Current status of terpenoids as inflammasome inhibitors

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Abstract

Increasing evidence supports NLRP3 inflammasome as a new target to control inflammation. Dysregulation of NLRP3 inflammasome has been reported to be involved in the pathogenesis of several human inflammatory diseases. However, no NLRP3 inflammasome inhibitors are available in clinic. Terpenoids are natural products with multi-target activities against inflammation. Recent studies have revealed that these compounds are capable of inhibiting the activation of NLRP3 inflammasome in several mouse models of NLRP3 inflammasome-related pathogenesis. Thus, terpenoids represent an interesting pharmacological approach for the treatment of inflammatory diseases as they are endowed with a dual mechanism of inhibition of NF-KB transcription factor and inflammasome activation, both critically involved in their anti-inflammatory effects. This work provides an overview of the current knowledge on the therapeutic potential of terpenoids as NLRP3 inflammasome inhibitors.

1. Introduction: overview of innate immune system and inflammation.

Innate immune system is one of the first line of defense of the organism against infections and tissue injury. Its activation after recognition of pathogen associated molecular patterns (PAMPs) or danger-associated molecules patterns (DAMPs) initiates the inflammatory response leading to the recruitment of inflammatory cells and the release of pro-inflammatory mediators. Pattern recognition receptors (PRRs) localized on the cell membrane or intracellular space, are responsible for the recognition of invading pathogens or molecules from tissue injury [1]. Several families of PRRs have been described including the Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoid acid-inducible gene I (RIG-I)-like receptors (RLRs), C-type lectin receptors (CLRs) and DNA sensors as absent in melanoma 2 (AIM2)-like receptors (ALRs), among others [2]. Whereas TLRs are mainly involved in PAMPs detection; NLRs, RLRs and ALRs sense the presence of pathogens via PAMPs but are also endogenous sensors of tissue damage through DAMPs. Upon recognition of these signals, PRRs trigger different signaling pathways, leading to the activation of nuclear factor-κB (NF-κB), interferon regulatory factors (IRFs) or inflammasome formation [2]. A misbalance in this complex machinery may lead to a chronic inflammatory state contributing to the progression of several pathologies. Recent studies have suggested that NLR family and inflammasome complexes play crucial roles in the pathogenesis of several inflammatory diseases [1], being considered a new promising target for development of novel therapies. In this commentary, we summarize the available evidences about the inhibitory potential of natural products on inflammasome activity, focusing on terpenoids since they are a traditional source of anti-inflammatory compounds.

2. PRRs and inflammasome.

2.1. NOD-like receptors (NLRs)

NLRs are a large family of intracellular receptors that participate in the recognition of pathogens and molecules from damaged cells to regulate the inflammatory response. They are mainly expressed in inflammatory cell types such as macrophages, neutrophils or dendritic cells [3, 4]. Members of the NLR family are structurally tri-domain proteins formed by an N-terminal protein-protein interaction domain such as caspase recruitment 'C' (CARD) or pyrin 'P' (PYD) domains, a central nucleotide-binding domain oligomerization domain (NOD) and a C-terminal leucine-rich repeats (LRRs) [5]. These domains exhibit different functions, thus, LRRs are essential for autoregulation and detention of PAMPs and DAMPs, leading to NLR activation; NOD region exerts regulatory and oligomerization functions and CARD and PYD domains have a signaling function through protein–protein interactions with downstream factors [5]. Over 22 NLR members have been described with important functions in signal transduction and inflammatory responses. Thus, some of those NLR family members such as NLRP1, NLRP3 and NLRC4 can form multiprotein complexes, called inflammasomes, leading to caspase-1 activation and subsequently secretion of pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 [6, 7].

2.2. ALR family

ALRs are a class of PRRs which recognizes cytosolic and nuclear pathogen DNA [8]. Members of this family include AIM2 and the interferon (IFN)-inducible protein 16 (IFI16) with act as dsDNA sensors activating inflammasome. ALRs are characterized by an N-terminal Pyrin signaling domain (PYD) and a C-terminal DNA-binding hematopoietic interferon (IFN)-inducible nuclear protein with a 200-amino-acid repeat (HIN200) domain. PYD domain mediates protein–protein bindings and interacts with

ASC, whereas the HIN domain is responsible for dsDNA recognition [9]. Correct binding and oligomerization require longer stretches of dsDNA, since both receptors lack from an ATP-dependent oligomerization NOD domain similar to that exhibited by members of the NLR family [10].

3. Inflammasome.

Inflammasomes are multiprotein complexes who play an essential role in the innate immune system, eliciting a defense response against pathogens and damaged cells [11]. Their structure consists in a sensor protein (a NLR, such as NLRP1, NLRP3 and NLRC4 or a ALR as AIM2), which recognizes PAMPs and DAMPs; an adaptor protein (ASC: apoptosis-associated speck-like protein containing a CARD domain), which acts as a bridge between NLRs/AIM2 and pro-caspase-1; and a effector molecule (precursor of caspase-1) [12]. Upon recognition of PAMPs and DAMPs by NLRs/AIM2, ASC is recruited to the complex via a pyrin-pyrin domain interaction, forming a very large supramolecular structure called pyroptosome. Complete inflammasome formation requires binding of pro-caspase-1 to ASC through CARD-CARD domains [13]. Inflammasome formation results in autocatalytic cleavage of pro-caspase 1 to form enzymatically active caspase 1. Activated caspase-1 leads to the maturation and secretion of pro-inflammatory cytokines IL-1β and IL-18 [14] or to the cleavage of gasdermin D protein which forms membrane pores to trigger pyroptosis, a class of programmed necrotic cell death [15, 16].

To date, five inflammasome complexes have been well established: NLRP1, NLRP3, NLRC4, AIM2, and the recently discovered Pyrin inflammasomes [17].

NLRP1, the first reported inflammasome, acts as a sensor for anthrax lethal toxin activity or *Toxoplasma gondii* infection [7]. It consists of a N-terminal pyrin domain (PYD), a NOD domain followed by LRRs, a functional-to-find (FIIND) domain, and a

C-terminal CARD domain. Since it contains both PYD and CARD domains, caspase 1 activation has been described in the absence of ASC through direct interaction between CARD motifs of NLRP1 and pro-caspase-1 [18].

NLRP3 inflammasome is the best studied and characterized of the inflammasomes. Importantly, NLRP3 inflammasome complex activation has been shown to involve a two-step process: a first signal or "priming" that lead to NF-κB activation, thus inducing NLRP3, pro-IL-1β and pro-IL-18 expression; and a second signal specific to the inflammasome. This can be triggered by many and diverse stimuli including bacteria pore-forming toxins, viruses, fungi, protozoa, reactive oxygen species (ROS) and non-microbial signals such as monosodium urate crystals or particulate matter as asbestos and silica, making NLRP3 the most versatile inflammasome [18-20].

Common molecular and cellular events must be responsible for the activation and assembly of the NLRP3 inflammasome, including K^+ efflux, Ca^{+2} mobilization, lysosomal rupture, ROS production and mitochondrial dysfunction. These mechanisms lead to the formation of the complex NLRP3, ASC, and pro-caspase-1. ASC is essential for interaction with pro-caspase-1 as NLRP3 has no a CARD domain. NLRP3 interacts with ASC through their PYD motifs and ASC with pro-caspase 1 via their CARD motifs. Besides, ATPase activity of NLRP3 is critical for the oligomerization of NLRP3 and its consequent activation [21].

NLRC4 is also an important sensor for inflammasome formation upon recognition of specific bacterial proteins, such as flagellin or type III secretion system proteins (T3SS) [22]. Among NLRs, NLRC4 exhibits special characteristics since it requires additional sensors, the NLR family apoptosis inhibitory proteins (NAIPs), that acts as cytosolic receptors for flagellin and T3SS [23]. Thus, members of this family as NAIP1 and NAIP2 interact with components of T3SS whereas NAIP5 and NAIP6 recognize

flagellin. Upon ligand binding, NAIPs recruit and co-oligomerize with NLRC4, and the resulting NAIP/NLRC4 inflammasome can activate caspase-1 [22, 23]. This activation may be mediated by ASC leading to IL-1β and IL-18 production and results in pyroptosis whether NLRC4 activates itself caspase-1 [24].

AIM2 has been described as the sensor for cytosolic dsDNA of several pathogens including cytomegalovirus, *Francisella tularensis* and vaccinia virus [25]. AIM2 is a member of the IFN-inducible PYHIN proteins that contain a C-terminal HIN-200 domain and an N-terminal pyrin domain (PYD) [26]. HIN200 domain binds dsDNA and PYD–PYD interactions result on ASC recruitment and caspase-1 activation.

4. Inflammasomes in inflammatory diseases.

Despite the enormous importance of inflammasomes in the physiological modulation of innate immune response, numerous evidences have demonstrated that inflammasome dysregulation is linked to a variety of inflammatory diseases. Thus, aberrant inflammasome activation resulting in excessive secretion of interleukins and induction of inflammatory cell death has been associated with the pathogenesis of cardiovascular disease as atherosclerosis, diabetes, neurological disorders as Alzheimer and Parkinson´s disease, gout, a group of rare autoinflammatory diseases named as cryopyrin-associated periodic syndromes (CAPS), kidney diseases, colitis, arthritis and psoriasis, among others [27-32].

Single nucleotide polymorphisms (SNPs) in the promoter and coding regions of NLRP1 have been linked to vitiligo, Addison's disease, and type I diabetes [27]. These mutations were related to conformational changes in NLRP1 resulting in an excessive production of the pro-inflammatory cytokines IL-1β and IL-18. Furthermore, NLRP1 upregulation in response to amyloid-β (Aβ) aggregates has been observed in

Alzheimer's disease (AD), producing caspase-1 activation and subsequent neuronal cell death [33].

Gain-of-function mutations in NLRP3 are associated with a group of rare autoinflammatory diseases known as CAPS, characterized by urticarial skin rashes and recurrent fever and inflammation. These fever syndromes included familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and chronic infantile neurological cutaneous and articular syndrome/neonatal onset multisystem inflammatory disease (CINCA/NOMID), ranging from the most mild to the most severe, respectively [27]. Similarly to NLRP1 mutations, NLPR3 changes also results in continuous caspase-1 activation leading to an excessive secretion of IL-1β production [34]. Opposite, an increased susceptibility to Crohn's disease has been associated with a reduced IL-1β production due to a defective NLPR3 expression [35]. Interestingly, contradictory evidences about the role of NLPR3 have been reported in ulcerative colitis, another inflammatory bowel disease (IBD). Thus, detrimental or protective effects have been described for NLRP3 in this pathology mainly dependent on differences in the composition of the microbiota or genetic background [36]. Additionally, recent evidences demonstrate that NLPR3 inflammasome is involved on atherosclerosis development or gout as cholesterol or monosodium urate (MSU) crystals act as inflammasome sensors [37, 38].

In the same line as the previously reported NLRP1 and NLRP3 mutations, defects on NLRC4 resulting in a hyperactivation of inflammasome are related with spontaneous autoinflammatory symptoms resulting in pathologies as syndrome of enterocolitis, macrophage activation syndrome (MAS) or atopic dermatitis [39-42].

Regarding AIM2 inflammasome, its ability to recognize its self-DNA contributes to the development of various autoimmune and autoinflammatory diseases including

psoriasis, arthritis, systemic lupus erythematosus, dermatitis and abdominal aortic aneurysm [43-46].

5. Natural compounds

Natural products and their derivatives represent a successful source in drug discovery and development of new therapeutic agents. Terpenoids, a structurally diverse group, are bioactive compounds widely distributed in nature and endowed with a wide range of biological activities. They have shown to exhibit potent anti-inflammatory and immunomodulatory properties in multiple inflammatory disorders [47-51]. Interestingly, these compounds have gained attention since they can act on specific and/or multiple molecular and cellular targets.

The great anti-inflammatory potential of these compounds has been largely associated to inhibition of classical inflammatory signaling pathways, as the activation of the NF-κB transcription factor and Mitogen-activated protein kinases (MAPKs) in various experimental disease models [49, 52, 53].

In recent years, inflammasome has emerged as a new therapeutic target in different inflammatory driven human diseases [27]. So far, one of the best studied inflammasomes is NLRP3. In the search of drugs targeting the inflammasome, several terpenoids have been evaluated against NLRP3 inflammasome complex. Accordingly, this work highlights and critically examines the current knowledge about terpenoids as potential NLRP3 inflammasome inhibitors (Fig. 1 and Table 1).

The first natural product found to inhibit inflammasome activity via direct targeting of caspase-1 and NLRP3 was parthenolide, a sesquiterpene lactone isolated from *Tanacetum parthenium* L. (Asteraceae) (feverfew) [54]. Since then, several terpenoids have demonstrated to exert inhibitory effects on this pathway.

Triptolide

Triptolide was isolated from the Chinese herb *Tripterygium wilfordii* Hook F (Celastraceae). Both *in vitro* and *in vivo* antifibrotic effects of this diterpenoid were reported in a mouse model of cardiac fibrosis [55]. Experiments revealed its molecular mechanism with insights into the NLRP3 inflammasome pathway, through a dual role, by downregulating the expression of NLRP3 inflammasome components as NLRP3 and ASC, and by interrupting NLRP3 inflammasome assembly. Triptolide also demonstrated therapeutic effects in a mouse model of myocardial remodeling induced by chronic pressure overload by decreasing cytokine (IL-1β, IL-18) serum levels and downregulating the profibrotic factor TGF- β1 activation [56]. In the same context of inhibition of inflammasome-mediated pro-inflammatory cytokines production, downregulation of NLRP3 and TLR4 expression with improvement of functional renal parameters by triptolide was observed in a rat kidney injury model [57].

Oridonin

Oridonin, an *ent*-kaurane diterpenoid, is the major active constituent from *Rabdosia rubescens* (Hemsl.) Hara (Lamiaceae). He *et al*. (2018) demonstrated that oridonin is a specific covalent inhibitor on NLRP3 inflammasome assembly and activation [58]. It acts as a Michael receptor thanks to the α,β-unsaturated carbonyl unit and covalently binds to Cys279 of NACHT domain of NLRP3 to block the interaction between NLRP3 and NEK7, a critical step for the subsequent NLRP3 oligomerization and recruitment of ASC. Dose-dependent inhibitory effects on caspase-1 cleavage, IL-1β secretion, and cell death after stimulation by different NLRP3 agonists [nigericin, MSU, ATP or cytosolic LPS (cLPS)] have been shown. Furthermore, this study also demonstrated that oridonin suppressed NLRP3-dependent inflammation in mouse models of chronic inflammatory

diseases such as peritonitis, gouty arthritis and type 2 diabetes (T2D) [58]. Additionally, oridonin exerted protective effects in a model of LPS-induced acute lung injury by downregulation of the expression of NLRP3-inflammasome components and inhibition of NF-κB pathway. Interestingly, oridonin protective effects in lung injury were due not only for its anti-inflammatory activity but also for its Nrf2-dependent antioxidative activity [59].

Andrographolide

Andrographolide is a labdane diterpenoid from *Andrographis paniculata* (Burm.f.) Nees (Acanthaceae). Inhibitory effects of andrographolide on NLRP3 inflammasome activation in experimental non-alcoholic steatohepatitis, lung injury, and colitis-associated cancer have been reported [60-62]. In these models, inhibitory effects of andrographolide have been related to the disruption of the NLRP3-CASP1 assembly, thereby inhibiting caspase-1 activation and leading to a decrease in the IL-1β secretion. Interestingly, in nonalcoholic steatohepatitis, andrographolide's modulatory effect was also mediated by a NF-κB-dependent mechanism [60]. Finally, similar mechanisms have been described in intracerebral hemorrhage (ICH)-induced secondary brain injury (SBI) [63]. Andrographolide reduced neuroinflammation via blocking the assembly of the NLRP3/ASC/CASP-1 together with the inhibition of pyroptosis.

Celastrol

The triterpenoid celastrol is the most promising bioactive compound isolated from *Tripterygium wilfordii* Hook F (Celastraceae). Protective effects of celastrol in a model of LPS-induced septic shock and dextran sulfate sodium (DSS)-induced colitis, identified this compound as a NLRP3 inhibitor [64, 65]. Interruption of ASC oligomerization and

autophagy activation by the triterpenoid were the main mechanisms involved, leading to the inhibition of caspase-1 activation and IL-1β and IL-18 release. Of note, this compound has been reported to inhibit NLRP3 inflammasome activation by reducing both the priming and activating signals. Thus, celastrol suppressed pyroptosis and reduced the secretion of IL-1β and IL-18 by inhibiting the expression of NLRP3 and the cleavage of caspase-1 in response to LPS/ATP induction, but also inhibited the NF-κB pathway and ROS production acting as a dual inhibitor [66, 67].

In contrast to the previous terpenoids, **genipin**, an iridoid monoterpenoid isolated from *Gardenia jasminoides* Ellis (Rubiaceae) fruits has been reported to inhibit not only NLRP3 but also NLRC4 inflammasome activation. Preincubation of macrophages with genipin specifically inhibited IL-1β secretion and caspase-1 cleavage, without affecting TNF-α, pro-IL-1β, pro-caspase-1 and ASC levels. Genipin prevented ASC-complex formation, but not NLRC4-mediated ASC oligomerization, suggesting that the compound may affect upstream signaling. In fact, it was demonstrated that genipin inhibited the autophagy signaling pathway and ROS production acting at the mitochondrial uncoupling protein-2 (UCP2) level, which leads to NLRP3 and NLRC4 inflammasome suppression [68, 69]. These results were confirmed *in vivo* in models of peritonitis, lung inflammation and hepatic injury [68, 70] .

Collectively, these findings identify NLRP3 inflammasome activation as a critical target involved in the anti-inflammatory mechanism of these terpenoids, suggesting the role of these natural products as promising therapeutic agents in various inflammatory diseases (Fig. 2). In addition, the well-known inhibitory effect of some of these compounds on NF-κB activation opens new strategies to develop dual inhibitors which can simultaneously suppress both inflammasome activation and NF-κB-dependent activation.

6. Other non-natural compounds as inhibitors

In addition to terpenoids, several small molecules have been identified as specific pharmacological inhibitors to NLRP3 inflammasome [71].

The vinyl sulfone **BAY 11-7082**, selectively inhibited activation of the NLRP3 inflammasome targeting its ATPase activity in an irreversible way. BAY 11-7082 showed to inhibit NLRP3-induced ASC pyroptosome formation and pyroptotic cell death in macrophages [54] and in a mouse model of psoriasis-like dermatitis [72].

MCC950, a diarylsulfonylurea-containing compound, impaired the activation of caspase-1 by NLRP3 and IL-1β release in both mouse and human macrophages, without altering pro-caspase-1, pro-IL-1β or ASC expression [73]. It blocked NLRP3 induced ASC oligomerization, but it did not prevent NLRP3-NLRP3 or NLRP3-ASC interactions. MCC950 specifically blocked NLRP3-dependent pyroptotic cell death, but had no effect on potassium efflux or calcium flux. MCC950 showed to reduced IL-1β and IL-18 levels *in vivo*, attenuating the severity of autoimmune encephalomyelitis, ulcerative colitis and CAPS disease models [73, 74]. Interestingly, new insights into the mechanism of action of MCC950 have shown that this molecule induces conformational changes on NLRP3, leading to an inactive state [75].

Tranilast is an analog of a tryptophan metabolite which has been traditionally used as an anti-allergic drug. Tranilast directly binds to NLRP3 and suppresses its oligomerization blocking NLRP3-NLRP3 interaction in an ATPase-independent manner, so no effects on the upstream events potassium efflux, mitochondrial damage or chloride efflux are detected. Inhibition of inflammasome activation is involved in the anti-inflammatory effects of tranilast on mouse models of gouty arthritis, CAPS diseases, and T2D [76].

In contrast to tranilast, **CY-09**, directly binds to the ATP-binding motif of NLRP3 NACHT domain and inhibits NLRP3 ATPase activity, resulting in the suppression of

NLRP3 inflammasome assembly and activation. Beneficial effects in mice models of T2D and CAPS, as well as *ex vivo* in monocytes from patients with gout were reported [77].

Finally, an orally active molecule called **OLT1177 or dapansutrile** has also been recently described to prevent NLRP3-ASC and NLRP3-caspase-1 interactions, blocking inflammasome oligomerization in macrophages. Potassium efflux and gene expression of pro-IL-1β were not affected by OLT1177. These effects were confirmed *ex vivo* in neutrophils and monocytes isolated from patients with CAPS and *in vivo* reducing the severity of LPS-induced systemic inflammation in mice [78]. Treatment with OLT1177 also reduced joint inflammation in reactive and gouty mouse arthritis, with a significant decrease of IL-1β and IL-6 secretion [79]. These results together with its favorable phase I safety profile, encouraged to the evaluation of oral treatment with OLT1177 in phase II clinical trials.

Conclusion and future directions

Inflammation has long been identified as a critical factor of many inflammatory conditions and autoimmunity. In recent years, significant progress has been made to understand the inflammasomes, specifically NLRP3, its regulation and role in diverse human diseases. Although inhibition of the NLRP3 inflammasome reduces inflammation in murine models of acute and chronic inflammatory diseases, there is currently no approved inflammasome inhibitor for human use.

A few compounds have been reported to possess potential inhibitory effects on NLRP3 inflammasome activation. Emerging evidence in the literature have revealed that some natural products, in particular terpenoids are capable of inhibiting the activation of NLRP3 inflammasome to modulate inflammatory disease pathogenicity, as detailed in

this commentary. The summarized studies revealed that terpenoids regulates the NLRP3 inflammasome with beneficial effects in several experimental models of chronic inflammation-associated diseases (colitis, CAPS, gouty arthritis….).

In addition, terpenoids have long been identified as potent inhibitors of the transcription factor NF-κB, a pivotal regulator for gene transcription of pro-inflammatory cytokines and chemokines implicated in numerous immune and chronic inflammatory disorders. To date, commercially available anti-inflammatory agents are potent inhibitors of cytokine production in these pathologies by interrupting NF-κB signaling pathway. Interestingly, NF-κB is also a first signal in NLRP3 inflammasome activation.

Although many challenges remain for elucidating the exact mechanisms by which terpenoids regulate the activation of NLRP3 inflammasome complex, recent studies suggest that terpenoids represent an interesting pharmacological approach as anti-inflammatory agents. These compounds could offer pleiotropic dual effects through blockade on both NF-κB signaling and NLRP3 inflammasome activation. This advantageous molecular mechanism opens new strategies to develop dual inhibitors. In conclusion, terpenoids are emerging as very promising candidates for the development of safe and efficacious novel therapeutics for the management of NLRP3-inflammatory diseases.

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Declaration of Competing Interest

None

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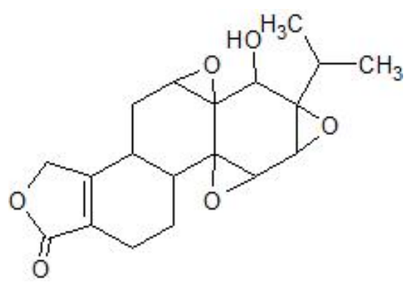
Figure legends

Fig. 1. Chemical structures of relevant terpenoids as NLPR3-inflammasome inhibitors.

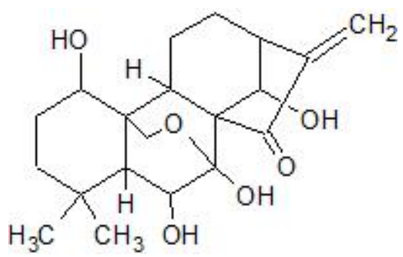
Table 1. Terpenoids inhibiting NLRP3 inflammasome activation in experimental models.

Fig. 2. Illustration for the mechanisms by which terpenoids impair NLRP3 inflammasome activation.

Figure



Triptolide



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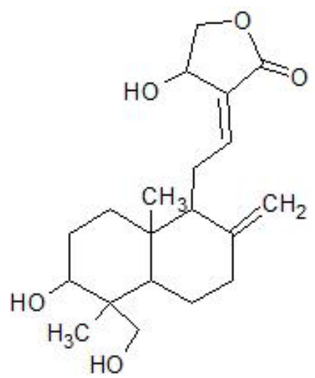

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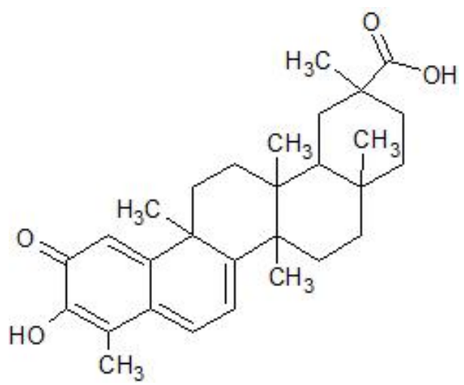
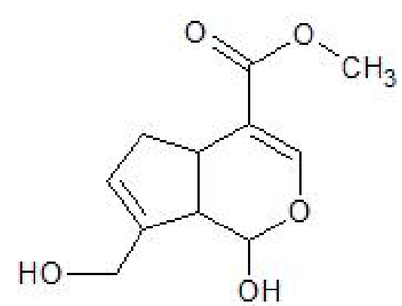


Celastrol



Genipin

Figure 1. Chemical structures of relevant terpenoids as NLPR3-inflammasome inhibitors

Table 1. Terpenoids inhibiting NLRP3 inflammasome activation in experimental models

Terpenoid	Molecular mechanism	Experimental models	References
Triptolide	Interruption of NLRP3-ASC assembly and suppression TLR4 expression	Cardiac fibrosis Myocardial remodeling Kidney injury	[55] [56] [57]
Oridonin	Block the interaction between NLRP3 and NEK-7	MSU-induced peritonitis and gouty arthritis Type 2 diabetes LPS-induced acute lung injury	[58] [59]
Andrographolide	Disruption of NLRP3-PYCARD-CASP1 complex assembly and reduction in the expression of inflammasome components through NF-κB inhibition	Nonalcoholic steatohepatitis (NASH) Lung injury Colitis-associated cancer (CAC)	[60] [61] [62]
Celastrol	Block ASC oligomerization and NLRP3 complex formation, autophagy, NFκB- signaling pathway and ROS production	Dextran sodium sulfate(DSS)-induced colitis LPS-induced septic shock	[64] [65] [64] [66] [67]
Genipin	Inhibition of NLRP3-mediated ASC oligomerization, autophagy signaling pathway and ROS production	LPS- and aluminium-induced peritonitis Flagellin-induced lung inflammation D-galactosamine and LPS-induced hepatic injury	[68] [68] [70] [69]

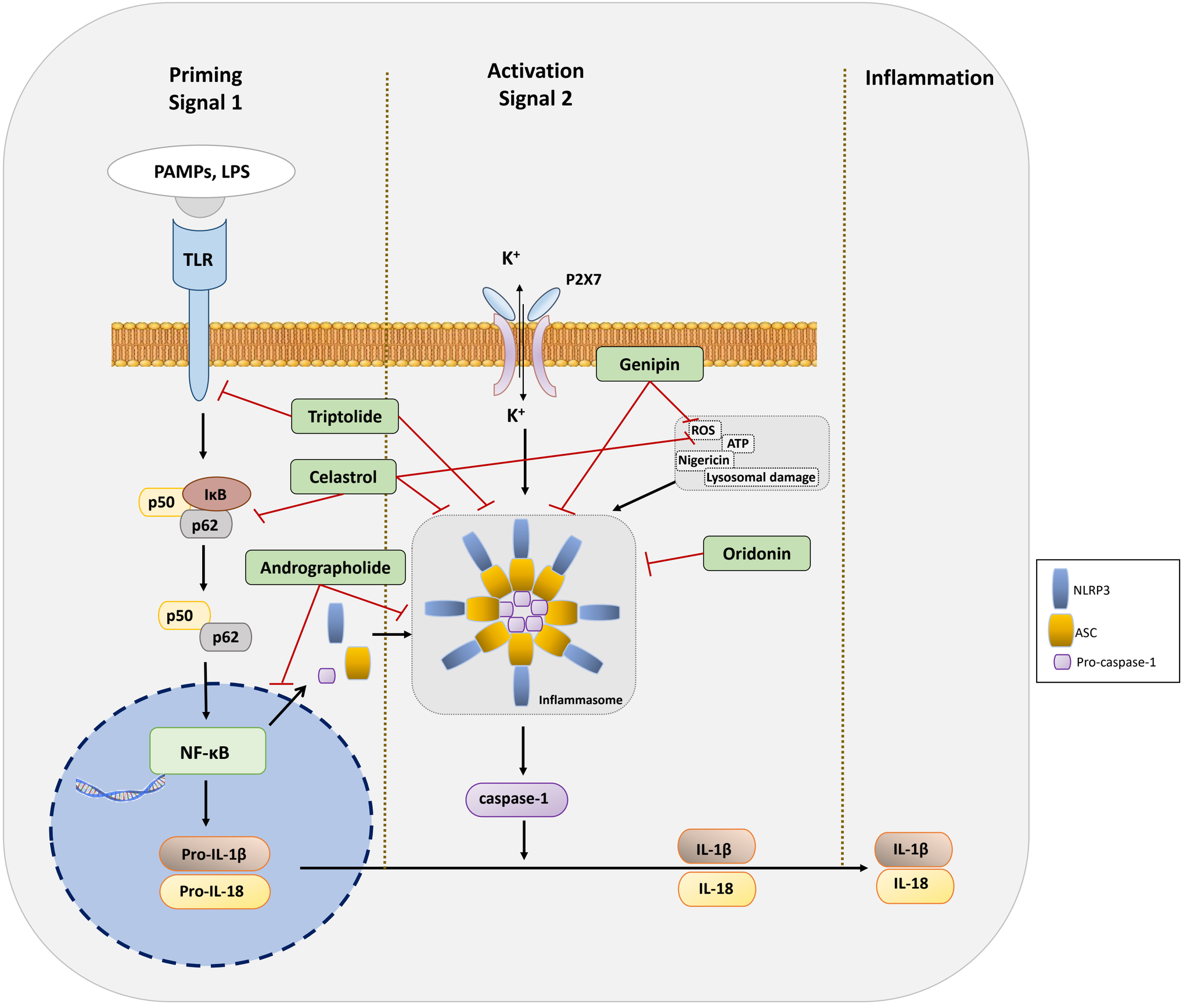


Figure 2. Illustration for the mechanisms by which terpenoids impair NLRP3 inflammasome activation