Acta Pharmaceutica Sinica B 2025;15(9):4497−4510

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

The role of SARS-CoV-2 main protease in innate immune regulation: From molecular mechanisms to therapeutic implications

Yumeng Gao, Jun Zhang*

Department of Immunology, School of Basic Medical Sciences, NHC Key Laboratory of Medical Immunology, Medicine Innovation Center for Fundamental Research on Major Immunology-related Diseases, Peking University, Beijing 100191, China

Received 14 January 2025; received in revised form 10 April 2025; accepted 19 May 2025

KEY WORDS

SARS-CoV-2;
Main protease;
Innate immunity;
Type I IFN;
Inhibitor

Abstract The main protease (M^{pro}) of SARS-CoV-2 plays a pivotal role in viral replication and immune evasion. Accumulating evidence highlights its significant role in suppressing innate immunity. In this review, we provide a comprehensive overview of how M^{pro} modulates host innate immune responses, including its interference with retinoic acid-inducible gene I (RIG-I)-like receptor (RLR) and cyclic GMP−AMP synthase (cGAS)−stimulator of interferon gene (STING) signaling pathways, inhibition of interferon production, and disruption of inflammasome activities. As a protease, M^{pro} cleaves a variety of host proteins to attenuate antiviral innate immunity, a process dependent on its catalytic dyad (Cys145−His41), which is crucial for its proteolytic activity. Meanwhile, M^{pro} also exerts innate immune regulatory functions in a protease-independent manner. Notably, inhibitors targeting M^{pro} have demonstrated efficacy in restoring immune functions and suppressing viral replication, offering potential therapeutic strategies against SARS-CoV-2 infection.

*Corresponding author.
E-mail address: junzhang@bjmu.edu.cn (Jun Zhang).

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2025.07.001

1. Introduction

The global pandemic of the coronavirus SARS-CoV-2 infection (COVID-19) has had a profound impact on human health. SARS-CoV-2 belongs to the β coronavirus family and shares approximately 80% genetic similarity with SARS-CoV[1]. Its genome is a positive-sense single-stranded RNA (+ssRNA) consisting of 14 open reading frames (ORFs) that encode 31 viral proteins, including 16 non-structural proteins (NSP1−16), 4 structural proteins (S, M, N, and E) and 11 accessory proteins (ORF3a, ORF3b, ORF3c, ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10) (Fig. 1)[2,3]. The entry of SARS-CoV-2 into the host cells is primarily mediated by the binding of the viral Spike (S) protein to its receptor angiotensin-converting enzyme 2 (ACE2)[4]. This process is further facilitated by proteolytic cleavage of the S protein by the serine protease TMPRSS2[5]. Other potential receptors or cofactors, including CD147, neuropilin-1, heparan sulfate, furin, etc., are also involved in the entry process[5−8].

Pathogen recognition receptors (PRRs) recognize the conserved motifs in pathogen-associated molecular patterns (PAMPs) from the viruses and initiate the innate immune response[9]. As an RNA virus, SARS-CoV-2 is primarily sensed in the cytoplasm by RLRs[10,11], including RIG-I and melanoma differentiation antigen 5 (MDA5), with MDA5 being the predominant sensor for SARS-CoV-2[12]. Upon recognition, the receptors then recruit the adaptor protein mitochondrial antiviral signaling (MAVS) to form the MAVS signalosome, which then activates TANK-binding kinase 1(TBK1) and inhibitor of κB kinase (IKK)[13]. This results in the phosphorylation of the transcription factors interferon regulatory factor 3 (IRF3) and nuclear factor-κB (NF-κB), driving the production of type I interferons (IFN-I) and pro-inflammatory cytokines[13]. Then IFN-I binds to the type I interferon receptor (interferon-α/β receptor (IFNAR)) to activate downstream signaling pathways, such as the JAK−STAT pathway, which ultimately induces the expression of interferon-stimulated genes (ISGs) to combat viral infection[13]. Interestingly, RIG-I can inhibit SARS-CoV-2 infection independently of IFN-I/III by recognizing the 3′ UTR of the viral genome and directly interfering with RNA-dependent RNA polymerase (RdRp) activity, bypassing RIG-I-induced signaling pathways[14]. Nevertheless, the RLR-induced signaling pathway remains the primary antiviral mechanism.

SARS-CoV-2 infection also triggers the translocation of chromatin DNA from the nucleus to the cytoplasm and causes mitochondrial damage and subsequent release of mitochondrial DNA (mtDNA) into the cytoplasm[15,16]. This cytoplasmic DNA is sensed by cGAS and activates the STING dependent signaling pathway, resulting in the activation of IRF3 and NF-κB[15,16]. SARS-CoV-2 infection also induces the formation of the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome and Gasdermin D (GSDMD)-induced pyroptosis[17−19]. Toll-like receptors (TLRs) also contribute to the innate immune response against SARS-CoV-2. TLR2 binds to the viral E protein and induces pro-inflammatory signaling pathways[20]. Activation of TLR3 and TLR7 is also observed to induce pro-inflammatory cytokines, such as IL-1α, IL-1β, IL-4, IL-6, and IFN[21].

Accumulating evidence highlights the critical role of SARS-CoV-2 viral proteins, both structural and non-structural proteins, in modulating innate immunity. For example, the M protein attenuates antiviral innate immune response by disrupting MAVS aggregation[22]. ORF9b suppresses the RIG-I-MAVS signaling by impeding the K63-linked ubiquitination of NF-κB essential modulator (NEMO)[23]. NSP6 suppresses innate immunity by binding to TBK1 and inhibits IRF3 phosphorylation[24]. Additionally, the NSP13, NSP14, NSP15, ORF6, and ORF8 also mitigate the interferon production and interferon-induced signaling[25−28].

NSP5, also known as 3-chymotrypsin-like protease (3CL^{pro}), is the main protease (M^{pro}) of SARS-CoV-2[2]. It is responsible for cleaving the two long polyproteins, pp1a and pp1ab, derived from the SARS-CoV-2 genome, and generating NSP5-NSP16, which are essential for the replication of SARS-CoV-2 in the host (Fig. 1)[2]. Extensive research has demonstrated that M^{pro} plays a pivotal role in the virus−host interaction. It may antagonize the innate immune response through mechanisms that are either dependent on or independent of its enzymatic activity.

Given the critical role of M^{pro} for viral replication and its inhibitory role in innate immunity, significant efforts have been directed toward developing M^{pro} inhibitors. In-silico techniques have demonstrated substantial utility in this pursuit, particularly for

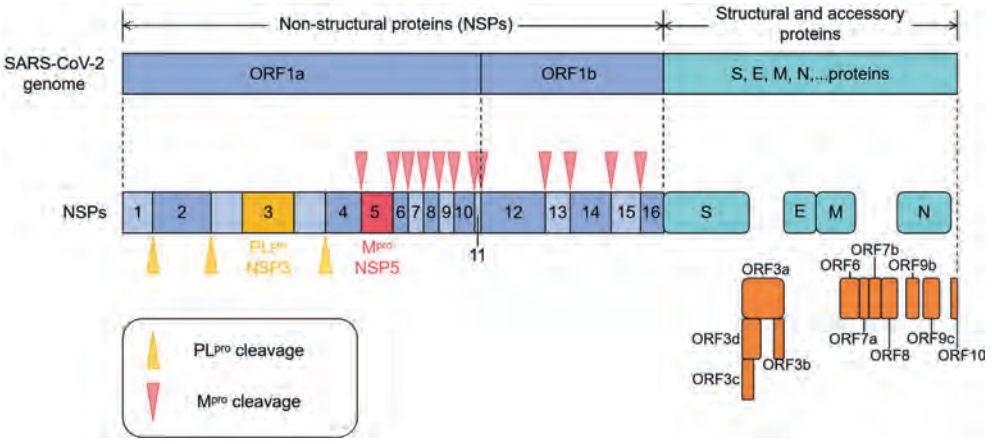

Figure 1 SARS-CoV-2 genome and proteins. The front part of the SARS-CoV-2 genome, ORF1a and ORF1b, encodes two polyproteins, pp1a and pp1ab, which can be cleaved into 16 non-structural proteins by the papain-like protease (PL^{pro}) and 3CL^{pro}, also called M^{pro}. The rest of the genome encodes 4 structural proteins (S, E, M, and N) and 11 accessory proteins (ORF3a, ORF3b, ORF3c, ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10).

predicting and evaluating inhibitors targeting SARS-CoV-2 proteins, including the S protein, N protein, RdRp, NSP3, NSP15, NSP16, and M^{pro}, as well as predicting substrates of M^{pro}[29−31]. These computational approaches include but are not limited to following methods: (1) molecular docking to screen potential ligands, (2) molecular dynamics simulations to assess protein-ligand interaction stability, and (3) molecular mechanics Poisson-Boltzmann surface area (MMPBSA) analysis to calculate binding free energy[30,31]. Compounds exhibiting strong binding affinity and stability, as indicated by high docking scores and low free energy values, are prioritized for further experimental validation. The development of nirmatrelvir, the first approved M^{pro} inhibitor, also engages in-silico techniques such as molecular docking[32].

In this review, we summarized the recent advancements in the interplay between M^{pro} and the host, with a focus on its pivotal role in modulating antiviral innate immunity. We further discussed the potential of M^{pro} as a promising target for the treatment of SARS-CoV-2 infection.

2. The structure and characteristics of SARS-CoV-2 M^{pro}

2.1. *Structure and catalytic mechanisms of M^{pro}*

M^{pro}, a cysteine protease, comprises three structural domains: a chymotrypsin-like domain I, a picornavirus 3C protease-like domain II, and a globular domain III formed by five α-helices (Fig. 2A)[33,34]. The substrate-binding pocket is located in a cleft between domain I and domain II[33,34], containing five key subsites: S1 and S2 (deeply buried), S3 (functionally tolerant), S4 (shallow and hydrophobic), and S1′, which collectively recognize substrate residues at P4−P1′ positions[33−35]. Notably, the S1 subsite exhibits a strong preference for glutamine, dictating cleavage specificity[2,35]. Catalytic activity is mediated by the Cys145−His41 dyad located near the S1′ subsite (Fig. 2B)[35]. Here, Cys145 acts as a nucleophile, attacking the substrate's carbonyl carbon to form an acyl-enzyme intermediate, while His41 facilitates the deprotonation of Cys145 to promote the nucleophilic attack[36]. The carbonyl oxygen of the intermediate interacts with the main chain nitrogen atoms of Gly143 and Cys145 through hydrogen bonds to form an oxyanion hole[37]. Different from the traditional catalytic Ser (Cys)−His−Asp(Glu) triad, M^{pro} employs a water molecule to form hydrogen bonds with His41 to stabilize the positive charge on His41, playing the role of the third catalytic residue[36]. M^{pro} functions as a homodimer, which is essential for its full enzymatic activity. This process depends on a salt−bridge interaction between Glu290 of one protomer and Arg4 from another protomer[33].

2.2. *The cleavage selectivity of M^{pro}*

M^{pro} exhibits distinct cleavage site preferences, with glutamine (Gln) at the P1 position (the first residue N-terminal to the cleavage site) being the most conserved and universally recognized motif across substrates[38−40]. However, emerging studies reveal additional flexibility: *in vitro* N-terminomics using LC−MS identified histidine (His) at P1, albeit less frequently[41], and glutamic acid (Glu) as a cleavage site is also reported[42]. The preferred cleavage sequences conform to X-(L/F/V)-Q↓(A/S)-X[39] or X-(A/S)-X-(L/M)-(Q/H)↓(G/A/S)-X[41] (X refers to any amino acid, ↓ marks the cleavage site). For example, the sequence between NSP4 and NSP5 is S-A-V-L-Q↓S-G, which is consistent with this pattern[43]. Overall, there are site preferences with some degree of versatility for M^{pro} substrate recognition, though the full scope of its cleavage selectivity remains incompletely characterized.

2.3. *The protease-independent roles of M^{pro}*

M^{pro} subverts host antiviral innate immunity through multifaceted mechanisms, leveraging both proteolytic and non-proteolytic activities. On the one hand, M^{pro} can cleave the host proteins and impede their functions to reduce antiviral innate immunity, a mechanism discussed in detail in subsequent sections. M^{pro} also downregulates antiviral proteins *via* indirect strategies, such as suppressing protein translation. For instance, SARS-CoV-2 M^{pro} cleaves eukaryotic translation initiation factor 4G (eIF4G) at Q658, effectively halting global protein synthesis and attenuating RLR signaling pathway[44]. On the other hand, M^{pro} also employs protease-independent mechanisms to evade innate immunity: (1) Ubiquitination modulation: M^{pro} can promote MAVS degradation via the ubiquitin proteasome system[45]. Some other reports demonstrated that M^{pro} exerts inhibitory effects on the ubiquitination levels of some innate immune components, such as reducing the K63-linked ubiquitination of RIG-I[46,47] and reducing total ubiquitinated MAVS[44], without elucidating whether protease activity is required. (2) Autophagy exploitation: Active M^{pro} (not the enzymatically inactive C145S mutant) facilitates the autophagic degradation of STAT1 by enhancing the association between STAT1 and autophagy receptor p62[47]. (3) Stress granule disruption: M^{pro} impedes stress granule (SG) formation through mechanisms independent of its proteolytic function[46]. (4) Scaffold: M^{pro} impairs RIG-I−TRIM25 interaction and RIG-I−MAVS interaction as a scaffold, rather than degrading them[46,47]. (5) SUMOylation modulation: M^{pro} promotes the SUMOylation of MAVS to increase its stability[48]. These findings will be elaborated later.

2.4. *Host regulation of M^{pro}*

The interplay between the host and the virus is a reciprocal process. Certain E3 ubiquitin ligases possess the ability to degrade M^{pro}. ZBTB25, a human E3 ubiquitin ligase that requires a C2H2 domain for activity, facilitates the ubiquitin-proteasome-dependent degradation of M^{pro}, whose Lys100 serves as the ubiquitin acceptor[49]. Parkin, another E3 ubiquitin ligase with a catalytic residue Cys in a C-terminal RING-between-RING domain, also induces the ubiquitination and degradation of M^{pro}[50]. Specifically, during cell mitophagy, the protein kinase PINK1 activates Parkin, thereby accelerating the degradation of M^{pro}[50].

2.5. *Brief comparisons of M^{pro} and other viral proteins*

There are seven kinds of coronaviruses that infect humans, all of which use NSP5 as their main protein. Among them, SARS-CoV, MERS-CoV, and SARS-CoV-2 are highly pathogenic and share many similarities in their genome structures, proteins, and pathogenesis[51]. Thus, their M^{pro} may play a similar role in defending innate immunity through cleaving NEMO and inhibiting IRF3 nuclear translocation, but there is little research about the first two[42,52,53]. Compared to SARS-CoV M^{pro}, SARS-CoV-2 M^{pro} demonstrates enhanced catalytic efficiency, polyprotein cleavage capacity, and interferon antagonism, largely due to serine (Ser) rather than alanine (Ala) in position 46[33,34,42]. This heightened

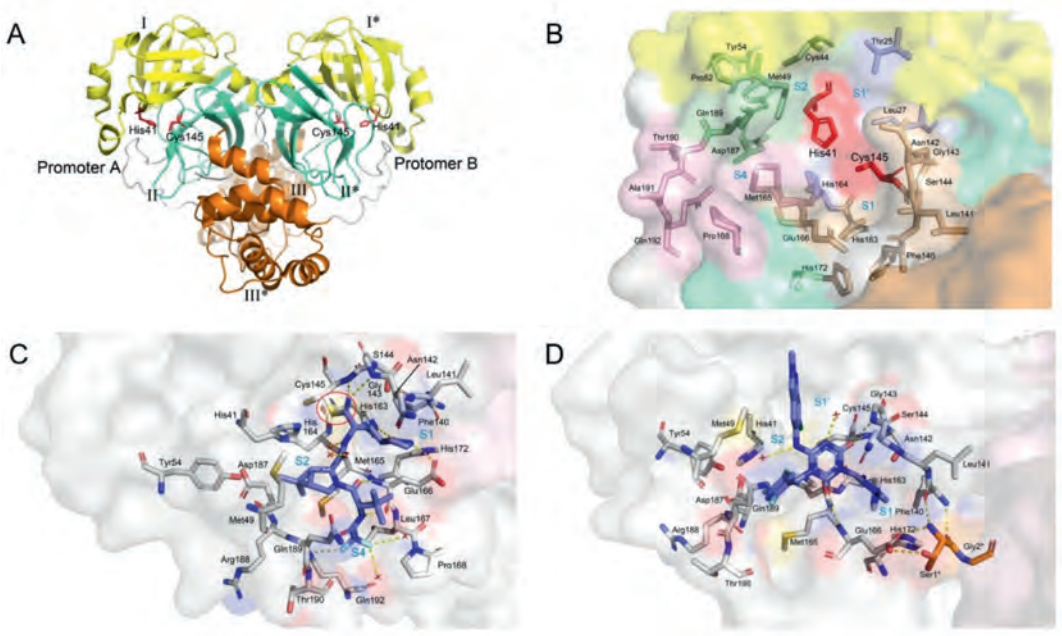

Figure 2 Structure of SARS-CoV-2 M^{pro} and its interactions with inhibitors. (A) Shows the dimer of M^{pro} (PDB ID: 6Y2E), including protomer A and protomer B. M^{pro} is a cysteine protease consisting of three domains: the chymotrypsin-like domain I (yellow), picornavirus 3C protease-like domain II (green), and domain III (orange), a globular cluster of five helices. (B) Shows the catalytic Cys145−His41 dyad (colored red) and the substrate-binding pocket. (C) Shows the interaction between nirmatrelvir and the substrate-binding pocket of M^{pro} (PDB ID: 7VH8). Nirmatrelvir is shown in purple, and M^{pro} is shown in gray. Red marks represent water molecules. Yellow dotted lines represent hydrogen bonds, which exist between nirmatrelvir and S1, S2, and S4 subsites. The C−S covalent bond is pointed out by the red circle. (D) Shows the interaction between ensitrelvir and the substrate-binding pocket of M^{pro} (PDB ID: 8HBK). Ensitrelvir is shown in purple, residues of protomer A are shown in orange, and residues of protomer B are shown in gray. Red marks represent water molecules. Yellow dotted lines represent hydrogen bonds, which exist between ensitrelvir and S1′, S1, and S2 subsites.

activity has dual implications. On the one hand, a stronger cleavage ability of SARS-CoV-2 M^{pro} at the N-terminal auto-cleavage site of viral polyproteins provides evidence that SARS-CoV-2 has a stronger replication ability than SARS-CoV[42]. On the other hand, the increased cleavage activity of host proteins induces stronger resistance to host antiviral immunity, which may contribute to asymptomatic transmission of COVID-19 patients[42]. Compared to MERS-CoV M^{pro}, SARS-CoV-2 M^{pro} shows higher thermostability and stronger stability in acidic environments[54]. More flexible cleavage sites of SARS-CoV-2 M^{pro}, rather than conservative ones like (L/M)-Q↓(S/A) of MERS-CoV M^{pro}[55], provide more potential for interfering with host immunity.

NSP3, also called papain-like protease (PL^{pro}), is another protease of SARS-CoV-2 cleaving pp1a into NSP1-NSP4. Different from M^{pro}, its catalytic site is a catalytic triad consisting of Cys, His, and Asp[56]. Despite its cleavage on the polyprotein pp1a, it also induces deubiquitinating (DUB) activity and deISGylating activity through cleaving ubiquitin tags and ISG15 tags from host proteins to defend host immunity, which also exists in other human-infecting coronaviruses[56,57]. By contrast, the pro-ubiquitination effect of M^{pro} is intriguing. It is interesting to explore the relationship between the opposite effects of these two proteases.

3. The modulation of M^{pro} on host innate immunity

3.1. M^{pro} acts at multiple steps of the RLR signaling pathway

RLR is the primary sensor of SARS-CoV-2, which induces potent antiviral signaling cascade reactions and can be modulated by stress granules (SGs)[10,58]. SG assembly is triggered by protein kinase R (PKR), which is activated by viral nucleic acids, through well-characterized mechanisms[46]. As the structural core of SGs, Ras GTPase-activating protein-binding protein 1 (G3BP1) facilitates RIG-I activation *via* direct interaction[58]. SARS-CoV-2 M^{pro} has been demonstrated to bind with G3BP1, decrease its level, and inhibit SG formation without any cleavage of SG components. The protease-independent mechanism is evidenced that comparable SG inhibition is observed with both wild-type M^{pro} and its catalytically inactive mutant (C145A) *in vitro* experiments[46]. The impairment of G3BP1-mediated signaling cascade leads to sequential suppression of RIG-I activation, IFN production, ISG expression, and enhanced viral replication. Mechanistically, M^{pro}-mediated disruption manifests through diminished phosphorylation of TBK1 and IRF3, impaired IRF3 nuclear translocation, and consequent reduction in IFN production[46].

Current evidence presents different paradigms for the regulation of M^{pro} on RIG-I: M^{pro} downregulates RIG-I by cleaving it at the N-terminal (residues 1−10), generating a dominant-negative fragment, which competes with WT RIG-I for dsRNA binding, loses the ability to form a heterodimer with MAVS and reduces IFN production in Caco-2 infection models[45]. Another study presents contradictory findings that no RIG-I and downstream molecules cleavage is observed, but M^{pro} decreases the RLR pathway proteins *via* cleaving eIF4G and repressing protein translation[44]. The methodological disparities may explain the discrepancies. The former is performed in Caco-2 cell lines infected with SARS-CoV-2 or transfected with M^{pro}[45], but the latter is conducted by incubating 293T whole cell extracts only

with M^{pro}[44]. This dichotomy underscores the necessity of distinguishing between *bona fide* virologic mechanisms and overexpression artifacts. N-terminal Edman sequencing of endogenous RIG-I in SARS-CoV-2-infected airway organoids in the future will be helpful to clarify this. M^{pro} is also demonstrated to physically interact with MDA5, but the exact function needs further exporation[46]. Ubiquitin E3 ligase TRIM25 promotes the K63-linked ubiquitination of RIG-I, which is essential for its oligomerization and subsequent MAVS activation[13]. M^{pro} employs multilayered strategies to modulate this process. M^{pro} directly impedes TRIM25−RIG-I complex formation[46,47], compromising TRIM25-mediated K63-linked ubiquitination of RIG-I. NLRP12 constitutively binds TRIM25, limiting its E3 ligase activity[59]. M^{pro} proteolytically cleaves NLRP12, ostensibly to relieve its inhibitory effect on TRIM25[60].

M^{pro} regulates the levels of MAVS in different ways. M^{pro} specifically enhances MAVS conjugation with SUMO2/3 (not SUMO1), stabilizing MAVS to amplify NF-κB-driven production of pro-inflammatory cytokines (IL-1β, IL-2, IL-6, TNF-α) *via* the RIG-I/MDA5−MAVS axis[48]. This facilitates hyperinflammatory responses while suppressing type I IFN production, aligning with COVID-19 immunopathology. Paradoxically, there is also evidence indicating the inhibitory effects of M^{pro} on MAVS. M^{pro} can catalyze K48-linked ubiquitination of MAVS at K136 to promote its degradation[45]. This apparent contradiction is further complicated by conflicting reports: While one study observes reduced global ubiquitination (including K48/K63 linkages) on ectopically expressed MAVS in M^{pro}-transfected cells[44], another confirms preserved K63-linked ubiquitination under similar conditions[61]. The degradation-supporting evidence derives from endogenous MAVS analysis in SARS-CoV-2-infected cells and ubiquitination analysis in ubiquitination-competent systems (E1/E2/M^{pro}/MAVS)[45], whereas contradictory studies rely on overexpression models without viral infection contexts[44,61]. Notably, the absence of SARS-CoV-2-infected cell models in the latter investigations raises critical questions about physiological relevance. These discordant findings highlight the context-dependent nature of MAVS regulation and underscore the need for integrated virological-biochemical approaches to resolve these mechanistic discrepancies.

The impact of M^{pro} on IRF3 activation presents complex mechanistic interpretations, with studies reporting conflicting mechanisms[45−47,62,63]. In Sendai virus-infected overexpressing M^{pro} A549 cells, IRF3 phosphorylation levels remain comparable to controls, suggesting M^{pro} does not directly cleave IRF3 or suppress its phosphorylation. Instead, M^{pro} blocks the nuclear translocation of phosphorylated IRF3, likely by interfering with transport machinery rather than degrading IRF3 itself[62]. In contrast, 293T cells co-transfected with M^{pro} and IRF3 exhibit reduced IRF3 levels, implying M^{pro} facilitates IRF3 degradation[63]. This discrepancy may arise from differences in cellular contexts: A549 (lung epithelial) *vs*. 293T (kidney epithelial), different expression/stimulation methods: viral infection (RIG-I/MAVS-dependent) *vs*. overexpression system.

Following the nuclear translocation of IRF3, phosphorylated IRF3 binds to IFN promoters and initiates the production of IFN-I (IFN-α/β)[13]. The host epigenetic factor histone deacetylase 2 (HDAC2) has been reported to bind to IRF3 and positively modulate IFN-β promoters[64]. M^{pro} disrupts the interaction between HDAC2 and IRF3, but this interference is not essential for the suppression of IFN-β expression[64] (Fig. 3A and Table 1[42,44-48,60-79]). Further studies are needed to explore whether M^{pro} disrupts the nuclear translocation signal or binds to nuclear import factors (*e.g*., KPNA/importin-α). The proteasome-*vs*. autophagy-mediated IRF3 turnover in different cell types also requires clarification.

3.2. *M^{pro} suppresses the cGAS−TING signaling pathway*

SARS-CoV-2 infection induces the translocation of chromatin DNA from the nucleus to the cytoplasm, along with the release of mtDNA into the cytoplasm, thereby activating the cGAS−STING pathway[15,16]. M^{pro} suppresses this pathway by directly binding to STING (not cGAS) (Fig. 3B and Table 1) and inhibiting its K63-linked ubiquitination—a critical modification essential for recruiting TBK1 and IKKβ to activate NF-κB and IRF3[80]. This inhibition requires the enzymatic active sites of M^{pro} and is evolutionarily conserved, observed in humans, mice, and chickens, suggesting a shared vertebrate targeting mechansim[61]. Notably, M^{pro} also blocks cGAS−STING-mediated nuclear translocation of p65 (NF-κB subunit), further attenuating proinflammatory effects[61]. Unlike its suppression of IRF3 in RIG-I/MDA5 pathways, M^{pro} has no effect on IRF3 and its nuclear translocation.

3.3. *M^{pro} in IFN-I-induced signaling pathway*

The RLR and cGAS−STING signaling pathways ultimately converge on the release of IFN-I, a pivotal pro-inflammatory cytokine critical for antiviral immunity[13,81]. IFN-I binds its receptor IFNAR1, activating JAK−STAT signaling to drive ISG expression[82]. Notably, Notably, LDL-receptor-related protein-associated protein 1 (LRPAP1) interacts with IFNAR1, promoting its ubiquitination and subsequent degradation. M^{pro} exacerbates this regulatory mechanism by upregulating the expression and secretion of LRPAP1[65].

M^{pro} can also reduce STAT1 protein levels, primarily through autophagy-mediated degradation, which is dependent on its enzymatic activity[47]. This is evidenced by the fact that the enzymatically inactive C145S mutant fails to enhance the interaction between STAT1 and autophagy receptor p62. Consequently, M^{pro} impairs the phosphorylation and the nuclear translocation of STAT1[47]. Following that, the expression of ISGs is decreased, resulting in reduced antiviral functions. Intriguingly, although M^{pro} broadly antagonizes IFN-I signaling, it is also observed that SARS-CoV-2-infected cells remain highly sensitive to exogenous IFN-I therapy[83], suggesting retained responsiveness to interferon supplementation.

All upstream inhibition results in a reduction of ISG expression. As mentioned above, HDAC2 is a known positive regulator of both IFN-β production and ISG transcription[64,66], and its role in M^{pro}-mediated immune evasion remains contentious. One study reports that M^{pro} inhibits the IFN-induced signaling pathway independent of HDAC2[64]. Conversely, another demonstrates that M^{pro} cleaves HDAC2 at Q261 and Q383, impairing IFN-α-stimulated ISG expression[66]. Similar cleavage has been extended to other HDAC family members[66]. Additionally, M^{pro} can cleave DNA polymerase delta interacting protein 3 (POLDIP3) at Q176, disrupting its role in modulating ISG transcription[67].

M^{pro} also directly targets antiviral proteins encoded by ISGs to mediate immune evasion. Ring Finger Protein 20 (RNF20), an E3 ligase that forms a complex with RNF40 to stabilize each other, restricts viral replication by inducing the degradation of sterol-regulatory element binding protein 1 (SREBP1). M^{pro} cleaves RNF20 at the conserved Q521 site, abolishing its antiviral

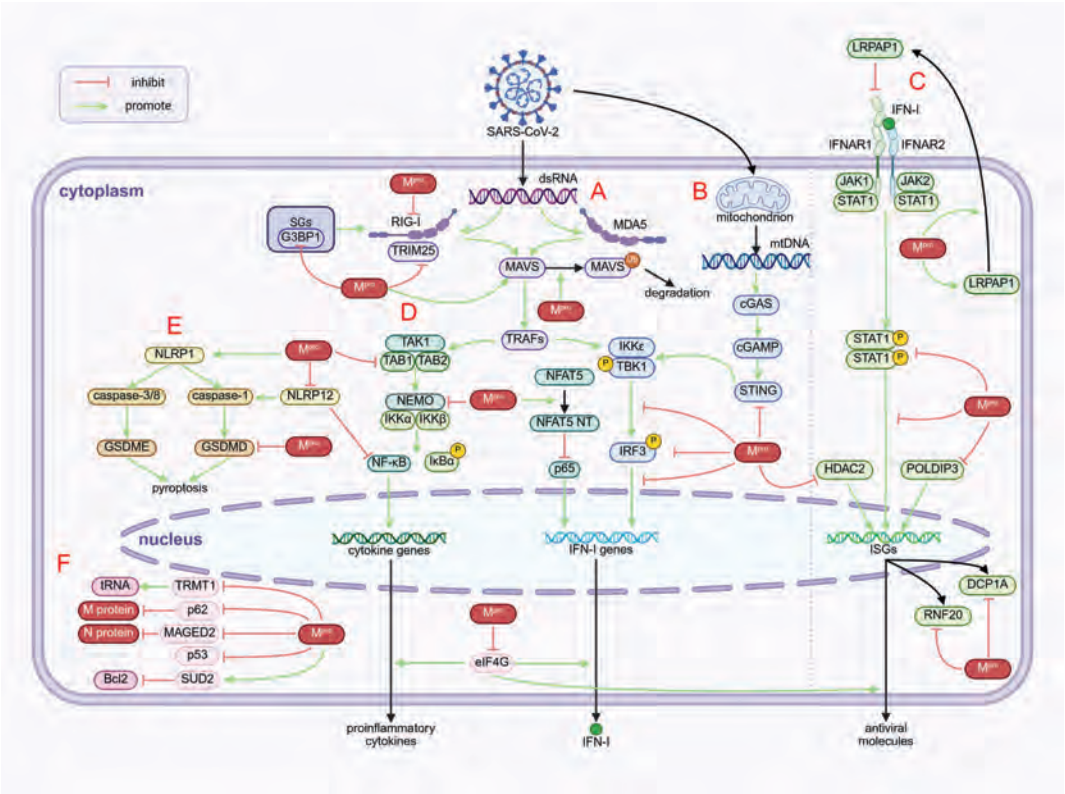

Figure 3 Regulatory functions of M^{pro} in innate immunity. M^{pro} regulates multiple signaling pathways involved in innate immune response. (A) RLR signaling pathway. (B) cGAS−STING signaling pathway. (C) IFN-I induced signaling pathway. (D) NF-κB pathway. (E) Inflammasome activation. (F) Other antiviral proteins.

function[68]. Another ISG, named mRNA-decapping enzyme 1a (DCP1A), is cleaved by M^{pro} at Q343 and loses its antiviral activity[66] (Fig. 3C and Table 1).

3.4. *M^{pro} in NF-κB pathway*

M^{pro} disrupts NF-κB signaling through multiple mechanisms to suppress pro-inflammatory cytokine production and interferon responses. M^{pro} cleaves TAB1 at Q129 (ASLQS motif) and Q441 (LTLQS motif), abolishing its ability to activate TAK1 and downstream IKK signaling[60]. NEMO (IKKγ), a critical NF-κB regulator, is cleaved at E152, Q205, and Q231 by M^{pro}, further blocking NF-κB activation[42]. In addition, M^{pro} cleaves NLRP12, downregulating the expression of NF-κB[60,84].

Conflicting reports exist on p65 localization: While one study suggests M^{pro} sequesters p65 in the cytoplasm[63], another indicates M^{pro} stabilizes MAVS to promote p65 nuclear translocation[48]. The unusual function of p65 is that it can bind to the positive regulatory domain II (PRDII) of IFN-β promoter to activate IFN-β production[85]. The nuclear factor of activated T cell 5 (NFAT5) is a transcription factor homologous to NF-κB. Intriguingly, M^{pro} is capable of cleaving NFAT5 at Q1127, resulting in the N-terminal fragment of NFAT5 (NFAT5 NT), which can compete with p65 at PRDII on the IFN-β promoter to repress IFN-β expression (Fig. 3D and Table 1)[73].

3.5. *M^{pro} in inflammasome activation*

The NLRs are a group of cytoplasmic pathogen sensors, some of which assemble to form inflammasomes[86]. Caspase-1 can be activated by inflammasomes to cleave pro-IL-1β, pro-IL-18, and GSDMD and then convert them into active forms[87]. Activation of GSDMD leads to a proinflammatory cell death called pyroptosis, which serves as a mechanism to inhibit pathogen replication. M^{pro} cleaves NLRP12 at two motifs: the LQA motif (residue 938) and the KLFQG sequence (residue 238), impairing NLRP12/NLRP3 mixed inflammasome assembly and suppressing NF-κB signaling[60]. M^{pro} cleaves GSDMD at H270, generating pore-forming fragments that trigger pyroptosis[72]. M^{pro} also cleaves GSDMD at Q29 and Q193, dismantling caspase-1-generated active fragments and attenuating GSDMD-induced pyroptosis[72,69,70]. Notably, GSDMD with Q29A, S30A, and Q193A confer resistance to M^{pro} cleavage but abolish its pyroptosis activity[69].

Unlike other NLRs, M^{pro} cleavage of NLRP1 at Q333 releases an active N-terminal fragment that promotes GSDME-dependent cell death (Fig. 3E and Table 1)[71]. Intriguingly, M^{pro} hijacks GSDMD/E pores for extracellular release, retaining enzymatic activity in serum to impair platelet activation and inactivate IFN-γ1[72]. This bidirectional regulation of pyroptosis shows the correlation between viral dissemination (*via* secretion) and immune evasion (*via* IFN suppression).

3.6. *M^{pro} and other proteins in antiviral innate immunity*

Furthermore, certain proteins cannot be classified into the above categories. One such example is eIF4G, which regulates protein translation and has been mentioned above. TRNA methyltransferase 1 (TRMT1) is responsible for catalyzing the formation of N^2-methylguanosine or N^2,N^2-dimethylguanosine at position 26

Table 1 Known targets of M^{pro} in innate immunity.

Pathway	Target	Effect	Mechanism	Ref.
RLR	G3BP1	↓	No protease activity required	46
	RIG-I	↓	Cleaving RIG-I at Q10 (LQA) as a protease	45
	MDA5	↓	/	46
	Interaction between RIG-I and TRIM25	↓	Reducing the K63-linked ubiquitination of RIG-I by TRIM25	46,47
	MAVS	↑	Promoting the SUMOylation of MAVS	48
		↓	Promoting the K48-linked ubiquitination and proteosome-mediated degradation of MAVS	45
	IRF3	↓	1. Inhibiting the phosphorylation of IRF3[a] 2. Inhibiting the nuclear translocation of IRF3 3. Promoting the degradation of IRF3 (no mention of protease activity required)	46,62,63
	Interaction between HDAC2 and IRF3	↓	/	64
cGAS	STING	↓	Reducing the K63-linked ubiquitination of STING (protease activity required)	61
IFN-I	LRPAP1	↑	Increasing the extracellular level of LRPAP1	65
	STAT1	↓	1. Promoting the autophagy degradation of STAT1 (protease activity required) 2. Inhibiting IFN-induced phosphorylation of STAT1 3. Inhibiting the nuclear translocation of STAT1	47
	HDAC2	↓	Cleaving HDAC2 at Q261 and Q383 as a protease	66
	POLDIP3	↓	Cleaving POLDIP3 at Q176 as a protease	67
	RNF20	↓	Cleaving RNF20 at Q521 as a protease	68
	DCP1A	↓	Cleaving DCP1A at Q343 as a protease	66
Inflammasome activation	NLRP12	↓	Cleaving NLRP12 at Q238 (KLFQG) and Q938 (LQA) as a protease	60
	GSDMD	↓	Cleaving GSDMD at Q29 and Q193 as a protease	69−71
		↑	Cleaving GSDMD at H270 as a protease to activate it	72
	NLRP1	↑	Cleaving NLRP1 at Q333 as a protease to activate it	71
NF-κB	TAB1	↓	Cleaving TAB1 at Q129 (ASLQS) and Q441 (LTLQS) as a protease	60
	p65	↓	Inhibiting the nuclear translocation of p65[b]	63
	NEMO	↓	Cleaving NEMO at E152, Q205 and Q231 as a protease	42
	NFAT5	[c]	Cleaving NFAT5 at Q1127 as a protease	73
Others	eIF4G	↓	Cleaving eIF4G at Q658 (LQGI) as a protease	44
	TRMT1	↓	Cleaving TRMT1 at Q530 as a protease	74
	p62	↓	Cleaving p62 at Q354 as a protease	75,76
	MAGED2	↓	Cleaving MAGED2 at Q263 as a protease	77
	p53	↓	Inhibiting the transcription activity of p53 (protease activity required)	78
	SUD2core	↑	Binding with SUD2core to increase the effect of SUD2core on *Bcl2G4*	79

↓ means inhibiting its function; ↑ means promoting its function.
[a]Another research[62] demonstrates that M^{pro} has no influence on the phosphorylation of IRF3.
[b]Another research[48] demonstrates that M^{pro} promotes the nuclear translocation of p65.
[c]The cleaved fragment competes away p65 at PRDII on the IFN-β promoter to suppress IFN-β production.

of the majority of human tRNAs. This process ensures that the tRNA is folded into the correct structure[88]. M^{pro} cleaves TRMT1 at Q530 to disrupt tRNA folding and global protein synthesis[74]. Whether this process suppresses the production of host antiviral proteins (*e.g.*, IFN) or hinders viral virion assembly remains unresolved, warranting further investigation. P62, also known as SQSTM1, is an autophagy receptor that can target the ubiquitinated SARS-CoV-2 M protein for autophagic degradation, but not S or E proteins[75]. M^{pro} cleaves p62 at Q354, disabling its ability to traffic M protein to autophagosomes[75]. By preserving M protein integrity, essential for viral envelope formation, M^{pro} ensures virion assembly while evading host autophagy. Concurrently, reduced expression of p62 in COVID-19 patients correlates with reduced TNF and IL-10[76]. Furthermore, melanoma-associated antigen D2 (MAGED2) has been proven to interact with SARS-CoV-2 N protein in the cytoplasm to block its binding to the viral genome[77]. M^{pro} cleaves MAGED2 at Q263, releasing an N-terminal fragment that translocates to the nucleus[77]. This cleavage restores N protein-viral genome interactions, enabling efficient viral genome packaging and replication.

Two additional targets associated with cell cycle and apoptosis are reported. P53 is a well-known regulator of the cell cycle and apoptosis. Intriguingly, it is also evidenced to restrict SARS-CoV-2 replication[78,89]. M^{pro} inhibits the transcriptional activity of p53 in a protease-dependent manner, thereby repressing p53-driven promoter activation and downregulating target genes such as *p21*. This suppression disrupts p53-mediated antiviral responses and cell cycle regulation[78]. The SARS unique domain 2 (SUD2) of NSP3 binds the antiapoptotic gene B cell lymphoma 2 (*Bcl2*) promoter G-quadruplex (G4) sequence (*Bcl2G4*)[90]. M^{pro} synergizes with

SUD2core (SUD2-N+M domains [K412 to S675]) to enhance binding affinity for *Bcl2G4*, suppressing *Bcl2* expression and promoting apoptosis[79] (Fig. 3F and Table 1).

While numerous putative M^{pro} substrates have been discovered through large-scale *in silico* prediction and *in vitro* experiments, the functional consequences of some cleaved proteins remain unvalidated, necessitating further mechanistic exploration by *in vitro* or *in vivo* experiments (Table 2[39,41,91]).

4. M^{pro} is a potential target for the treatment of SARS-CoV-2 infection

4.1. M^{pro} inhibitors in clinical practice

M^{pro} is a pivotal drug target due to its dual role in viral replication and immune evasion. While early breakthroughs highlight M^{pro}'s druggability, thousands of additional candidates, spanning covalent, non-covalent, and allosteric inhibitors, have been identified through high-throughput screening and computational modeling. Inhibitors of M^{pro} have emerged as promising therapeutic agents, with many targeting its catalytic dyad (Cys145−His41) to block enzymatic activity.

Several inhibitors are currently in clinical use or clinical trials (Table 3[92-108]). The best-known pharmaceutical agent is nirmatrelvir (Paxlovid, PF-07321332) from Pfizer. Nirmatrelvir is a representative of reversible covalent inhibitors, targeting M^{pro} by forming a C−S covalent bond with Cys145 and engaging subsites S1, S2, and S4 (Fig. 2C)[109]. Clinical trials and real-world data confirm its efficacy in reducing viral load and improving patient outcomes, making it the only FDA-approved oral antiviral for COVID-19[110-112]. Another inhibitor, ensitrelvir, is a non-covalent small-molecule inhibitor, stabilizing the binding to the M^{pro}'s S1, S2, and S1′ subsites through a hydrogen bond network (Fig. 2D)[113]. Ensitrelvir has demonstrated antiviral efficacy and the ability to relieve symptoms in clinical trials[95].

Preclinical studies suggest that combining nirmatrelvir with other antivirals may yield synergistic effects. For instance, RdRp inhibitors (*e.g.*, molnupiravir, remdesvir) and HCB NS5A inhibitors (*e.g.*, ombitasvir) have been shown to increase suppression of SARS-CoV-2 compared to nimatrelvir monotherapy[114-116]. However, large-scale clinical evidence remains limited. A target trial emulation (n = 18,196) reports no added benefit from combining Paxlovid with remdesivir[110]. Systematic reviews caution that multidrug regimens may increase the risk of adverse events[117]. In contrast, a randomized controlled trial (RCT) (n = 312) demonstrates that combining Paxlovid with Huashi Baidu granule (an antiviral Chinese patent medicine) significantly improved the therapeutic efficacy against Omicron variant compared to monotherapies[118]. One review highlights that traditional Chinese medicine may synergize with Paxlovid through multi-target and multi-pathway mechanisms, and specific drug interactions arise from its influences on the pharmacokinetics of Paxlovid, which still needs further research to clarify[119]. These findings underscore the potential of integrating traditional Chinese medicine with known antiviral inhibitors. Among other M^{pro} inhibitors, only ensitrelvir has shown enhanced efficacy when combined with corticosteroids in a hamster model[120]. Moving forward, well-designed RCTs are essential to validate combination strategies and address mechanistic uncertainties.

Table 2 Potential substrates of protease M^{pro}.

Substrate	Function	Probable cleavage site	Ref.
OPTN	Ubiquitin-binding protein	Q151 (LQAE) and Q165 (LQLK)	41
CWC22	Component of the RNA spliceosome	RSVLQAQSAS	91
FANCD2	Associated with DNA damage	PSRLQASQVK& EQLLQSTFSV	91
CTBP1	Transcriptional co-repressor protein	REGTRVQ& SVEQIRE	39
IRAK1	Activating NF-κB pathway	/	39
ITCH	E3 ligase	/	41
UBE3A	E3 ligase	/	41

4.2. Resistance of M^{pro} against inhibitors

Although drugs are currently available for COVID-19 infection, viral resistance to M^{pro} inhibitors, driven by genetic mutations, diminishes therapeutic efficacy. Nimatrelvir resistance arises *via* three mutation classes: (1) Reduced binding affinity: Mutations in S1/S4 subsites (F140L, S144A, E166V, L167F, and A193P) impair inhibitor binding. Notably, E166V mutation disrupts a critical hydrogen bond with the lactam ring of nirmatrelvir[121]; (2) Enhanced protease activity: Mutations (T21I in S4′, L50F in S2′, and P252L) increase substrate binding and cleavage efficiency, not only strengthening the protease's ability to cleave viral polyproteins and interfere with host immunity, but also compensating for the reduced protease activity caused by E166V in double (T21I/E166V, L50F/E166V) or triple mutants (L50F/E166V/L167F)[122,123]; (3) Indirect resistance: T304I at the P3 position of the NSP5/6 cleavage site enhances auto-cleavage, indirectly promoting resistance[121]. As for ensitrelvir, it has reduced antiviral effects in M49L or E166V mutants, in which a disrupted hydrogen bond with E166 weakens its interaction with M^{pro}[113,124]. Although an epidemiological study indicates that resistance to M^{pro} inhibitors is currently rare in clinical settings[125], this emergence underscores the need for next-generation inhibitors, which target conserved regions or resistant mutant-specific pockets, and combination therapies to mitigate resistance through multi-target approaches.

4.3. Anti-resistance M^{pro} inhibitors and drugs

Newly developed anti-resistant drugs still include covalent inhibitors, non-covalent inhibitors, and allosteric inhibitors, as well as agents inducing degradation of M^{pro} or targeting host proteins. Covalent inhibitors target M^{pro}'s catalytic Cys145 *via* reactive warheads. Oridonin, a novel covalent inhibitor, forms C−S covalent bonds with Cys145 of M^{pro} through the Michael acceptors without relying on nirmatrelvir-binding subsites, retaining efficacy against nirmatrelvir-resistant mutants (*e.g.*, E166 V/A)[122,126]. It also suppresses NLRP3 and NF-κB pathways, offering dual antiviral and anti-inflammatory effects[127]. TMP1 and ML2006a4 are two inhibitors with α-ketoamide demonstrating antiviral activity against E166 V/A mutants[128,129]. ML2006a4 may extend its P1′ position to provide additional hydrogen bonds and enhanced affinity with M^{pro} mutants[129]. AB-343, with a nitrile group, mimics nirmatrelvir, but shows reduced interactions with nirmatrelvir-resistant mutation sites except E166 to show anti-resistance effects[130].

Table 3 M^{pro} inhibitors in clinical trials[a].

Drug name	R&D company	R&D stage	Listed countries	Indication	Status	Clinical outcome	Ref.
Paxlovid [nirmatrelvir (PF07321332) + ritonavir]	Pfizer	Approval for listing	USA, South Korea, UK, Australia, New Zealand, EU, Canada, Japan, China	COVID-19	/	Significantly improves patients' symptoms	92-94
Ensitrelvir (S-217622)	Shionogi, Inc.	Approval for listing	Japan	COVID-19	/	Significantly improves patients' symptoms	95-97
Leritrelvir (RAY1216)	Guangdong Raynovent Biotech Co., Ltd.	Approval for listing	China	COVID-19	/	Significantly improves patients' symptoms	98,99
Simnotrelvir (SIM-0417 or SSD8432) + ritonavir	Jiangsu Simcere Pharmaceutical Co., Ltd.	Approval for listing	China	COVID-19	/	Significantly improves patients' symptoms	100,101
Atilotrelvir (GST-HG171) + ritonavir	Fujian Guangshengtang Pharmaceutical Co., Ltd.	Approval for listing	China	COVID-19	/	Significantly improves patients' symptoms	102,103
Olgotrelvir (STI-1558)	Sorrento Therapeutics, Inc.	Phase III	/	Mild or moderate COVID-19	Completed	Promising results	104,105
Montelukast	Susanna Naggie, MD, Duke University	Phase III	/	Mild to moderate COVID-19	Completed	Does not reduce duration of patients' symptoms	106
WPV01	Westlake University (Westlake Pharmaceuticals (Hangzhou) Co., Ltd.)	Phase III	/	Mild or moderate COVID-19	Completed	/	/
FB-2001	Frontier Biotechnologies Inc.	Phase III	/	COVID-19	Unknown status	/	/
QLS-1128	Qilu Pharmaceutical Co., Ltd.	Phase III	/	Mild or moderate COVID-19	Unknown status	/	/
PBI-0451 (Pomotrelvir)	Pardes Biosciences, Inc.	Phase II	/	COVID-19	Completed	Does not relieve patients' symptoms	107
EDP-235	Enanta Pharmaceuticals, Inc.	Phase II	/	COVID-19	Completed	/	/
Ibuzatrelvir (PF-07817883)	Pfizer	Phase II	/	COVID-19	Completed	Shows robust antiviral activity	108
HS-10517	Jiangsu Hansoh Pharmaceutical Co., Ltd.	Phase II	/	COVID-19	Unknown status	/	/
Masitinib	AB Science	Phase II	/	Mild or moderate COVID-19	Unknown status	/	/
Ebselen (SPI-1005)	Sound Pharmaceuticals, Inc.	Phase II	/	Moderate to severe COVID-19	Enrolling by invitation	/	/
Pentarlandir™ UPPTA	SyneuRx International (Taiwan) Corp.	Phase II	/	COVID-19	Unknown status	/	/
CDI-988	Cocrystal Pharma, Inc.	Phase I	/	COVID-19	Recruiting	/	/
ASC-11	Ascletis Pharmaceuticals Co., Ltd.	Phase I	/	COVID-19	Completed	/	/
ALG-097558	Aligos Therapeutics	Phase I	/	COVID-19	Completed	/	/

[a]Data from Cinicaltrials.gov and published literature.

As for non-covalent inhibitors, they circumvent catalytic site mutations through alternative binding modes. Besides ensitrelvir, some molecules adopt a macrocyclization strategy to become macrocyclic peptides or macrocyclic lactams[131,132]. NMI-003, C5N17B, and glycyrrhizic acid derivatives are also non-covalent inhibitors and show resistance to some M^{pro} mutants, especially E166V[133−135]. All of them don't form hydrogen bonds with E166[132,134] or even strongly interact with V166 of mutants[133,135]. The above inhibitors effectively inhibit the proliferation of SARS-CoV-2 *in vitro*, no matter whether mutations exist. Some drugs employ a dual-target strategy to resist M^{pro} mutations, such as targeting Spike-ACE2 interaction or TMPRSS2[128,136]. This strategy reduces viral escape probability through complementary targeting. Finally, some allosteric inhibitors have been developed, such as methyl rosmarinate and the compound ZINC4497834, which bind non-catalytic pockets (*e.g.*, dimerization interface), disrupting M^{pro} activity without direct competition[137,138].

In addition, novel degradation strategies, including PROTACs and host target approaches, are also developed. HP211206 and MPD2 (M^{pro} PROTAC degraders 2) recruit E3 ligases (*e.g.*, CRBN) to ubiquitinate and degrade M^{pro} *via* the proteasome, even in E166 V/A mutants[139,140]. Mutations in M^{pro} occur frequently. Thus, developing drugs that target host molecules could lead to unexpected effects. For example, it has been demonstrated that α2M, an inhibitor of LRPAP1, suppresses viral replication[65]. To sum up, the strategies of newly developed inhibitors against M^{pro} mutations mainly include avoiding interactions with mutation sites, changing drug targets, and exploring multi-targets.

While a considerable number of M^{pro} inhibitors demonstrate potential in preclinical development, their clinical efficacy and safety profiles against circulating SARS-CoV-2 variants require rigorous validation. Advances in understanding the structural plasticity, substrate specificity, and host interaction networks of M^{pro} provide critical frameworks for designing next-generation therapies capable of overcoming resistance mechanisms and achieving clinical impacts. The structural and mechanistic diversity of M^{pro} inhibitors also underscores their potential as broad-spectrum antivirals. Future efforts should not only prioritize optimizing pharmacokinetics, selectivity, and resistance profiles to advance clinical candidates, but also optimize drug delivery systems and explore combination therapies to improve clinical efficacy.

5. Summary and perspective

M^{pro} is indispensable for viral replication, cleaving SARS-CoV-2 polyproteins pp1a/pp1ab into functional non-structural proteins. Beyond this role, M^{pro} actively subverts host antiviral innate immunity by targeting antiviral innate immune signaling components. While several host substrates or targets have been identified, key questions remain unsolved about substrate specificity and cleavage dynamics: M^{pro} exhibits context-dependent selectivity, preferentially cleaving substrates with Gln at the P1 position but tolerating flexibility at other subsites (*e.g.*, S4 hydrophobic pocket). However, a detailed understanding of its substrate specificity and the kinetics of these cleavage events in the regulation of innate immunity will be of great interest. The large-scale prediction suggests numerous unvalidated host proteins as potential substrates of M^{pro}. There is an urgent need to elucidate how M^{pro} interacts with unidentified host proteins and the related signaling pathways to suppress or manipulate the immune response.

Although M^{pro} is primarily characterized as a protease, emerging evidence highlights its enzymatic activity-independent roles in subverting antiviral innate immunity. The following issues remain to be resolved: How M^{pro} alters ubiquitin-dependent immune signaling functions by acting as a ubiquitin E3 ligase or a deubiquitinase mimic; whether M^{pro} exploits autophagy receptors to degrade antiviral proteins; how M^{pro} disrupts host protein−protein interactions; and how the non-catalytic regions of M^{pro} (such as dimerization domains or flexible loops) contribute to immune evasion functions. It is also necessary to address whether M^{pro} modulates host epigenetic regulation (*e.g.*, histone modifications, DNA methylation) of antiviral genes to mediate immune evasion and how M^{pro} interferes with crosstalk between innate immunity and adaptive immunity. Future studies should prioritize native infection systems over M^{pro} overexpression models. This can be achieved by utilizing polarized airway/organoid cultures and primary human cell panels. Additionally, integrating time-resolved multi-omics approaches (*e.g.*, phosphoproteomics, ubiquitinomics) with spatial transcriptomics will provide a comprehensive understanding of the kinetic functions of M^{pro} in innate immunity regulation. These strategies will help overcome the limitations of previous studies and yield more reliable and insightful conclusions.

With the ongoing evolution of SARS-CoV-2, how mutations in M^{pro} affect its function and resistance to inhibitors has been partly explained. It is yet unknown if M^{pro} co-evolves with other viral proteins to maximize immune evasion. More research is required to determine their impact on host−pathogen interplay, such as altered virulence, enhanced immune evasion, and elevated drug resistance.

Therefore, future research on SARS-CoV-2 M^{pro} should prioritize integrating structural insights, host−pathogen interactomics, and translational innovation to address unresolved mechanisms of immune evasion and drug resistance. Next-generation therapies have the potential to achieve broader efficacy against current and emerging coronaviruses by targeting both viral replication and immune suppression. It will take coordinated efforts from the fields of virology, immunology, and drug discovery to accomplish this aim.

Acknowledgments

This study was supported by Beijing Municipal Natural Science Foundation (M23006, China). Graphical abstract and Fig. 3 were created with BioRender.com.

Author contributions

Jun Zhang and Yumeng Gao discussed and determined the theme together. Yumeng Gao wrote the review, and Jun Zhang revised the review.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. *Nature* 2020;**579**:265−9.
2. Yang H, Rao Z. Structural biology of SARS-CoV-2 and implications for therapeutic development. *Nat Rev Microbiol* 2021;**19**:685−700.

3. Redondo N, Zaldívar-López S, Garrido JJ, Montoya M. SARS-CoV-2 accessory proteins in viral pathogenesis: knowns and unknowns. *Front Immunol* 2021;**12**:708264.
4. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;**181**:271−80.e8.
5. Essalmani R, Jain J, Susan-Resiga D, Andréo U, Evagelidis A, Derbali RM, et al. Distinctive roles of furin and TMPRSS2 in SARS-CoV-2 infectivity. *J Virol* 2022;**96**:e0012822.
6. Wang K, Chen W, Zhang Z, Deng Y, Lian JQ, Du P, et al. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduct Targeted Ther* 2020;**5**:283.
7. Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* 2020;**370**:856−60.
8. Clausen TM, Sandoval DR, Spliid CB, Pihl J, Perrett HR, Painter CD, et al. SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. *Cell* 2020;**183**:1043−57.e15.
9. Chow KT, Gale M Jr, Loo YM. RIG-I and other RNA sensors in antiviral immunity. *Annu Rev Immunol* 2018;**36**:667−94.
10. Thorne LG, Reuschl A, Zuliani-Alvarez L, Whelan MVX, Turner J, Noursadeghi M, et al. SARS-CoV-2 sensing by RIG-I and MDA5 links epithelial infection to macrophage inflammation. *EMBO J* 2021;**40**:e107826.
11. Yin X, Riva L, Pu Y, Martin-Sancho L, Kanamune J, Yamamoto Y, et al. MDA5 governs the innate immune response to SARS-CoV-2 in lung epithelial cells. *Cell Rep* 2021;**34**:108628.
12. Yang DM, Geng TT, Harrison AG, Wang PH. Differential roles of RIG-I like receptors in SARS-CoV-2 infection. *Mil Med Res* 2021;**8**: 49.
13. Rehwinkel J, Gack MU. RIG-I-like receptors: their regulation and roles in RNA sensing. *Nat Rev Immunol* 2020;**20**:537−51.
14. Yamada T, Sato S, Sotoyama Y, Orba Y, Sawa H, Yamauchi H, et al. RIG-I triggers a signaling-abortive anti-SARS-CoV-2 defense in human lung cells. *Nat Immunol* 2021;**22**:820−8.
15. Zhou Z, Zhang X, Lei X, Xiao X, Jiao T, Ma R, et al. Sensing of cytoplasmic chromatin by cGAS activates innate immune response in SARS-CoV-2 infection. *Signal Transduct Targeted Ther* 2021;**6**:382.
16. Shteinfer-Kuzmine A, Verma A, Bornshten R, Ben Chetrit E, Ben-Ya'acov A, Pahima H, et al. Elevated serum mtDNA in COVID-19 patients is linked to SARS-CoV-2 envelope protein targeting mitochondrial VDAC1, inducing apoptosis and mtDNA release. *Apoptosis* 2024;**29**:2025−46.
17. Pan P, Shen M, Yu Z, Ge W, Chen K, Tian M, et al. SARS-CoV-2 N protein promotes NLRP3 inflammasome activation to induce hyperinflammation. *Nat Commun* 2021;**12**:4664.
18. Liang S, Bao C, Yang Z, Liu S, Sun Y, Cao W, et al. SARS-CoV-2 spike protein induces IL-18-mediated cardiopulmonary inflammation *via* reduced mitophagy. *Signal Transduct Targeted Ther* 2023;**8**:108.
19. Junqueira C, Crespo Â, Ranjbar S, de Lacerda LB, Lewandrowski M, Ingber J, et al. FcγR-mediated SARS-CoV-2 infection of monocytes activates inflammation. *Nature* 2022;**606**:576−84.
20. Planès R, Bert JB, Tairi S, BenMohamed L, Bahraoui E. SARS-CoV-2 envelope (E) protein binds and activates TLR2 pathway: a novel molecular target for COVID-19 interventions. *Viruses* 2022;**14**:999.
21. Bortolotti D, Gentili V, Rizzo S, Schiuma G, Beltrami S, Strazzabosco G, et al. TLR3 and TLR7 RNA sensor activation during SARS-CoV-2 infection. *Microorganisms* 2021;**9**:1820.
22. Fu YZ, Wang SY, Zheng ZQ, Huang Yi, Li WW, Xu ZS, et al. SARS-CoV-2 membrane glycoprotein M antagonizes the MAVS-mediated innate antiviral response. *Cell Mol Immunol* 2021;**18**:613−20.
23. Wu J, Shi Y, Pan X, Wu S, Hou R, Zhang Y, et al. SARS-CoV-2 ORF9b inhibits RIG-I-MAVS antiviral signaling by interrupting K63-linked ubiquitination of NEMO. *Cell Rep* 2021;**34**:108761.
24. Xia H, Cao Z, Xie X, Zhang X, Chen JY, Wang H, et al. Evasion of type I interferon by SARS-CoV-2. *Cell Rep* 2020;**33**:108234.
25. Yuen CK, Lam JY, Wong WM, Mak LF, Wang X, Chu H, et al. SARS-CoV-2 nsp 13, nsp14, nsp15 and orf6 function as potent interferon antagonists. *Emerg Microb Infect* 2020;**9**:1418−28.
26. Li JY, Liao CH, Wang Q, Tan YJ, Luo R, Qiu Y, et al. The ORF6, ORF8 and nucleocapsid proteins of SARS-CoV-2 inhibit type I interferon signaling pathway. *Virus Res* 2020;**286**:198074.
27. Lei X, Dong X, Ma R, Wang W, Xiao X, Tian Z, et al. Activation and evasion of type I interferon responses by SARS-CoV-2. *Nat Commun* 2020;**11**:3810.
28. Miorin L, Kehrer T, Sanchez-Aparicio MT, Zhang K, Cohen P, Patel RS, et al. SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. *Proc Natl Acad Sci U S A* 2020;**117**:28344−54.
29. Singh R, Purohit R. Multi-target approach against SARS-CoV-2 by stone apple molecules: a master key to drug design. *Phytother Res* 2024;**38**:7−10.
30. Singh R, Bhardwaj VK, Purohit R. Potential of turmeric-derived compounds against RNA-dependent RNA polymerase of SARS-CoV-2: an *in-silico* approach. *Comput Biol Med* 2021;**139**:104965.
31. Bhardwaj VK, Singh R, Sharma J, Rajendran V, Purohit R, Kumar S. Identification of bioactive molecules from tea plant as SARS-CoV-2 main protease inhibitors. *J Biomol Struct Dyn* 2021; **39**:3449−58.
32. Tuttle JB, Allais C, Allerton CMN, Anderson AS, Arcari JT, Aschenbrenner LM, et al. Discovery of nirmatrelvir (PF-07321332): a potent, orally active inhibitor of the severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) main protease. *J Med Chem* 2025;**68**:7003−30.
33. Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science* 2020;**368**: 409−12.
34. Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, et al. Structure of M^{pro} from SARS-CoV-2 and discovery of its inhibitors. *Nature* 2020;**582**: 289−93.
35. Xiong M, Su H, Zhao W, Xie H, Shao Q, Xu Y. What coronavirus 3C-like protease tells us: from structure, substrate selectivity, to inhibitor design. *Med Res Rev* 2021;**41**:1965−98.
36. Kneller DW, Phillips G, O'Neill HM, Jedrzejczak R, Stols L, Langan P, et al. Structural plasticity of SARS-CoV-2 3CL Mpro active site cavity revealed by room temperature X-ray crystallography. *Nat Commun* 2020;**11**:3202.
37. Lee J, Worrall LJ, Vuckovic M, Rosell FI, Gentile F, Ton AT, et al. Crystallographic structure of wild-type SARS-CoV-2 main protease acyl-enzyme intermediate with physiological C-terminal autoprocessing site. *Nat Commun* 2020;**11**:5877.
38. Lee J, Kenward C, Worrall LJ, Vuckovic M, Gentile F, Ton AT, et al. X-ray crystallographic characterization of the SARS-CoV-2 main protease polyprotein cleavage sites essential for viral processing and maturation. *Nat Commun* 2022;**13**:5196.
39. Miczi M, Golda M, Kunkli B, Nagy T, Tőzsér J, Mótyán JA. Identification of host cellular protein substrates of SARS-COV-2 main protease. *IJMS* 2020;**21**:9523.
40. Scott BM, Lacasse V, Blom DG, Tonner PD, Blom NS. Predicted coronavirus Nsp5 protease cleavage sites in the human proteome. *BMC Genom Data* 2022;**23**:25.
41. Koudelka T, Boger J, Henkel A, Schönherr R, Krantz S, Fuchs S, et al. *N*-Terminomics for the identification of *in vitro* substrates and cleavage site specificity of the SARS-CoV-2 main protease. *Proteomics* 2021;**21**:e2000246.
42. Chen J, Li Z, Guo J, Xu S, Zhou J, Chen Q, et al. SARS-CoV-2 nsp5 exhibits stronger catalytic activity and interferon antagonism than its SARS-CoV ortholog. *J Virol* 2022;**96**:e0003722.
43. Song S, Kim Y, Kwak K, Lee H, Park H, Kim YB, et al. The N-terminal peptide of the main protease of SARS-CoV-2, targeting dimer interface, inhibits its proteolytic activity. *BMB Rep* 2023;**56**: 606−11.

44. Liang W, Gu M, Zhu L, Yan Z, Schenten D, Herrick S, et al. The main protease of SARS-CoV-2 downregulates innate immunity *via* a translational repression. *Signal Transduct Targeted Ther* 2023;**8**:162.
45. Liu Y, Qin C, Rao Y, Ngo C, Feng JJ, Zhao J, et al. SARS-CoV-2 Nsp5 demonstrates two distinct mechanisms targeting RIG-I and MAVS to evade the innate immune response. *mBio* 2021;**12**:e0233521.
46. Zheng Y, Deng J, Han L, Zhuang MW, Xu Y, Zhang J, et al. SARS-CoV-2 NSP5 and N protein counteract the RIG-I signaling pathway by suppressing the formation of stress granules. *Signal Transduct Targeted Ther* 2022;**7**:22.
47. Wu Y, Ma L, Zhuang Z, Cai S, Zhao Z, Zhou L, et al. Main protease of SARS-CoV-2 serves as a bifunctional molecule in restricting type I interferon antiviral signaling. *Signal Transduct Targeted Ther* 2020; **5**:221.
48. Li W, Qiao J, You Q, Zong S, Peng Q, Liu Y, et al. SARS-CoV-2 Nsp5 activates NF-κB pathway by upregulating SUMOylation of MAVS. *Front Immunol* 2021;**12**:750969.
49. Lear TB, Boudreau ÁN, Lockwood KC, Chu E, Camarco DP, Cao Q, et al. E3 ubiquitin ligase ZBTB25 suppresses beta coronavirus infection through ubiquitination of the main viral protease MPro. *J Biol Chem* 2023;**299**:105388.
50. Zhou L, Liu R, Pathak H, Wang X, Jeong GH, Kumari P, et al. Ubiquitin ligase parkin regulates the stability of SARS-CoV-2 main protease and suppresses viral replication. *ACS Infect Dis* 2024;**10**: 879−89.
51. Fani M, Teimoori A, Ghafari S. Comparison of the COVID-2019 (SARS-CoV-2) pathogenesis with SARS-CoV and MERS-CoV infections. *Future Virol* 2020;**15**:317−23.
52. Zhang Y, Kandwal S, Fayne D, Stevenson NJ. MERS-CoV-nsp5 expression in human epithelial BEAS 2b cells attenuates type I interferon production by inhibiting IRF3 nuclear translocation. *Cell Mol Life Sci* 2024;**81**:433.
53. Martiáñez-Vendrell X, van Kasteren PB, Myeni SK, Kikkert M. HCoV-229E Mpro suppresses RLR-mediated innate immune signalling through cleavage of NEMO and through other mechanisms. *Int J Mol Sci* 2025;**26**:1197.
54. Almutairi GO, Malik A, Alonazi M, Khan JM, Alhomida AS, Khan MS, et al. Expression, purification, and biophysical characterization of recombinant MERS-CoV main (Mpro) protease. *Int J Biol Macromol* 2022;**209**:984−90.
55. Wu A, Wang Y, Zeng C, Huang X, Xu S, Su C, et al. Prediction and biochemical analysis of putative cleavage sites of the 3C-like protease of Middle East respiratory syndrome coronavirus. *Virus Res* 2015;**208**:56−65.
56. Xiong Y, Huang B, Yang Y, Fu X, Fu Z, Xu H, et al. The substrate selectivity of papain-like proteases from human-infecting coronaviruses correlates with innate immune suppression. *Sci Signal* 2023;**16**: eade1985.
57. Shin D, Mukherjee R, Grewe D, Bojkova D, Baek K, Bhattacharya A, et al. Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. *Nature* 2020;**587**:657−62.
58. Kim SS, Sze L, Liu C, Lam KP. The stress granule protein G3BP1 binds viral dsRNA and RIG-I to enhance interferon-β response. *J Biol Chem* 2019;**294**:6430−8.
59. Chen ST, Chen L, Lin DS, Chen SY, Tsao YP, Guo H, et al. NLRP12 regulates anti-viral RIG-I activation *via* interaction with TRIM25. *Cell Host Microbe* 2019;**25**:602−16.e7.
60. Moustaqil M, Ollivier E, Chiu HP, Van Tol S, Rudolffi-Soto P, Stevens C, et al. SARS-CoV-2 proteases PLpro and 3CLpro cleave IRF3 and critical modulators of inflammatory pathways (NLRP12 and TAB1): implications for disease presentation across species. *Emerg Microb Infect* 2021;**10**:178−95.
61. Rui Y, Su J, Shen S, Hu Y, Huang D, Zheng W, et al. Unique and complementary suppression of cGAS−STING and RNA sensing-triggered innate immune responses by SARS-CoV-2 proteins. *Signal Transduct Targeted Ther* 2021;**6**:123.
62. Fung SY, Siu KL, Lin H, Yeung ML, Jin DY. SARS-CoV-2 main protease suppresses type I interferon production by preventing nuclear translocation of phosphorylated IRF3. *Int J Biol Sci* 2021;**17**: 1547−54.
63. Zhang W, Ma Z, Wu Y, Shi X, Zhang Y, Zhang M, et al. SARS-CoV-2 3C-like protease antagonizes interferon-beta production by facilitating the degradation of IRF3. *Cytokine* 2021;**148**: 155697.
64. Naik NG, Lee SC, Veronese BHS, Ma Z, Toth Z. Interaction of HDAC2 with SARS-CoV-2 NSP5 and IRF3 is not required for NSP5-mediated inhibition of type I interferon signaling pathway. *Microbiol Spectr* 2022;**10**:e0232222.
65. Li H, Wang X, Wang Y, Li Y, Chen Y, Wong YT, et al. Secreted LRPAP1 binds and triggers IFNAR1 degradation to facilitate virus evasion from cellular innate immunity. *Signal Transduct Targeted Ther* 2023;**8**:374.
66. Song L, Wang D, Abbas G, Li M, Cui M, Wang J, et al. The main protease of SARS-CoV-2 cleaves histone deacetylases and DCP1A, attenuating the immune defense of the interferon-stimulated genes. *J Biol Chem* 2023;**299**:102990.
67. Wu Y, Li M, Tian J, Yan H, Pan Y, Shi H, et al. Broad antagonism of coronaviruses nsp5 to evade the host antiviral responses by cleaving POLDIP3. *PLoS Pathog* 2023;**19**:e1011702.
68. Zhang S, Wang J, Cheng G. Protease cleavage of RNF20 facilitates coronavirus replication *via* stabilization of SREBP1. *Proc Natl Acad Sci U S A* 2021;**118**:e2107108118.
69. Shen S, Guo H, Li Y, Zhang L, Tang Y, Li H, et al. SARS-CoV-2 and oncolytic EV-D68-encoded proteases differentially regulate pyroptosis. *J Virol* 2024;**98**:e0190923.
70. Shi F, Lv Q, Wang T, Xu J, Xu W, Shi Y, et al. Coronaviruses Nsp5 antagonizes porcine Gasdermin D-mediated pyroptosis by cleaving pore-forming p30 fragment. *mBio* 2022;**13**:e0273921.
71. Planès R, Pinilla M, Santoni K, Hessel A, Passemar C, Lay K, et al. Human NLRP1 is a sensor of pathogenic coronavirus 3CL proteases in lung epithelial cells. *Mol Cell* 2022;**82**:2385−400.e9.
72. Grin PM, Baid K, de Jesus HC, Kozarac N, Bell PA, Jiang SZ, et al. SARS-CoV-2 3CLpro (main protease) regulates caspase activation of gasdermin-D/E pores leading to secretion and extracellular activity of 3CLpro. *Cell Rep* 2024;**43**:115080.
73. Park H, Lee SM, Jeong SJ, Kweon YC, Shin GW, Kim WY, et al. A gain-of-function cleavage of TonEBP by coronavirus NSP5 to suppress IFN-β expression. *Cells* 2024;**13**:1614.
74. Lu JL, Zhou XL. SARS-CoV-2 main protease Nsp5 cleaves and inactivates human tRNA methyltransferase TRMT1. *J Mol Cell Biol* 2023;**15**:mjad024.
75. Zhang Y, Liu S, Xu Q, Li H, Lu K. Cleavage of the selective autophagy receptor SQSTM1/p62 by the SARS-CoV-2 main protease NSP5 prevents the autophagic degradation of viral membrane proteins. *Mol Biomed* 2022;**3**:17.
76. Paunovic V, Vucicevic L, Misirkic Marjanovic M, Perovic V, Ristic B, Bosnjak M, et al. Autophagy receptor p62 regulates SARS-CoV-2-induced inflammation in COVID-19. *Cells* 2023;**12**: 1282.
77. Ju X, Wang Z, Wang P, Ren W, Yu Y, Yu Y, et al. SARS-CoV-2 main protease cleaves MAGED2 to antagonize host antiviral defense. *mBio* 2023;**14**:e0137323.
78. Kumar A, Grams TR, Bloom DC, Toth Z. Signaling pathway reporter screen with SARS-CoV-2 proteins identifies nsp5 as a repressor of p53 activity. *Viruses* 2022;**14**:1039.
79. Li Y, Yu Q, Huang R, Chen H, Ren H, Ma L, et al. SARS-CoV-2 SUD2 and Nsp5 conspire to boost apoptosis of respiratory epithelial cells *via* an augmented interaction with the G-quadruplex of BclII. *mBio* 2023;**14**:e0335922.
80. Dunphy G, Flannery SM, Almine JF, Connolly DJ, Paulus C, Jønsson KL, et al. Non-canonical activation of the DNA sensing adaptor STING by ATM and IFI16 mediates NF-κB signaling after nuclear DNA damage. *Mol Cell* 2018;**71**:745−760.e5.
81. Hopfner KP, Hornung V. Molecular mechanisms and cellular functions of cGAS−STING signalling. *Nat Rev Mol Cell Biol* 2020;**21**: 501−21.

82. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 2014;**32**: 513−45.
83. Shemesh M, Aktepe TE, Deerain JM, McAuley JL, Audsley MD, David CT, et al. SARS-CoV-2 suppresses IFNβ production mediated by NSP1, 5, 6, 15, ORF6 and ORF7b but does not suppress the effects of added interferon. *PLoS Pathog* 2021;**17**:e1009800.
84. Tuladhar S, Kanneganti TD. NLRP12 in innate immunity and inflammation. *Mol Aspects Med* 2020;**76**:100887.
85. Honda K, Takaoka A, Taniguchi T. Type I inteferon gene induction by the interferon regulatory factor family of transcription factors. *Immunity* 2006;**25**:349−60.
86. Ting JP, Duncan JA, Lei Y. How the non-inflammasome NLRs function in the innate immune system. *Science* 2010;**327**:286−90.
87. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 2015;**526**:660−5.
88. Dewe JM, Fuller BL, Lentini JM, Kellner SM, Fu D. TRMT1-catalyzed tRNA modifications are required for redox homeostasis to ensure proper cellular proliferation and oxidative stress survival. *Mol Cell Biol* 2017;**37**:e00214-17.
89. Rivas C, Aaronson SA, Munoz-Fontela C. Dual role of p53 in innate antiviral immunity. *Viruses* 2010;**2**:298−313.
90. Lavigne M, Helynck O, Rigolet P, Boudria-Souilah R, Nowakowski M, Baron B, et al. SARS-CoV-2 Nsp3 unique domain SUD interacts with guanine quadruplexes and G4-ligands inhibit this interaction. *Nucleic Acids Res* 2021;**49**:7695−712.
91. Meyers JM, Ramanathan M, Shanderson RL, Beck A, Donohue L, Ferguson I, et al. The proximal proteome of 17 SARS-CoV-2 proteins links to disrupted antiviral signaling and host translation. *PLoS Pathog* 2021;**17**:e1009412.
92. Owen DR, Allerton CMN, Anderson AS, Aschenbrenner L, Avery M, Berritt S, et al. An oral SARS-CoV-2 M^{pro} inhibitor clinical candidate for the treatment of COVID-19. *Science* 2021;**374**:1586−93.
93. Hammond J, Leister-Tebbe H, Gardner A, Abreu P, Bao W, Wisemandle W, et al. Oral nirmatrelvir for high-risk, nonhospitalized adults with COVID-19. *N Engl J Med* 2022;**386**:1397−408.
94. Lamb YN. Nirmatrelvir plus ritonavir: first approval. *Drugs* 2022;**82**: 585−91.
95. Shimizu R, Sonoyama T, Fukuhara T, Kuwata A, Matsuo Y, Kubota R. A phase 1 study of ensitrelvir fumaric acid tablets evaluating the safety, pharmacokinetics and food effect in healthy adult populations. *Clin Drug Invest* 2023;**43**:785−97.
96. Mukae H, Yotsuyanagi H, Ohmagari N, Doi Y, Imamura T, Sonoyama T, et al. A randomized phase 2/3 study of ensitrelvir, a novel oral SARS-CoV-2 3C-like protease inhibitor, in Japanese patients with mild-to-moderate COVID-19 or asymptomatic SARS-CoV-2 infection: results of the phase 2a part. *Antimicrob Agents Chemother* 2022;**66**:e0069722.
97. Yotsuyanagi H, Ohmagari N, Doi Y, Yamato M, Bac NH, Cha BK, et al. Efficacy and safety of 5-day oral ensitrelvir for patients with mild to moderate COVID-19. *JAMA Netw Open* 2024;**7**:e2354991.
98. Wang B, Li H, Cai M, Lin Z, Ou X, Wu S, et al. Antiviral efficacy of RAY1216 monotherapy and combination therapy with ritonavir in patients with COVID-19: a phase 2, single centre, randomised, double-blind, placebo-controlled trial. *eClinicalMedicine* 2023;**63**: 102189.
99. Zhan Y, Lin Z, Liang J, Sun R, Li Y, Lin B, et al. Leritrelvir for the treatment of mild or moderate COVID-19 without co-administered ritonavir: a multicentre randomised, double-blind, placebo-controlled phase 3 trial. *eClinicalMedicine* 2023;**67**:102359.
100. Yang XM, Yang Y, Yao BF, Ye PP, Xu Y, Peng SP, et al. A first-in-human phase 1 study of simnotrelvir, a 3CL-like protease inhibitor for treatment of COVID-19, in healthy adult subjects. *Eur J Pharmaceut Sci* 2023;**191**:106598.
101. Cao B, Wang Y, Lu H, Huang C, Yang Y, Shang L, et al. Oral simnotrelvir for adult patients with mild-to-moderate Covid-19. *N Engl J Med* 2024;**390**:230−41.
102. Zhang H, Zhou J, Chen H, Mao J, Tang Y, Yan W, et al. Phase I study, and dosing regimen selection for a pivotal COVID-19 trial of GST-HG171. *Antimicrob Agents Chemother* 2024;**68**:e0111523.
103. Lu H, Zhang G, Mao J, Chen X, Zhan Y, Lin L, et al. Efficacy and safety of GST-HG171 in adult patients with mild to moderate COVID-19: a randomised, double-blind, placebo-controlled phase 2/3 trial. *eClinicalMedicine* 2024;**71**:102582.
104. Mao L, Shaabani N, Zhang X, Jin C, Xu W, Argent C, et al. Olgotrelvir, a dual inhibitor of SARS-CoV-2 Mpro and cathepsin L, as a standalone antiviral oral intervention candidate for COVID-19. *Med* 2024;**5**:169−71.
105. Jiang R, Han B, Xu W, Zhang X, Peng C, Dang Q, et al. Olgotrelvir as a single-agent treatment of nonhospitalized patients with Covid-19. *NEJM Evid* 2024;**3**:EVIDoa2400026.
106. Rothman RL, Stewart TG, Mourad A, Boulware DR, McCarthy MW, Thicklin F, et al. Time to sustained recovery among outpatients with COVID-19 receiving montelukast *vs* placebo: the ACTIV-6 randomized clinical trial. *JAMA Netw Open* 2024;**7**:e2439332.
107. Borroto-Esoda K, Wilfret D, Tong X, Plummer A, Kearney B, Kwong AD. SARS-CoV-2 viral dynamics in a placebo-controlled phase 2 study of patients infected with the SARS-CoV-2 Omicron variant and treated with pomotrelvir. *Microbiol Spectr* 2024;**12**:e0298023.
108. Mortezavi M, Sloan A, Singh RSP, Chen LF, Kim JH, Shojaee N, et al. Virologic response and safety of ibuzatrelvir, a novel SARS-CoV-2 antiviral, in adults with COVID-19. *Clin Infect Dis* 2025;**80**:673−80.
109. Zhao Y, Fang C, Zhang Q, Zhang R, Zhao X, Duan Y, et al. Crystal structure of SARS-CoV-2 main protease in complex with protease inhibitor PF-07321332. *Protein Cell* 2022;**13**:689−93.
110. Choi MH, Wan EY, Wong IC, Chan EW, Chu WM, Tam AR, et al. Comparative effectiveness of combination therapy with nirmatrelvir−ritonavir and remdesivir *versus* monotherapy with remdesivir or nirmatrelvir−ritonavir in patients hospitalised with COVID-19: a target trial emulation study. *Lancet Infect Dis* 2024;**24**:1213−24.
111. Jiang J, Li Y, Jiang Q, Jiang Y, Qin H, Li Y. Early use of oral antiviral drugs and the risk of post COVID-19 syndrome: a systematic review and network meta-analysis. *J Infect* 2024;**89**:106190.
112. Rubin R. The latest research about paxlovid: effectiveness, access, and possible long COVID benefits. *JAMA* 2024;**332**:1040−2.
113. Lin M, Zeng X, Duan Y, Yang Z, Ma Y, Yang H, et al. Molecular mechanism of ensitrelvir inhibiting SARS-CoV-2 main protease and its variants. *Commun Biol* 2023;**6**:694.
114. Moon C, Porges E, Roberts A, Bacon J. A combination of nirmatrelvir and ombitasvir boosts inhibition of SARS-CoV-2 replication. *Antiviral Res* 2024;**225**:105859.
115. Fiaschi L, Biba C, Varasi I, Bartolini N, Paletti C, Giammarino F, et al. *In vitro* combinatorial activity of direct acting antivirals and monoclonal antibodies against the ancestral B.1 and BQ.1.1 SARS-CoV-2 viral variants. *Viruses* 2024;**16**:168.
116. Gidari A, Sabbatini S, Schiaroli E, Bastianelli S, Pierucci S, Busti C, et al. Synergistic activity of remdesivir−nirmatrelvir combination on a SARS-CoV-2 *in vitro* model and a case report. *Viruses* 2023;**15**:1577.
117. Hirsch C, Kreuzberger N, Skoetz N, Monsef I, Kluge S, Spinner CD, et al. Efficacy and safety of antiviral therapies for the treatment of persistent COVID-19 in immunocompromised patients since the Omicron surge: a systematic review. *J Antimicrob Chemother* 2025;**80**: 633−44.
118. Yu Z, Zheng Y, Chen B, Lv J, Zhu X, Shang B, et al. Efficacy and safety of Huashi Baidu granule plus Nirmatrelvir-Ritonavir combination therapy in patients with high-risk factors infected with Omicron (B.1.1.529): a multi-arm single-center, open-label, randomized controlled trial. *Phytomedicine* 2023;**120**:155025.
119. Ye L, Fan S, Zhao P, Wu C, Liu M, Hu S, et al. Potential herb−drug interactions between anti-COVID-19 drugs and traditional Chinese medicine. *Acta Pharm Sin B* 2023;**13**:3598−637.
120. Sasaki M, Sugi T, Iida S, Hirata Y, Kusakabe S, Konishi K, et al. Combination therapy with oral antiviral and anti-inflammatory drugs improves the efficacy of delayed treatment in a COVID-19 hamster model. *EBioMedicine* 2024;**99**:104950.

121. Iketani S, Mohri H, Culbertson B, Hong SJ, Duan Y, Luck MI, et al. Multiple pathways for SARS-CoV-2 resistance to nirmatrelvir. *Nature* 2023;**613**:558−64.
122. Duan Y, Zhou H, Liu X, Iketani S, Lin M, Zhang X, et al. Molecular mechanisms of SARS-CoV-2 resistance to nirmatrelvir. *Nature* 2023; **622**:376−82.
123. Lewandowski EM, Zhang X, Tan H, Jaskolka-Brown A, Kohaal N, Frazier A, et al. Distal protein−protein interactions contribute to nirmatrelvir resistance. *Nat Commun* 2025;**16**:1266.
124. Kiso M, Yamayoshi S, Iida S, Furusawa Y, Hirata Y, Uraki R, et al. *In vitro* and *in vivo* characterization of SARS-CoV-2 resistance to ensitrelvir. *Nat Commun* 2023;**14**:4231.
125. Ip JD, Wing-Ho Chu A, Chan WM, Cheuk-Ying Leung R, Umer Abdullah SM, Sun Y, et al. Global prevalence of SARS-CoV-2 3CL protease mutations associated with nirmatrelvir or ensitrelvir resistance. *EBioMedicine* 2023;**91**:104559.
126. Zhong B, Peng W, Du S, Chen B, Feng Y, Hu X, et al. Oridonin inhibits SARS-CoV-2 by targeting its 3C-like protease. *Small Sci* 2022;**2**:2270012.
127. Zhang Z, Zhang H, Zhang Y, Zhang Q, Liu Q, Hu Y, et al. Oridonin inhibits SARS-CoV-2 replication by targeting viral proteinase and polymerase. *Virol Sin* 2023;**38**:470−9.
128. Chu H, Shuai H, Qiao J, Yoon C, Zhang G, Hou Y, et al. An orally available Mpro/TMPRSS2 bispecific inhibitor with potent anti-coronavirus efficacy *in vivo*. *Res Sq* 2024. Available from: https://doi.org/10.21203/rs.3.rs-5454588/v1.
129. Westberg M, Su Y, Zou X, Huang P, Rustagi A, Garhyan J, et al. An orally bioavailable SARS-CoV-2 main protease inhibitor exhibits improved affinity and reduced sensitivity to mutations. *Sci Transl Med* 2024;**16**:eadi0979.
130. McGovern-Gooch KR, Mani N, Gotchev D, Ardzinski A, Kowalski R, Sheraz M, et al. Biological characterization of AB-343, a novel and potent SARS-CoV-2 Mpro inhibitor with pan-coronavirus activity. *Antivir Res* 2024;**232**:106038.
131. Tan Y, Yang J, Wang M, Peng Q, Li Y, Fu L, et al. *De novo* discovery of a noncovalent cell-penetrating bicyclic peptide inhibitor targeting SARS-CoV-2 main protease. *J Med Chem* 2024;**67**:20258−74.
132. Wang X, Gotchev D, Fan KY, Vega MM, Mani N, McGovern-Gooch K, et al. Rational design of macrocyclic noncovalent inhibitors of SARS-CoV-2 Mpro from a DNA-encoded chemical library screening hit that demonstrate potent inhibition against pan-coronavirus homologues and nirmatrelvir-resistant variants. *J Med Chem* 2024;**67**:19623−67.
133. Yang J, Fu B, Gou R, Lin X, Wu H, Xue W. Molecular mechanism-driven discovery of novel small molecule inhibitors against drug-resistant SARS-CoV-2 M^{pro} variants. *J Chem Inf Model* 2024;**64**: 7998−8009.
134. Yang M, Lee MK, Gao S, Song L, Jang H, Jo I, et al. Miniaturized modular click chemistry-enabled rapid discovery of unique SARS-CoV-2 M^{pro} inhibitors with robust potency and drug-like profile. *Adv Sci (Weinh)* 2024;**11**:e2404884.
135. Le UN, Chang YJ, Lu CH, Chen Y, Su WC, Chao ST, et al. Glycyrrhizic acid conjugates with amino acid methyl esters target the main protease, exhibiting antiviral activity against wild-type and nirmatrelvir-resistant SARS-CoV-2 variants. *Antiviral Res* 2024;**227**:105920.
136. Matos-Hernández ML, Samples R, Dyer G, Casimir Montán VM, Morales-Colón CA, Salvino JM, et al. Metabolomic analysis and antiviral screening of a marine algae library yield jobosic acid (2,5-dimethyltetradecanoic acid) as a selective inhibitor of SARS-CoV-2. *J Nat Prod* 2024;**87**:1513−20.
137. Li H, Sun M, Lei F, Liu J, Chen X, Li Y, et al. Methyl rosmarinate is an allosteric inhibitor of SARS-CoV-2 3 CL protease as a potential candidate against SARS-cov-2 infection. *Antiviral Res* 2024;**224**: 105841.
138. Fatima A, Geethakumari AM, Ahmed WS, Biswas KH. A potential allosteric inhibitor of SARS-CoV-2 main protease (M^{pro}) identified through metastable state analysis. *Front Mol Biosci* 2024;**11**: 1451280.
139. Sang X, Wang J, Zhou J, Xu Y, An J, Warshel A, et al. A chemical strategy for the degradation of the main protease of SARS-CoV-2 in cells. *J Am Chem Soc* 2023;**145**:27248−53.
140. Alugubelli YR, Xiao J, Khatua K, Kumar S, Sun L, Ma Y, et al. Discovery of first-in-class PROTAC degraders of SARS-CoV-2 main protease. *J Med Chem* 2024;**67**:6495−507.