Lanostane Tetracyclic Triterpenoids as Important Sources for Anti-Inflammatory Drug Discovery

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Abstract

Inflammation is a defensive reaction of the human body to numerous detrimental stimuli, including physical trauma, noxious chemicals, as well as microbial agents. Uncontrolled inflammation is the pathological basis of multiple diseases, such as rheumatoid arthritis (RA), neurodegenerative diseases, liver disease, and lung inflammation. Lanostane triterpenoids are natural tetracyclic triterpenoids with significant anti-inflammatory activity. An extensive review of the published literature regarding the phytochemistry and anti-inflammatory pharmacology of lanostane triterpenoids has been performed and analyzed using several search engines, such as SciFinder, Web of Science, Scopus, PubMed, Google Scholar, and ScienceDirect. This review is devoted to naturally occurring lanostane‑type triterpenes with anti‑inflammatory activity, including their sources, biosynthesis, and mechanism of action. This review also discusses the inflammation‑related diseases and the clinical significance of traditional Chinese medicine as multi‑target therapeutic agents for the prevention and treatment of inflammatory diseases. In the past 30 years, more than 100 new lanostane‑type triterpenes have been reported from the families Schisandraceae, Ganodermataceae, and Polyporaceae. Six compounds, fomitiporic acid A, fomitoxides E and F, obtusifoliol, 4α, 14α‑dimethyl‑5α‑ergosta‑7,9(11), 24(28)‑trien‑3β‑ol, and gramisterol exhibited the most potent anti‑inflammatory activity against cyclooxygenase‑1 (COX‑1) and COX‑2, with IC₅₀ values ranging from 0.087 to 1.15 µM. Some of these compounds exhibited significant activity by mediating the inhibition of the pro‑inflammatory cytokines, inducible nitric oxide synthase, and COX‑2 expression. This review provides a basis for identifying anti‑inflammatory drugs with high selectivity, high potency, and few adverse effects from lanostane‑type triterpenes.

Keywords: Inflammation, inflammatory diseases, lanostane triterpenoids, rheumatoid arthritis

INTRODUCTION

Inflammation is a protective response of the human body to various harmful stimuli, including physical trauma, noxious chemicals, and microbes. The inflammatory reaction is initiated by a series of harmful exogenous and endogenous signals resulting from mechanically, chemically, or biologically induced tissue damage. Inflammation is closely related to the occurrence and development of many major diseases and is mainly characterized by local redness, swelling, pain, edema, and temperature increase caused by reactions of the vascular system. Failure to resolve recurrent acute events of inflammation may lead to chronic inflammatory diseases, such as arthritis, colitis, or asthma, which are associated with irreversible tissue damage and increased risk for the development of cardiovascular disease, cancers, rheumatoid arthritis (RA), and osteoporosis. Chronic inflammation includes severe inflammatory autoimmune diseases, which can lead to severe tissue damage. During chronic inflammatory states, pro-inflamatory cytokines such tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β persist for long periods. Inflammation is caused by a prolonged immune response, consisting of pro-inflammatory macrophages, microglia, and neutrophils, together with cytokines...
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referred from other cell types, such as endothelial cells, oligodendrocytes, and fibroblasts.[14] The reaction mechanism of inflammatory diseases forms a complex network of relationships.

Many enzymes and factors in cell signaling pathways are involved, such as nuclear factor (NF)-κB,[15] mitogen-activated protease (p38MAPK),[16] matrix metalloproteinases,[17] and γ-interferon[18] and the STAT nuclear protein family[19] that play a crucial role in the growth hormone signaling pathway. In general, drug treatment can ameliorate inflammatory responses by blocking the production or release of inflammatory mediators. Clinical treatment of inflammation uses three major types of drugs, nonsteroidal anti-inflammatory drugs,[20] steroidal anti-inflammatory drugs, and traditional Chinese medicine.[21-23]

Lanostane triterpenoids are a type of tetracyclic triterpenic acids whose skeletons contain one to three carboxyl groups. Natural lanostane-type triterpenoids are common in fungi and marine organisms and occur in higher plants, but so far are mainly isolated from the families Ganodermataceae, Polyporaceae, and Schisandraceae. Compounds isolated from these sources showed pharmacologically diverse activities, including anti-inflammatory, anti-tumor, cytotoxicity, anti-bacterial, and analgesia, among others.

This review systematically summarizes all previously isolation and synthesis work performed with lanostane triterpenoids and discusses the anti-inflammatory effects of lanostane triterpenoids. This review can serve as a reference for future research on the lanostane triterpenoids.

**Inflammation-Related Diseases**

Inflammation is a complicated biological interaction that arises in response to a series of injuries caused by physical trauma, noxious chemical stimuli, or microbiological toxins. Clinically, many diseases are associated with inflammation, including RA, neurodegenerative diseases, liver and lung inflammation, cardiovascular diseases, cancer, and colitis.

RA is a common chronic disease, in which the failure of spontaneous resolution of inflammation causes the disease to persist in patients throughout their lives.[24,25] Extensive investigations of the pathogenetic mechanisms of inflammation and autoimmunity, and the resulting increased understanding of cytokine networks and cellular players in RA, have led to the development of agents that block TNF-α, IL-1, and IL-6 signaling, or target pathogenic cells such as B cells[27] and osteoclasts.[28] Bari summarized recent insights into the roles of mesenchymal stem cells (MSCs) in RA and discussed the potential to harness the immunomodulatory properties of MSCs for the treatment of RA.[29] Numerous studies have shown that fibroblast-like synoviocytes (FLS) are significant contributors to joint inflammation in RA.[30] Brandstetter’s research identified that the inactivation of forkhead box O3 (FOXO3) results from TNF-α-induced downregulation of phosphoinositide-3-kinase-interacting protein 1 (PI3KIP1). Reduced PI3KIP3 results in increased phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) pathway activity in FLS. This finding provides novel insights into the molecular mechanism underlying TNF-driven inflammatory diseases.[31]

Neurodegenerative disorders such as Alzheimer’s disease and Parkinson’s disease are related to neuroinflammation and most commonly occur in the elderly. Their pathogenesis is related to microglia-mediated neuroinflammation. Many inflammatory factors, such as IL-1β and TNF-α, are detected in neurodegenerative disease patients.[32] Numerous studies have suggested that neuroinflammation plays an essential role in neurodegenerative disorders. Toll-like receptor 4 (TLR4) is an important pattern recognition receptor expressed in microglia and is responsible for microglial activation.[32] Nair et al. reported that the DNA damage-linked inflammatory cascade is mediated by expression-level changes in G protein-coupled receptors and can be targeted to counteract inflammation during anticancer therapies as well as aging.[33]

Inflammation is also associated with various diseases. It can suppress pro-inflammatory cytokine production, including TNF-α, IL-1β, and IL-6 via inhibition of the NF-kB pathway, and lead to chronic obstructive pulmonary disease.[34] The previous study showed that TLRs play critical roles in liver inflammation.[35,36] Inflammation is also a significant cause of disease in patients with colitis. Singh et al.[37] found that the conditional deletion of HDAC2 in CD4+ T cells results in elevated IL-17 expression and causes severe colitis in a mouse model. The identification of the Ubc9/ROR-γt/HDAC2 axis that governs IL-17 expression may open new avenues for the development of therapeutic measures for inflammatory disorders.[37] However, the development of fibrosis and inflammation may not be interlinked with inflammatory bowel diseases elucidated by Hünerwadel et al.[38]

**Lanostane-Type Triterpenoids**

Triterpenes comprise a large number of compounds which, according to the diverse features of their skeletons, can be classified as acyclic, mono-, bi-, tri-, tetra-, and pentacyclic triterpenes.[39] More than 20,000 triterpenes have been discovered in nature, many of them occurring in their free form, while others occur as glycosides (saponins) or other combinations.[40] Triterpenoids are compounds of wide occurrence and structural diversity that have gained attention for their pharmacological activities.[41] Since 1985, Connolly and Hill have annually reviewed newly isolated triterpenoids from the plant kingdom.[42] The most studied triterpenes are tetracyclic triterpenes such as lanostanes, protostanes, tirucallanes, cucurbitanes, meliacanes, cycloartanes, dammaranes, and euphanes and pentacyclic triterpenes including ursanes, gamaeracanes, lupanes, oleananes, and hopanes.[43,44] Among numerous inhibitory activities of such derivatives, inhibition of tumor promotion, inflammation, and lipid peroxidation are the most significant.

Lanostane-type triterpenoids are tetracyclic triterpenoids. They can be divided into two subtypes: intact lanostanes and...
seco lanostanes [Figure 1]. The structural characteristics of triterpenoids are that the C-3 position is mainly substituted by a carbonyl group or a hydroxy group, and the double bonds are mostly located at C-8 (9) and C-9 (11), and the OH or OAc is substituted at the C-12 position, the side chain is mainly 24(Z)-ene-26-acid or a 22, 26 lactone ring.\[86,47\]

Natural lanostane-type triterpenoids have mostly been reported in fungi and marine organisms, but also occur in higher plants.\[80\] Through literature investigation, it was found that triterpenoids are mainly isolated from fungi belonging to the genera *Ganoderma*, *Scilla*, *Fomitopsis*, *Astraeus*, *Tricholoma*, *Antrodia*, *Naematoloma*, *Piptoporus*, *Euphoria*, *Abies*, and the families *Ganodermataceae*, *Liliaceae*, *Polyporaceae*, *Astraeaceae*, *Tricholomataceae*, *Strophariaceae*, *Euphorbiaceae*, and *Pinaceae*.\[49-73\] In fungi, most have been identified in the families *Ganodermataceae* and *Polyoporaceae*. The skeletal structural characteristics of this class include five types, “3 β-hydroxy-8-ene,” “3 β-hydroxy-7,9-diene,” “3 β-hydroxy-8-ene-7-ketone,” “3 β-hydroxy-9-ene-7-ketone,” and “3 β-hydroxy-7-ene-11-ketone.” The C-17 side chains are usually cyclized, most are C-21 and C-24, and connected to form a cyclopentane, form an ester ring at C-21 with C-23 or C-24, or forming an ether ring between C-22 and C-25. Substituents such as aldehydes, carboxyls, hydroxyls, and ketones are primarily present at C-1, C-7, C-11, C-21, C-22, C-24, and C-25.

In higher plants, lanostane-type triterpenoids are reported from the family Schisandraceae, which has two genera, Kadsura\[74-86\] and Schisandra.\[87-93\] Previous phytochemical investigations showed that the lanostane triterpenoids, which contain the intact and 3,4-seco tetracyclic core structural skeleton. The structural characteristics of them are that C3 is mainly substituted by carbonyl or hydroxy groups, the double bonds are usually located at C7/C8, C8/C9, or C9/C11, the hydroxy or carbonyl groups are mostly substituted at C12, and the C17 side chain is mainly 24 (Z)ene26acid or 22, 26 lactone ring [Table 1].

**Anti-Inflammatory Activity of Natural Lanostane-Type Triterpenoids**

Natural lanostane-type triterpenoids can be isolated from fungi and marine organisms and also from higher plants. Through literature investigations, it was found that they were mainly isolated from fungi and higher plants.

Two new lanostane triterpenoids and ten new lanostane triterpene glycosides have been isolated from the fruit bodies of *Fomitopsis pinicola* by Yoshikawa et al. All compounds were tested for anti-inflammatory activity against cyclooxygenase-1 (COX-1) and COX-2 and compared to the positive control drug indomethacin (IC\(_{50}\) of 0.60 μM). The results showed that fomitopinic acid A (1) [Figure 2], and the fomitosides E (2) and F (3), showed significant activities with IC\(_{50}\) values of 1.15, 0.15, and 0.13 μM, respectively.\[94\] Zhang et al.\[95\] reported eight undescribed lanostane triterpenoids and one known; all of the compounds also were evaluated for inhibition of nitric oxide (NO) production. Pardinols B and E-H exhibited inhibition of NO production, with IC\(_{50}\) values ranging from 5.3 to 14.7 μM. Among them, pardinol B (4), E (5), and H (6) showed significant inhibition, with IC\(_{50}\) values of 5.3 ± 1.22, 6.8 ± 0.53, and 5.6 ± 0.26 μM, respectively. Eight new highly oxygenated lanostane triterpenes and gibbosic acids A–H and nine known compounds were isolated by Pu et al.\[96\] These compounds were screened for immunoregulatory effects and anti-inflammatory activities in a murine lymphocyte proliferation assay and lipopolysaccharide (LPS)-stimulated RAW-264.7 macrophages. Gibbosic acid B (7) exhibited immunostimulatory effects both in the lymphocyte proliferation assay without any induction and in ConA-induced mitogenic activity of T-lymphocytes, and the proportion of lymphocyte proliferation at a concentration of 0.1 μM was 20.01% and 21.40%, respectively. These compounds did not influence the production of NO in LPS-induced RAW-264.7 macrophages. Twenty-nine new lanostane triterpenoids were obtained from the EtOH extract of fruiting bodies of *Ganoderma curtisii* by Jiao et al.\[97\] All of these compounds were evaluated for their inhibition of NO production in BV-2 microglia cells activated by LPS. The results showed that 3 β,7,12 β-trihydroxy-11, 15,23-trioxo-lanost-8,20-dien-26-oic acid (8), 3 β,7,15α-tri-hydroxy-4-(hydroxymethyl)-11,23-dioxo-lanost-8-en-26-oic acid (9), 7 β,12 β-dihydroxy-3,11,15,23-tetraoxo-5α-lanost-8-e n-26-oic acid (10), 3 β,15α-dihydroxy-7,11,23-trioxo-lanost-8-dien-26-oic acid (11), methyl ganoderate A (12), and ganoderic acid E (13), G (14), and K (15) inhibited NO production, with IC\(_{50}\) values of 9.55 ± 0.43, 4.15 ± 0.12, 5.91 ± 0.21, 6.50 ± 0.97, 8.01 ± 1.56, 9.41 ± 0.30, 5.77 ± 0.48, and 3.65 ± 0.41 μM, respectively, while none of them showed significant cytotoxicity in BV-2 microglia cells. Lee et al.\[98,99\] reported 22 compounds, including four new lanostane triterpenoids, named coriacoic acids A–D, isolated from the EtOH extract of the sclerotia of *Poria cocos*. All compounds were tested for their inhibition of NO and prostaglandin E\(_2\) production in LPS-stimulated Raw 264.7 cells as well as on the expression of inducible NO synthase (iNOS) and COX-2. Coriacoic acid A and B, dehydroeburiconic acid, acetyl eburicic acid, and poricoic acid C (16) inhibited NO production, with IC\(_{50}\) values ranging from 49.43 to 82.32 μM. Among them, 16 was the most active compound with an IC\(_{50}\) value of 49.43 μM; western blotting analyses revealed that 16 effectively suppressed iNOS, COX-2, and NF-κB at the protein level in a dose-dependent manner. These results suggest that the compound with the
most highly active anti-inflammatory effect, 16, was mediated through the inhibition of iNOS and COX-2 expression via the downregulation of NF-κB. Poricoic acid A (17) produced the greatest inhibition and inhibited iNOS protein expression in a dose-dependent manner, exerted anti-inflammatory activity and reduced PGE₂ levels via the downregulation of COX-2 protein expression, and exhibited significant NO-reducing activity with an IC₅₀ value of 18.12 µM. Lee et al. also elucidated the structure–activity relationship (SAR), which suggests that a ring-opened seco-lanostane skeleton rather than the intact lanostane skeleton may hold promise for inhibiting NO production. Two new lanostane-type triterpenoids,
named (3S, 4S, 7S, 9R)-4-methyl-3,7-dihydroxy-7 (8 → 9) abeo-lanost-24(28)-en-8-one and 24-hydroperoxylanost-7,25-dien-3β-ol, together with 15 known triterpene derivatives, were isolated from Euphorbia maculata by Sun et al. Among them, 11 lanostane triterpenoids were evaluated for their anti-inflammatory effects in the test of TPA-induced inflammation in mice. The results showed that obtusifoliol (18), 4α, 14α-dimethyl-5α-ergosta-7,9(11),24(28)-triien-3β-ol (19), and gramisterol (20) exhibited the most potent anti-inflammatory activity, with IC50 values of 87.1, 363.1, and 204 nM, respectively. Due to these three compounds (18–20) having one methyl substitution at C-4 and a double bond at C-24 in the side chain, their anti-inflammatory activity is stronger than the other tetracyclic triterpenoids, which infers that methyl and double bond groups located at the side chain might be an active part of this type of triterpenoid. Kim et al. isolated a rearranged lanostane-type triterpenoid, namely holophyllane A (21), which impeded NO production with the IC50 value of 12.74 µM in LPS-stimulated murine microglia [Table 2].

**Synthetic Lanostane-Type Triterpenoids**

Triterpenoids are natural products with thirty carbon atoms, biosynthetically derived from the cyclization of squalene. Lanostane triterpenoids are reported to possess various biological, pharmacological, or medicinal activities, including anti-inflammatory and anticancer effects. However, in many cases, the potency of these triterpenoids is relatively weak. At present, only a few studies have presented research on synthetic intact lanostane triterpenoids [Figure 3]. Wada et al. synthesized eight intact lanostane-type triterpenoids with various functional groups (-Cl, -Br, -OMe, -CHO, -CN, -COOH, and -COOMe) at the C-2 position, according to the substrate compound 3-oxolanost-9(11)-en-24S, 25-diol isolated from Pinus luchuensis. All the derivatives showed DNA topoisomerase II inhibitory effects, with IC50 values ranging from 1.86 to 149.97 µM. Seventeen intact lanostane-type triterpenoid derivatives, including 11 N-glycosides, were synthesized from the natural triterpenoid, lanosterol, by Ukiya et al. Honda et al. revised and confirmed the regiochemistry of these isoxazoles and subsequently synthesized a new intact lanostane triterpenoid with a cyano-enone functionality in ring A from lanost-8-en-3-one. Six compounds were synthesized from the intact lanostane-type tetracyclic triterpenoid of trametenolic acid B by Ding et al.

**Discussion**

The lanostane triterpenoids are mostly isolated from fungi and higher plants, which are represented by more than thirty different species. This phytochemical investigation has identified 85 new compounds isolated from the family Schisandraceae, 56 new compounds in the family Ganodermataceae, and 48 new compounds have been identified from the family Polyporaceae. Twenty-one of these compounds exhibited...
significant anti-inflammatory activity; particularly, fomitopinic acid A and fomitosides E and F showed significant activities with IC_{50} values of 1.15, 0.15, and 0.13 µM, respectively. Obtusifoliol, 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-tri-en-3β-ol, and gramisterol exhibited the most potent anti-inflammatory activity, with IC_{50} values of 87.1, 363.1, and 204 nM, respectively. The anti-inflammatory mechanism was found to be mediated by the inhibition of pro-inflammatory cytokines, iNOS, and COX-2 expression via the regulation of the NF-κB signaling pathway. SARs showed that the activity of a ring-opened seco-lanostane skeleton is much better than an intact lanostane triterpenoid, and the compound structure can be modified to increase its activity effectively.

The triterpenoids are economically and medicinally important and include many species with a variety of uses, which is attractive for pharmacological researchers and pharmaceutical industries. In recent years, new chemical entities derived...
from natural products have gained importance in the search for therapeutic drugs. Lanostane-type triterpenoids are considered to be promising agents for the development of anti-inflammatory drugs, and their derivatives have been shown to have anti-inflammatory activities. There is increasing evidence that lanostane triterpenoid compounds are widely found in nature and are a rich resource for the discovery of anti-inflammatory drugs.

**CONCLUSION**

The lanostane tetracyclic triterpenoids represent a promising and growing platform for anti-inflammatory biologically active natural compounds whose potential currently has yet to be developed into drugs by the pharmaceutical industry. Some recently studied lanostane triterpenoids showed significant anti-inflammatory activity. At present, the mechanism of anti-inflammatory effects of some of these lanostane tetracyclic triterpenoids has already been explained from different aspects at the cellular and molecular level. However, the molecular mechanism of the anti-inflammatory effects of most lanostane tetracyclic triterpenoids is not sufficiently elucidated or comprehensive for the specific propagation of action, and research at the genetic level is still uncommon. We conclude that the systemic evaluation of the anti-inflammatory activities of these compounds in vivo and in vitro is still needed. We also anticipate further progress in improvements in the methods of isolation and identification of lanostane triterpenoids, which open the way to targeted pharmacological modeling and resulting synthetic modifications. Targeted delivery systems, structural modifications, and the mechanisms of action of these compounds are essential in the design of novel derivatives with reduced cytotoxicity, improved efficacy, and increased therapeutic index. Research and development of drugs based on these triterpenoids, therefore, would be a key program in research institutions and pharmaceutical companies.

**References**


18. Wong CK, Ho CY, Ko FW, Chan CH, Ho AS, Hui DS, et al. Prolinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN-γ, IL-4, IL-10 and IL-13) in patients with allergic asthma. Clin Exp Immunol 2001;121:177-83.


29. de Bari C. Are mesenchymal stem cells in rheumatoid arthritis the good or bad guys? Arthritis Res Ther 2015;17:113.


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