

Inhibitors of the NLRP3 inflammasome pathway as promising therapeutic candidates for inflammatory diseases (Review)

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Abstract. The inflammasome regulates innate immunity by serving as a signaling platform. The Nod-like receptor protein 3 (NLRP3) inflammasome, equipped with NLRP3, the adaptor protein apoptosis-associated speck-like protein (ASC) and pro-caspase-1, is by far the most extensively studied and well-characterized inflammasome. A variety of stimuli can activate the NLRP3 inflammasome. When activated, the NLRP3 protein recruits the adaptor ASC protein and activates pro-caspase-1, resulting in inflammatory cytokine maturation and secretion, which is associated with inflammation

and pyroptosis. However, the aberrant activation of the NLRP3 inflammasome has been linked to various inflammatory diseases, including atherosclerosis, ischemic stroke, Alzheimer's disease, diabetes mellitus and inflammatory bowel disease. Therefore, the NLRP3 inflammasome has emerged as a promising therapeutic target for inflammatory diseases. In the present review, systematic searches were performed using 'NLRP3 inhibitor(s)' and 'inflammatory disease(s)' as key words. By browsing the literature from 2012 to 2022, 100 articles were retrieved, of which 35 were excluded as they were reviews, editorials, retracted or unavailable online, and 65 articles were included. According to the retrieved literature, the current understanding of NLRP3 inflammasome pathway activation in inflammatory diseases was summarize, and inhibitors of the NLRP3 inflammasome pathway targeting the NLRP3 protein and other inflammasome components or products were highlighted. Additionally, the present review briefly discusses the current novel efforts in clinical research.

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Abbreviations: ATPase, adenosine triphosphate; AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein; A β , amyloid β ; AD, Alzheimer's disease; BBB, blood-brain barrier; CAPS, cryopyrin-associated periodic syndrome; CVD, cardiovascular disease; CIRI, cerebral ischemia/reperfusion injury; DAMPs, danger-associated molecular patterns; DMF, dimethyl fumarate; DCA, directional coronary atherectomy; DM, diabetes mellitus; DSS, dextran sulfate sodium; ER, endoplasmic reticulum; GSDMD, gasdermin D; HFD, high-fat diet; IL, interleukin; IBD, inflammatory bowel disease; LRR, leucine-rich repeat; LPS, lipopolysaccharide; LDLR, low-density lipoprotein receptor; mtROS, mitochondrial reactive oxygen species; MI, myocardial infarction; MNS, 3,4-methylenedioxy- β -nitrostyrene; MCNs, mouse cortical neurons; NLRs, NOD-like receptors; NF- κ B, nuclear factor- κ B; NSA, necrosulfonamide; PAMPs, pathogen-associated molecular patterns; PRRs, pattern recognition receptors; ROS, reactive oxygen species; TLR, Toll like receptor; T2DM, type 2 diabetes mellitus; TXNIP, thioredoxin-interacting protein

Key words: inflammatory diseases, pyroptosis, inflammation, Nod-like receptor protein 3 inflammasome inhibitors, clinical perspectives

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1. Introduction

The innate immune response, often known as non-specific immunity, is the body's first line of defense (1) and recognizes pathogen-associated molecular patterns (PAMPs) and host-derived danger-associated molecular patterns (DAMPs) via pattern recognition receptors (PRRs) (2). NOD-like receptors (NLRs), which belong to the evolutionarily well conserved PRR family are located in the cytoplasm. By recruiting downstream adaptor proteins, they can form inflammasome complexes that promote the maturation and secretion of inflammatory mediators, including interleukin (IL)-1 β and

IL-18, resulting in inflammatory reactions. A total of five major inflammasomes currently exist, i.e., NLRP1, NLRP3, NLRC4, NLRP6 and absent in melanoma 2 (AIM2), which are activated to promote inflammasome-dependent immune responses when they recognize PAMPs and DAMPs from pathogenic microorganisms (3). The activation of the majority of inflammasomes is dependent on a few highly specific agonists; however, the NLRP3 inflammasome can be activated by various unrelated stimuli, including K^+ , Cl^- , Ca^{2+} , lysosomal destruction, mitochondrial dysfunction and metabolic alterations (4). Additionally, inflammasomes are activated when PAMP receptors, including Toll-like receptors, recognize their ligands. Concomitant with the cleavage of IL-1 β and IL-18, gasdermin D (GSDMD) is cleaved by activated caspase-1, resulting in a lytic-regulated cell death mode, termed pyroptosis. Upon cleavage, the N-terminus of GSDMD binds to membrane lipids and forms micropores, causing cell rupture and the occurrence of an inflammatory cascade (5). Accordingly, apart from being crucial to the resistance to pathogen invasion, the NLRP3 inflammasome modulates inflammation (6). There is increasing evidence to suggest that inflammatory diseases can be treated more effectively by targeting the NLRP3 inflammasome (7), including atherosclerosis (8), ischemic stroke (9), Alzheimer's disease (AD) (10), diabetes mellitus (DM) (11) and inflammatory bowel disease (IBD) (12). A therapeutic strategy for inflammatory disorders focuses on recombinant cytokine receptor antagonists and neutralizing antibodies targeting the IL-1 family (7). Nonetheless, there is an increased risk of infection associated with cytokine therapy. Inhibitors targeting the NLRP3 inflammasome pathway rather than effector molecules currently exhibit desired prevention or therapeutic effects in animal models of inflammatory diseases, as discussed below. In the present review, systematic searches in the title, key words and abstract of articles were performed using the PubMed and Web of Science databases with 'NLRP3 inhibitor(s)' and 'inflammatory disease(s)' as key words at initial retrieval. By browsing the literature from 2012 to 2022, 100 articles were retrieved, of which 35 were excluded as they were reviews, editorials, retracted, or unavailable online, and 65 articles were included. NLRP3 inhibitors were shown to relieve inflammatory diseases. Therefore, when discussing specific inflammatory diseases, the present review included further literature by combining 'Atherosclerosis', 'ischemic stroke', 'Alzheimer's disease', 'Diabetes mellitus' and 'Inflammatory bowel disease', respectively with 'NLRP3 inhibitor(s)' as key words. According to the retrieved literature, a brief review of studies on LRP3 inflammasome inhibitors is presented herein, in an aim to aid the development of NLRP3 inflammasome-related disease drugs.

2. Biology of the NLRP3 inflammasome

Due to inflammatory stimuli, the NLRP3 inflammasome is predominantly found in immune and inflammatory cells (13,14), and is equipped with NLRP3, the adaptor protein apoptosis-associated speck-like protein (ASC) and pro-caspase-1 (15). The NLRP3 protein comprises three main components, i.e., a leucine-rich repeat (LRR), a central nucleotide-binding oligomerization domain (NOD), also known as NACHT, in the carboxy terminus, and a pyrin domain (PYD)

in the amino-terminal. Similar to Toll-like receptor (TLR), LRR recognizes and binds PAMP or DAMP stimuli; PYD is the functional region connecting downstream bridging proteins to effector molecules; NOD is the core part of NLRs that undergoes oligomerization when the LRR recognizes and binds PAMPs or DAMPs and exerts adenosine triphosphate (ATPase) activity for the self-association and function of NLRP3 (4).

NLRP3 inflammasome activation is tightly regulated, due to a two-step process known as priming and assembly (16) (Fig. 1). The priming step, indicated as 'the first signal', is initiated by TLR and nuclear factor- κ B (NF- κ B) to increase the intracellular transcript levels of pro-IL-1 β , pro-IL-18 and NLRP3 (17,18). Once primed, subsequent NLRP3 inflammasome activation by NLRP3 oligomerization and the later NLRP3 inflammasome assembly is termed 'the second signal' (19).

Studies have reported four possible models [K^+ efflux, lysosomal damage, reactive oxygen species (ROS) and Ca^{2+} mobilization] (20) for NLRP3 inflammasome activation (Fig. 1), which may not be exclusive. i) K^+ efflux: Multiple signaling pathways initiated by PAMPs/DAMPs can converge on K^+ efflux (21), resulting in NLRP3-NEK (NIMA-related kinase) interaction, further activating the NLRP3 inflammasome. As several NLRP3 activators reduce intracellular K^+ concentrations, K^+ efflux is a key function in NLRP3 inflammasome activation (21). Research has indicated that the incubation of bone marrow-derived macrophages in potassium-free buffer induces potent mitochondrial damage and ROS production to promote NLRP3 inflammasome activation (22). By contrast, NLRP3 inflammasome activation has been shown to be suppressed by the increasing extracellular K^+ concentration (22). There is a well-conserved serine or threonine kinase known as NIMA-related kinase (NEK)7, a key component of the NLRP3 inflammasome (23). As a downstream component of the K^+ efflux, NEK7 participates in NLRP3 activation (23). ii) Lysosomal damage: Due to the phagocytosis of crystals or specific ligands, including monosodium urate (24), silica (25) and amyloid- β (A β) (26), lysosomal damage occurs, releasing its contents. Lysosomal contents, specifically cathepsin B, can activate the NLRP3 inflammasome via a direct interaction (23). iii) ROS: A majority of NLRP3 stimuli, including ATP and asbestos, generate ROS, directly causing the combination of thioredoxin-interacting protein (TXNIP) with NLRP3 and activating it (27). Several small molecules targeting the mitochondria produce mitochondrial ROS (mtROS), which activates the NLRP3 inflammasome complex (28). Previous research, however, has revealed that while N-acetyl cysteine (NAC) suppresses NLRP3 activation by blocking ROS in wild-type macrophages stimulated with lipopolysaccharide (LPS)/silica or LPS/nigericin, caspase-1 activation is not inhibited when NLRP3 expression is uncoupled from the priming signal by stable overexpression. As such, ROS affects NLRP3 inflammasome activation only during priming, but not during activation (4,29). As a key molecular upstream regulator of the NLRP3 inflammasome, ROS are able to activate the NLRP3 inflammasome; however, their role in this process has not yet been fully elucidated. iv) Ca^{2+} mobilization: Increased cytosolic Ca^{2+} concentration (Ca^{2+} overload)

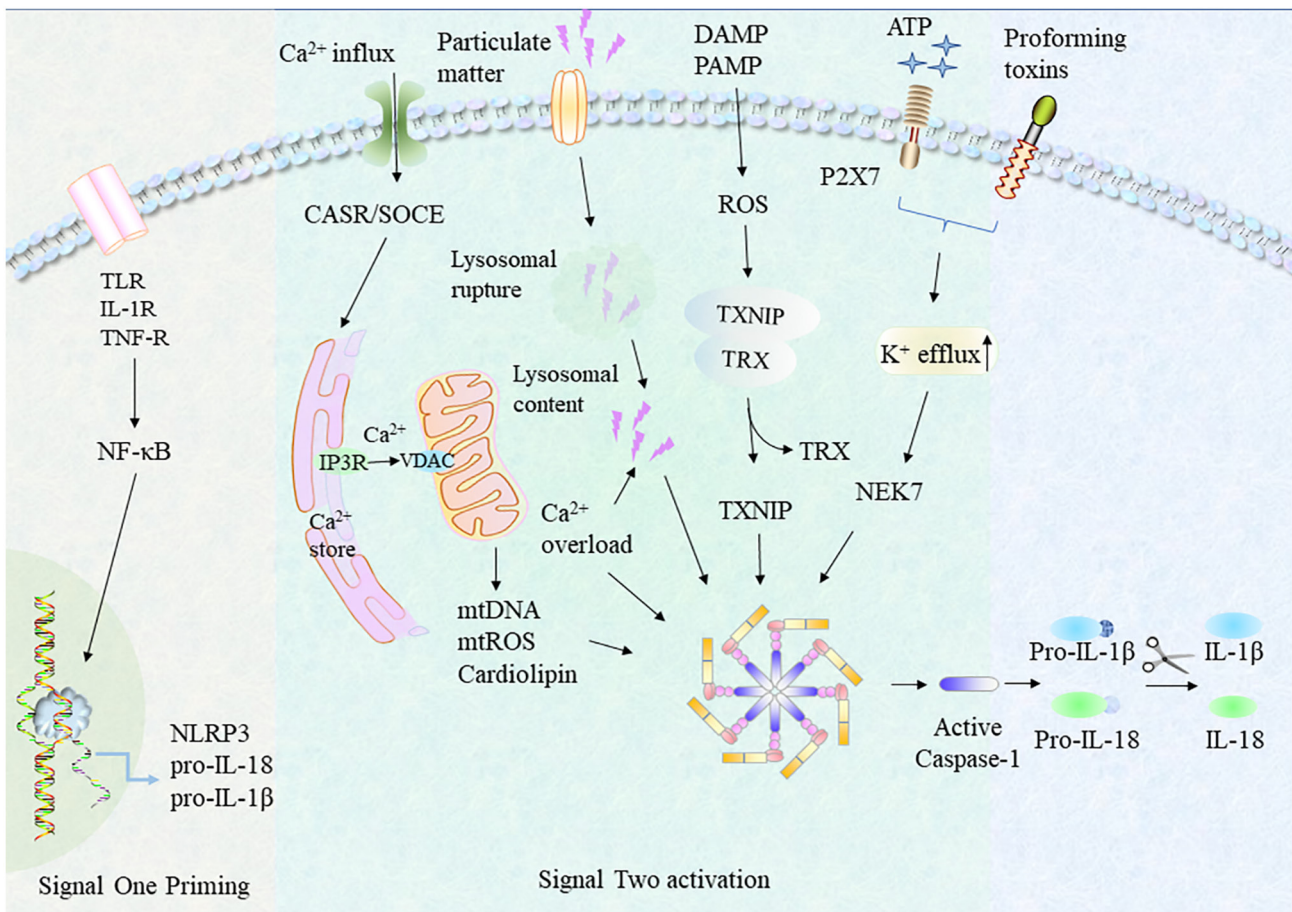


Figure 1. Model and regulation of NLRP3 inflammasome activation. The priming step involves the transcriptional of NLRP3 inflammasome key components via TLR IL-1R and TNF-R and subsequent NF-κB signaling. At the activation step, the NLRP3 inflammasome is activated by diverse stimuli, including K⁺ efflux, Ca²⁺ overload, ROS, and lysosomal rupture to trigger NLRP3 inflammasome assembly, resulting in IL-1β and IL-18 release. NLRP3, Nod-like receptor protein 3; TLR, Toll-like receptor; IL, interleukin; DAMP, danger-associated molecular pattern; PAMP, pathogen-associated molecular pattern; ATP, adenosine triphosphate; CASR, calcium-sensing receptor; ROS, reactive oxygen species; TXNIP, thioredoxin-interacting protein; TRX, thioredoxin; NEK7, NIMA-related kinase 7; mtDNA, mitochondrial DNA; mtROS, mitochondrial ROS.

results from NLRP3 agonists inducing the mobilization of Ca²⁺ from endoplasmic reticulum (ER) Ca²⁺ stores or extracellular milieu. Through Ca²⁺ channels, calcium ions are released from the ER, the intracellular calcium storage pool, when cells are stimulated (30). Researchers have demonstrated that intracellular Ca²⁺ levels are increased by calcium-sensing receptor (31,32). It has been shown that both calcium influx and ER calcium release are required for essential NLRP3 activation (33), causing the assembly of the NLRP3 inflammasome complex (Fig. 1). NLRP3 inflammasome activation is a complex process, including protein transcription and translation, post-translational modification and protein-protein interaction (34). Although research has been documented on the activation process of NLRP3 inflammasomes, the specific mechanisms remain unclear in different diseases.

3. Pathophysiological role of the NLRP3 inflammasome in inflammatory diseases

The NLRP3 inflammasome is crucial for innate immunity; however, its aberrant activation promotes various inflammatory disorders, including atherosclerosis, ischemic stroke, AD, DM and IBD (Fig. 2).

Atherosclerosis. Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality across the globe (35). Ischemic CVD is largely caused by atherosclerosis, a chronic inflammatory disease of the arterial wall caused by lipids (36). In patients with atherosclerosis, the NLRP3 inflammasome is highly expressed in the aorta (37). NLRP3 activation significantly increases macrophage lipid deposition susceptibility and migration capacity, hence promoting atherosclerosis. In advanced atherosclerosis, the NLRP3 inflammasome is crucial for necrotic core formation, and its silencing increases plaque stability (38). However, other researchers have observed opposite effects. NLRP3 also promotes the proliferation and migration of vascular smooth muscle cells (VSMCs) in vessels (39), which may contribute to vascular remodeling and plaque stability. Smoking affects the stages of atherosclerosis, and is hence one of the major independent risk factors. Cigarette smoke extracts impair the cardiovascular system *in vitro* by activating the nuclear factor erythroid2-related factor 2 pathway and inducing ROS generation, hence activating the NLRP3 inflammasome (40,41). Furthermore, cigarette smoke condensate induces THP-1 monocyte differentiation into macrophages (42), which, in combination with a high-fat diet (HFD), exert a synergistic promoting effect

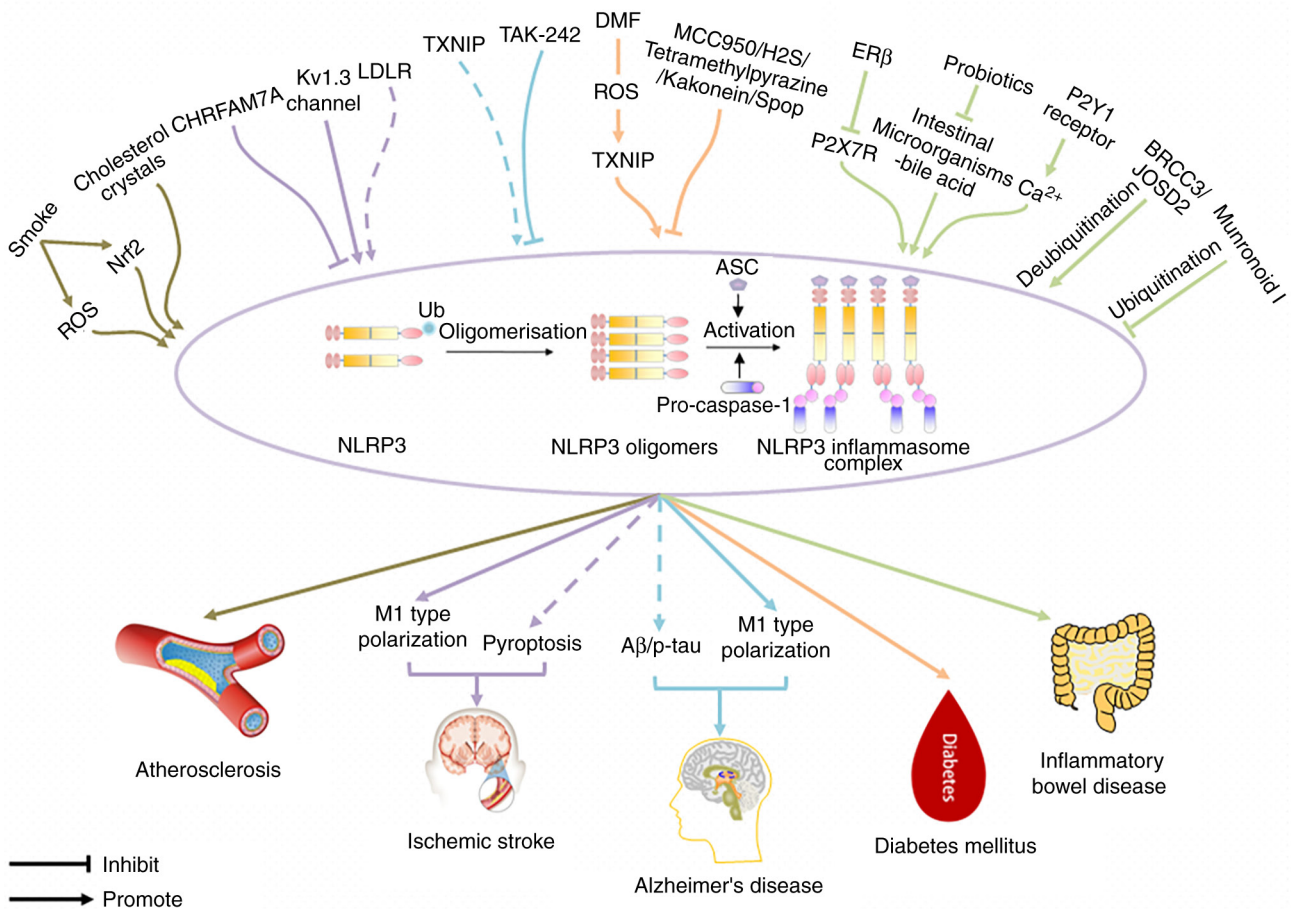


Figure 2. Pathophysiological role of the NLRP3 inflammasome in inflammatory diseases. Different pathological factors can cause the aberrant activation of the NLRP3 inflammasome, leading to the development of various inflammatory diseases, such as atherosclerosis, ischemic stroke, Alzheimer's disease, diabetes mellitus and inflammatory bowel disease. The same color represents the same disease. The dotted and solid lines in the same disease represent different mechanisms. NLRP3, Nod-like receptor protein 3; BRCC3, BRCA1/BRCA2-containing complex 3; DMF, dimethyl fumarate; ERβ, estrogen receptor β; JOSD2, Josephin domain containing 2; LDLR, low density lipoprotein receptor; Nrf2, nuclear factor erythroid2-related factor 2; TXNIP, thioredoxin-interacting protein; ROS, reactive oxygen species.

on atherosclerosis (43). During atherogenesis, the formation of cholesterol crystals in the vessel wall initiates plaque inflammation by activating the NLRP3 inflammasome during atherogenesis (44). Similarly, the NLRP3 inflammasome is involved in hyperglycemia-induced endothelial inflammation and diabetes-related atherosclerosis (45).

Ischemic stroke. Ischemic stroke is caused by cerebral ischemia, which eventually causes lifelong disability or mortality (46). It is characterized by an inflammatory response responsible for its pathophysiology (47). Neuroinflammation due to ischemic stroke is controlled by microglia, which are categorized into M1-like (pro-inflammatory) and M2-like (anti-inflammatory pro-regenerative) phenotypes (48). An increased number of M1 microglia is caused by dysregulated microglia polarization dynamics, resulting in post-stroke injury expansion (49,50). The NLRP3 inflammasome promotes the development of ischemic stroke, primarily by promoting microglial polarization. For instance, ischemic stroke increases NLRP3 inflammasome expression and activation (51). Liu *et al* (52) and Zhao *et al* (53) found that the NLRP3 inflammasome was activated in the microglia and astrocytes affected by cerebral ischemia/reperfusion injury (CIRI). The Kv1.3 channel,

a transmembrane protein, is involved in the production of inflammatory cytokines and ROS (49), and even promotes neuronal death (54). Cerebral ischemic injury is alleviated by inhibiting Kv1.3 channels, which may be related to the remodeling of the microglial phenotypic response from M1 to M2 as well as inhibiting NLRP3 inflammasome activation and IL-1β release (49). In addition, the injection of salvianolic acids has been found to generate similar effects, altering the microglial phenotype from M1 to M2 by suppressing the pyroptosis mediated by the NLRP3 inflammasome (55). ChRFAM7A, a dominant-negative inhibitor of α7 nicotinic acetylcholine receptor (α7nAChR, coded by CHRNA7), causes brain disorders (56). ChRFAM7A overexpression attenuates CIRI by inhibiting microglial pyroptosis via the NLRP3/caspase-1 pathway and promoting M2 microglial polarization (57). In addition to microglia, NLRP3 expression is upregulated in endothelial cells and neurons following stroke (58,59). Low-density lipoprotein (LDL) receptors (LDLRs) regulate cholesterol uptake and exhibit anti-inflammatory properties (47). Research indicates that LDLRs play a modulatory role in LRP3-mediated neuronal pyroptosis and inflammation following ischemic stroke (47). Furthermore, LDLR knockout increases caspase-1/GSDMD expression, resulting in severe

neuronal pyroptosis (47). By contrast, opposite findings have also been reported, demonstrating that ischemic brain injury is reduced in *ASC^{-/-}*, *AIM2^{-/-}* and *NLRC4^{-/-}* mice and not in mice deficient for the canonical sensor of sterile injury, NLRP3 (60).

AD. AD is the most prevalent type of dementia among the elderly, characterized by hyper-phosphorylated tau protein and A β accumulation (61). Moreover, numerous inflammatory markers are present in the AD-affected brain, including inflammatory cytokines and chemokines (62). Senile plaques activate microglia, contributing to cerebral neuroinflammation, which is termed the third core pathological characteristic of AD (63). TLR4 functions as a 'priming' signal for the NLRP3 inflammasome activation (64), unlike TLR4, whose inhibitor (TAK-242) provides neuroprotection and promotes microglial M2-like phenotype in AD (65). Trained microglia respond to subsequent unspecific stimuli in an enhanced manner and microglial training is a major pro-AD factor, augmenting the subsequent inflammatory response (66). In a previous study, in mice with sporadic AD injected with streptozotocin, microglial training worsened A β accumulation, neuronal loss and cognitive impairment, effects which were attenuated by the microglial NLRP3 inhibitor (66). Moreover, increased ER stress has been observed in AD (67). TXNIP, an endogenous inhibitor of thioredoxin, is a key antioxidant reductive protein and anti-apoptotic protein (66), which may also represent a connection between ER stress and neuroinflammation (67). According to a previous study, using double immunofluorescence staining, TXNIP and IL-1 β were shown to be co-localized near A β plaques and p-tau (68). TXNIP also directly interacts with the NLRP3 inflammasome in AD-affected brains, modulating inflammatory cascades. Therefore, inhibiting the NLRP3 inflammasome activation may help to control AD. However, Tang and Harte (69) indicated that the levels of NLRP3 activation markers were not significantly altered in the temporal cortex of patients with AD, and in age- and sex-matched controls.

DM. DM is a prevalent metabolic disorder characterized by hyperglycemia, marked by a chronic state of low-grade inflammation (70); it is a highly prevalent disease with high morbidity and mortality rates (71). Several common clinical complications of DM have been reported, including CVD, stroke, diabetic nephropathy and diabetic retinopathy (72,73), which are all closely associated with NLRP3 inflammasome activation (74). Additionally, there is evidence to indicate that hyperglycemic conditions cause endothelial cell dysfunction and NLRP3 inflammasome activation (75). MCC950, an NLRP3 inhibitor (11), tetramethylpyrazine (76), hydrogen sulfide (77) and Kakonein (78) have been shown to improve endothelial dysfunction by suppressing NLRP3 inflammasome activation or the production of its effectors, caspase-1 and IL-1 β . Moreover, MCC950 targets NLRP3-mediated inflammation, and reduces plaque development, promotes plaque stability and improves diabetes-associated vascular disease (79). Similarly, MCC950 is a promising treatment that prevents neurovascular remodeling and cognitive impairment in diabetic patients following stroke (80). Dimethyl fumarate exerts vasculoprotective effects on diabetic aortas by suppressing the activation of the ROS/TXNIP/NLRP3

inflammasome pathway (81). Furthermore, NLRP3 inflammasome activation has been shown to exacerbate cardiac dysfunction following ischemic stroke in diabetic mice (82). By contrast, sodium-glucose cotransporter 2 inhibitor exerts cardioprotective effects by suppressing the NLRP3 inflammasome (83). Differing from NLRP3, IL-1 β has a more complex effect on systemic glucose metabolism. It has been shown that IL-1 β contributes to the postprandial stimulation of insulin secretion (84). Moreover, the deletion of IL-1R impairs glucose tolerance and reduces the insulin-positive area in pancreatic tissue of db/db and C57BL/6 mice (85).

Diabetic nephropathy is a prevalent complication of DM and a major cause of end-stage renal disease. The inflammatory response induced by NLRP3 inflammasome activation modulates the pathological process of diabetic nephropathy (86). Curcumin, a principal and most active curcuminoid (87), attenuates the progression of diabetic nephropathy by limiting the activation of the NLRP3 inflammasome (88). Similarly, the E3 ubiquitin ligase speckle BTB-POZ protein, a suppressor of the NLRP3 inflammasome, promotes NLRP3 degradation by improving the K48-linked polyubiquitin of NLRP3, thereby suppressing renal dysfunction and pathological changes to ameliorate diabetic nephropathy (89). Moreover, the NLRP3 inflammasome may promote pathological neovascularization in the advanced stages of diabetic retinopathy (90). Li *et al* (91) discovered that quercetin, a bioactive flavonoid pigment in several fruits, had therapeutic potential in diabetic retinopathy-associated retinal neovascularization by suppressing the NLRP3 inflammasome. Furthermore, isoflurane pre-treatment has been shown to inhibit NLRP3 inflammasome activation in the retina and provide substantial retinal protection against retinal injury induced by stroke associated with DM (92). Taken together, these findings demonstrate that NLRP3 inflammasome participates in the development and progression of DM and its related complications.

IBD. IBD is an idiopathic disease of the gut characterized by chronic, recurrent inflammation (93). Its pathogenesis is directly associated with changes in the immune environment (94,95). It has been demonstrated that the NLRP3 inflammasome in childhood IBD may be involved in the regulation of immune mechanisms by upregulating caspase-1 and IL-1 β expression (96). There is evidence to suggest that the NLRP3 inflammasome is persistently activated and plays a key role in IBD (97). Consequently, it is a potential therapeutic target for the treatment of IBD. Adenosine diphosphate, which is abundant in injured colonic tissue, activates the NLRP3 inflammasome by regulating P2Y1 receptor-mediated Ca²⁺ signaling, resulting in the maturation and secretion of IL-1 β , further aggravating the progression of colitis (98). On the other hand, dextran sulfate sodium salt (DSS)-induced colitis and endotoxic shock have been shown to be significantly ameliorated by genetic ablation or the pharmacological blockade of the P2Y1 receptor (98). Additionally, the BRCA1/BRCA2-containing complex 3 and Josephin domain containing 2 mediate NLRP3-R779C deubiquitination (99) and the interaction between NEK7 and NLRP3 (100), both of which promote NLRP3 inflammasome activation and an increased risk of IBD. Munronoid I is a novel diterpenoid isolated and purified from the Meliaceae family. In mice with DSS-induced IBD, NLRP3 has been

found to be ubiquitinated and degraded to regulate canonical pyroptosis (101). Moreover, estrogen receptor β is a crucial anti-inflammatory agent in rats with IBD, related to P2X7R downregulation, the inhibition of NLRP3 inflammasome activation, as well as the release of IL-1 β from macrophages via the JAK2/STAT3 signaling pathway (102). A disrupted intestinal microbiota is also a feature of IBD (103). Notably, probiotics alleviate IBD by modulating the intestinal microorganisms-bile acid-NLRP3 inflammasome pathway (104).

4. Inhibitors of the NLRP3 inflammasome pathway

Currently available clinical treatment agents for NLRP3-related diseases include drugs targeting IL-1 β , including anakinra, canakinumab and rilonacept (105). However, there are some concerns that these treatments may increase the risks of infection (106). Therefore, inhibitors targeting the NLRP3 inflammasome may be more effective than those targeting IL-1 β in the treatment of NLRP3-driven diseases (12). In recent years, researchers have suppressed the NLRP3 inflammasome through various targets by exploiting their complex signaling pathways, including the priming step of NLRP3 inflammasome activation, the content of K⁺, Ca²⁺, Cl⁻ and ROS in the microenvironment, the assembly of NLRP3 the inflammasome and GSDMD cleavage. As such, the present review summarizes recent inhibitors of the NLRP3 inflammasome pathway and their roles in inflammatory diseases (Table I and Fig. 3).

NLRP3 inflammasome pathway inhibitors targeting the priming step of NLRP3 inflammasome activation. LPS, oxidized LDL (ox-LDL) and cholesterol are recognized by TLR4 and IL-1R to mediate NF- κ B entry and upregulated the expression of pro-caspase-1, NLRP3, pro-IL-1 β and pro-IL-18, which is defined as the priming step.

Inhibitors of TLR4. TLRs are a type of transmembrane protein, which can be combined with a corresponding ligand to trigger intracellular signal transduction cascade responses, hence stimulating chemokines and proinflammatory cytokines (107). As the PRR, TLR4 regulates neuroinflammation. In the priming step of NLRP3 inflammasome activation, TLR4 signals are activated by LPS via myeloid differentiation primary response 88 (MyD88), which ultimately activates NF- κ B, thereby upregulating pro-IL-1 β , pro-IL-18 and NLRP3 expression (108,109). Therefore, it is possible to develop chronic/sustained inflammation caused by a vicious circle of NLRP3 inflammasome activation via TLR4 signaling (110). Consequently, the development of small molecule pharmacological antagonists for TLR4 is a novel molecular therapeutic approach. TAK-242, or resatorvid, is a TLR4 inhibitor that binds to the TIR domain of TLR4 and competes with TLR4 interacting molecules, thereby suppressing the TLR4-mediated release of several cytokines (111). TAK-242 penetrates the blood-brain barrier (BBB) and is an effective inhibitor of congenital inflammation (112), as well as neuroinflammation (112-114). TAK-242 inhibits the TLR4/NLRP3 inflammasome signaling pathway induced by A β in microglia (115). A similar mechanism is adopted by TAK-242 to provide neuroprotection and promote M2 microglial polarization by suppressing the TLR4/MyD88/NF- κ B/NLRP3 signaling pathway (65).

Moreover, a HFD exacerbates the extent and severity of acute pancreatitis via the inflammatory response. The inhibition of TLR4 signaling by TAK-242 decreases inflammatory reaction, exerting a protective effect during acute pancreatitis in HFD rats (116). Moreover, TAK-242 improves symptoms of myocardial infarction (MI) (117), periodontitis (118), renal/retinal lesions (107,119), ischemia-reperfusion and acute lung injury by inhibiting TLR4 and its downstream inflammatory markers (120).

Inhibitors of NF- κ B. BAY 11-7082 is an NF- κ B inhibitor that targets the phosphorylation of I κ B α (inhibitor of NF- κ B) (121). BAY 11-7082 suppresses the phosphorylation of I κ B α and NF- κ B translocation to the nucleus induced by TNF- α , thereby suppressing NLRP3 inflammasome activation (122). Following oxygen-glucose deprivation and re-oxygenation, BAY 11-7082 decreases the levels of the NLRP3 inflammasome and cleaved caspase-1 protein in BV2 microglial cells, presenting a pharmacological effect in stroke (123). Moreover, chronic cold stress activates the microglia, causing neuroinflammation that can be significantly inhibited by BAY 11-7082 by targeting the GABA-induced NLRP3 inflammasome (124). Sulfasalazine is a drug used in the treatment of rheumatoid arthritis and ulcerative colitis. It can also inhibit NF- κ B activity (125). Sulfasalazine significantly inhibits NF- κ B expression to dose-dependently ameliorate acetic acid-induced inflammasome activation by reducing NLRP3 and caspase-1 expression, thereby reducing ulcerative colitis in rats (126). Moreover, the therapeutic administration of sulfasalazine effectively downregulates NF- κ B activation, as well as IL-1 β and IL-8 mRNA expression, whereas I κ B α levels have been shown to be stable in biopsy specimens from patients with ulcerative colitis (125). Analogous data were also obtained when sulfasalazine was used to attenuate oxazolone-induced ulcerative colitis in mice (127).

NLRP3 inflammasome pathway inhibitors targeting the microenvironment of NLRP3 inflammasome assembly. As the priming signal, intracellular K⁺ efflux, increased ROS generation and lysosomal damage disrupt the local microenvironment, they promote the assembly of NLRP3 and ASC, and recruit pro-caspase-1 to complete the assembly of the NLRP3 inflammasome. As such, maintaining a balanced internal environment is critical for the inhibition of NLRP3 inflammasome activation. The K⁺ efflux is an upstream signaling event that causes NLRP3 inflammasome activation (128). The inflammasome activators that trigger NLRP3 inflammasome reduce the intracellular K⁺ levels (129). ATP, a P2X7 purinoceptor agonist, induces a significant K⁺ efflux in LPS-primed cells, which is substantially diminished by 11Cha1, a chalcone derivative. Additionally, 11Cha1 exerts a concentration-dependent inhibitory effect on LPS/ATP-induced LDH release, further suppressing pyroptosis (129). NEK7 also functions downstream of K⁺ efflux during NLRP3 inflammasome activation (23). As one of the major components of licorice, licochalcone B specifically inhibits the NLRP3 inflammasome and directly binds to NEK7 to inhibit its interaction with NLRP3, thereby suppressing NLRP3 inflammasome activation (130).

Apart from K⁺ efflux, ROS are a contributing factor for NLRP3 inflammasome activation (131). Nicotine is involved in the development of atherosclerosis-related endothelial

Table I. NLRP3 inflammasome inhibitors targeting NLRP3 protein and NLRP3 inflammasome assembly.

Inhibitor	NLRP3	ASC	NLRP3- NEK7	NLRP3- ASC	Casp-1	IL-1 β	NLRC4	AIM2	k ⁺	CL ⁻	mtROS	Ca ²⁺	NF- κ B	GSDMD
Tranilast	+						-	-	-	-	-			
MNS	+	+					-	-	-					
Parthenolide	+				+		+	+					+	
INF39	+		+	+		+	-	-	-		-			-
CY-09	+						-	-	-		-			
MCC950	+		-	-					-	+	-	-		
OLT1177	+		+	+		+	-	-	-				+	
Oridonin	+	+	+	+			-	-	-		-		+	
BHB		+							+					
X-11-5-27		+		+							+			
BOT-4-one	+													
FC11A-2					+									-
JC124		+			+	+								

The '+' symbol indicates inhibition, and the '-' symbol indicates no effect. NLRP3, Nod-like receptor protein 3; ASC, apoptosis-associated speck-like protein; NEK7, NIMA-related kinase 7; IL, interleukin; GSDMD, gasdermin D; mtROS, mitochondrial reactive oxygen species.

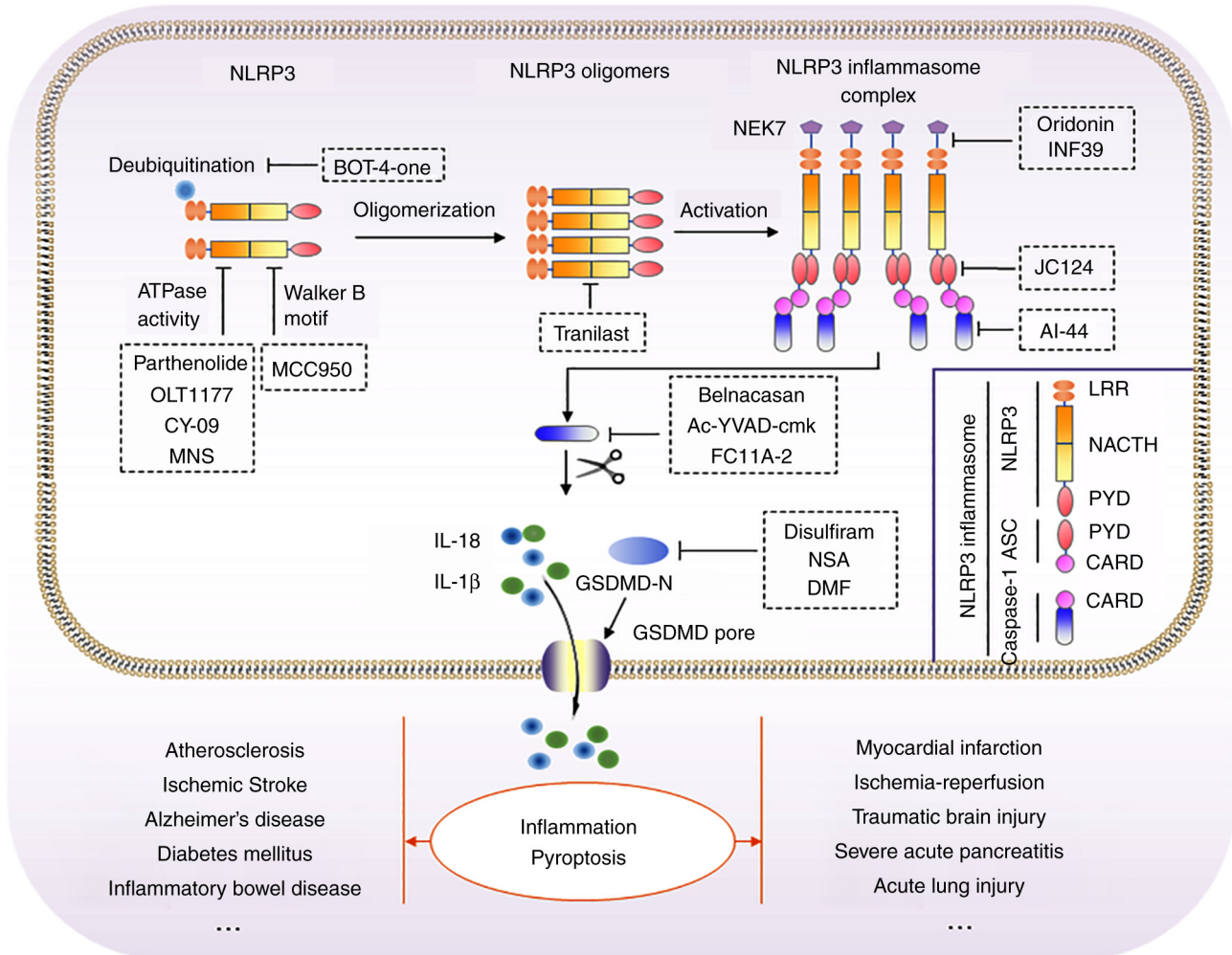


Figure 3. NLRP3 inflammasome pathway inhibitors. In the activation step of the NLRP3 inflammasome, NLRP3 oligomerizes via the NACHT domains following stimulation by DAMP and PAMP. It also recruits ASC and caspase-1 to form the NLRP3 inflammasome, which mediates GSDMD to cause cell membrane rupture and release IL-1 β and IL-18, causing inflammation and pyroptosis. NLRP3 inflammasome inhibitors target the priming and activation step in the NLRP3 inflammasome signaling pathway. Arrows indicate activation, whereas blunted lines indicate inhibition by selective compounds. NLRP3, Nod-like receptor protein 3; DAMP, danger-associated molecular pattern; PAMP, pathogen-associated molecular pattern; ASC, apoptosis-associated speck-like protein; GSDMD, gasdermin D; NEK7, NIMA-related kinase 7.

cell pyroptosis via ROS/NLRP3 signaling, whereas the ROS scavenger, NAC, exerts opposite effects (40). Thioredoxin and its endogenous inhibitor, TXNIP, play crucial roles in oxidative stress (132). In mice with DSS-induced colitis, flavonoid VI-16 has been shown to reduce oxidative stress by suppressing the TXNIP/NLRP3 inflammasome pathway (133). CLIC-dependent chloride efflux is also a proximal upstream regulator of NLRP3 inflammasome activation (22). IAA94 and anthracene-9-carboxylic acid can suppress NLRP3 agonist-induced CL⁻ efflux (134), whereas the latter is a CLIC inhibitor. Furthermore, Ca²⁺ also regulates NLRP3 inflammasome activation. The IP3R-mediated increase in the release of Ca²⁺ stimulates NLRP3 inflammasome activation via ER stress and mitochondrial dysfunction, involved in the inflammatory pathophysiology of ventilator-induced lung injury (135). The IP3R inhibitor, 2-aminoethoxydiphenyl borate, and the Ca²⁺ chelator, BAPTA-AM, can maintain Ca²⁺ homeostasis to suppress NLRP3 inflammasome activation (135). Collectively, various molecular or cellular events, including K⁺ efflux, ROS production, CLIC-dependent chloride efflux and Ca²⁺ release,

disrupt the local microenvironment and may promote NLRP3 inflammasome assembly.

NLRP3 inflammasome pathway inhibitors targeting NLRP3 protein and NLRP3 inflammasome assembly. NLRP3 inflammasome complex formation is dependent on NLRP3 oligomerization and the recruitment of ASC to NLRP3 oligomers (136) (Fig. 3). NLRP3 is oligomerized by the ATPase activity of NLRP3 NACHT domain to recruit and oligomerize ASC, hence activating caspase-1 (137,138). Consequently, NLRP3 inflammasome-specific inhibitors targeting NLRP3 are considered attractive targets (Table I).

MCC950. MCC950 (CP-456,773, CRID3), an inhibitor of the NLRP3 inflammasome, has demonstrated excellent *in vivo* efficacy in several species and disease models. There is ample evidence to suggest that MCC950 inhibits ATP hydrolysis, ASC oligomerization and chloride efflux by directly interacting with the Walker B motif of the NLRP3 NACHT domain, thereby suppressing NLRP3 inflammasome activation (139). However, its inhibitory effects are independent of NLRP3

inflammasome priming, calcium signaling, potassium efflux, mitochondrial respiration, ROS production, NLRP3-NLRP3, NLRP3-ASC and NEK7-NLRP3 interaction (140-142). It has been demonstrated that MCC950 is responsible for the treatment of inflammatory-based diseases and their complications. For instance, MCC950 attenuates macrophage inflammation and pyroptosis to prevent atherosclerosis (8). Similarly, MCC950 significantly reduces plaque sizes in hyperlipidemic murine models, suggesting that NLRP3 inhibitors may be candidates for the treatment of atherosclerosis (143). The oral administration of MCC950 is a recently identified approach for reducing the severity of spontaneous chronic colitis in Winnie mice (144) and for suppressing human retinal endothelial cell dysfunction for the treatment of diabetic retinopathy induced by high glucose conditions (145). Furthermore, NLRP3 inflammasome activation in neurons mediates neuroinflammation in acute ischemic stroke, whereas MCC950 reduces CIRI by mitigating inflammation and preserving BBB integrity (146). Similarly, MCC950 treatment significantly improves insulin sensitivity to alleviate diabetic encephalopathy in db/db mice (147). Additionally, MCC950 ameliorates diabetic kidney injury in db/db mice by decreasing the fibrosis markers in high glucose-induced mesangial cells to prevent diabetic nephropathy progression (148). Due to its effects on inflammation, MCC950 may be effective in treating such disorders.

Oridonin. Oridonin, an ent-kaurane diterpenoid, is a primary active component of *Rabdosia rubescens* (149) that exerts anti-inflammatory effects against NLRP3. Oridonin blocks NLRP3 inflammasome assembly by covalently bonding to cysteine (Cys)279 of NLRP3 in the NACHT domain (150). However, oridonin does not affect NLRP3 and NLRC4 ATPase activity, AIM2 activation, or upstream signaling events that trigger the activation of the NLRP3 inflammasome, including K⁺ efflux and mitochondrial damage. Notably, oridonin exerts preventive or therapeutic effects against MI, CIRI, traumatic brain injury (TBI) and insulin resistance by inhibiting NLRP3 inflammasome activation (150). In a mouse model of MI, oridonin was shown to inhibit myocardial fibrosis, reduce the myocardial infarct size, and improve cardiac function (151). Oridonin also suppresses BV2 microglial cells stimulated by oxygen-glucose deprivation/reoxygenation, particularly upon the activation of the NLRP3 inflammasome (152). In mice with TBI, oridonin has been found to prevent the inflammatory response and neuronal apoptosis, maintain the BBB integrity and attenuates neurological deficits (153). In addition, oridonin causes insulin resistance partially by inhibiting macrophage infiltration into the islets and NLRP3 activation induced by chronic unpredictable mild stress (154).

OLT1177. OLT1177 is an orally active β -sulfonyl cyanide molecule (155), whose pharmacokinetic and safety analyses have been conducted with healthy volunteers following an oral administration in a phase 1 trial (156). By directly binding to NLRP3, OLT1177 reduces ATPase activity and suppresses ASC oligomerization (28), but not NLRC4 or AIM2 inflammasome activation (157). Moreover, OLT1177 prevents the NLRP3-ASC interaction to inhibit NLRP3 inflammasome assembly. However, OLT1177 does not affect, the K⁺ efflux or synthesis of the pro-IL-1 β (157). As previously reported by Lonnemann *et al* (158) in a mouse model of AD, OLT1177 reduces the activation of microglia, reduces cerebral cortex

plaques, and normalizes the levels of plasma metabolic markers in a dose-dependent manner. Moreover, the prophylactic oral administration of OLT1177 has been shown to significantly reduce the infiltration of CD4⁺ T-cells and macrophages in the spinal cord, hence ameliorating the clinical signs of experimental autoimmune encephalomyelitis (159). Oizumi *et al* (160) demonstrated that OLT1177 administration early in the disease phase, prevented inflammation in mice with DSS-induced colitis. In addition to reducing the myocardial infarct size in mice, OLT1177 has been shown to prevent left ventricular dysfunction following ischemia-reperfusion injury (within 60 min) (161). Due to its low toxicity and limited side-effects, OLT1177 is an orally bioavailable drug with a significant benefit for inflammatory diseases.

INF39. INF39 is a non-toxic, irreversible, specific inhibitor of the NLRP3 inflammasome, which specifically inhibits NLRP3 activation, but not the NLRC4 or AIM2 inflammasomes (162). However, INF39 affects neither the upstream events of NLRP3 inflammasome activation, including K⁺ efflux, ROS generation or mitochondrial membrane potential, nor the downstream signal, GSDMD (162). The inhibition of NEK7-NLRP3 interaction is a major mechanism of the anti-inflammatory effects of INF39, followed by the inhibition of NLRP3 oligomerization, NLRP3-ASC, ASC oligomerization and speckle formation (162). According to bioluminescence resonance energy transfer analyses, INF39 suppresses the release of IL-1 β from macrophages by directly interfering with NLRP3 activation (163). In rats with type 2 DM (T2DM), INF39 has been shown to effectively suppress the expression of ICAM-1, NLRP3, as well as other inflammatory factors, and to reduce intimal-media thickness, as well as platelet activation (76). INF39 also promotes the effect of Arctigenin on DSS-induced acute colitis by suppressing the NLRP3 inflammasome (164). Pellegrini *et al* (165) also demonstrated that directly inhibiting NLRP3, reduced systemic and bowel inflammation more effectively than inhibiting caspase-1 or IL-1 receptors.

Tranilast. Tranilast is a tryptophan metabolite that suppresses NLRP3 inflammasome activation; it is also used in the treatment of allergies and asthma, without affecting AIM2 or NLRC4 inflammasome activation (166). Tranilast suppresses NLRP3 oligomerization by binding to the NACHT domain and subsequent NLRP3 inflammasome assembly, caspase-1 activation and IL-1 β production with no effects on its ATPase activity, K⁺ efflux, mitochondrial damage, or Cl⁻ efflux (166,167). In recent research, tranilast was shown to inhibit NLRP3 oligomerization in an ATPase-independent manner and exert profound treatment and preventive effects in mouse models of gout, cryopyrin-associated periodic syndrome (CAPS), and T2DM (28). According to Cao and Peng (168), tranilast ameliorated the symptoms of gestational DM, including hyperglycemia, insulin deficiency, glucose intolerance and insulin resistance by suppressing NLRP3 inflammasome activation, as well as inflammatory responses. Furthermore, tranilast has been shown to inhibit NLRP3 inflammasome activation by improving NLRP3 ubiquitination to reduce vascular inflammation, and ameliorate atherosclerosis in both LDLR- and apolipoprotein E-deficient mice (169).

CY-09. There is evidence to indicate that CY-09 suppresses the NLRP3 inflammasome (12). It inhibits NLRP3 ATPase activity by directly binding to the ATP binding motif of the

NACHT domain, which is specific as it does not affect NLRC4, NLRP1, NOD2 or RIG-I ATPase activity. Notably, CY-09 does not affect mitochondrial damage, potassium, or chloride ion efflux during NLRP3 inflammasome activation (12). More importantly, Jiang *et al.* (12) revealed that CY-09 directly targeted NLRP3 to inhibit NLRP3 inflammasome activation *in vivo* and was particularly effective in treating T2DM and CAPS caused by NLRP3. A recent study noted that CY-09 therapy attenuated IL-1 β secretion and astrocyte activation, which was effective in reducing neuronal loss (170).

JC124. JC124 is an active and selective NLRP3 inflammasome inhibitor that targets ASC oligomerization in macrophages, and constitutively expresses active NLRP3 inflammasome (171). A previous photoaffinity labeling probe experiment indicated that JC124 directly targets NLRP3 inflammasome complex without changing its ATPase activity (172). Furthermore, JC124 targets the NLRP3 inflammasome and exerts beneficial effects in APP/PS1 mice, significantly decreasing A β accumulation and improving cognitive function (173,174). Moreover, JC124 significantly decreases the number of degenerating neurons, the inflammatory response, and cortical lesion volume post-injury (172). In TBI, JC124 substantially downregulates NLRP3, ASC, IL-1 β , TNF- α , inducible nitric oxide synthase and caspase-1 expression (172). SAR adds more information to the JC124 structure, leading to the discovery of two novel lead compounds, i.e., 14 and 17, with improved inhibitory potency (175).

3,4-Methylenedioxy- β -nitrostyrene (MNS), parthenolide and BOT-4-one. MNS, parthenolide and BOT-4-one impair NLRP3 ATPase activity, thereby suppressing NLRP3 inflammasome activation. MNS does not inhibit K⁺ efflux or influence NLRC4 or AIM2 inflammasome activation, suggesting that it specifically inhibits NLRP3 inflammasome (176). Apart from targeting NLRP3, parthenolide is a direct inhibitor of caspase-1 protease activity (177). Moreover, BOT-4-one increases NLRP3 ubiquitination and suppresses NLRP3 inflammasome activation (178). Summarily, for NLRP3 inflammasome inhibitors targeting NLRP3 protein and NLRP3 inflammasome assembly, tranilast, CY-09 and BOT-4-one can only suppress NLRP3 oligomerization by binding to the NACHT domain or affect its ATPase activity; hence, they are NLRP3 inflammasome-specific inhibitors. Additionally, INF39, OLT1177, oridonin and X-11-5-27 inhibit NLRP3 inflammasome assembly. Among all the inhibitors, only parthenolide can inhibit NLRP3, NLRC4 and AIM2, whereas others exhibit NLRP3 specificity.

NLRP3 inflammasome pathway inhibitors targeting caspase-1. As the protease that matures IL-1 β , IL-18 and GSDMD, caspase-1 is a key initial event at the onset of NLRP3 inflammasome activation and canonical caspase-1-dependent pyroptosis (179). Therefore, the pharmaceutical industry has focused on developing clinical-grade molecules that suppress caspase protease activity.

Belnacasan. Belnacasan (VX-765), an efficient and selective caspase-1 inhibitor, can hinder the development and progression of atherosclerosis at least by targeting ox-LDL-induced VSMC pyroptosis (180). Caspase-1 inhibition with VX-765 has been shown to significantly reduce neuropathological damage and pyroptosis following prolonged

ketamine exposure (181). It has also been shown that VX-765 significantly attenuates cerebral ischemic injury and cerebral edema, as well as reduces ischemia-associated BBB permeability in rats subjected to middle cerebral artery occlusion by suppressing pyroptosis and the RAGE/MAPK pathway (182). Similarly, it has been shown that VX-765 not only attenuates brain injury, but also suppresses microglial pyroptosis and neuroinflammation by downregulating GSDMD, TNF- α and MPO in an *in vivo* model of intracerebral hemorrhage (ICH) (183). In addition, overactivated N9 microglia treated with VX-765 are responsible for the reduction in the NLRP3 inflammasome and pyroptosis-associated proteins expression *in vitro* (184). Other research has shown that VX-765 inhibits silica nanoparticle-induced cardiomyocytic pyroptosis and cardiac hypertrophy (185).

Ac-YVAD-cmk. Ac-YVAD-cmk is a peptide whose sequence is homologous to a known caspase substrate sequence, confirming its capacity in suppressing caspase-1 activation (186). As a selective caspase-1 inhibitor, Ac-YVAD-cmk effectively inhibits pyroptosis, and IL-1 β and IL-18 expression in numerous diseases (187). As previously demonstrated, in rat H9C2 cardiomyocytes, LPS pre-treatment can efficiently mediate pyroptosis by activating the NLRP3 inflammasome, exacerbating high glucose and hypoxia/reoxygenation injury. Ac-YVAD-cmk responds by counteracting these effects (188). Ac-YVAD-cmk reduces the expression of mature IL-1 β /IL-18, improves behavioral performance, and alleviates microglia in the perihematoma region in rats with ICH (189). The SARS-CoV-2 N protein promotes NLRP3 inflammasome activation and generates excessive inflammatory responses, which are blocked by Ac-YVAD-cmk (190).

AI-44 and FC11A-2. AI-44, a curcumin analogue, binds to peroxiredoxin 1 (PRDX1) and promotes the interaction of PRDX1 with pro-caspase-1, thereby demonstrating an association between pro-caspase-1 and ASC (191). However, the inhibitory effect of AI-44 on NLRP3 inflammasome is markedly diminished after PRDX1 is knocked out (192). FC11A-2, another caspase-1 inhibitor, has been shown to significantly attenuate experimental colitis in mice induced by DSS, primarily by targeting caspase-1 activation prior to IL-1 β /IL-18 production in macrophages (193). VX-765 and Ac-YVAD-cmk are the most broadly used inhibitors. Both can reduce the related inflammatory diseases by suppressing caspase-1 expression to varying degrees, such as atherosclerosis, CVD (cerebral ischemia injury, cerebral edema and cerebral hemorrhage), myocardial pyroptosis and myocardial hypertrophy.

NLRP3 inflammasome pathway inhibitors targeting GSDMD. As a candidate for pyroptotic pore formation, GSDMD is a downstream effector of caspase-1 that not only regulates pyroptosis, but also releases IL-1 β and IL-18 to the extracellular space (194). As described above, caspase-1 cleaves GSDMD following NLRP3 inflammasome activation, allowing GSDMD-N-mediated pore formation in the plasma membrane to promote pyroptosis (195). In total, 12 Cys residues are present in the sequence of GSDMD, and the reactivity of Cys191/192 (human/mouse) is crucial for pore formation, since it is well exposed and is highly reactive in the protein structure. Suppressing GSDMD can alleviate

inflammasome-induced pyroptosis (196), thus suggesting that GSDMD may be an attractive novel target for regulating inflammation.

Necrosulfonamide (NSA). NSA was initially identified as an inhibitor in mixed lineage kinase domain-like protein-mediated necroptotic cell death (197). However, Rathkey *et al* (198) found that the NSA also bound to GSDMD via Cys191, thereby inhibiting the oligomerization of p30-GSDMD and preventing pyroptosis through both primary and immortalized macrophages. Additionally, pre-treatment with NSA was shown to suppress A β -142-induced mouse cortical neuron (MCN) pyroptosis, primarily by targeting the permeability of cell membrane and inflammatory factor release (199). Notably, the inhibition of p30-GSDMD oligomerization blocks the opening of membrane pores, confirming its importance in MCN pyroptosis and its potential as an NSA target (199). In A549 and H1299 cells, Teng *et al* (200) similarly found that NSA inhibited the polyphyllin VI-induced activation of the NLRP3 inflammasome (200).

Disulfiram. Disulfiram inhibits membrane pore formation in GSDMD, but not in other GSDMDs families, hence covalently modifying GSDMD in Cys191/Cys192 of human/mice to inhibit pore formation. Disulfiram and its metabolism exhibit anti-inflammatory activities, which can alleviate inflammation *in vitro* and *in vivo* (201). Disulfiram relieves severe acute pancreatitis induced by caerulein and related lung injury, and inhibits IL-1 β and IL-18 production by targeting GSDMD cleavage (202). In both human and mouse monocyte/macrophage cells, disulfiram has been shown to inhibit the release of IL-1 β and pyroptosis (203). Similarly, disulfiram has been shown to block pyroptosis and cytokine release in phorbol 12-myristate 13-acetate-differentiated, as well as LPS-primed human THP-1 cells and LPS-induced sepsis-associated mortality in mice (204). It has also been demonstrated that disulfiram significantly promotes macrophage M2 phenotype polarization based on a small-molecule compound library (205). Mechanistically, disulfiram targets GSDMD to attenuate macrophagic pyroptosis, IL-1 β and high mobility group box 1 protein release (205). In mouse experiments, Hu *et al* revealed that disulfiram inhibited the function of GSDMD by covalently modifying its cys192, hence blocking the IL-1 β release without affecting caspase-1 and pro-IL-1 β expression (204). Thus, disulfiram does not affect IL-1 β production and maturation, but rather blocks pores formation in the cell membrane to prevent IL-1 β release and pyroptosis (204,206).

Dimethyl fumarate (DMF). As an ester of fumaric acid, DMF exerts anti-inflammatory effects (207). DMF delivered to cells or endogenous fumarate reacts with GSDMD at Cys191/Cys192 to form S-(2-succinyl)-cysteine, further preventing its interaction with Caspase-1, hence limiting its capacity to process, oligomerize and pyroptosis (208). GSDMD is distributed into NK92 cell membranes following LPS stimulation, a process suppressed by DMF in NK92 cells (209). Furthermore, DMF inhibits GSDMD production by targeting DNA methyltransferases, preventing them from hypermethylating the promoter region of the gene (209). Moreover, DMF effectively reduces GSDMD-N and inflammatory factors, including IL-1 β and IL-18 in the hippocampus following status epilepticus (210). NSA, disulfiram and DMF can inhibit

pore formation by reacting with GSDMD at Cys191/Cys192, thereby suppressing the inflammatory reaction.

5. Clinical perspectives

As described above, the treatment efficacy of NLRP3 inhibitors in inflammatory diseases has been largely documented in animal and cellular experiments. However, their practical application in treating these diseases is limited because of insufficient clinical research. Tranilast, OLT1177, NAC, DMF and disulfiram have been tested thus far in clinical trials (Table II). For instance, tranilast at a dose of 300 mg/day for 1 year, is safe for patients with both early-stage and advanced diabetic nephropathy. Increased mesangial cell proliferation, and the accumulation of extracellular matrix components, such as collagen in the glomeruli, is one of the pathologic features during in the early stages of diabetic nephropathy (211). Tranilast treatment may suppress collagen accumulation in renal tissue and may be therapeutically beneficial in reducing the progression of advanced diabetic nephropathy. Moreover, tranilast may be therapeutically beneficial for early-stage diabetic nephropathy (212,213). In another study, tranilast was administered to patients with coronary artery disease following a successful directional coronary atherectomy (DCA) at a dose of 600 mg daily for 3 months. Consequently, the oral administration of tranilast significantly prevented restenosis following DCA (214). In a phase I study, OLT1177 was analyzed for safety in patients with heart failure and reduced ejection fraction; as a result, it was found to be safe and well-tolerated after 14 days of treatment (215). Furthermore, the trial demonstrated that NAC was safe when preoperatively administered; however, its efficacy as an antioxidant and anti-inflammatory agent was not statistically significant and thus, additional investigations using a larger sample are warranted (trial no. NCT03589495). In patients ICH with oxidative stress, was shown to NAC substantially reduce perihematomal edema volume and shorten intensive care unit stay (216). Furthermore, NAC has been shown to significantly improve remission maintenance in ulcerative colitis patients receiving 800 mg NAC for 16 weeks unlike the placebo (217). Alcohol-dependent patients are subjected to disulfiram-treatment to discourage alcohol-consumption. Besides, disulfiram can increase A disintegrin and metalloprotease 10 expression (218), which inhibits the production of A β , the hallmarks of AD pathology (219). Therefore, NLRP3 inflammasome pathway inhibitors have been demonstrated *in vitro* or *in vivo* and in clinical trials. Among these candidate molecules are tranilast and OLT1177, which are safe and effective in both clinical and basic studies. Furthermore, tranilast is the most extensively studied, with apparent treatment effects on early or late diabetes nephropathy. Additionally, tranilast can also prevent stenosis following DCA in patients with coronary artery disease.

6. Conclusion and future perspectives

The NLRP3 inflammasome is present at low levels under normal circumstances, which is important for innate immunity regulation. However, NLRP3 inflammasome activation ultimately results in inflammation and pyroptosis. Therefore,

Table II. Summary of the clinical trial data of NLRP3 inflammasome inhibitors for the treatment of inflammatory diseases.

NLRP3 inflammasome pathway inhibitor	Trail phase	Trail design	Trail length	Sample size	Patients recruited	Dosage	Main results		(Refs.) trail identifier
							Yes/No	Details	
Tranilast	Phase 2	Placebo-controlled trial	1 year	8	Advanced diabetic nephropathy	100 mg, 3 times daily	Yes	Tranilast slowed the progression of advanced diabetic nephropathy	(212)
	Phase 2	Randomized, placebo-controlled trial	1 year	20	Advanced diabetic nephropathy	100 mg, 3 times daily	Yes	Tranilast could be therapeutically beneficial in early-stage DN	(213)
	/	Placebo-controlled trial	3 months	192	Patients with coronary artery disease after successful directional coronary atherectomy (DCA)	600 mg tranilast daily from the day after successful DCA to 3 months later	Yes	Oral administration of tranilast strongly prevents restenosis after directional coronary atherectomy	(214)
OLT1177	Phase I	Randomized, double-blind, dose escalation, single-center, repeat dose safety and pharmacodynamics study	14 days	30	Patients with heart failure and reduced ejection fraction	Placebo, OLT1177 500 mg/day, OLT1177 1000mg/day, or OLT1177 2,000 mg/day	Yes	Treatment with dapansutril for 14 days was safe and well tolerated	(215)
NAC	Phase 4	Randomized, double-blinded, and controlled clinical trial	48 h	60	Systemic inflammatory response syndrome	Group 1: 100 mg/kg acetyl cysteine dissolved in dextrose 5%; Group 2: dextrose 5%	Yes	Preoperative administration of NAC was safe, but its efficacy as an antioxidant and anti-inflammatory	NCT03589495

Table II. Continued.

NLRP3 inflammasome pathway inhibitor	Trail phase	Trail design	Trail length	Sample size	Patients recruited	Dosage	Main results		(Refs.) trail identifier
							Yes/No	Details	
	Phase2 Phase3	Randomized	3 months	60	Diabetic nephropathy chronic kidney disease diabetes type 2	600 mg effervescent NAC tablet twice per day for three months	No	agent was not statistically significant and requires further investigation in a larger sample	NCT00556465
	/	Multicenter, single-blind, randomized study	14 days	123	Patients with intracerebral hemorrhage have oxidative stress	NAC was given for 14 days with a dose of 2,000 mg/d and selenium 1,600 μ g/d intravenously	Yes	NAC showed a significantly reduced perihematomal edema volume and shortened length of intensive care unit stay in patients with acute intracerebral hemorrhage	(216)
	/	Double-blind randomized controlled clinical trial	16 weeks	168	Ulcerative colitis	The patients received 800 mg NAC or placebo for 16 weeks	Yes	NAC had a significantly more positive effect on the maintenance of remission compared	(217)

Table II. Continued.

NLRP3 inflammasome pathway inhibitor	Trail phase	Trail design	Trail length	Sample size	Patients recruited	Dosage	Main results		(Refs.) trail identifier
							Yes/No	Details	
Dimethyl fumarate	Phase I	Randomized, double-blind, crossover study	48 h	80	Healthy	Single dose 240 mg	No	with placebo in patients with ulcerative colitis that were in the steroid-tapering phase of therapy No results posted	NCT02171208
Disulfiram	/	Case-control	14 days	17	Alcohol addiction	A dosage of 1,500, 1,000 and 500 mg was used for the first three days, and then a dose of 500 mg three times a week was prescribed	Yes	Disulfiram increased ADAM10 production and could be repurposed as a treatment drug for Alzheimer's disease	(218)

NLRP3, Nod-like receptor protein 3; NAC, N-acetyl cysteine; ADAM10, A disintegrin and metalloproteinase domain-containing protein 10.

the NLRP3 inflammasome may provide novel targets for the treatment of various inflammatory diseases. Notably, both priming and activation steps are crucial for NLRP3 inflammasome activation. Therefore, beginning from the priming step, the present review summarized the related TLR4 and NF- κ B inhibitors, among which TAK-242, BAY11-7082 and sulfasalazine inhibit inflammatory diseases caused by the NLRP3 inflammasome, without any notable adverse toxic side-effects. Subsequently, the present review also summarized the associated ion inhibitors to preserve the associated ion homeostasis during NLRP3 inflammasome activation. Of note, NLRP3, ASC and caspase-1 inhibitors for the NLRP3 inflammasome itself were also described. NLRP3-induced pyroptosis is an important mechanism causing inflammatory disease. Therefore, the present review described the related inhibitors of pyroptosis executor, GSDMD, which may serve as an effective target for inflammatory diseases. In conclusion, the present review comprehensively described the inhibitors that can trigger NLRP3 inflammasome activation from the priming step to the activation step, illustrating their promising roles in the treatment of NLRP3 inflammasome-induced inflammatory diseases. However, future research is necessary to elucidate certain issues. First, TLR4 and NF- κ B, as common membrane receptors and transcription factors activate the NLRP3 inflammasome. Secondly, K⁺ and Cl⁻ efflux are two independent, yet indispensable events that activate the NLRP3 inflammasome. The small-molecule inhibitors of the NLRP3 inflammasome documented thus far have not been confirmed in clinical trials or approved by the FDA or other institutions. Therefore, their pharmacokinetic characteristics and comprehensive mechanisms warrant further investigation, given their promising prospects as NLRP3 inflammasome inhibitors.

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Availability of data and material

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Authors' contributions

XZ drafted the manuscript. XJ designed and supervised the study. JG verified the contents and revised the manuscript. ZW, YZ, QY, MZ and LB critically revised the manuscript. LY and MG edited the manuscript. All authors reviewed, and have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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