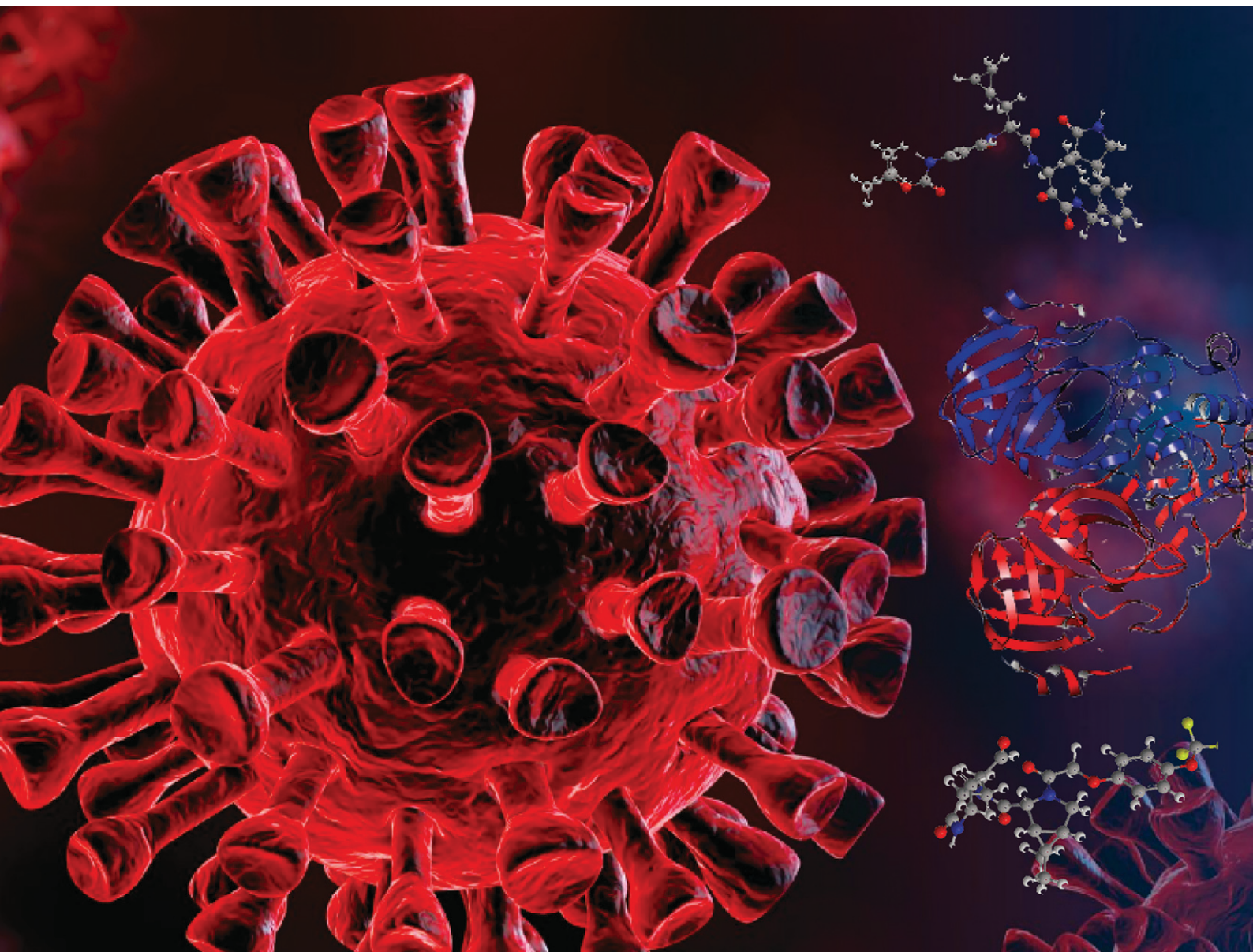


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SARS-COV inhibitors

REVIEW



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A review of the latest research on M^{Pro} targeting SARS-COV inhibitors

Huihui Yang^b and Jinfei Yang^{id}*^{ab}

Since the outbreak of COVID-19, the pandemic caused by SARS-CoV-2 infection is still spreading at an alarming rate and has caused huge loss of life and economic damage worldwide. Although more than one year has passed, effective treatments for COVID-19 and other pathogenic coronaviruses have not yet been developed. Therefore, the development of SARS-CoV-2 inhibitors is an urgent priority. Given that the M^{Pro} sequences of SARS-CoV-2 and SARS-CoV-1 are 100% identical in the catalytic domain for protein cleavage, the viral main protease (M^{Pro}) is one of the most extensive drug targets in all the drug targets being investigated for SARS-CoV-2. To provide scientific researchers with timely anti-SARS-CoV drug development information for M^{Pro}, we focus on the past and current drug design and development strategies for M^{Pro} in this review. We believe that this review will provide meaningful guidance for the design and development of innovative drugs against COVID-19 and other pathogenic coronaviruses in the future.

1 Introduction

Since the first case was detected in December 2019, coronavirus disease 2019 (COVID-19) has rapidly evolved into a global pandemic.^{1,2} This rapidly spreading pathogenic virus was soon identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive-sense single-stranded RNA virus consisting of four structural proteins and an RNA genome.³ Up to now, the origin of SARS-CoV-2 is still under investigation. According to the past reports, the closest strain is the bat coronavirus RaTG13, which has 96% sequence similarity with SARS-CoV-2.³ Humans have encountered many similar fatal virus outbreaks, such as Ebola virus,⁴ Zika virus⁵ and Nipah virus,⁶ as well as various coronaviruses (CoV) including SARS-CoV⁷ and MERS-CoV.⁸ However, the recent outbreak of SARS-CoV-2 is the most serious public health crisis since the Spanish influenza pandemic.^{9,10} As of January 12, 2021, there have been more than 91 million positive cases with nearly 2 million deaths in the world. After in-depth research by scientists, the SARS-CoV-2 genome was shown to encode two large polyproteins, pp1a and pp1ab, which contain overlapping sequences and the main viral protease (M^{Pro}).¹¹ The function of this internally encoded M^{Pro} is essential to the processing of these proteins and critical for viral replication.¹² SARS-CoV-2 M^{Pro} is very similar to SARS-CoV-1 M^{Pro}. Specifically, SARS-CoV-1 and SARS-CoV-2 share

96% identity between their respective M^{Pro} sequences and 100% identity in the active site.¹³

2 Drug design targets

The target of drug action is very important to the design of drug molecules during the development of small molecule drugs. SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus belonging to the genus β -coronavirus, which also includes SARS-CoV, MERS-CoV, HCoV-OC43 and HCoV-HKU1. Given that SARS-CoV-2 shares about 80% sequence similarity with SARS-CoV-1, most of the antiviral drugs for SARS-CoV-2 were originally developed against SARS-CoV-1 or other related coronaviruses.¹⁴ To know the target of SARS-CoV-2, it is necessary to clearly understand its specific life cycle. Specifically, SARS-CoV-2 infects ACE2-expressing cells and enters the cell through either direct cell surface fusion or the endosomal pathway. For direct cell surface fusion, the host membrane protease TMPRSS2 cleaves the viral spike protein, triggering viral membrane fusion with the host cell membrane.¹⁵ For endosomal entry, cathepsin L mediates the cleavage of the viral spike protein.¹⁶ Once the viral RNA is released in the cytoplasm, it undergoes translation into viral polyproteins pp1a and pp1ab, which are subsequently cleaved by two viral proteases, the main protease (M^{Pro}) and the papain-like protease (PL^{Pro}), the main protease also being called 3-chymotrypsin-like protease (3CL^{Pro}). Then, the released viral proteins can be assembled to form a viral polymerase RdRp complex to catalyze the replication of viral RNA. Finally, the progeny virions are released from the infected cells through exocytosis and are ready for the next round of infection.

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Based on the above introduction to the SARS-CoV-2 infection route, we can clearly know that M^{Pro}, PL^{Pro}, ACE2 and RdRp play a very critical role in the replication of coronavirus. Among these key targets, M^{Pro} has become the most important target for anti-COVID-19 drug design due to its highly conservative nature and specificity.

3 Main protease (M^{Pro})

The SARS-CoV main protease is excised from the polyprotein by its own proteolytic activity, and then cleaves the polyprotein together with the papain-like protease, resulting in a total of 16 functional non-structural proteins (nsps). According to reports, the M^{Pro} of SARS-CoV specifically acts at 11 cleavage sites of large polyprotein 1ab (790 kDa),¹⁷ and no human protease has been found to have similar cleavage specificity. The cleaved nsps play essential roles in assembling the viral replication transcription complex (RTC) to initiate the viral replication. Although some companies have developed vaccines that can prevent COVID-19, their preventive effect is not good enough. Therefore, the development of small molecule antiviral drugs that can effectively inhibit SARS-CoV-2 is still a top priority.

In view of the high specificity of M^{Pro} and the extremely low toxicity of M^{Pro} inhibitors to host cells, M^{Pro} has become one of the most intriguing drug targets for antiviral drug development.^{13,18}

4 SARS-CoV M^{Pro} inhibitors

Since the SARS outbreak in 2003,^{19,20} and later on with the COVID-19 outbreak in late 2019,^{1,2} most of the scientific research teams studying SARS-CoV have devoted themselves to the development of peptidomimetic and non-peptidomimetic M^{Pro} and PL^{Pro} inhibitors.^{21–24} Due to its highly conservative nature and specificity, M^{Pro} has become one of the most popular research targets. Specifically, M^{Pro} is a cysteine protease that cleaves the viral polyprotein at more than 11 sites. It has a unique substrate preference for glutamine at the P1 position, while no host protease is known to have such a preference.^{25–27} Since the structure of M^{Pro} was published, it has greatly promoted the research on M^{Pro} inhibitors as new antiviral drugs.^{18,28} From the structural point of view, M^{Pro} has three domains: I, II and III. The active site region spans domains I and II, which are β barrel domains, while domain III presents an α -helical structure. In the active site region, Cys145 acts as a nucleophile, while His41 acts as a general acid/base. Based on the above information on M^{Pro}, scientists have developed many M^{Pro} inhibitors. Although only a small number of inhibitors that have been developed show good affinity for the M^{Pro} target, they provide a good starting point for further M^{Pro}-targeted antiviral drug development.^{29–32}

4.1 SARS-CoV-1 M^{Pro} inhibitors

In 2005, Ghosh and collaborators reported the design, synthesis and biological evaluation of some peptidomimetic

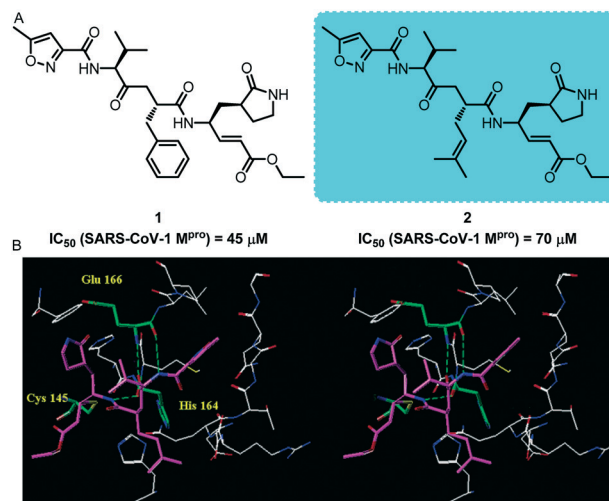


Fig. 1 A, Chemical structures of compounds 1 and 2. B, X-ray crystal structure of compound 2 (thick stick with magenta carbon) with SARS-CoV-1 M^{Pro}. Hydrogen bonds between the inhibitor and SARS-CoV-1 M^{Pro} are shown as green dotted lines.

SARS-CoV-1 M^{Pro} inhibitors. Biological experimental evaluation results show that this type of inhibitor can be used to treat severe acute respiratory syndrome.³³ These inhibitors exhibited antiviral activity against SARS-CoV-1 in infected cells in the micromolar range; compounds 1 and 2 exhibited excellent inhibitory activity with an IC₅₀ value of 45 μ M and 70 μ M, respectively. The X-ray crystal structure shows that lead inhibitor 2 has a good binding effect with SARS-CoV-1 M^{Pro}. The results of this study provide an important drug design template for the design of SARS-CoV-1 M^{Pro} small molecule inhibitors (Fig. 1). Meanwhile, it provides reference for the follow-up study of SARS-CoV M^{Pro} related inhibitors.

One year later, Hsu and his collaborators discovered an effective SARS-CoV-1 M^{Pro} inhibitor (TG-0205221, $K_i = 53$ nM), which showed good anti-SARS coronavirus activity (Fig. 2).³⁴ Analysis of the crystal structure showed that the inhibitor exerts its antiviral activity through the interaction of its 16 carbon atoms with 10 residues on the enzyme, and the extensive hydrophobic contact with the enzyme. The inhibitor binds to SARS-CoV-1 M^{Pro} through 10 hydrogen bonds and 1 covalent bond, forming a very strong binding conformation. The crystal structure of TG-0205221 and SARS-CoV-1 M^{Pro}

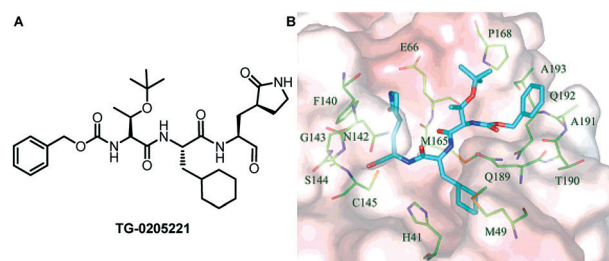


Fig. 2 A, Chemical structure of TG-0205221. B, Stereo-view of SARS-CoV-1 M^{Pro} bound with the TG-0205221 inhibitor shown by electrostatic potential.

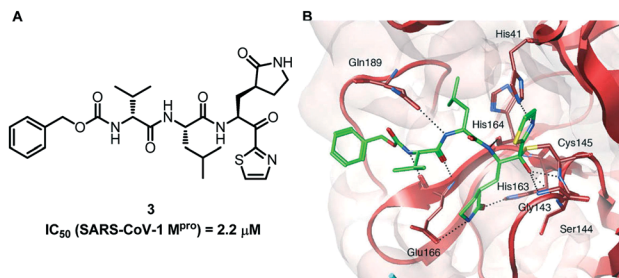


Fig. 3 A, Chemical structure of compound 3. B, Molecular dynamics simulated pose of compound 3 (green stick) bound to SARS-CoV-1 M^{pro} (PDB 1WOF, with red and cyan ribbons with molecular surfaces).

(resolution = 1.93 Å) shows a unique binding mode, including covalent bonds, hydrogen bonds and many hydrophobic interactions. The results of this study provide a basis for the further development of structure-based peptidomimetic SARS-CoV-1 M^{pro} inhibitors.

In 2009, Hayashi and his colleagues developed a series of peptidomimetic SARS-CoV-1 M^{pro} inhibitors containing trifluoromethyl, benzothiazolyl and thiazolyl ketones. Some of the compounds show good biological activity.³⁵ Among all the small molecule inhibitors, compound 3 containing P1-pyrrolidone and a warhead thiazolyl unit showed the best inhibitory efficacy against SARS-CoV-1 M^{pro} with an IC₅₀ value of 2.2 μM. It provides a new direction for the subsequent rapid development of more effective inhibitors (Fig. 3).

On the basis of previous research work on SARS-CoV-1 M^{pro} inhibitors, four years later, the same authors reported the design and synthesis of a series of dipeptide inhibitors with novel P3 scaffolds. These compounds containing P3 scaffolds are effective against SARS-CoV-1 M^{pro} and also show

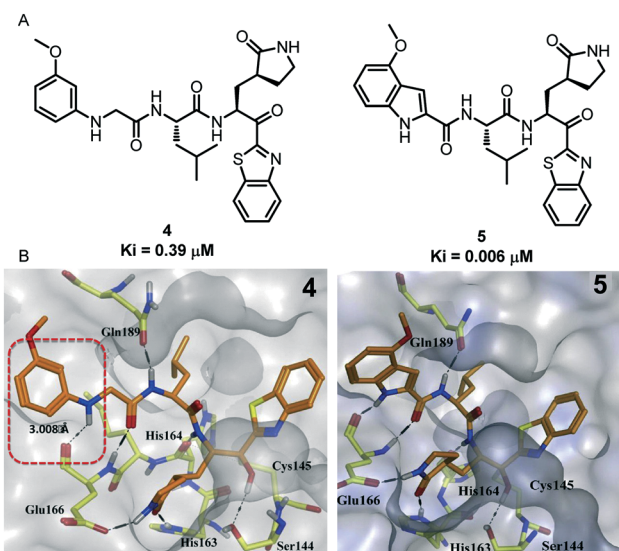


Fig. 4 A, Chemical structures of compounds 4 and 5. B, Molecular docking pose and binding interactions of compounds 4 and 5 (orange sticks) bound to SARS-CoV-1 M^{pro} (PDB ID: 1WOF). Only the residues (yellow color), which are engaged in binding to the ligands (orange sticks), are highlighted. Dotted black lines represent the hydrogen bonding interaction.

good inhibitory activity.^{36–38} In particular, compound 5 showed the best inhibitory activity with a K_i value of 0.006 mM. Happily, its inhibitory potency against SARS-CoV-1 M^{pro} is 65 times higher than that of the lead compound 4 (K_i = 0.39 μM). In addition, they used isothermal titration calorimetry (ITC) to verify the experimental results (Fig. 4).

While developing peptidomimetic SARS-CoV-1 M^{pro} inhibitors, many medicinal chemists have also made a lot of effort in the design of non-peptidomimetic drug molecules. Vederas and collaborators designed and synthesized many non-peptidyl inhibitors containing pyridyl ester structures.^{39–41} Some of these compounds have shown high inhibitory activity against SARS-CoV-1 M^{pro}, and the IC₅₀ value of compound 8 can be up to 50 nM (Fig. 5). They used electrospray mass spectrometry to reveal the mechanism of action of such small molecule inhibitors on SARS-CoV-1 M^{pro}. These results provide a template for the further development of non-peptidomimetic SARS-CoV-1 inhibitors.

Subsequently, Ghosh and his co-workers reported a series of 5-chloropyridinyl indolecarboxylates as potent non-peptidyl SARS-CoV-1 M^{pro} inhibitors (Fig. 6).⁴² It is worth noting that the position of the ester functional group is critical to the effectiveness of the inhibitor. After investigating the positions of a large number of functional groups, they found that a compound containing 5-chloropyridyl ester 15 showed the most effective inhibitory effect. Its SARS-CoV-1 M^{pro} IC₅₀ value was 30 nM and its antiviral EC₅₀ value was 6.9 μM. Subsequently, they proved the possible combination of these inhibitors with SARS-CoV-1 M^{pro} through molecular docking experiments.

Collectively, since the SARS outbreak in 2003, many scientists have made many contributions to the development of SARS-CoV-1 M^{pro} inhibitors. They have developed many peptidomimetic and non-peptidomimetic SARS-CoV-1 M^{pro} inhibitors. Here, we only list some representative works for your reference. These efforts in the development of SARS-CoV-1 M^{pro} inhibitors have laid a solid foundation for the subsequent SARS-CoV-2 M^{pro} inhibitors.

4.2 SARS-CoV-2 M^{pro} inhibitors

In view of the high similarity between SARS-CoV-1 M^{pro} and SARS-CoV-2 M^{pro}, the former provides a lot of reference for the

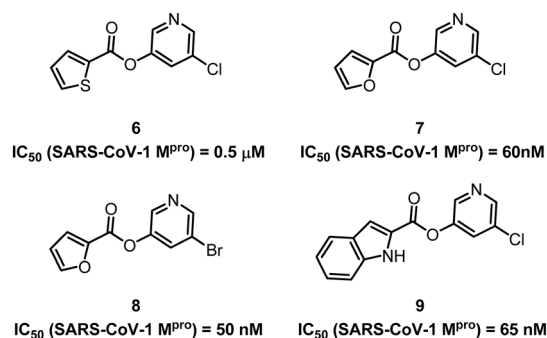


Fig. 5 Chemical structures of compounds 6–9.

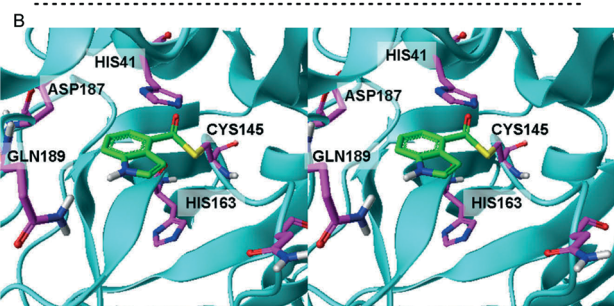
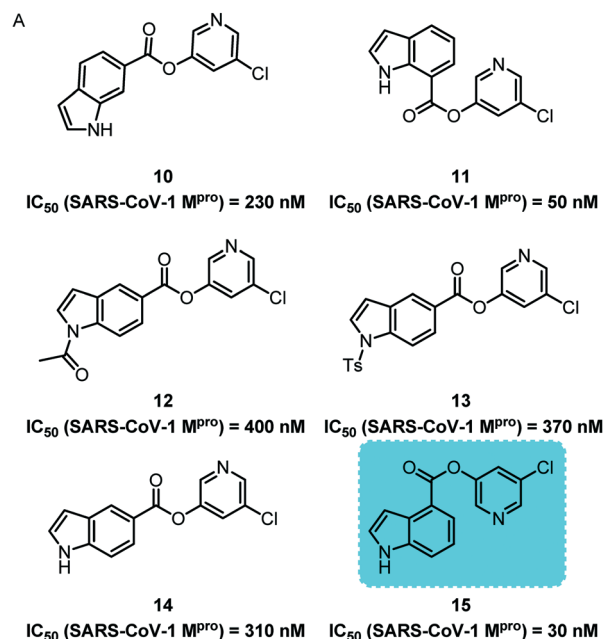


Fig. 6 A, Chemical structures of compounds 10–15. B, GOLD docked conformation of 15 (green), covalently linked to Cys-145 of M^{Pro} based on the 2V6N M^{Pro} structure.

development of SARS-CoV-2 M^{Pro} inhibitors. Like SARS-CoV-1, SARS-CoV-2 M^{Pro} inhibitors can be classified as peptoids and non-peptidomimetics, and the mechanism of action of peptide inhibitors includes two steps. Peptidomimetics that mimic natural peptide substrates initially bind to M^{Pro} and form a noncovalent complex, and the warhead group, which is spatially very close to the catalytic residue of the target protein, undergoes a nucleophilic attack to catalyse the formation of cysteine-participating covalent bonds.^{43,44} These warheads mainly contain Michael receptors, aldehydes and different types of ketones,^{45–47} which covalently bind to the Cys145 residue in the M^{Pro} S1' pocket to exert an inhibitory effect.^{48,49} At the present moment, many research papers and reviews related to SARS-CoV-2 M^{Pro} inhibitors have been reported.^{50–52} Next, we will select some representative research work to introduce to you.

Mitsuya and his collaborators developed a series of non-peptidyl SARS-CoV-2 M^{Pro} inhibitors.⁵³ In particular, two indole chloropyridinyl-ester derivatives, GRL-0820 and GRL-0920, exerted potent activity against SARS-CoV-2 in cell-based assays performed using VeroE6 cells and TMPRSS2-

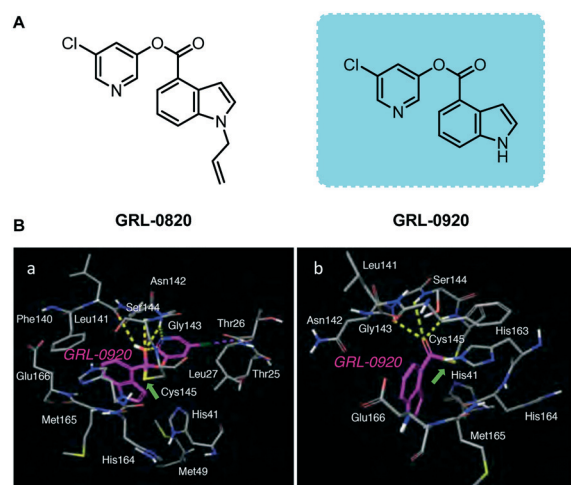


Fig. 7 A, Chemical structures of compounds GRL-0820 and GRL-0920. B, Molecular models of interactions of GRL-0920 with SARS-CoV-2 M^{Pro} .

overexpressing VeroE6 cells (Fig. 7). They pointed out in the article that although GRL-0820 prevented SARS-CoV-2 infection, viral breakthrough occurred. GRL-0920 not only exerts effective activity on SARS-CoV-2 (EC_{50} = 2.8 μ M), but also significantly reduces the infectivity, replication ability and cytopathic effects of SARS-CoV-2, and no obvious cytotoxicity is seen. Subsequently, structural modeling showed that the indole and chloropyridinyl of the derivatives interact with two catalytic dyad residues of M^{Pro} , Cys145 and His41, resulting in covalent bonding, which was verified by high-performance liquid chromatography–mass spectrometry (HPLC/MS). The research results show that the indole moiety is essential for the anti-SARS-CoV-2 activity of the derivative. This also proves that this type of indole chloropyridine containing active ester has a good anti-SARS-CoV-2 effect.

While developing non-peptidomimetic SARS-CoV-2 M^{Pro} inhibitors, many researchers have also made great progress

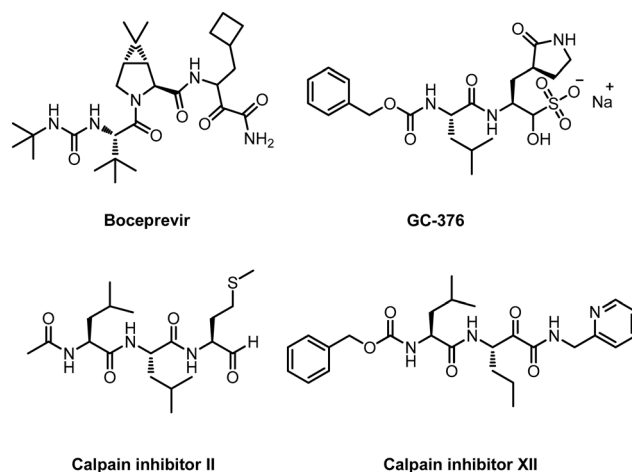


Fig. 8 Chemical structures of boceprevir, GC-376, calpain inhibitors II and XII.

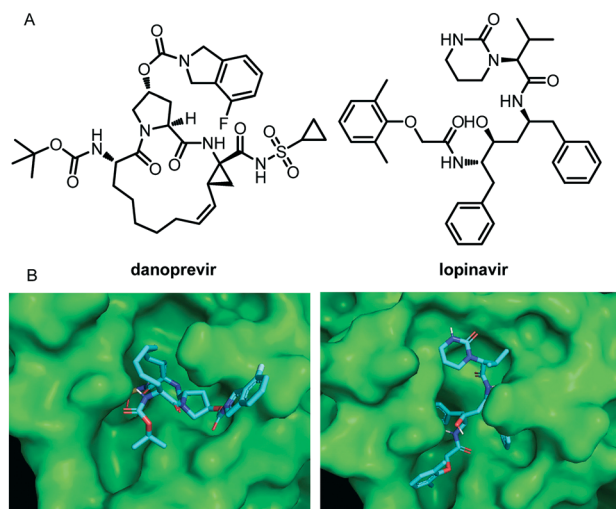


Fig. 11 A, Chemical structures of danoprevir and lopinavir. B, Danoprevir and lopinavir docked in the binding site of SARS-CoV-2 M^{Pro}.

temperature for at least 3 days; 2) significantly improved respiratory symptoms; (3) lung imaging shows obvious absorption and recovery of acute exudative lesion; 4) two consecutive RT-PCR negative tests of SARS-CoV-2 nucleotide acid (respiratory track sampling with an interval of at least one day). These clinical experimental data provide strong evidence for the potential of the two molecules in SARS-CoV-2 therapy.

Yang and his co-workers also did a lot of beautiful work in the development of anti-SARS-CoV-2 M^{Pro} inhibitors since the outbreak of the pandemic. In early 2020, based on the discovery of the N3 inhibitor against SARS-CoV-1 M^{Pro} by Rao *et al.*,⁵⁸ they identified an N3 inhibitor also suitable for SARS-CoV-2, and then determined the crystal structure of M^{Pro} of SARS-CoV-2 in complex with this compound (Fig. 12).¹¹ In addition, Yang and his colleagues used structure-based virtual and high-throughput screening to analyze more than 10 000 compounds including candidate drugs in clinical trials, approved drugs and other pharmacologically active compounds. The test results show that six drug molecules (ebiselen, disulfiram, tideglusib, carmofur, shikonin and PX-12) have strong inhibitory effects on SARS-CoV-2 M^{Pro}, showing half-maximal inhibitory concentration values that ranged from 0.67 to 21.4 μM (Fig. 13). Among them, ebiselen exhibited promising antiviral activity in cell-based assays. Subsequently, they also characterized the X-ray crystal structure of the composite of M^{Pro} and carmofur, and the crystal structure reveals that the carbonyl reactive group of carmofur showed an antiviral effect by covalently binding to the catalytic Cys145.⁵⁹

To explore the mechanism of action of the above six SARS-CoV-2 M^{Pro} inhibitors, subsequently, Wang and his co-workers investigated the mechanism of action of the six M^{Pro} inhibitors using a consortium of techniques including the FRET-based enzymatic assay, thermal shift assay, native mass spectrometry, cellular antiviral assays, and molecular

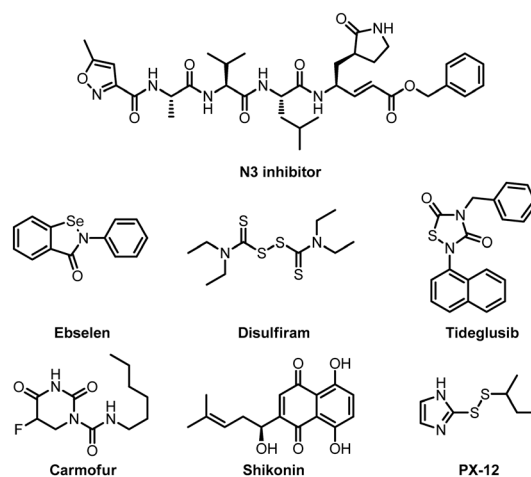


Fig. 12 Chemical structures of the N3 inhibitor, ebselen, disulfiram, tideglusib, carmofur, shikonin, and PX-12.

dynamics simulations.⁶⁰ Collectively, the results of this study show that the inhibitory effects of these six compounds on SARS-CoV-2 M^{Pro} are non-specific, and their inhibitory effects can be eliminated or greatly reduced by adding the reducing agent dithiothreitol (DTT). Without DTT, these six compounds inhibit not only M^{Pro} but also a panel of viral cysteine proteases including SARS-CoV-2 PL^{Pro} and 2A^{Pro} and 3C^{Pro} from enterovirus A71 (EV-A71) and EV-D68.

High-throughput drug screening technology is an effective way to obtain active molecules relatively quickly in drug development. Recently, Zeng and his colleagues used the SARS-CoV-2 M^{Pro} analysis method to perform quantitative high-throughput screening (qHTS) on 10 755 compounds including approved drugs, drugs under investigation, and other biologically active molecules.⁶¹ Finally, they obtained 23 SARS-CoV-2 M^{Pro} small molecule inhibitors with IC₅₀ ranging from 0.26 to 28.85 μM . Among them, Walrycin B (IC₅₀ = 0.26 μM), LLL-12 (IC₅₀ = 9.84 μM) and Z-FA-FMK (IC₅₀ = 11.39 μM) are the most effective M^{Pro} inhibitors (Fig. 13). Notably, they used the SARS-CoV-2 cytopathic effect assay to confirm their activity against SARS-CoV-2 virus infection.

To speed up drug discovery and development, Xu and his co-workers investigated the inhibition of SARS-CoV-2 M^{Pro} by natural products derived from traditional Chinese medicines.⁶² In this study, baicalin and baicalein were identified as the first non-covalent, non-peptidomimetic inhibitors of SARS-CoV-2 M^{Pro} and exhibited potent antiviral activities in a cell-based

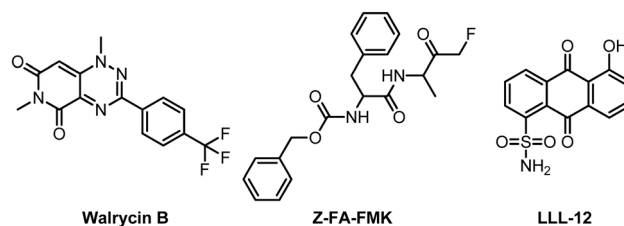


Fig. 13 Chemical structures of Walrycin B, LLL-12 and Z-FA-FMK.

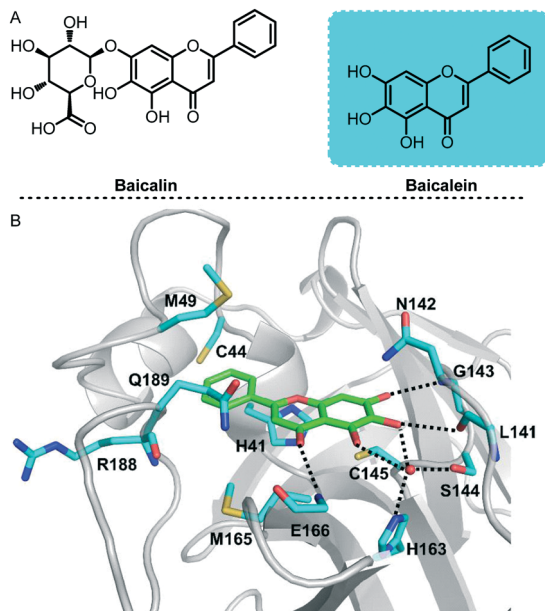


Fig. 14 A, Chemical structures of baicalin and baicalein. B, Interactions formed between baicalein (green) and surrounding residues (cyan). Residues as well as the ligand are shown as sticks and hydrogen bonds are represented by black-dashed lines.

system (Fig. 14). Remarkably, X-ray protein crystallography results show that the binding mode of baicalein with SARS-CoV-2 M^{pro} is distinctly different from those of known inhibitors. Baicalein is perfectly ensconced in the core of the substrate-binding pocket by interacting with two catalytic residues, the crucial S1/S2 subsites and the oxyanion loop, acting as a “shield” in front of the catalytic dyad to prevent the peptide substrate from approaching the active site. This binding mode is significantly different from those of known inhibitors. Baicalein’s unique mode of action, good *in vitro* antiviral activity and good safety data from clinical trials all proved its great potential as an anti-coronavirus drug. This also proves why traditional Chinese medicines played an important role in the outbreak of this pandemic.

Recently, Yang and his collaborators designed and synthesized 32 new M^{pro} inhibitors containing bicyclic proline.⁶³ All the compounds inhibit SARS-CoV-2 M^{pro} activity *in vitro*, with IC₅₀ values between 7.6 and 748.5 nM. After evaluating the anti-SARS-CoV-2 virus activity, rat pharmacokinetic properties and safety of these molecules at the cellular level, they finally selected two small molecule compounds (MI-09 and MI-30) with high activity and safety to carry out *in vivo* antiviral activity tests (Fig. 15). In a transgenic mouse model of SARS-CoV-2 infection, oral or intraperitoneal injection of MI-09 or MI-30 can significantly reduce lung viral load and lung pathological damage. This is the first publicly reported experimental data of M^{pro} inhibitors in a mouse model of SARS-CoV-2 infection.

Mitsuya and his collaborators have long been engaged in the development of antiviral drugs. After continuous exploration, they discovered two small molecule compounds

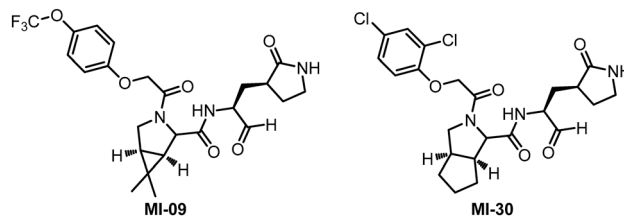


Fig. 15 Chemical structures of MI-09 and MI-30.

GRL-1720 and **20** that target SARS-CoV-2 M^{pro} (Fig. 16).⁶⁴ Based on the detection of VeroE6 cells and RNA-qPCR, the results of cytopathic detection and immunocytochemistry experiments show that these two compounds can prevent SARS-CoV-2 infection. The EC₅₀ values of GRL-1720 and **20** are 15 ± 4 and 4.2 ± 0.7 μM, respectively. Compound **20** completely blocked SARS-CoV-2 infection *in vitro*, and there was no viral breakthrough or detectable cytotoxicity. In addition, the combination of **20** and remdesivir showed a synergistic effect against SARS-CoV-2. Therefore, they believe that compound **20** can be used as the main inhibitor of M^{pro} for the development of therapeutic drugs for SARS-CoV-2 infection.

Interestingly, Hoffman and his collaborators reported a hydroxymethyl ketone derivative PF-00835231, which is structurally similar to compound **20** and shows effective inhibition of SARS-CoV-2 in M^{pro} and antiviral tests.⁶⁵ Preclinical experiments show that PF-00835231 can be used as an effective inhibitor of SARS-CoV-2 M^{pro} and has the potential to become an intravenous treatment drug for COVID-19.

4.3 SARS-CoV-2 M^{pro} inhibitors from computer-aided design

Computer-aided virtual screening technology has played an indispensable role in this COVID-19 pandemic. Many scientists have used this technology to screen out a large number of M^{pro} inhibitors with potential anti-SARS-CoV-2 effects. For example, Lee and his co-workers identified potential inhibitors against COVID-19, using an amalgam of virtual screening, molecular dynamics (MD) simulations, and binding free energy approaches from the Korea Chemical Bank drug repurposing database.⁶⁶ The screening of the Korea Chemical Bank drug repurposing database resulted in 149 binders. They studied the kinetics of the protein–drug complex formation of the seven

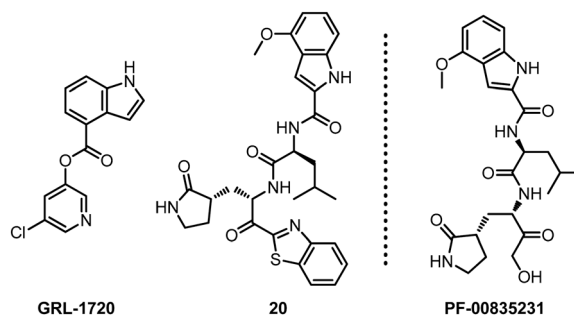


Fig. 16 Chemical structures of GRL-1720, **20** and PF-00835231.

highest-scoring drugs through MD simulations. The test results showed that six drugs exhibited good interactions with the active site of SARS-CoV-2 M^{PRO}. Furthermore, binding free energy calculations suggested that the community-acquired bacterial pneumonia drugs ceftaroline fosamil and telaprevir are potent inhibitors against SARS-CoV-2 M^{PRO} (Fig. 17). Molecular dynamics and interaction analysis revealed that ceftaroline fosamil and telaprevir form hydrogen bonds with important active site residues such as Thr24, Thr25, His41, Thr45, Gly143, Ser144, Cys145, and Glu166, which is supported by crystallographic information of known inhibitors.

The study of Mesecar and his co-workers also proved that telaprevir is a potent inhibitor against M^{PRO}.⁶⁷ In their study, telaprevir showed significant inhibition of M^{PRO} from SARS-CoV-2 with an IC₅₀ value of 10.7 ± 0.4 μM.

Almost simultaneously, Kang and his co-workers used their pre-trained deep learning-based drug-target interaction model called Molecule Transformer-Drug Target Interaction (MT-DTI) to screen for inhibitors that have a better inhibitory effect on the SARS-CoV-2 main protein.⁶⁸ The test results showed that atazanavir (Fig. 17), an antiretroviral medication used to treat and prevent the human immunodeficiency virus (HIV), is the best compound, showing an inhibitory potency with a K_d of 94.94 nM against the SARS-CoV-2 main proteinase.

For finding more potential drugs as inhibitors of SARS-CoV-2 M^{PRO}, Zhu and his colleagues conducted molecular docking experiments on 1903 small molecule drugs by establishing a homology model based on the structure of SARS-CoV-2 M^{PRO}.⁶⁹ Based on the docking score and the 3D similarity of the binding mode to the known SARS-CoV-2 M^{PRO} ligands, four drugs were selected for binding free energy calculations. The

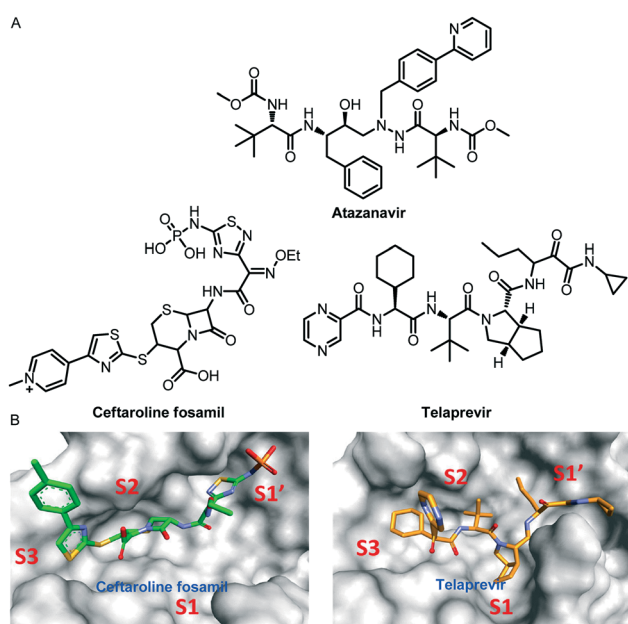


Fig. 17 A, Chemical structures of atazanavir, ceftaroline fosamil and telaprevir. B, The binding of drugs at subsites S1, S1', S2, and S3 of SARS-CoV-2 M^{PRO}. Ceftaroline fosamil and telaprevir are shown in stick forms in green and orange, respectively.

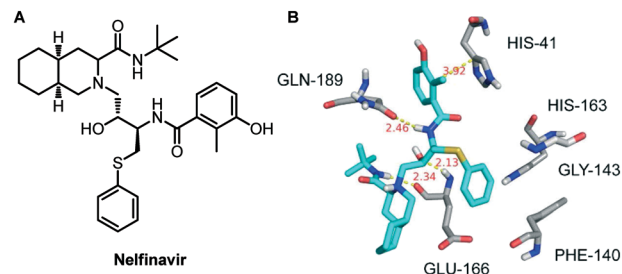


Fig. 18 A, Chemical structure of nelfinavir. B, Interactions between nelfinavir and associated residues in the homology model of SARS-CoV-2 M^{PRO}. The data in red are the interaction distances (Å).

calculation results show that both the MM/GBSA and SIE methods vote for nelfinavir, with a binding free energy of -24.69 ± 0.52 kcal mol⁻¹ and -9.42 ± 0.04 kcal mol⁻¹, respectively. Therefore, they proposed that nelfinavir might be a potential inhibitor against SARS-CoV-2 M^{PRO} (Fig. 18).

Riva *et al.* conducted a virtual screening of 12 000 clinical-stage and FDA-approved small molecules in the drug database. The test results showed that six molecules have the characteristics of a cell dose-activity relationship, and showed an effective concentration that may be equivalent to the patient's therapeutic dose.^{70,71} The six molecules include the PIKfyve kinase inhibitor apilimod, cysteine protease inhibitors MDL-28170, Z LVG CHN2, VBY-825, and ONO 5334, and the CCR1 antagonist MLN-3897. Since many of these molecules have advanced into the clinic, the known pharmacological and human safety profiles of these compounds will accelerate their preclinical and clinical evaluation for COVID-19 therapy. Among them, Z LVG CHN2 exerted its anti-COVID-19 effects by inhibiting the SARS-CoV-2 main protease (Fig. 19).

5 Summary and perspective

The COVID-19 pandemic caused by the highly transmissible SARS-CoV-2 has become the world's largest public health crisis. The pandemic is still spreading at an alarming rate and has caused fatal deaths, especially for the elderly and immunocompromised patients. According to BBC reports, countries such as the United Kingdom and China have found mutant strains that spread faster. Although some people have begun to be vaccinated with vaccines developed by China and the United States, the immunization efficiency is still not high enough. Therefore, the development of antiviral drugs for the treatment of COVID-19 is still urgent. In our current

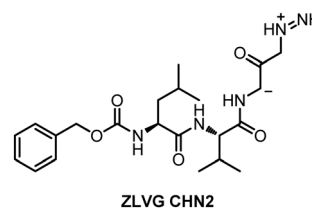


Fig. 19 Chemical structure of Z LVG CHN2.

pandemic situation, effective antiviral treatments may have a major impact on reducing morbidity and mortality. At the same time, antiviral drugs can also be used as cheap prevention methods. Computer-aided drug design, especially artificial intelligence, is rapidly emerging. As the accuracy of artificial intelligence in assisted drug design improves, it will provide more help to the development of SARS-COV inhibitors. As a highly conservative SARS-COV target, M^{Pro} should be continuously of concern and studied. The research on M^{Pro} inhibitors is of great significance both for the present and the future development of preventive drugs. In addition, we believe that strengthening the interdisciplinary integration of computer, chemistry and biomedicine will accelerate the development of M^{Pro} inhibitors, and our research team is also working towards this goal.

This review summarizes many representative works in the development of SARS-COV main protease drugs, and focuses on the structure of many small lead compounds and the active sites of action. We hope that this review will provide ideas and help for the future development of anti-COVID-19 and other coronavirus drugs, and help develop a broad-spectrum antiviral drug for the treatment of coronavirus diseases as soon as possible.

Conflicts of interest

There are no conflicts to declare.

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