Review

Targeting the NLRP3 Inflammasome: Novel Inhibitors for Cardiovascular Disease Management

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Review

Targeting the NLRP3 Inflammasome: Novel Inhibitors for Cardiovascular Disease Management

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Abstract: The innate immune system is the first line of defense against pathogens and intracellular danger signals, providing a rapid non-specific response to eliminate the infection and maintain tissue homeostasis. NLRP3 inflammasome as a critical component of the innate immune system plays a pivotal role in the inflammatory response. Many research studies have highlighted the implication of NLRP3 in the pathogenesis of various cardiovascular diseases including atherosclerosis, myocardial infarction, hypertension and heart failure. Activation of NLRP3 elicits a robust inflammatory response represented in proteolytic cleavage and release of pro-inflammatory cytokines such as IL-1β and IL-18, which contribute to the progression of vascular inflammation and myocardial damage. This review aims to comprehensively examine the current understanding of NLRP3 inflammasome activation in cardiovascular diseases, exploring the molecular mechanisms underlying its role in inflammation and tissue injury. Furthermore, we will discuss the evolution of NLRP3 inflammasome inhibitors, focusing on the most novel small synthetic inhibitors of NLRP3 protein and phytoestrogens targeting NLRP3, providing a thorough overview of the potential of targeting NLRP3 as a therapeutic strategy for cardiovascular disease management.

Keywords: cardiovascular diseases; Novel NLRP3 inhibitors; phytoestrogens

1. Introduction

The innate immunity represented in tissue-resident macrophages, dendritic cells, granulocytes as well as marrow and blood monocytes, acts as a primary defense mechanism, detecting extracellular and intracellular danger signals or microbes through pattern recognition receptors (PRRs) [1]. The membrane-bound and cytosolic PRRs can recognize nonspecific pathogen-associated molecular patterns (PAMPs), which are derived from microorganisms and self-derived molecules, defined as damage-associated molecular patterns (DAMPs). The activation of PRRs triggers a swift and potent inflammatory response, both locally and systemically, leading to the production of several pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and IL-18 and ultimately results in the elimination of the pathogen, maintaining tissue homeostasis and correct orang function [2,3].

Inflammasome is a newly identified cytosolic PRR that was first described in detail in 2002. So far, five well-characterized inflammasomes have been identified: nucleotide-binding domain leucine-rich repeat (NLR) and pyrin domain-containing receptor 1 (NLRP1), NLRP3, NLR and caspase recruitment domain-containing receptor 4 (NLRC4), the AIM2-like receptor (ALR) family, which includes absent in melanoma 2 (AIM2), and interferon gamma-inducible protein 16 (IFI16) [3–6]. Among them, NLRP3 has been extensively studied and was reported to be implicated in the pathogenesis of many chronic inflammatory diseases including gout, atherosclerosis, obesity, type 2 diabetes, neurodegenerative disorders such as Alzheimer's disease and autoimmune diseases such as Crohn's disease, rheumatoid arthritis and systemic lupus erythematosus [7,8]. Besides, NLRP3-gain-of-function mutation has been linked to cryopyrin associated periodic syndromes (CAPS) [9]. Therefore, pharmacological targeting of NLRP3 addresses unmet medical needs. With this review, we outline the mechanisms underlying NLRP3 inflammasome formation and activation and explore

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the role of NLRP3 in cardiovascular diseases (CVDs). Additionally, we will discuss the evolution of NLRP3 inhibitors elaborating the different classes of the small synthetic molecules that can inhibit NLRP3 protein per se, focusing on the most novel ones as well as their preclinical and clinical usage, the most recent phytochemicals and phytoestrogens with NLRP3 inhibitory activity.

2. An Overview of NLRP3 Inflammasome Structure, Assembly and Activation

The NLRP3 inflammasome is an intracellular complex consisting of 3 major components: the sensor NLRP3, the adaptor apoptosis-associated speck-like protein (ASC), and the effector Caspase1. The NLRP3 is composed of three domains: a pyrin domain (PYD) at the amino (N) terminal, NAIP, CIITA, HETE, and TP1 (NACHT) domain in the center, and leucine-rich repeat (LRR) domain at the carboxyl (C) terminal. The PYD domain plays a role in recruiting the ASC protein once the NLRP3 is activated while the NACHT domain functions as an ATPase in which the Walker A motif contains an ATP-binding site and the Walker B motif is essential to ATPase activity with subsequent NLRP3 oligomerization and function. Consisting of 12 repeats, the LRR domain has more complex functions. Previous research had indicated that LRR plays an autoinhibitory role through folding back onto the NACHT domain. On the other hand, some studies showed that LRR domain can undergo several posttranslational modifications such deubiquitination and phosphorylation upon sensing the danger signal, playing a role in NLRP3 activation [10–14]. In addition, previous structural studies have demonstrated that the NLRP3- NEK7 (NIMA-related kinase 7) interaction and the creation of the NLRP3 cage structure, that disperses the trans-Golgi network in the first stage of the inflammasome pathway, are controlled by the LRR domain [15,16]. Within that context, NEK7 is a serine-threonine kinase involved in mitosis specifically interacts with NLRP3 but not with NLRC4 or AIM2 via its catalytic domain, independently of its kinase activity. More importantly, NLRP3-NEK7 interaction is also crucial for enhancing ASC speck formation and procaspase-1 activation, subsequently to sensing the danger signal by NLRP3. Thereby, NEK7 is a crucial mediator for full NLRP3 activation [17–19].

Once NLRP3 sensed the danger signal and became activated, it recruits ASC, a bipartite protein consists of two protein interaction domains: N-terminal PYD and C-terminal caspase-recruitment domain (CARD), via homotypic PYD-PYD interactions, initiating the formation of helical ASC filaments, which in turn merge into single macromolecular fibrillary structure, known as ASC speck [20,21]. Upon assembly and oligomerization of ASC, procaspase-1 is recruited by CARD-CARD interactions and activated by proximity-induced self-cleavage into caspase-1 [22]. Caspase-1 by its turn cleaves and activates cytosolic pro-interleukin (IL)-1β and pro-interleukin (IL)-18, converted into their mature biologically active form. Simultaneously with the cleavage of IL-1β and IL-18, Gasdermin D (GSDMD) is cleaved by activated caspase-1, leading to a regulated form of cell death known as pyroptosis. After cleavage, the N-terminal fragment of GSDMD binds to membrane lipids and forms micropores, resulting in cell rupture and release of the inflammatory cytokines, recruiting other immune cells to the site of infection, thereby helping in the eradication of pathogens [23–25].

NLRP3 inflammasome activation is mediated by canonical pathway which is a finely regulated two-step process involving priming and activation. Priming aims to increase the genes expression of NLRP3 and pro-IL-1β to elevate their cellular levels on contrary to ASC, procaspase-1 and pro-IL-18, which under basic conditions, are abundant in the cell cytoplasm [26,27]. NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is the transcription factor which induces the expression of NLRP3 and pro-IL-1β upon its activation downstream of many PAMPs, DAMPs and cytokine receptors, such as Toll-like receptors (TLRs), IL-1 receptor 1 (IL-1R1), NOD-like receptors and tumor necrosis factor receptor 1 and 2 (TNFR1, TNFR2). Once primed, cytosolic NLRP3 is kept in an inactive state due to the ubiquitination of its LRR domain; therefore, deubiquitination is necessary before NLRP3 can become activated [10,26,28].

The observation that NLRP3 is assembled and activated by a vast diversity of stimuli including elevated extracellular ATP levels, PAMPs, bacterial ionophores, pore-forming toxins, RNA viruses, and lysosome-damaging agents such as crystalline silica, cholesterol, and monosodium urate (MSU) crystals, proposed the notion that NLRP3 activation is mediated by a common second messenger that

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is recognized by NLRP3 [29,30]. Interestingly, most agents that activate NLRP3 are known to damage the plasma membrane, resulting in a decrease in cytosolic potassium K^+ levels, calcium mobilization, chloride efflux, translocation of oxidized double-stranded DNA (dsDNA) from damaged mitochondria into the cytosol, and lysosome rupture [31,32]. However, NLRP3 activation can also occur independently of K^+ efflux and without plasma membrane damage in response to specific stimuli, such as in human monocytes exposed to the TLR7 ligand imiquimod or extracellular lipopolysaccharide (LPS), suggesting NLRP3 may have multiple parallel activation pathways [33,34].

Recently, other pathways for NLRP3 inflammasome activation, including non-canonical and alternative mechanisms, have been reported. In the non-canonical NLRP3 inflammasome activation, lipopolysaccharide (LPS) of gram-negative bacteria is detected by human caspases-4 and -5, as well as murine caspase-11, triggering its oligomerization and activation through auto-proteolytic cleavage upon the direct interaction between LPS and different caspases [35–38]. This activation subsequently results in the cleavage of GSDMD by caspases-4, -5, or -11, inducing pyroptosis [39,40]. As a result of pyroptosis, K^+ efflux stimulates NLRP3-caspase-1-dependent secretion of IL-1β. Additionally, the oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC) can also bind to caspase-11, further activating this non-canonical pathway [41]. Besides, the alternative NLRP3 inflammasome activation is not yet fully understood; however, evidence suggests involvement of toll like receptor 4 (TLR4) along with TRIF, RIPK-1 (receptor-interacting serine/threonine-protein kinase 1), and FADD (Fas-associated protein with a death domain), with caspase-8 as upstream signaling that directly activates NLRP3, independently of K^+ efflux and without induction of GSDMD as a pyroptotic effector [34,42].

3. NLRP3 Implication in the Pathogenesis of Cardiovascular Diseases

NLRP3 inflammasome activation has been reported to be implicated in the pathogenesis of cardiovascular diseases (CVDs). However, the dynamics of NLRP3 activation vary between acute and chronic injuries. DAMPs mediate the priming phase of NLRP3 following acute cardiac injuries such as myocardial infarction and ischemic/reperfusion injury. On the other hand, in the chronic conditions associated with CVDs such as hypertension, atherosclerosis and diabetes, the metabolites and/or neurohormonal activation (e.g., angiotensin II, trimethylamine N-oxide, and glucose) enhances the expression of NLRP3 inflammasome components and substrates [43,44].

3.1. Atherosclerosis

Atherosclerosis is a chronic inflammatory pathological process that starts with accumulation and trapping of low-density lipoprotein (LDL) in the subendothelial intimal layer of arteries. Then, LDL is undergoing oxidation, converted into oxidized LDL which in turn causes activation of endothelial cells and infiltration of monocytes and T lymphocytes into vascular intima. Monocyte-to-macrophage differentiation allows them to engulf the oxidized LDL, forming foam cells, which accumulate in the intima and release inflammatory cytokines. The Outcome of the inflammatory cytokines release is recruitment and proliferation of smooth muscle cells as well as endothelial dysfunction. Over time, the atherosclerotic plaque is formed due to accumulation of lipid-laden foam cells and the gradually enlarged plaque reduce the blood flow into the organ/tissue, resulting in ischemia and infarction [45,46].

Interestingly, NLRP3 activation has been implicated in atherosclerotic plaque formation with several stimuli contributing to its activation. In macrophages, cholesterol and calcium phosphate crystals cause lysosomal destabilization, resulting in the release of cathepsin B, which subsequently leads to K^+ efflux and activates the NLRP3 inflammasome, triggering the release of IL-1β [47–49]. Additionally, NEK7 detects alterations in intracellular K^+ levels and facilitates the activation of the NLRP3 inflammasome [17].

Numerous clinical and experimental studies have indicated the involvement of NLRP3-mediated inflammatory cytokines in atherosclerosis. Some clinical studies have demonstrated elevated NLRP3 expression in aortic tissue samples from patients with atherosclerosis, and this

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increase was associated with disease severity [50,51]. Elevated levels of inflammasome components, such as NLRP3, ASC, IL-1β, and IL-18, were also observed in human carotid atherosclerotic plaques [52]. Moreover, mRNA expression of NLRP3, ASC, caspase-1, IL-1β, and IL-18 was significantly higher in atherosclerotic plaques from symptomatic patients compared to asymptomatic individuals [53]. Collectively, these findings suggest that atherosclerotic patients exhibit activation of NLRP3 pathways in both atherosclerotic plaques and the bloodstream, which correlates with disease severity. This underscores the importance of NLRP3 inflammasome signaling in the development and progression of atherosclerosis.

On the other hand, Duewell et al. were the first to directly demonstrate the significance of NLRP3 in atherogenesis in vivo. They used LDL receptor-deficient ($LDLR^{-/-}$) mice as a model and transplanted bone marrow from wild-type, $NLRP3^{-/-}$, $ASC^{-/-}$, or IL-1$\alpha^{-/-}$/IL-1$\beta^{-/-}$ mice, subsequently feeding them a Western diet containing 0.15% cholesterol. The results indicated a reduction in atherosclerotic lesions in mice transplanted with $NLRP3^{-/-}$, $ASC^{-/-}$, or IL-1$\alpha^{-/-}$/IL-1$\beta^{-/-}$ marrow compared to those with wild-type marrow [48]. In 2012, Usui et al. crossed $Apoe^{-/-}$ mice with $Casp1^{-/-}$ mice to generate $Apoe^{-/-}$ $Casp1^{-/-}$ mice. When fed a Western diet, these mice also exhibited reduced lesions without changes in total serum cholesterol levels or lipoprotein-cholesterol distribution [54]. Similarly, Gage et al. in the same year fed $Apoe^{-/-}$ $Casp1^{-/-}$ mice a high fat diet (HFD) and observed comparable results [55]. These findings suggest that the inflammatory response mediated by NLRP3 plays a significant role in atherosclerotic lesion development.

3.2. Hypertension

Hypertension, or high blood pressure, is a prevalent risk factor for cardiovascular, cerebrovascular, and chronic kidney diseases. It is defined by consistently elevated systolic and/or diastolic blood pressure in the systemic arteries. There are two determinants that affect blood pressure directly, including poor vasodilation capacity and increased volume of intravascular fluid to which many factors are contributing including genetic predisposition, excess dietary salt intake, alcohol abuse and obesity [56,57]. Interestingly, the observation that serum IL-1β level was elevated in patients with high blood pressure suggested the contribution of inflammation in hypertension pathogenesis and aroused the possibility that activation of NLRP3 is involved in hypertension pathogenesis [58].

The first study to examine the relationship between NLRP3 and hypertension focused on alleviating high blood pressure in preeclampsia [59]. To identify the pathogenic contribution of NLRP3 activation in preeclampsia's hypertension, Shirasuna et al. developed a preeclampsia-induced hypertension model by administering angiotensin II to pregnant $NLRP3^{-/-}$ and $ASC^{-/-}$ mice [60]. Hypertension was prevented in $NLRP3^{-/-}$ mice, but no significant reduction in blood pressure was observed in $ASC^{-/-}$ mice, suggesting that NLRP3 contributes to hypertension development through a pathway independent of the inflammasome. Additionally, IL-6 levels, rather than IL-1β, were reduced in $NLRP3^{-/-}$ mice, indicating that NLRP3 plays a broad role in various inflammatory responses [60]. Therefore, targeting NLRP3 could provide additional benefits in mitigating auto-inflammation associated with hypertension.

Besides, upregulation of NLRP3 and IL-1β in the heart has been observed in two distinct mouse models of hypertension: transverse aortic constriction, which is a pressure overload model that leads to myocardial fibrosis and remodeling, and the angiotensin II-infusion model of hypertension. In both models, inhibiting or deleting NLRP3 improved cardiac remodeling by reducing inflammation and fibrosis [61–64]. However, the precise mechanisms behind inflammasome activation in the absence of ischemic damage and cell death remain unclear. Recent research suggests that in response to pressure overload, the priming and activation of cardiac NLRP3 are mediated by Ca^{2+}/calmodulin-dependent protein kinase II δ (CaMKIIδ) [64].

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3.3. Myocardial Infarction and Ischemic Reperfusion Injury

Myocardial infarction is defined as the death of heart muscle cells due to low oxygen supply caused by prolonged severe ischemia upon narrowing of coronary arteries by unstable atherosclerotic plaque. Prompt diagnosis and reperfusion therapy greatly enhance the survival rate of MI patients. However, cardiac ischemia-reperfusion (I/R) injury following reperfusion therapy exacerbates substantial myocardium damage through induction of sterile inflammatory responses, driven by NLRP3-inflammasome activation [65,66]. The NLRP3 is strongly activated upon sensing DAMPs and alarmins released from cells damaged by ischemia, which in turn can also recruit highly active inflammatory cells at the site of injury [67,68].

In experimental models of acute myocardial infarction with and without reperfusion, the NLRP3 inflammasome reaches peak activation at 1 day and 3 days post-ischemia, respectively. In addition, NLRP3 inflammasome specks are identifiable in leukocytes, endothelial cells, fibroblasts, and cardiomyocytes [67,69,70]. Furthermore, the implementation of animal models with gene deletions of inflammasome components, such as NLRP3, ASC, and caspase-1, has enhanced our understanding of the role inflammasome pathways play in the onset and progression of I/R injury. Sandanger et al. demonstrated that hearts from NLRP3$^{-/-}$ mice, which were perfused and exposed to I/R injury, exhibited significantly improved cardiac function, reduced hypoxic damage, and smaller infarct sizes [71]. These beneficial effects were not observed in hearts from ASC$^{-/-}$ mice, indicating potential ASC-independent roles for NLRP3 in I/R injury [72]. In addition, other studies revealed that a wide range of proinflammatory and oxidative signals, such as IL-17, ATF4, and TRPV1, initiate intracellular processes that facilitate both the assembly of the canonical NLRP3 inflammasome complex and the activation of NLRP3 signaling pathways independent of the inflammasome, following the myocardial I/R injury [73–75].

Besides, some clinical studies have reported elevated ASC expression in the heart tissues of patients with myocardial I/R injury. This increased expression was specifically observed in cardiac fibroblasts, rather than in cardiomyocytes, suggesting that the activation of inflammasome pathways may play a role in the cardiac dysfunction and remodeling that accompany I/R injury [76]. Cardiac fibroblasts are essential for maintaining normal heart function, as they regulate the synthesis and deposition of the extracellular matrix and interact with myocytes, endothelial cells, and other fibroblasts [77]. Bai et al. also reported elevated serum levels of IL-1β and IL-18 in patients with myocardial I/R injury, suggesting that NLRP3 inflammasome activation influences the pathogenesis of myocardial I/R injury [78].

3.4. Pericarditis

Pericarditis is defined as an intense inflammatory response because of infectious or noninfectious injury to the mesothelial cells forming the pericardial sac. NLRP3 inflammasome activation has been reported to play a role in the pathogenesis of pericarditis whereas inflammasome components, such as NLRP3, ASC, and caspase-1, were detected in pericardial samples from patients with chronic pericarditis during an acute exacerbation [79]. Interestingly, the inhibition of NLRP3 effectively reduced the risk of recurrent pericarditis in patients. While colchicine is considered the first-line therapy for both acute and recurrence pericarditis, Anakinra can effectively alleviate pericarditis in colchicine-resistant patients [80,81].

3.5. Cardiotoxicity

Many cancer chemotherapeutic agents have been reported with cardiotoxicity as a side effect of which doxorubicin is one of the most well-known for causing significant damage to cardiac tissue [82]. Doxorubicin causes dose-dependent acute and chronic cardiotoxicity, varying from occult alterations in myocardial structure and function to irreversible heart failure. The pathogenesis of doxorubicin-induced cardiomyopathy is complex and multifactorial, among them mitochondrial dysfunction and ROS production can directly activate NLRP3 inflammasome [83–85].

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Experimentally, mice treated with doxorubicin exhibit left ventricular dilation, impaired cardiac function, and increased cardiac fibrosis [86,87]. This decline in cardiac performance is associated with elevated expression of NLRP3, caspase-1, IL-1β, and IL-18 in cardiomyocytes, along with significant pyroptosis [88,89]. Pharmacological inhibition of NLRP3 and NLRP3$^{-/-}$ or Casp1$^{-/-}$ mice have been shown to reduce cardiac dysfunction and myocardial damage resulting from pyroptosis [88,90]. Considering the positive effects of IL-1β and IL-18 inhibition in radiation-induced cardiomyopathy, the NLRP3 inflammasome is suggested to play a direct role in the onset and progression of cardiac damage caused by radiation [91,92].

3.6. Diabetic Cardiomyopathy

Diabetic cardiomyopathy is a severe complication of diabetes mellitus describes structural alterations in the hearts of individuals with diabetes exhibiting left ventricular hypertrophy, myocardial cell death and myocardial fibrosis, all eventually lead to diastolic dysfunction and heart failure [93,94]. Type 2 diabetes (T2D) is associated with chronic low-grade inflammation, attributed to NLRP3 inflammasome activation which is also involved in the pathogenesis of diabetic cardiomyopathy. While Type 1 diabetes and T2D cause hyperglycemia, T2D additionally causes hyperlipidemia. Both glucotoxicity and lipotoxicity are significant contributors to the priming and activation of NLRP3 inflammasome pathways. Several studies have shown that elevated glucose and lipid levels in the blood can lead to increased production of ROS, which in turn causes activation of NF-κB, thereby enhancing the priming of NLRP3, IL-1β and IL-18 [95,96]. In addition, ROS upregulates the expression of thioredoxin interacting/inhibiting protein (TXNIP), which in turn directly binds to NLRP3, enhancing its oligomerization [97]. In mice model of streptozotocin (STZ)-induced diabetes mellitus, the deficiency of Sirtuin 3 aggravated hyperglycemia-induced mitochondrial damage, led to increased ROS accumulation, which in turn activated the NLRP3 inflammasome, and ultimately exacerbated diabetic cardiomyopathy (DCM) [98]. Moreover, the genetic deletion of NLRP3 and ASC alleviated the HFD-induced cardiac remodeling, and heart failure with preserved ejection fraction in mice [99]. In other study, mitochondrial oxidative damage and mtDNA release activated the cGAS-STING pathway, which in turn promotes NLRP3 inflammasome-dependent pyroptosis and inflammatory responses in cardiomyocytes, contributing to myocardial hypertrophy and the overall progression of diabetic cardiomyopathy [100].

Besides, diabetic conditions were reported to reduce the activity of sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2a), an enzyme maintains Ca^{2+} transport between reticulum and cytoplasm [101]. As a result of its dysfunction, Ca^{2+} transport is disrupted, leading to NLRP3 activation and subsequent pyroptosis; a finding that was observed in diabetic rats [102]. Taken together, these findings indicate that diabetic cardiomyopathy is mediated by NLRP3 inflammasome activation because of ROS production and Ca^{2+} disturbance.

3.7. Heart Failure

Heart failure represents the end stage of nearly all forms of severe cardiovascular diseases (CVDs) including hypertension, myocardial infarction, ischemic reperfusion injury and cardiomyopathies, in which NLRP3 inflammasome activation contributes to the pathogenesis. Heart failure is defined as the heart's inability to maintain adequate blood output due to either contractile or diastolic dysfunction, which significantly increases the mortality rate associated with CVDs [103,104]. The underlying pathophysiologic mechanism of HF is widely believed to involve myocardial remodeling, a process in which inflammatory cytokines are key contributors. Two critical physiological events central to this remodeling process are myocardial injury resulting from pyroptosis and the activation of cardiac fibroblasts [105].

The inflammatory cytokines cause cardiomyocytes death by induction of nitric oxide (NO) production. While IL-1β strongly induces the production of inducible nitric oxide synthase (iNOS), IL-18 enhances iNOS overexpression, which in turn results in excessive NO production, leading to cardiomyocytes death and tissue remodeling, as well as the generation of small, uncharged NO

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molecules [106–108]. These NO molecules can transform into reactive nitrogen species (RNS), functioning similarly to reactive oxygen species (ROS). Notably, the maturation of both cytokines depends on caspase-1 cleavage within the NLRP3 inflammasome [109–111]. Besides, Tumor necrosis factor α (TNF-α) is another inflammatory cytokine that significantly contributes to cardiomyocytes hypertrophy, apoptosis and decreasing cardiac contractility by decreasing intracellular Ca^{2+} release. Interestingly, IL-18, a downstream product of NLRP3 activation, promotes the production of TNF-α. In turn, TNF-α can activate the NF-κB pathway, which subsequently drives the transcription of NLRP3, IL-18 and IL-1β. This vicious cycle of inflammatory cytokines production intensifies myocardial tissue damage [112,113].

Fibrosis is a key component of ventricular remodeling and derived by cardiac fibroblasts, which constitute about two-thirds of the heart tissue. Hypoxia stimulates the generation of reactive oxygen species (ROS) and K^+ efflux from cardiac fibroblasts, both of which are major activators of the NLRP3 inflammasome, which serves as the primary sensor for damage-associated molecular patterns (DAMPs) following oxygen deprivation [76,114]. Through NLRP3 inflammasome activation, fibroblasts contribute to and amplify myocardial inflammatory damage. Beyond inflammation, hypoxia also induces a fibrogenic response in cardiac fibroblasts, leading to their differentiation into myofibroblasts and increased collagen synthesis. This process ultimately results in the proliferation of fibroblasts, myocardial fibrosis, and ventricular remodeling. Independently of its inflammasome activity, NLRP3 was found to facilitate cardiac fibroblast differentiation by modulating mitochondrial ROS levels and enhance R-Smad signaling, which drives the expression of pro-fibrotic genes. This identifies a novel mechanism by which NLRP3 contributes to myocardial fibrosis and remodeling, ultimately leading to heart failure [114,115].

Consistent with that, clinical studies reported elevated levels of proinflammatory cytokines, including IL-1β, in patients with heart failure, and these levels have been linked to the severity and prognosis of heart failure [116]. Moreover, reduced methylation of ASC has been associated with poorer outcomes in heart failure. Notably, another clinical study found that patients with heart failure exhibited decreased ASC methylation and increased plasma IL-1β and ASC mRNA levels, an observation that was reversed by an aerobic exercise program [117]. These findings suggest that the epigenetic regulation of ASC may be a biological mechanism through which exercise improves outcomes in HF.

4. Inhibitors of NLRP3 Inflammasome

Mechanistically, inhibition of NLRP3 inflammasome can be achieved by 3 strategies: firstly, by targeting the upstream signaling that upregulates the expression of NLRP3, caspase-1 and IL-1β genes. Secondly, by inhibition of NLRP3 inflammasome assembly, and thirdly by inhibition of NLRP3 downstream signaling. On one hand, inhibition of NLRP3 priming (downregulation of NLRP3 expression) can be achieved by TLR4 inhibitors such as TAK-242, and NF-κB, IKKβ as well as IRAK4 inhibitors; all have been extensively reviewed in [118–120]. In addition, the inhibition of NLRP3 activation by upstream signal 2 can be achieved by targeting various upstream processes, including ion flux such as K^+ efflux, Cl^- efflux, and Ca^{2+} influx, $P2X_7$ signaling, and the production of mitochondrial ROS [121,122]. On the other hand, several review articles [119,120,123] have already covered many caspase-1 inhibitors as Ac-YVAD-cmk, pralnacasan (VX-740), emricasan, and VX-765, as well as GSDMD inhibitors such as necrosulfonamide, dimethyl fumarate and disulfiram to inhibit NLRP3 downstream signaling. Within this context, targeting ASC as downstream target of NLRP3 would be a relatively nonspecific method for blocking NLRP3 activation since ASC is a component shared by most inflammasomes, and also because the mechanisms by which ASC is undergoing oligomerization are not fully understood [124,125]. Despite being potential effective strategies to inhibit NLRP3, those two strategies likely involve cellular mechanisms unrelated to the NLRP3 inflammasome, potentially leading to significant off-target effects if inhibited. For instance, inhibiting NF-κB can reduce the induction of NLRP3 protein by LPS, but it would also inhibit numerous other NF-κB-dependent processes that are essential for both innate and adaptive immune responses.

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Therefore, targeting the NLRP3 protein per se and its assembly offers a potentially more direct strategy for inhibiting the NLRP3 inflammasome. In the following lines, we enumerate the different classes of synthetic NRLP3 inflammasome inhibitors and the novel phytochemicals with anti-NLRP3 activity, all are presented in Table1.

4.1. Sulphonylurea NLRP3 Inhibitors

4.1.1. Glyburide

Glyburide, also known as glibenclamide, is an antidiabetic Sulphonylurea drug that works by suppressing ATP-sensitive K^+ channels in pancreatic β-cells and stimulating insulin release in type 2 diabetic patients [126]. Interestingly, in 1997, Glyburide was found to prevent the release of IL-1β from human and murine macrophages activated by LPS and ATP before the discovery of inflammasomes [127]. Then, in 2009, glyburide was shown to selectively inhibit NLRP3-mediated caspase-1 activation, IL-1β secretion, and pyroptosis but not NLRC4, NLRP1b, and AIM2 inflammasomes-driven caspase-1 activation, hence providing a proof-of-concept for specific pharmacological inhibition of NLRP3, albeit the exact mechanism needs further studies [128]. In addition, other sulfonylureas such as sulofenur and glimepiride have been tested for their ability to inhibit the NLRP3 inflammasome, but they showed weak inhibition [129]. Despite its potent inhibition of NLRP3 activation in vitro, high doses of glyburide are required in vivo to inhibit NLRP3, which is associated with hypoglycemia and perturbations of glucose metabolism, hindering its use beyond diabetes [130]. Therefore, developing novel analogs of glyburide that lack its hypoglycemic effect while inhibiting NLRP3 activation could provide a targeted therapeutic strategy for treating inflammatory diseases.

4.1.2. Second-Generation glyburide-Based Inhibitors: JC121, JC124, JC171, YQ128

Using glyburide as a starting point, many inhibitors have been developed including JC121 (16673-34-0) and its methylated (JC124) and hydroxylated (JC171) analogs that lack the cyclohexylurea group, responsible for hypoglycemic activity while retaining the benzamide and the sulfonyl moieties, required for NLRP3 inflammasome inhibition [131]. Mechanistically, JC171 was reported to inhibit the oligomerization of NACHT domain upon direct interaction with allosteric site of NLRP3 nest to ATP binding site. Although, whether it acts on NLRP3 ATPase activity needs to be elucidated [132]. In cardiomyocytes exposed to ATP and nigericin, JC121 was found to inhibit ASC aggregation, and Caspase-1 activity. Besides, it decreases the expression of NLRP3, caspase-1, and IL-1β production, reducing the inflammatory cell death [133–135]. JC121 has also shown positive cardiac effects in vivo in many mice models. It reduced the infarct size by inhibition of cardiac caspase-1 activity in mice subjected to myocardial ischemia followed by 24 hours reperfusion when administered in a clinically relevant scenario; 60 minutes after reperfusion [133,136]. In addition, JC121 limited the ischemic damage and improved cardiac contractility in the circulatory death model while it enhanced the cardiac function in a model of permanent coronary artery ligation [90,137]. Furthermore, JC121 reduced interstitial fibrosis, improving the cardiac function in mouse models of doxorubicin-induced and Western diet-induced cardiomyopathy [90,138]. To overcome the solubility issues associated with JC121, JC124 was designed at Virginia Commonwealth University by incorporating a methylated sulfonamide into the structure, thereby enhancing the solubility. Interestingly, JC124 showed higher potency, albeit the same selective inhibition for NLRP3 inflammasome of JC121, inhibiting IL-1β release with an IC_{50} of 3.25 μM [131,139]. This increased potency is attributed to the sulfonamide moiety. More importantly, JC124 remains active against NLRP3 mutants associated with genetic forms of cryopyrin-associated diseases [90,131]. Besides showing a cardioprotective effect in acute myocardial infarction model, JC124 was found to be neuroprotective, mitigating AD-related deficits in two different transgenic animal models of AD and traumatic brain injury by inhibition of brain NLRP3 [131,139,140]. In addition, based on JC124 structure, many sulfonamide inhibitors with nanomolar potency that can more effectively enter the

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blood-brain barrier to block NLRP3 inflammasome activity in the central nervous system (CNS) have been developed such as YQ-II-128 (YQ128), resulting in the creation of NLRP3 inhibitors suitable for clinical use. However, YQ128 and other sulfonamide inhibitors showed poor oral bioavailability, addressing the need for SAR studies to improve not only the potency but also the pharmacokinetics of these compounds [141,142]. Interestingly, Sun et al. developed 2,3-dihydro-1H-indene-5-sulfonamide analogues as novel NLRP3 inflammasome inhibitors by structural modification of YQ128, resulting in lower toxicity and enhanced efficacy. Among these newly developed compounds is 15z which directly binds to NLRP3, blocking its assembly and activation. In addition, 15z inhibited DSS-induced colitis and relieved NLRP3-mediated inflammatory bowel disease [143].

More recently, Huang et al. have developed novel biphenyl-sulfonamide derivatives of which compound H28 has been identified as a potent and specific inhibitor of the NLRP3 inflammasome, with an IC_{50} value of 0.57 μM. Mechanistically, H28 directly binds to the NLRP3 protein, effectively preventing the assembly and activation of the inflammasome. In addition, in a mouse model of acute peritonitis, H28 was shown to effectively inhibit the NLRP3 inflammasome pathway, demonstrating its anti-inflammatory effects. These findings strongly support the further development of H28 as a potential lead compound for the treatment of NLRP3-related diseases [144].

4.1.3. CRID3

In an attempt to find structurally related compounds more potent than glyburide in blocking IL-1β release, Gabel et al. at Pfizer conducted a screening for a focused library of structurally related diaryl-sulfonylureas and discovered cytokine release inhibitory drugs (CRIDs), which block IL-1β release with nanomolar potency [145,146]. One of those CRIDs is the Pfizer compound CRID3 (also named MCC950 or CP-456773) which was found to inhibit classical and non-classical NLRP3 activation with an IC50 of 7.5 nM in BMDMs and 8.1 nM in HMDMs [147]. The inhibitory activity of CRID3 on NLRP3 was demonstrated in many preclinical models of cardiovascular disease, including myocardial infarction, atherosclerosis, stroke, and allergic airway inflammation, as well as inflammatory arthritis. Interestingly, CRID3 suppresses NLRP3-dependent neuroinflammation in APP/PS1 model of Alzheimer disease and other NLRP3-mediated neurodegenerative disorders including multiple sclerosis, Parkinson's disease and Amyotrophic lateral sclerosis in animal models [147–149]. By maintaining the closed conformation and obstructing its transition to the open, inflammasome-competent active conformation, CRID3 effectively suppresses NLRP3 activation. Besides, it does not affect NLRP3's active hydrolysis of ATP [150–153]. This demonstrates that CRID3 cannot target NLRP3 in its active state, providing a plausible mechanistic explanation for why specific NLRP3 mutations linked with CAPS in and around the central NACHT make the mutant protein less susceptible to CRID3 suppression [154]. Consistent with that, the serum levels of IL-1β and IL-18 in LPS-challenged mice expressing the Muckle-Wells syndrome (MWS)-associated NLRP3A350V mutation were efficiently suppressed by high doses of CRID3, while being resistant to CRID3 suppression in mice expressing the Familial cold Autoinflammatory (FCAS)-associated NLRP3L351P mutation [154]. Additionally, a subgroup of patients with CAPS may require greater plasma concentrations of CRID3-based medicines to adequately cure illness symptoms than patients with disorders driven by wild-type NLRP3 [155]. Besides, Pfizer has halted the clinical development of CRID3 due to its induced liver injury, especially with higher doses in healthy volunteers and also for its off-target carbonic anhydrases I and II inhibition [156]. Taken together, the reported CRID3-induced liver injury and inefficacy towards mutant-NLRP3 induced diseases have spurred several companies to develop second-generation CRID3-based NLRP3 inhibitors with improved potency and pharmacological profiles.

4.1.4. Second-Generation CRID3-Based Inhibitors

In 2019, Agarwal and his colleagues in Zydus lifesciences research center have developed alkenyl Sulphonylurea derivatives using CRID3 as a scaffold, replacing the furan moiety of CRID3, the cause behind liver toxicity, with many bioisosteric heterocyclic rings including thiophene,

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pyridine, and thiazole. Among those, the thiazole derivative (compound 7 in this study) showed good potency (IC_{50} 35nM) compared with (IC_{50} 8nM) of CRID3 with good pharmacokinetics profile in vitro and in vivo, upon testing on MSU- and nigericin-stimulated THP-1 cells [157]. In 2020, the same research group has also designed N-cyano-sulfoximine urea derivatives of CRID3; among these, ZY19800 demonstrated equipotent efficacy to CRID3 in both in vitro and in vivo contexts [158]. Eventually, Zydus lifesciences has developed a CRID3 analog named ZYIL1 in which the furan head is replaced by a substituted pyrrolidine ring. ZYIL1 is the first NLRP3 inhibitor to effectively finish a proof-of-concept phase II clinical trial in CAPS patients who showed rapid improvement in the clinical markers and achieved clinical remission days after starting treatment. Previously, the inhibitor was found to be safe and well-tolerated in both first-in-human single-ascending dosage (NCT04731324) and multiple-ascending dose (NCT04972188) phase I clinical trials [159,160].

Emlenoflast (previously known as inzomelid/MCC7840) and selnoflast (formerly somalix/RG6418/IZD334) are CRID3-derived analogues developed by Inflazome, now part of Roche. In these compounds, the isopropyl furan group of CRID3 has been replaced with a substituted pyrazole and piperidine groups, respectively [161]. Both emlenoflast and selnoflast, which are orally available, have successfully completed phase I safety and tolerability studies in healthy volunteers and demonstrated promising clinical efficacy in adults with CAPS (emlenoflast, NCT04015076; selnoflast, NCT04086602). Emlenoflast, designed to treat neurodegenerative diseases such as Alzheimer's and Parkinson's, is a brain-penetrant NLRP3 inhibitor but is no longer part of Roche's clinical development [162]. On contrast, selnoflast is a peripherally restricted NLRP3 inhibitor under development for systemic inflammatory diseases and is currently in phase I trials for ulcerative colitis and chronic obstructive pulmonary disease (COPD), according to the Roche development pipeline. Besides, Roche's subsidiary Genentech acquired Jecure Therapeutics in 2018, obtaining rights to Jecure's CRID3-based NLRP3 inhibitor RG6338. However, Roche has recently announced that RG6338 has been removed from its clinical pipeline [163].

DFV890 (formerly IFM-2427) is a peripherally restricted CRID3-based NLRP3 inhibitor, originally discovered at IFM Therapeutics prior to its acquisition by Novartis [164]. This inhibitor successfully completed phase I clinical trials and was recently assessed in a phase II trial for COVID-19-associated pneumonia (NCT04382053), but the results indicated it did not enhance treatment efficacy beyond the standard of care. Currently, DFV890 is being evaluated in phase II trials for FCAS (NCT04868968) and knee osteoarthritis (NCT04886258) [165].

Besides, some CRID3-based inhibitors are still in preclinical development, among these NDT-30805 which has been developed by NodThera as one of series of CRID3-related NLRP3 inhibitors by replacing the central sulfonylurea group of CRID3 with a thiocarbonyl group [166]. Additionally, it was also reported that NodThera research group designed novel NLRP3 inhibitors using CRID3 as a starting point, developing ester-substituted urea compounds in a trial to increase the cell permeability of these NLRP3 inhibitors which will be activated inside the cell by the action of carboxylesterase [167]. Furthermore, Aikelin et al. developed MCC950 analogue that lack the toxicophoric furan ring and thereby its liver toxicity, JT002. JT002 has shown selective inhibition for NLRP3 inflammasome by canonical and alternative pathways, albeit more efficacy in inhibition of the alternative pathway. Moreover, the daily administration of JT002 at dose of 30 mg/kg resulted in attenuation of inflammation in a mice model of MWS. Lastly, JT002 inhibited neutrophilic airway inflammation and asthma by suppressing NLRP3 inflammasome upon direct binding to the NACHT domain, in two different in vivo models [168].

4.2. NLRP3-Inhibiting Compounds (NIC)

Interestingly, continuous research on developing new NLRP3 inhibitors resulted in evolution of a novel chemical class of NLRP3-inhibiting compounds (NIC) including NIC11 and NIC12 which are structurally based on CRID3 but lacking its off-target activity against carbonic anhydrases I and II. Both compounds suppressed the release of IL-1β and pyroptosis in LPS-primed and nigericin-stimulated mouse macrophages with IC_{50} value of 69 nM for NIC11and IC_{50} value of 11 nM for NIC12.

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In addition, NIC12 selectively lowers circulating IL-1ß levels in a mouse model of LPS-induced endotoxemia and inhibits NLRP3 inflammasome activation in monocytes from CAPS patients, demonstrating approximately tenfold greater potency than CRID3. Therefore, NIC11 and NIC12 can be furtherly used as a nucleus for developing promising NLRP3 inhibitors [169].

4.3. Boron-Based NLRP3 Inflammasome Inhibitors

Despite its unique chemistry, Boron is an often overlooked element in medicinal chemistry and is infrequently used in pharmaceutical compounds, with Bortezomib (Velcade®), tavaborole, and crisaborole being the primary examples in clinical application for treatment of multiple myeloma, onychomycosis and atopic dermatitis, respectively [170]. Similarly to Bortezomib, Ixazomib was approved by FDA for the treatment of multiple myeloma, but it is the first orally bioavailable proteasome inhibitor. Interestingly, Ixazomib showed good inhibitory rate (77.0% at 1μM) against IL-1β release, an effect that was furtherly optimized upon substitution on the phenyl ring with 2,6-difluoro, resulting in development of NIC-0102 [171]. NIC-0102 is the first orally available proteasome inhibitor that selectively inhibit NLRP3 inflammasome by enhancing its polyubiquitination. As a result, NIC-0102 disrupts NLRP3-ASC interaction with consequent blocking of ASC oligomerization and NLRP3 deactivation, a mechanism by which NIC-0102 alleviated DSS-induced colitis in mice [172]. Notably, a promising class of NLRP3 inhibitors has been both documented and patented, utilizing the boron semimetal framework of 2-aminoethoxy diphenylborinate (2APB). 2APB showed a cardioprotective effect against ischemic/reperfusion injury by ROS scavenging, suggesting the possibility of inhibiting NLRP3 inflammasome activation [173]. Interestingly, 2APB was found to inhibit the NLRP3 inflammasome in LPS-primed peritoneal macrophages, stimulated by ATP, nigericin, sphingosine, MSU, calcium pyrophosphate dihydrate crystals (CPPD), or alum [174]. However, the major drawback of 2APB is its non-selective effects on cellular Ca^{2+} homeostasis by targeting inositol 1,4,5-trisphosphate ($InsP_3$)-dependent Ca^{2+} release, store-operated Ca^{2+} entry, Ca^{2+} pumps and mitochondria as well as transient receptor potential (TRP) family of ion channels; effects which are use-dependent and not easily reversible. Therefore, Baldwin et al. created a series of Boron-containing (BC) NLRP3 inflammasome inhibitors that do not impact Ca^{2+} homeostasis, using 2APB as a scaffold. The synthesis of a range of BC compounds led to the discovery of two highly effective candidates, BC7 and BC23, each featuring a diphenyl-substituted oxazaborine ring and demonstrated inhibition of IL-1β with IC_{50} values of 1.2 and 2.3 μM, respectively, in LPS/ATP-stimulated bone marrow-derived macrophages (BMDMs) [174]. Later, three series of oxazaborines, dioxaborines, and diazaborines with various substitutions were synthesized and biologically screened, resulting in the identification of the oxazaborine derivative novel boron compound 6 (NBC6) as the most promising compound. NBC6 demonstrated inhibition of NLRP3-dependent IL-1β release with an IC_{50} of 0.574 μM in LPS/nigericin-stimulated THP-1 cells, and its action was independent of intracellular calcium levels or direct caspase-1 inhibition [175]. Structure-activity relationship (SAR) studies revealed that the electron-withdrawing, lipophilic trichloromethyl group at position 4 of the ring was crucial for its activity. In general, the substitution of trichloromethyl group with its bioisosteric trifluoromethyl (CF3) group renders the NBCs inactive. In addition, phenyl ring substitution, particularly with more steric such as methyl group and lipophilic substituents such as Cl or CF_3, is unlikely to enhance the activity of the NBCs [175]. NBC6 effectively blocked both canonical and non-canonical NLRP3 activation as well as NLRC4 inflammasome, but did not affect AIM2 inflammasome, while showing no cytotoxicity in HEK293 and HepG2 cells. Mechanistically, NBC6 prevented ASC-speck formation, a marker of inflammasome assembly, and irreversibly inhibited NLRP3-dependent IL-1β release from iBMDMs, similar to the behavior of the nitrostyrene derivative MNS used as a reference. Additionally, NBC6 inhibited IL-1β release from neutrophils at 10 μM. NBC13, an analogue of NBC6 with similar in vitro potency (95% IL-1β release inhibition at 10 μM in LPS/nigericin-stimulated THP-1 cells), was selected for in vivo testing in LPS-driven peritonitis model. Administered orally at 50 mg/kg, NBC13 reduced IL-1β levels in peritoneal lavage fluid and both IL-1β and IL-1α levels in plasma, comparable to the

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reference compound MCC950 [176]. Additionally, NBC19 is another analogue of NBC6 which showed more potency in comparison with NBC6 based on previously reported IC_{50}s, albeit it has not been tested in vivo [174,177,178]. These results indicate that cyclic diarylboron derivatives hold significant promise for future development as irreversible NLRP3 inhibitors.

4.4. Acrylic Acid Derivatives (INF Compounds)

Coco et al. have designed a group of NLRP3 inflammasome inhibitors based on the acrylic acid scaffold having electrophilic warheads; reactive groups that can form covalent bonds with amino acid residues in proteins [179]. Those electrophilic compounds have been screened for their cytotoxicity using human renal epithelial (HK-2) cells as well as for their ability to NLRP3-dependent pyroptotic cell death. Notably, INF4E inhibited pyroptosis by 75% at a concentration of 10 μM and exhibited a tolerable TC_{50} of 67 μM. As expected for a covalent irreversible drug, the antipyroptotic effect of INF4E was time-dependent, with maximum efficacy observed after 60 minutes of pre-incubation before the NLRP3-activating stimulus. At this pre-incubation time, dose-dependent reductions in pyroptosis induced by ATP and nigericin were achieved, with EC_{50} values of 0.12 μM and 0.16 μM, respectively. A positive correlation was identified between cysteamine reactivity, measured by the second-order rate constant (k_2), and antipyroptotic activity within this compound series. INF4E inhibited ATPase activity in recombinant human NLRP3 (rhNLRP3) and partially and irreversibly inhibited caspase-1, with a k_i of 9.6 ± 3.3 μM and a k_{inact} of 3.2 ± 1.1 s^{-1} [179,180]. INF4E was evaluated in an ex vivo model of cardiac ischemia/reperfusion injury, where the formation of an NLRP3 complex was detected. In this model, INF4E reduced the formation of the NLRP3 complex in a time-dependent manner and significantly decreased infarct size and LDH release, while also enhancing post-ischemic left ventricular pressure. Additionally, the hearts of animals pre-treated with INF4E showed a substantial improvement in the pro-survival RISK (Reperfusion Injury Salvage Kinase) pathway, along with enhanced mitochondrial function [181,182].

By the chemical modulation of the lead compound INF4E, a series of electrophilic compounds were designed and synthesized to carefully adjust the reactivity and minimize the cytotoxicity [180]. Screening results have identified INF39 as a potent specific NLRP3 inhibitor but not for the NLRC4 or AIM2 inflammasomes with no cytotoxic effects, acting by irreversible blocking of the ATPase activity of NLRP3 and hindering NEK7-NLRP3 interaction, followed by the inhibition of NLRP3 oligomerization, NLRP3-ASC interaction, and ASC oligomerization. Consistent with that, INF39 was found to effectively reduce IL-1β release in LPS/ATP- and LPS/nigericin-stimulated BMDMs and partially inhibited NF-κB signaling without directly affecting caspase-1 [180,183].

Unlike its precursor, INF4E, INF39 remained stable in human serum, with no detectable binding to human serum albumin. In addition, the in vitro and ex vivo ADME experiments showed that INF39 is stable in simulated gastric and intestinal fluids, absorbed through the intestinal epithelium but it was rapidly metabolized within cells, converted into its active acid metabolite. As a result, INF39 was selected for in vivo studies using a rat model of experimental colitis, which mimics human Crohn's disease, with oral administration chosen for these experiments. In dextran sodium sulphate (DSS) and 2,4-dinitrobenzenesulfonic acid-induced colitis, INF39 administration led to a reduction in both local and systemic inflammatory markers, such as IL-1β, TNF-α, and tissue myeloperoxidase (MPO), along with a decrease in spleen weight. Morphological and anatomical examination revealed dose-dependent prevention of colon damage. Overall, INF39 (at 25 mg/kg) exhibited a protective effect comparable to that of the reference drug dexamethasone (1 mg/kg), without significant reduction in body weight, a common side effect of chronic steroidal anti-inflammatory drug use [184,185]. Additionally, INF39 mitigated the pathological inflammatory response in caerulein-induced acute pancreatitis and associated lung injury animal model [186]. Furthermore, it was reported that INF39-mediated NLRP3 inhibition ameliorated liver injury induced by Rifampicin and Isoniazid [187,188].

Further trials for optimization of NLRP3-directed electrophilic compounds resulting in the development of acrylamide derivatives which are less reactive compared to their acrylates

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counterparts. Among these acrylamide derivatives, INF58 was found to prevent NLRP3-dependent pyroptosis with IC_{50} value of 23.2 μM after 1 hour of preincubation, even though it was less reactive than INF4E [189]. In addition, INF58 successfully inhibited the ATPase activity of rhNLRP3 and reduced IL-1β release in iBMDMs, primary BMDMs, primary peritoneal mouse macrophages, and in macrophages harboring typical mutations (R258W, A350V, L351P) observed in CAPS pathology, without affecting the release of TNF. Furthermore, a homology model of the NLRP3 NACHT domain, containing the ATP-binding region, was developed using the resolved structure of NLRC4 (PDB ID: 4KXF), and a potential binding mode for INF58 was proposed. According to this hypothesis, Cys419 is believed to be the nucleophilic residue closest to the molecule bound in the ATP-binding pocket, enabling it to approach and covalently bind to the terminal position of the double bond in the acrylamide pharmacophore [179,189]. However, additional experimental studies are needed to prove the effectiveness of INF58 in CVDs.

4.5. Nitrostyrene Analogs

In studies conducted by He et al. and Gan et al. for screening a library of several kinase inhibitors, 3,4-Methylenedioxy-β-nitrostyrene (MNS) was identified as specific NLRP3 inhibitor but not for NLRC4 and AIM2 inflammasomes, independently of its Syk and Src tyrosine-kinase inhibition [61,190,191]. Upon binding to the LRR and NACHT domains and cysteine modification via its nitrovinyl group, MNS directly inhibits the ATPase activity of NLRP3. As a result, MNS block NLRP3-mediated ASC speck formation and aggregation without affecting K^+ efflux [190]. Interestingly, MNS was found to alleviate DSS-induced colitis in mice, induce apoptosis in osteosarcoma cell line, and significantly inhibit NLRP3 inflammasome activation and inflammatory cytokine production in burn wounds [192–194]. Nevertheless, its severe toxicity impeded the continuation of research [195,196]. Subsequent research demonstrated that a MNS analogue, NPe, exhibited a potent anti-inflammasome effect, completely reducing TNF-α at a concentration of 5 mg/ml through the NF-κB and ERK pathways [132].

4.6. Phenyl Vinyl Sulfones

BAY 11-7082, a vinyl sulfone first synthesized in 1968, was initially believed to inhibit the nuclear translocation of NF-κB by blocking IKK activity and the phosphorylation of IκB in response to upstream signal [197]. Subsequent studies revealed that its anti-inflammatory effects are mediated through interactions with multiple targets, including a group of protein tyrosine phosphatases (PTPs) that function upstream of IKK and play a role in activating IκB kinase. The mechanism of action of BAY 11-7082 involves its ability to covalently and irreversibly bind to the conserved nucleophilic Cys215 within the active sites of various PTPs, as evidenced by its interaction with PTPB1 [198,199]. Later, Juliana et al. found that BAY 11–7082 has NLRP3 inflammasome repressive activity, effectively suppressing the production of pro-inflammatory cytokines IL-1β and IL-18 in macrophages due to irreversible alkylating the cysteine residues of NLRP3 ATPase, independent of NF-κB pathways [200]. BAY 11–7082 specifically inhibited NLRP3 inflammasome activity, with minimal to no effect on NLRP1 and NLRC4 inflammasomes. Studies using NG5 cells, a stable $NLRP3^{-/-}$ bone marrow macrophage line with constitutive NLRP3 expression controlled by a murine stem cell virus promoter, confirmed that BAY 11–7082 can impede ATP-, nigericin-, and sodium monourate (MSU)-induced caspase-1 activation [200]. Moreover, BAY 11–7082 demonstrated a dose-dependent inhibition of LPS/ATP- and LPS/nigericin-stimulated IL-1β release from BMDMs with completely suppression at a concentration of 100 μM [180].

Interestingly, BAY 11-7082 has been tested in many in vivo mice models. In an experimental mouse model of myocardial ischemia-reperfusion, administering BAY 11-7082 10 minutes prior to coronary artery reperfusion significantly reduces leukocyte infiltration in the infarcted region and enhances outcomes related to cardiomyocyte apoptosis and infarct size [70]. Similarly, pre-treatment with BAY 11-7082 mitigates myocardial injury, maintains contractile function, and curtails subsequent fibrosis [201]. In diabetic rats subjected to cardiac ischemia-reperfusion, BAY 11-7082 was

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found to reduce NLRP3 activation, caspase-1 and IL-1β expression, and pyroptosis [202]. In addition, BAY 11-7082 was tested in a diabesity mice model, generated by high fructose diet whereas it was effective in lowering inflammatory mediators, including IL-1β, IL-18, and TNF-α, at both systemic (plasma) and local (liver and kidney) levels. Additionally, BAY 11–7082 counteracted diet-induced metabolic disturbances and enhanced insulin signaling by restoring the IRS-1/Akt/GSK-3β pathway, which is compromised by NLRP3 activation [203]. Interestingly, treatment of mice with implanted fibroid xenografts by BAY 11-7082 for 2 months resulted in fibroids shrinkage and inflammation reduction [204]. In imiquimod (IMQ)-induced mouse models of psoriasis, treatment with BAY 11-7082 markedly alleviated symptoms such as scaling, erythema, and increased epidermal thickness [205]. Lastly, BAY 11-7082 attenuated burn-induced acute pulmonary injury by reducing NLRP3-related inflammatory cytokines, accompanied by a corresponding decrease in myeloperoxidase levels. Additionally, the histopathological features of the injury, including neutrophil infiltration, edema, alveolar wall thickening, and hemorrhage, were all alleviated [206]. Besides, a series of structurally related vinylsulfones (BAY 11–7085, IMPSPN, CPSMB, ESMB, PV-sulfone) have been developed of which one sulfonylpropanenitrile (MBSPN) and one sulfide (PV-sulfide) were inactive, suggesting that the conjugated vinyl group is essential for the activity of BAY 11–7082 and related vinylsulfones [182].

4.7. Benzoxathiole Derivatives

Initial research on benzoxathiole-one derivatives in the 1970s recognized this class of compounds as potential anti-psoriatic agents, of which BOT-4-one has been reported with antiproliferative and immunomodulatory activities [207,208]. While the blockade of Janus kinase 3 (JAK3)/signal transducer and activator of transcription 3 (STAT3) signaling mediates the antiproliferative action of BOT-4-one, the mechanism of BOT-4-one's immunomodulatory action is mediated by its alkylation of Cys179 in the activation loop of the IKKβ kinase, thereby it fully inhibits IKKβ activity at a concentration of 30 μM [209,210]. This inhibition leads to the downregulation of the NF-κB signaling pathway. Computational studies predict that BOT-4-one alkylates Cys179 in the activation loop of the IKKβ kinase [210]. Regarding its mechanism of action, BOT-4-one causes alkylation of NLRP3 resulting in reducing its ATPase and blocking the oligomerization of NLRP3 and ASC. Furthermore, the NLRP3 alkylation increases the NLRP3 ubiquitination which inhibits the NLRP3 inflammasome activation. The researchers discovered that BOT-4-one effectively suppresses both canonical and non-canonical activation of the NLRP3 inflammasome. In addition, it was observed that BOT-4-one inhibited caspase-1 activation, IL-1β release, and pyroptosis in a dose-dependent manner (0.75–3 μM) when stimulated by LPS/ATP, LPS/nigericin, and LPS/silica [211,212]. However, the inhibition of pyroptosis was less effective when induced by silica. In both BMDMs and THP-1 cells, nearly complete inhibition was achieved at 3 μM concentration. Lastly, it was reported that BOT-4-one specifically targets NLRP3, without inhibiting AIM2 and only partially inhibiting NLRC4 [212].

The antiinflammatory activity of BOT-4-one was also evaluated in in vivo model of MSU-induced peritonitis whereas it significantly lowered IL-1β levels in the peritoneal lavage fluid and reduced neutrophil infiltration at the site of inflammation [211]. In addition, BOT-4-one inhibited $CD4^+$ T-cell polarization into Th1- and Th17-cell subsets and infiltration of the immune cells, thereby it alleviated 2,4,6-trinitrochlorobenzene-induced dermatitis as well as IL-23-induced psoriasis-like skin inflammation [210]. In addition, BOT-4-one showed antiinflammatory activity in collagen-induced arthritis in mice [213]. In summary, BOT-4-one is a noteworthy addition to the classes of covalent inhibitors targeting NLRP3.

4.8. Benzimidazoles Derivatives (Fc11a-2, TBZ-09, TBZ-21)

Fc11a-2, a benzimidazole derivative, reduced the release of IL-1β and IL-18 from LPS-primed ATP-stimulated THP-1 cells, achieving an IC_{50} of approximately 10 μM. Fc11a-2 prevented NLRP3 activation by blocking the autocleavage of inactive procaspase-1 into its active form, caspase-1,

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thereby reducing the release of inflammatory cytokines. Notably, Fc11a-2 did not fully suppress cytokine secretion even at the highest concentration tested (30 μM). In addition, the expression of NF-κB protein and its phosphorylation remained unaffected at concentrations up to 30 μM [214].

Interestingly, Fc11a-2 has showed antiinflammatory activity in vivo. In murine colitis models induced by DSS, treatment with Fc11a-2 at 30 mg/kg mitigated pathological changes, including weight loss and colon shortening. The treatment also reduced MPO activity and macrophage infiltration in the colon tissues. Furthermore, protein and mRNA levels of DSS-induced proinflammatory cytokines such as IFN-γ, TNF-α, IL-1β, IL-18, IL-17A, VCAM1, and ICAM1 in the colon were significantly decreased. Fc11a-2 also inhibited the phosphorylation of ERK, JNK, and STAT1 in DSS-colitis, suggesting its anti-inflammatory action across multiple signaling pathways [214].

In 2017, thiabendazole analogs bearing the same benzimidazole core as Fc11a-2 were developed by Pan et al. Among these, TBZ-09 and TBZ-21 demonstrated a 30% inhibitory effect on IL-1β at a concentration of 10 μM in ATP-stimulated THP-1 macrophages, primed with LPS. Both compounds feature an electron-withdrawing group at the C5 position of the benzimidazole core, and benzyl substitution at the C1 position further enhanced their inhibitory potency [215]. However, further studies are required to elucidate the precise mechanism by which TBZ-09 and TBZ-21 inhibit NLRP3 activation and to test their anti-NLRP3 activity in various in vivo inflammatory diseases models.

4.9. Benzo[d]imidazol-2-One Compounds (HS-203873, HS-206461)

In 2019, Liao et al. identified a novel benzo[d]imidazol-2-one molecules that specifically interfere with the ATP-binding and hydrolysis functions of the NLRP3 protein [216]. HS203873, a benzo[d]imidazol-2-one derivative, was found to compete with ATP on binding to the Walker A within the NLRP3 NACHT domain, inhibiting the ATPase activity of NLRP3 and its oligomerization and interaction with ASC Among the compounds developed, HS203873 was the most effective in diminishing ATP-induced IL-1β secretion, lowering it to approximately 35% of the vehicle control. Additionally, HS206461 displayed some inhibitory effect on ATP-induced IL-1β secretion [217]. Interestingly, Gastaldi et al. developed a series of novel noncovalent NLRP3 inhibitors including INF156, INF120, INF148, and INF172 by merging INF39 and HS203873 following pharmacophore-hybridization strategy, inhibiting NLRP3-dependent IL-1β and pyroptosis in LPS-primed/ATP-stimulated macrophages [218]. As further optimization of the activity without changing the noncovalent binding mode, a short series of compounds lacking the benzimidazol-2-one was developed, of which INF195 showed protective effect against ischemic reperfusion injury upon inhibition of NLRP3-mediated pyroptosis and IL-1β release [219].

4.10. Glitazones (CY-09)

The class of thiazolidinone derivatives was first discovered in 2017 and reported as strong NLRP3 inhibitors by Jiang *et al.* [220]. The first-in-class is C172, identified through the screening of a library of cystic fibrosis transmembrane conductance regulator (CFTR) channel inhibitors, and inhibited caspase-1-mediated IL-1β release in LPS/nigericin-stimulated bone marrow-derived macrophages (BMDMs), in a dose dependent manner without affecting LPS priming. The NLRP3 inhibition was shown to be independent of CFTR inhibition, as confirmed by studies using *cftr*$^{-/-}$ BMDMs. Subsequently, the authors evaluated a series of previously synthesized C172 derivatives. Among them, CY-09 was identified as a potent NLRP3 inhibitor with minimal activity on CFTR [220,221].

The mechanism of CY-09 action is the same as HS-203873 whereas it competes with ATP on Walter A motif within the NLRP3 NACHT domain, thereby it prevents ATPase activity of NLRP3 and consequent oligomerization with ASC protein. Notably, this inhibition is specific for NLRP3, but not for NLRC4 and AIM2. Consistent with that, CY-09 inhibits the ATP, nigericin- and MSU-induced NLRP3 activation with consequent Caspase-1 activation and IL-1β secretion in LPS-primed BMDMs,

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human macrophages and in peripheral blood mononuclear cells (PBMC) in a dose dependent manner [220].

Interestingly, CY-09 showed a good pharmacokinetics profile whereas it did not inhibit CYP1A2, CYP3A4, CYP2C9, CYP2C19 or CYP2D6 in human and liver microsomes in vitro. Additionally, in vivo, CY-09 showed that maximal plasma concentration (C_{max}) of 3.25 ng/mL, 72% bioavailability, and half-life 5.1 h after 30 min of single oral dose in mice [123,220]. Based on the observed pharmacokinetics, the antiinflammatory activity of CY-09 has been tested in many animal models. In mice, CY-09 was able to protect from cardiac dysfunction associated with diabetic ischemic stroke [222]. In addition, CY-09 exhibits promising efficacy in mice carrying the NLRP3 (A350V neoR) mutation, which is linked to human MWS. In this model, oral administration of CY-09 at a dose of 40 mg/kg significantly extended the survival of the mutant mice, indicating that the severe inflammation resulting from the NLRP3 gain-of-function mutation was effectively suppressed. Additionally, CY-09 shows therapeutic efficacy in a mouse model of MSU-induced peritonitis [220]. Besides, CY-09 was evaluated in a HFD-induced mouse model of Type 2 Diabetes (T2D). In this model, intraperitoneal administration of CY-09 (2.5 mg/kg daily) for six weeks resulted in improvements in body weight, insulin sensitivity, and blood glucose levels in HFD-fed wild-type (WT) mice, compared to HFD-fed NLRP3$^{-/-}$ mice, highlighting its NLRP3-dependent effects in vivo. Importantly, CY-09 showed no adverse effects in untreated mice maintained on a standard diet [223]. Lastly, CY-09 also demonstrated activity ex vivo on synovial fluid cells obtained from patients with gout whereas the incubation of CY-09 with synovial fluid cells inhibited NLRP3-mediated caspase-1 activation and IL-1β production in a dose-dependent manner [220]. Despite its in vivo antiinflammatory activity, CY-09 is still in the preclinical stage.

4.11. Sulfonyl Nitrile Derivatives (OLT1177 Dapansutrile)

OLT1177 is a 3-methyl-β-sulfonylpropionitrile compound, also known as Dapansutrile, and first developed by Olatec therapeutics. The mechanism by which OLT1177 inhibits NLRP3 activation depends on its inhibition of NACHT domain ATPase activity. In addition, it blocks NLRP3-ASC and NLRP3-caspase-1 interaction. OLT1177 showed specific suppression of both canonical and non-canonical NLRP3 activation with consequent release of IL-1β without affecting NLRC4 or AIM2 activation in nigericin- stimulated human monocyte-derived macrophages, primed with LPS [224,225]. Besides, OLT1177 has showed antiinflammatory activity in LPS/ATP stimulated microglia cells, inhibiting the release of proinflammatory cytokines as IL-1β, IL-6 and TNF-α [226]. However, there is a study showing that OLT1177 did not directly engage with and inhibit NLRP3 in LPS/nigericin stimulated PBMCs, Human embryonic kidney cells and mouse J774A.1 macrophages as well as partial deactivation of ATPase, suggesting possible other mechanism of action for OLT1177 as antiinflammatory agent [178].

Interestingly, OLT1177 was reported to alleviate many NLRP3- dependent pathologies such as neurodegenerative disorders, cardiovascular diseases and ulcerative colitis in vivo mice models. Six-month-old wild type and APP/PS1 mice were fed with standard mouse chow or OLT1177-enriched chow (3.75gm/kg and 7.5 gm/kg) for 3 months. OLT1177 enhanced the behavioral and cognitive functions indicated by improved results of Morris water maze test. Additionally, OLT1177 decreased the formation of Aβ plaques in the parenchyma of the CNS, the cortex and the hippocampus, evaluated by immunohistochemistry and the release of inflammatory Cytokines in brain tissue lysates [226]. In a model of myocardial infarction whereas the mice underwent Left carotid artery ligation without reperfusion, the administration of OLT1177 in chow diet for 9 weeks, significantly decreased the size of died cardiac muscle and it improved the diastolic dysfunction and maintained the cardiac contractility indicated by improved left ventricular end diastolic pressure [227]. Moreover, OLT1177 mitigated folic acid-induced acute kidney injury by suppressing NLRP3-mediated Caspase-1 and IL-1β activation [228]. Lastly, OLT1177 alleviated the DSS-induced colitis decreasing the mRNA and gene expression of NF-κB and IL-1β in colon tissues and improving DSS-induced histological changes of colon [229].

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Based on its safety profile in healthy volunteers, OLT1177 was undergoing phase II trials for Schnitzler syndrome (NCT03595371) and for patients with moderate COVID-19 symptoms who exhibit early signs of cytokine release syndrome (NCT04540120). Previously, it completed phase II trials for osteoarthritis (NCT01768975) and a phase IIa trial for acute gout (EudraCT number 2016-000943-14) [230,231].

4.12. Tryptophane Derivatives (Tranilast)

Tranilast (N-[3′,4′-dimethoxycinnamoyl]-anthranilic acid, TR), a tryptophan metabolite, is clinically approved for the treatment of asthma and other inflammatory diseases in South Korea and Japan due to its ability to inhibit cytokines-induced NF-κB activation [232]. Tranilast was found to inhibit NLRP3 inflammasome activation in LPS-primed BMDMs by binding to NACHT domain, blocking NLRP3 oligomerization without affecting neither its ATPase activity or the upstream events such as K^+ efflux, mitochondrial damage and reactive oxygen species production. Notably, it did not affect the expression of NLRP3 and the IL-1β [232]. In addition, NLRP3 is the preferential target of Tranilast as it did not show inhibitory effect on NLRC4 and AIM2 inflammasomes [220]. Besides, Tranilast inhibited NLRP3 activation by enhancing its ubiquitination in LPS-primed ATP-stimulated low-density lipoprotein receptor– and apolipoprotein E–deficient macrophages. In addition, it blunted the initiation of atherosclerosis in $ApoE^{-/-}$ and $Ldlr^{-/-}$ mice fed with HFD indicated by enhanced Aortic sinus analysis in terms of atherosclerotic lesion area, necrotic area and collagen content [233]. Tranilast also showed downregulation of TGF-β1 in hepatocytes, thereby it ameliorated the progression of nonalcoholic steatohepatitis in rats [234]. In addition, Tranilast showed protective effects against 2,4,6-trinitrobenzenesulfonic acid-induced colitis in rats by decreasing neutrophils and macrophages infiltration into colon tissues indicating by reduced myeloperoxidase activity in colon, thereby it alleviated the ulcerative colitis [235].

Given its established clinical safety and tolerability at a dosage of 300 mg/day over a one-year period in patients with diabetic nephropathy, Tranilast offers beneficial effects for individuals with T2DM [236,237]. In addition, Tranilast is presently being assessed in a phase 2 open-label clinical trial for its effectiveness and safety in patients with CAPS (NCT03923140).

4.13. Other Synthetic NLRP3 Inhibitors (Quinazolin-4(3H)-Ones, Oxazole, Triazinone, and Tetrahydroquinoline Derivatives & ODZ10117)

Abdullaha et al. have synthesized a series of quinazolin-4(3H)-ones, of which compound 7 demonstrated an IC_{50} of 5 mM for NLRP3 inhibitory activity, while compounds 9 and 10 exhibited modest inhibitory effects, reducing IL-1β release by 28.8% and 21.3% at a concentration of 10 mM, respectively. Besides, compound 8 which showed inhibitory effect on both expression and activation of pro-IL-1β in J774A.1 cells in a dose-dependent manner, was proposed to bind to the ATP-binding domain of the NLRP3, similar to MCC950 and INF 58, inhibiting its ATPase activity and the consequent assembly of NLRP3 [238].

On the other side, Ohba et al. synthesized oxazole analogs (compounds 9 and 10 in this study) and the acylsulfamide-bearing compound 18 which showed inhibitory IL-1β activity in nigericin-stimulated THP-1 cells, primed with LPS. Additionally, one of those oxazole analogs (compound 32) showed in vivo anti-NLRP3 activity, attenuating the kidney injury in Adriamycin-induced glomerulonephritis in mice. Mechanistically, compound 32 was shown to bind to NACHT domain of NLRP3, suppressing its activation by X-ray cocrystal analysis [239].

Dai et al. have developed novel tetrahydroquinoline inhibitors of NLRP3 inflammasome that did not inhibit NLRC4 or AIM2 inflammasomes. Mechanistically, tetrahydroquinoline derivatives bind to the NACTH domain of NLRP3, inhibiting its ATPase activity. As a result, ASC oligomerization is blocked with inhibition of NLRP3 assembly, suppressing DSS-induced colitis in vivo [240]. More recently, Li et al. has designed and patented triazinone derivatives as novel specific NLRP3 inflammasome inhibitors, among them the compound L38 has shown great efficacy and metabolic stability. Mechanistically, compound L38 directly binds to NACHT domain of NLRP3,

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hindering NLRP3-ASC interaction and ASC oligomerization without affecting other upstream activators of NLRP3 as Cl^-/ K^+ or lysosomal damage. Similar to the tetrahydroquinoline derivatives, compound L38 showed therapeutic effect against mice model of ulcerative colitis [241].

Interestingly, ODZ10117, initially discovered as STAT3 inhibitor, was found to selectively inhibit NLRP3-mediated IL-1β and pyroptosis in LPS-primed BMDMs stimulated by ATP, nigericin, silica crystals, and imiquimod in a dose-dependent manner. In addition, ODZ10117 improved mortality and IL-1β release MSU-induced peritonitis model and LPS-induced sepsis model in mice, suggesting that it can be used to treat NLRP3 inflammasome-mediated diseases. Beside its ability to inhibit the ATPase activity of NLRP3 upon binding to the NACHT domain, ODZ10117 can also inhibit NRLP3-NEK7 interaction, suppressing NLRP3 inflammasome activation [242].

4.14. Novel Phytochemicals and Phytoestrogen Targeting NLRP3 Inhibitors

Previous review articles have documented the anti-NLRP3 activity of various phytochemicals such as alkaloids, diterpenes (e.g., Oridonin), triterpenes, sesquiterpene lactones, flavonoids, quinones, stilbenoids, chalcones, limonoid substrates, pentacyclic natural products, steroids, and glucosinolates [123,243,244]. More recently, britannin, a sesquiterpene lactone isolated from *Inula japonica Thunb*, has shown a dose-dependent inhibition of NLRP3-dependent caspase 1 and IL-1β release in ATP stimulated BMDMs, primed with LPS, with IC_{50} value of 3.63 μM. Mechanistically, britannin binds directly to NACHT domain of NLRP3, interrupting the NLRP3 assembly step especially its interaction with NEK7. This effect was independent on inhibition of NLRP3 ATPase activity. Furthermore, britannin inhibited NLRP3-mediated gouty arthritis and acute lung injury in mice [245].

Besides, Zhao *et al.* have conducted pioneering research reporting the anti-NLRP3 activity of *Inula racemosa Hook. f.* and identifying isoalantolactone, the principle bioactive component, as responsible for the observed NLRP3-inhibitory activity. In the same study, they found that isoalantolactone inhibited nigericin-induced IL-1β release in THP-1 cells, with an IC_{50} value of 7.86 μM. However, its poor metabolic stability, and limited solubility have impeded further exploration and development in drug research, the need to develop new analogs. Having the same α, β-unsaturated carbonyl group as natural NLRP3 inhibitors oridonin and costunolide, isoalantolactone is proposed to covalently bind to the NACHT domain of NLRP3, suggesting that it may share a similar binding mechanism to inhibit NLRP3 inflammasome activation [246]. Interestingly, isoalantolactone has shown an antiinflammatory effect in many in vitro and in vivo studies. By NF-κB inactivation, isoalantolactone mitigated LP-mediated production of nitric oxide and inflammatory cytokines (IL-6, TNF-α) in peritoneal macrophages, in RAW 264.7 macrophages and in vivo sepsis model in mice, suggesting its ability to inhibit NLRP3 upstream priming signal [247]. In addition, isoalantolactone showed cardioprotective anti-atherosclerotic effect in HFD-fed mice, reversing the HFD-induced coronary artery changes and the Aortic lesion [248]. In addition, isoalantolactone was also found to be responsible for the phytoestrogenic effects of *Inula racemosa*, inferred from upregulation of estrogen-dependent pS2 gene expression in human breast cancer MCF-7 cells [249].

Recent studies suggest that estrogen is linked to disease progression. While estrogen can alleviate certain conditions including sepsis, neurological disorders as Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, multiple sclerosis, heart diseases as myocardial ischemia/reperfusion injury and atherosclerosis, osteoarthritis, inflammatory bowel disease, liver diseases as well as renal fibrosis by inhibiting the NLRP3 inflammasome, it can, on the other hand, facilitate the progression of ovarian endometriosis, dry eye disease, and systemic lupus erythematosus by upregulating the NLRP3 inflammasome [250]. Therefore, most recent research efforts are focused on utilizing phytoestrogens to inhibit the NLRP3 inflammasome. Erianin is a phytoestrogen and the primary active constituent of the traditional Chinese medicine *Dendrobll caulis*, responsible for its pharmacological effects including anti-tumor, anti-diabetic retinopathy, antibacterial, antipsoriatic effects and antiinflammatory effect in DSS-induced colitis, collagen-induced arthritis and HFD-induced diabetes in mice [251–255]. Mechanistically, Erianin directly

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binds to NLRP3, upon interaction with Walker A motif within the NACHT domain, thereby it suppresses the ATPase activity of NLRP3 and inhibits NLRP3 inflammasome assembly [255].

Besides, formononetin (7-hydroxy-4′-methoxyisoflavone), is an isoflavone phytoestrogen that has been identified in various plants, most notably in *Trifolium pratense* (red clover) and the traditional Chinese medicinal herb *Astragalus membranaceus* [256,257]. By suppression of mitochondrial ROS-TXNIP, formononetin mitigated NLRP3 inflammasome activation in LPS-primed nigericin-stimulated neonatal rat cardiomyocytes (NRCMs), an effect that also was proven in vivo upon administering formononetin (30 mg/kg) for 24 hours, alleviating myocardial ischemia/reperfusion (I/R) injury ischemic myocardial infarction injury in rats [258]. A recent study demonstrated that administering the phytoestrogen formononetin (50 mg/kg) for six weeks can mitigate cognitive dysfunction in diabetic mice. This effect is achieved by inhibiting NLRP3 inflammasome expression and reducing inflammatory cytokine levels (IL-1β and IL-6) in the serum and hippocampus, which occurs through the downregulation of the HMGB1/TLR4/NF-κB signaling pathway [257]. Another study showed that formononetin can downregulate the expression of NLRP3 and caspase3 upon inhibition of NF-κB, alleviating autoimmune hepatitis and suggesting that formononetin can inhibit NLRP3 activation indirectly by targeting its upstream signal [259].

Another isoflavone estrogen is biochanin A which is abundant in the seeds of Glycine max belonging to the family Fabaceae. Biochanin A also showed anti-NLRP3 activity in many in vivo models. For example, administration of biochanin A (50 mg/kg) for seven days can reduce myocardial ischemia-reperfusion injury (MIRI) in a rat model by modulating the TLR4/NF-κB/NLRP3 signaling pathway [260]. Another study found that treating rats with the phytoestrogen biochanin A (64 mg/kg) for seven days provided neuroprotection to dopaminergic neurons by reducing the expression of NLRP3, ASC, Caspase-1, IL-1β, IL-6, IL-18, and TNF-α in an angiotensin II-induced Parkinson's disease model [261]. Additionally, biochanin A was shown to alleviate liver injury by inhibiting NLRP3 inflammasome activation and the expression of TNF-α, IL-1β, and TXNIP, through the activation of the Nrf2 pathway [262]. Furthermore, a recent study demonstrated that a 8-week treatment with dietary estrogen biochanin A (10 and 20 mg/kg) reduced the expression of the NLRP3 inflammasome, IL-1β, and IL-18 by inhibiting NF-κB signaling and blunted transforming growth factor (TGF)-β/Smad signaling and production of collagen I, collagen III, fibronectin, and alfa-smooth muscle actin (α-SMA) in HFD/STZ-induced experimental model of diabetic nephropathy [263].

Lastly, Syringaresinol is a phytoestrogen which is abundant in medicinal plants, such as *Sargentodoxa cuneata* and *Rubia philippinensis* [264]. It has been shown to inhibit the NLRP3 inflammasome by upregulating estrogen receptor β (ERβ) expression. Several studies have demonstrated that Syringaresinol can prevent sepsis-induced organ damage, including lung and myocardial injury, by suppressing the NLRP3 inflammasome and reducing pyroptosis through the activation of ER/SIRT1/NLRP3/GSDMD signaling pathway. Consequently, estrogen or ER activation may alleviate organ dysfunction in sepsis by inhibiting inflammatory processes [265,266].

From the aforementioned information, it is concluded that phytoestrogens can be effective against NLRP3-dependent pathologies and development of estrogen-related drugs or analogs as well as drugging phytoestrogens can help in overcoming many inflammatory diseases, involving NLRP3 activation as cardiovascular diseases.

5. Conclusions

The sterile inflammatory response elicited by NLRP3 inflammasome activation plays a critical role in the development of the CVDs. Understanding the molecular mechanisms underlying NLRP3 activation has shed light on its contribution to disease progression and opened new avenues for therapeutic intervention. The previously mentioned NLRP3 inflammasome inhibitors have shown promising potential in preclinical models for reducing inflammation, thereby mitigating cardiovascular damage and other inflammatory diseases. These inhibitors represent a new frontier in cardiovascular disease therapy, offering targeted approaches that could improve patient outcomes by directly targeting NLRP3 protein. Moving forward, further research is needed to optimize these

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compounds for clinical use and to explore their efficacy and safety in human studies, paving the way for innovative treatments that could transform the management of cardiovascular conditions.

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