounds based on ursolic acid and open the way to its use in the pharmaceutical field.



(**19**)

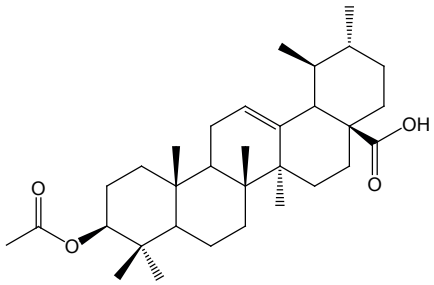


(**20**)



(**21**)

Batra et al. isolated ursolic acid from the holy basil plant (*Ocimum sanctum*) and synthesized three new derivatives based on this compound. These derivatives were obtained by introducing various functional groups at the C-3, C-11 and C-28 positions of ursolic acid and were evaluated for antitumor activity. A series of derivatives (**22-24**) decreased DHFR activity and increased extracellular homocysteine levels. Compound (**23**) showed significant antiproliferative activity in cancer cells (Batra et al, 2013).



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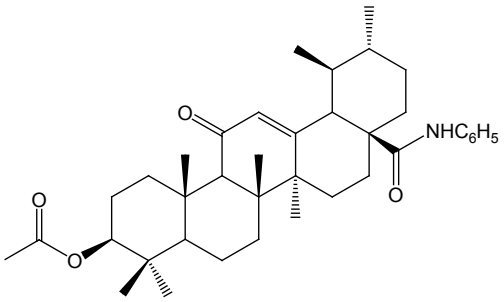

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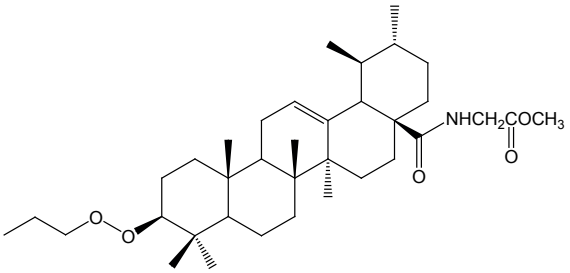

(24)

Conclusion. Ursolic acid is a biologically active substance belonging to the group of natural triterpenoids. The main functional groups include the hydroxyl group, carboxyl group, and double bond. These groups are chemically active, so ursolic acid is easily subjected to various chemical modifications. Such modifications are aimed at improving its properties such as solubility, bioavailability, and pharmacological activity.

The chemical structure of ursolic acid is amenable to modification at various positions, which in turn allows it to significantly increase its biological activity. The C-3 position is one of the most frequently modified regions. The hydroxyl group at this position easily undergoes various chemical reactions, improving the lipophilicity and biological activity of the molecule. Significant modifications have also been made at the C-28 position. Here, the antitumor activity of the derivatives has been significantly increased by the addition of various fragments. Modification of the double bonds at positions C-12 and C-13 is also of interest. In particular, reactions such as the introduction of a fluorine atom or the formation of a lactone ring enhance the activity of ursolic acid against cancer cells. These changes contribute to cell cycle arrest, apoptosis activation, and cytotoxicity.

Overall, this review has shown that chemical modifications at different positions of the ursolic acid molecule significantly affect its pharmacological properties. The clinical efficacy and safety of the resulting derivatives need to be further evaluated through in vivo and clinical studies. Work in this direction will pave the way for the development of new, effective, and safe drugs based on ursolic acid.

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