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Small molecule modulators of immune pattern recognition receptors†

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Pattern recognition receptors (PRRs) represent a re-emerging class of therapeutic targets for vaccine adjuvants, inflammatory diseases and cancer. In this review article, we summarize exciting developments in discovery and characterization of small molecule PRR modulators, focusing on Toll-like receptors (TLRs), NOD-like receptors (NLRs) and the cGAS-STING pathway. We also highlight PRRs that are currently lacking small molecule modulators and opportunities for chemical biology and therapeutic discovery.

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Introduction

The ability of animals and plants to sense microbes provides an important means to discriminate self from non-self and initiate immune responses to establish homeostasis as well as defend against potentially lethal infections. To do so, animals and plants have acquired and evolved pattern recognition receptors (PRRs) that detect and respond to common microbe, pathogen and damage associated molecular patterns (MAMPs, PAMPs and DAMPs, respectively).[1,2] These molecular patterns are composed of molecules foreign to a healthy host yet common among different microbes or damaged host cells. These molecules vary drastically in structure and complexity. MAMPs and PAMPs can include components of the bacterial cell wall and membrane, bacterial and viral nucleic acids, particulates, and potassium efflux in the host. DAMPs include molecules associated with the breakdown vital cellular components like organelles and the extracellular matrix. The ability to sense diverse types of molecules allow the host to mount an immune response and initiate clearance of infected or damaged cells.

Several classes of PRRs are expressed in humans that signal for MAMPs, PAMPs and DAMPs. These include but are not limited to toll-like receptors (TLRs),[3] NOD-like receptors (NLRs),[4] and the cGAMP signaling (cGAS-STING) pathway.[5] Activation of these receptors leads to downstream production of inflammatory cytokines, antimicrobial factors, and cell death factors. Disfunction of these receptors can lead to immune disorders and diseases resulting in chronic inflammation. For example, improper function of the NLR, nucleotide-binding oligomerization domain-containing protein 2 (NOD2), is associated with Crohn's disease and Blau syndrome,[6,7] and disfunction of the NLR-family pyrin domain containing 3 (NLRP3) inflammasome is associated with a myriad of disorders ranging from diabetes to Alzheimer's disease.[8]

Agonists of PRRs can be powerful immunostimulants and are used as adjuvants. One of the earliest discovered immune activators is Freund's adjuvant, which is composed of heat inactivated mycobacteria in a water/oil emulsion.[9] The active molecule of Freund's adjuvant was later identified to be muramyl dipeptide (MDP), which is the ligand of NOD2. MDP and its derivatives that activate NOD2 have been found to be potent immunostimulants that serve important roles in pathogen clearance and increasing the efficacy of cancer immunotherapy.[10,11] Numerous immunologic adjuvants exist that target several PRRs. For example, aluminum acts as a NLRP3 agonist, Lipid A derivatives activate TLR4, and CpG oligonucleotides activate TLR9.[12]

As each PRR has evolved to signal for specific MAMPs and PAMPS, structural models of these receptors deepen the understanding of ligand specificity and activation mechanisms. Reliable structures of PRRs can be used to assist the development of next generation immunotherapies. This review seeks to summarize natural PRR agonists from a structural and chemical perspective and describe synthetic activators and inhibitors of these receptors. Additionally, this review will highlight opportunities for chemical biology and therapeutic discovery and complement existing reviews on this topic.[13,14]

Toll-like receptors

There are 10 TLR genes in humans and 12 in mice.[2,3] TLRs 1–9 are conserved between the two species. TLR10 is expressed in humans but is a pseudogene in mice. TLRs 11–13 are expressed

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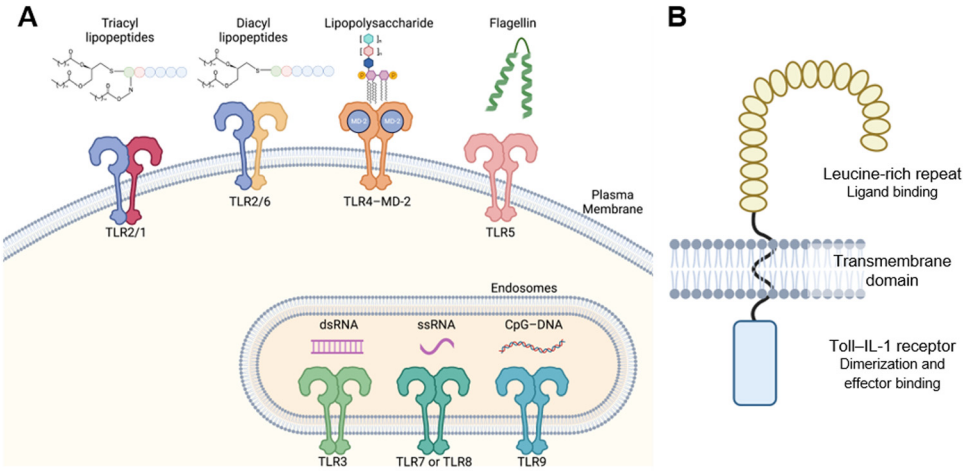

Fig. 1 Toll-like receptors. (A) Sub-cellular localization and MAMPs, PAMPs and/or DMAPs of TLRs. (B) Domain structure of TLRs.

in mice but are pseudo genes in humans. Each TLR recognizes a distinct set of molecular patterns that are atypical of healthy host cells. TLR10 remains to be an orphan receptor. TLRs-1, 2, 4, 5, and 6 are localized on the plasma membrane, while TLRs-3, 7, 8, and 9 are localized on the endosomal membrane (Fig. 1A). TLRs are single-pass membrane proteins consisting of an ectodomain, a transmembrane domain, and a Toll–IL-1 receptor (TIR) domain (Fig. 1B). The ectodomain contains 18–25 copies of leucine rich repeat (LRR) and typically binds ligands. The TIR domain interfaces the cytoplasm and interacts with other TIR-type domains in signaling proteins. Agonistic ligands induce receptor dimerization and bring the two TIR domains together, which allows them to interact with the TIR domains of cytoplasmic adapter molecules to trigger intracellular signaling. There are four such adaptors used by TLRs: MyD88, MAL (also known as TIRAP), TRIF, and TRAM. Most TLRs interact with MyD88 except for TLR3, which interacts with TRIF. All TLRs induce NF-κB–mediated cytokine production, whereas endosomal TLRs also induce IRF-mediated production of type-I interferons. TLRs are intricately involved in various aspects of health and disease and are important therapeutic targets for sepsis, lupus, and vaccine adjuvants to name a few.

TLR2. TLR2 is expressed on the plasma membrane and recognizes bacterial lipopeptides.[2] Diacyl and triacyl lipopeptides induce the formation of TLR2–TLR6 and TLR2–TLR1 heterodimers, respectively, which brings the cytoplasmic TIR domains closer to initiate signal transduction.[15,16] The most studied TLR2 ligands are Pam_2Cys and Pam_3Cys (Fig. 2A). Pam_2Cys is a synthetic analogue of the 2 kDa molecule called Macrophage-Activating Lipopeptide-2 (MALP2) which was isolated from *Mycoplasma fermentans*.[17] On the other hand, Pam_3Cys is a conserved motif on the N-termini of Braun's lipoprotein, which was prepared from *Escherichia coli*, and most bacterial lipoproteins.[18,19] In addition to lipopeptides, TLR2 is also activated by various microbial metabolites such as lipoteichoic acids and zymosan.[20] For example, a recent study identified a lipid from *Akkermansia muciniphila*'s cell membrane that recapitulates the immunomodulatory activity of *A. muciniphila* in cell-based assays.[21] The isolated immunogen, a diacyl phosphatidylethanolamine with two branched chains, activates TLR2-TLR1 heterodimer.

Co-crystal structures of Pam_2CSK_4 or Pam_3CSK_4 bound to the TLR2–TLR6 or TLR2–TLR1 heterodimers show exactly how lipopeptides induce dimerization. The TLR2–TLR1 heterodimer assumes an "m" shape with the two C-termini converging at the middle and binds a single Pam_3CSK_4 at the dimerization interface which serves as a molecular glue (Fig. 2B).[22] The two ester-bound lipid chains of Pam_3CSK_4 insert into an internal pocket of TLR2 from its opening on the convex surface and the amide-bound lipid chain interacts with a channel formed on the convex surface of TLR1. In addition, the glycerol and Cys-Ser backbone of the ligand fits tightly in the dimerization interface. Amino acids from TLR2 and TLR1 also form hydrophobic, hydrogen-bonding, and ionic interactions to further stabilize the heterodimer. Similarly, the TLR2-TLR6 heterodimer binds a single Pam_2CSK_4 at the dimerization interface (Fig. 2C).[23] The lack of the amide-bound lipid chain appears to be compensated by stronger protein–protein interactions between TLR2 and TLR6. These structure analyses advance further understanding and development of the structure–activity relationship of lipopeptide analogues.[24]

Recent studies have identified small molecule TLR2 agonists that are structurally unrelated to the lipopeptides (Fig. 2A).[25] For example, high-throughput screening with reporter gene assays yielded several compounds containing the 1,4-diphenyl-1*H*-imidazole core such as CU-T12-9[26,27] and SMU-Z1.[28] CU-T12-9 and SMU-Z1 specifically activated TLR2-TLR1 heterodimer and presumably bind the same site as Pam_3Cys as suggested by *in vitro* competitive binding assays. Similar effort also led to the identification of tricyclic dihydropyridine-quinolone compounds.[29] Interestingly, the agonist activity was highly dependent on the chirality of the methyl substituent. Diprovocim is a class of potent synthetic TLR2–TLR1 agonists that emerged from the screening of a unique chemical library designed for promoting cell surface receptor dimerization.[30] Interestingly, co-crystal structure analysis of diprovocim-1–bound TLR2 homodimer revealed that two molecules of diprovocim-1 bind the ligand-binding pocket formed by the two TLR2 ectodomains, which offers the first insight into how

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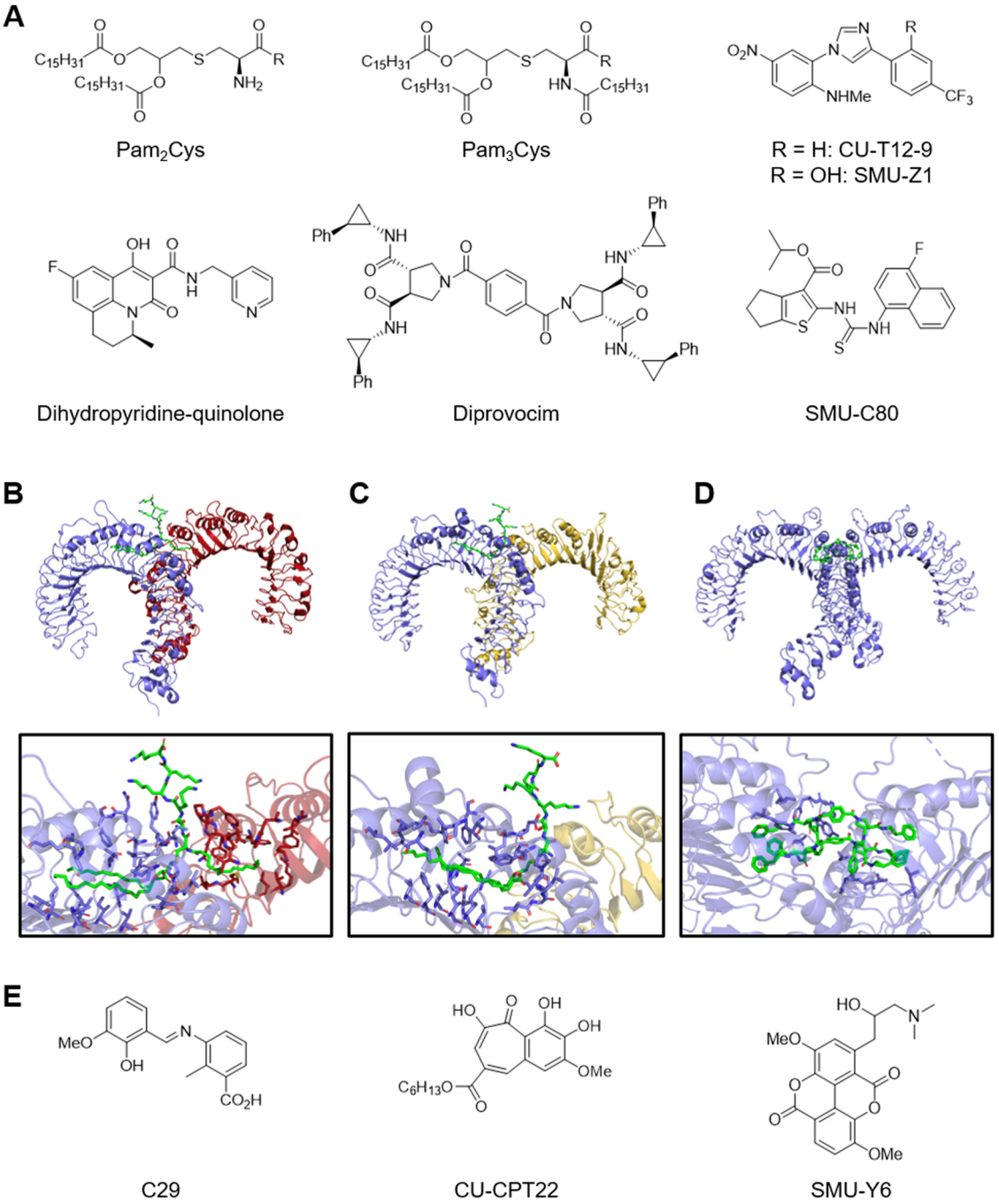

Fig. 2 TLR2 modulators. (A) Representative TLR2 agonists. Co-crystal structures of Pam_3CSK_4–TLR2–TLR1 complex (B), Pam_3CSK_4–TLR2–TLR6 complex (C), and diprovocim–TLR2–TLR2 complex (D). (E) Representative TLR2 inhibitors.

non-lipopeptide ligands interact with TLR2 (Fig. 2D).[31] In addition, diprovocim-1 synergized with anti–PD-L1 treatment to inhibit melanoma growth in mice.[32] Alternatively, structure-based virtual screening of the ZINC database against the Pam_3CSK-bound TLR2–TLR1 heterodimer led to the identification of SMU127 and the potency-optimized compound SMU-C80.[33,34] It is noteworthy that SMU-C80 contains the *N*-aryl-*N*′-(thiophen-2-yl)thiourea core that had been previously identified through a high-throughput screening campaign.[26] These studies demonstrate that TLR2 is a tractable target for small and medium-sized molecules.

Recent studies have also identified several TLR2 antagonists (Fig. 2E). High-throughput screening led to the identification of the first TLR2-selective antagonist CU-CPT22.[35] CU-CPT22 competitively binds TLR2–TLR1 dimer *in vitro* and inhibits Pam_3CSK-induced NO and inflammatory cytokine production in RAW 264.7 macrophages. Similar effort led to the identification of taspine and the derivative SMU-Y6.[36] Virtual screening

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approaches yielded several TLR2 antagonists.[37–39] For example, docking screening against a pocket located in the TLR2 TIR domain led to the identification of C29.[39] Interestingly, *ortho*-vanillin which is generated from C29 imine hydrolysis turned out to be the active species. Another study reported structure–activity relationship of related *N*-benzylideneaniline derivatives.[40] However, readers should note that most TLR2 antagonists in literature contain alert structures for assay interference and stability.[41–43]

TLR4. TLR4 was the first human ortholog of fruit fly Toll to be cloned.[44] TLR4 is expressed by several immune cells such as dendritic cells and macrophages on the plasma membrane and functions as a receptor for lipopolysaccharide (LPS).[45–47] Mechanistically, TLR4 and myeloid differentiation factor 2 (MD-2) form a complex that recognizes LPS.[48,49] Co-crystal structure shows that the TLR4–MD-2–LPS complex is an m-shaped dimer of TLR4–MD-2 dimers which are bridged by two molecules of LPS (Fig. 3A).[50] Five of the six lipid chains of lipid A are buried in the hydrophobic binding pocket inside MD-2 and the remaining chain is partially exposed to the surface of MD-2 and interacts with TLR4. The phosphate groups of lipid A form ionic interactions with positively charged residues of TLR4 and MD-2. Eritoran is structurally similar to lipid A but possesses only four lipid chains and functions antagonistically.[51] Eritoran binds MD-2 with all the lipid chains occupying the hydrophobic pocket and substantially shifts the disaccharide scaffold relative to lipid A, which precludes formation of effective interactions with TLR4.[52,53]

Structure–activity relationship of lipid A analogues has been extensively studied, delineating the impacts of the number and length of lipid chains as well as the identity and substitution pattern of disaccharide scaffold (Fig. 3B).[54] For example, monophosphoryl lipid A (MPLA) lacks the 1-phosphono and 3-*O*-(*R*)-3-hydroxytetradecanoyl groups. MPLA is a milder TLR4 agonist and is less endotoxic compared to LPS, perhaps due to TRIF-biased downstream signaling.[55] MPLA is clinically approved by FDA for the use as a vaccine adjuvant in humans. It is noteworthy that lipid A analogues inspired the development of non-glycolipid amphiphilic TLR4 agonists such as E6020 as a vaccine adjuvant.[56] In contrast, E5531[57] and Eritoran,[51] which is also known as E5564 and B1287, are penta- and tetra-lipidated analogues, respectively, and potent TLR4 antagonists. Both compounds entered clinical trials to treat sepsis but were discontinued. In addition, a recent study identified a complex glucorhamnan polysaccharide from a culture medium of *Ruminococcus gnavus* that activates TLR4 and induces inflammatory cytokines.[58] This molecule may contribute to the association between *R. gnavus* and Crohn's disease.

Recent studies have identified small molecule TLR4 agonists that are structurally unrelated to the lipopeptides (Fig. 3C). For example, high-throughput screening with a reporter gene assay led to the identification of pyrimido[5,4-*b*]indoles and 4-aminoquinazolines as TLR4 agonists.[59,60] Optimization of the pyrimido[5,4-*b*]indole series achieved ~20-fold improvement in activity.[61] The pyrimido[5,4-*b*]indoles and 4-aminoquinazolines require MD-2 for activity and are presumed to bind the hydrophobic pocket inside MD-2 based on docking studies. Similarly, high-throughput screening of a nontraditional compound libraries including an α-helix mimetic library led to the discovery of neoseptins.[62] Neoseptins exhibit flat structure–activity relationship and low potency *in vitro* but are effective adjuvants *in vivo*. Co-crystal structure analysis revealed that the overall conformation of the TLR4–MD-2 complex and the local conformation of the MD-2 ligand-binding pocket were similar between neoseptin-3 and lipid A (Fig. 3D).[63] Interestingly, two molecules of neoseptin-3 bound to each 1 : 1 TLR4–MD-2 heterodimer with each molecule adopting different conformations and interacting with different areas of TLR4 and MD-2. In addition, the two neoseptins bind to each other *via* π–π interaction and two hydrogen bonds. Screening a small library of 750 pure natural products led to the identification of Euodenine A.[64] Structure–activity relationship development around the cyclobutane ring resulted in a 10-fold increase in potency. Alternatively, a cascade of ligand-based and structure-based virtual screenings yielded 2,3-(9,10-dihydroanthracene-9,10-diyl)succinimides.[65] These studies highlight a unique and unexpected binding mechanism of a synthetic ligand with little structural similarity with the natural ligand and should inspire new approaches in library design and computer-assisted drug design.

Small molecule TLR4 antagonist have also been pursued.[66] For example, the 3-(indol-5-yl)-indazole weakly 22m binds TLR4 and MD-2 *in vitro* and inhibits LPS-induced cytokine release (Fig. 3E). The authors demonstrated selectivity against kinases with the KinomeScan assay.[67] Another interesting example is TAK-242.[68] TAK-242 potently inhibits the production of inflammatory cytokines and nitric oxide induced by LPS *in vitro* and *in vivo* and protects mice against LPS-induced lethality.[68,69] Mechanistically, TAK-242 is a non-competitive, covalent inhibitor and binds the intracellular domain of TLR4 *via* Cys747.[70] TAK-242 does not affect the dimerization of TLR4 but instead disrupts interactions between TLR4 and the adapter proteins TIRAP and TRAM.[71] This case study highlights the potential of modulating pattern recognition receptor functions through covalent mechanisms.

TLR5. TLR5 is expressed on the cell surface of macrophages, dendritic cells, and epithelial cells. TLR5 senses bacterial flagellin protein and triggers Myd88-dependent signaling.[72,73] Crystal structure analysis shows that TLR5 forms a symmetric 2 : 2 complex with flagellin and recognizes the ligand mostly on the lateral surface (Fig. 4A).[74] Entolimod also known as CBLB502 is a pharmacologically-optimized flagellin derivative.[75] Numerous studies demonstrated its anti-tumour efficacy in animal models.[76] Development of small molecule TLR5 modulators remains challenging. The antagonist TH1020 is the only compound that has been reported in literature (Fig. 4B).[77] Its mechanism of action remains unclear.

TLR3. TLR3 is expressed by macrophages, conventional dendritic cells, and intestinal epithelial cells. TLR3 recognizes double-stranded RNA (dsRNA), which is indicative of viral genome and replication intermediates.[78] Upon ligand engagement, TLR3 dimerizes and activates TRIF-dependent NF-κB and IRF3 pathways.[79] Crystal structure analysis shows that two molecules of TLR3 sandwich one molecule of dsRNA.[80] Both N- and C-terminal regions of the ectodomain make sideway contacts with the sugar–phosphate backbone of dsRNA. TLR3

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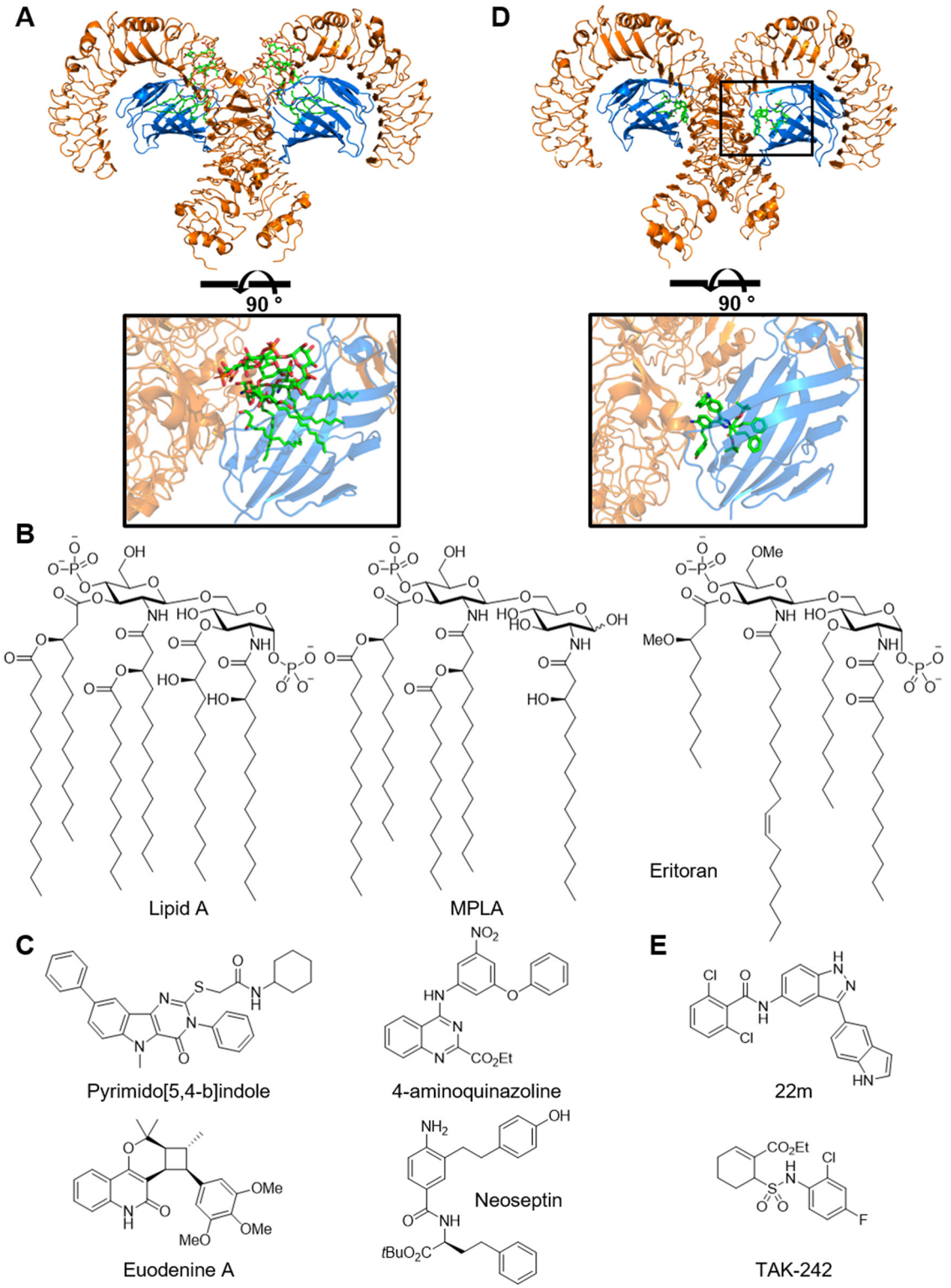

Fig. 3 TLR4 modulators. (A) Co-crystal structures of Lipid A–MD-2–TLR4 complex. (B) Lipid A and analogues. (C) Representative TLR4 agonists. (D) Co-crystal structure of neoseptin–MD-2–TLR4 complex. (E) TLR4 inhibitor TAK-242.

can be activated by poly I:C, which is a synthetic polymer composed inosinic acid and cytidylic acid.

TLR3 remains a challenging target for small molecule modulator development (Fig. 5A). Structure-based virtual screening led to the identification of compound 4a as TLR3-selective antagonist.[81] Compound 4a competed with poly I:C for TLR3 binding and inhibited poly I:C–induced NO and inflammatory cytokine production. High-throughput screening with a cell-

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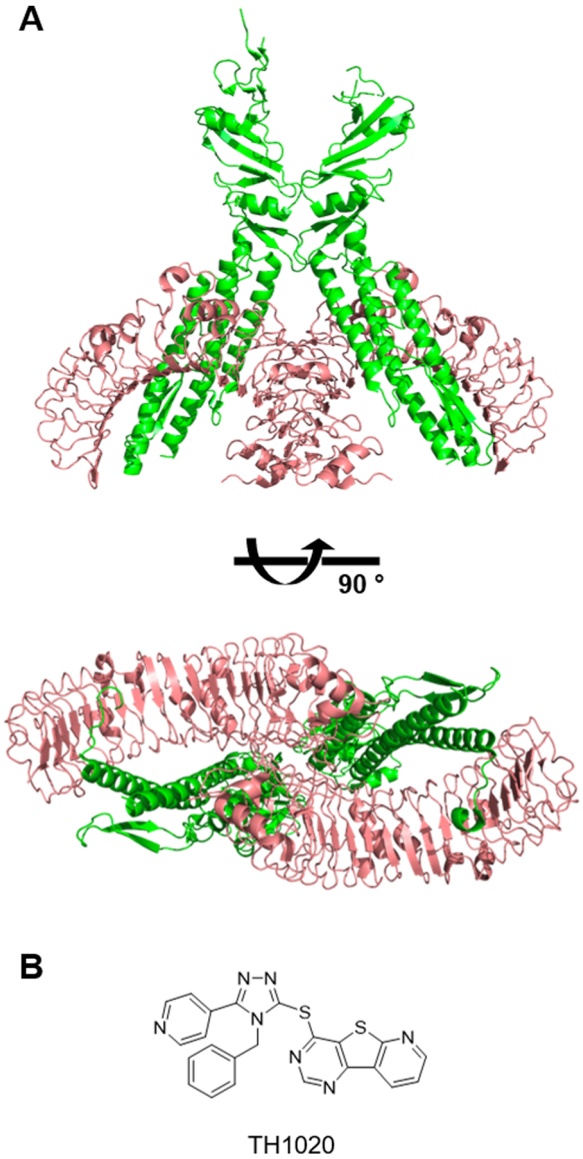

Fig. 4 TLR5 modulators. (A) Co-crystal structure of flagellin–TLR5 complex. (B) TLR5 inhibitor TH1020.

based assay led to the identification of the potent TLR3-selective antagonist SMU-CX24.[82] Notably, SMU-CX24 directly bound TLR3 with high affinity ($K_d \sim 20$ nM) and exhibited *in vivo* efficacy in an atherosclerosis model. Similarly, high-throughput screening with a reporter gene assay yielded a chromene chemical series such as CU-CPT17e that represented the first and only small molecule TLR3 agonists.[83] CU-CPT17e also activated TLR7 and TLR8. However, a comprehensive structure–activity relationship study of the series by another group failed to identify any analogues that displayed significant activity and to reproduce the activity for CU-CPT17e that was initially reported.[84]

TLR7 and TLR8. TLR7 and TLR8 are localised in the endosomal membrane and recognise viral single-stranded ribonucleotides (ssRNAs).[2,85,86] In addition, TLR7 and TLR8 senses imiquimod and related antiviral small molecules, which led to their characterization as antiviral TLRs (Fig. 5B).[3,87–90] Upon agonist ligand binding, TLR7 and TLR8 trigger MyD88-dependent production of interferons and pro-inflammatory mediators.[87] Synthetic agonists of TLR7 and TLR8 have been extensively studied in the past several decades and summarized elsewhere.[91,92] In contrast, understanding of activation and inhibition mechanisms is still evolving and will inspire new approaches to ligand development.

Recent structural analyses have revealed mechanisms of TLR7 and TLR8 activation. TLR8 exists as a pre-formed dimer and binds synthetic agonists such as CL097 at a dimerization interface.[93] This site also binds uridine derived from ssRNAs, whereas another site located at the concave surface binds short oligonucleotides.[94] Agonist ligands induce a conformational change that brings the two C-terminal TIR domains closer and enables downstream signaling. On the other hand, TLR7 exists as a monomer in the absence of ligands and forms a dimer upon binding synthetic agonists such as R-848 or upon binding guanosine and polyuridine.[95] Synthetic agonists and guanosine occupy a site at the dimerization surface in TLR7 that corresponds to TLR8's binding site for synthetic agonists and guanosine. Polyuridine binds another site in TLR7 consisting of the concave surface and a dimerization interface, which is distinct from TLR8's binding site for short oligonucleotides. These studies will facilitate not only further understanding of functional roles of TLR7 and TLR8 but also facilitate the development of synthetic ligands.

Development of selective antagonists for TLR7 and TLR8 lagged compared to agonist development (Fig. 5C). A recent study identified the potent and selective TLR8 antagonist CU-CPT8m through a high-throughput screening with a reporter gene assay and structural optimisation.[96] Co-crystal structure analysis revealed that CU-CPT8m binds a hydrophobic site located at a dimerization interface and stablises the resting-state conformation. This binding site is nearby, but distinct from, the synthetic agonist–binding site and is only formed by the pre-formed dimer in the resting state. Another recent study identified the potent and selective TLR7 antagonist Cpd-7 through structure-based design.[97] Co-crystal structure analysis of the agonist ligand 8-oxadenine derivative suggested that the substitution of the 8-oxo group to fill an additional space at the dimerization interface would convert the agonist into an antagonist. The resulting compounds exhibited antagonistic activity in reporter gene assay and blocked TLR7-dependent IFN-α secretion from PBMC. Surprisingly, crystallographic analysis revealed that the antagonist-bound complex adopted an activated dimeric structure with the antagonist occupying the same site as the original agonist. Interestingly, cryo-EM analysis revealed that the antagonist-bound dimer adopted two major forms: a closed form which was also observed in the crystal structure and an open form in which the two C-termini were separated from each other. These studies highlight implications of conformational dynamics in ligand design.

TLR9. TLR9 is an endosomal nucleic acid sensor that is expressed by plasmacytoid dendritic cells, B cells, and eosinophiles. TLR9 recognises unmethylated cytosine–phosphate–guanine (CpG) deoxynucleotide motifs, which are common in

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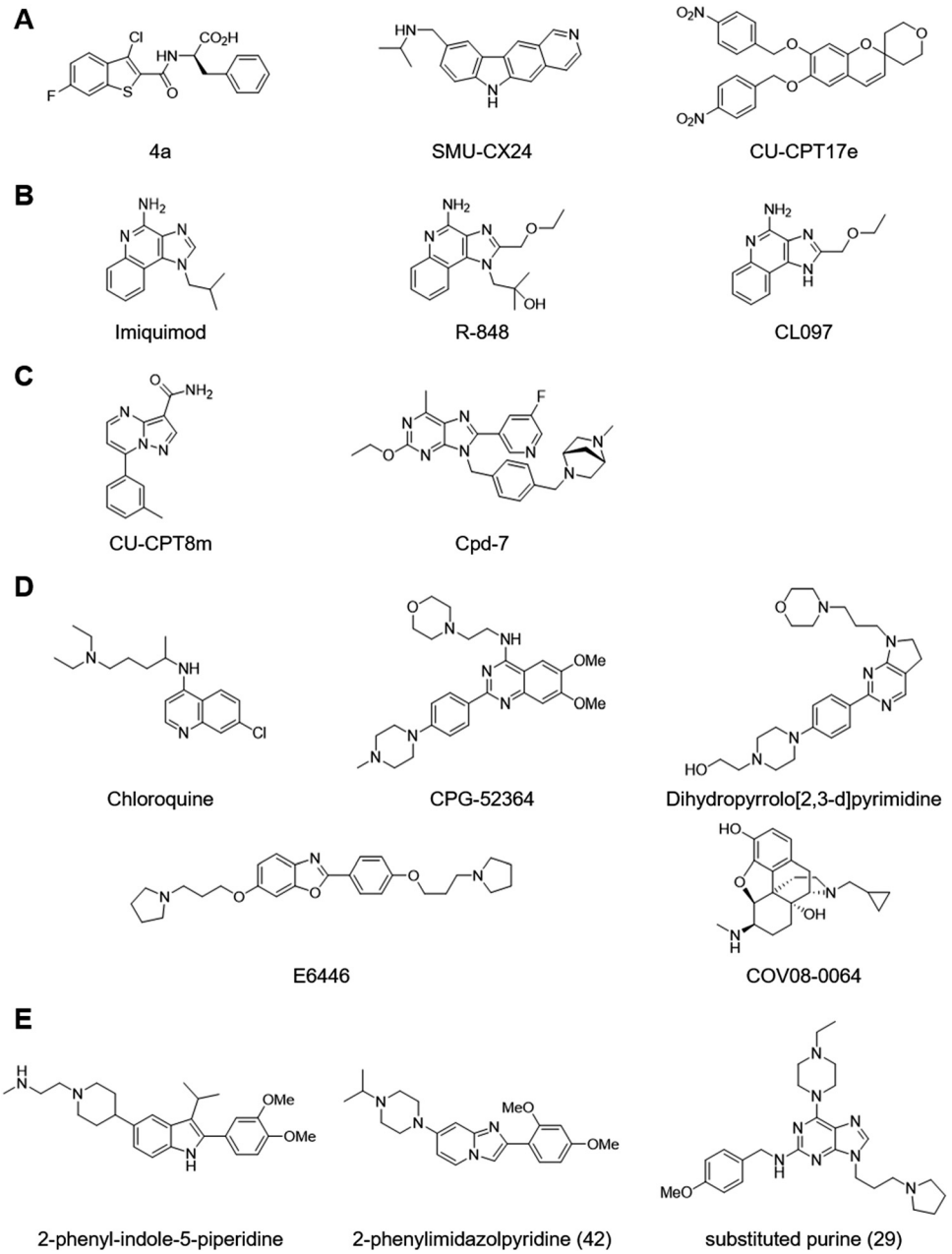

Fig. 5 Small molecule modulators of TLRs 3, 7, 8 and 9. (A) Representative TLR3 inhibitors. (B) Representative TLR7 and TLR8 agonists. (C) Representative TLR7 and TLR8 inhibitors. (D) Representative TLR9 inhibitors. (E) Representative dual inhibitors of TLR7 and TLR9.

bacteria and herpesvirus, by directly binding CpG-containing DNA (CpG DNA).[98–100] Agonistic ligand engagement triggers TLR9 dimerization and downstream signaling through MyD88-dependent NF-κB and IRF7 pathways.[101] Accordingly, various synthetic oligodeoxynucleotides containing the CpG motif entered clinical trials as adjuvants for vaccines targeting infectious diseases and cancer.[102]

Recent crystal structure analysis revealed that CpG DNA induces the formation of a symmetric TLR9-CpG DNA complex with 2 : 2 stoichiometry.[103] In the active complex, the CpG DNA wraps around the N-terminal region of one LRR domain from the lateral surface to the concave surface and extends to the C-terminal region of the other LRR domain, acting as a molecular glue to bridge the two TLR9 molecules. The CpG motif is accommodated in the groove on the lateral surface and recognized *via* interactions with multiple amino acids and *via* water-mediated hydrogen bonds. On the other hand, inhibitory DNA binds the concave surface that overlaps with the binding site for CpG DNA and prevents dimerization. Another study identified a second DNA-binding site in TLR9 that binds DNA containing cytosine at the second position from the 5′ end.[104] This site corresponds to the nucleoside-binding site in TLR7 and TLR8 and cooperatively regulates receptor dimerization and activation.

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Recent studies identified several small molecule TLR9-selective antagonists (Fig. 5D). For example, the antimalarial drug chloroquine has inspired the development of multiple TLR9 antagonists. Chloroquine inhibits TLR9 selectively over TLR7 and TLR8.[89] Chloroquine directly blocks TLR9–CpG DNA interaction *in vitro*, perhaps by interacting with the nucleic acid.[100,105] Regardless of exact mechanism of action, chloroquine's evolved into more potent and selective CPG-52364 and derivatives with the quinazoline core,[106] and further optimization led to dihydropyrrolo[2,3-*d*]pyrimidines.[107] Similarly, CpG DNA is a proposed target for benzoxazoles such as E6446.[108,109] Alternatively, benzoxazole derivatives may bind the inhibitory DNA site of TLR9, although experimental evidence is lacking.[110] Other TLR9-selective antagonists include COV08-0064, which emerged from a screening of morphinans using a reporter gene assay, and thiophene derivatives.[111]

Dual-inhibition of TLR7 and TLR9 has potential clinical benefit in autoimmune disorders such as lupus.[112] High-throughput screening with TLR7 and TLR9 reporter gene assays yielded a 2-pheny-indole-5-piperidine chemical series (Fig. 5E).[113] Optimisation efforts led to the lead compound 7f with potent TLR7 and TLR8 inhibitory activity and modest TLR9 inhibitory activity as well as desirable pharmacokinetic and pharmacodynamic profiles. Co-crystal structure analysis of 7f–TLR8 complex revealed that 7f stabilizes an inactive conformation which aligns well with the apo conformation. The lead compound demonstrated efficacy in rodent disease models for psoriasis and lupus. Interestingly, an analogous 2-phenylimidazopyridine chemical series was developed and optimized into the lead compound 42 with potent TLR7 and TLR9 inhibitory activity and modest TLR8 inhibitory activity.[114] Alternatively, a purine-based TLR7 agonist was converted to the dual TLR7 and TLR9 antagonist 29.[115] Compounds 29 and 42 did not interact with CpG DNA to a detectable level by isothermal titration calorimetry. It remains unclear whether any of small molecule TLR9 antagonists directly bind TLR9 *in vitro* or in cells.

NOD-like receptors

There are 22 NLR genes in humans.[116] Although several human NLR genes have multiple murine paralogs, some human NLR genes lack murine counterparts (Fig. 6A). NLRs are intracellular receptors and respond to diverse PAMPs and DAMPs (Fig. 6B). Some NLRs activate NF-κB to initiate inflammatory responses just like TLRs, while other NLRs trigger a distinct pathway that induces cell death and the production of pro-inflammatory cytokines. NLR typically consists of three functional domains, namely N-terminal signaling, central [NAIP, CIITA, HETE, TP1 (NACHT)], and leucine-rich repeat (LRR) domains. NLRs are classified into subfamilies according to the type of N-terminal signaling domain: those with acidic activation domain (AAD) are called the NLR AAD containing family (NLRA); those with baculovirus inhibitor of apoptosis repeat (BIR) are called the NLR BIR containing family (NLRB); those with caspase recruitment domain (CARD) are called the NLR CARD containing (NLRC) family; those with pyrin domain (PYD) are called the NLR pyrin domain containing (NLRP) family. For example, an NLRC such as NOD1 and NOD2 recruits RIP2 *via* its CARD upon activation and triggers proinflammatory cytokine production through NF-κB and MAPK activation.[2] In contrast, NLRP members interact with ASC *via* its PYD to recruit procaspase-1 and form oligomers called inflammasomes.[117] Inflammasome formation leads to the auto-cleavage of procaspase-1 to release the active caspase-1, which subsequently proteolytically processes the inflammatory cytokines IL-1β and IL-18. Caspase-1 also induces the inflammatory cell death called pyroptosis by cleaving gasdermin D.

NOD1 and NOD2. NOD1 and NOD2 are intracellular pattern recognition receptors that sense bacterial peptidoglycan metabolites and ER stress.[118,119] NOD1 recognizes fragments of the peptide stem containing γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP),[120,121] while NOD2 recognizes muramyl dipeptide (MDP)[122,123] (Fig. 7A). NOD1 is ubiquitously expressed by various cell types such as endothelial cells and stromal cells, while NOD2 expression is confined to monocytes, dendritic cells, intestinal epithelial cells, Paneth cells and intestinal stem cells.[118] In addition, NOD1 and NOD2 are interferon-stimulated genes whose expression in tissues may only become detectable under particular conditions, such as viral and bacterial infections.[124]

Biochemical evidence supports that NOD1 and NOD2 directly bind iE-DAP and MDP. For example, a biotinylated MDP (of unknown structure) was used to isolate recombinant NOD2 from cell lysate.[125] This study suggested that MDP binds to the NOD2 NBD based on a domain truncation analysis, though the specificity of this interaction remains to be evaluated with a diastereomer control and/or competition with unmodified MDP. Another study demonstrated direct binding using surface plasmon resonance employing an MDP-functionalized chip.[126] The active MDP-L,D isomer or an inactive MDP-L,L isomer was covalently immobilized to the chip *via* the 6-amino group and purified full-length NOD2 was flowed over the chip. Surprisingly, NOD2 was found to bind both isomers with similar affinities: K_D = 51 nM for the active isomer and K_D = 150 nM for the inactive isomer. Subsequent studies from the same group demonstrated that a LRR domain construct binds 6-amino-MDP (K_D = 213 nM), 6-amino-GlcNAc (K_D = 354 nM), and the dipeptide (K_D = 428 nM) using the same setup.[127] Binding affinities were weaker for constructs carrying mutations on the concave surface of LRR that had been reported to diminish NOD2 activation, suggesting these residues may be directly involved in MDP binding. In addition, modification *via* the 1- or 2-positions on MurNAc decreased to affinity to ~1 μM.[128,129] A similar SPR study has also been performed for iE-DAP analogs.[130] However, the biotinylated analogues and surface immobilization affect cellular uptake and activity, which precluded target engagement analysis in cells. In this regard, we have developed a series of photo-activatable chemical reporters mimicking iE-DAP and MDP (Fig. 7B).[131] We demonstrated diastereo-selective and ligand-competitive crosslinking of NOD1 and NOD2 with these chemical reporters in HEK293T cells and bone marrow-derived macrophages. In addition, chemical proteomics revealed the membrane associated GTPase ARF6 as an unpredicted target of MDP which is recruited to NOD2 upon activation. A recent study

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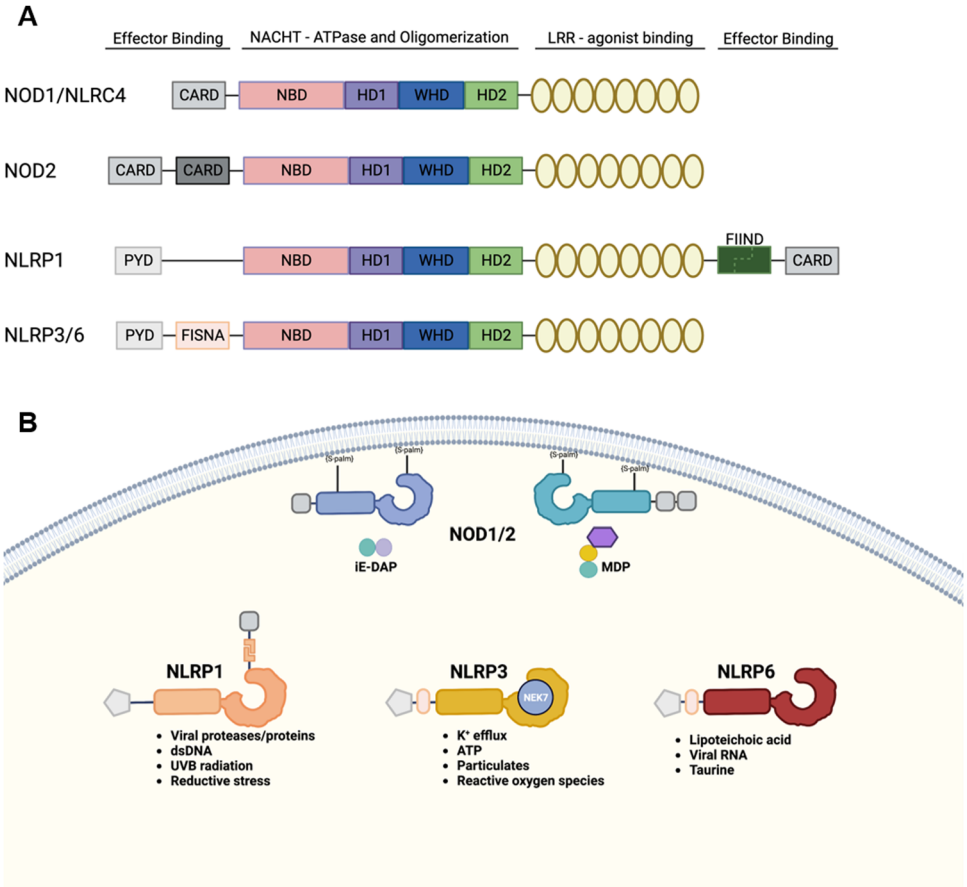

Fig. 6 NOD-like receptors. (A) Domain architectures of representative members of the NLR family. (B) Sub-cellular localization and PAMPs/DAMPs of NLRs.

demonstrated *N*-acetylglucosamine kinase (NAGK) is required for the immunostimulatory activity of MDP.[132] Mechanistically, NAGK phosphorylates MDP at the C6-hydroxyl group to yiled 6-*O*-phospho-MDP. While 6-*O*-phospho-MDP activates NOD2 in $NAGK^{-/-}$ cells, whether it engages NOD2 remains unclear.

Membrane association of NOD1 and NOD2 is critical for their function and is indirectly mediated by post-translational modification.[133,134] Indeed, a recent study identified multiple *S*-palmitoylated cysteine residues and demonstrated that these post-translational modifications are necessary for their membrane localization and ligand-induced signaling.[135] The palmitoyltransferase ZDHHC5 was found to be responsible for this critical post-translational modification. In addition, we recently identified the membrane-associated GTPase ARF6 as a component of the MDP–NOD2 complex with the photo-activatable chemical reporter.[131] The mutation of an N-terminus myristoylation site of ARF6 from glycine to alanine diminishes MDP–NOD2 binding, presumably due to mis-localization off the plasma membrane. In a follow-up study, we identified the conserved aromatic triad of ARF is necessary for this interaction.[136] Interestingly, the lipid-modified MDP analogue L18-MDP induces stronger ARF6–NOD2 association and NF-κB activation than MDP. These studies suggest that membrane-targeting is potentially an effective design strategy for NOD2 ligand development.

NOD1 and NOD2 are attractive targets for therapeutic development.[137] For example, our recent studies on the *Enterococcus* species revealed peptidoglycan remodeling and NOD2 activation as key mechanisms for microbiota-mediated enhancement of immune checkpoint inhibitor therapy.[10] Agonist ligand development for these receptors has remained focused on analog design and derivatisation of the peptidoglycan metabolites over the past several decades.[137] For example, FK-156 was isolated and synthesized as an immunostimulatory component of Gram-positive *Streptomyces olivaceogriseus* and *S. violaceus* strains in 1981 (Fig. 7C).[138–142] FK-156 was found to induce proliferation of murine splenocytes, protect against lethal challenge with *Escherichia coli*, and improve carbon clearance from the blood, an early assay for phagocytic activity *in vivo*. Through a reductionist approach, a subsequent study identified iE-DAP is the minimal prerequisite structure of FK-156 that elicits activity in 1982, revealing the minimal NOD1 ligand 20 years before the discovery of the receptor.[143] Further synthetic studies yielded several lipophilic analogues of iE-DAP including FK-565 which improved overall activity.[144,145] Subsequent studies delineated structural elements required for NOD1 activation including the terminal carboxyl and amine groups of DAP and the terminal carboxyl group of glutamic acid.[146,147] More recently, rigidification of DAP *via* the introduction of a double bond retained

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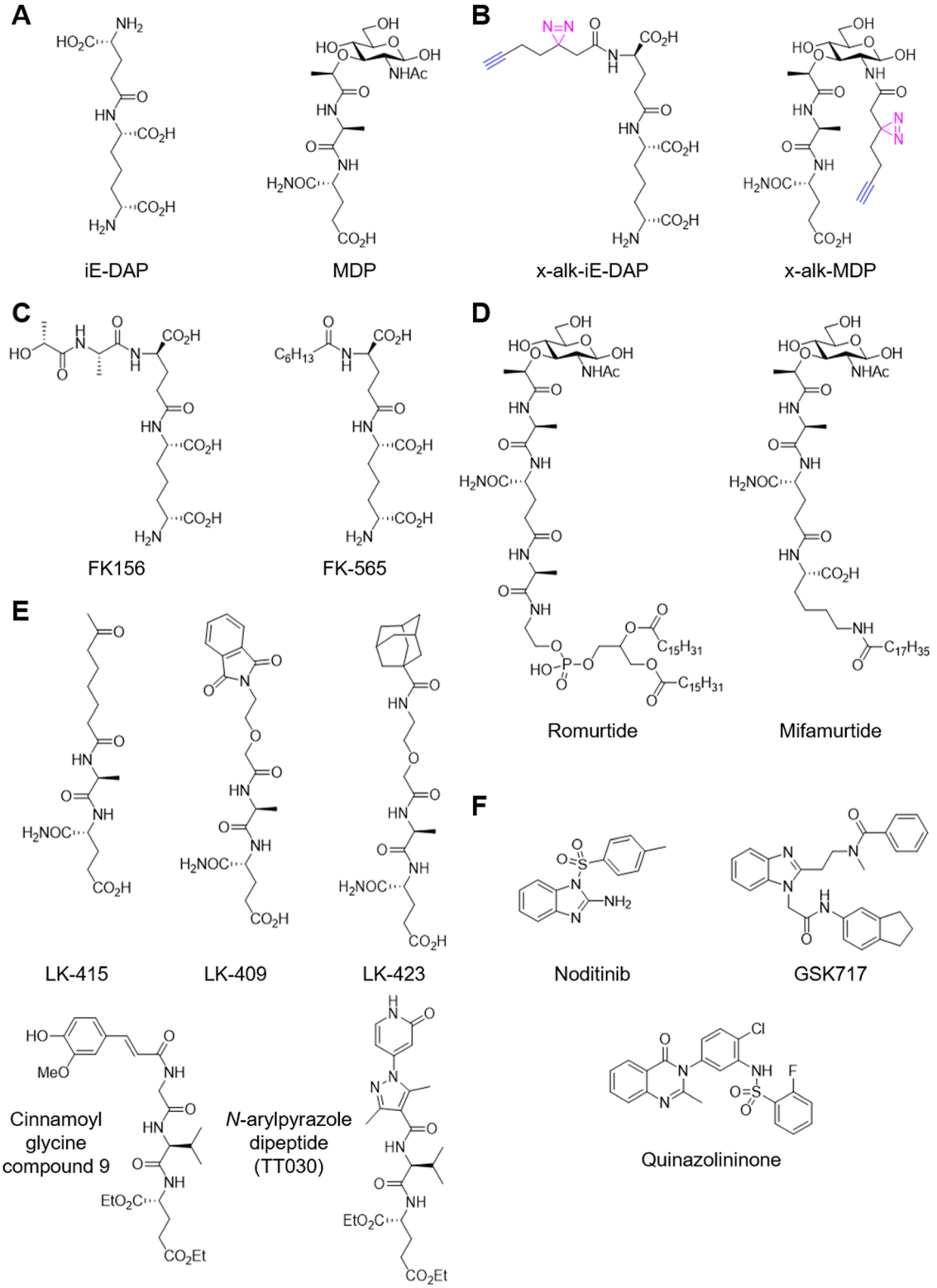

Fig. 7 Small molecule modulators of NOD1 and NOD2. (A) Minimal activating ligands of NOD1 and NOD2. (B) iE-DAP and MDP–based chemical reporters. (C) Synthetic derivatives of iE-DAP. (D) Synthetic derivatives of MDP. (E) Representative desmuramyl dipeptides. (F) Representative inhibitors of NOD1 and NOD2.

activity relative to lauroyl iE-DAP while rigidification *via* cyclisation involving the terminal amine abrogated activity.[148,149] Similarly, MDP was found to be the minimal adjuvant molecule from Freund's adjuvant in 1974.[150] Over the next decade, its structure–activity relationship was defined and various lipophilic derivatives were synthesized to improve the pharmacological profile.[151]

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These efforts yielded clinical molecules such as romurtide and mifamurtide (Fig. 7D).[152] Alternatively, the *N*-acetylmuramic acid moiety was replaced with various lipophilic surrogates containing adamantyl,[153] oxo fatty acid,[154] phthalimido,[155] or carbocyclic[156] groups to yield desmuramyldipeptides (Fig. 7E). More recently, cinnamoylglycine emerged as a promising scaffold to replace MurNAc.[157–160] The authors identified a hit compound from a small panel of saccharine- and indole-based desmuramyl dipeptides and empirically morphed it into the optimized scaffold to achieve high nanomolar NOD2 activity *in vitro*. Further optimization of the series led to lipophilic analogues with the adjuvant activity promoting antibody production *in vivo*[161] and single nanomolar NOD2 activity *in vitro*.[162] In addition, we recently disclosed *N*-arylpyrazole NOD2 agonists that promote immune checkpoint therapy.[163] We identified the *N*-arylpyrazole dipeptides from a structure-based virtual screening and empirically optimized the hit compounds. Importantly, our *N*-arylpyrazole NOD2 agonist is enantiomer-specific, effective at promoting immune checkpoint inhibitor therapy and requires NOD2 for activity *in vivo*.

In contrast, the development of antagonists for NOD1 and NOD2 relies on high-throughput screening (Fig. 7F).[164] For example, noditinib-1 emerged from a HTS campaign and is selective for NOD1 over NOD2.[165] Similarly, selective inhibitors based on the benzimidazole diamide and several other scaffolds are published.[166,167] Protein target of these inhibitors remain unclear.

NLRP1. Human NLRP1 was the first protein identified to form inflammasome.[168] Mice carry several *Nlrp1* paralogs; however, the *Nlrp1b* allele, which encodes NLRP1B protein, is best characterized.[169] Both human NLRP1 and mouse NLRP1B have a similar domain architecture consisting of a disordered domain, NACHT, LRR, FIIND, and CARD. Human NLRP1 has an additional PYD at the N-terminus. FIIND domain undergoes constitutive autocleavage and generates N- and C-terminal fragments that remain non-covalently associated.[170] Activation signals lead to the proteasomal degradation of the N-terminal fragment to release the C-terminal fragment which subsequently recruits ASC and caspase-1 to form the inflammasome.[171–173] This activation mechanism is termed functional degradation. Humans, but not mice, possess another FIIND-containing and inflammasome-forming protein called CARD8. In humans, it appears that NLRP1 functions primarily in epithelial cells[174] whereas CARD8 functions primarily in myeloid and lymphoid cells.[175–177]

Decoding danger signals that activate human and/or mouse NLRP1 remains an active research area. An early study reconstituted the human NLRP1 inflammasome *in vitro* and demonstrated that MDP induced oligomerization and dependent caspase-1 activation.[178] Another study also demonstrated that recombinantly expressed human NLRP1 LRR domain binds MDP-functionalized chip surface.[129] However, no follow-up studies substantiated MDP-dependent human NLRP1 activation.[179] Instead, MDP-driven inflammasome response from THP-1 cells, a monocytic cell line, could stem from NLRP3.[180] Rather, recent studies have demonstrated that human NLRP1 senses viral double-stranded RNA (dsRNA),[181] viral protein,[182] viral protease,[183,184] ultraviolet B irradiation,[185] and reductive stress,[186] whereas mouse NLRP1B senses bacterial and protozoan toxins.[187–189] Many of these danger signals such as protease,[183,184,188] ultraviolet B irradiation,[190] and reductive stress[186] lead to the destabilization and accelerated proteasomal degradation of the N-terminal fragment of NLRP1 through post-translational modification of NLRP1 (proteolysis, hyperphosphorylation) or alteration of stabilizing protein–protein interaction. On the other hand, dsRNA directly binds the NACHT-LRR domain and induces ATPase activity.[181] dsRNA-induced human NLRP1 activation requires FIIND autoproteolysis and proteasome activity and thus appears to involve the functional degradation mechanism. In contrast, the tegument protein ORF45 from Kaposi sarcoma–associated herpesvirus induces proteasome-independent human NLRP1 activation.[182] ORF45 directly binds the disordered domain and drags the N-terminal fragment to the nucleus, which enables the C-terminal fragment to form the inflammasome.

Synthetic inhibitors of DPP8 and DPP9 such as Val-boroPro also known as talabostat and PT-100 activate both human[191] and mouse[192,193] NLRP1 as well as CARD8[175,193] (Fig. 8A). DPP8

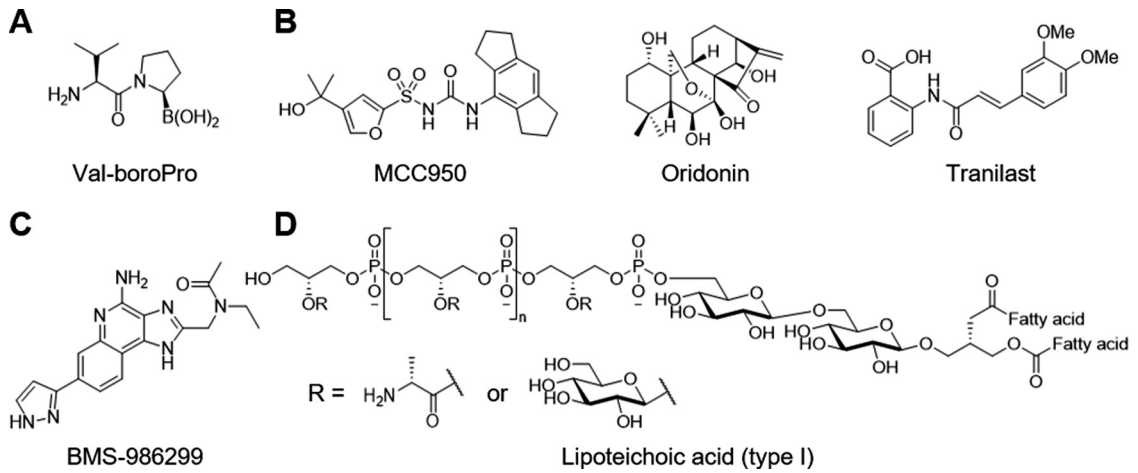

Fig. 8 Small molecule modulators of NLRP1, 3 and 6. (A) DPP8 and 9 inhibitor Val-boroPro triggers NLRP1 and CARD8 inflammasomes. (B) Representative NLRP3 inhibitors. (C) NLRP3 agonist BMS-986299. (D) NLRP6 agonist lipoteichoic acid.

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and DPP9 are members of the S9B/DPPIV (DPP4) serine protease family, which had been implicated in immune responses.[194] These chemical genetics studies employing Val-boroPro revealed their physiological function as a checkpoint that holds innate immune system at bay. Mechanistically, DPP9 bind the NLRP1 FIIND domain and cleave unknown substrates to prevent the inflammasome formation.[191] Indeed, cryo-EM analysis shows that DPP9 forms a ternary complex with a full-length NLRP1 and a C-terminal fragment of NLRP1.[195,196] The N-terminus of the NLRP1 C-terminal fragment inserts into the DPP9 active site, which is disrupted by Val-boroPro. Similarly, DPP9 forms an inhibitory ternary complex with CARD8; however, the N-terminus of the CARD8 C-terminal fragment does not closely interact with the DPP9 active site.[197] Interestingly, Val-boroPro does not dissociate DPP9 from CARD8 *in vitro*, and it remains unclear whether DPP8 and DPP9 inhibitors need to destabilize the DPP9-CARD8 ternary complex.[198] Differences in the regulatory mechanisms of NLRP1 and CARD8 such as this may potentially be exploited to develop selective agonists.[199] Alternatively, high-throughput screening with a scintillation proximity assay led to the identification of several ATP-competitive inhibitors.[200] These hit compounds were confirmed with fluorescence polarization assay but lacked on-target efficacy in cells.

NLRP3. NLRP3 responds to various damage- and pathogen-associated molecular patterns such as ATP, bacterial toxin, and alum.[201] These activators cause cellular stress often mediated by ion flux change, lysosomal rapture, and mitochondrial dysfunction. How NLRP3 senses cellular stress remains unclear. Recent studies identified NIMA-related kinase 7 (NEK7), a serine-threonine kinase, as an essential and unique component of NLRP3 inflammasome.[202–204] NEK7 directly binds NLRP3 and, perhaps, facilitates conformational change.

The active structure of NLRP3 is predicted to be similar to the disc-shaped oligomers observed in active NLRC4.[205,206] Activated NLRC4 forms a flat disc with each NLRC4 monomer interacting at the nucleotide binding domain (NBD). The cryo-EM structure of monomeric NLRP3 and NEK7 revealed the binding interfaces in which the C-terminal lobe of NEK7 forms interactions with both the leucine-rich repeat domain (LRR) and helical domain 2 (HD2) of NLRP3.[207] Mutations that disrupt binding at either interface diminished inflammasome activation. Although this structure of NLRP3 was in an inactive conformation, the potential interface of active NLRP3 along the NBD surface would not affect binding of NEK7. Inactive oligomeric structures of NLPR3 were also solved by cryo-EM.[208,209] Unlike active NLRC4 discs which make inter-NBD interactions, the inactive NLRP3 cages make inter-LRR interactions forming cage-like dodecameric structures that sterically block the N-terminal pyrin domain (PYD) from binding with ASC.

Most recently, the cryo-EM structure of active NLRP3 bound to NEK7 and ASC was solved.[210] Active NLRP3 forms a disc structure composed of 10 or 11 NLPR3 monomers similar to the active structures of NLRC4.[205,206] NEK7 sits in the concave surface of the LRR, which likely dissolves the inter-LRR interactions observed in the inactive cage structures. Therefore, binding to NEK7 directly breaks up NLRP3 cages freeing the NBD domains to form the flat disc structure of active inflammasomes, which position the PYD to bind ASC and activate caspase 1.

NLRP3 antagonists have broad therapeutic potential in a wide array of autoinflammatory and chronic inflammatory diseases from gout and nonalcoholic steatohepatitis (NASH) to neurovegetative diseases such as the Parkinson's and the Alzheimer's.[8] The best characterized NLRP3 antagonist MCC950 emerged from a phenotypic screening of a diarysulfonylurea library against IL-1β secretion from human monocytes (Fig. 8B).[211] Initial mechanism of action study proposed GST Omega 1–1 as a functionally relevant target based on affinity labelling and affinity chromatography.[212] Further work led to the discovery that these compounds function through NLRP3 inhibition.[213] One of these compounds was renamed from CRID3 or CP456,773 to MCC950. MCC950 engages NLRP3 and stabilizes an inactive conformation in cells as demonstrated by various techniques including drug affinity responsive target stabilization, photoaffinity labelling, and bioluminescence resonance energy transfer.[214–216] Binding site for MCC950 is located in the NACHT domain. Indeed, crystal structure analysis shows that the binding pocket is formed by the four subdomains of the NACHT domain and that MCC950 acts as an intramolecular glue to lock the protein in an inactive conformation.[217] The characterization of the antagonist binding interactions will facilitate the interpretation of structure–activity relationship and structure-based design.[218] Alternatively, the natural product oridonin covalently binds to Cys279 in the NLRP3 NACHT domain to block the interaction between NLRP3 and NEK7.[219] Mutation of Cys279 to alanine confers resistance to oridonin, which supports this mechanism of action. Similarly, tranilast, which is approved for the treatment of allergy and asthma in Japan, blocks NLRP3 inflammasome formation without affecting the ATPase activity, although exact mechanism of action remains unclear.[220] Unlike oridonin and tranilast, most electrophilic NLRP3 antagonists target the ATPase activity.[221]

In contrast, small molecules that directly bind and activate NLRP3 are currently lacking. Some examples include the TLR7 and TLR8 ligand imiquimod and the related compound CL097, which activate the NLRP3 inflammasome in myeloid cells (Fig. 5B).[222] Surprisingly, CL097 activates NLRP3 independently of potassium efflux unlike most NLRP3 agonists.[223] Initially proposed mechanism of action is that CL097 inhibits mitochondrial complex I to trigger reactive oxygen species (ROS) production. A recent study confirmed the requirement of the complex I inhibition but found no evidence that mitochondrial ROS are necessary for CL097-induced NLRP3 activation.[224] It is plausible that CL097 targets complex I and other unknown molecules to activate the NLRP3 inflammasome. Further mechanism of action study would advance the understanding of NLRP3 regulation. The related imidazoquinoline derivative BMS-986299 entered a phase-I clinical in patients with advanced solid tumour (Fig. 8C).[225] This trial was terminated early due to the COVID-19 pandemic but demonstrated modest clinical activity in combination with immune checkpoint inhibitors and carried a manageable toxicity profile.

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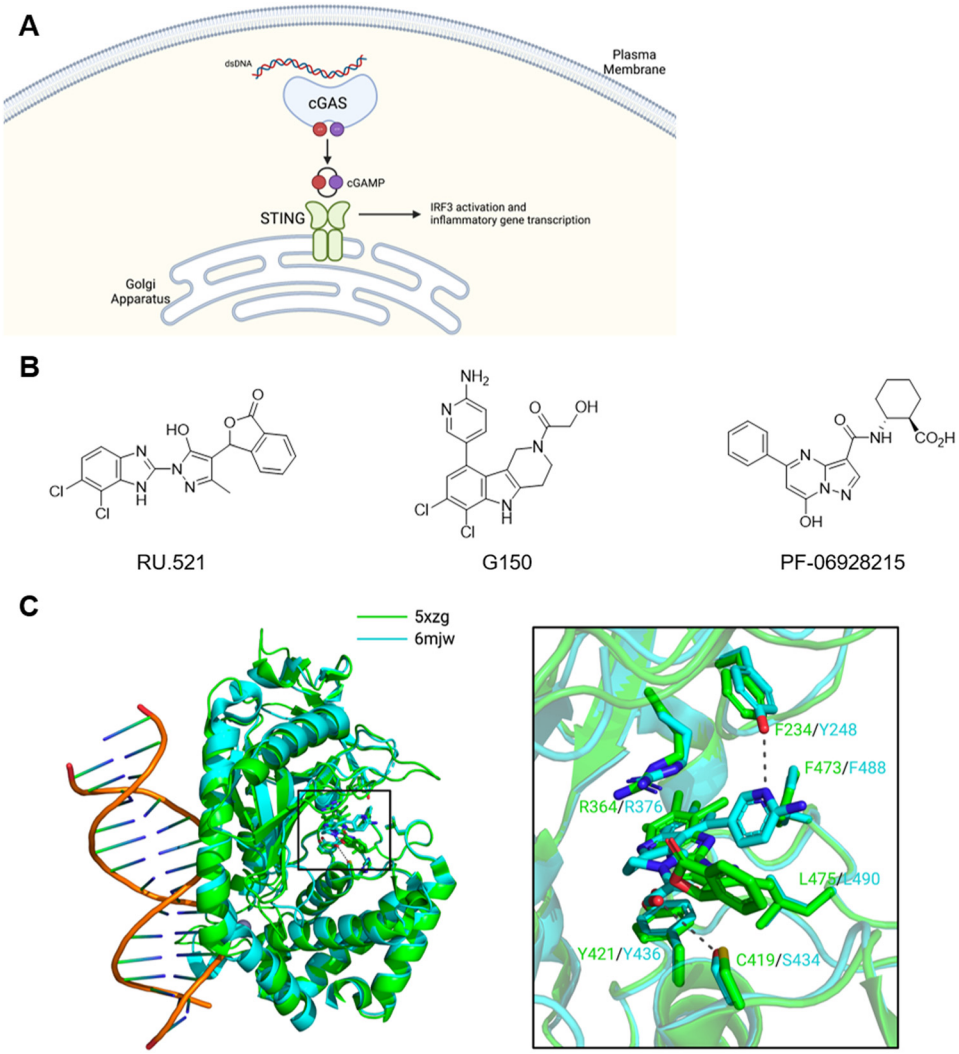

Fig. 9 cGAS–STING pathway. (A) Sub-cellular localization and PAMPs/DAMPs of cGAS and STING. (B) Representative cGAS inhibitors. (C) Co-crystal structures of RU.521–cGAS and G150–cGAS complexes.

NLRP6. NLRP6 plays a role in host defense in the intestines.[226] In intestinal epithelial cells, NLRP6 has been reported to respond to various microbiota-associated metabolites and form an inflammasome for the secretion of IL-18 and anti-microbial peptides.[227] For example, bile acid–derived taurine activates NLRP6 while histamine and spermine inhibit NLRP6, thereby serving as feedback mechanisms to shape microbiome. Meanwhile, other studies using littermate controls found no role for NLRP6 in regulating gut microbiome.[228,229] Another study proposed that lipoteichoic acid derived from the cell wall of Gram-positive bacteria is a ligand for NLRP6 in bone marrow–derived macrophages (Fig. 8C).[230] Lipoteichoic acid or infection with *Listeria monocytogenes* induce NLRP6-dependent activation of caspase-11 and caspase-1. Biochemical evidence suggests lipoteichoic acid directly binds the NLRP6 LRR domain. Alternatively, NLRP6 regulates intestinal viral immunity by sensing viral RNA.[231] A recent study proposed that NLRP6 forms a liquid condensate with its ligands including lipoteichoic acid and viral RNA instead of an ordered assembly that is typical for inflammasomes.[232] Understanding the chemical properties of these phase-separating ligands may inspire design of small molecule NLRP6 ligands.[233]

cGAS-STING pathway

The STING pathway responds to cytoplasmic double-stranded DNA that originates from pathogen and self-DNA damage (Fig. 9A).[234,235] Cytoplasmic DNA activate the enzyme cyclic GMP–AMP synthase (cGAS) to produce the mixed phosphodiester-linked 2′3′-cyclic-GMP–AMP (cGAMP).[236,237] Subsequently, cGAMP binds the ligand binding domain (LBD) of STING and triggers STING activation.[238] Bacterial cyclic dinucleotides can also directly activate STING. Upon ligand engagement, STING undergoes conformational change and polymerization[239,240] to recruit and activate the downstream effectors TBK1 and IRF3, leading to the production of type-I interferons. The components of this pathway are expressed in a wide variety of cell types and implicated in various physiological processes. STING activation has potential roles in vaccine adjuvants and, more recently, cancer immunotherapy.[241,242] On the other hand, aberrant

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regulation of the STING pathway is associated with autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and STING-associated vasculopathy with onset in infancy.[5] As such, there has been strong interest in the development of both STING agonists and antagonists.

Studies have explored various sites of cGAS as potential target for inhibitory ligand development (Fig. 9B). For example, RU.521 emerged from a biochemical high-throughput screening.[243] Crystal structure analysis revealed RU.521 binds the active site and enabled structure-guided optimization. While RU.521 is a potent inhibitor of mouse cGAS both biochemically and in mouse macrophages, it is a poor inhibitor of human cGAS perhaps due to low conservation of active site sequence between the two species. Thus, the same group adapted the biochemical assay for human cGAS and developed G150 (Fig. 9C).[244] Interestingly, substitutions on the 4-position of indole differentially impacted the inhibition of human and mouse cGAS. Similarly, PF-06928215 targets the active site but lacks cellular activity.[245] The interaction with DNA is an alternative target for inhibition. The DNA binding site contains several key lysine residues that mediate the interaction. Mutation of these residues to glutamine to mimic acetylation or acetyl lysine *via* amber suppression block cGAS activation and the DNA binding.[246]

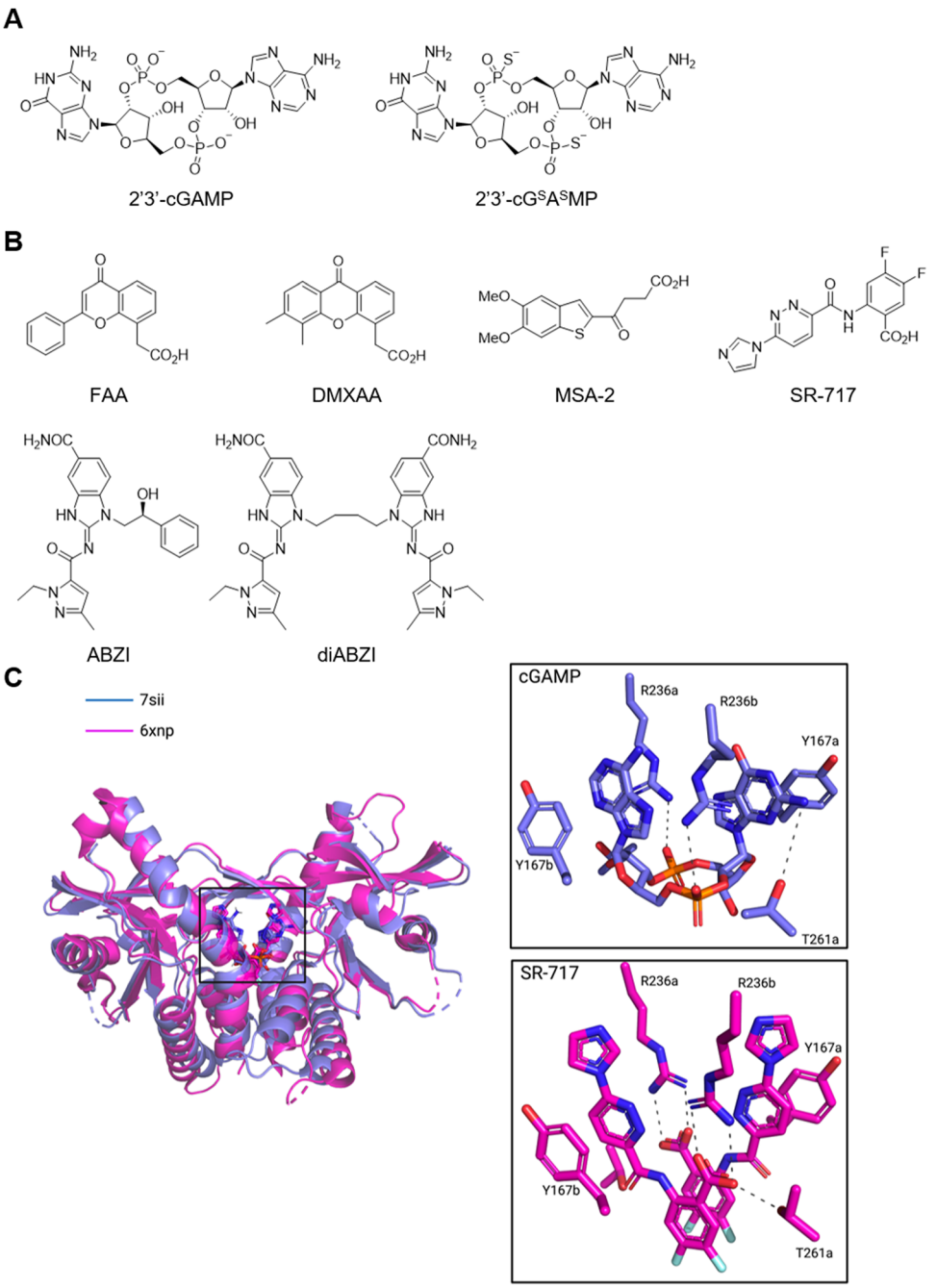

Fig. 10 STING ligands. (A) Representative cyclic dinucleotide analogues. (B) Representative synthetic agonist ligands of STING. (C) Co-crystal structures of cGAMP–STING and SR-717–STING complexes.

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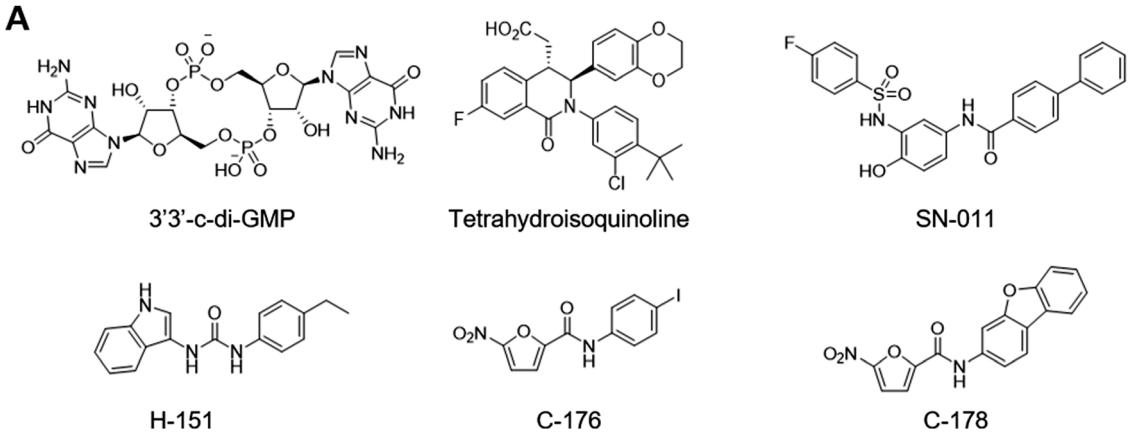

Fig. 11 STING inhibitors.

This study also demonstrated that aspirin can acetylate these residues and inhibit cGAS. Several DNA-intercalating compounds such as hydroxychloroquine reportedly inhibit cGAS by blocking the DNA binding.[247] The poly-pharmacology of aspirin, hydroxychloroquine, and others[248,249] makes it challenging to rationally develop these compounds to achieve cGAS-selective inhibition.

Derivatisation of cGAMP yielded a number of nucleotide-based STING agonists to eliminate some of its liabilities such as poor membrane permeability and susceptibility to enzymatic hydrolysis.[250] For example, a bisphosphothioate analogue, 2′3′-cG^{S}A^{S}MP, is among the earliest cGMAP analogues (Fig. 10A).[251] It is resistant to hydrolysis by the cGAMP hydrolase ENPP1 while retaining a similar affinity for human STING as cGAMP. The related compound cA^{S}A^{S}MP (also known as ADU-S100 and MIW815) entered dose-escalation phase I clinical trials as a monotherapy[252] or a combination therapy with an inhibitory anti-PD-1 mAb[253] in patients with advanced/metastatic solid tumour or lymphoma. These treatments were well tolerated but resulted in minimal anti-tumour response. To overcome the poor permeability and instability of nucleotide analogues, several small molecule ligands have been developed (Fig. 10B). For example, the flavonoid compounds such as flavone acetic acid (FAA) and 5,6-dimethylxanthenone-4-acetic acid (DMXAA) possess remarkable anti-tumor activity in mice models.[250] DMXAA failed phase III clinical trials due to lack of efficacy. Subsequent studies revealed that these compounds bind mouse STING but not human STING.[254] Interestingly, two molecules of DMXAA bind one molecule of STING in the closed conformation.[255] On the other hand, amidobenzimidazole (ABZI)-based compounds are the first synthetic small molecule STING agonists that are human active.[256] Crystal structure analysis inspired the design of linked compounds with 1000-fold improvement in potency. Similarly, MSA-2[257] and SR-717 (Fig. 10C)[258] bind STING as non-covalent dimers. These synthetic ligands demonstrated remarkable anti-tumor effects in mouse models and are suitable for systemic administration—a key advantage over cGAMP analogues.

The development of STING antagonists remains challenging (Fig. 11). This is because antagonists targeting the ligand binding domain would have to stabilize the inactive open conformation and prevent it from binding cGAMP and adopting the active closed conformation. For example, cyclic-di-GMP binds STING in the open conformation and acts as a partial antagonist.[239] The open conformer stacks poorly with the closed conformers and interferes with the polymerization and activation. Similarly, a tetrahydroisoquinoline compound binds the open conformation. This compound was designed and crystallographically characterized to capture the 2 : 1 binding stoichiometry observed for DMXAA[255] and required two-dimensional optimization of protein–ligand and ligand–ligand interactions.[259] Additionally, structure-based virtual screening based on the open conformer yielded SN-011.[260] The authors confirmed the engagement of LBD with a biotinylated analog. On the other hand, ABZI-based agonists also bind the open conformation but activate STING.[256] These observations indicate that the relationship between conformation and activation is more nuanced and merits further research. Alternatively, the S-palmitoylation of transmembrane cysteine residues is necessary for STING activation. The nitrofuran compounds C-176 and C-178 as well as the indolylurea compound H-151 reportedly form covalent adducts with these cysteine residues and inhibit STING activation.[261] However, nitrofuran is highly reactive in cellular environment and C-176 and C-178 lack specificity. In addition, the proposed adduct for C-178 appears to be unstable and unlikely. Furthermore, reaction mechanism for H-151 remains unclear. Similarly, nitro fatty acids inhibit STING but lack specificity.[262] Nevertheless, these studies proposed an interesting idea of targeting key post-translational modifications with covalent ligands.

Conclusions

Early efforts to develop PRR-targeting therapeutics predated the discovery of their PRR targets. Isolation and characterization of immunostimulatory microbial metabolites led to the synthesis of numerous analogues and derivatives including several compounds that continue to be valuable therapeutics today. Genotype analysis, knockout experiments, and co-transfection assay later revealed that PRRs recognize these microbial metabolites. However, several PRRs still lack ligands and their functions remain unclear. Recent studies have begun to dissect mechanisms of

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host–microbiota interactions and reveal roles of microbiota-derived metabolites.[263] Further microbiome analysis may lead to discovery of novel metabolite–receptor pairs involving PRRs. In this regard, chemical proteomics combined with metabolite-based chemical reporters represents a powerful approach.[264] Beyond metabolites and their synthetic analogues, PRR ligand development has relied on functional screening of chemical libraries and empirical optimization of screening hits. Non-traditional chemical libraries including dimeric compound collections may lead to interesting discovery.[30] In addition, recent studies revealed critical roles of various post-translational modifications in immune regulation[265] and ligandability of cysteine residues that could be exploited for immune modulation.[266] Covalent ligand may be advantageous to target less druggable sites on PRRs especially for inhibitor campaigns. Recent advance in structural characterization of PRRs has revealed molecular interactions between PRRs and their ligands and illuminated mechanisms of PRR regulation at the atomic level. These studies will continue to inspire structure-based drug design efforts. Finally, target engagement and off-target profile of PRR modulators often remains unestablished. Target engagement and off-target profiling study is not only an important aspect of hit-to-lead effort[267] but may also lead to surprising discovery and illuminate new aspects of PRR regulation.[131] Chemical proteomics and thermal proteome profiling will facilitate this effort. In summary, discovery and characterization of small molecule ligands for PRRs offers new opportunities for therapeutic development.

Conflicts of interest

The authors declare the following competing financial interest(s): T. T. and H. C. H. have filed a patent application for the commercial use of *N*-arylpyrazole NOD2 agonists for immunotherapy.

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Notes and references

1 Y. Kumagai and S. Akira, *J. Allergy Clin. Immunol.*, 2010, **125**, 985–992.
2 S. W. Brubaker, K. S. Bonham, I. Zanoni and J. C. Kagan, *Annu. Rev. Immunol.*, 2015, **33**, 257–290.
3 L. A. J. O'Neill, D. Golenbock and A. G. Bowie, *Nat. Rev. Immunol.*, 2013, **13**, 453–460.
4 J. M. Platnich and D. A. Muruve, *Arch. Biochem. Biophys.*, 2019, **670**, 4–14.
5 A. Decout, J. D. Katz, S. Venkatraman and A. Ablasser, *Nat. Rev. Immunol.*, 2021, **21**, 548–569.
6 L. Beaugerie, P. Seksik, I. Nion-Larmurier, J. P. Gendre and J. Cosnes, *Gastroenterology*, 2006, **130**, 650–656.
7 P. Sfriso, F. Caso, S. Tognon, P. Galozzi, A. Gava and L. Punzi, *Autoimmun. Rev.*, 2012, **12**, 44–51.
8 M. S. J. Mangan, E. J. Olhava, W. R. Roush, H. M. Seidel, G. D. Glick and E. Latz, *Nat. Rev. Drug Discovery*, 2018, **17**, 588–606.
9 J. Freund, J. Casals and E. P. Hosmer, *Exp. Biol. Med.*, 1937, **37**, 509–513.
10 M. E. Griffin, J. Espinosa, J. L. Becker, J. D. Luo, T. S. Carroll, J. K. Jha, G. R. Fanger and H. C. Hang, *Science*, 2021, **373**, 1040–1046.
11 K. J. Rangan, V. A. Pedicord, Y. C. Wang, B. Kim, Y. Lu, S. Shaham, D. Mucida and H. C. Hang, *Science*, 2016, **353**, 1434–1437.
12 B. Pulendran, P. S. Arunachalam and D. T. O'Hagan, *Nat. Rev. Drug Discovery*, 2021, **20**, 454–475.
13 X. Cao, A. F. Cordova and L. Li, *Chem. Rev.*, 2022, **122**, 3414–3458.
14 D. H. O. ' Donovan, Y. Mao and D. A. Mele, *Curr. Med. Chem.*, 2019, **27**, 5654–5674.
15 O. Takeuchi, S. Sato, T. Horiuchi, K. Hoshino, K. Takeda, Z. Dong, R. L. Modlin and S. Akira, *J. Immunol.*, 2002, **169**, 10–14.
16 O. Takeuchi, T. Kawai, P. F. Mühlradt, M. Morr, J. D. Radolf, A. Zychlinsky, K. Takeda and S. Akira, *Int. Immunol.*, 2001, **13**, 933–940.
17 P. F. Mühlradt, M. Kieß, H. Meyer, R. Süßmuth and G. Jung, *J. Exp. Med.*, 1997, **185**, 1951–1958.
18 W. G. Bessler, M. Cox, A. Lex, B. Suhr, K. H. Wiesmüller and G. Jung, *J. Immunol.*, 1985, **135**, 1900–1905.
19 V. Braun and K. Rehn, *Eur. J. Biochem.*, 1969, **10**, 426–438.
20 U. Zähringer, B. Lindner, S. Inamura, H. Heine and C. Alexander, *Immunobiology*, 2008, **213**, 205–224.
21 M. Bae, C. D. Cassilly, X. Liu, S. M. Park, B. K. Tusi, X. Chen, J. Kwon, P. Filipčík, A. S. Bolze, Z. Liu, H. Vlamakis, D. B. Graham, S. J. Buhrlage, R. J. Xavier and J. Clardy, *Nature*, 2022, **608**, 168–173.
22 M. S. Jin, S. E. Kim, J. Y. Heo, M. E. Lee, H. M. Kim, S. G. Paik, H. Lee and J. O. Lee, *Cell*, 2007, **130**, 1071–1082.
23 J. Y. Kang, X. Nan, M. S. Jin, S. J. Youn, Y. H. Ryu, S. Mah, S. H. Han, H. Lee, S. G. Paik and J. O. Lee, *Immunity*, 2009, **31**, 873–884.
24 B. L. Lu, G. M. Williams and M. A. Brimble, *Org. Biomol. Chem.*, 2020, **18**, 5073–5094.
25 A. Kaur, D. Kaushik, S. Piplani, S. K. Mehta, N. Petrovsky and D. B. Salunke, *J. Med. Chem.*, 2021, **64**, 233–278.
26 Y. Guan, K. Omueti-Ayoade, S. K. Mutha, P. J. Hergenrother and R. I. Tapping, *J. Biol. Chem.*, 2010, **285**, 23755–23762.
27 K. Cheng, M. Gao, J. I. Godfroy, P. N. Brown, N. Kastelowitz and H. Yin, *Sci. Adv.*, 2015, **1**, e1400139.
28 X. Cen, G. Zhu, J. Yang, J. Yang, J. Guo, J. Jin, K. S. Nandakumar, W. Yang, H. Yin, S. Liu and K. Cheng, *Adv. Sci.*, 2019, **6**, 1802042.
29 Z. Hu, J. Banothu, M. Beesu, C. J. Gustafson, M. J. H. Brush, K. L. Trautman, A. C. D. Salyer, B. Pathakumari and S. A. David, *ACS Med. Chem. Lett.*, 2019, **10**, 132–136.

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30 M. Morin, Y. Wang, B. T. Jones, Y. Mifune, L. Su, H. Shi, E. M. Y. Moresco, H. Zhang, B. Beutler and D. L. Boger, *J. Am. Chem. Soc.*, 2018, **140**, 14440–14454.
31 L. Su, Y. Wang, J. Wang, Y. Mifune, M. D. Morin, B. T. Jones, E. M. Y. Moresco, D. L. Boger, B. Beutler and H. Zhang, *J. Med. Chem.*, 2019, **62**, 2938–2949.
32 Y. Wang, L. Su, M. D. Morin, B. T. Jones, Y. Mifune, H. Shi, K. Wen Wang, X. Zhan, A. Liu, J. Wang, X. Li, M. Tang, S. Ludwig, S. Hildebrand, K. Zhou, D. J. Siegwart, E. M. Y. Moresco, H. Zhang, D. L. Boger and B. Beutler, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E8698–E8706.
33 Z. Chen, X. Cen, J. Yang, X. Tang, K. Cui and K. Cheng, *Chem. Commun.*, 2018, **54**, 11411–11414.
34 Z. Chen, L. Zhang, J. Yang, L. Zheng, F. Hu, S. Duan, K. S. Nandakumar, S. Liu, H. Yin and K. Cheng, *J. Med. Chem.*, 2021, **64**, 7371–7389.
35 K. Cheng, X. Wang, S. Zhang and H. Yin, *Angew. Chem., Int. Ed.*, 2012, **51**, 12246–12249.
36 J. Yang, Y. Pan, X. Zeng, S. Liu, Z. Chen and K. Cheng, *Acta Pharm. Sin. B*, 2023, **13**, 3782–3801.
37 M. S. Murgueitio, P. Henneke, H. Glossmann, S. Santos-Sierra and G. Wolber, *ChemMedChem*, 2014, **9**, 813–822.
38 Z. Zhong, L. J. Liu, Z. Q. Dong, L. Lu, M. Wang, C. H. Leung, D. L. Ma and Y. Wang, *Chem. Commun.*, 2015, **51**, 11178–11181.
39 P. Mistry, M. H. W. Laird, R. S. Schwarz, S. Greene, T. Dyson, G. A. Snyder, T. S. Xiao, J. Chauhan, S. Fletcher, V. Y. Toshchakov, A. D. MacKerell and S. N. Vogel, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 5455–5460.
40 S. Cai, G. Zhu, X. Cen, J. Bi, J. Zhang, X. Tang, K. Chen and K. Cheng, *Bioorganic Med. Chem.*, 2018, **26**, 2041–2050.
41 M. Bermudez, M. Grabowski, M. S. Murgueitio, M. Tiemann, P. Varga, T. Rudolf, G. Wolber, G. Weindl and J. Rademann, *ChemMedChem*, 2020, **15**, 1364–1371.
42 J. Baell and M. A. Walters, *Nature*, 2014, **513**, 481–483.
43 C. Aldrich, C. Bertozzi, G. I. Georg, L. Kiessling, C. Lindsley, D. Liotta, K. M. Merz, A. Schepartz and S. Wang, *ACS Cent. Sci.*, 2017, **3**, 143–147.
44 R. Medzhitov, P. Preston-Hurlburt and C. A. Janeway, *Nature*, 1997, **388**, 394–397.
45 S. T. Qureshi, L. Larivière, G. Leveque, S. Clermont, K. J. Moore, P. Gros and D. Malo, *J. Exp. Med.*, 1999, **189**, 615–625.
46 A. Poltorak, X. He, I. Smirnova, M. Y. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton and B. Beutler, *Science*, 1998, **282**, 2085–2088.
47 K. Hoshino, O. Takeuchi, T. Kawai, H. Sanjo, T. Ogawa, Y. Takeda, K. Takeda and S. Akira, *J. Immunol.*, 1999, **162**, 3749–3752.
48 R. Shimazu, S. Akashi, H. Ogata, Y. Nagai, K. Fukudome, K. Miyake and M. Kimoto, *J. Exp. Med.*, 1999, **189**, 1777–1782.
49 A. B. Schromm, E. Lien, P. Henneke, J. C. Chow, A. Yoshimura, H. Heine, E. Latz, B. G. Monks, D. A. Schwartz, K. Miyake and D. T. Golenbock, *J. Exp. Med.*, 2001, **194**, 79–88.
50 B. S. Park, D. H. Song, H. M. Kim, B. S. Choi, H. Lee and J. O. Lee, *Nature*, 2009, **458**, 1191–1195.
51 R. R. Ingalls, B. G. Monks, R. Savedra, W. J. Christ, R. L. Delude, A. E. Medvedev, T. Espevik and D. T. Golenbock, *J. Immunol.*, 1998, **161**, 5413–5420.
52 H. M. Kim, B. S. Park, J. I. Kim, S. E. Kim, J. Lee, S. C. Oh, P. Enkhbayar, N. Matsushima, H. Lee, O. J. Yoo and J. O. Lee, *Cell*, 2007, **130**, 906–917.
53 U. Ohto, K. Fukase, K. Miyake and T. Shimizu, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 7421–7426.
54 J. Gao and Z. Guo, *Med. Res. Rev.*, 2018, **38**, 556–601.
55 C. R. Casella and T. C. Mitchell, *Cell. Mol. Life Sci.*, 2008, **65**, 3231–3240.
56 S. T. Ishizaka and L. D. Hawkins, *Expert Rev. Vaccines*, 2007, **6**, 773–784.
57 W. J. Christ, O. Asano, A. L. C. Robidoux, M. Perez, Y. Wang, G. R. Dubuc, W. E. Gavin, L. D. Hawkins, P. D. McGuinness, M. A. Mullarkey, M. D. Lewis, Y. Kishi, T. Kawata, J. R. Bristol, J. R. Rose, D. P. Rossignol, S. Kobayashi, L. Hishinuma, A. Kimura, N. Asakawa, K. Katayama and I. Yamatsu, *Science*, 1995, **268**, 80–83.
58 M. T. Henke, D. J. Kenny, C. D. Cassilly, H. Vlamakis, R. J. Xavier and J. Clardy, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 12672–12677.
59 A. Nour, T. Hayashi, M. Chan, S. Yao, R. I. Tawatao, B. Crain, I. F. Tsigelny, V. L. Kouznetsova, A. Ahmadiiveli, K. Messer, M. Pu, M. Corr, D. A. Carson and H. B. Cottam, *Bioorganic Med. Chem. Lett.*, 2014, **24**, 4931–4938.
60 M. Chan, T. Hayashi, R. D. Mathewson, A. Nour, Y. Hayashi, S. Yao, R. I. Tawatao, B. Crain, I. F. Tsigelny, V. L. Kouznetsova, K. Messer, M. Pu, M. Corr, D. A. Carson and H. B. Cottam, *J. Med. Chem.*, 2013, **56**, 4206–4223.
61 M. Chan, Y. Kakitsubata, T. Hayashi, A. Ahmadi, S. Yao, N. M. Shukla, S. Y. Oyama, A. Baba, B. Nguyen, M. Corr, Y. Suda, D. A. Carson, H. B. Cottam and M. Wakao, *J. Med. Chem.*, 2017, **60**, 9142–9161.
62 M. D. Morin, Y. Wang, B. T. Jones, L. Su, M. M. R. P. Surakattula, M. Berger, H. Huang, E. K. Beutler, H. Zhang, B. Beutler and D. L. Boger, *J. Med. Chem.*, 2016, **59**, 4812–4830.
63 Y. Wang, L. Su, M. D. Morin, B. T. Jones, L. R. Whitby, M. M. R. P. Surakattula, H. Huang, H. Shi, J. H. Choi, K. W. Wang, E. M. Y. Moresco, M. Berger, X. Zhan, H. Zhang, D. L. Boger and B. Beutler, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, E884–E893.
64 J. E. Neve, H. P. Wijesekera, S. Duffy, I. D. Jenkins, J. A. Ripper, S. J. Teague, M. Campitelli, A. Garavelas, G. Nikolakopoulos, P. V. Le, P. De, A. Leone, N. B. Pham, P. Shelton, N. Fraser, A. R. Carroll, V. M. Avery, C. McCrae, N. Williams and R. J. Quinn, *J. Med. Chem.*, 2014, **57**, 1252–1275.
65 J. Honegr, D. Malinak, R. Dolezal, O. Soukup, M. Benkova, L. Hroch, O. Benek, J. Janockova, K. Kuca and R. Prymula, *Eur. J. Med. Chem.*, 2018, **146**, 38–46.

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66 Y. Zhang, X. Liang, X. Bao, W. Xiao and G. Chen, *Eur. J. Med. Chem.*, 2022, **235**, 114291.
67 Z. Liu, L. Chen, P. Yu, Y. Zhang, B. Fang, C. Wu, W. Luo, X. Chen, C. Li and G. Liang, *J. Med. Chem.*, 2019, **62**, 5453–5469.
68 M. Ii, N. Matsunaga, K. Hazeki, K. Nakamura, K. Takashima, T. Seya, O. Hazeki, T. Kitazaki and Y. Iizawa, *Mol. Pharmacol.*, 2006, **69**, 1288–1295.
69 T. Sha, M. Sunamoto, T. Kitazaki, J. Sato, M. Ii and Y. Iizawa, *Eur. J. Pharmacol.*, 2007, **571**, 231–239.
70 K. Takashima, N. Matsunaga, M. Yoshimatsu, K. Hazeki, T. Kaisho, M. Uekata, O. Hazeki, S. Akira, Y. Iizawa and M. Ii, *Br. J. Pharmacol.*, 2009, **157**, 1250–1262.
71 N. Matsunaga, N. Tsuchimori, T. Matsumoto and M. Ii, *Mol. Pharmacol.*, 2011, **79**, 34–41.
72 A. T. Gewirtz, T. A. Navas, S. Lyons, P. J. Godowski and J. L. Madara, *J. Immunol.*, 2001, **167**, 1882–1885.
73 F. Hayashi, K. D. Smith, A. Ozinsky, T. R. Hawn, E. C. Yi, D. R. Goodlett, J. K. Eng, S. Akira, D. M. Underhill and A. Aderem, *Nature*, 2001, **410**, 1099–1103.
74 S. Il Yoon, O. Kurnasov, V. Natarajan, M. Hong, A. V. Gudkov, A. L. Osterman and I. A. Wilson, *Science*, 2012, **335**, 859–864.
75 L. G. Burdelya, V. I. Krivokrysenko, T. C. Tallant, E. Strom, A. S. Gleiberman, D. Gupta, O. V. Kurnasov, F. L. Fort, A. L. Osterman, J. A. Didonato, E. Feinstein and A. V. Gudkov, *Science*, 2008, **320**, 226–230.
76 C. M. Brackett, B. Kojouharov, J. Veith, K. F. Greene, L. G. Burdelya, S. O. Gollnick, S. I. Abrams and A. V. Gudkov, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, E874–E883.
77 L. Yan, J. Liang, C. Yao, P. Wu, X. Zeng, K. Cheng and H. Yin, *ChemMedChem*, 2016, **11**, 822–826.
78 L. Alexopoulou, A. Holt, R. Medzhitov and R. Flavell, *Nature*, 2001, **413**, 732–738.
79 K. A. Fitzgerald, D. C. Rowe, B. J. Barnes, D. R. Caffrey, A. Visintin, E. Latz, B. Monks, P. M. Pitha and D. T. Golenbock, *J. Exp. Med.*, 2003, **198**, 1043–1055.
80 L. Liu, I. Botos, Y. Wang, J. N. Leonard, J. Shiloach, D. M. Segal and D. R. Davies, *Science*, 2008, **320**, 379–381.
81 K. Cheng, X. Wang and H. Yin, *J. Am. Chem. Soc.*, 2011, **133**, 3764–3767.
82 X. Cen, B. Wang, Y. Liang, Y. Chen, Y. Xiao, S. Du, K. S. Nandakumar, H. Yin, S. Liu and K. Cheng, *Acta Pharm. Sin. B*, 2022, **12**, 3667–3681.
83 L. Zhang, V. Dewan and H. Yin, *J. Med. Chem.*, 2017, **60**, 5029–5044.
84 A. Sarkar, A. C. G. Kankanamalage, Q. Zhang, H. Cheng, P. Sivaprakasam, J. Naglich, C. Xie, S. Gangwar and D. L. Boger, *Med. Chem. Res.*, 2021, **30**, 1377–1385.
85 S. S. Diebold, T. Kaisho, H. Hemmi, S. Akira and C. Reise Sousa, *Science*, 2004, **303**, 1529–1531.
86 F. Heil, H. Hemmi, H. Hochrein, F. Ampenberger, C. Kirschning, S. Akira, G. Lipford, H. Wagner and S. Bauer, *Science*, 2004, **303**, 1526–1529.
87 H. Hemmi, T. Kaisho, O. Takeuchi, S. Sato, H. Sanjo, K. Hoshino, T. Horiuchi, H. Tomizawa, K. Takeda and S. Akira, *Nat. Immunol.*, 2002, **3**, 196–200.
88 M. Jurk, F. Heil, J. Vollmer, C. Schetter, A. M. Krieg, H. Wagner, G. Lipford and S. Bauer, *Nat. Immunol.*, 2002, **3**, 499.
89 J. Lee, T. H. Chuang, V. Redecke, L. She, P. M. Pitha, D. A. Carson, E. Raz and H. B. Cottam, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 6646–6651.
90 F. Heil, P. Ahmad-Nejad, H. Hemmi, H. Hochrein, F. Ampenberger, T. Gellert, H. Dietrich, G. Lipford, K. Takeda, S. Akira, H. Wagner and S. Bauer, *Eur. J. Immunol.*, 2003, **33**, 2987–2997.
91 A. Talukdar, D. Ganguly, S. Roy, N. Das and D. Sarkar, *J. Med. Chem.*, 2021, **64**, 8010–8041.
92 D. Kaushik, A. Kaur, N. Petrovsky and D. B. Salunke, *RSC Med. Chem.*, 2021, **12**, 1065–1120.
93 H. Tanji, U. Ohto, T. Shibata, K. Miyake and T. Shimizu, *Science*, 2013, **339**, 1426–1429.
94 H. Tanji, U. Ohto, T. Shibata, M. Taoka, Y. Yamauchi, T. Isobe, K. Miyake and T. Shimizu, *Nat. Struct. Mol. Biol.*, 2015, **22**, 109–116.
95 Z. Zhang, U. Ohto, T. Shibata, E. Krayukhina, M. Taoka, Y. Yamauchi, H. Tanji, T. Isobe, S. Uchiyama, K. Miyake and T. Shimizu, *Immunity*, 2016, **45**, 737–748.
96 S. Zhang, Z. Hu, H. Tanji, S. Jiang, N. Das, J. Li, K. Sakaniwa, J. Jin, Y. Bian, U. Ohto, T. Shimizu and H. Yin, *Nat. Chem. Biol.*, 2018, **14**, 58–64.
97 S. Tojo, Z. Zhang, H. Matsui, M. Tahara, M. Ikeguchi, M. Kochi, M. Kamada, H. Shigematsu, A. Tsutsumi, N. Adachi, T. Shibata, M. Yamamoto, M. Kikkawa, T. Senda, Y. Isobe, U. Ohto and T. Shimizu, *Nat. Commun.*, 2020, **11**, 4–6.
98 H. Hemmi, O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda and S. Akira, *Nature*, 2000, **408**, 740–745.
99 J. Lund, A. Sato, S. Akira, R. Medzhitov and A. Iwasaki, *J. Exp. Med.*, 2003, **198**, 513–520.
100 M. Rutz, J. Metzger, T. Gellert, P. Luppa, G. B. Lipford, H. Wagner and S. Bauer, *Eur. J. Immunol.*, 2004, **34**, 2541–2550.
101 T. Kawai, S. Sato, K. J. Ishii, C. Coban, H. Hemmi, M. Yamamoto, K. Terai, M. Matsuda, J. I. Inoue, S. Uematsu, O. Takeuchi and S. Akira, *Nat. Immunol.*, 2004, **5**, 1061–1068.
102 J. Scheiermann and D. M. Klinman, *Vaccine*, 2014, **32**, 6377–6389.
103 U. Ohto, T. Shibata, H. Tanji, H. Ishida, E. Krayukhina, S. Uchiyama, K. Miyake and T. Shimizu, *Nature*, 2015, **520**, 702–705.
104 U. Ohto, H. Ishida, T. Shibata, R. Sato, K. Miyake and T. Shimizu, *Immunity*, 2018, **48**, 649–658.
105 A. Kužnik, M. Benčina, U. Švajger, M. Jeras, B. Rozman and R. Jerala, *J. Immunol.*, 2011, **186**, 4794–4804.
106 B. Paul, O. Rahaman, S. Roy, S. Pal, S. Satish, A. Mukherjee, A. R. Ghosh, D. Raychaudhuri, R. Bhattacharya, S. Goon, D. Ganguly and A. Talukdar, *Eur. J. Med. Chem.*, 2018, **159**, 187–205.
107 M. Watanabe, M. Kasai, H. Tomizawa, M. Aoki, K. Eiho, Y. Isobe and S. Asano, *ACS Med. Chem. Lett.*, 2014, **5**, 1235–1239.

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108 B. S. Franklin, S. T. Ishizaka, M. Lamphier, F. Gusovsky, H. Hansen, J. Rose, W. Zheng, M. A. Ataíde, R. B. De Oliveira, D. T. Golenbock and R. T. Gazzinelli, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 3689–3694.

109 M. Lamphier, W. Zheng, E. Latz, M. Spyvee, H. Hansen, J. Rose, M. Genest, H. Yang, C. Shaffer, Y. Zhao, Y. Shen, C. Liu, D. Liu, T. R. Mempel, C. Rowbottom, J. Chow, N. C. Twine, M. Yu, F. Gusovsky and S. T. Ishizaka, *Mol. Pharmacol.*, 2014, **85**, 429–440.

110 S. Roy, A. Mukherjee, B. Paul, O. Rahaman, S. Roy, G. Maithri, B. Ramya, S. Pal, D. Ganguly and A. Talukdar, *Eur. J. Med. Chem.*, 2017, **134**, 334–347.

111 R. Hoque, A. Farooq, A. Malik, B. N. Trawick, D. W. Berberich, J. P. McClurg, K. P. Galen and W. Mehal, *J. Immunol.*, 2013, **190**, 4297–4304.

112 S. R. Christensen, J. Shupe, K. Nickerson, M. Kashgarian, R. A. A. Flavell and M. J. Shlomchik, *Immunity*, 2006, **25**, 417–428.

113 C. P. Mussari, D. S. Dodd, R. K. Sreekantha, L. Pasunoori, H. Wan, S. L. Posy, D. Critton, S. Ruepp, M. Subramanian, A. Watson, P. Davies, G. L. Schieven, L. M. Salter-Cid, R. Srivastava, D. M. Tagore, S. Dudhgaonkar, M. A. Poss, P. H. Carter and A. J. Dyckman, *ACS Med. Chem. Lett.*, 2020, **11**, 1751–1758.

114 N. Das, P. Bandopadhyay, S. Roy, B. P. Sinha, U. G. Dastidar, O. Rahaman, S. Pal, D. Ganguly and A. Talukdar, *J. Med. Chem.*, 2022, **65**, 11607–11632.

115 B. Kundu, D. Raychaudhuri, A. Mukherjee, B. P. Sinha, D. Sarkar, P. Bandopadhyay, S. Pal, N. Das, D. Dey, K. Ramarao, K. Nagireddy, D. Ganguly and A. Talukdar, *J. Med. Chem.*, 2021, **64**, 9279–9301.

116 J. P. Y. Ting, R. C. Lovering, E. S. Alnemri, J. Bertin, J. M. Boss, B. K. Davis, R. A. Flavell, S. E. Girardin, A. Godzik, J. A. Harton, H. M. Hoffman, J. P. Hugot, N. Inohara, A. MacKenzie, L. J. Maltais, G. Nunez, Y. Ogura, L. A. Otten, D. Philpott, J. C. Reed, W. Reith, S. Schreiber, V. Steimle and P. A. Ward, *Immunity*, 2008, **28**, 285–287.

117 P. Broz and V. M. Dixit, *Nat. Rev. Immunol.*, 2016, **16**, 407–420.

118 R. Caruso, N. Warner, N. Inohara and G. Núñez, *Immunity*, 2014, **41**, 898–908.

119 S. K. Kuss-Duerkop and A. M. Keestra-Gounder, *Infect. Immun.*, 2020, **88**, 1–19.

120 S. E. Girardin, I. G. Boneca, L. A. M. Carneiro, A. Antignac, M. Jéhanno, J. Viala, K. Tedin, M. K. Taha, A. Labigne, U. Zähringer, A. J. Coyle, P. S. DiStefano, J. Bertin, P. J. Sansonetti and D. J. Philpott, *Science*, 2003, **300**, 1584–1587.

121 M. Chamaillard, M. Hashimoto, Y. Horie, J. Masumoto, S. Qiu, L. Saab, Y. Ogura, A. Kawasaki, K. Fukase, S. Kusumoto, M. A. Valvano, S. J. Foster, T. W. Mak, G. Nuñez and N. Inohara, *Nat. Immunol.*, 2003, **4**, 702–707.

122 N. Inohara, Y. Ogura, A. Fontalba, O. Gutierrez, F. Pons, J. Crespo, K. Fukase, S. Inamura, S. Kusumoto, M. Hashimoto, S. J. Foster, A. P. Moran, J. L. Fernandez-Luna and G. Nuñez, *J. Biol. Chem.*, 2003, **278**, 5509–5512.

123 S. E. Girardin, I. G. Boneca, J. Viala, M. Chamaillard, A. Labigne, G. Thomas, D. J. Philpott and P. J. Sansonetti, *J. Biol. Chem.*, 2003, **278**, 8869–8872.

124 Y. G. Kim, J. H. Park, T. Reimer, D. P. Baker, T. Kawai, H. Kumar, S. Akira, C. Wobus and G. Núñez, *Cell Host Microbe*, 2011, **9**, 496–507.

125 J. Mo, J. P. Boyle, C. B. Howard, T. P. Monie, B. K. Davis and J. A. Duncan, *J. Biol. Chem.*, 2012, **287**, 23057–23067.

126 C. L. Grimes, L. D. Z. Ariyananda, J. E. Melnyk and E. K. O'Shea, *J. Am. Chem. Soc.*, 2012, **134**, 13535–13537.

127 M. L. Lauro, E. A. D'Ambrosio, B. J. Bahnson and C. L. Grimes, *ACS Infect. Dis.*, 2017, **3**, 264–270.

128 K. M. Lazor, J. Zhou, K. E. DeMeester, E. A. D'Ambrosio and C. L. Grimes, *ChemBioChem*, 2019, **20**, 1369–1375.

129 E. A. D'Ambrosio, K. L. Bersch, M. L. Lauro, C. L. Grimes and C. L. Grimes, *J. Am. Chem. Soc.*, 2020, **142**, 10926–10930.

130 H. Laroui, Y. Yan, Y. Narui, S. A. Ingersoll, S. Ayyadurai, M. A. Charania, F. Zhou, B. Wang, K. Salaita, S. V. Sitaraman and D. Merlin, *J. Biol. Chem.*, 2011, **286**, 31003–31013.

131 Y. C. Wang, N. P. Westcott, M. E. Griffin and H. C. Hang, *ACS Chem. Biol.*, 2019, **14**, 405–414.

132 C. A. Stafford, A.-M. Gassauer, C. C. de Oliveira Mann, M. C. Tanzer, E. Fessler, B. Wefers, D. Nagl, G. Kuut, K. Sulek, C. Vasilopoulou, S. J. Schwojer, A. Wiest, M. K. Pfautsch, W. Wurst, M. Yabal, T. Fröhlich, M. Mann, N. Gisch, L. T. Jae and V. Hornung, *Nature*, 2022, **609**, 590–596.

133 N. Barnich, J. E. Aguirre, H. C. Reinecker, R. Xavier and D. K. Podolsky, *J. Cell Biol.*, 2005, **170**, 21–26.

134 A. K. Schaefer, J. E. Melnyk, M. M. Baksh, K. M. Lazor, M. G. Finn and C. L. Grimes, *ACS Chem. Biol.*, 2017, **12**, 2216–2224.

135 Y. Lu, Y. Zheng, É. Coyaud, C. Zhang, A. Selvabaskaran, Y. Yu, Z. Xu, X. Weng, J. S. Chen, Y. Meng, N. Warner, X. Cheng, Y. Liu, B. Yao, H. Hu, Z. Xia, A. M. Muise, A. Klip, J. H. Brumell, S. E. Girardin, S. Ying, G. D. Fairn, B. Raught, Q. Sun and D. Neculai, *Science*, 2019, **366**, 460–467.

136 C. W. Hespen, X. Zhao and H. C. Hang, *Chem. Commun.*, 2022, **58**, 6598–6601.

137 M. E. Griffin, C. W. Hespen, Y. C. Wang and H. C. Hang, *Clin. Transl. Immunol.*, 2019, **8**, 1–18.

138 K. Hemmi, H. Takeno, S. Okada, O. Nakaguchi, Y. Kitaura and M. Hashimoto, *J. Am. Chem. Soc.*, 1981, **103**, 7026–7028.

139 T. Gotoh, K. Nakahara, T. Nishiura, M. Hashimoto, T. Kino, Y. Kuroda, M. Okuhara, M. Kohsaka, H. Aoki and H. Imanaka, *J. Antibiot.*, 1982, **35**, 1286–1292.

140 T. Gotoh, K. Nakahara, M. Iwami, H. Aoki and H. Imanaka, *J. Antibiot.*, 1982, **35**, 1280–1285.

141 Y. Kawai, K. Nakahara, T. Gotoh, I. Uchida, H. Tanaka and H. Imanaka, *J. Antibiot.*, 1982, **35**, 1293–1299.

142 K. Hemmi, M. Aratani, H. Takeno, S. Okada, Y. Miyazaki, O. Nakaguchi, Y. Kitaura and M. Hashimoto, *J. Antibiot.*, 1982, **35**, 1300–1311.

143 Y. Kitaura, O. Nakaguchi, H. Takeno, S. Okada, S. Yonishi, K. Hemmi, J. Mori, H. Senoh, Y. Mine and M. Hashimoto, *J. Med. Chem.*, 1982, **25**, 335–337.

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144 Y. Kitaura, H. Takeno, M. Aratani, S. Okada, S. Yonishi, K. Hemmi, O. Nakaguchi and M. Hashimoto, *Experientia*, 1982, **38**, 1101–1103.
145 Y. Fujimoto, A. R. Pradipta, N. Inohara and K. Fukase, *Nat. Prod. Rep.*, 2012, **29**, 568–579.
146 M. A. Wolfert, A. Roychowdhury and G. J. Boons, *Infect. Immun.*, 2007, **75**, 706–713.
147 G. Agnihotri, R. Ukani, S. S. Malladi, H. J. Warshakoon, R. Balakrishna, X. Wang and S. A. David, *J. Med. Chem.*, 2011, **54**, 1490–1510.
148 Ž. Jakopin, M. Gobec, J. Kodela, T. Hazdovac, I. Mlinarič-Raščan and M. Sollner Dolenc, *Eur. J. Med. Chem.*, 2013, **69**, 232–243.
149 S. Guzelj and Ž. Jakopin, *Molecules*, 2020, **25**, 5228.
150 F. Ellouz, A. Adam, R. Ciorbaru and E. Lederer, *Biochem. Biophys. Res. Commun.*, 1974, **59**, 1317–1325.
151 A. Adam and E. Lederer, *Med. Res. Rev.*, 1984, **4**, 111–152.
152 S. V. Guryanova and R. M. Khaitov, *Front. Immunol.*, 2021, **12**, 607178.
153 B. Vranešić, J. Tomašić, S. Smerdel, D. Kantoci and F. Benedetti, *Helv. Chim. Acta*, 1993, **76**, 1752–1758.
154 M. Sollner, V. Kotnik Pečar, A. Štalc, S. Simčič, L. Povšič, B. Herzog-Wraber, L. Klampfer, A. Ihan and P. Grosman, *Agents Actions*, 1993, **38**, 273–280.
155 U. Urleb, A. Krbavčič, M. Sollner, D. Kikelj and S. Pečar, *Arch. Pharm.*, 1995, **328**, 113–117.
156 D. Kikelj, S. Pečar, V. Kotnik, A. Štalc, B. Wraber-Herzog, S. Simčič, A. Ihan, L. Klamfer, L. Povšič, R. Grahek, E. Suhadolc, M. Hočevar, H. Hönig and R. Rogi-Kohlenprath, *J. Med. Chem.*, 1998, **41**, 530–539.
157 Ž. Jakopin, E. Corsini, M. Gobec, I. Mlinarič-Raščan and M. S. Dolenc, *Eur. J. Med. Chem.*, 2011, **46**, 3762–3777.
158 Ž. Jakopin, M. Gobec, I. Mlinarič-Raščan and M. Sollner Dolenc, *J. Med. Chem.*, 2012, **55**, 6478–6488.
159 M. Gobec, I. Mlinarič-Raščan, M. S. Dolenc and Ž. Jakopin, *Eur. J. Med. Chem.*, 2016, **116**, 1–12.
160 M. Gobec, T. Tomašič, A. Štimac, R. Frkanec, J. Trontelj, M. Anderluh, I. Mlinarič-Raščan and Ž. Jakopin, *J. Med. Chem.*, 2018, **61**, 2707–2724.
161 S. Guzelj, S. Nabergoj, M. Gobec, S. Pajk, V. Klančič, B. Slütter, R. Frkanec, A. Štimac, P. Šket, J. Plavec, I. Mlinarič-Raščan and Ž. Jakopin, *J. Med. Chem.*, 2021, **64**, 7809–7838.
162 S. Guzelj, Š. Bizjak and Ž. Jakopin, *ACS Med. Chem. Lett.*, 2022, **13**, 1270–1277.
163 M. E. Griffin, T. Tsukidate and H. C. Hang, *ACS Chem. Biol.*, 2023, **18**, 1368–1377.
164 Ž. Jakopin, *J. Med. Chem.*, 2014, **57**, 6897–6918.
165 P. M. Khan, R. G. Correa, D. B. Divlianska, S. Peddibhotla, E. H. Sessions, G. Magnuson, B. Brown, E. Suyama, H. Yuan, A. Mangravita-Novo, M. Vicchiarelli, Y. Su, S. Vasile, L. H. Smith, P. W. Diaz, J. C. Reed and G. P. Roth, *ACS Med. Chem. Lett.*, 2011, **2**, 780–785.
166 D. J. Rickard, C. A. Sehon, V. Kasparcova, L. A. Kallal, X. Zeng, M. N. Montoute, T. Chordia, D. D. Poore, H. Li, Z. Wu, P. M. Eidam, P. A. Haile, J. Yu, J. G. Emery, R. W. Marquis, P. J. Gough and J. Bertin, *PLoS One*, 2013, **8**, e69619.
167 D. J. Rickard, C. A. Sehon, V. Kasparcova, L. A. Kallal, P. A. Haile, X. Zeng, M. N. Montoute, D. D. Poore, H. Li, Z. Wu, P. M. Eidam, J. G. Emery, R. W. Marquis, P. J. Gough and J. Bertin, *PLoS One*, 2014, **9**, e96737.
168 F. Martinon, K. Burns and J. Tschopp, *Mol. Cell*, 2002, **10**, 417–426.
169 J. Lilue, A. G. Doran, I. T. Fiddes, M. Abrudan, J. Armstrong, R. Bennett, W. Chow, J. Collins, S. Collins, A. Czechanski, P. Danecek, M. Diekhans, D. D. Dolle, M. Dunn, R. Durbin, D. Earl, A. Ferguson-Smith, P. Flicek, J. Flint, A. Frankish, B. Fu, M. Gerstein, J. Gilbert, L. Goodstadt, J. Harrow, K. Howe, X. Ibarra-Soria, M. Kolmogorov, C. J. Lelliott, D. W. Logan, J. Loveland, C. E. Mathews, R. Mott, P. Muir, S. Nachtweide, F. C. P. Navarro, D. T. Odom, N. Park, S. Pelan, S. K. Pham, M. Quail, L. Reinholdt, L. Romoth, L. Shirley, C. Sisu, M. Sjoberg-Herrera, M. Stanke, C. Steward, M. Thomas, G. Threadgold, D. Thybert, J. Torrance, K. Wong, J. Wood, B. Yalcin, F. Yang, D. J. Adams, B. Paten and T. M. Keane, *Nat. Genet.*, 2018, **50**, 1574–1583.
170 A. D'Osualdo, C. X. Weichenberger, R. N. Wagner, A. Godzik, J. Wooley and J. C. Reed, *PLoS One*, 2011, **6**, e27396.
171 A. Sandstrom, P. S. Mitchell, L. Goers, E. W. Mu, C. F. Lesser and R. E. Vance, *Science*, 2019, **364**, eaau1330.
172 A. J. Chui, M. C. Okondo, S. D. Rao, K. Gai, A. R. Griswold, D. C. Johnson, D. P. Ball, C. Y. Taabazuing, E. L. Orth, B. A. Vittimberga and D. A. Bachovchin, *Science*, 2019, **364**, 82–85.
173 H. Xu, J. Shi, H. Gao, Y. Liu, Z. Yang, F. Shao and N. Dong, *EMBO J.*, 2019, **38**, 1–11.
174 F. L. Zhong, O. Mamaï, L. Sborgi, L. Boussofara, R. Hopkins, K. Robinson, I. Szeverényi, T. Takeichi, R. Balaji, A. Lau, H. Tye, K. Roy, C. Bonnard, P. J. Ahl, L. A. Jones, P. J. Baker, L. Lacina, A. Otsuka, P. R. Fournie, F. Malecaze, E. B. Lane, M. Akiyama, K. Kabashima, J. E. Connolly, S. L. Masters, V. J. Soler, S. S. Omar, J. A. McGrath, R. Nedelcu, M. Gribaa, M. Denguezli, A. Saad, S. Hiller and B. Reversade, *Cell*, 2016, **167**, 187–202.
175 D. C. Johnson, C. Y. Taabazuing, M. C. Okondo, A. J. Chui, S. D. Rao, F. C. Brown, C. Reed, E. Peguero, E. de Stanchina, A. Kentsis and D. A. Bachovchin, *Nat. Med.*, 2018, **24**, 1151–1156.
176 A. Linder, S. Bauernfried, Y. Cheng, M. Albanese, C. Jung, O. T. Keppler and V. Hornung, *EMBO J.*, 2020, **39**, 1–16.
177 D. C. Johnson, M. C. Okondo, E. L. Orth, S. D. Rao, H.-C. Huang, D. P. Ball and D. A. Bachovchin, *Cell Death Dis.*, 2020, **11**, 628.
178 B. Faustin, L. Lartigue, J. M. Bruey, F. Luciano, E. Sergienko, B. Bailly-Maitre, N. Volkmann, D. Hanein, I. Rouiller and J. C. Reed, *Mol. Cell*, 2007, **25**, 713–724.
179 S. Bauernfried and V. Hornung, *J. Exp. Med.*, 2021, **219**, 1–8.

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180 F. Martinon, L. Agostini, E. Meylan and J. Tschopp, *Curr. Biol.*, 2004, **14**, 1929–1934.
181 S. Bauernfried, M. J. Scherr, A. Pichlmair, K. E. Duderstadt and V. Hornung, *Science*, 2021, **371**, eabd0811.
182 X. Yang, J. Zhou, C. Liu, Y. Qu, W. Wang, M. Z. X. Xiao, F. Zhu, Z. Liu and Q. Liang, *Nat. Immunol.*, 2022, **23**, 916–926.
183 B. V. Tsu, C. Beierschmitt, A. P. Ryan, R. Agarwal, P. S. Mitchell and M. D. Daugherty, *eLife*, 2021, **10**, 1–76.
184 R. Planès, M. Pinilla, K. Santoni, A. Hessel, C. Passemar, K. Lay, P. Paillette, A. L. C. Valadão, K. S. Robinson, P. Bastard, N. Lam, R. Fadrique, I. Rossi, D. Pericat, S. Bagayoko, S. A. Leon-Icaza, Y. Rombouts, E. Perouzel, M. Tiraby, Q. Zhang, P. Cicuta, E. Jouanguy, O. Neyrolles, C. E. Bryant, A. R. Floto, C. Goujon, F. Z. Lei, G. Martin-Blondel, S. Silva, J. L. Casanova, C. Cougoule, B. Reversade, J. Marcoux, E. Ravet and E. Meunier, *Mol. Cell*, 2022, **82**, 2385–2400.
185 G. Fenini, S. Grossi, E. Contassot, T. Biedermann, E. Reichmann, L. E. French and H. D. Beer, *J. Invest. Dermatol.*, 2018, **138**, 2644–2652.
186 D. P. Ball, L. P. Tsamouri, A. E. Wang, H.-C. Huang, C. D. Warren, Q. Wang, I. H. Edmondson, A. R. Griswold, S. D. Rao, D. C. Johnson and D. A. Bachovchin, *Sci. Immunol.*, 2022, **7**, 1–16.
187 S. E. Ewald, J. Chavarria-Smith and J. C. Boothroyd, *Infect. Immun.*, 2014, **82**, 460–468.
188 J. Chavarría-Smith and R. E. Vance, *PLoS Pathog.*, 2013, **9**, e1003452.
189 K. M. Cirelli, G. Gorfu, M. A. Hassan, M. Printz, D. Crown, S. H. Leppla, M. E. Grigg, J. P. J. Saeij and M. Moayeri, *PLoS Pathog.*, 2014, **10**, e1003927.
190 K. S. Robinson, G. A. Toh, P. Rozario, R. Chua, S. Bauernfried, Z. Sun, M. J. Firdaus, S. Bayat, R. Nadkarni, Z. S. Poh, K. C. Tham, C. R. Harapas, C. K. Lim, W. Chu, C. W. S. Tay, K. Y. Tan, T. Zhao, C. Bonnard, R. Sobota, J. E. Connolly, J. Common, S. L. Masters, K. W. Chen, L. Ho, B. Wu, V. Hornung and F. L. Zhong, *Science*, 2022, **377**, 328–335.
191 F. L. Zhong, K. Robinson, D. E. T. Teo, K. Y. Tan, C. Lim, C. R. Harapas, C. H. Yu, W. H. Xie, R. M. Sobota, V. B. Au, R. Hopkins, A. D'Osualdo, J. C. Reed, J. E. Connolly, S. L. Masters and B. Reversade, *J. Biol. Chem.*, 2018, **293**, 18864–18878.
192 M. C. Okondo, S. D. Rao, C. Y. Taabazuing, A. J. Chui, S. E. Poplawski, D. C. Johnson and D. A. Bachovchin, *Cell Chem. Biol.*, 2018, **25**, 262–267.
193 M. C. Okondo, D. C. Johnson, R. Sridharan, E. Bin Go, A. J. Chui, M. S. Wang, S. E. Poplawski, W. Wu, Y. Liu, J. H. Lai, D. G. Sanford, M. O. Arciprete, T. R. Golub, W. W. Bachovchin and D. A. Bachovchin, *Nat. Chem. Biol.*, 2017, **13**, 46–53.
194 H. Zhang, Y. Chen, F. M. Keane and M. D. Gorrell, *Mol. Cancer Res.*, 2013, **11**, 1487–1496.
195 M. Huang, X. Zhang, G. A. Toh, Q. Gong, J. Wang, Z. Han, B. Wu, F. Zhong and J. Chai, *Nature*, 2021, **592**, 773–777.
196 L. R. Hollingsworth, H. Sharif, A. R. Griswold, P. Fontana, J. Mintseris, K. B. Dagbay, J. A. Paulo, S. P. Gygi, D. A. Bachovchin and H. Wu, *Nature*, 2021, **592**, 778–783.
197 H. Sharif, L. R. Hollingsworth, A. R. Griswold, J. C. Hsiao, Q. Wang, D. A. Bachovchin and H. Wu, *Immunity*, 2021, **54**, 1392–1404.
198 A. R. Griswold, D. P. Ball, A. Bhattacharjee, A. J. Chui, S. D. Rao, C. Y. Taabazuing and D. A. Bachovchin, *ACS Chem. Biol.*, 2019, **14**, 2424–2429.
199 S. D. Rao, Q. Chen, Q. Wang, E. L. Orth-He, M. Saoi, A. R. Griswold, A. Bhattacharjee, D. P. Ball, H. C. Huang, A. J. Chui, D. J. Covelli, S. You, J. R. Cross and D. A. Bachovchin, *Nat. Chem. Biol.*, 2022, **18**, 565–574.
200 P. A. Harris, C. Duraiswami, D. T. Fisher, J. Fornwald, S. J. Hoffman, G. Hofmann, M. Jiang, R. Lehr, P. M. McCormick, L. Nickels, B. Schwartz, Z. Wu, G. Zhang, R. W. Marquis, J. Bertin and P. J. Gough, *Bioorganic Med. Chem. Lett.*, 2015, **25**, 2739–2743.
201 K. V. Swanson, M. Deng and J. P. Y. Ting, *Nat. Rev. Immunol.*, 2019, **19**, 477–489.
202 J. L. Schmid-Burgk, D. Chauhan, T. Schmidt, T. S. Ebert, J. Reinhardt, E. Endl and V. Hornung, *J. Biol. Chem.*, 2016, **291**, 103–109.
203 Y. He, M. Y. Zeng, D. Yang, B. Motro and G. Núñez, *Nature*, 2016, **530**, 354–357.
204 H. Shi, Y. Wang, X. Li, X. Zhan, M. Tang, M. Fina, L. Su, D. Pratt, C. Hui Bu, S. Hildebrand, S. Lyon, L. Scott, J. Quan, Q. Sun, J. Russell, S. Arnett, P. Jurek, D. Chen, V. V. Kravchenko, J. C. Mathison, E. M. Y. Moresco, N. L. Monson, R. J. Ulevitch and B. Beutler, *Nat. Immunol.*, 2016, **17**, 250–258.
205 L. Zhang, S. Chen, J. Ruan, J. Wu, A. B. Tong, Q. Yin, Y. Li, L. David, A. Lu, W. L. Wang, C. Marks, Q. Ouyang, X. Zhang, Y. Mao and H. Wu, *Science*, 2015, **350**, 404–409.
206 J. L. Tenthorey, E. M. Kofoed, M. D. Daugherty, H. S. Malik and R. E. Vance, *Mol. Cell*, 2014, **54**, 17–29.
207 H. Sharif, L. Wang, W. L. Wang, V. G. Magupalli, L. Andreeva, Q. Qiao, A. V. Hauenstein, Z. Wu, G. Núñez, Y. Mao and H. Wu, *Nature*, 2019, **570**, 338–343.
208 L. Andreeva, L. David, S. Rawson, C. Shen, T. Pasricha, P. Pelegrin and H. Wu, *Cell*, 2021, **184**, 6299–6312.
209 U. Ohto, Y. Kamitsukasa, H. Ishida, Z. Zhang, K. Murakami, C. Hirama, S. Maekawa and T. Shimizu, *Proc. Natl. Acad. Sci. U. S. A.*, 2022, **119**, e2121353119.
210 L. Xiao, V. G. Magupalli and H. Wu, *Nature*, 2023, **613**, 595–600.
211 D. G. Perregaux, P. Mcniff, R. Laliberte, N. Hawryluk, H. Peurano, E. Stam, J. Eggler, R. Griffiths, M. A. Dombroski and C. A. Gabel, *J. Pharmacol. Exp. Ther.*, 2001, **299**, 187–197.
212 R. E. Laliberte, D. G. Perregaux, L. R. Hoth, P. J. Rosner, C. K. Jordan, K. M. Peese, J. F. Eggler, M. A. Dombroski, K. F. Geoghegan and C. A. Gabel, *J. Biol. Chem.*, 2003, **278**, 16567–16578.
213 R. C. Coll, A. A. B. Robertson, J. J. Chae, S. C. Higgins, R. Muñoz-Planillo, M. C. Inserra, I. Vetter, L. S. Dungan, B. G. Monks, A. Stutz, D. E. Croker, M. S. Butler, M. Haneklaus, C. E. Sutton, G. Núñez, E. Latz, D. L. Kastner, K. H. G. Mills, S. L. Masters, K. Schroder,

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M. A. Cooper and L. A. J. O'Neill, *Nat. Med.*, 2015, **21**, 248–257.

214 A. Tapia-Abellán, D. Angosto-Bazarra, H. Martínez-Banaclocha, C. de Torre-Minguela, J. P. Cerón-Carrasco, H. Pérez-Sánchez, J. I. Arostegui and P. Pelegrin, *Nat. Chem. Biol.*, 2019, **15**, 560–564.

215 R. C. Coll, J. R. Hill, C. J. Day, A. Zamoshnikova, D. Boucher, N. L. Massey, J. L. Chitty, J. A. Fraser, M. P. Jennings, A. A. B. Robertson and K. Schroder, *Nat. Chem. Biol.*, 2019, **15**, 556–559.

216 L. Vande Walle, I. B. Stowe, P. Šácha, B. L. Lee, D. Demon, A. Fossoul, F. Van Hauwermeiren, P. H. V. Saavedra, P. Šimon, V. Šubrt, L. Kostka, C. E. Stivala, V. C. Pham, S. T. Staben, S. Yamazoe, J. Konvalinka, N. Kayagaki and M. Lamkanfi, *PLoS Biol.*, 2019, **17**, 1–24.

217 C. Dekker, H. Mattes, M. Wright, A. Boettcher, A. Hinniger, N. Hughes, S. Kapps-Fouthier, J. Eder, P. Erbel, N. Stiefl, A. Mackay and C. J. Farady, *J. Mol. Biol.*, 2021, **433**, 167309.

218 C. McBride, L. Trzoss, D. Povero, M. Lazic, G. Ambrus-Aikelin, A. Santini, R. Pranadinata, G. Bain, R. Stansfield, J. A. Stafford, J. Veal, R. Takahashi, J. Ly, S. Chen, L. Liu, M. Nespi, R. Blake, A. Katewa, T. Kleinheinz, S. Sujatha-Bhaskar, N. Ramamoorthi, J. Sims, B. McKenzie, M. Chen, M. Ultsch, M. Johnson, J. Murray, C. Ciferri, S. T. Staben, M. J. Townsend and C. E. Stivala, *J. Med. Chem.*, 2022, **65**, 14721–14739.

219 H. He, H. Jiang, Y. Chen, J. Ye, A. Wang, C. Wang, Q. Liu, G. Liang, X. Deng, W. Jiang and R. Zhou, *Nat. Commun.*, 2018, **9**, 1–12.

220 Y. Huang, H. Jiang, Y. Chen, X. Wang, Y. Yang, J. Tao, X. Deng, G. Liang, H. Zhang, W. Jiang and R. Zhou, *EMBO Mol. Med.*, 2018, **10**, 1–15.

221 M. Bertinaria, S. Gastaldi, E. Marini and M. Giorgis, *Arch. Biochem. Biophys.*, 2019, **670**, 116–139.

222 T. D. Kanneganti, N. Özören, M. Body-Malapel, A. Amer, J. H. Park, L. Franchi, J. Whitfield, W. Barchet, M. Colonna, P. Vandenabeele, J. Bertin, A. Coyle, E. P. Grant, S. Akira and G. Núñez, *Nature*, 2006, **440**, 233–236.

223 C. J. Groß, R. Mishra, K. S. Schneider, G. Médard, J. Wettmarshausen, D. C. Dittlein, H. Shi, O. Gorka, P. A. Koenig, S. Fromm, G. Magnani, T. Ćiković, L. Hartjes, J. Smollich, A. A. B. Robertson, M. A. Cooper, M. Schmidt-Supprian, M. Schuster, K. Schroder, P. Broz, C. Traidl-Hoffmann, B. Beutler, B. Kuster, J. Ruland, S. Schneider, F. Perocchi and O. Groß, *Immunity*, 2016, **45**, 761–773.

224 L. K. Billingham, J. S. Stoolman, K. Vasan, A. E. Rodriguez, T. A. Poor, M. Szibor, H. T. Jacobs, C. R. Reczek, A. Rashidi, P. Zhang, J. Miska and N. S. Chandel, *Nat. Immunol.*, 2022, **23**, 692–704.

225 B. E. Nelson, R. Sheth, K. Kelly, H. Park, S. P. Patel, D. McKinley, S. O'Brien, J. L. Parra Palau and S. A. Piha-Paul, *J. Clin. Oncol.*, 2023, **41**, e14584–e14584.

226 D. Angosto-Bazarra, C. Molina-López and P. Pelegrín, *Commun. Biol.*, 2022, **5**, 1–8.

227 M. Levy, C. A. Thaiss, D. Zeevi, L. Dohnalová, G. Zilberman-Schapira, J. A. Mahdi, E. David, A. Savidor, T. Korem, Y. Herzig, M. Pevsner-Fischer, H. Shapiro, A. Christ, A. Harmelin, Z. Halpern, E. Latz, R. A. Flavell, I. Amit, E. Segal and E. Elinav, *Cell*, 2015, **163**, 1428–1443.

228 P. Lemire, S. J. Robertson, H. Maughan, I. Tattoli, C. J. Streutker, J. M. Platnich, D. A. Muruve, D. J. Philpott and S. E. Girardin, *Cell Rep.*, 2017, **21**, 3653–3661.

229 M. Mamantopoulos, F. Ronchi, F. Van Hauwermeiren, S. Vieira-Silva, B. Yilmaz, L. Martens, Y. Saeys, S. K. Drexler, A. S. Yazdi, J. Raes, M. Lamkanfi, K. D. McCoy and A. Wullaert, *Immunity*, 2017, **47**, 339–348.

230 H. Hara, S. S. Seregin, D. Yang, K. Fukase, M. Chamaillard, E. S. Alnemri, N. Inohara, G. Y. Chen and G. Núñez, *Cell*, 2018, **175**, 1651–1664.

231 P. Wang, S. Zhu, L. Yang, S. Cui, W. Pan, R. Jackson, Y. Zheng, A. Rongvaux, Q. Sun, G. Yang, S. Gao, R. Lin, F. You, R. Flavell and E. Fikrig, *Science*, 2015, **350**, 826–830.

232 C. Shen, R. Li, R. Negro, J. Cheng, S. M. Vora, T. M. Fu, A. Wang, K. He, L. Andreeva, P. Gao, Z. Tian, R. A. Flavell, S. Zhu and H. Wu, *Cell*, 2021, **184**, 5759–5774.

233 H. R. Kilgore and R. A. Young, *Nat. Chem. Biol.*, 2022, **18**, 1298–1306.

234 H. Ishikawa, Z. Ma and G. N. Barber, *Nature*, 2009, **461**, 788–792.

235 H. Ishikawa and G. N. Barber, *Nature*, 2008, **455**, 674–678.

236 A. Ablasser, M. Goldeck, T. Cavlar, T. Deimling, G. Witte, I. Röhl, K. P. Hopfner, J. Ludwig and V. Hornung, *Nature*, 2013, **498**, 380–384.

237 L. Sun, J. Wu, F. Du, X. Chen and Z. J. Chen, *Science*, 2013, **339**, 786–791.

238 X. Zhang, H. Shi, J. Wu, X. Zhang, L. Sun, C. Chen and Z. J. Chen, *Mol. Cell*, 2013, **51**, 226–235.

239 S. L. Ergun, D. Fernandez, T. M. Weiss and L. Li, *Cell*, 2019, **178**, 290–301.

240 G. Shang, C. Zhang, Z. J. Chen, X. Chen Bai and X. Zhang, *Nature*, 2019, **567**, 389–393.

241 C. Vanpouille-Box, J. A. Hoffmann and L. Galluzzi, *Nat. Rev. Drug Discovery*, 2019, **18**, 845–867.

242 K. M. Garland, T. L. Sheehy and J. T. Wilson, *Chem. Rev.*, 2022, **122**, 5977–6039.

243 J. Vincent, C. Adura, P. Gao, A. Luz, L. Lama, Y. Asano, R. Okamoto, T. Imaeda, J. Aida, K. Rothamel, T. Gogakos, J. Steinberg, S. Reasoner, K. Aso, T. Tuschl, D. J. Patel, J. F. Glickman and M. Ascano, *Nat. Commun.*, 2017, **8**, 1–12.

244 L. Lama, C. Adura, W. Xie, D. Tomita, T. Kamei, V. Kuryavyi, T. Gogakos, J. I. Steinberg, M. Miller, L. Ramos-Espiritu, Y. Asano, S. Hashizume, J. Aida, T. Imaeda, R. Okamoto, A. J. Jennings, M. Michino, T. Kuroita, A. Stamford, P. Gao, P. Meinke, J. F. Glickman, D. J. Patel and T. Tuschl, *Nat. Commun.*, 2019, **10**, 1–14.

245 J. Hall, A. Brault, F. Vincent, S. Weng, H. Wang, D. Dumlao, A. Aulabaugh, D. Aivazian, D. Castro, M. Chen, J. Culp, K. Dower, J. Gardner, S. Hawrylik, D. Golenbock, D. Hepworth, M. Horn, L. Jones, P. Jones, E. Latz, J. Li, L. L. Lin, W. Lin, D. Lin, F. Lovering, N. Niljanskul,

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R. Nistler, B. Pierce, O. Plotnikova, D. Schmitt, S. Shanker, J. Smith, W. Snyder, T. Subashi, J. Trujillo, E. Tyminski, G. Wang, J. Wong, B. Lefker, L. Dakin and K. Leach, *PLoS One*, 2017, **12**, 1–16.

246 J. Dai, Y. J. Huang, X. He, M. Zhao, X. Wang, Z. S. Liu, W. Xue, H. Cai, X. Y. Zhan, S. Y. Huang, K. He, H. Wang, N. Wang, Z. Sang, T. Li, Q. Y. Han, J. Mao, X. Diao, N. Song, Y. Chen, W. H. Li, J. H. Man, A. L. Li, T. Zhou, Z. G. Liu, X. M. Zhang and T. Li, *Cell*, 2019, **176**, 1447–1460.

247 J. An, J. J. Woodward, T. Sasaki, M. Minie and K. B. Elkon, *J. Immunol.*, 2015, **194**, 4089–4093.

248 M. Wang, M. A. Sooreshjani, C. Mikek, C. Opoku-Temeng and H. O. Sintim, *Future Med. Chem.*, 2018, **10**, 1301–1317.

249 R. Padilla-Salinas, L. Sun, R. Anderson, X. Yang, S. Zhang, Z. J. Chen and H. Yin, *J. Org. Chem.*, 2020, **85**, 1579–1600.

250 H. Zhang, Q. You and X. Xu, *J. Med. Chem.*, 2020, **63**, 3785–3816.

251 L. Li, Q. Yin, P. Kuss, Z. Maliga, J. L. Millán, H. Wu and T. J. Mitchison, *Nat. Chem. Biol.*, 2014, **10**, 1043–1048.

252 F. Meric-Bernstam, R. F. Sweis, F. S. Hodi, W. A. Messersmith, R. H. I. Andtbacka, M. Ingham, N. Lewis, X. Chen, M. Pelletier, X. Chen, J. Wu, S. M. McWhirter, T. Müller, N. Nair and J. J. Luke, *Clin. Cancer Res.*, 2022, **28**, 677–688.

253 F. Meric-Bernstam, R. F. Sweis, S. Kasper, O. Hamid, S. Bhatia, R. Dummer, A. Stradella, G. V. Long, A. Spreafico, T. Shimizu, N. Steeghs, J. J. Luke, S. M. McWhirter, T. Müller, N. Nair, N. Lewis, X. Chen, A. Bean, L. Kattenhorn, M. Pelletier and S. Sandhu, *Clin. Cancer Res.*, 2023, **29**, 110–121.

254 S. Kim, L. Li, Z. Maliga, Q. Yin, H. Wu and T. J. Mitchison, *ACS Chem. Biol.*, 2013, **8**, 1396–1401.

255 P. Gao, M. Ascano, T. Zillinger, W. Wang, P. Dai, A. A. Serganov, B. L. Gaffney, S. Shuman, R. A. Jones, L. Deng, G. Hartmann, W. Barchet, T. Tuschl and D. J. Patel, *Cell*, 2013, **154**, 748–762.

256 J. M. Ramanjulu, G. S. Pesiridis, J. Yang, N. Concha, R. Singhaus, S. Y. Zhang, J. L. Tran, P. Moore, S. Lehmann, H. C. Eberl, M. Muelbaier, J. L. Schneck, J. Clemens, M. Adam, J. Mehlmann, J. Romano, A. Morales, J. Kang, L. Leister, T. L. Graybill, A. K. Charnley, G. Ye, N. Nevins, K. Behnia, A. I. Wolf, V. Kasparcova, K. Nurse, L. Wang, Y. Li, M. Klein, C. B. Hopson, J. Guss, M. Bantscheff, G. Bergamini, M. A. Reilly, Y. Lian, K. J. Duffy, J. Adams, K. P. Foley, P. J. Gough, R. W. Marquis, J. Smothers, A. Hoos and J. Bertin, *Nature*, 2018, **564**, 439–443.

257 B.-S. Pan, S. A. Perera, J. A. Piesvaux, J. P. Presland, G. K. Schroeder, J. N. Cumming, B. W. Trotter, M. D. Altman, A. V. Buevich, B. Cash, S. Cemerski, W. Chang, Y. Chen, P. J. Dandliker, G. Feng, A. Haidle, T. Henderson, J. Jewell, I. Kariv, I. Knemeyer, J. Kopinja, B. M. Lacey, J. Laskey, C. A. Lesburg, R. Liang, B. J. Long, M. Lu, Y. Ma, E. C. Minnihan, G. O'Donnell, R. Otte, L. Price, L. Rakhilina, B. Sauvagnat, S. Sharma, S. Tyagarajan, H. Woo, D. F. Wyss, S. Xu, D. J. Bennett and G. H. Addona, *Science*, 2020, **369**, 993–999.

258 E. N. Chin, C. Yu, V. F. Vartabedian, Y. Jia, M. Kumar, A. M. Gamo, W. Vernier, S. H. Ali, M. Kissai, D. C. Lazar, N. Nguyen, L. E. Pereira, B. Benish, A. K. Woods, S. B. Joseph, A. Chu, K. A. Johnson, P. N. Sander, F. Martínez-Peña, E. N. Hampton, T. S. Young, D. W. Wolan, A. K. Chatterjee, P. G. Schultz, H. M. Petrassi, J. R. Teijaro and L. L. Lairson, *Science*, 2020, **369**, 993–999.

259 T. Siu, M. D. Altman, G. A. Baltus, M. Childers, J. M. Ellis, H. Gunaydin, H. Hatch, T. Ho, J. Jewell, B. M. Lacey, C. A. Lesburg, B. S. Pan, B. Sauvagnat, G. K. Schroeder and S. Xu, *ACS Med. Chem. Lett.*, 2019, **10**, 92–97.

260 Z. Hong, J. Mei, C. Li, G. Bai, M. Maimaiti, H. Hu, W. Yu, L. Sun, L. Zhang, D. Cheng, Y. Liao, S. Li, Y. You, H. Sun, J. Huang, X. Liu, J. Lieberman and C. Wang, *Proc. Natl. Acad. Sci. U. S. A.*, 2021, **118**, e2105465118.

261 S. M. Haag, M. F. Gulen, L. Reymond, A. Gibelin, L. Abrami, A. Decout, M. Heymann, F. G. Van Der Goot, G. Turcatti, R. Behrendt and A. Ablasser, *Nature*, 2018, **559**, 269–273.

262 A. L. Hansen, G. J. Buchan, M. Rühl, K. Mukai, S. R. Salvatore, E. Ogawa, S. D. Andersen, M. B. Iversen, A. L. Thielke, C. Gunderstofte, M. Motwani, C. T. Møller, A. S. Jakobsen, K. A. Fitzgerald, J. Roos, R. Lin, T. J. Maier, R. Goldbach-Mansky, C. A. Miner, W. Qian, J. J. Miner, R. E. Rigby, J. Rehwinkel, M. R. Jakobsen, H. Arai, T. Taguchi, F. J. Schopfer, D. Olagnier and C. K. Holm, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E7768–E7775.

263 K. A. Krautkramer, J. Fan and F. Bäckhed, *Nat. Rev. Microbiol.*, 2021, **19**, 77–94.

264 T. Tsukidate, Q. Li and H. C. Hang, *Curr. Opin. Chem. Biol.*, 2020, **54**, 19–27.

265 C. Diskin, T. A. J. Ryan and L. A. J. O'Neill, *Immunity*, 2021, **54**, 19–31.

266 E. V. Vinogradova, X. Zhang, D. Remillard, D. C. Lazar, R. M. Suciu, Y. Wang, G. Bianco, Y. Yamashita, V. M. Crowley, M. A. Schafroth, M. Yokoyama, D. B. Konrad, K. M. Lum, G. M. Simon, E. K. Kemper, M. R. Lazear, S. Yin, M. M. Blewett, M. M. Dix, N. Nguyen, M. N. Shokhirev, E. N. Chin, L. L. Lairson, B. Melillo, S. L. Schreiber, S. Forli, J. R. Teijaro and B. F. Cravatt, *Cell*, 2020, **182**, 1009–1026.

267 J. Stefaniak and K. V. M. Huber, *ACS Med. Chem. Lett.*, 2020, **11**, 403–406.

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