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

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Received November 10, 2022; Accepted March 6, 2023

DOI: 10.3892/ijmm.2023.5238

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; Aβ, 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-κB, nuclear factor-κB; 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

Contents

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)-1β 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-1β 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-κB (NF-κB) to increase the intracellular transcript levels of pro-IL-1β, 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 (Aβ) (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)

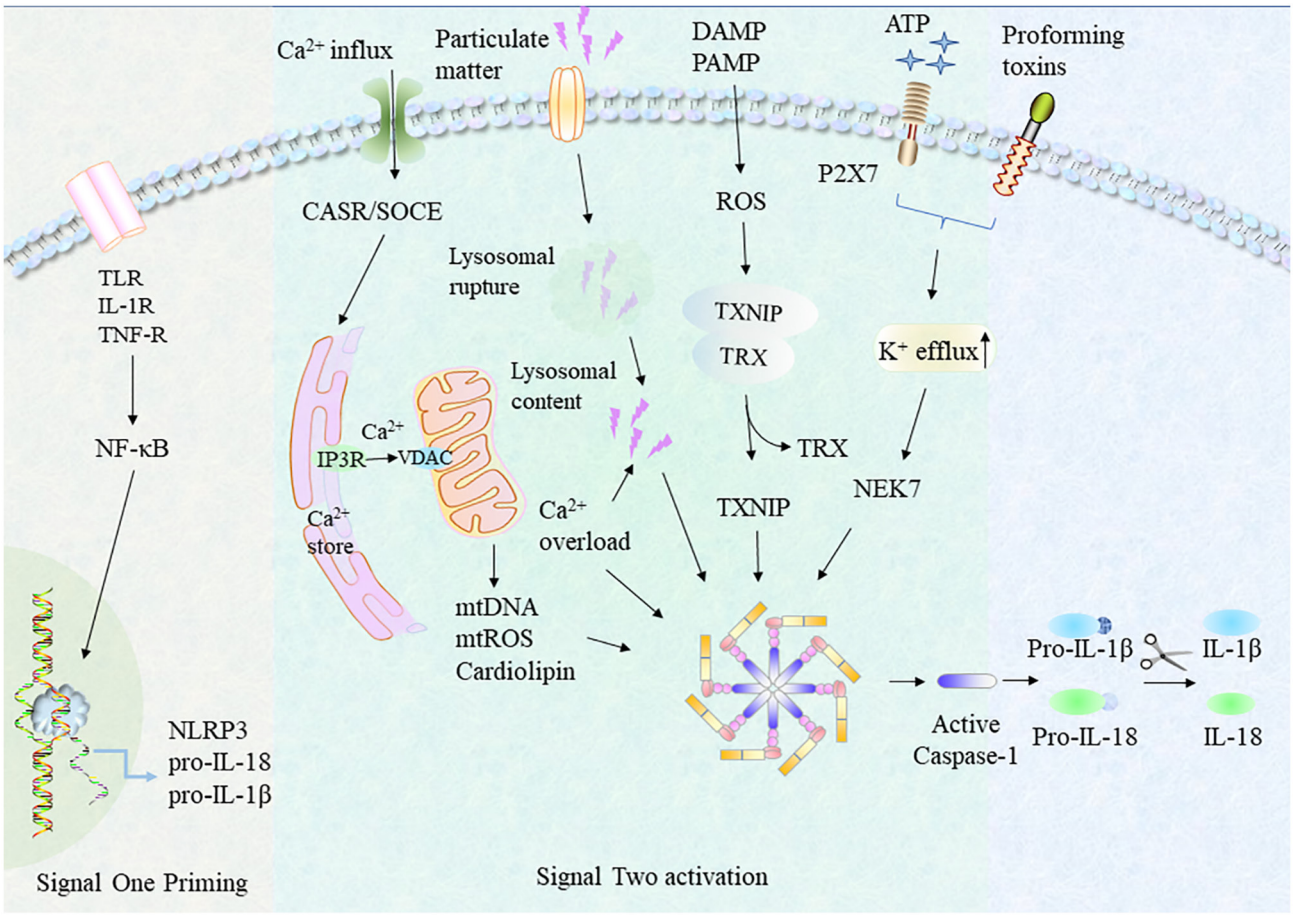

Figure 1. Model and regulation of NLRP3 inflammasome activation. The priming step involves the transcriptional of NLRP3 inflammasome key components via TLR IL-1R and TNF-R and subsequent NF-κB signaling. At the activation step, the NLRP3 inflammasome is activated by diverse stimuli, including K^+ efflux, Ca^{2+} 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 Ca^{2+} from endoplasmic reticulum (ER) Ca^{2+} stores or extracellular milieu. Through Ca^{2+} channels, calcium ions are released from the ER, the intracellular calcium storage pool, when cells are stimulated (30). Researchers have demonstrated that intracellular Ca^{2+} 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

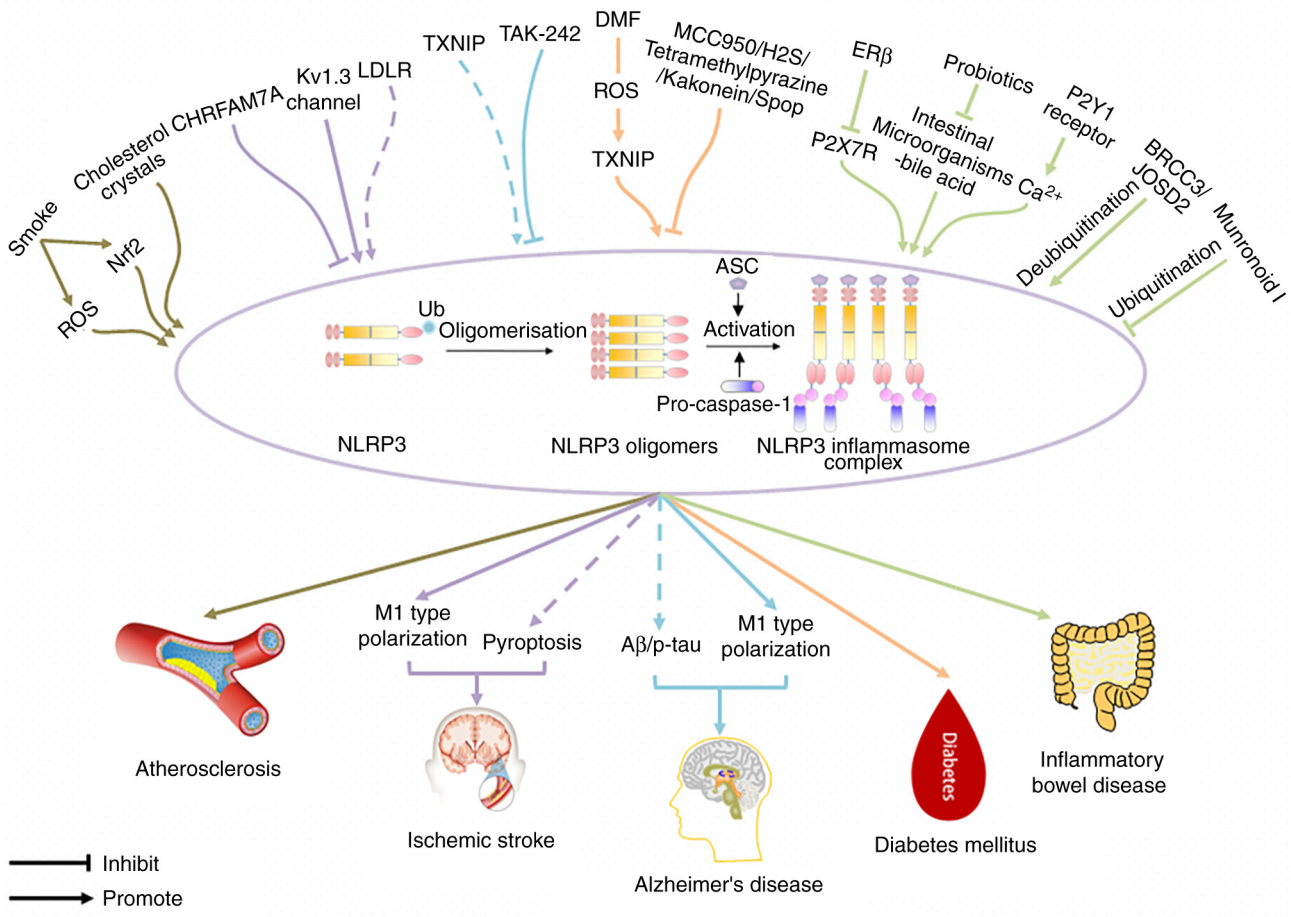

Figure 2. Pathophysiological role of the NLRP3 inflammasome in inflammatory diseases. Different pathological factors can cause the aberrant activation of the NLRP3 inflammasome, leading to the development of various inflammatory diseases, such as atherosclerosis, ischemic stroke, Alzheimer's disease, 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 Aβ 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 Aβ 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-1β were shown to be co-localized near Aβ 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 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-1β. 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-1β has a more complex effect on systemic glucose metabolism. It has been shown that IL-1β 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-1β 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 Ca^{2+} signaling, resulting in the maturation and secretion of IL-1β, 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-1β 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-1β, 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-1β 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^+, Ca^{2+}, 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-κB entry and upregulated the expression of pro-caspase-1, NLRP3, pro-IL-1β 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-κB, thereby upregulating pro-IL-1β, 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 Aβ 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-κB/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-κB. BAY 11-7082 is an NF-κB inhibitor that targets the phosphorylation of IκBα (inhibitor of NF-κB) (121). BAY 11-7082 suppresses the phosphorylation of IκBα and NF-κB 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-κB activity (125). Sulfasalazine significantly inhibits NF-κB 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-κB activation, as well as IL-1β and IL-8 mRNA expression, whereas IκBα 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-1β	NLRC4	AIM2	k^+	CL^-	mtROS	Ca^{2+}	NF-κB	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.

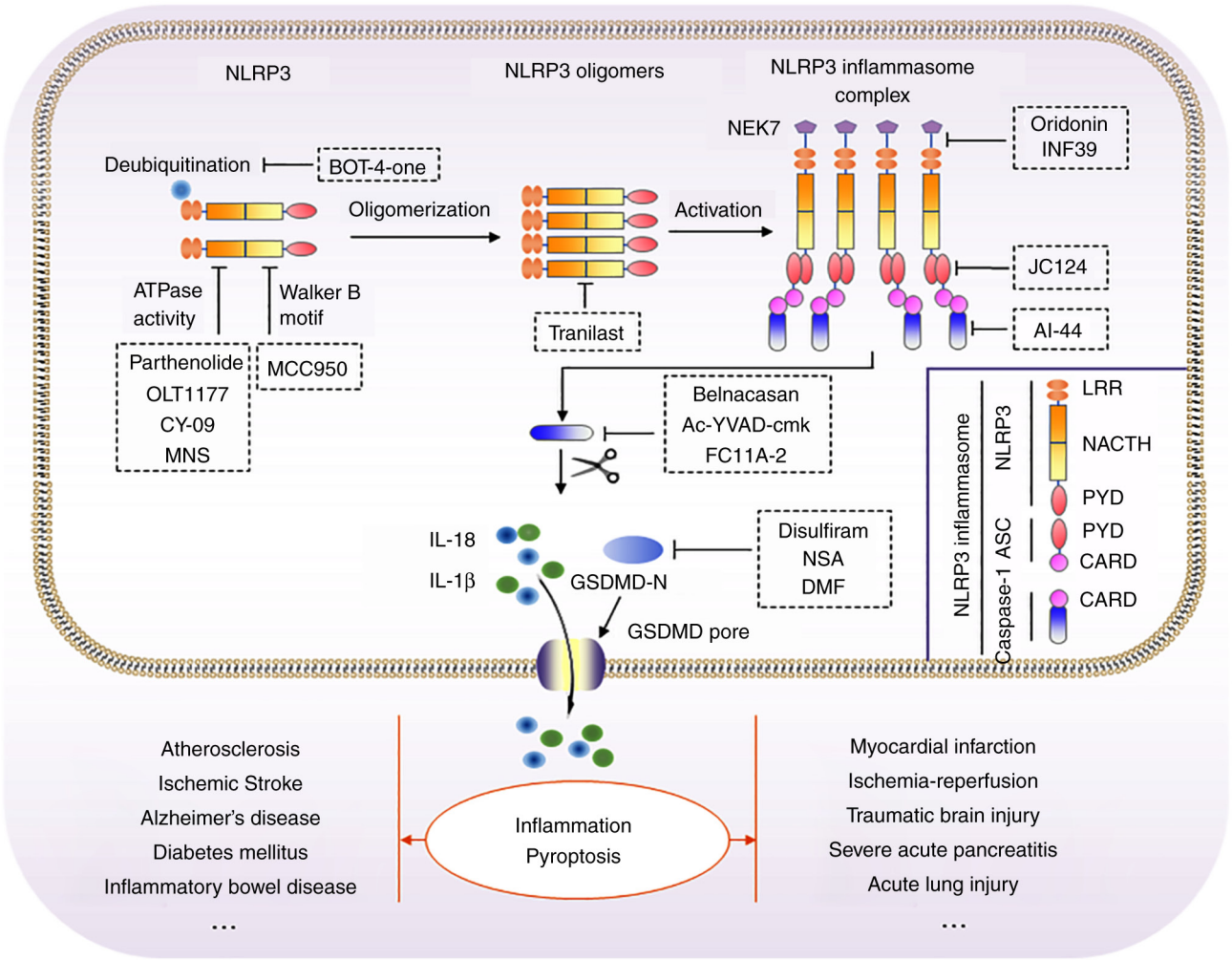

Figure 3. NLRP3 inflammasome pathway inhibitors. In the activation step of the NLRP3 inflammasome, NLRP3 oligomerizes via the NACHT domains following stimulation by DAMP and PAMP. It also recruits ASC and caspase-1 to form the NLRP3 inflammasome, which mediates GSDMD to cause cell membrane rupture and release IL-1β 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, Ca^{2+} also regulates NLRP3 inflammasome activation. The IP3R-mediated increase in the release of Ca^{2+} 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 Ca^{2+} chelator, BAPTA-AM, can maintain Ca^{2+} homeostasis to suppress NLRP3 inflammasome activation (135). Collectively, various molecular or cellular events, including K^+ efflux, ROS production, CLIC-dependent chloride efflux and Ca^{2+} 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-1β (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-1β 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-1β 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-1β 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 Aβ 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-1β, 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 parathenolide can inhibit NLRP3, NLRC4 and AIM2, whereas others exhibit NLRP3 specificity.

NLRP3 inflammasome pathway inhibitors targeting caspase-1. As the protease that matures IL-1β, 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 sujected 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-1β 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-1β/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-1β/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-1β 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 Aβ-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 GSDMs 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-1β 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-1β 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-1β 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-1β release without affecting caspase-1 and pro-IL-1β expression (204). Thus, disulfiram does not affect IL-1β production and maturation, but rather blocks pores formation in the cell membrane to prevent IL-1β 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-1β 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 Aβ, 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	Phase1	Randomized, double-blind, dose escalation, single-center, repeat dose safety and pharma-codynamics 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 dapansutrile 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	
								agent was not statistically significant and requires further investigation in a larger sample	
	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 posted	No results	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 μg/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	
								with placebo in patients with ulcerative colitis that were in the steroid-tapering phase of therapy	
Dimethyl fumarate	Phase 1	Randomized, double-blind, crossover study	48 h	80	Healthy	Single dose 240 mg	No	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-κB 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-κB, 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.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant nos. 82074211, 81873130 and 82174470), the Tianjin Natural Science Foundation (grant no. 21JCQNJC01170) and the 2019 Annual Graduate Students Innovation Fund, School of Integrative Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, China (grant no. ZXYCXLX201902).

Availability of data and material

Not applicable.

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.

References

1. Fu C, Ye S, Liu Y and Li S: Role of CARD region of MDA5 gene in canine influenza virus infection. Viruses 12: 307, 2020.
2. Zhang Y and Okamoto CT: Nucleotide binding domain and leucine-rich repeat pyrin domain-containing protein 12: Characterization of its binding to hematopoietic cell kinase. Int J Biol Sci 16: 1507-1525, 2020.
3. Zhao C and Zhao W: NLRP3 inflammasome-A key player in antiviral responses. Front Immunol 11: 211, 2020.
4. Swanson KV, Deng M and Ting JP: The NLRP3 inflammasome: Molecular activation and regulation to therapeutics. Nat Rev Immunol 19: 477-489, 2019.
5. Zuo Y, Chen L, Gu H, He X, Ye Z, Wang Z, Shao Q and Xue C: GSDMD-mediated pyroptosis: A critical mechanism of diabetic nephropathy. Expert Rev Mol Med 23: e23, 2021.
6. Arioz BI, Tarakcioglu E, Olcum M and Genc S: The role of melatonin on NLRP3 inflammasome activation in diseases. Antioxidants (Basel) 10: 1020, 2021.
7. Vong CT, Tseng H, Yao P, Yu H, Wang S, Zhong Z and Wang Y: Specific NLRP3 inflammasome inhibitors: Promising therapeutic agents for inflammatory diseases. Drug Discov Today 26: 1394-1408, 2021.
8. Zeng W, Wu D, Sun Y, Suo Y, Yu Q, Zeng M, Gao Q, Yu B, Jiang X and Wang Y: The selective NLRP3 inhibitor MCC950 hinders atherosclerosis development by attenuating inflammation and pyroptosis in macrophages. Sci Rep 11: 19305, 2021.
9. Feng YS, Tan ZX, Wang MM, Xing Y, Dong F and Zhang F: Inhibition of NLRP3 inflammasome: A prospective target for the treatment of ischemic stroke. Front Cell Neurosci 14: 155, 2020.
10. Holbrook JA, Jarosz-Griffiths HH, Caseley E, Lara-Reyna S, Poulter JA, Williams-Gray CH, Peckham D and McDermott MF: Neurodegenerative disease and the NLRP3 inflammasome. Front Pharmacol 12: 643254, 2021.
11. Ferreira NS, Bruder-Nascimento T, Pereira CA, Zanotto CZ, Prado DS, Silva JF, Rassi DM, Foss-Freitas MC, Alves-Filho JC, Carlos D, *et al*: NLRP3 inflammasome and mineralocorticoid receptors are associated with vascular dysfunction in type 2 diabetes mellitus. Cells 8: 1595, 2019.
12. Jiang H, He H, Chen Y, Huang W, Cheng J, Ye J, Wang A, Tao J, Wang C, Liu Q, *et al*: Identification of a selective and direct NLRP3 inhibitor to treat inflammatory disorders. J Exp Med 214: 3219-3238, 2017.
13. Guarda G, Zenger M, Yazdi AS, Schroder K, Ferrero I, Menu P, Tardivel A, Mattmann C and Tschopp J: Differential expression of NLRP3 among hematopoietic cells. J Immunol 186: 2529-2534, 2011.
14. Zhong Y, Kinio A and Saleh M: Functions of NOD-like receptors in human diseases. Front Immunol 4: 333, 2013.
15. Park WJ and Han JS: *Gryllus bimaculatus* extract protects against lipopolysaccharide and palmitate-induced production of proinflammatory cytokines and inflammasome formation. Mol Med Rep 23: 206, 2021.
16. Flores-Costa R, Duran-Guell M, Casulleras M, Lopez-Vicario C, Alcaraz-Quiles J, Diaz A, Lozano JJ, Titos E, Hall K, Sarno R, *et al*: Stimulation of soluble guanylate cyclase exerts antiinflammatory actions in the liver through a VASP/NF-κB/NLRP3 inflammasome circuit. Proc Natl Acad Sci USA 117: 28263-28274, 2020.
17. Dowling JK and O'Neill LA: Biochemical regulation of the inflammasome. Crit Rev Biochem Mol 47: 424-443, 2012.
18. Ulland TK, Ferguson PJ and Sutterwala FS: Evasion of inflammasome activation by microbial pathogens. J Clin Invest 125: 469-477, 2015.

19. Trojan E, Tylek K, Leskiewicz M, Lason W, Brandenburg LO, Leopoldo M, Lacivita E and Basta-Kaim A: The N-Formyl peptide receptor 2 (FPR2) agonist MR-39 exhibits anti-inflammatory activity in LPS-stimulated organotypic hippocampal cultures. Cells 10: 1524, 2021.
20. Ming SL, Zeng L, Guo YK, Zhang S, Li GL, Ma YX, Zhai YY, Chang WR, Yang L, Wang J, *et al*: The human-specific STING agonist G10 activates type I interferon and the NLRP3 inflammasome in porcine cells. Front Immunol 11: 575818, 2020.
21. Munoz-Planillo R, Kuffa P, Martinez-Colon G, Smith BL, Rajendiran TM and Nunez G: K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. Immunity 38: 1142-1153, 2013.
22. Tang T, Lang X, Xu C, Wang X, Gong T, Yang Y, Cui J, Bai L, Wang J, Jiang W and Zhou R: CLICs-dependent chloride efflux is an essential and proximal upstream event for NLRP3 inflammasome activation. Nat Commun 8: 202, 2017.
23. He Y, Zeng MY, Yang D, Motro B and Nunez G: NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. Nature 530: 354-357, 2016.
24. Maejima I, Takahashi A, Omori H, Kimura T, Takabatake Y, Saitoh T, Yamamoto A, Hamasaki M, Noda T, Isaka Y, *et al*: Autophagy sequesters damaged lysosomes to control lysosomal biogenesis and kidney injury. EMBO J 32: 2336-2347, 2013.
25. Otsuki T, Holian A and Di Gioacchino M: Immunological effects of environmental factors: Focus on the fibrous and particulated materials. J Immunol Res 2014: 697438, 2014.
26. Wang Y, Qin X and Paudel HK: Amyloid beta peptide promotes lysosomal degradation of clusterin via sortilin in hippocampal primary neurons. Neurobiol Dis 103: 78-88, 2017.
27. Ismael S, Ahmed HA, Adris T, Parveen K, Thakor P and Ishrat T: The NLRP3 inflammasome: A potential therapeutic target for traumatic brain injury. Neural Regen Res 16: 49-57, 2021.
28. Paik S, Kim JK, Silwal P, Sasakawa C and Jo EK: An update on the regulatory mechanisms of NLRP3 inflammasome activation. Cell Mol Immunol 18: 1141-1160, 2021.
29. Bauernfeind F, Bartok E, Rieger A, Franchi L, Nunez G and Hornung V: Cutting edge: Reactive oxygen species inhibitors block priming, but not activation, of the NLRP3 inflammasome. J Immunol 187: 613-617, 2011.
30. Wang C, Fu KK, Dai J, Lacey SD, Yao Y, Pastel G, Xu L, Zhang J and Hu L: Inverted battery design as ion generator for interfacing with biosystems. Nat Commun 8: 15609, 2017.
31. Ma C, Liu S, Zhang S, Xu T, Yu X, Gao Y, Zhai C, Li C, Lei C, Fan S, *et al*: Evidence and perspective for the role of the NLRP3 inflammasome signaling pathway in ischemic stroke and its therapeutic potential (Review). Int J Mol Med 42: 2979-2990, 2018.
32. Lee GS, Subramanian N, Kim AI, Aksentijevich I, Goldbach-Mansky R, Sacks DB, Germain RN, Kastner DL and Chae JJ: The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca2+ and cAMP. Nature 492: 123-127, 2012.
33. Zhong Z, Zhai Y, Liang S, Mori Y, Han R, Sutterwala FS and Qiao L: TRPM2 links oxidative stress to NLRP3 inflammasome activation. Nat Commun 4: 1611, 2013.
34. Jo EK, Kim JK, Shin DM and Sasakawa C: Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 13: 148-159, 2016.
35. Hosen MR, Goody PR, Zietzer A, Nickenig G and Jansen F: MicroRNAs as master regulators of atherosclerosis: From pathogenesis to novel therapeutic options. Antioxid Redox Sign 33: 621-644, 2020.
36. Saigusa R, Winkels H and Ley K: T cell subsets and functions in atherosclerosis. Nat Rev Cardiol 17: 387-401, 2020.
37. Martinet W, Coornaert I, Puylaert P and De Meyer G: Macrophage death as a pharmacological target in atherosclerosis. Front Pharmacol 10: 306, 2019.
38. Xu H, Jiang J, Chen W, Li W and Chen Z: Vascular macrophages in atherosclerosis. J Immunol Res 2019: 4354786, 2019.
39. Ren XS, Tong Y, Ling L, Chen D, Sun HJ, Zhou H, Qi XH, Chen Q, Li YH, Kang YM and Zhu GQ: NLRP3 Gene deletion attenuates Angiotensin II-Induced phenotypic transformation of vascular smooth muscle cells and vascular remodeling. Cell Physiol Biochem 44: 2269-2280, 2017.
40. Wu X, Zhang H, Qi W, Zhang Y, Li J, Li Z, Lin Y, Bai X, Liu X, Chen X, *et al*: Nicotine promotes atherosclerosis via ROS-NLRP3-mediated endothelial cell pyroptosis. Cell Death Dis 9: 171, 2018.
41. Zhao Z, Wang X, Zhang R, Ma B, Niu S, Di X, Ni L and Liu C: Melatonin attenuates smoking-induced atherosclerosis by activating the Nrf2 pathway via NLRP3 inflammasomes in endothelial cells. Aging (Albany NY) 13: 11363-11380, 2021.
42. Mehta S and Dhawan V: Exposure of cigarette smoke condensate activates NLRP3 inflammasome in THP-1 cells in a stage-specific manner: An underlying role of innate immunity in atherosclerosis. Cell Signal 72: 109645, 2020.
43. Mehta S, Srivastava N, Bhatia A and Dhawan V: Exposure of cigarette smoke condensate activates NLRP3 inflammasome in vitro and in vivo: A connotation of innate immunity and atherosclerosis. Int Immunopharmacol 84: 106561, 2020.
44. Keping Y, Yunfeng S, Pengzhuo X, Liang L, Chenhong X and Jinghua M: Sestrin1 inhibits oxidized low-density lipoprotein-induced activation of NLRP3 inflammasome in macrophages in a murine atherosclerosis model. Eur J Immunol 50: 1154-1166, 2020.
45. Ma Q, Yang Q, Chen J, Yu C, Zhang L, Zhou W and Chen M: Salvianolic acid A ameliorates early-stage atherosclerosis development by inhibiting NLRP3 inflammasome activation in zucker diabetic fatty rats. Molecules 25: 1089, 2020.
46. Li W, Liu D, Xu J, Zha J, Wang C, An J, Xie Z and Qiao S: Astrocyte-Derived TNF-alpha-Activated platelets promote cerebral Ischemia/Reperfusion injury by regulating the RIP1/RIP3/AKT signaling pathway. Mol Neurobiol 59: 5734-5749, 2022.
47. Sun R, Peng M, Xu P, Huang F, Xie Y, Li J, Hong Y, Guo H, Liu Q and Zhu W: Low-density lipoprotein receptor (LDLR) regulates NLRP3-mediated neuronal pyroptosis following cerebral ischemia/reperfusion injury. J Neuroinflamm 17: 330, 2020.
48. Shimizu T, Smits R and Ikenaka K: Microglia-induced activation of non-canonical Wnt signaling aggravates neurodegeneration in demyelinating disorders. Mol Cell Biol 36: 2728-2741, 2016.
49. Ma DC, Zhang NN, Zhang YN and Chen HS: Kv1.3 channel blockade alleviates cerebral ischemia/reperfusion injury by reshaping M1/M2 phenotypes and compromising the activation of NLRP3 inflammasome in microglia. Exp Neurol 332: 113399, 2020.
50. Pozzo ED, Tremolanti C, Costa B, Giacomelli C, Milenkovic VM, Bader S, Wetzel CH, Rupprecht R, Taliani S, Settimo FD and Martini C: Microglial Pro-Inflammatory and Anti-Inflammatory phenotypes are modulated by translocator protein activation. Int J Mol Sci 20: 4467, 2019.
51. Chen C, Ai Q, Chu S, Zhang Z, Zhou X, Luo P, Liu Y and Chen N: IMM-H004 protects against oxygen-glucose deprivation/reperfusion injury to BV2 microglia partly by modulating CKLF1 involved in microglia polarization. Int Immunopharmacol 70: 69-79, 2019.
52. Liu H, Wu X, Luo J, Zhao L, Li X, Guo H, Bai H, Cui W, Guo W, Feng D and Qu Y: Adiponectin peptide alleviates oxidative stress and NLRP3 inflammasome activation after cerebral ischemia-reperfusion injury by regulating AMPK/GSK-3β. Exp Neurol 329: 113302, 2020.
53. Zhao J, Piao X, Wu Y, Liang S, Han F, Liang Q, Shao S and Zhao D: Cepharanthine attenuates cerebral ischemia/reperfusion injury by reducing NLRP3 inflammasome-induced inflammation and oxidative stress via inhibiting 12/15-LOX signaling. Biomed Pharmacother 127: 110151, 2020.
54. Kaushal V, Koeberle PD, Wang Y and Schlichter LC: The Ca2+-activated K+ channel KCNN4/KCa3.1 contributes to microglia activation and nitric oxide-dependent neurodegeneration. J Neurosci 27: 234-244, 2007.
55. Ma DC, Zhang NN, Zhang YN and Chen HS: Salvianolic Acids for injection alleviates cerebral ischemia/reperfusion injury by switching M1/M2 phenotypes and inhibiting NLRP3 inflammasome/pyroptosis axis in microglia in vivo and in vitro. J Ethnopharmacol 270: 113776, 2021.
56. Li ZG, Shui SF, Han XW and Yan L: NLRP10 ablation protects against ischemia/reperfusion-associated brain injury by suppression of neuroinflammation. Exp Cell Res 389: 111912, 2020.
57. Cao X, Wang Y and Gao L: CHRFAM7A overexpression attenuates cerebral ischemia-reperfusion injury via inhibiting microglia pyroptosis mediated by the NLRP3/Caspase-1 pathway. Inflammation 44: 1023-1034, 2021.
58. Bellut M, Papp L, Bieber M, Kraft P, Stoll G and Schuhmann MK: NLPR3 inflammasome inhibition alleviates hypoxic endothelial cell death in vitro and protects blood-brain barrier integrity in murine stroke. Cell Death Dis 13: 20, 2021.

59. Fu C, Zhang X, Zeng Z, Tian Y, Jin X, Wang F, Xu Z, Chen B, Zheng H and Liu X: Neuroprotective effects of qingnao dripping pills against cerebral ischemia via Inhibiting NLRP3 inflammasome signaling pathway: In vivo and in vitro. Front Pharmacol 11: 65, 2020.
60. Denes A, Coutts G, Lenart N, Cruickshank SM, Pelegrin P, Skinner J, Rothwell N, Allan SM and Brough D: AIM2 and NLRC4 inflammasomes contribute with ASC to acute brain injury independently of NLRP3. Proc Natl Acad Sci USA 112: 4050-4055, 2015.
61. Severini C, Barbato C, Di Certo MG, Gabanella F, Petrella C, Di Stadio A, de Vincentiis M, Polimeni A, Ralli M and Greco A: Alzheimer's disease: New concepts on the role of autoimmunity and NLRP3 inflammasome in the pathogenesis of the disease. Curr Neuropharmacol 19: 498-512, 2021.
62. Lee YJ, Han SB, Nam SY, Oh KW and Hong JT: Inflammation and Alzheimer's disease. Arch Pharm Res 33: 1539-1556, 2010.
63. Li G, Dong Y, Liu D, Zou Z, Hao G, Gao X, Pan P and Liang G: NEK7 coordinates rapid neuroinflammation after subarachnoid hemorrhage in mice. Front Neurol 11: 551, 2020.
64. Liang S, Zhong Z, Kim SY, Uchiyama R, Roh YS, Matsushita H, Gottlieb RA and Seki E: Murine macrophage autophagy protects against alcohol-induced liver injury by degrading interferon regulatory factor 1 (IRF1) and removing damaged mitochondria. J Biol Chem 294: 12359-12369, 2019.
65. Cui W, Sun C, Ma Y, Wang S, Wang X and Zhang Y: Inhibition of TLR4 induces M2 microglial polarization and provides neuroprotection via the NLRP3 inflammasome in Alzheimer's disease. Front Neurosci 14: 444, 2020.
66. He XF, Xu JH, Li G, Li MY, Li LL, Pei Z, Zhang LY and Hu XQ: NLRP3-dependent microglial training impaired the clearance of amyloid-beta and aggravated the cognitive decline in Alzheimer's disease. Cell Death Dis 11: 849, 2020.
67. Ismael S, Wajidunnisa, Sakata K, McDonald MP, Liao FF and Ishrat T: ER stress associated TXNIP-NLRP3 inflammasome activation in hippocampus of human Alzheimer's disease. Neurochem Int 148: 105104, 2021.
68. Li L, Ismael S, Nasoohi S, Sakata K, Liao FF, McDonald MP and Ishrat T: Thioredoxin-interacting protein (TXNIP) associated NLRP3 inflammasome activation in human Alzheimer's disease brain. J Alzheimers Dis 68: 255-265, 2019.
69. Tang H and Harte M: Investigating markers of the NLRP3 inflammasome pathway in Alzheimer's disease: A human post-mortem study. Genes (Basel) 12: 1753, 2021.
70. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, Griep A, Axt D, Remus A, Tzeng TC, *et al*: NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493: 674-678, 2013.
71. Garcia-Serrano AM and Duarte J: Brain metabolism alterations in type 2 diabetes: What did we learn from diet-induced diabetes models? Front Neurosci 14: 229, 2020.
72. An X, Jin D, Duan L, Zhao S, Zhou R, Lian F and Tong X: Direct and indirect therapeutic effect of traditional Chinese medicine as an add-on for non-proliferative diabetic retinopathy: A systematic review and meta-analysis. Chin Med 15: 99, 2020.
73. Omar SM, Musa IR, ElSouli A and Adam I: Prevalence, risk factors, and glycaemic control of type 2 diabetes mellitus in eastern Sudan: A community-based study. Ther Adv Endocrinol 10: 1906186649, 2019.
74. Yu ZW, Zhang J, Li X, Wang Y, Fu YH and Gao XY: A new research hot spot: The role of NLRP3 inflammasome activation, a key step in pyroptosis, in diabetes and diabetic complications. Life Sci 240: 117138, 2020.
75. Wanrooy BJ, Kumar KP, Wen SW, Qin CX, Ritchie RH and Wong C: Distinct contributions of hyperglycemia and high-fat feeding in metabolic syndrome-induced neuroinflammation. J Neuroinflamm 15: 293, 2018.
76. Zhang H, Chen H, Wu X, Sun T, Fan M, Tong H, Zhu Y, Yin Z, Sun W, Zhang C, *et al*: Tetramethylpyrazine alleviates diabetes-induced high platelet response and endothelial adhesion via inhibiting NLRP3 inflammasome activation. Phytomedicine 96: 153860, 2022.
77. Zheng Q, Pan L and Ji Y: H_2S protects against diabetes-accelerated atherosclerosis by preventing the activation of NLRP3 inflammasome. J Biomed Res 34: 94-102, 2019.
78. Lian D, Liu J, Han R, Jin J, Zhu L, Zhang Y, Huang Y, Wang X, Xian S and Chen Y: Kakonein restores diabetes-induced endothelial junction dysfunction via promoting autophagy-mediated NLRP3 inflammasome degradation. J Cell Mol Med 25: 7169-7180, 2021.
79. Sharma A, Choi J, Stefanovic N, Al-Sharea A, Simpson DS, Mukhamedova N, Jandeleit-Dahm K, Murphy AJ, Sviridov D, Vince JE, *et al*: Specific NLRP3 inhibition protects against diabetes-associated atherosclerosis. Diabetes 70: 772-787, 2021.
80. Ward R, Li W, Abdul Y, Jackson L, Dong G, Jamil S, Filosa J, Fagan SC and Ergul A: NLRP3 inflammasome inhibition with MCC950 improves diabetes-mediated cognitive impairment and vasoneuronal remodeling after ischemia. Pharmacol Res 142: 237-250, 2019.
81. Amin FM, Abdelaziz RR, Hamed MF, Nader MA and Shehatou G: Dimethyl fumarate ameliorates diabetes-associated vascular complications through ROS-TXNIP-NLRP3 inflammasome pathway. Life Sci 256: 117887, 2020.
82. Lin HB, Wei GS, Li FX, Guo WJ, Hong P, Weng YQ, Zhang QQ, Xu SY, Liang WB, You ZJ, *et al*: Macrophage-NLRP3 inflammasome activation exacerbates cardiac dysfunction after ischemic stroke in a mouse model of diabetes. Neurosci Bull 36: 1035-1045, 2020.
83. Kim SR, Lee SG, Kim SH, Kim JH, Choi E, Cho W, Rim JH, Hwang I, Lee CJ, Lee M, *et al*: SGLT2 inhibition modulates NLRP3 inflammasome activity via ketones and insulin in diabetes with cardiovascular disease. Nat Commun 11: 2127, 2020.
84. Dror E, Dalmas E, Meier DT, Wueest S, Thevenet J, Thienel C, Timper K, Nordmann TM, Traub S, Schulze F, *et al*: Postprandial macrophage-derived IL-1beta stimulates insulin, and both synergistically promote glucose disposal and inflammation. Nat Immunol 18: 283-292, 2017.
85. Burke SJ, Batdorf HM, Burk DH, Martin TM, Mendoza T, Stadler K, Alami W, Karlstad MD, Robson MJ, Blakely RD, *et al*: Pancreatic deletion of the interleukin-1 receptor disrupts whole body glucose homeostasis and promotes islet β-cell de-differentiation. Mol Metab 14: 95-107, 2018.
86. Yang M, Wang X, Han Y, Li C, Wei L, Yang J, Chen W, Zhu X and Sun L: Targeting the NLRP3 inflammasome in diabetic nephropathy. Curr Med Chem 28: 8810-8824, 2021.
87. Den Hartogh DJ, Gabriel A and Tsiani E: Antidiabetic properties of Curcumin II: Evidence from in vivo studies. Nutrients 12: 58, 2019.
88. Oltean S, Coward R, Collino M and Baelde H: Diabetic nephropathy: Novel molecular mechanisms and therapeutic avenues. Biomed Res Int 2017: 3146524, 2017.
89. Wang B, Dai Z, Gao Q, Liu Y, Gu G and Zheng H: Spop ameliorates diabetic nephropathy through restraining NLRP3 inflammasome. Biochem Bioph Res Commun 594: 131-138, 2022.
90. Tassetto M, Scialdone A, Solini A and Di Virgilio F: The P2X7 receptor: A promising pharmacological target in diabetic retinopathy. Int J Mol Sci 22: 7110, 2021.
91. Li R, Chen L, Yao GM, Yan HL and Wang L: Effects of quercetin on diabetic retinopathy and its association with NLRP3 inflammasome and autophagy. Int J Ophthalmol 14: 42-49, 2021.
92. Lin HB, Lin YH, Zhang JY, Guo WJ, Ovcjak A, You ZJ, Feng ZP, Sun HS, Li FX and Zhang HF: NLRP3 inflammasome: A potential target in isoflurane pretreatment alleviates Stroke-induced retinal injury in diabetes. Front Cell Neurosci 15: 697449, 2021.
93. Shujun W, Huijie Z, Xia B and Hongjian W: Cerebral venous sinus thrombosis in patients with inflammatory bowel disease: A retrospective study. Sci Rep 11: 17004, 2021.
94. Fu Y, Lee CH and Chi CC: Association of psoriasis with inflammatory bowel disease: A systematic review and Meta-analysis. JAMA Dermatol 154: 1417-1423, 2018.
95. Ginwala R, Bhavsar R, Chigbu DI, Jain P and Khan ZK: Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants (Basel) 8: 35, 2019.
96. Wang H and Ma YC: Role of NLRP1 and NLRP3 inflammasome signaling pathways in the immune mechanism of inflammatory bowel disease in children. Zhongguo Dang Dai Er Ke Za Zhi 22: 854-859, 2020 (In Chinese).
97. Zhou W, Liu X, Zhang X, Tang J, Li Z, Wang Q and Hu R: Oroxylin A inhibits colitis by inactivating NLRP3 inflammasome. Oncotarget 8: 58903-58917, 2017.
98. Zhang C, Qin J, Zhang S, Zhang N, Tan B, Siwko S, Zhang Y, Wang Q, Chen J, Qian M, *et al*: ADP/$P2Y_1$ aggravates inflammatory bowel disease through ERK5-mediated NLRP3 inflammasome activation. Mucosal Immunol 13: 931-945, 2020.

99. Zhou L, Liu T, Huang B, Luo M, Chen Z, Zhao Z, Wang J, Leung D, Yang X, Chan KW, *et al*: Excessive deubiquitination of NLRP3-R779C variant contributes to very-early-onset inflammatory bowel disease development. J Allergy Clin Immun 147: 267-279, 2021.
100. Chen X, Liu G, Yuan Y, Wu G, Wang S and Yuan L: NEK7 interacts with NLRP3 to modulate the pyroptosis in inflammatory bowel disease via NF-κB signaling. Cell Death Dis 10: 906, 2019.
101. Ma X, Di Q, Li X, Zhao X, Zhang R, Xiao Y, Li X, Wu H, Tang H, Quan J, *et al*: Munronoid I ameliorates DSS-induced mouse colitis by inhibiting NLRP3 inflammasome activation and pyroptosis via modulation of NLRP3. Front Immunol 13: 853194, 2022.
102. Jiang Q, Li W, Zhu X, Yu L, Lu Z, Liu Y, Ma B and Cheng L: Estrogen receptor β alleviates inflammatory lesions in a rat model of inflammatory bowel disease via down-regulating P2X7R expression in macrophages. Int J Biochem Cell Biol 139: 106068, 2021.
103. Sang H, Xie Y, Su X, Zhang M, Zhang Y, Liu K and Wang J: Mushroom Bulgaria Inquinans modulates host immunological response and gut microbiota in mice. Front Nutr 7: 144, 2020.
104. Liu Z and Wang H: Probiotics alleviate inflammatory bowel disease in mice by regulating intestinal microorganisms-bile acid-NLRP3 inflammasome pathway. Acta Biochim Pol 68: 687-693, 2021.
105. Dinarello CA, Simon A and van der Meer JW: Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov 11: 633-652, 2012.
106. Liu M, Saredy J, Zhang R, Shao Y, Sun Y, Yang WY, Wang J, Liu L, Drummer CT, Johnson C, *et al*: Approaching inflammation paradoxes-proinflammatory cytokine blockages induce inflammatory regulators. Front Immunol 11: 554301, 2020.
107. Liu J, Zhang N, Zhang M, Yin H, Zhang X, Wang X, Wang X and Zhao Y: N-acetylserotonin alleviated the expression of interleukin-1beta in retinal ischemia-reperfusion rats via the TTLR4/NF-κB/NLRP3 pathway. Exp Eye Res 208: 108595, 2021.
108. Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, Fernandes-Alnemri T, Wu J, Monks BG, Fitzgerald KA, *et al*: Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J Immunol 183: 787-791, 2009.
109. Fernandes-Alnemri T, Kang S, Anderson C, Sagara J, Fitzgerald KA and Alnemri ES: Cutting edge: TLR signaling licenses IRAK1 for rapid activation of the NLRP3 inflammasome. J Immunol 191: 3995-3999, 2013.
110. Yang J, Wise L and Fukuchi KI: TLR4 cross-talk with NLRP3 inflammasome and complement signaling pathways in Alzheimer's disease. Front Immunol 11: 724, 2020.
111. Matsunaga N, Tsuchimori N, Matsumoto T and Ii M: TAK-242 (resatorvid), a small-molecule inhibitor of Toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. Mol Pharmacol 79: 34-41, 2011.
112. Plunk MA, Alaniz A, Olademehin OP, Ellington TL, Shuford KL and Kane RR: Design and catalyzed activation of Tak-242 prodrugs for localized inhibition of TLR4-induced inflammation. Acs Med Chem Lett 11: 141-146, 2020.
113. Feng Y, Gao J, Cui Y, Li M, Li R, Cui C and Cui J: Neuroprotective effects of resatorvid against traumatic brain injury in rat: Involvement of neuronal autophagy and TLR4 signaling pathway. Cell Mol Neurobiol 37: 155-168, 2017.
114. Karimy JK, Reeves BC and Kahle KT: Targeting TLR4-dependent inflammation in post-hemorrhagic brain injury. Expert Opin Ther Tar 24: 525-533, 2020.
115. Liu Y, Dai Y, Li Q, Chen C, Chen H, Song Y, Hua F and Zhang Z: Beta-amyloid activates NLRP3 inflammasome via TLR4 in mouse microglia. Neurosci Lett 736: 135279, 2020.
116. Hong YP, Yu J, Su YR, Mei FC, Li M, Zhao KL, Zhao L, Deng WH, Chen C and Wang WX: High-fat diet aggravates acute pancreatitis via TLR4-mediated necroptosis and inflammation in rats. Oxid Med Cell Longev 2020: 8172714, 2020.
117. Xu M, Ye Z, Zhao X, Guo H, Gong X and Huang R: Deficiency of tenascin-C attenuated cardiac injury by inactivating TLR4/NLRP3/caspase-1 pathway after myocardial infarction. Cell Signal 86: 110084, 2021.
118. Huang X, Shen H, Liu Y, Qiu S and Guo Y: Fisetin attenuates periodontitis through FGFR1/TLR4/NLRP3 inflammasome pathway. Int Immunopharmacol 95: 107505, 2021.
119. Zhu Y, Zhu WP, Li W, Zhang HT, Chen BH, Ding A, Yang H and Zhang H: Implications of EET in renal ischemia/reperfusion by regulating NLRP3 expression and pyroptosis. Zhonghua Yi Xue Za Zhi 100: 779-784, 2020 (In Chinese).
120. Xu Q, Wang M, Guo H, Liu H, Zhang G, Xu C and Chen H: Emodin alleviates severe acute pancreatitis-associated acute lung injury by inhibiting the cold-inducible RNA-binding protein (CIRP)-mediated activation of the NLRP3/IL-1β/CXCL1 signaling. Front Pharmacol 12: 655372, 2021.
121. Pierce JW, Schoenleber R, Jesmok G, Best J, Moore SA, Collins T and Gerritsen ME: Novel inhibitors of cytokine-induced IkappaBalpha phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. J Biol Chem 272: 21096-21103, 1997.
122. Irrera N, Vaccaro M, Bitto A, Pallio G, Pizzino G, Lentini M, Arcoraci V, Minutoli L, Scuruchi M, Cutroneo G, *et al*: BAY 11-7082 inhibits the NF-κB and NLRP3 inflammasome pathways and protects against IMQ-induced psoriasis. Clin Sci (Lond) 131: 487-498, 2017.
123. Chen X, Wang Y, Yao N and Lin Z: Immunoproteasome modulates NLRP3 inflammasome-mediated neuroinflammation under cerebral ischaemia and reperfusion conditions. J Cell Mol Med 26: 462-474, 2022.
124. Lang L, Xu B, Yuan J, Li S, Lian S, Chen Y, Guo J and Yang H: GABA-mediated activated microglia induce neuroinflammation in the hippocampus of mice following cold exposure through the NLRP3 inflammasome and NF-κB signaling pathways. Int Immunopharmacol 89: 106908, 2020.
125. Gan HT, Chen YQ and Ouyang Q: Sulfasalazine inhibits activation of nuclear factor-kappaB in patients with ulcerative colitis. J Gastroen Hepatol 20: 1016-1024, 2005.
126. Hafez HM, Ibrahim MA, Yehia AW, Gad AA, Mohammed NAHS and Abdel-Gaber SA: Protective effect of mirtazapine against acetic acid-induced ulcerative colitis in rats: Role of NLRP3 inflammasome pathway. Int Immunopharmacol 101: 108174, 2021.
127. Ullah H, Saba E, Lee YY, Hong SB, Hyun SH, Kwak YS, Park CK, Kim SD and Rhee MH: Restorative effects of Rg3-enriched Korean Red Ginseng and Persicaria tinctoria extract on oxazolone-induced ulcerative colitis in mice. J Ginseng Res 46: 628-635, 2022.
128. Zhang Y and Shi Y: Intracellular potassium ion measurements by inductively coupled plasma optical emission spectrometer (ICP-OES). Methods Mol Biol 2459: 85-92, 2022.
129. Leu WJ, Chu JC, Hsu JL, Du CM, Jiang YH, Hsu LC, Huang WJ and Guh JH: Chalcones display anti-NLRP3 inflammasome activity in macrophages through inhibition of both priming and activation steps-structure-activity-relationship and mechanism studies. Molecules 25: 5960, 2020.
130. Li Q, Feng H, Wang H, Wang Y, Mou W, Xu G, Zhang P, Li R, Shi W, Wang Z, *et al*: Licochalcone B specifically inhibits the NLRP3 inflammasome by disrupting NEK7-NLRP3 interaction. EMBO Rep 23: e53499, 2022.
131. Abais JM, Xia M, Zhang Y, Boini KM and Li PL: Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? Antioxid Redox Sign 22: 1111-1129, 2015.
132. Lan XF, Zhang XJ, Lin YN, Wang Q, Xu HJ, Zhou LN, Chen PL and Li QY: Estradiol regulates Txnip and prevents intermittent hypoxia-induced vascular injury. Sci Rep 7: 10318, 2017.
133. Zhao Y, Guo Q, Zhu Q, Tan R, Bai D, Bu X, Lin B, Zhao K, Pan C, Chen H, *et al*: Flavonoid VI-16 protects against DSS-induced colitis by inhibiting Txnip-dependent NLRP3 inflammasome activation in macrophages via reducing oxidative stress. Mucosal Immunol 12: 1150-1163, 2019.
134. Al KH, Brown LJ, Hossain KR, Hudson AL, Sinclair-Burton AA, Ng JP, Daniel EL, Hare JE, Cornell BA, Curmi PM, *et al*: Members of the chloride intracellular ion channel protein family demonstrate glutaredoxin-like enzymatic activity. PLoS One 10: e115699, 2015.
135. Ye L, Zeng Q, Ling M, Ma R, Chen H, Lin F, Li Z and Pan L: Inhibition of IP3R/Ca2+ Dysregulation Protects mice from ventilator-induced lung injury via endoplasmic reticulum and mitochondrial pathways. Front Immunol 12: 729094, 2021.
136. Davis BK, Wen H and Ting JP: The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu Rev Immunol 29: 707-735, 2011.
137. Dick MS, Sborgi L, Ruhl S, Hiller S and Broz P: ASC filament formation serves as a signal amplification mechanism for inflammasomes. Nat Commun 7: 11929, 2016.

138. Duncan JA, Bergstralh DT, Wang Y, Willingham SB, Ye Z, Zimmermann AG and Ting JP: Cryopyrin/NALP3 binds ATP/dATP, is an ATPase, and requires ATP binding to mediate inflammatory signaling. Proc Natl Acad Sci USA 104: 8041-8046, 2007.
139. Wu D, Chen Y, Sun Y, Gao Q, Li H, Yang Z, Wang Y, Jiang X and Yu B: Target of MCC950 in inhibition of NLRP3 inflammasome activation: A literature review. Inflammation 43: 17-23, 2020.
140. Dempsey C, Rubio AA, Bryson KJ, Finucane O, Larkin C, Mills EL, Robertson A, Cooper MA, O'Neill L and Lynch MA: Inhibiting the NLRP3 inflammasome with MCC950 promotes non-phlogistic clearance of amyloid-beta and cognitive function in APP/PS1 mice. Brain Behav Immun 61: 306-316, 2017.
141. Coll RC, Robertson AA, Chae JJ, Higgins SC, Munoz-Planillo R, Inserra MC, Vetter I, Dungan LS, Monks BG, Stutz A, *et al*: A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat Med 21: 248-255, 2015.
142. Gross CJ, Mishra R, Schneider KS, Medard G, Wettmarshausen J, Dittlein DC, Shi H, Gorka O, Koenig PA, Fromm S, *et al*: K^+ Efflux-independent NLRP3 inflammasome activation by small molecules targeting mitochondria. Immunity 45: 761-773, 2016.
143. van der Heijden T, Kritikou E, Venema W, van Duijn J, van Santbrink PJ, Slutter B, Foks AC, Bot I and Kuiper J: NLRP3 inflammasome inhibition by MCC950 reduces atherosclerotic lesion development in apolipoprotein E-deficient Mice-brief report. Arterioscl Throm Vas 37: 1457-1461, 2017.
144. Perera AP, Fernando R, Shinde T, Gundamaraju R, Southam B, Sohal SS, Robertson A, Schroder K, Kunde D and Eri R: MCC950, a specific small molecule inhibitor of NLRP3 inflammasome attenuates colonic inflammation in spontaneous colitis mice. Sci Rep 8: 8618, 2018.
145. Ye J, Li L, Wang M, Ma Q, Tian Y, Zhang Q, Liu J, Li B, Zhang B, Liu H, *et al*: Diabetes mellitus promotes the development of atherosclerosis: The role of NLRP3. Front Immunol 13: 900254, 2022.
146. Franke M, Bieber M, Kraft P, Weber A, Stoll G and Schuhmann MK: The NLRP3 inflammasome drives inflammation in ischemia/reperfusion injury after transient middle cerebral artery occlusion in mice. Brain Behav Immun 92: 223-233, 2021.
147. Zhai Y, Meng X, Ye T, Xie W, Sun G and Sun X: Inhibiting the NLRP3 inflammasome activation with MCC950 ameliorates diabetic encephalopathy in db/db Mice. Molecules 23: 522, 2018.
148. Zhang C, Zhu X, Li L, Ma T, Shi M, Yang Y and Fan Q: A small molecule inhibitor MCC950 ameliorates kidney injury in diabetic nephropathy by inhibiting NLRP3 inflammasome activation. Diabetes Metab Syndr Obes 12: 1297-1309, 2019.
149. Kuo LM, Kuo CY, Lin CY, Hung MF, Shen JJ and Hwang TL: Intracellular glutathione depletion by oridonin leads to apoptosis in hepatic stellate cells. Molecules 19: 3327-3344, 2014.
150. He H, Jiang H, Chen Y, Ye J, Wang A, Wang C, Liu Q, Liang G, Deng X, Jiang W, *et al*: Oridonin is a covalent NLRP3 inhibitor with strong anti-inflammasome activity. Nat Commun 9: 2550, 2018.
151. Gao RF, Li X, Xiang HY, Yang H, Lv CY, Sun XL, Chen HZ, Gao Y, Yang JS, Luo W, *et al*: The covalent NLRP3-inflammasome inhibitor Oridonin relieves myocardial infarction induced myocardial fibrosis and cardiac remodeling in mice. Int Immunopharmacol 90: 107133, 2021.
152. Jia Y, Tong Y, Min L, Li Y and Cheng Y: Protective effects of oridonin against cerebral ischemia/reperfusion injury by inhibiting the NLRP3 inflammasome activation. Tissue Cell 71: 101514, 2021.
153. Yan C, Yan H, Mao J, Liu Y, Xu L, Zhao H, Shen J, Cao Y, Gao Y, Li K, *et al*: Neuroprotective effect of oridonin on traumatic brain injury via inhibiting NLRP3 inflammasome in experimental mice. Front Neurosci 14: 557170, 2020.
154. Liang L, Zheng Y, Xie Y, Xiao L and Wang G: Oridonin ameliorates insulin resistance partially through inhibition of inflammatory response in rats subjected to chronic unpredictable mild stress. Int Immunopharmacol 91: 107298, 2021.
155. Marchetti C, Swartzwelter B, Koenders MI, Azam T, Tengesdal IW, Powers N, de Graaf DM, Dinarello CA and Joosten L: NLRP3 inflammasome inhibitor OLT1177 suppresses joint inflammation in murine models of acute arthritis. Arthritis Res Ther 20: 169, 2018.
156. Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, Copeland NG, Lee MK, Younkin LH, Wagner SL, *et al*: Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: Evidence for augmentation of a 42-specific gamma secretase. Hum Mol Genet 13: 159-170, 2004.
157. Marchetti C, Swartzwelter B, Gamboni F, Neff CP, Richter K, Azam T, Carta S, Tengesdal I, Nemkov T, D'Alessandro A, *et al*: OLT1177, a β-sulfonyl nitrile compound, safe in humans, inhibits the NLRP3 inflammasome and reverses the metabolic cost of inflammation. Proc Natl Acad Sci USA 115: E1530-E1539, 2018.
158. Lonnemann N, Hosseini S, Marchetti C, Skouras DB, Stefanoni D, D'Alessandro A, Dinarello CA and Korte M: The NLRP3 inflammasome inhibitor OLT1177 rescues cognitive impairment in a mouse model of Alzheimer's disease. Proc Natl Acad Sci USA 117: 32145-32154, 2020.
159. Sanchez-Fernandez A, Skouras DB, Dinarello CA and Lopez-Vales R: OLT1177 (Dapansutrile), a selective NLRP3 inflammasome inhibitor, ameliorates experimental autoimmune encephalomyelitis pathogenesis. Front Immunol 10: 2578, 2019.
160. Oizumi T, Mayanagi T, Toya Y, Sugai T, Matsumoto T and Sobue K: NLRP3 Inflammasome inhibitor OLT1177 suppresses onset of inflammation in mice with dextran sulfate sodium-induced colitis. Digest Dis Sci 67: 2912-2921, 2022.
161. Toldo S, Mauro AG, Cutter Z, Van Tassell BW, Mezzaroma E, Del BM, Prestamburgo A, Potere N and Abbate A: The NLRP3 inflammasome inhibitor, OLT1177 (Dapansutrile), reduces infarct size and preserves contractile function after ischemia reperfusion injury in the mouse. J Cardiovasc Pharm 73: 215-222, 2019.
162. Shi Y, Lv Q, Zheng M, Sun H and Shi F: NLRP3 inflammasome inhibitor INF39 attenuated NLRP3 assembly in macrophages. Int Immunopharmacol 92: 107358, 2021.
163. Cocco M, Pellegrini C, Martinez-Banaclocha H, Giorgis M, Marini E, Costale A, Miglio G, Fornai M, Antonioli L, Lopez-Castejon G, *et al*: Development of an acrylate derivative targeting the NLRP3 inflammasome for the treatment of inflammatory bowel disease. J Med Chem 60: 3656-3671, 2017.
164. Pu Z, Han C, Zhang W, Xu M, Wu Z, Liu Y, Wu M, Sun H and Xie H: Systematic understanding of the mechanism and effects of Arctigenin attenuates inflammation in dextran sulfate sodium-induced acute colitis through suppression of NLRP3 inflammasome by SIRT1. Am J Transl Res 11: 3992-4009, 2019.
165. Pellegrini C, Fornai M, Colucci R, Benvenuti L, D'Antongiovanni V, Natale G, Fulceri F, Giorgis M, Marini E, Gastaldi S, *et al*: A Comparative study on the efficacy of NLRP3 inflammasome signaling inhibitors in a Pre-clinical model of bowel inflammation. Front Pharmacol 9: 1405, 2018.
166. Huang Y, Jiang H, Chen Y, Wang X, Yang Y, Tao J, Deng X, Liang G, Zhang H, Jiang W, *et al*: Tranilast directly targets NLRP3 to treat inflammasome-driven diseases. EMBO Mol Med 10: e8689, 2018.
167. Saeedi-Boroujeni A, Mahmoudian-Sani MR, Nashibi R, Houshmandfar S, Tahmaseby GS and Khodadadi A: Tranilast: A potential anti-Inflammatory and NLRP3 inflammasome inhibitor drug for COVID-19. Immunopharm Immunot 43: 247-258, 2021.
168. Cao J and Peng Q: NLRP3 inhibitor Tranilast attenuates gestational diabetes mellitus in a genetic mouse model. Drugs R D 22: 105-112, 2022.
169. Chen S, Wang Y, Pan Y, Liu Y, Zheng S, Ding K, Mu K, Yuan Y, Li Z, Song H, *et al*: Novel role for Tranilast in regulating NLRP3 ubiquitination, vascular inflammation, and atherosclerosis. J Am Heart Assoc 9: e15513, 2020.
170. Shen K, Jiang W, Zhang C, Cai L, Wang Q, Yu H, Tang Z, Gu Z and Chen B: Molecular mechanism of a specific NLRP3 inhibitor to alleviate seizure severity induced by pentylenetetrazole. Curr Mol Pharmacol 14: 579-586, 2021.
171. Marchetti C, Toldo S, Chojnacki J, Mezzaroma E, Liu K, Salloum FN, Nordio A, Carbone S, Mauro AG, Das A, *et al*: Pharmacologic inhibition of the NLRP3 inflammasome preserves cardiac function after ischemic and nonischemic injury in the mouse. J Cardiovasc Pharm 66: 1-8, 2015.
172. Kuwar R, Rolfe A, Di L, Xu H, He L, Jiang Y, Zhang S and Sun D: A novel small molecular NLRP3 inflammasome inhibitor alleviates neuroinflammatory response following traumatic brain injury. J Neuroinflamm 16: 81, 2019.

173. Kuwar R, Rolfe A, Di L, Blevins H, Xu Y, Sun X, Bloom GS, Zhang S and Sun D: A novel inhibitor targeting NLRP3 inflammasome reduces neuropathology and improves cognitive function in Alzheimer's disease transgenic mice. J Alzheimers Dis 82: 1769-1783, 2021.
174. Yin J, Zhao F, Chojnacki JE, Fulp J, Klein WL, Zhang S and Zhu X: NLRP3 Inflammasome inhibitor ameliorates amyloid pathology in a mouse model of Alzheimer's disease. Mol Neurobiol 55: 1977-1987, 2018.
175. Fulp J, He L, Toldo S, Jiang Y, Boice A, Guo C, Li X, Rolfe A, Sun D, Abbate A, *et al*: Structural insights of Benzenesulfonamide analogues as NLRP3 inflammasome inhibitors: Design, synthesis, and biological characterization. J Med Chem 61: 5412-5423, 2018.
176. He Y, Varadarajan S, Munoz-Planillo R, Burberry A, Nakamura Y and Nunez G: 3,4-methylenedioxy-β-nitrostyrene inhibits NLRP3 inflammasome activation by blocking assembly of the inflammasome. J Biol Chem 289: 1142-1150, 2014.
177. Juliana C, Fernandes-Alnemri T, Wu J, Datta P, Solorzano L, Yu JW, Meng R, Quong AA, Latz E, Scott CP, *et al*: Anti-inflammatory compounds parthenolide and Bay 11-7082 are direct inhibitors of the inflammasome. J Biol Chem 285: 9792-9802, 2010.
178. Shim DW, Shin WY, Yu SH, Kim BH, Ye SK, Koppula S, Won HS, Kang TB and Lee KH: BOT-4-one attenuates NLRP3 inflammasome activation: NLRP3 alkylation leading to the regulation of its ATPase activity and ubiquitination. Sci Rep 7: 15020, 2017.
179. Zheng D, Liwinski T and Elinav E: Inflammasome activation and regulation: Toward a better understanding of complex mechanisms. Cell Discov 6: 36, 2020.
180. Li Y, Niu X, Xu H, Li Q, Meng L, He M, Zhang J, Zhang Z and Zhang Z: VX-765 attenuates atherosclerosis in ApoE deficient mice by modulating VSMCs pyroptosis. Exp Cell Res 389: 111847, 2020.
181. Zhang Z, Bai H, Ma X, Shen M, Li R, Qiu D, Li S and Gao L: Blockade of the NLRP3/caspase-1 axis attenuates ketamine-induced hippocampus pyroptosis and cognitive impairment in neonatal rats. J Neuroinflamm 18: 239, 2021.
182. Liang Y, Song P, Chen W, Xie X, Luo R, Su J, Zhu Y, Xu J, Liu R, Zhu P, *et al*: Inhibition of Caspase-1 Ameliorates Ischemia-Associated Blood-Brain barrier dysfunction and integrity by suppressing pyroptosis activation. Front Cell Neurosci 14: 540669, 2020.
183. Gu L, Sun M, Li R, Zhang X, Tao Y, Yuan Y, Luo X and Xie Z: Didymin suppresses microglia pyroptosis and neuroinflammation through the Asc/Caspase-1/GSDMD pathway following experimental intracerebral hemorrhage. Front Immunol 13: 810582, 2022.
184. Tian DD, Wang M, Liu A, Gao MR, Qiu C, Yu W, Wang WJ, Zhang K, Yang L, Jia YY, *et al*: Antidepressant effect of paeoniflorin is through inhibiting pyroptosis CASP-11/GSDMD pathway. Mol Neurobiol 58: 761-776, 2021.
185. Wang F, Liang Q, Ma Y, Sun M, Li T, Lin L, Sun Z and Duan J: Silica nanoparticles induce pyroptosis and cardiac hypertrophy via ROS/NLRP3/Caspase-1 pathway. Free Radical Bio Med 182: 171-181, 2022.
186. Xu S, Li X, Liu Y, Xia Y, Chang R and Zhang C: Inflammasome inhibitors: Promising therapeutic approaches against cancer. J Hematol Oncol 12: 64, 2019.
187. Wang F, Li G, Ning J, Chen L, Xu H, Kong X, Bu J, Zhao W, Li Z, Wang X, *et al*: Alcohol accumulation promotes esophagitis via pyroptosis activation. Int J Biol Sci 14: 1245-1255, 2018.
188. Qiu Z, He Y, Ming H, Lei S, Leng Y and Xia ZY: Lipopolysaccharide (LPS) aggravates high glucose- and Hypoxia/Reoxygenation-induced injury through activating ROS-dependent NLRP3 inflammasome-mediated pyroptosis in H9C2 Cardiomyocytes. J Diabetes Res 2019: 8151836, 2019.
189. Liang H, Sun Y, Gao A, Zhang N, Jia Y, Yang S, Na M, Liu H, Cheng X, Fang X, *et al*: Ac-YVAD-cmk improves neurological function by inhibiting caspase-1-mediated inflammatory response in the intracerebral hemorrhage of rats. Int Immunopharmacol 75: 105771, 2019.
190. Pan P, Shen M, Yu Z, Ge W, Chen K, Tian M, Xiao F, Wang Z, Wang J, Jia Y, *et al*: SARS-CoV-2 N protein promotes NLRP3 inflammasome activation to induce hyperinflammation. Nat Commun 12: 4664, 2021.
191. Zheng X, Chen W, Gong F, Chen Y and Chen E: The role and mechanism of pyroptosis and potential therapeutic targets in sepsis: A review. Front Immunol 12: 711939, 2021.
192. Liu W, Guo W, Zhu Y, Peng S, Zheng W, Zhang C, Shao F, Zhu Y, Hang N, Kong L, *et al*: Targeting Peroxiredoxin 1 by a Curcumin analogue, AI-44, inhibits NLRP3 inflammasome activation and attenuates lipopolysaccharide-induced sepsis in mice. J Immunol 201: 2403-2413, 2018.
193. Liu W, Guo W, Wu J, Luo Q, Tao F, Gu Y, Shen Y, Li J, Tan R, Xu Q and Sun Y: A novel benzo[d]imidazole derivate prevents the development of dextran sulfate sodium-induced murine experimental colitis via inhibition of NLRP3 inflammasome. Biochem Pharmacol 85: 1504-1512, 2013.
194. Sborgi L, Ruhl S, Mulvihill E, Pipercevic J, Heilig R, Stahlberg H, Farady CJ, Muller DJ, Broz P and Hiller S: GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. EMBO J 35: 1766-1778, 2016.
195. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, Zhuang Y, Cai T, Wang F and Shao F: Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 526: 660-665, 2015.
196. Liu Z, Gan L, Xu Y, Luo D, Ren Q, Wu S and Sun C: Melatonin alleviates inflammasome-induced pyroptosis through inhibiting NF-κB/GSDMD signal in mice adipose tissue. J Pineal Res: 63, 2017 doi: 10.1111/jpi.12414.
197. Sun L, Wang H, Wang Z, He S, Chen S, Liao D, Wang L, Yan J, Liu W, Lei X and Wang X: Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell 148: 213-227, 2012.
198. Rathkey JK, Zhao J, Liu Z, Chen Y, Yang J, Kondolf HC, Benson BL, Chirieleison SM, Huang AY, Dubyak GR, *et al*: Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell death and sepsis. Sci Immunol 3: eaat2738, 2018.
199. Han C, Yang Y, Guan Q, Zhang X, Shen H, Sheng Y, Wang J, Zhou X, Li W, Guo L, *et al*: New mechanism of nerve injury in Alzheimer's disease: β-amyloid-induced neuronal pyroptosis. J Cell Mol Med 24: 8078-8090, 2020.
200. Teng JF, Mei QB, Zhou XG, Tang Y, Xiong R, Qiu WQ, Pan R, Law BY, Wong VK, Yu CL, *et al*: Polyphyllin VI induces Caspase-1-mediated Pyroptosis via the induction of ROS/NF-κB/NLRP3/GSDMD signal axis in Non-small cell lung cancer. Cancers (Basel) 12: 193, 2020.
201. Guo W, Chen S, Li C, Xu J and Wang L: Application of Disulfiram and its metabolites in treatment of inflammatory disorders. Front Pharmacol 12: 795078, 2021.
202. Wu J, Zhang J, Zhao J, Chen S, Zhou T and Xu J: Treatment of severe acute pancreatitis and related lung injury by targeting gasdermin D-mediated pyroptosis. Front Cell Dev Biol 9: 780142, 2021.
203. Pandeya A, Li L, Li Z and Wei Y: Gasdermin D (GSDMD) as a new target for the treatment of infection. Medchemcomm 10: 660-667, 2019.
204. Hu JJ, Liu X, Xia S, Zhang Z, Zhang Y, Zhao J, Ruan J, Luo X, Lou X, Bai Y, *et al*: FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. Nat Immunol 21: 736-745, 2020.
205. Zhou Q, Wang W, Yang F, Wang H, Zhao X, Zhou Y, Fu P and Xu Y: Disulfiram suppressed Peritendinous fibrosis through inhibiting macrophage accumulation and its pro-inflammatory properties in tendon bone healing. Front Bioeng Biotech 10: 823933, 2022.
206. Yan H, Yang H, Wang L, Sun X, Han L, Cong P, Chen X, Lu D and Che C: Disulfiram inhibits IL-1β secretion and inflammatory cells recruitment in Aspergillus fumigatus keratitis. Int Immunopharmacol 102: 108401, 2022.
207. Cattani-Cavalieri I, Da MVH, Moraes JA, Brito-Gitirana L, Romana-Souza B, Schmidt M and Valenca SS: Dimethyl fumarate attenuates lung inflammation and oxidative stress induced by chronic exposure to diesel exhaust particles in mice. Int J Mol Sci 21: 9658, 2020.
208. Humphries F, Shmuel-Galia L, Ketelut-Carneiro N, Li S, Wang B, Nemmara VV, Wilson R, Jiang Z, Khalighinejad F, Muneeruddin K, *et al*: Succination inactivates gasdermin D and blocks pyroptosis. Science 369: 1633-1637, 2020.
209. Muhammad JS, Jayakumar MN, Elemam NM, Venkatachalam T, Raju TK, Hamoudi RA and Maghazachi AA: Gasdermin D Hypermethylation inhibits pyroptosis and LPS-Induced IL-1β release from NK92 cells. Immunotargets Ther 8: 29-41, 2019.

210. Xia L, Liu L, Cai Y, Zhang Y, Tong F, Wang Q, Ding J and Wang X: Inhibition of Gasdermin D-mediated pyroptosis attenuates the severity of seizures and astroglial damage in kainic Acid-induced epileptic mice. Front Pharmacol 12: 751644, 2021.
211. Sohn E, Kim J, Kim CS, Jo K and Kim JS: Osteomeles schwerinae extract prevents diabetes-induced renal injury in spontaneously diabetic Torii rats. Evid Based Complement Alternat Med 2018: 6824215, 2018.
212. Soma J, Sugawara T, Huang YD, Nakajima J and Kawamura M: Tranilast slows the progression of advanced diabetic nephropathy. Nephron 92: 693-698, 2002.
213. Soma J, Sato K, Saito H and Tsuchiya Y: Effect of Tranilast in early-stage diabetic nephropathy. Nephrol Dial Transpl 21: 2795-2799, 2006.
214. Kosuga K, Tamai H, Ueda K, Hsu YS, Ono S, Tanaka S, Doi T, Myou-U W, Motohara S and Uehata H: Effectiveness of Tranilast on restenosis after directional coronary atherectomy. Am Heart J 134: 712-718, 1997.
215. Wohlford GF, Van Tassell BW, Billingsley HE, Kadariya D, Canada JM, Carbone S, Mihalick VL, Bonaventura A, Vecchie A, Chiabrando JG, *et al*: Phase 1B, randomized, Double-blinded, dose escalation, single-center, repeat dose safety and pharmacodynamics study of the oral NLRP3 inhibitor dapansutrile in subjects with NYHA II-III systolic heart failure. J Cardiovasc Pharm 77: 49-60, 2020.
216. Kim M, Byun J, Chung Y, Lee SU, Park JE, Park W, Park JC, Ahn JS and Lee S: Reactive Oxygen species scavenger in acute intracerebral hemorrhage patients: A multicenter, randomized controlled trial. Stroke 52: 1172-1181, 2021.
217. Masnadi SK, Sotoudeh S, Masnadi SA, Moaddab SY, Nourpanah Z and Nikniaz Z: Effect of N-acetylcysteine on remission maintenance in patients with ulcerative colitis: A randomized, double-blind controlled clinical trial. Clin Res Hepatol Gas 45: 101532, 2021.
218. Reinhardt S, Stoye N, Luderer M, Kiefer F, Schmitt U, Lieb K and Endres K: Identification of disulfiram as a Secretase-modulating compound with beneficial effects on Alzheimer's disease hallmarks. Sci Rep 8: 1329, 2018.
219. Yuan XZ, Sun S, Tan CC, Yu JT and Tan L: The role of ADAM10 in Alzheimer's disease. J Alzheimers Dis 58: 303-322, 2017.