2026, 66(3): 1007-1025
CSTR: 32112.14.j.AMS.20250733
DOI: 10.13343/j.cnki.wsxb.20250733

微生物学报
Acta Microbiologica Sinica
http://journals.im.ac.cn/actamicrocn

Review 综述

冠状病毒主蛋白酶功能及抑制剂筛选方法研究进展

熊谋康，朱君海，王玉娥*

西南大学 动物医学院，重庆

熊谋康, 朱君海, 王玉娥. 冠状病毒主蛋白酶功能及抑制剂筛选方法研究进展[J]. 微生物学报, 2026, 66(3): 1007-1025.
XIONG Moukang, ZHU Junhai, WANG Yu'e. Advances in coronavirus main protease functions and inhibitor screening methods[J]. Acta Microbiologica Sinica, 2026, 66(3): 1007-1025.

摘　要：冠状病毒是严重威胁人类与动物健康的病原体。主蛋白酶(main protease, Mpro)在病毒生命周期及宿主免疫调节中均发挥着重要作用，是开发广谱抗冠状病毒药物的关键靶点。Mpro 的核心作用在于特异性切割病毒多聚蛋白 pp1a 和 pp1ab，以释放功能性非结构蛋白(non-structural proteins, NSPs)，进而驱动病毒复制/转录复合体的组装。此外，Mpro 能够靶向切割宿主免疫信号通路中的关键分子，有利于病毒实现免疫逃逸。鉴于其功能的双重性以及催化中心的高度保守性，针对 Mpro 的研究已成为该领域的热点。本文系统概述了 Mpro 的结构特征与功能多样性，重点论述了其在病毒复制周期中的催化机制及介导免疫抑制的作用，进而详细探讨了 Mpro 抑制剂的筛选方法与设计策略，为针对该靶点的抗冠状病毒药物研发提供了理论依据和新思路。

关键词：冠状病毒；主蛋白酶；抑制剂筛选方法；抗病毒策略

Advances in coronavirus main protease functions and inhibitor screening methods

XIONG Moukang, ZHU Junhai, WANG Yu'e*

College of Veterinary Medicine, Southwest University, Chongqing, China

Abstract: Coronaviruses pose a serious threat to human and animal health. Their main protease (Mpro) plays a critical role in both the viral life cycle and host immune regulation, serving as a key target for the development of broad-spectrum anti-coronavirus drugs. The core function of Mpro

资助项目：重庆市自然科学基金(CSTB2024NSCQ-MSX0467)；中央高校基本科研业务费专项资金(SWU-KR22036)
This work was supported by the Natural Science Foundation of Chongqing (CSTB2024NSCQ-MSX0467) and the Fundamental Research Funds for the Central Universities (SWU-KR22036).
*Corresponding author. E-mail: vetyuewang@163.com
Received: 2025-09-27; Accepted: 2025-10-28; Published online: 2025-11-17

lies in its specific cleavage of viral polyproteins pp1a and pp1ab to release functional non-structural proteins (NSPs), thereby driving the assembly of the viral replication/transcription complex. Additionally, Mpro can target and cleave key molecules in host immune signaling pathways, facilitating viral immune evasion. Given its dual roles and highly conserved catalytic center, the research on Mpro has become a major focus in the field. This review systematically outlines the structural features and functional diversity of Mpro, with an emphasis on its catalytic mechanism in the viral replication cycle and its role in mediating immune suppression. Furthermore, this article details the screening methods and design strategies for Mpro inhibitors, aiming to offer theoretical foundations and novel insights for the development of anti-coronavirus drugs targeting this critical protein.

Keywords: coronavirus; main protease; inhibitor screening methods; antiviral strategies

冠状病毒是严重威胁人类与动物健康的重要病原体，其引发的疾病谱系广泛，对全球公共卫生构成持续性挑战[1]。在病毒与宿主的相互作用过程中，宿主的先天免疫反应是抵御病毒入侵的首道防线，而冠状病毒则进化出复杂的策略以逃逸免疫清除，二者间的动态博弈是决定感染进程的关键因素。在这一过程中，病毒编码的主蛋白酶(main protease, Mpro)，又称 3C 样蛋白酶(3-chymotrypsin-like protease, 3CLpro)，发挥着枢纽作用：一方面，它通过切割病毒多聚蛋白 pp1a 和 pp1ab，产生病毒复制与转录所必需的非结构蛋白(non-structural proteins, NSPs)，是病毒生命周期的核心执行者；另一方面，它还可直接靶向切割宿主天然免疫信号通路中的关键分子[如核因子 κB 必需调节因子(nuclear factor kappa-B essential modulator, NEMO)、线粒体抗病毒信号蛋白(mitochondrial antiviral signaling protein, MAVS)等]，从而抑制干扰素的产生与信号转导，成为病毒实现免疫逃逸的新型“武器”[2-4]。鉴于 Mpro 在病毒复制与免疫调节方面具有双重功能，且其活性位点在冠状病毒属内高度保守，它被公认为开发广谱、高效抗病毒药物的理想靶点。本文系统阐述了 Mpro 的结构特征与功能多样性，重点剖析了其在病毒复制周期中的催化机制及免疫调节功能，并深入探讨了 Mpro 抑制剂的筛选策略与研发进展，以期为抗冠状病毒药物研发提供理论依据和新思路。

1 Mpro 的概述

目前，多种高致病性冠状病毒持续存在且存在新发风险，如人类冠状病毒 229E (human coronavirus 229E, HCoV-229E)、严重急性呼吸综合征冠状病毒(severe acute respiratory syndrome coronavirus, SARS-CoV)、中东呼吸综合征冠状病毒(Middle East respiratory syndrome coronavirus, MERS-CoV)、猪德尔塔冠状病毒(porcine deltacoronavirus, PDCoV)、猪流行性腹泻病毒(porcine epidemic diarrhea virus, PEDV)、猪传染性胃肠炎病毒(porcine transmissible gastroenteritis virus, TGEV)、猫传染性腹膜炎病毒(feline infectious peritonitis virus, FIPV)、传染性支气管炎病毒(infectious bronchitis virus, IBV)等，这些病毒对全球公共卫生构成严峻挑战，也凸显了开发广谱抗病毒药物的紧迫性。任何能够精准干预病毒生命周期的环节均有望成为治疗的突破口[5]。本课题组 Xiong 等[6]前期研究证实，Ouabain 和 PST2238 可减少 PEDV 对靶细胞的识别，进而抑制病毒复制。在众多病毒蛋白中 Mpro 因在冠状病毒属中序列和功能高度保守，

且在病毒多聚蛋白加工与免疫逃逸中发挥核心作用，成为广谱药物开发的理想靶点[7]。因此，系统解析 Mpro 的结构与功能机制，推进靶向 Mpro 药物/抑制剂的研发，不仅有助于深化对冠状病毒致病机制的理解，也为应对当前及未来冠状病毒威胁提供了重要的策略方向。

1.1 Mpro 的结构与催化机制

冠状病毒 Mpro 是约 33 kDa 的关键病毒蛋白酶，其三维结构的解析为理解其功能与药物设计奠定了基础[8]。Jin 等[9]首次通过 X 射线衍射法解析了 SARS-CoV-2 Mpro 的晶体结构，分辨率达 2.16 Å (PDB ID：6LU7)。如图 1 所示，Mpro 由 3 个结构域组成：结构域 I (氨基酸残基 8−101)和结构域 II (残基 102−184)以 β-折叠结构为主，负责维持蛋白质的稳定与功能；结构域 III (残基 201−303)由 α-螺旋簇构成，通过一段长环区(残基 185−200)与结构域 II 连接，参与调控蛋白二聚化及稳定性；Mpro 的活性中心位于结构域 I 和 II 之间的沟槽内，包含由 Cys 和 His 组成的催化二元体，以及 S1′、S1、S2 和 S4 等底物结合口袋[10] (冠状病毒 Mpro 结构域及活性位点见表 1)。这些口袋中的关键残基，如 S1′口袋的 Leu27 和 Gly142，S1 口袋的 Phe139、His163、Glu166 和 His172，S2 口袋的 His41、Tyr53 和 Asp187，S4 口袋的 Leu167 和 Gln192，在感染人的冠状病毒 Mpro 中高度保守[19]。Zhou 等[20]对冠状病毒 Mpro 序列进行比对表明，S2 口袋中第 49、54 和 189 位(按 SARS-CoV-2 Mpro 编号)的氨基酸在 α-CoVs 中为 T、Y 和 P，

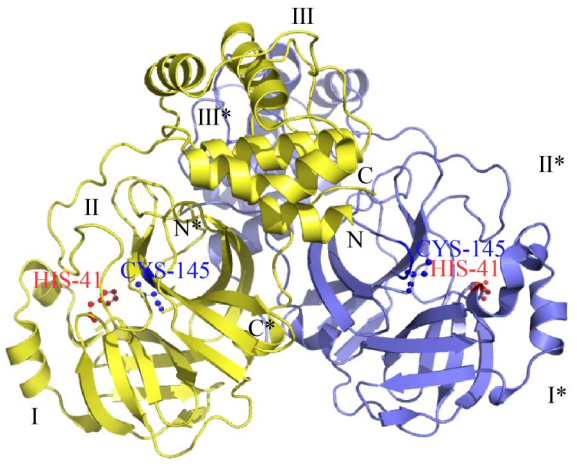

图1 SARS-CoV-2 Mpro三维结构。从2个不同视角呈现，二聚体中一个单体以黄色显示，另一个单体则以浅蓝显示并标注星号(*)；PDB ID: 7EN8。

Figure 1 The 3D structure of SARS-CoV-2 Mpro. Presented from two distinct perspectives, one monomer in the dimer is displayed in yellow, while the other monomer is shown in light blue and marked with an asterisk (*); PDB ID: 7EN8.

表1 冠状病毒Mpro结构域及活性位点

Table 1 Structural domains and the active site of the coronavirus Mpro

Virus genus	Virus	Domain	Active site
α	HCoV-229E	I (8−99), II (100−183), long loop (184−199), III (200−300)	His41 and Cys144[10-13]
	FIPV	−	
	PEDV	−	
	TGEV	I (8−100), II (101−183), long loop (184−199), III (200−302)	
β	MERS-CoV	I (8−101), II (102−184), long loop (185−200), III (201−303)	His41 and Cys148[14]
	SARS-CoV		His41 and Cys145[15-16]
	SARS-CoV-2		
γ	IBV	I (3−99), II (100−182), long loop (183−198), III (199−307)	His41 and Cys143[17]
δ	PDCoV	I (1−97), II (98−186), long loop (187−199), III (200−304)	His41 and Cys144[18]

−表示引用的文献未报道。

− indicates not reported in the cited literature.

在 β-CoVs 中为 M/L、Y 和 Q，在 γ-CoVs 和 δ-CoVs 中则为 K、W 和 E；上述特征在冠状病毒属内高度保守，但在属间存在差异，体现出一定的属特异性。Mpro 不仅在冠状病毒复制中发挥重要作用，更是连接病毒复制与药物干预策略的关键枢纽。理解 Mpro 结构是开发应对当前乃至未来冠状病毒威胁的广谱特效药物的基石。

从催化机制来看，Mpro 催化活性依赖于 Cys145-His41 催化对的协同作用[21]。其基本过程包括：His41 促进 Cys145 的−SH 基团去质子化(图 2)，形成活化的硫醇盐中间体[21,24-25]；该中间体随后对底物肽键的羰基碳发起亲核攻击，形成四面体过渡态；在 His41 的催化作用下促进 N 端产物的释放；最终涉及硫酯键裂解生成含 C 端的多肽水解产物[26-27]。这种催化机制在不同冠状病毒的 Mpro 中高度保守，确保了它们对病毒多聚蛋白的有效加工[28]。

1.2 Mpro 切割方式及成熟机制

Mpro 的成熟是一个精密的级联自切割过程。它以无活性的前体形式存在于多聚蛋白 pp1a/pp1ab 中，必须通过自切割释放自身才能获得活性，进而反式切割多聚蛋白上的其他位点，产生功能性 NSPs。冠状病毒 Mpro 的剪切机制可分为 2 类：(1) Mpro 在成熟过程中的自剪切机制，Mpro 位于多聚蛋白上，在切割多聚蛋白前首先在 N 端进行自切割，随后在 C 端进行切割，进而从多聚蛋白中释放出来，形成具有完整活性的成熟 Mpro[29]；(2) Mpro 的反式剪切机制，即 Mpro 在成熟以后，切割多聚蛋白使 NSPs 从多聚蛋白上释放[30]。

冠状病毒 Mpro 的自切割过程需要膜结构的参与[31-32]。Mpro 形成二聚体时具有最佳的催化功能，但在对多聚蛋白进行切割时可能以单体

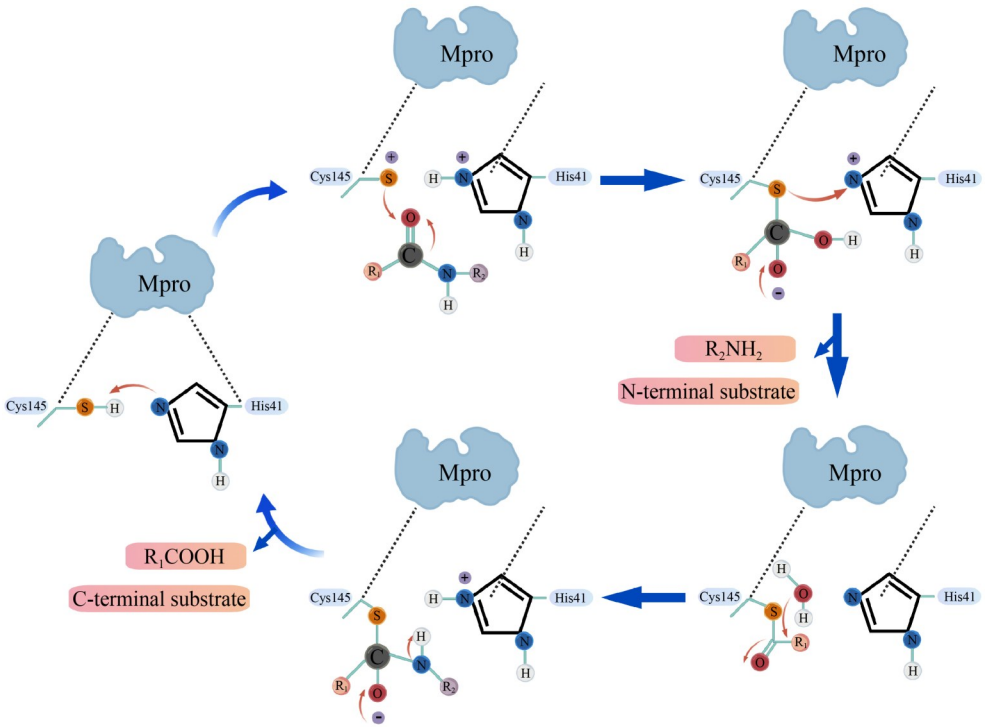

图2 冠状病毒Mpro的催化机制[22]。催化二元组与底物氨基酸分别用蓝色和红色标示。

Figure 2 Hydrolysis mechanism of SARS-CoV-2 Mpro[22]. Amino acids of the catalytic dyad and the substrate are depicted in blue and red, respectively. Created with BioGDP.com[23].

发挥作用[33]。其成熟机制涉及顺式与反式切割的协同：首先，2 个未成熟的 Mpro 单体通过其 N 端前体结构域相互作用形成初始二聚体，从而介导 N 端自切割，此过程可能不依赖于完全成熟的酶[34-36]。对 SARS-CoV-2 Mpro 的高分辨率结构生物学研究为此提供了更清晰的证据。Noske 等[37]发现，未成熟 Mpro 的 N 末端延伸会破坏 S1−S3 活性位点的正确构象并抑制二聚化；该研究证实，初始的 N 端切割可由单体通过顺式作用完成，而有效的二聚体形成则显著增强了这一过程，并伴随 C 端的进一步切割，最终产生结构稳定、功能完备的成熟 Mpro 二聚体；Mpro 与 C145S 突变体共同作用可显著增强 N 末端切割与二聚体的形成，表明 Mpro 初始成熟过程涉及顺式与反式切割；最终 N 末端区域被完全切割并整合至多聚蛋白内部。包含 C 末端的活性位点区域完成成熟，Mpro 多蛋白的 C 末端会采取特殊的旋转构象并与另一个成熟或半成熟 Mpro 活性位点结合；随后，完全成熟的 Mpro 形成并进一步加工病毒多聚蛋白[22]。

Mpro 是一个经历精密自激活的酶，从无活性前体到活性二聚体的成熟过程是整个病毒复制链的关键步骤。Mpro 的二聚化效率与成熟速度直接调控病毒复制的组装进度，进而影响病毒载量和疾病进程，破坏 Mpro 成熟过程能从根本上削弱病毒的致病能力。针对这些环节进行干预，为开发更高效、更广谱、更安全的抗冠状病毒药物开辟了新途径。

1.3 Mpro 在病毒复制周期中的作用

冠状病毒 NSPs 具有多种生理功能，在病毒复制与转录过程中发挥着关键作用[38]。木瓜样蛋白酶(papain-like proteases, PLpro)和 Mpro 通过将多聚蛋白切割为成熟 NSPs，进而在病毒复制和免疫逃逸中发挥核心作用[39-40]。具体而言，Mpro 主要负责切割 nsp4/nsp5、nsp5/nsp6、nsp6/nsp7、nsp7/nsp8、nsp8/nsp9、nsp9/nsp10、nsp10/nsp12、nsp12/nsp13、nsp13/nsp14、nsp14/nsp15、nsp15/nsp16 在内的 11 个位点，这些切割位点在冠状病毒中保守[41]。这种通过多聚蛋白水解产生功能元件的机制是 RNA 病毒调控生理功能的重要策略。鉴于 NSPs 是构成复制转录复合体(replication-transcription complex, RTC)的关键组分，抑制 Mpro 活性可有效阻止 RTC 形成，从而抑制冠状病毒复制。

从作用机制上看，Mpro 的切割序列以切割位点为中心，向 N 端和 C 端延伸形成 P1、P2、P3、P4、P5 及 P1′、P2′、P3′、P4′、P5′等位点，这些位点与 S2′、S1′、S1、S2、S4 等多个底物结合口袋相互作用；其中，P1、P2 和 P1′位点保守性最高，决定底物特异性[42]。Mpro 特异性识别并切割多聚蛋白中的典型序列 Leu-Gln ↓ (Ser, Ala, Gly)。特别需指出的是，P1 位点需为 Gln 残基，P1′位点偏好小侧链氨基酸，P2 位点需具有长侧链的疏水残基(偏好 Leu)[11,43]。基于此，Mpro 抑制剂通过抑制 Mpro 切割活性可显著影响病毒复制[44]。此外，由于人类和动物体内不存在 Mpro 同源蛋白，针对 Mpro 的药物开发具有较低的脱靶风险。

鉴于 Mpro 在冠状病毒复制中不可或缺的地位及切割特性，它被视为一个极具前景的药物靶点。通过解析 Mpro 的功能与底物特异性能够指导药物的合理设计，这不仅对当前冠状病毒感染的治疗至关重要，也为应对未来新发冠状病毒疫情提供了抗病毒药物开发策略。

1.4 Mpro 抑制干扰素产生

病毒感染会触发宿主免疫应答，其中 I 型干扰素(type I interferon, IFN-I)通过快速诱导产生以建立抗病毒状态。IFN-I 在抑制病毒过程中起到关键作用，当宿主模式识别受体(pattern recognition receptors, PRRs)识别病毒组分后可迅速诱导其产生[45]。IFN-I 信号通路通过激活转录因子，进而诱导干扰素刺激基因(interferon-stimulated genes, ISGs)的表达，从而抑制病毒复制并招募和激活免疫细胞[46-47]。然而，冠状病毒采用多种策略逃逸先天免疫，这些策略构成病毒免疫逃逸和适应宿主的基础。关键逃逸机

制包括：(1) 形成双层膜囊泡隔离病毒核酸，避免其被 PRRs 识别；(2) 通过病毒蛋白直接降解天然免疫信号分子[48-49]。研究表明，Mpro 介导的对 IFN 信号转导的抑制在冠状病毒中具有进化保守性[50-52]。表 2 系统阐述了不同冠状病毒 Mpro 对宿主免疫信号关键介导因子的切割作用。综上所述，Mpro 既调节冠状病毒复制，又通过调控宿主信号通路实现免疫逃逸[13]。作为病毒复制过程中至关重要的多功能酶，靶向 Mpro 的蛋白酶活性已成为治疗冠状病毒疾病的重要策略。

2 靶向 Mpro 抑制剂以及筛选方法研究进展

2.1 Mpro 抑制剂

Mpro 抑制剂根据结构特征可分为共价抑制剂、非共价抑制剂两大类。大多数肽类共价抑制剂具有肽骨架与高反应性弹头相结合的特征[59]。过渡态类似物通过模拟酶催化过程中形成的过渡态中间体来抑制 Mpro 活性，这类抑制剂通常具有较高的结合亲和力，能有效阻断酶催化过程。例如，基于 α-酮酰胺的抑制剂与 Cys145 形成半硫缩醛中间体，从而抑制 Mpro 活性[19]。

研究人员对冠状病毒 Mpro 抑制剂进行了深入研究，已提出多种候选药物方案，这些药物需满足低毒性、高溶解性和可逆性等要求[60]。共价抑制剂主要包括 α-酮酰胺类[16]、腈类[61]、磺酸酯类[62-63]、迈克尔受体类[9]、醛类[64]及有机硒化合物[65]。这些抑制剂包含多种亲电弹头，如环氧环[66]、氟甲基[67]、肉桂酸酯[68]和乙烯基酯[69]。

在已开发的共价抑制剂中帕昔洛韦(nirmatrelvir 和 ritonavir)将活性 Mpro 抑制剂与增强剂联合使用，协同提高药物疗效，从而有效抑制 SARS-CoV-2 复制[70-71]。奈玛特韦(nirmatrelvir，PF-07321332) 的前体化合物为 PF-07304814，是一种口服有效的 Mpro 抑制剂；该抑制剂对多种 α 和 β 冠状病毒 Mpro 均显示出高效的抑制活性，包括 SARS-CoV、HKU1、OC43、MERS、229E 和 NL63[72-73]。值得注意的是，一些丙型肝炎病毒(hepatitis C virus, HCV)的抗病毒药物，如 telaprevir、narlaprevir 和 boceprevir 也显示出对 Mpro 的抑制能力[22]。在动物疾病治疗方面，靶向 Mpro 的肽类化合物 GC376 已成功用于抑制 FIPV 复制，并对鼬冠状病毒和貂冠状病毒表现出广谱抑制效果[74-76]。

共价抑制剂通过形成稳定共价键延长药物作用时间，但伴随着潜在的毒性和脱靶效应；非共价抑制剂通过可逆的非共价相互作用与 Mpro 结合，降低了不良反应的风险，并可能提高对 Mpro 的选择性[77-78]。非共价抑制剂如 S-217622 (ensitrelvir) [79]、Masitinib[80]、ML188[81]、WU-04 (mprosevir) [82] 已成为靶向 Mpro 的具有前景的候选药物。对 Mpro 抑制剂

表2 冠状病毒对宿主蛋白切割位点分析

Table 2 Analysis of host protein cleavage sites by coronavirus

Virus genus	Virus	Host protein	Cleavage site
α	HCoV-229E	NEMO[4]	Q83, Q205, and Q231
	FIPV	NEMO[53]	Q132, Q205, and Q231
	PEDV	NEMO[51]	Q231
		HDAC6[54]	Q519
	TGEV	HDAC6[54]	Q519
β	MERS-CoV	MAVS[55]	–
	SARS-CoV	NEMO[52]	E152, Q205, and Q231
	SARS-CoV-2	NEMO[52]	E152, Q205, and Q231
		RIG-I[56]	Q10
δ	PDCoV	NEMO[50]	Q231
		STAT2[57]	Q685 and Q758
		IFIT3[58]	Q406
		HDAC6[54]	Q519

–表示引用文献未报道切割位点。

– indicates that the cleavage site has not been reported in the cited literature.

的研究为抗冠状病毒药物的发现奠定了科学基础，并为未来可能出现的冠状病毒变种提供了潜在治疗策略。鉴于 Mpro 抑制剂的重要应用前景，高效、精准的抑制剂筛选方法已成为加速此类药物研发的关键环节。下文将系统综述目前广泛应用的 Mpro 抑制剂筛选策略。

2.2 Mpro 抑制剂筛选方法

Mpro 抑制剂的筛选方法包括但不限于虚拟筛选(virtual screening)以及表型筛选等方法，这些方法在新抑制剂的发现和优化中发挥着重要作用。为拓展筛选高效广谱 Mpro 抑制剂的策略，本节将在戚海燕等[83]工作的基础上，重点介绍基于活性的蛋白质分析(activity-based protein profiling, ABPP)与有限蛋白水解-质谱法(limited proteolysis-mass spectrometry, LiP-MS)等新方法的应用。

2.2.1 虚拟筛选

虚拟筛选作为计算机辅助药物设计(computer-aided drug design, CADD)的关键技术，在丰富先导化合物结构骨架的同时，降低了新药研发的成本和周期。虚拟筛选通过构建化合物活性与结构的关系，运用计算机程序精准筛选大量天然产物库、上市药物库及小分子库，以发现潜在先导化合物。虚拟筛选主要分为基于结构的虚拟筛选和基于配体的虚拟筛选(图 3)：基于结构的虚拟筛选依赖 Mpro 的三维结构，通过分子对接模拟化合物与 Mpro 活性位点的相互作用，采用对接软件和评分函数来预测结合亲和力，并筛选出具有潜力的抑制剂[84-85]；基于配体的虚拟筛选需已知活性分子的构效关系和药效团模型，寻找具有相似特征的化合物[86]。因此，虚拟筛选的关键在于理解 Mpro 的分子结

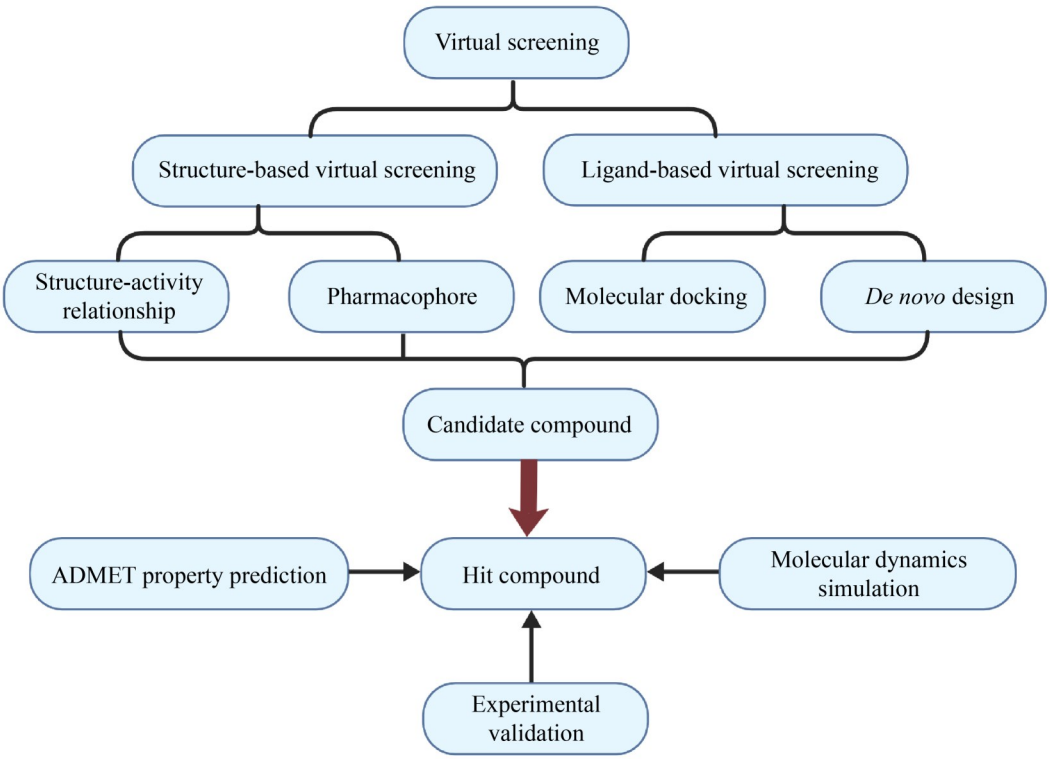

图3 虚拟筛选法筛选Mpro抑制剂

Figure 3 The virtual screening for identifying Mpro inhibitors. ADMET: Absorption, distribution, metabolism, excretion, and toxicity. Created with BioGDP.com.

构特征，并精准设定配体结合的筛选标准。具体而言，需整合分子对接评分、药效团匹配和分子动力学模拟等多维参数，以设定结合能阈值和构象稳定性的评判标准。基于 Mpro 的活性位点，采用虚拟筛选技术鉴定出 ebselen 和 nirmatrelvir 等具有潜在活性的化合物[9,87]。此外，Sharma 等[88]基于 SARS-CoV-2 Mpro 的活性位点进行同源建模，并通过虚拟筛选方法发现多种已上市药物 cobicistat、iopromide、cangrelor 等可作为 Mpro 抑制剂，表明这些药物可能具有抗 SARS-CoV-2 的潜力。

2.2.2 有限蛋白水解质谱技术

有限蛋白水解质谱技术(LiP-MS)是一种用于鉴定小分子化合物与蛋白质相互作用位点的经典方法。LiP-MS 的原理基于小分子药物与靶蛋白结合后会稳定蛋白结构，减少表面肽段被蛋白酶酶切的几率；通过对比药物处理组与对照组经非特异性蛋白酶处理后的酶切结果，LiP-MS 能够精准鉴定与化合物结合的特异性肽段，并通过肽段比对与组装将这些肽段归属至特定靶蛋白(图 4)[89]。LiP-MS 方法适用于小分子的蛋白质及多肽靶标发现，为药物研发与机制研究提供了有力工具。

2.2.3 基于活性的蛋白质组学分析法

基于活性的蛋白质分析(ABPP)是一种由 Cravatt 团队开创的蛋白质组学技术，旨在研究酶的活性状态(图 5)[90]。该技术通过评估酶活性来识别在疾病中失调的关键酶，进而指导针对这些酶活性的抑制剂开发[91]。该技术结合质谱和定量蛋白质组学，已广泛应用于药物靶标的

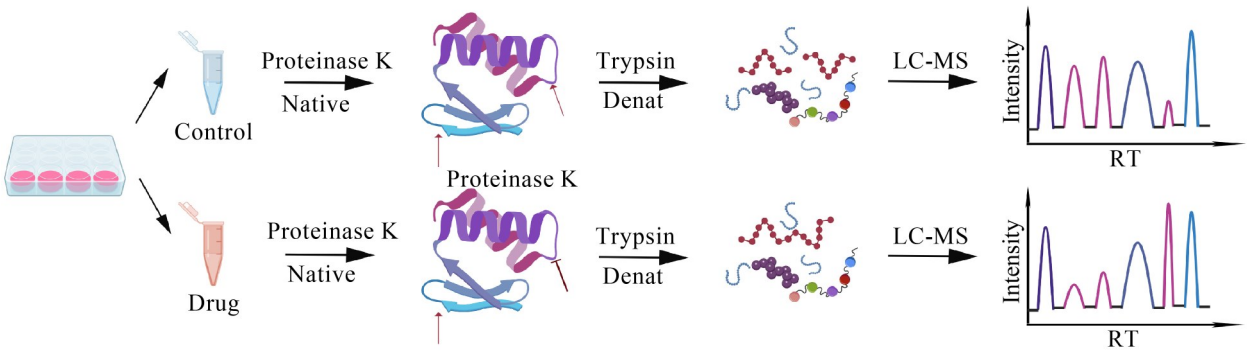

图4 LiP-MS筛选Mpro抑制剂

Figure 4 LiP-MS screening for Mpro inhibitors. Created with BioGDP.com.

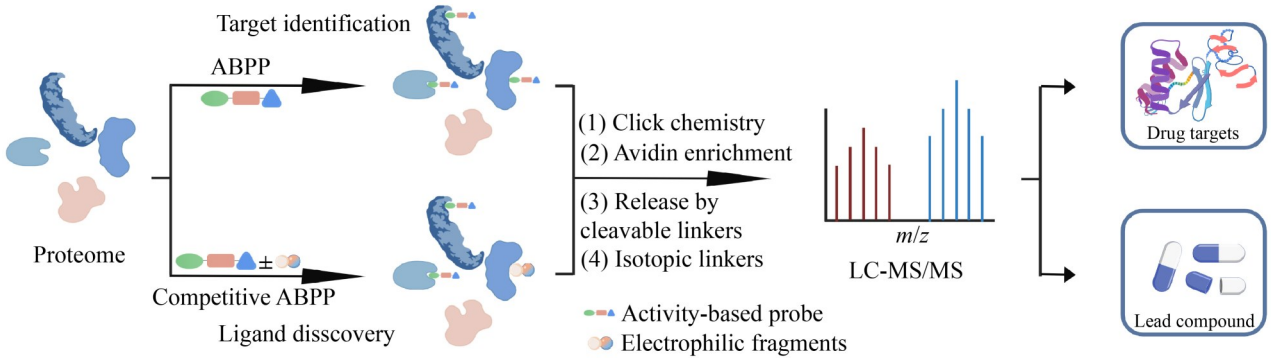

图5 ABPP鉴定Mpro药物靶点

Figure 5 ABPP identifies drug targets on Mpro. Created with BioGDP.com.

发现。主要采用 2 种策略：直接富集策略通过活性探针与靶蛋白的共价结合，经点击化学连接报告基团后，利用质谱或荧光技术鉴定标记蛋白，从而分析氨基酸残基的反应活性，用于发现潜在的药物靶标；间接富集策略通过候选小分子与蛋白质组的预孵育，随后使用广谱活性探针标记未结合位点，并结合同位素定量技术评估小分子与氨基酸的结合特性，进而筛选先导化合物及其靶标蛋白。ABPP 方法尤其适用于共价抑制剂和小分子药物靶蛋白的研究[92]。Moon 等[93]利用 ABPP 成功发现并优化了一种靶向半胱氨酸的吡唑啉类共价抑制剂，该抑制剂能有效抑制多种冠状病毒 Mpro 的活性，凸显了 ABPP 在抗病毒药物研发中的潜力。

2.2.4 荧光共振能量转移法

荧光共振能量转移法(fluorescence resonance energy transfer, FRET)是一种非辐射过程，处于激发态的供体荧光团通过偶极-偶极相互作用将能量转移给受体发色团[94]。该方法具有操作简单、分析快速以及灵敏度高等优势，在抑制剂筛选研究中得到广泛运用。研究人员基于 Mpro 的天然切割位点设计了用 7-甲氧基香豆素-4-乙酸(7-methoxycoumarin-4-acetic acid, MCA)和淬灭基团 2,4-二硝基苯酚(2,4-dinitropheno, Dnp)进行标记的肽(MCA-substrate)作为 Mpro 切割的底物，MCA 与 Dnp 通过 Mpro 的切割位点序列(AVLQ)分隔形成 FRET 对；当 Mpro 水解 MCA 标记的肽底物时，由于 FRET 效应消失，MCA-AVLQ 肽段产生增强的荧光信号[95]。因此，能够抑制 Mpro 活性的化合物会在 FRET 分析中表现为相对较低的荧光值(图 6)。利用 FRET 筛选方法，已鉴定出 Lead E24 和 4′-*O*-methylscutellarein 等化合物，这些化合物对 Mpro 表现出良好抑制活性，并能有效抑制 SARS-CoV-2 复制[96-97]。Su 等[98]证实中药成分通过靶向 Mpro 表现出 SARS-CoV-2 抑制活性，

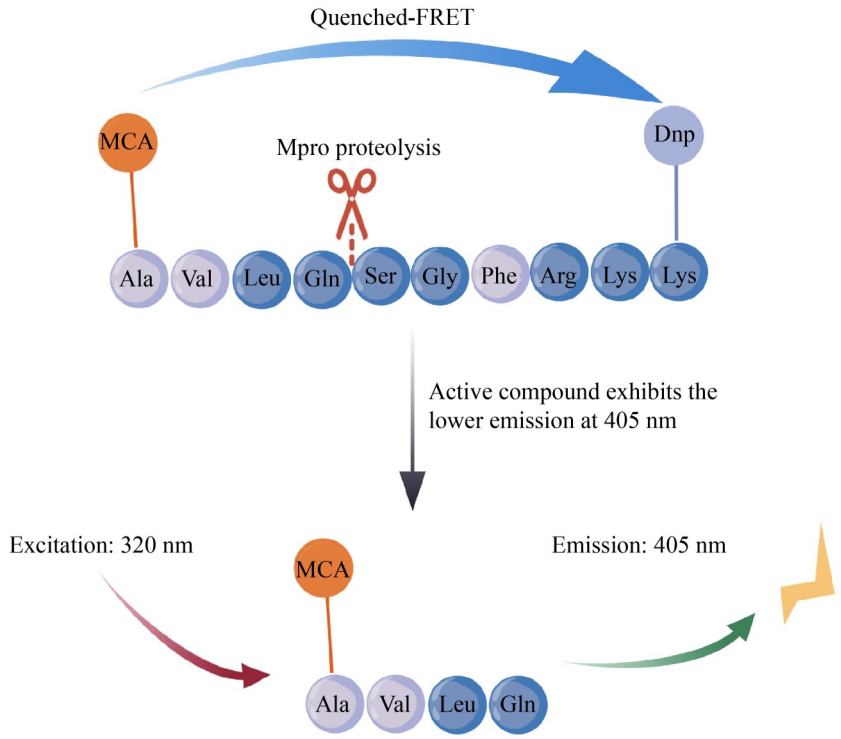

图6 基于FRET原理的Mpro抑制剂筛选法

Figure 6 Mpro inhibitor screening method based on the FRET principle. Created with BioGDP.com.

从黄芩中分离的生物活性成分黄芩苷和黄芩素已在肝炎和呼吸道疾病治疗中展现出显著抑制Mpro的能力[99]。

2.2.5 荧光素酶报告基因筛选法

Lin等[100]建立了一种用于高通量筛选冠状病毒Mpro抑制剂的荧光素酶报告基因筛选(luciferase reporter screening assay)系统。该方法的核心是构建一个整合SARS-CoV Mpro基因、含有切割位点的底物(S)以及荧光素酶(Luc)基因的重组质粒，随后将质粒转染至非洲绿猴肾细胞(Vero)中，并通过G418抗性筛选获得稳定表达的细胞系[83]。由于荧光素酶的N端融合了相对分子质量较大的底物蛋白，通常会抑制荧光素酶活性，导致荧光信号减弱；而当SARS-CoV Mpro高效切割底物蛋白时荧光素酶活性得以释放，荧光信号随之增强。因此，在该筛选体系中具有抑制SARS-CoV Mpro切割活性的化合物会导致较低的荧光值(图7)[83]。通过此筛选策略，证实了nirmatrelvir、GC376和lopinavir抑制SARS-CoV-2 Mpro功能，为新型Mpro抑制剂的发现与开发提供了有力工具[101]。

2.2.6 表型筛选法

表型筛选法(phenotype screening assay)是一种基于细胞的分析方法，用于评估化合物抑制SARS-CoV-2病毒感染的潜力，旨在发现具有抗病毒活性的先导化合物。该方法的核心是通过

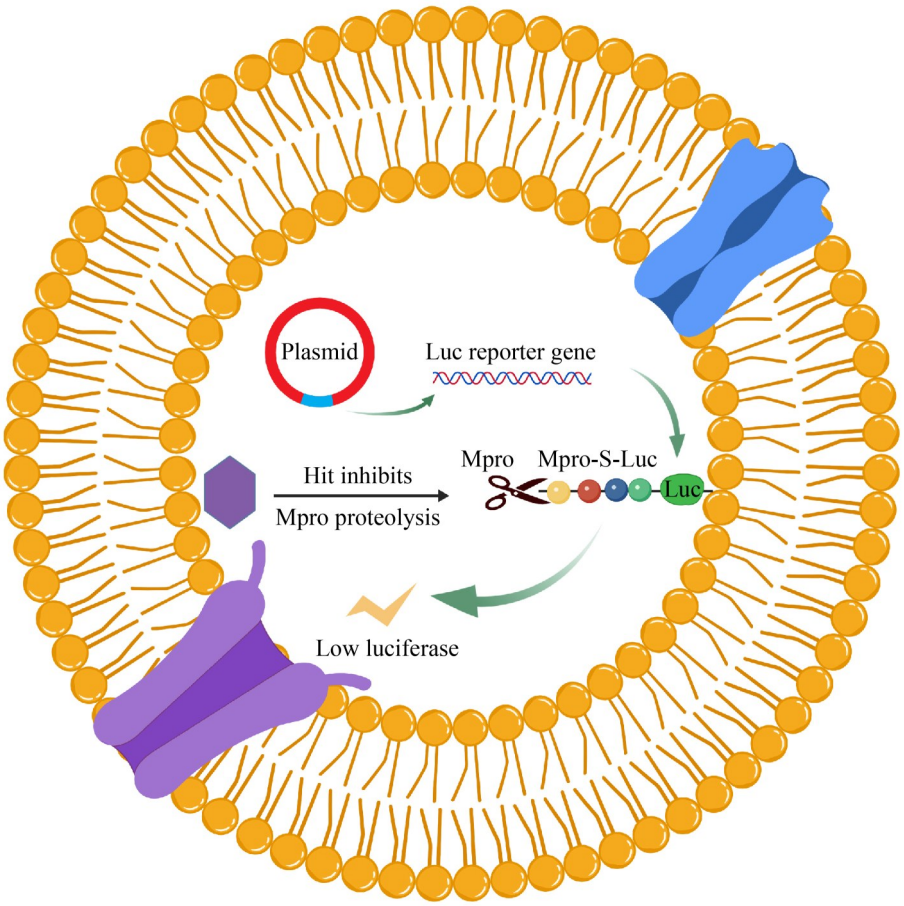

图7 基于荧光素酶报告基因法筛选Mpro抑制剂[83]

Figure 7 Screening of Mpro inhibitors based on the luciferase reporter screening assay[83]. Created with BioGDP.com.

观察化合物预处理后对病毒感染细胞的保护程度来评估化合物抗病毒效果，具体操作流程为：在培养基中预先用待筛选化合物处理非洲绿猴肾上皮细胞(Vero E6)，随后感染 SARS-CoV-2 病毒；通过观察和分析细胞病变效应(cytopathic effect, CPE)、空斑减少以及病毒 RNA 拷贝数变化等关键指标，可有效评估待测化合物对 SARS-CoV-2 的抗病毒活性(图 8)[83]。此方法的优势在于能直接反映化合物对病毒感染细胞的作用效果，从而为化合物抗病毒活性提供直接证据。通过表型筛选法，Riva 等[102]鉴定出多种化合物(MDL-28170、ONO-5334、VBY-825 及 apilimod)表现出显著抗 SARS-CoV-2 活性并能有效抑制病毒复制。

3 总结与展望

通过对 Mpro 底物识别模式的分析，研究已确定 P2、P1 和 P1′三个关键位点的特定氨基酸残基对酶活性起关键决定作用[103-104]。值得注意的是，Mpro 以二聚体形式发挥功能，不仅介导复制酶多聚蛋白的成熟，也可切割宿主蛋白以帮助病毒逃逸先天免疫反应[105-106]。这种双重功能在 SARS-CoV-2 Mpro 中表现尤为突出：它通过切割 MAGED2、TRMT1、RNF20 和 SQSTM1/p62 等宿主蛋白，进而促进病毒感染[107-110]。类似地，SADS-CoV Mpro 能够切割 DCP1A[111]，PEDV Mpro 则切割 gasdermin D[112]，这些切割机制均在病毒致病机制中发挥重要作

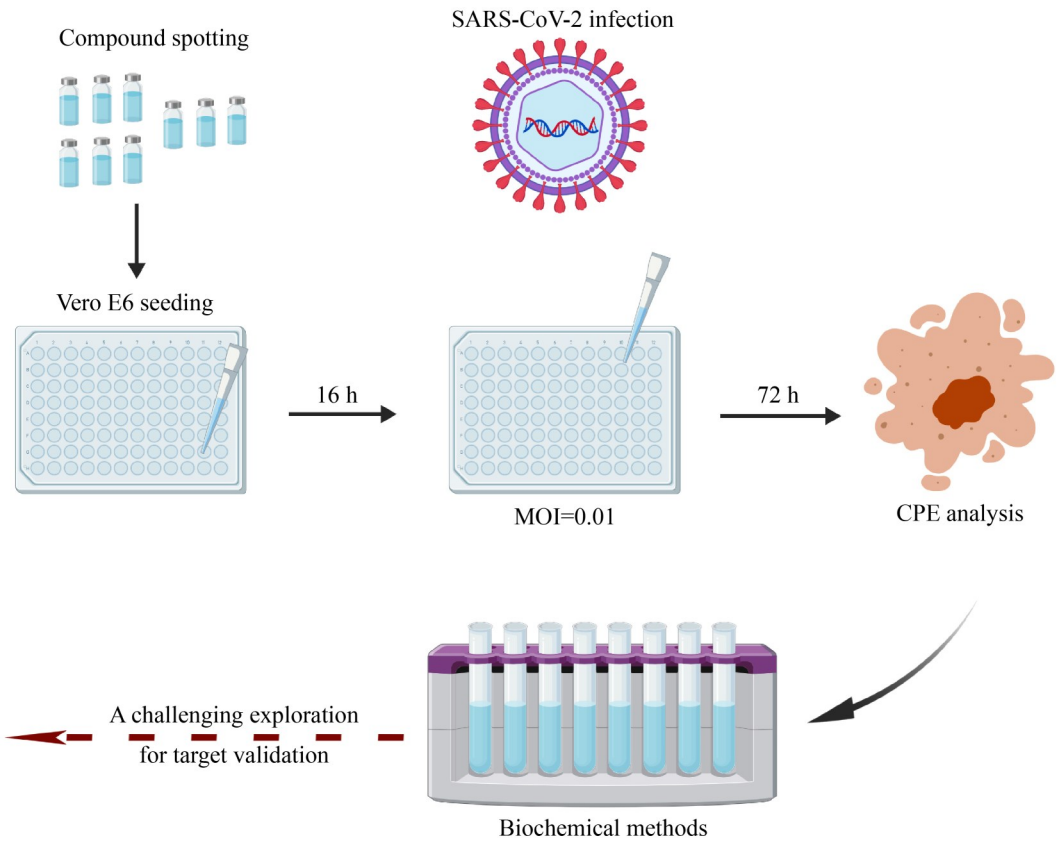

图8 Mpro抑制剂的表型筛选法[83]。MOI是感染复数(multiplicity of infection)，是指病毒颗粒数与靶细胞数的比值。

Figure 8 Phenotypic screening method for Mpro inhibitors[83]. The multiplicity of infection (MOI) is defined as the ratio of virus particles to target cells. Created with BioGDP.com.

用。尽管 Mpro 在冠状病毒科中高度保守，但存在其切割宿主蛋白的特异性差异，如何调控病毒的致病性及免疫逃逸能力仍有待阐明。

Mpro 抑制 IFN-I 信号通路的机制较为复杂，且在冠状病毒科内具有保守性[50-52]。Mpro 能够靶向通路中的多个关键组分，包括视黄酸诱导基因 I (retinoic acid inducible gene I, RIG-I)、MAVS、干扰素基因刺激因子(stimulator of interferon genes, STING)和信号转导和转录激活因子 1 (signal transducer and activator of transcription 1, STAT1)[56,113]。值得注意的是，与 SARS-CoV 相比，SARS-CoV-2 Mpro 表现出更强的催化活性和干扰素拮抗能力[52]。此外，SARS-CoV-2 Mpro 还通过阻止磷酸化干扰素调节因子 3 (interferon regulatory factor 3, IRF3)的核转位，进而抑制 IFN-I 产生[114]。其他冠状病毒如 PDCoV、PEDV 和 FIPV 也分别通过切割 NEMO 或其他关键分子干扰 IFN-I 信号通路[50-51,53]。然而，为精确解析 Mpro 与免疫信号组分的相互作用，亟需采用高分辨率结构生物学技术以明确其结合界面并系统阐明蛋白切割的分子机制，这是开发新型抑制剂的关键基础。此类抑制剂通过破坏上述相互作用，从而有效恢复宿主的抗病毒免疫应答。

Mpro 抑制剂研发已取得重大进展，已涌现出共价抑制剂和非共价抑制剂等多种类型的抑制剂[59]。近年来报道的 Mpro 抑制剂以共价抑制剂最为常见，其分子结构中通常含有反应性弹头，可与催化位点的 Cys145 发生共价结合。然而，高反应性弹头的存在不仅引发对靶向安全性的担忧，还可能降低口服生物利用度[19]。非共价抑制剂则通过可逆结合方式与 Mpro 催化口袋中的氨基酸残基相互作用。尽管这种结合方式有利于减少潜在副作用和脱靶效应，但由于其与催化口袋的结合相对较弱，常需长期给药或增加剂量，从而可能增加耐药风险[115]。因此，兼具小分子特性和共价抑制剂弹头优势的非共价抑制剂展现出较好的开发前景[116]。然而，将这些抑制剂从实验室推向临床仍面临诸多挑战。在药物递送方面，开发适宜制剂以实现抑制剂向靶细胞或组织的高效递送；在分子设计层面，共价与非共价抑制剂的设计需要系统评估关键特性，包括吸收、分布、代谢、排泄和毒性性质优化、联合治疗策略、可开发性评估及耐药屏障建立[117-119]。此外，潜在的脱靶效应可能导致不良反应和毒性。因此，必须通过动物模型进行体内研究和最终临床试验全面评估这些抑制剂的安全性与有效性。

在筛选方法方面，各技术均存在其优缺点。FRET 筛选由于操作简便、灵敏度高而备受青睐，但存在成本高和假阳性风险[120]；虚拟筛选法高效经济，但需通过实验验证以排除假阳性；荧光素酶报告基因筛选法可提供化合物活性的详细信息，但成本高昂且流程复杂；ABPP 能精确定位共价化合物作用位点，但需对化合物结构进行修饰，增加了实验复杂度；LiP-MS 是鉴定蛋白-靶标结合序列的有效工具，但数据解读可能存在困难；表型筛选法可直接评估化合物抗病毒活性，但耗时且成本高。因此，联合运用多种方法可提高抑制剂鉴定的准确性。

除传统策略外，大环化合物、多价配体和蛋白降解靶向嵌合体(proteolysis targeting chimera, PROTAC)等新策略展现出巨大潜力[121-124]。其中，PROTAC 技术能精确靶向蛋白降解，对开发抗 Mpro 病毒药物具有重要意义。传统抑制剂可能由于靶蛋白发生突变产生耐药性，而促进靶蛋白降解的 PROTAC 技术有望降低耐药风险[125]，这为应对冠状病毒快速进化提供了灵活策略。近年来，硒元素因潜在治疗价值受到关注。研究表明在小分子中引入硒原子可增强生物活性[126]。含硒化合物 ebselen 及其衍生物对 SARS-CoV-2 Mpro 表现出更好抑制效果[127]。为高效筛选具有潜力的 Mpro 抑制剂，建议采用联合筛选策略：首先对大规模有机硒化合物库进行虚拟筛选以缩小候选范围，继而通过 FRET 法进行验证以排除假阳性，最后利用 LiP-MS 技术

解析 Mpro 与候选药物的作用域及结合位点。该策略可显著缩短筛选时间、降低化合物采购成本，并有效鉴定具有开发前景的新型 Mpro 抑制剂，从而拓展抗冠状病毒治疗的策略边界。

为应对冠状病毒的持续突变，相关策略不应仅停留于被动应对新变种，而需转向实施前瞻性的预测方法。可以利用计算建模和机器学习算法分析 Mpro 突变趋势，通过整合不同冠状病毒 Mpro 的结构与功能数据，预测潜在突变位点和保守区域，从而指导广谱抑制剂的合理设计。此外，开发具有新型作用机制的抑制剂，可为应对耐药病毒变种提供重要的替代治疗策略。

另一方面，宿主因素对 Mpro 功能及抑制剂效力的影响也不容忽视。宿主遗传多态性可能调节 Mpro 与抑制剂的相互作用，进而影响治疗反应[19]。宿主免疫状态同样发挥关键作用，激活的免疫系统可能增强抑制剂的抗病毒效果，而免疫抑制状态则可能削弱其疗效[22]。深入理解这些宿主相关因素有助于制定个性化治疗策略，提高抗病毒治疗的整体有效性。

综上所述，尽管本文为理解 Mpro 及其抑制剂奠定了坚实基础，上述多个方向仍亟需深入探索。通过对这些关键问题的持续研究，不仅可进一步阐明 Mpro 的生物学机制，也能为开发针对冠状病毒持续进化威胁的有效抗病毒策略提供重要支撑。此类研究既能为创新疗法设计提供理论依据，也有助于为未来病毒大流行的防控构建科学、前瞻的公共卫生策略。

作者贡献声明

熊谋康：构思与设计、撰写初稿和修改；朱君海：监督指导；王玉娥：资金获取、论文修改和监督指导。

作者利益冲突公开声明

作者声明不存在任何可能会影响本文所报告工作的已知经济利益或个人关系。

参考文献

[1] GORBALENYA AE, BAKER SC, BARIC RS, de GROOT RJ, DROSTEN C, GULYAEVA AA, HAAGMANS BL, LAUBER C, LEONTOVICH AM, NEUMAN BW, PENZAR D, PERLMAN S, POON LLM, SAMBORSKIY DV, SIDOROV IA, SOLA I, ZIEBUHR J. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2[J]. Nature Microbiology, 2020, 5(4): 536-544.

[2] DUAN YK, WANG HF, YUAN ZH, YANG HT. Structural biology of SARS-CoV-2 Mpro and drug discovery[J]. Current Opinion in Structural Biology, 2023, 82: 102667.

[3] KENWARD C, VUCKOVIC M, PAETZEL M, STRYNADKA NCJ. Kinetic comparison of all eleven viral polyprotein cleavage site processing events by SARS-CoV-2 main protease using a linked protein FRET platform[J]. Journal of Biological Chemistry, 2024, 300(6): 107367.

[4] 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[J]. International Journal of Molecular Sciences, 2025, 26(3): 1197.

[5] LI GD, HILGENFELD R, WHITLEY R, de CLERCQ E. Therapeutic strategies for COVID-19: progress and lessons learned[J]. Nature Reviews Drug Discovery, 2023, 22(6): 449-475.

[6] XIONG MK, LIU XH, LIANG TR, BAN YF, LIU YL, ZHANG LY, XU Z, SONG CX. The alpha-1 subunit of the Na^+/K^+-ATPase (ATP1A1) is a host factor involved in the attachment of porcine epidemic diarrhea virus[J]. International Journal of Molecular Sciences, 2023, 24(4): 4000.

[7] YANG HT, XIE WQ, XUE XY, YANG KL, MA J, LIANG WX, ZHAO Q, ZHOU Z, PEI DQ, ZIEBUHR J, HILGENFELD R, YUEN KY, WONG L, GAO GX, CHEN SJ, CHEN Z, MA DW, BARTLAM M, RAO ZH. Design of wide-spectrum inhibitors targeting coronavirus main proteases[J]. PLoS Biology, 2005, 3(10): e324.

[8] PLAVEC Z, PÖHNER I, POSO A, BUTCHER SJ. Virus structure and structure-based antivirals[J]. Current Opinion in Virology, 2021, 51: 16-24.

[9] JIN ZM, DU XY, XU YC, DENG YQ, LIU MQ, ZHAO Y, ZHANG B, LI XF, ZHANG LK, PENG C, DUAN YK, YU J, WANG L, YANG KL, LIU FJ, JIANG RD, YANG XL, YOU T, LIU XC, YANG XN, et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors[J]. Nature, 2020, 582(7811): 289-293.

[10] ANAND K, PALM GJ, MESTERS JR, SIDDELL SG, ZIEBUHR J, HILGENFELD R. Structure of coronavirus main proteinase reveals combination of a chymotrypsin fold with an extra alpha-helical domain[J]. EMBO Journal, 2002, 21(13): 3213-3224.

[11] ZIEBUHR J, HEUSIPP G, SIDDELL SG. Biosynthesis, purification, and characterization of the human

coronavirus 229E 3C-like proteinase[J]. Journal of Virology, 1997, 71(5): 3992-3997.
[12] LU J, CHEN SA, KHAN MB, BRASSARD R, ARUTYUNOVA E, LAMER T, VUONG W, FISCHER C, YOUNG HS, VEDERAS JC, LEMIEUX MJ. Crystallization of feline coronavirus Mpro with GC376 reveals mechanism of inhibition[J]. Frontiers in Chemistry, 2022, 10: 852210.
[13] WANG FH, CHEN C, YANG KL, XU Y, LIU XM, GAO F, LIU H, CHEN X, ZHAO Q, LIU X, CAI Y, YANG HT. Michael acceptor-based peptidomimetic inhibitor of main protease from porcine epidemic diarrhea virus[J]. Journal of Medicinal Chemistry, 2017, 60(7): 3212-3216.
[14] HO BL, CHENG SC, SHI L, WANG TY, HO KI, CHOU CY. Critical assessment of the important residues involved in the dimerization and catalysis of MERS coronavirus main protease[J]. PLoS One, 2015, 10(12): e0144865.
[15] YANG HT, YANG MJ, DING Y, LIU YW, LOU ZY, ZHOU Z, SUN L, MO LJ, YE S, PANG H, GAO GF, ANAND K, BARTLAM M, HILGENFELD R, RAO ZH. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor[J]. Proceedings of the National Academy of Sciences of the United States of America, 2003, 100(23): 13190-13195.
[16] LIN C, ZHU ZM, JIANG HH, ZOU XF, ZENG XY, WANG J, ZENG P, LI WW, ZHOU XL, ZHANG J, WANG QS, LI J. Structural basis for coronaviral main proteases inhibition by the 3CLpro inhibitor GC376[J]. Journal of Molecular Biology, 2024, 436(6): 168474.
[17] XUE XY, YU HW, YANG HT, XUE F, WU ZX, SHEN W, LI J, ZHOU Z, DING Y, ZHAO Q, ZHANG XC, LIAO M, BARTLAM M, RAO ZH. Structures of two coronavirus main proteases: implications for substrate binding and antiviral drug design[J]. Journal of Virology, 2008, 82(5): 2515-2527.
[18] WANG FH, CHEN C, WANG ZF, HAN X, SHI PD, ZHOU KX, LIU XM, XIAO YJ, CAI Y, HUANG JH, ZHANG L, YANG HT. The structure of the porcine deltacoronavirus main protease reveals a conserved target for the design of antivirals[J]. Viruses, 2022, 14(3): 486.
[19] SHAWKY AM, ALMALKI FA, ALI ALZAHRANI H, ABDALLA AN, YOUSSIF BGM, IBRAHIM NA, GAMAL M, EL-SHERIEF HAM, ABDEL-FATTAH MM, HEFNY AA, ABDELAZEEM AH, GOUDA AM. Covalent small-molecule inhibitors of SARS-CoV-2 Mpro: insights into their design, classification, biological activity, and binding interactions[J]. European Journal of Medicinal Chemistry, 2024, 277: 116704.
[20] ZHOU JW, SUN P, YANG ZX, WANG TQ, GUO JH, QIU RH, LI Z, WEI DG, ZHENG JS, PENG GQ, FANG LR, XIAO SB. The S2 pocket governs the genus-specific substrate selectivity of coronavirus 3C-like protease[J]. Advanced Science, 2024, 11(44): 2407766.
[21] MENGIST HM, DILNESSA T, JIN TC. Structural basis of potential inhibitors targeting SARS-CoV-2 main protease[J]. Frontiers in Chemistry, 2021, 9: 622898.
[22] REN JY, ZHANG ZF, XIA Y, ZHAO DQ, LI DQ, ZHANG SJ. Research progress on the structure and function, immune escape mechanism, antiviral drug development methods, and clinical use of SARS-CoV-2 Mpro[J]. Molecules, 2025, 30(2): 351.
[23] JIANG S, LI HQ, ZHANG L, MU WP, ZHANG Y, CHEN TJ, WU JX, TANG HY, ZHENG SX, LIU YF, WU YX, LUO XT, XIE YB, REN J. Generic Diagramming Platform (GDP): a comprehensive database of high-quality biomedical graphics[J]. Nucleic Acids Research, 2025, 53(D1): D1670-D1676.
[24] JIN ZM, WANG HF, DUAN YK, YANG HT. The main protease and RNA-dependent RNA polymerase are two prime targets for SARS-CoV-2[J]. Biochemical and Biophysical Research Communications, 2021, 538: 63-71.
[25] CITARELLA A, SCALA A, PIPERNO A, MICALE N. SARS-CoV-2 Mpro: a potential target for peptidomimetics and small-molecule inhibitors[J]. Biomolecules, 2021, 11(4): 607.
[26] HUANG CK, WEI P, FAN KQ, LIU Y, LAI LH. 3C-like proteinase from SARS coronavirus catalyzes substrate hydrolysis by a general base mechanism[J]. Biochemistry, 2004, 43(15): 4568-4574.
[27] PILLAIYAR T, MANICKAM M, NAMASIVAYAM V, HAYASHI Y, JUNG SH. An overview of severe acute respiratory syndrome-coronavirus (SARS-CoV) 3CL protease inhibitors: peptidomimetics and small molecule chemotherapy[J]. Journal of Medicinal Chemistry, 2016, 59(14): 6595-6628.
[28] ZHANG LL, LIN DZ, SUN X, CURTH U, DROSTEN C, SAUERHERING L, BECKER S, ROX K, HILGENFELD R. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors[J]. Science, 2020, 368(6489): 409-412.
[29] ANIANA A, NASHED NT, GHIRLANDO R, COATES L, KNELLER DW, KOVALEVSKY A, LOUIS JM. Insights into the mechanism of SARS-CoV-2 main protease autocatalytic maturation from model precursors[J]. Communications Biology, 2023, 6(1): 1159.
[30] YADAV R, COUROUBLE VV, DEY SK, HARRISON JJEK, TIMM J, HOPKINS JB, SLACK RL, SARAFIANOS SG, RUIZ FX, GRIFFIN PR, ARNOLD E. Biochemical and structural insights into SARS-CoV-2 polyprotein processing by Mpro[J]. Science Advances, 2022, 8(49): eadd2191.
[31] PIÑÓN JD, MAYREDDY RR, TURNER JD, KHAN FS, BONILLA PJ, WEISS SR. Efficient autoproteolytic processing of the MHV-A59 3C-like proteinase from the flanking hydrophobic domains requires membranes[J]. Virology, 1997, 230(2): 309-322.
[32] TIBBLES KW, BRIERLEY I, CAVANAGH D, BROWN TD. Characterization *in vitro* of an autocatalytic processing activity associated with the predicted 3C-like proteinase domain of the coronavirus avian infectious bronchitis virus[J]. Journal of Virology, 1996, 70(3): 1923-1930.
[33] ZHANG J, GUO LJ, XU YF, YANG LJ, SHI HY, FENG L, WANG Y. Characterization of porcine epidemic diarrhea virus infectivity in human embryonic kidney

cells[J]. Archives of Virology, 2017, 162(8): 2415-2419.
[34] HSU MF, KUO CJ, CHANG KT, CHANG HC, CHOU CC, KO TP, SHR HL, CHANG GG, WANG AH, LIANG PH. Mechanism of the maturation process of SARS-CoV 3CL protease[J]. The Journal of Biological Chemistry, 2005, 280(35): 31257-31266.
[35] LI CM, QI YF, TENG X, YANG ZC, WEI P, ZHANG CS, TAN L, ZHOU L, LIU Y, LAI LH. Maturation mechanism of severe acute respiratory syndrome (SARS) coronavirus 3C-like proteinase[J]. Journal of Biological Chemistry, 2010, 285(36): 28134-28140.
[36] YANG YH, CHANG CJ, CHUANG YH, HSU HY, CHEN PP, CHIANG BL. Identification of anti-prothrombin antibodies in the anti-phospholipid syndrome that display the prothrombinase activity[J]. Rheumatology, 2010, 49(1): 34-42.
[37] NOSKE GD, NAKAMURA AM, GAWRILJUK VO, FERNANDES RS, LIMA GMA, ROSA HVD, PEREIRA HD, ZERI ACM, NASCIMENTO AFZ, FREIRE MCLC, FEARON D, DOUANGAMATH A, von DELFT F, OLIVA G, GODOY AS. A crystallographic snapshot of SARS-CoV-2 main protease maturation process[J]. Journal of Molecular Biology, 2021, 433(18): 167118.
[38] SNIJDER EJ, DECROLY E, ZIEBUHR J. The nonstructural proteins directing coronavirus RNA synthesis and processing[J]. Advances in Virus Research, 2016, 96: 59-126.
[39] DIOGO MA, CABRAL AGT, de OLIVEIRA RB. Advances in the search for SARS-CoV-2 Mpro and PLpro inhibitors[J]. Pathogens, 2024, 13(10): 825.
[40] MALONE B, URAKOVA N, SNIJDER EJ, CAMPBELL EA. Structures and functions of coronavirus replication-transcription complexes and their relevance for SARS-CoV-2 drug design[J]. Nature Reviews Molecular Cell Biology, 2021, 23(1): 21-39.
[41] ZIEBUHR J. The coronavirus replicase[J]. Current Topics in Microbiology and Immunology, 2005, 287: 57-94.
[42] HEGYI A, ZIEBUHR J. Conservation of substrate specificities among coronavirus main proteases[J]. Journal of General Virology, 2002, 83(Pt 3): 595-599.
[43] CUI W, YANG KL, YANG HT. Recent progress in the drug development targeting SARS-CoV-2 main protease as treatment for COVID-19[J]. Frontiers in Molecular Biosciences, 2020, 7: 616341.
[44] KIM JC, SPENCE RA, CURRIER PF, LU X, DENISON MR. Coronavirus protein processing and RNA synthesis is inhibited by the cysteine proteinase inhibitor E64d[J]. Virology, 1995, 208(1): 1-8.
[45] STREICHER F, JOUVENET N. Stimulation of innate immunity by host and viral RNAs[J]. Trends in Immunology, 2019, 40(12): 1134-1148.
[46] CROUSE J, KALINKE U, OXENIUS A. Regulation of antiviral T cell responses by type I interferons[J]. Nature Reviews Immunology, 2015, 15(4): 231-242.
[47] MAKRIS S, PAULSEN M, JOHANSSON C. Type I interferons as regulators of lung inflammation[J]. Frontiers in Immunology, 2017, 8: 259.
[48] KINDLER E, THIEL V, WEBER F. Interaction of SARS and MERS coronaviruses with the antiviral interferon response[J]. Advances in Virus Research, 2016, 96: 219-243.
[49] SNIJDER EJ, van der MEER Y, ZEVENHOVEN-DOBBE J, ONDERWATER JJM, van der MEULEN J, KOERTEN HK, MOMMAAS AM. Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex[J]. Journal of Virology, 2006, 80(12): 5927-5940.
[50] ZHU XY, FANG LR, WANG D, YANG YT, CHEN JY, YE X, FODA MF, XIAO SB. Porcine deltacoronavirus nsp5 inhibits interferon-β production through the cleavage of NEMO[J]. Virology, 2017, 502: 33-38.
[51] WANG D, FANG LR, SHI YL, ZHANG H, GAO L, PENG GQ, CHEN HC, LI K, XIAO SB. Porcine epidemic diarrhea virus 3C-like protease regulates its interferon antagonism by cleaving NEMO[J]. Journal of Virology, 2016, 90(4): 2090-2101.
[52] CHEN JY, LI Z, GUO JH, XU SG, ZHOU JW, CHEN Q, TONG X, WANG D, PENG GQ, FANG LR, XIAO SB. SARS-CoV-2 nsp5 exhibits stronger catalytic activity and interferon antagonism than its SARS-CoV ortholog[J]. Journal of Virology, 2022, 96(8): e0003722.
[53] CHEN S, TIAN J, LI ZJ, KANG HT, ZHANG JK, HUANG JP, YIN H, HU XL, QU LD. Feline infectious peritonitis virus Nsp5 inhibits type I interferon production by cleaving NEMO at multiple sites[J]. Viruses, 2020, 12(1): 43.
[54] LI Z, XIAO WW, YANG ZX, GUO JH, ZHOU JW, XIAO SB, FANG PX, FANG LR. Cleavage of HDAC6 to dampen its antiviral activity by nsp5 is a common strategy of swine enteric coronaviruses[J]. Journal of Virology, 2024, 98(2): e0181423.
[55] Van HUIZEN M, VENDRELL XM, de GRUYTER HLM, BOOMAARS-VAN der ZANDEN AL, van der MEER Y, SNIJDER EJ, KIKKERT M, MYENI SK. The main protease of middle east respiratory syndrome coronavirus induces cleavage of mitochondrial antiviral signaling protein to antagonize the innate immune response[J]. Viruses, 2024, 16(2): 256.
[56] LIU YZ, QIN C, RAO YL, NGO C, FENG JJ, ZHAO J, ZHANG S, WANG TY, CARRIERE J, SAVAS AC, ZARINFAR M, RICE S, YANG H, YUAN WM, CAMARERO JA, YU JH, CHEN XS, ZHANG C, FENG PH. SARS-CoV-2 Nsp5 demonstrates two distinct mechanisms targeting RIG-I and MAVS to evade the innate immune response[J]. mBio, 2021, 12(5): e0233521.
[57] ZHU XY, WANG D, ZHOU JW, PAN T, CHEN JY, YANG YT, LV MT, YE X, PENG GQ, FANG LR, XIAO SB. Porcine deltacoronavirus nsp5 antagonizes type I interferon signaling by cleaving STAT2[J]. Journal of Virology, 2017, 91(10): e00003-17.
[58] HUANG HX, LEI XX, ZHAO CC, QIN Y, LI YY, ZHANG XY, LI CK, LAN T, ZHAO BP, SUN WC, LU HJ, JIN NY. Porcine deltacoronavirus nsp5 antagonizes type I interferon signaling by cleaving IFIT3[J]. Journal of Virology, 2024, 98(2): e0168223.
[59] ZAGÓRSKA A, CZOPEK A, FRYC M, JOŃCZYK J.

Inhibitors of SARS-CoV-2 main protease (Mpro) as anti-coronavirus agents[J]. Biomolecules, 2024, 14(7): 797.
[60] DRAG M, SALVESEN GS. Emerging principles in protease-based drug discovery[J]. Nature Reviews Drug Discovery, 2010, 9(9): 690-701.
[61] HOFFMAN RL, KANIA RS, BROTHERS MA, DAVIES JF, FERRE RA, GAJIWALA KS, HE MY, HOGAN RJ, KOZMINSKI K, LI LY, LOCKNER JW, LOU JH, MARRA MT, MITCHELL LJ Jr, MURRAY BW, NIEMAN JA, NOELL S, PLANKEN SP, ROWE T, RYAN K, et al. Discovery of ketone-based covalent inhibitors of coronavirus 3CL proteases for the potential therapeutic treatment of COVID-19[J]. Journal of Medicinal Chemistry, 2020, 63(21): 12725-12747.
[62] MA CL, SACCO MD, HURST B, TOWNSEND JA, HU YM, SZETO T, ZHANG XJ, TARBET B, MARTY MT, CHEN Y, WANG J. Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2 viral replication by targeting the viral main protease[J]. Cell Research, 2020, 30(8): 678-692.
[63] VUONG W, KHAN MB, FISCHER C, ARUTYUNOVA E, LAMER T, SHIELDS J, SAFFRAN HA, McKAY RT, van BELKUM MJ, JOYCE MA, YOUNG HS, TYRRELL DL, VEDERAS JC, LEMIEUX MJ. Feline coronavirus drug inhibits the main protease of SARS-CoV-2 and blocks virus replication[J]. Nature Communications, 2020, 11(1): 4282.
[64] DAI WH, ZHANG B, JIANG XM, SU HX, LI J, ZHAO Y, XIE X, JIN ZM, PENG JJ, LIU FJ, LI CP, LI Y, BAI F, WANG HF, CHENG X, CEN XB, HU SL, YANG XN, WANG J, LIU X, et al. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease[J]. Science, 2020, 368(6497): 1331-1335.
[65] NOGUEIRA CW, BARBOSA NV, ROCHA JBT. Toxicology and pharmacology of synthetic organoselenium compounds: an update[J]. Archives of Toxicology, 2021, 95(4): 1179-1226.
[66] MARZI M, VAKIL MK, BAHMANYAR M, ZARENEZHAD E. Paxlovid: mechanism of action, synthesis, and *in silico* study[J]. BioMed Research International, 2022, 2022(1): 7341493.
[67] KALLAM SR, EDA VR, SEN S, DATRIKA R, RAPOLU RK, KHOBARE S, GAJARE V, BANDA M, KHAN RAR, SINGH M, LLOYD M, KANDAGATLA B, JANAGILI MB, TADIKONDA VP, VIDAVALUR S, IQBAL J, FOX ME, DAHANUKAR VH, ORUGANTI S. A diastereoselective synthesis of boceprevir's gem-dimethyl bicyclic [3.1.0] proline intermediate from an insecticide ingredient *Cis*-cypermethric acid[J]. Tetrahedron, 2017, 73(30): 4285-4294.
[68] ZHAO Y, FANG C, ZHANG Q, ZHANG RX, ZHAO XB, DUAN YK, WANG HF, ZHU Y, FENG L, ZHAO JY, SHAO ML, YANG XN, ZHANG LK, PENG C, YANG KL, MA DW, RAO ZH, YANG HT. Crystal structure of SARS-CoV-2 main protease in complex with protease inhibitor PF-07321332[J]. Protein & Cell, 2022, 13(9): 689-693.
[69] PRESCHEL HD, OTTE RT, ZHUO Y, RUSCOE RE, BURKE AJ, KELLERHALS R, HORST B, HENNIG S, JANSSEN E, GREEN AP, TURNER NJ, RUIJTER E. Multicomponent synthesis of the SARS-CoV-2 main protease inhibitor nirmatrelvir[J]. Journal of Organic Chemistry, 2023, 88(17): 12565-12571.
[70] ANASTASSOPOULOU C, HATZIANTONIOU S, BOUFIDOU F, PATRINOS GP, TSAKRIS A. The role of oral antivirals for COVID-19 treatment in shaping the pandemic landscape[J]. Journal of Personalized Medicine, 2022, 12(3): 439.
[71] DUAN XH, LACKO LA, CHEN SB. Druggable targets and therapeutic development for COVID-19[J]. Frontiers in Chemistry, 2022, 10: 963701.
[72] OWEN DR, ALLERTON CMN, ANDERSON AS, ASCHENBRENNER L, AVERY M, BERRITT S, BORAS B, CARDIN RD, CARLO A, COFFMAN KJ, DANTONIO A, DI L, ENG H, FERRE R, GAJIWALA KS, GIBSON SA, GREASLEY SE, HURST BL, KADAR EP, KALGUTKAR AS, et al. An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19[J]. Science, 2021, 374(6575): 1586-1593.
[73] VANDYCK K, DEVAL J. Considerations for the discovery and development of 3-chymotrypsin-like cysteine protease inhibitors targeting SARS-CoV-2 infection[J]. Current Opinion in Virology, 2021, 49: 36-40.
[74] KIM Y, MANDADAPU SR, GROUTAS WC, CHANG KO. Potent inhibition of feline coronaviruses with peptidyl compounds targeting coronavirus 3C-like protease[J]. Antiviral Research, 2013, 97(2): 161-168.
[75] PERERA KD, GALASITI KANKANAMALAGE AC, RATHNAYAKE AD, HONEYFIELD A, GROUTAS W, CHANG KO, KIM Y. Protease inhibitors broadly effective against feline, ferret and mink coronaviruses[J]. Antiviral Research, 2018, 160: 79-86.
[76] PERERA KD, RATHNAYAKE AD, LIU HW, PEDERSEN NC, GROUTAS WC, CHANG KO, KIM Y. Characterization of amino acid substitutions in feline coronavirus 3C-like protease from a cat with feline infectious peritonitis treated with a protease inhibitor[J]. Veterinary Microbiology, 2019, 237: 108398.
[77] KITAMURA N, SACCO MD, MA CL, HU YM, TOWNSEND JA, MENG XZ, ZHANG FS, ZHANG XJ, BA M, SZETO T, KUKULJAC A, MARTY MT, SCHULTZ D, CHERRY S, XIANG Y, CHEN Y, WANG J. Expedited approach toward the rational design of noncovalent SARS-CoV-2 main protease inhibitors[J]. Journal of Medicinal Chemistry, 2022, 65(4): 2848-2865.
[78] ZHANG CH, STONE EA, DESHMUKH M, IPPOLITO JA, GHAHREMANPOUR MM, TIRADO-RIVES J, SPASOV KA, ZHANG S, TAKEO Y, KUDALKAR SN, LIANG ZB, ISAACS F, LINDENBACH B, MILLER SJ, ANDERSON KS, JORGENSEN WL. Potent noncovalent inhibitors of the main protease of SARS-CoV-2 from molecular sculpting of the drug perampanel guided by free energy perturbation calculations[J]. ACS Central

Science, 2021, 7(3): 467-475.
[79] UNOH Y, UEHARA S, NAKAHARA K, NOBORI H, YAMATSU Y, YAMAMOTO S, MARUYAMA Y, TAODA Y, KASAMATSU K, SUTO T, KOUKI K, NAKAHASHI A, KAWASHIMA S, SANAKI T, TOBA S, UEMURA K, MIZUTARE T, ANDO S, SASAKI M, ORBA Y, et al. Discovery of S-217622, a noncovalent oral SARS-CoV-2 3CL protease inhibitor clinical candidate for treating COVID-19[J]. Journal of Medicinal Chemistry, 2022, 65(9): 6499-6512.
[80] DRAYMAN N, DeMARCO JK, JONES KA, AZIZI SA, FROGGATT HM, TAN KM, MALTSEVA NI, CHEN SQ, NICOLAESCU V, DVORKIN S, FURLONG K, KATHAYAT RS, FIRPO MR, MASTRODOMENICO V, BRUCE EA, SCHMIDT MM, JEDRZEJCZAK R, MUÑOZ-ALÍA MÁ, SCHUSTER B, NAIR V, et al. Masitinib is a broad coronavirus 3CL inhibitor that blocks replication of SARS-CoV-2[J]. Science, 2021, 373(6557): 931-936.
[81] JACOBS J, GRUM-TOKARS V, ZHOU Y, TURLINGTON M, SALDANHA SA, CHASE P, EGGLER A, DAWSON ES, BAEZ-SANTOS YM, TOMAR S, MIELECH AM, BAKER SC, LINDSLEY CW, HODDER P, MESECAR A, STAUFFER SR. Discovery, synthesis, and structure-based optimization of a series of *N*-(tert-butyl)-2-(*N*-arylamido)-2-(pyridin-3-yl) acetamides (ML188) as potent noncovalent small molecule inhibitors of the severe acute respiratory syndrome coronavirus (SARS-CoV) 3CL protease[J]. Journal of Medicinal Chemistry, 2013, 56(2): 534-546.
[82] HOU NK, SHUAI L, ZHANG LJ, XIE XP, TANG KM, ZHU YK, YU Y, ZHANG WY, TAN QZ, ZHONG GX, WEN ZY, WANG C, HE XJ, HUO H, GAO HS, XU Y, XUE J, PENG C, ZOU J, SCHINDEWOLF C, et al. Development of highly potent noncovalent inhibitors of SARS-CoV-2 3CLpro[J]. ACS Central Science, 2023, 9(2): 217-227.
[83] 戚海燕, 闫干干, 付正豪, 刘晓平, 陈云雨. 新型冠状病毒主蛋白酶抑制剂的筛选方法研究进展[J]. 生命的化学, 2021, 41(2): 207-214.
QI HY, YAN GG, FU ZH, LIU XP, CHEN YY. Miniaturized high-throughput screening assays for the discovery of SARS-CoV-2 main protease inhibitors[J]. Chemistry of Life, 2021, 41(2): 207-214 (in Chinese).
[84] LUTTENS A, GULLBERG H, ABDURAKHMANOV E, VO DD, AKABERI D, TALIBOV VO, NEKHOTIAEVA N, VANGEEL L, de JONGHE S, JOCHMANS D, KRAMBRICH J, TAS A, LUNDGREN B, GRAVENFORS Y, CRAIG AJ, ATILAW Y, SANDSTRÖM A, MOODIE LWK, LUNDKVIST Å, van HEMERT MJ, et al. Ultralarge virtual screening identifies SARS-CoV-2 main protease inhibitors with broad-spectrum activity against coronaviruses[J]. Journal of the American Chemical Society, 2022, 144(7): 2905-2920.
[85] FOMINA AD, UVAROVA VI, KOZLOVSKAYA LI, PALYULIN VA, OSOLODKIN DI, ISHMUKHAMETOV AA. Ensemble docking based virtual screening of SARS-CoV-2 main protease inhibitors[J]. Molecular Informatics, 2024, 43(8): e202300279.
[86] KHANFAR MA, SALEH M. Virtual screening identifies inhibitors of SARS-CoV-2 main protease through pharmacophore and similarity approaches[J]. Current Pharmaceutical Design, 2025, 31(18): 1461-1473.
[87] CLYDE A, GALANIE S, KNELLER DW, MA H, BABUJI Y, BLAISZIK B, BRACE A, BRETTIN T, CHARD K, CHARD R, COATES L, FOSTER I, HAUNER D, KERTESZ V, KUMAR N, LEE H, LI ZZ, MERZKY A, SCHMIDT JG, TAN L, et al. High-throughput virtual screening and validation of a SARS-CoV-2 main protease noncovalent inhibitor[J]. Journal of Chemical Information and Modeling, 2022, 62(1): 116-128.
[88] SHARMA P, VIJAYAN V, PANT P, SHARMA M, VIKRAM N, KAUR P, SINGH TP, SHARMA S. Identification of potential drug candidates to combat COVID-19: a structural study using the main protease (Mpro) of SARS-CoV-2[J]. Journal of Biomolecular Structure & Dynamics, 2021, 39(17): 6649-6659.
[89] CAPPELLETTI V, HAUSER T, PIAZZA I, PEPELNJAK M, MALINOVSKA L, FUHRER T, LI YZ, DÖRIG C, BOERSEMA P, GILLET L, GROSSBACH J, DUGOURD A, SAEZ-RODRIGUEZ J, BEYER A, ZAMBONI N, CAFLISCH A, de SOUZA N, PICOTTI P. Dynamic 3D proteomes reveal protein functional alterations at high resolution *in situ*[J]. Cell, 2021, 184(2): 545-559.e22.
[90] LIU Y, PATRICELLI MP, CRAVATT BF. Activity-based protein profiling: the serine hydrolases[J]. Proceedings of the National Academy of Sciences of the United States of America, 1999, 96(26): 14694-14699.
[91] GYENIS L, TUROWEC JP, BRETNER M, LITCHFIELD DW. Chemical proteomics and functional proteomics strategies for protein kinase inhibitor validation and protein kinase substrate identification: applications to protein kinase CK2[J]. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 2013, 1834(7): 1352-1358.
[92] SPRADLIN JN, ZHANG E, NOMURA DK. Reimagining druggability using chemoproteomic platforms[J]. Accounts of Chemical Research, 2021, 54(7): 1801-1813.
[93] MOON P, ZAMMIT CM, SHAO Q, DOVALA D, BOIKE L, HENNING NJ, KNAPP M, SPRADLIN JN, WARD CC, WOLLEB H, FULLER D, BLAKE G, MURPHY JP, WANG F, LU YP, MOQUIN SA, TANDESKE L, HESSE MJ, McKENNA JM, TALLARICO JA, et al. Discovery of potent pyrazoline-based covalent SARS-CoV-2 main protease inhibitors[J]. ChemBioChem, 2023, 24(11): e202300116.
[94] CENTONZE VE, SUN M, MASUDA A, GERRITSEN H, HERMAN B. Fluorescence resonance energy transfer imaging microscopy[M]//Biophotonics: Part A. Amsterdam: Elsevier, 2003: 542-560.

[95] COELHO C, GALLO G, CAMPOS CB, HARDY L, WÜRTELE M. Biochemical screening for SARS-CoV-2 main protease inhibitors[J]. PLoS One, 2020, 15(10): e0240079.
[96] HUFF S, KUMMETHA IR, TIWARI SK, HUANTE MB, CLARK AE, WANG SB, BRAY W, SMITH D, CARLIN AF, ENDSLEY M, RANA TM. Discovery and mechanism of SARS-CoV-2 main protease inhibitors[J]. Journal of Medicinal Chemistry, 2022, 65(4): 2866-2879.
[97] WU QQ, YAN SQ, WANG YJ, LI MT, XIAO YB, LI YX. Discovery of 4′-*O*-methylscutellarein as a potent SARS-CoV-2 main protease inhibitor[J]. Biochemical and Biophysical Research Communications, 2022, 604: 76-82.
[98] SU H, YAO S, ZHAO W, LI JL. Discovery of baicalin and baicalein as novel, natural product inhibitors of SARS-CoV-2 3CL protease *in vitro*[J/OL]. bioRxiv, 2020. https://doi.org/10.1101/2020.04.13.038687.
[99] DINDA B, DINDA S, DasSHARMA S, BANIK R, CHAKRABORTY A, DINDA M. Therapeutic potentials of baicalin and its aglycone, baicalein against inflammatory disorders[J]. European Journal of Medicinal Chemistry, 2017, 131: 68-80.
[100] LIN CW, TSAI FJ, TSAI CH, LAI CC, WAN L, HO TY, HSIEH CC, CHAO PL. Anti-SARS coronavirus 3C-like protease effects of Isatis indigotica root and plant-derived phenolic compounds[J]. Antiviral Research, 2005, 68(1): 36-42.
[101] VLACHOU A, NCHIOUA R, REGENSBURGER K, KIRCHHOFF F, KMIEC D. A Gaussia luciferase reporter assay for the evaluation of coronavirus Nsp5/3CLpro activity[J]. Scientific Reports, 2024, 14: 20697.
[102] RIVA L, YUAN SF, YIN X, MARTIN-SANCHO L, MATSUNAGA N, PACHE L, BURGSTALLER-MUEHLBACHER S, de JESUS PD, TERIETE P, HULL MV, CHANG MW, CHAN JF, CAO JL, POON VK, HERBERT KM, CHENG KY, NGUYEN TH, RUBANOV A, PU Y, NGUYEN C, et al. Author correction: discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing[J]. Nature, 2024, 631(8019): E3.
[103] HEGYI A, FRIEBE A, GORBALENYA AE, ZIEBUHR J. Mutational analysis of the active centre of coronavirus 3C-like proteases[J]. Journal of General Virology, 2002, 83(Pt 3): 581-593.
[104] HSU WC, CHANG HC, CHOU CY, TSAI PJ, LIN PI, CHANG GG. Critical assessment of important regions in the subunit association and catalytic action of the severe acute respiratory syndrome coronavirus main protease[J]. Journal of Biological Chemistry, 2005, 280(24): 22741-22748.
[105] CHEN S, ZHANG J, HU TC, CHEN KX, JIANG HL, SHEN X. Residues on the dimer interface of SARS coronavirus 3C-like protease: dimer stability characterization and enzyme catalytic activity analysis[J]. Journal of Biochemistry, 2008, 143(4): 525-536.
[106] SHI JH, SIVARAMAN J, SONG JX. Mechanism for controlling the dimer-monomer switch and coupling dimerization to catalysis of the severe acute respiratory syndrome coronavirus 3C-like protease[J]. Journal of Virology, 2008, 82(9): 4620-4629.
[107] JU XH, WANG ZQ, WANG PC, REN WL, YU YY, YU Y, YUAN B, SONG JW, ZHANG XC, ZHANG Y, XU C, TIAN BX, SHI Y, ZHANG R, DING Q. SARS-CoV-2 main protease cleaves MAGED2 to antagonize host antiviral defense[J]. mBio, 2023: e01373-23.
[108] LU JL, ZHOU XL. SARS-CoV-2 main protease Nsp5 cleaves and inactivates human tRNA methyltransferase TRMT1[J]. Journal of Molecular Cell Biology, 2023, 15(4): mjad024.
[109] ZHANG SL, WANG JF, CHENG GH. Protease cleavage of RNF20 facilitates coronavirus replication via stabilization of SREBP1[J]. Proceedings of the National Academy of Sciences of the United States of America, 2021, 118(37): e2107108118.
[110] ZHANG YB, LIU SY, XU QJ, LI HH, LU KF. Cleavage of the selective autophagy receptor SQSTM1/p62 by the SARS-CoV-2 main protease NSP5 prevents the autophagic degradation of viral membrane proteins[J]. Molecular Biomedicine, 2022, 3(1): 17.
[111] HUANG HX, ZHAO CC, LEI XX, ZHANG XY, LI YY, LAN T, ZHAO BP, LU JY, SUN WC, LU HJ, JIN NY. Swine acute diarrhoea syndrome coronavirus (SADS-CoV) Nsp5 antagonizes type I interferon signaling by cleaving DCP1A[J]. Frontiers in Immunology, 2023, 14: 1196031.
[112] SHI FS, LV Q, WANG TJ, XU JD, XU W, SHI YH, FU XY, YANG TM, YANG Y, ZHUANG LN, FANG WH, GU JY, LI XL. Coronaviruses Nsp5 antagonizes porcine gasdermin D-mediated pyroptosis by cleaving pore-forming p30 fragment[J]. mBio, 2022, 13(1): e0273921.
[113] RUI YJ, SU JM, SHEN S, HU Y, HUANG DB, ZHENG WW, LOU M, SHI YF, WANG M, CHEN SQ, ZHAO N, DONG Q, CAI Y, XU RZ, ZHENG S, YU XF. Unique and complementary suppression of cGAS-STING and RNA sensing- triggered innate immune responses by SARS-CoV-2 proteins[J]. Signal Transduction and Targeted Therapy, 2021, 6: 123.
[114] FUNG SY, SIU KL, LIN HY, YEUNG ML, JIN DY. SARS-CoV-2 main protease suppresses type I interferon production by preventing nuclear translocation of phosphorylated IRF3[J]. International Journal of Biological Sciences, 2021, 17(6): 1547-1554.
[115] SHE ZX, YAO YN, WANG CL, LI Y, XIONG XH, LIU YY. Mpro-targeted anti-SARS-CoV-2 inhibitor-based drugs[J]. Journal of Chemical Research, 2023, 47(4): 17475198231184799.
[116] SHI YF, DONG LT, JU Z, LI QF, CUI YR, LIU YR, HE JY, DING XP. Exploring potential SARS-CoV-2 Mpro non-covalent inhibitors through docking, pharmacophore profile matching, molecular dynamic simulation, and MM-GBSA[J]. Journal of Molecular Modeling, 2023, 29(5): 138.
[117] DROŻDŻAL S, ROSIK J, LECHOWICZ K, MACHAJ

F, SZOSTAK B, PRZYBYCIŃSKI J, LORZADEH S, KOTFIS K, GHAVAMI S, ŁOS MJ. An update on drugs with therapeutic potential for SARS-CoV-2 (COVID-19) treatment[J]. Drug Resistance Updates, 2021, 59: 100794.
[118] TAN B, JOYCE R, TAN HZ, HU YM, WANG J. SARS-CoV-2 main protease drug design, assay development, and drug resistance studies[J]. Accounts of Chemical Research, 2023, 56(2): 157-168.
[119] van de WATERBEEMD H, GIFFORD E. ADMET in silico modelling: towards prediction paradise[J]. Nature Reviews Drug Discovery, 2003, 2(3): 192-204.
[120] IMANI M, MOHAJERI N, RASTEGAR M, ZARGHAMI N. Recent advances in FRET-Based biosensors for biomedical applications[J]. Analytical Biochemistry, 2021, 630: 114323.
[121] de WISPELAERE M, DU GY, DONOVAN KA, ZHANG TH, ELEUTERI NA, YUAN JC, KALABATHULA J, NOWAK RP, FISCHER ES, GRAY NS, YANG PL. Small molecule degraders of the hepatitis C virus protease reduce susceptibility to resistance mutations[J]. Nature Communications, 2019, 10: 3468.
[122] LIANG JS, WU YH, LAN K, DONG CE, WU SW, LI S, ZHOU HB. Antiviral PROTACs: opportunity borne with challenge[J]. Cell Insight, 2023, 2(3): 100092.
[123] MA Y, FRUTOS-BELTRÁN E, KANG DW, PANNECOUQUE C, de CLERCQ E, MENÉNDEZ-ARIAS L, LIU XY, ZHAN P. Medicinal chemistry strategies for discovering antivirals effective against drug-resistant viruses[J]. Chemical Society Reviews, 2021, 50(7): 4514-4540.
[124] YAMINI G, NESTOROVICH EM. Multivalent inhibitors of channel-forming bacterial toxins[J]. Current Topics in Microbiology and Immunology, 2017, 406: 199-227.
[125] ALUGUBELLI YR, XIAO J, KHATUA K, KUMAR S, SUN L, MA YY, MA XR, VULUPALA VR, ATLA S, BLANKENSHIP LR, COLEMAN D, XIE XP, NEUMAN BW, LIU WR, XU SQ. Discovery of first-in-class PROTAC degraders of SARS-CoV-2 main protease[J]. Journal of Medicinal Chemistry, 2024, 67(8): 6495-6507.
[126] ZHAO KS, ZHANG Y, SUI WH. Association between whole blood selenium levels and triglyceride-to-high-density lipoprotein cholesterol ratio among the general population[J]. Biological Trace Element Research, 2024, 202(5): 1998-2007.
[127] AMPORNDANAI K, MENG XL, SHANG WJ, JIN Z, ROGERS M, ZHAO Y, RAO ZH, LIU ZJ, YANG HT, ZHANG LK, O'NEILL PM, SAMAR HASNAIN S. Inhibition mechanism of SARS-CoV-2 main protease by ebselen and its derivatives[J]. Nature Communications, 2021, 12: 3061.