Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

European Journal of Medicinal Chemistry 257 (2023) 115491

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: www.elsevier.com/locate/ejmech

Review article

The research progress of SARS-CoV-2 main protease inhibitors from 2020 to 2022

Xiaojing Pang [a,1], Wei Xu [b,1], Yang Liu [a], Hua Li [a,b,**], Lixia Chen [a,*]

[a] Wuya College of Innovation, Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang, 110016, China

[b] Institute of Structural Pharmacology & TCM Chemical Biology, College of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou, 350122, China

ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
M^{pro} inhibitors
Drug repurposing
Drug design

ABSTRACT

The novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide. The main protease (M^{pro}) of SARS-CoV-2 plays a central role in viral replication and transcription and represents an attractive drug target for fighting COVID-19. Many SARS-CoV-2 M^{pro} inhibitors have been reported, including covalent and noncovalent inhibitors. The SARS-CoV-2 M^{pro} inhibitor PF-07321332 (Nirmatrelvir) designed by Pfizer has been put on the market. This paper briefly introduces the structural characteristics of SARS-CoV-2 M^{pro} and summarizes the research progress of SARS-CoV-2 M^{pro} inhibitors from the aspects of drug repurposing and drug design. These information will provide a basis for the drug development of treating the infection of SARS-CoV-2 and even other coronaviruses in the future.

1. Introduction

At the end of 2019, COVID-19 caused by SARS-CoV-2 brought about an unprecedented public health emergency around the globe. The severity of infection varies from asymptomatic to severe fatal disease [1,2]. SARS-CoV-2 mainly spreads through small droplets discharged by infected individuals when they breathe or cough [3,4]. As of February 18, 2023, 756 million confirmed cases and 6.8 million deaths have been reported globally (https://www.who.int/emergencies/diseases/novel-coronavirus-2019).

Coronaviruses (CoVs) are positive single-stranded RNA viruses being diversely prevalent in humans and wildlife [5]. According to the International Committee on Taxonomy of Viruses, Coronaviruses belong to the subfamily Coronavirinae in the family Coronaviridae of the order Nidovirales. The Coronavirinae are divided into four genera, including α-, β-, γ- and δ-coronaviruses (Fig. 1). There are seven coronaviruses that can infect humans. HCoV-229E and HCoV-NL63 belong to α-coronavirus, and HCoV-OC43, HCoV-HKU1, SARS-CoV-1, Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2 belong to type β-coronavirus [6–8]. Coronavirus has posed a persistent threat to human. In 2002, SARS-CoV-1 infected 8096 people, of whom 774 died. MERS-CoV, which appeared in 2012, affected a total of 1841 individuals, 652 of whom died [9,10]. Therefore, it is an urgent task to develop more efficient antiviral drugs [11].

Recently, a large number of drug targets of SARS-CoV-2 have been identified through relevant studies on COVID-19. The main protease (M^{pro}) is vital for the replication of SARS-CoV-2, so SARS-CoV-2 M^{pro} is considered as a prominent drug target of COVID-19 therapy. This review briefly introduces the structural characteristics of SARS-CoV-2 M^{pro} and focuses on the research progress of SARS-CoV-2 M^{pro} inhibitors in recent years from all sources, including drug repurposing and drug design. These information will provide a basis for the drug development of treating the infection of SARS-CoV-2 and even other coronaviruses in the future.

Abbreviations: 3CLpro, 3-chymotrypsin like protease; ACE2, angiotensin converting enzyme 2; COVID-19, coronavirus disease 2019; FRET, fluorescence resonance energy transfer; FIP, feline infectious peritonitis; Mpro, main protease; MERS-CoV, Middle East respiratory syndrome coronavirus; NSP, nonstructural proteins; ORF, open reading frame; PLpro, papain-like protease; SARS-CoV-1, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

* Corresponding author.

** Corresponding author. Wuya College of Innovation, Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang, 110016, China.

E-mail addresses: 2022041@fjtcm.edu.cn (H. Li), syzyclx@163.com (L. Chen).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ejmech.2023.115491
Received 21 March 2023; Received in revised form 6 May 2023; Accepted 14 May 2023
Available online 22 May 2023

2. Structure and function of SARS-CoV-2 M^{pro}

SARS-CoV-2 is composed of genomic RNA complexes and membrane, which consists of at least three viral proteins: (a) Spinous protein (S); (b) Transmembrane membrane protein (M); and (c) Envelope protein (E) (Fig. 2A) [12–14]. The spike protein (S) of SARS-CoV-2 is responsible for binding to its host cell surface receptor, angiotensin converting enzyme 2 (ACE2) [15]. After entering the host cells, viral RNA encodes two overlapping open-reading frames (ORF1a and ORF1b), which are translated into two large polyproteins, pp1a and pp1ab. These polyproteins are further processed by the main protease (M^{pro}) and papain-like protease (PL^{pro}) to generate 16 nonstructural proteins (NSPs) (Fig. 2B) [16,17]. The replicase genes encoding 16 NSPs occupy the most of the genome, approximately two thirds [18]. These NSP4 -NSP16 released by cleavage with M^{pro} are responsible for viral genome replication and transcription [19,20]. Therefore, effectively blocking M^{pro} can stop the viral RNA replication and transcription, thereby reducing the virus proliferation.

M^{pro}, also known as 3-chymotrypsin like protease ($3CL^{pro}$), is a cysteine protease composed of about 300 amino acids and contains three domains [19,21]. M^{pro}, with a nonclassical Cys-His catalytic binary (Cys145 and His41) in the gap between domains I and II, cleaves the polyproteins at 11 positions with stringent substrate specificity and shows self-proteolytic activity [13,20,22]. In the Cys-His catalytic binary, Cys145 acts as a nucleophile and His41 plays a general acid-base role in the proteolytic process(Fig. 2C) [23]. M^{pro} is embedded in the polyproteins as the nsp5 domain, thus M^{pro} must use its own proteolytic activity to separate itself from the polyproteins in order to release the mature protease [24–27]. The active site of M^{pro} is composed of four sites (S1′, S1, S2, and S4), which often accommodate four fragments (P1′, P1, P2, and P3, respectively) of peptidomimetic inhibitors. Notably, the S1′ is formed by His41, Gly143, Ser144, and Cys145 [28,29]. M^{pro} exclusively cleaves polypeptides after a glutamine (Gln) residue, and no known human protease displays the same cleavage specificity as M^{pro} [28,30], so M^{pro} inhibitors have extremely low toxicity to host cells. M^{pro} has become one of the ideal specific drug targets in the development of antiviral drugs.

3. The development of SARS-CoV-2 M^{pro} inhibitors

Because of the crucial role of M^{pro} in viral replication and transcription, it has been considered as a promising target for fighting COVID-19 drug development. Based on drug repurposing and drug design, scientists and researchers have developed a number of SARS-CoV-2 M^{pro} inhibitors. This section summarizes the research progress of SARS-CoV-2 M^{pro} inhibitors.

3.1. *Approved SARS-CoV-2 M^{pro} inhibitors*

After the outbreak of SARS in 2003, **PF-00835231** (**2**) was identified as an effective inhibitor of SARS-CoV-1 M^{pro} [31]. In 2021, in order to improve the low passive absorptive permeability and poor oral absorption of **PF-00835231** (**2**) in animals [32], Owen and his colleagues reported an oral SARS-CoV-2 M^{pro} inhibitor (**PF-07321332** (**1**), K_i = 0.003 μM) (Fig. 3). At the same time, **PF-07321332** (**1**) had strong Vero E6 antiviral activity with the half maximum effective concentration (EC_{50}) values of 0.074 μM and exhibited excellent selectivity and safety *in vivo* [33]. Furthermore, in order to block the rapid metabolism of **PF-07321332** (**1**) by CYP3A, the HIV protease inhibitor Ritonavir was added to form a new drug, Paxlovid. It is used to treat adults with mild and moderate COVID-19 symptoms authorized by the United States Food and Drug Administration on December 22, 2021. The data showed an 89% reduction in the risk of COVID-19 related death or hospitalization in adults treated with Paxlovid, compared to placebo, within three days of symptom onset.

In addition to Paxlovid, Japan approved Ensitrelvir (**S-217622**) developed by Hokkaido University and Shionogi & Co, Ltd on November 22, 2022. **S-217622** (**6**) was discovered via virtual screening followed by biological assay, and optimization of the hit compound **4** using a structure-based drug design strategy(Fig. 3) [34,35]. The specific steps are as following: first, they optimized the P1' ligand on the basis of compound **4** to obtain compound **5**. The P1′ ligand of compound **5** is 6-chloro-2-methyl-2*H*-indayzole, and its enzyme inhibitory activity is increased by 90 times. Next, the methylamide moiety of P1 was substituted by a series of heterocyclic compounds to obtain **S-217622** (**6**). **S-217622** (**6**) exhibited significant inhibitory activity against SARS-CoV-2 M^{pro} with IC_{50} value of 0.013 μM, and effective antiviral activity with EC_{50} value of 0.37 μM. In addition, **S-217622** (**6**) showed antiviral activity against a series of SARS-CoV-2 variants and coronavirus family *in vitro*, favorable drug metabolism and pharmacokinetic (DMPK) profiles for the oral dosing, such as high metabolic stability (96% and 88% in human and rat liver microsomes, respectively), high oral absorption (97%), and low clearance (1.70 mL/min/kg) in rats [34].

The National Medical Products Administration (NMPA) approved the SARS-CoV-2 M^{pro} inhibitor **SIM0417**, which is used to treat adults with mild and moderate COVID-19 symptoms on January 28, 2023. The structure of **SIM0417** has not yet been disclosed. **SIM0417** and **PF-07321332** (**1**) need to be combined with low-dose Ritonavir, which helps to delay their metabolism *in vivo* and improve the antiviral effect.

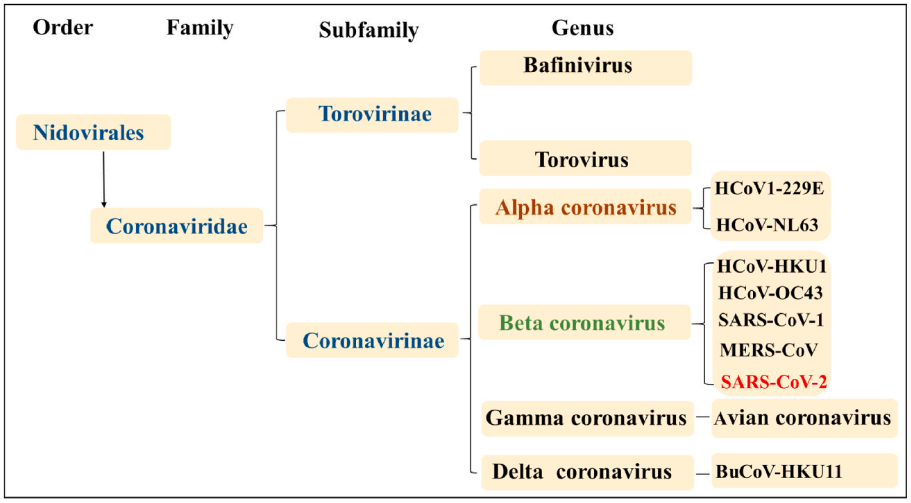


Fig. 1. Schematic representation of the taxonomy of Coronaviruses.

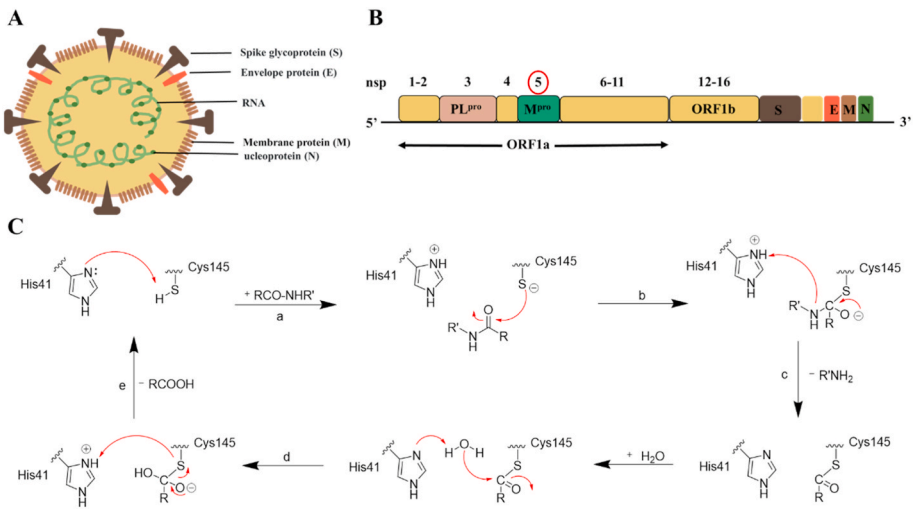


Fig. 2. Structure and function of SARS-CoV-2 M^{pro}. (A) The structure of coronavirus. (B) Coronavirus (SARS-CoV-2) genome. (C) Overall scheme of natural amide substrate hydrolysis by Cys145 and His41 at the active site of M^{pro}: a. The first step in the process is the deprotonation of the Cys145-thiol; b. In the second step, the anionic sulfur next attacks the substrate carbonyl carbon; c. Then, the product with an amino terminus is released, while the His41 is restored its deprotonated form; d. Next, the generated thioester is hydrolyzed to release the carboxylic acid. e. In the last step, the Cys-His catalytic binary is formed again for the next proteolytic cycle.

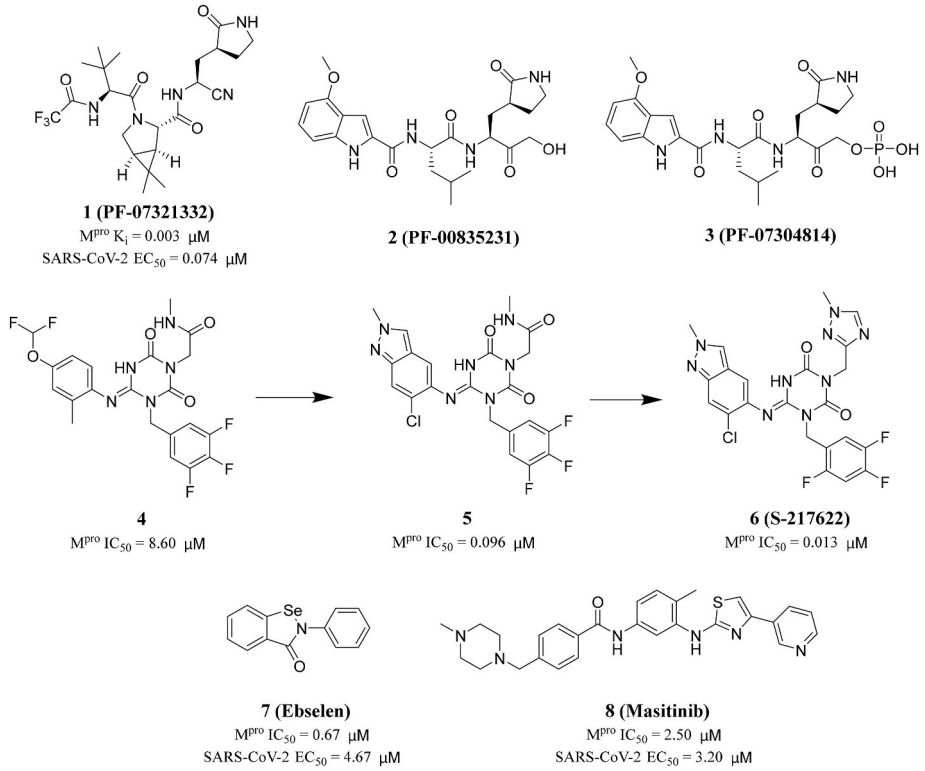


Fig. 3. Approved and clinical drugs of SARS-CoV-2.

PF-07321332 (**1**) is a covalent peptidomimetic inhibitor with a nitrile warhead. Conversely, **S-217622** (**6**) is a noncovalent, nonpeptidic inhibitor. The nonpeptidic character provides metabolic stability, and the lack of a covalent warhead reduces potential off-target toxicity issues. Most of the antiviral protease (HIV and HCV) inhibitors in clinic are peptidic, with short plasma half-life and easy to be filtered and cleared by the kidney. The method adopted by Unoh et al. eliminates the bias towards molecules containing amide bond, thus avoiding potential metabolic degradation.

3.2. *SARS-CoV-2 M^{pro} inhibitors in clinical trials*

Following the outbreak of SARS in 2003, Boras and his colleagues found a potential SARS-CoV-1 M^{pro} inhibitor **PF-00835231** (**2**) based on structure-based drug design, and its phosphate prodrug is **PF-07304814** (**3**) (Fig. 3). Then, **PF-00835231** (**2**), as a single agent, showed potent antiviral activity against SARS-CoV-2 *in vitro* [31,36]. Currently, NCT04627532, NCT04535167 and NCT05050682 are in clinical trials (Table 1).

Jin and his colleagues screened a large number of compounds (about 10000) by using fluorescence resonance energy transfer (FRET) analysis, including natural products, approved drugs and candidate drugs for clinical trials. Among them, **Ebselen** (**7**) had the strongest inhibitory effect on M^{pro} activity, with an IC_{50} value of 0.67 μM [37] (Fig. 3). In addition, **Ebselen** (**7**) has extremely low cytotoxicity (the median lethal dose in rats is > 4600 mg/kg), and its safety in humans has been evaluated in many clinical trials [38–40]. Considering the therapeutic potential of **Ebselen** (**7**) in COVID-19, two clinical trials have been

Table 1
SARS-CoV-2 M^{pro} inhibitors in clinical trials (www.clinicaltrials.gov).

Inhibitor	Sponsor	Phase	Status	Identifier
PF-07304814	Pfizer	1	Completed	NCT04627532
PF-07304814	Pfizer	1	Completed	NCT04535167
PF-07304814	Pfizer	1	Completed	NCT05050682
Ebselen	Sound Pharmaceuticals, Incorporated	2	Enrolling by invitation	NCT04484025
Ebselen	Sound Pharmaceuticals, Incorporated	2	Enrolling by invitation	NCT04483973
Masitinib	AB Science	2	Recruiting	NCT04622865
Masitinib	AB Science	2	Recruiting	NCT05047783

registered to evaluate the safety and efficacy of this drug in patients with moderate and severe COVID-19 (Table 1) [41].

In 2021, Drayman and his colleagues screened a library of 1900 clinically used drugs that were either approved for human use or had extensive safety data in humans (phase 2 or 3 clinical trials). Eight of these drugs inhibited the activity of SARS-CoV-2 M^{pro}, of which **Masitinib** (**8**) is the most effective (Fig. 3). After receiving **Masitinib** (**8**) treatment, the virus titer in the lungs and nose of mice infected with SARS-CoV-2 decreased by more than 200 times, and the lung inflammation was also alleviated. The two phase 2 clinical trials have been registered with clinicaltrials.gov (identifier: NCT04622865 and NCT05047783) to test the effect of the combination of **Masitinib** (**8**) and isoquercetin on COVID-19 hospitalized patients [42] and evaluate the anti-viral efficacy of three different dosages of **masitinib**in patients with mild symptom (Table 1).

Ebselen (**7**) is a glutathione peroxidase mimic that can permeate the blood-brain barrier and has anti-inflammatory, anti-tumor and antiviral activities, making it a novel anti-inflammatory agent approved by FDA. **Masetinib** (**8**), an oral tyrosine kinase inhibitor, was approved by FDA in 2009 and 2015, respectively, for the treatment of pancreatic cancer and amyotrophic lateral sclerosis (ALS). The security of **Ebselen** (**7**) and **Masitinib** (**8**) have been proved repeatedly, thus the treatment of COVID-19 may become their new mission.

3.3. SARS-CoV-2 M^{pro} inhibitors obtained by drug repurposing

The high infectivity and pathogenicity of SARS-CoV-2 have made it a global pandemic, so it is urgent to develop effective drugs to treat COVID-19. Drug repurposing is considered to be one of the most practical and rapid methods to find such therapeutic drugs. Drug repurposing has the advantages of lower cost and higher safety, especially for the drugs that have been studied their clinical safety, which is attracting people's attention.

In 2020, Jin and his colleagues used molecular docking to observe whether **N3** (**9**) could target SARS-CoV-2 M^{pro} (Table 2). A docking result indicates that **N3** (**9**) could fit inside the substrate-binding pocket [37]. To evaluate the efficacy of **N3** (**9**) against SARS-CoV-2 M^{pro}, they performed kinetic analysis. The results showed that **N3** (**9**) was a time-dependent irreversible inhibitor and showed a very potent inhibitory effect on SARS-CoV-2 M^{pro}. They determined the value of $k_{obs}/[I]$ of **N3** (**9**) for SARS-CoV-2 M^{pro} as 11,300 ± 880 $M^{-1}s^{-1}$, and the $k_{obs}/[I]$ was used as an approximation of the pseudo second-order rate constant (k_3/k_i) to evaluate the inhibitory effect.

In addition, Jin and his colleagues found seven hits by screening with FRET analysis, including approved drugs (**Disulfiram** (**10**) and **Carmofur** (**11**)) and preclinical or clinical trial candidate drugs (**Ebselen** (**7**), **Shikonin** (**12**), **Tideglusib** (**13**), **PX-12** (**14**) and **TDZD-8** (**15**)) (Table 2). The IC_{50} values of these seven compounds were determined as 0.67–21.4 μM. Besides, the tandem mass spectrometry data showed that **Ebselen** (**7**), **PX-12** (**14**) and **Carmofur** (**11**) could covalently bind to Cys145 of SARS-CoV-2 M^{pro} catalytic binary. **Ebselen** (**7**) and **N3** (**9**) at the concentration of 10 μM showed the strongest antiviral effect on Vero cells infected with SARS-CoV-2. They further evaluated the efficacy of these two compounds in protecting cells. **Ebselen** (**7**) and **N3** (**9**) showed inhibitory effect on SARS-CoV-2, with EC_{50} values of 4.67 and 16.77 μM, respectively [37,43].

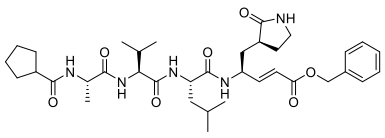
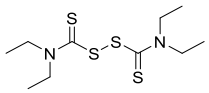
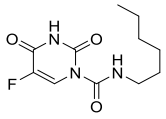
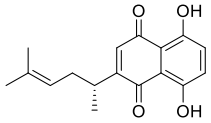
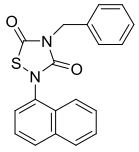
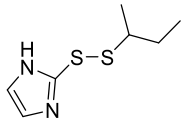
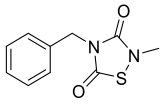
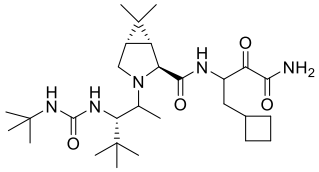
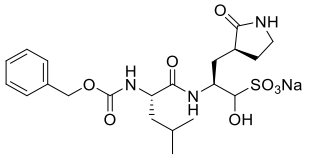
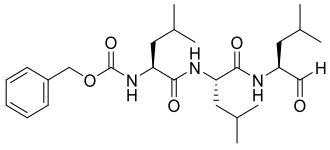
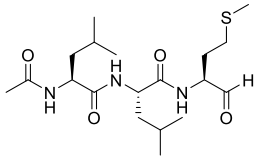
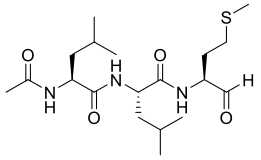
In 2020, Ma and his colleagues found several potent SARS-CoV-2 M^{pro} inhibitors with effective cellular antiviral activity (Table 2). **Boceprevir** (**16**), an FDA approved HCV drug, inhibited SARS-COV-2 M^{pro} with an IC_{50} of 4.13 μM. In the cellular viral CPE assay, the EC_{50} against SARS-CoV-2 is 1.90 μM. Because **Boceprevir** (**16**) is a drug approved by FDA, the dose, toxicity, dosage form and pharmacokinetic properties have been clearly defined, which will greatly accelerate the design of follow-up studies [44,45]. **GC-376** (**17**) is a research veterinary drug that is being developed the treatment of feline infectious peritonitis (FIP). **GC-376** (**17**) showed promising antiviral activity against SARS-CoV-2 virus (EC_{50} = 3.37 μM). The enzyme inhibition of M^{pro} was the strongest, with IC_{50} value of 0.03 μM. Besides, it has promising *in vivo* therapeutic effect on cats infected with FIP and favorable *in vivo* pharmacokinetic properties. Therefore, **GC-376** (**17**) can be detected in relevant animal models of SARS-CoV-2 at any time [44–47]. Three calpain/cathepsin inhibitors, **MG-132** (**18**), calpain inhibitor **II** (**19**) and **XII** (**20**), are effective inhibitors of M^{pro}, with single digit to submicromolar efficacy in enzyme detection. Calpain inhibitors **II** (**19**) and **XII** (**20**) inhibited SARS-CoV-2 in CPE assay, with EC_{50} values of 2.07 and 0.49 μM, respectively [44]. This result indicates that calpain/cathepsin inhibitors are rich sources of drug candidates for SARS-CoV-2, and also suggests that it may be feasible to design dual inhibitors against viral M^{pro} and host calpains/cathepsins. Further development based on these drugs may generate clinically useful COVID-19 antiviral drugs.

Konno et al. repositioned and investigated the potential of their SARS-CoV-1 inhibitors as anti-SARS-CoV-2 drugs [48]. In particular, **YH-53** (**21**) contains an indole group at the P3- position and benzothiazolyl ketone as the reactive warhead, which demonstrated strong inhibitory activity against SARS-CoV-2 M^{pro} (K_i = 0.034 μM) (Table 2). X-ray structure analysis showed that **YH-53** (**21**) formed multiple hydrogen bond interactions with the main chain amino acids and a covalent bond with the active site of M^{pro}.

Tripathi and his colleagues tested the different classes of drugs-nucleoside analogues, antiretroviral drugs, HIV protease inhibitors and neuraminidase inhibitors to research their potential antiviral effects. They found that **Teicoplanin** (**22**) was an effective drug against SARS-CoV-2 M^{pro} with an IC_{50} value of 1.5 μM [49] (Table 2). **Teicoplanin** is an effective glycopeptide antibiotic and has been reported to have anti-MERS-CoV activity [50,51]. Zhou et al. pointed out that **Teicoplanin** (**22**) acts at the early stage of the life cycle of coronavirus virus, and its mechanism of action is to inhibit the low-pH cleavage of the viral spike protein by late endosomal cathepsin L, thereby preventing the release of genomic viral RNA and viral replication [52]. In addition, Zhang and his colleagues showed that **Teicoplanin** blocked virus entry by specifically inhibiting the activity of cathepsin L of SARS-CoV-2 virus [53].

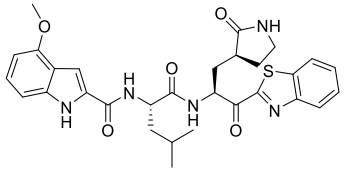
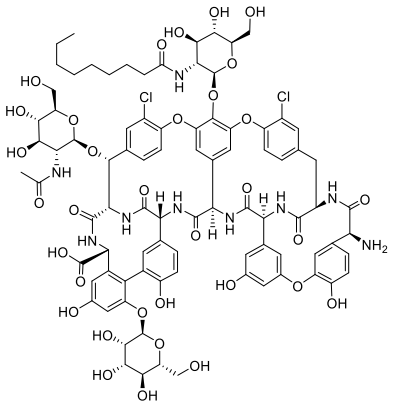
Zhu et al. performed a quantitative high-throughput screening (qHTS) of 10755 compounds using a self-quenching fluorescent peptide substrates, including approved drugs, candidate drugs for clinical studies and bioactive compounds. They identified twenty-three SARS-CoV-2 M^{pro} inhibitors with IC_{50} values ranging from 0.26 to 28.85 μM. Among these inhibitors, **Walrycin B** (**24**) showed the best enzyme inhibitory activity with IC_{50} value of 0.26 μM (Table 3).

Table 2
Structures and biological activity data of compounds **9**–**22**.

Drug	Structure	Class	IC_{50}[a] (μM)
N3 (9)		M^{pro} inhibitor	–
Disulfiram (10)		Alcohol deterrent	9.35
Carmofur (11)		Antineoplastic agent	1.82
Shikonin (12)		Pyruvate kinase M2 inhibitor	15.75
Tideglusib (13)		Anti-inflammatory and neuroprotective agents	1.55
PX-12 (14)		Thioredoxin-1 inhibitor	21.39
TDZD-8 (15)		GSK-3β inhibitor	2.15
Boceprevir (16)		Hepatitis C virus NS3/4A protease inhibitor	4.13
GC-376 (17)		M^{pro} inhibitor	0.03
MG-132 (18)		Proteasome inhibitor	3.90
19		Calpain inhibitors II	0.97
20		Calpain inhibitors XII	0.45

(*continued on next page*)

Table 2 (*continued*)

Drug	Structure	Class	IC_{50}[a] (μM)
YH-53 (21)		SARS-CoV-1 M^{pro} inhibitor	–
Teicoplanin (22)		Glycopeptide antibiotic	1.50

[a] SARS-CoV-2 M^{pro} IC_{50}.

Hydroxycobalamin (**23**) (IC_{50} = 3.29 μM), **Z-DEVD-FMK** (**25**) (IC_{50} = 6.81 μM), **LLL-12** (**26**) (IC_{50} = 9.84 μM), **Suramin sodium** (**27**) (IC_{50} = 6.5 μM) and **Z-FA-FMK** (**28**) (IC_{50} = 11.39 μM) are also potent inhibitors of SARS-CoV-2 M^{pro} [54].

The utilization of FDA approved drug library is an efficient tool for drug reuse in antiviral research [55–57]. In 2021, Zhao and his colleagues screened a library containing 774 FDA approved drugs to find potential SARS-CoV-2 M^{pro} inhibitors. Among them, seven drugs have superior inhibitory activity against SARS-CoV-2 M^{pro} (Table 3), namely, **Ethacrynic acid** (**29**), **Naproxen** (**30**), **Allopurinol** (**31**), **Butenafine hydrochloride** (**32**), **Raloxifene hydrochloride** (**33**), **Tranylcypromine hydrochloride** (**34**) and **Saquinavir mesylate** (**35**) [58].

Steuten and his colleagues screened a library of approximately 650 diverse covalent inhibitor scaffolds against the two major SARS-CoV-2 cysteine proteases, M^{pro} and PL^{pro}. They identified seven inhibitors containing various electrophiles, of which six exhibited time-dependent inhibition of recombinant SARS-CoV-2 M^{pro}. Notably, they did not find any viable PL^{pro} inhibitors. In the cellular infectivity assays of A549 epithelial lung cells, only chloromethyl ketone **JCP400** (**36**) and acyloxymethyl ketone **JCP403** (**37**) were active (Table 3). The two compounds demonstrated relatively weak potency with greater than 75% inhibition only when applied at concentrations above 20 μM. The two most effective inhibitors against SARS-CoV-2 M^{pro} *in vitro*, **JCP474** (**38**) and **JCP543** (**39**), were not active in the cellular infectivity assays [59].

Coelho et al. performed biochemical high-throughput screening (HTS) on the recombinantly expressed SARS-CoV-2 M^{pro}. A fluorescent assay was used to identify inhibitors from a compound library containing known drugs, bioactive molecules and natural products [60]. The screening led to the identification of 13 compounds with IC_{50} values ranging from 0.2 to 23 μM, and several known SARS-CoV-1 M^{pro} inhibitors were identified as inhibitors of SARS-CoV-2 M^{pro}, such as the organomercury compounds **Thiomersal** (**40**) and **Phenylmercury acetate** (**41**) (Table 4). Benzophenone derivative (**42**) was identified as the most effective screening hits. In addition, **Evans blue** (**43**), a sulfonic acid-containing dye, was also identified as a SARS-CoV-2 M^{pro} inhibitor [60].

Iketani and his colleagues also identified that compound **44**, **GC376** (**17**) and **MAC-5576** (**45**) were inhibitors of SARS-CoV-2 M^{pro} (Table 4). In the cell-based assay, compound **44** and **GC376** (**17**) also inhibited the SARS-CoV-2 virus, while **MAC-5576** (**45**) did not [47].

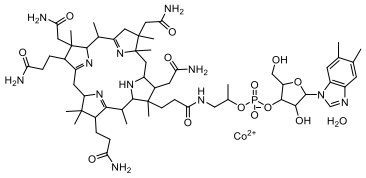
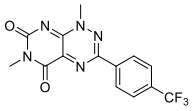
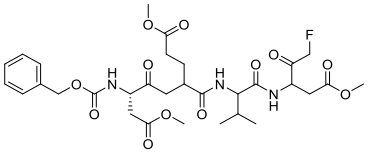
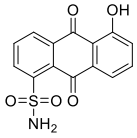
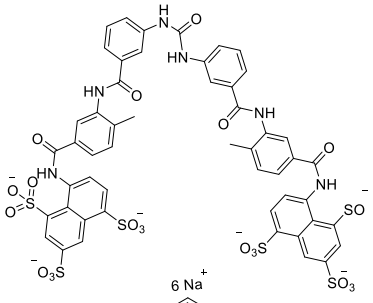
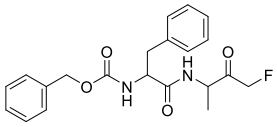
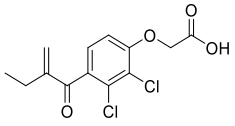
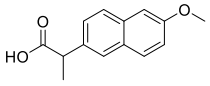
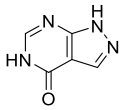
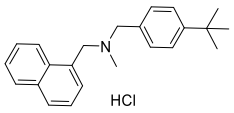
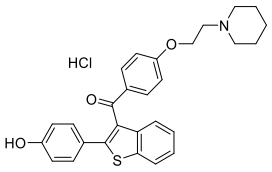
Liu and his colleagues reported that **Baicalein** (**46**) (Table 4)and the four active Baicalein analogues compounds **47**–**50** could effectively inhibit the SARS-CoV-2 M^{pro} *in vitro*, and **Baicalein** (**46**) had the strongest inhibitory activity, with an IC_{50} value of 0.39 μM [61,62]. These compounds can be used as lead compounds to develop more effective drugs for COVID-19 disease through further optimization and modification.

In 2022, Chen research group established an enzymatic assay that used a fluorogenic substrate to screen the M^{pro} inhibitors [63]. The results showed that **Acriflavine** (**51**) and **Proflavine Hemisulfate** (**52**) had good inhibitory activity against M^{pro} with IC_{50} values of 5.60 and 2.07 μM, respectively (Table 4). They also exhibited nanomolar activities against SARS-CoV-2, which were superior to **GC376** (**17**) for anti-HCoV-43. **Acriflavine** (**51**) has previously been reported as a PL^{pro} inhibitor [64], therefore these two compounds might be dual-targeted inhibitors against coronaviruses.

In 2021, Yang and his colleagues used FRET experiments to identify potential inhibitors of M^{pro}. A total of thirty-six compounds were screened, with IC_{50} values ranging from 0.074 to 0.91 μM. Among them, compounds **53** and **54** had the strongest inhibitory effect on M^{pro}, with IC_{50} values of 0.074 and 0.11 μM, and K_i values of 0.031 and 0.078 μM, respectively (Table 4). The preincubation, jump dilution assays and fluorescent labeling experiments showed that the two compounds were covalently and irreversibly bound to M^{pro}, and molecular docking suggested that compound **54** formed an S–S bond with Cys145 at the active site of the enzyme [65]. The research of Yang and his colleagues provides two very effective scaffolds **Ebsulfur** and **Ebselen** (**7**) for the development of M^{pro} covalent inhibitors to combat COVID-19.

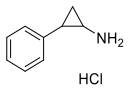
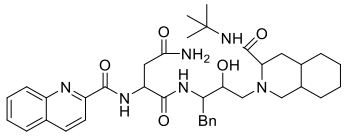
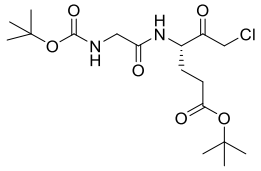
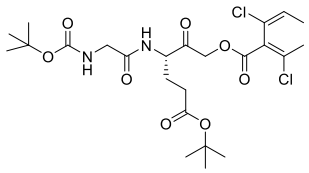
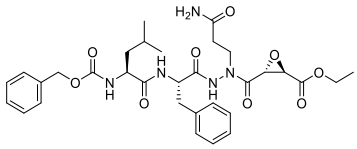
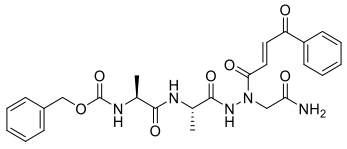
Most of the above compounds with potential SARS-CoV-2 M^{pro} inhibitory activity were obtained by scientists and researchers screening approved, clinical, and preclinical trials drugs. These compounds usually have different biological significance, such as anticancer, anti-inflammatory, antivirus, alcohol abstinence activities, *etc*. **Baicalin** (**46**) shows stronger antiviral effects and higher clinical efficacy than Ribavirin in the treatment of hand-foot-mouth disease [66]. **N3** (**9**) and **GC376** (**17**) have effect on SARS-CoV and MERS-CoV, two zoonotic coronaviruses infecting human beings [67]. **GC376** (**17**) could also inhibit the main protease of ferret and mink coronavirus [68].

Table 3
Structures and biological activity data of compounds **23**–**39**.

Drug	Structure	Class	IC_{50}[a] (μM)
Hydroxycobalamin (23)		Vitamin B12a	3.29
Walrycin B (24)		Antibacterial agent	0.26
Z-DEVD-FMK (25)		Caspase-3 inhibitor	6.81
LLL-12 (26)		Signal transducer and activator of transcriptionl 3 inhibitor	9.48
Suramin sodium (27)		Antiprotozoal drug	6.5
Z-FA-FMK (28)		Cysteine protease inhibitor	11.39
Ethacrynic acid (29)		Diuretic	1.11
Naproxen (30)		Anti-inflammatory drug	3.45
Allopurinol (31)		Xanthine oxidase inhibitor	3.77
Butenafine hydrochloride (32)		Antifungal	5.40
Raloxifene hydrochloride (33)		Estrogen receptor modulator	5.61

(*continued on next page*)

Table 3 (*continued*)

Drug	Structure	Class	IC_{50}[a] (μM)
Tranylcypromine hydrochloride (34)		Antidepressant drug	8.64
Saquinavir mesylate (35)		HIV protease inhibitor	9.92
JCP400 (36)		cysteine proteases inhibitor	1.74
JCP403 (37)		cysteine proteases inhibitor	2.99
JCP474 (38)		cysteine proteases inhibitor	0.96
JCP543 (39)		cysteine proteases inhibitor	0.49

[a] SARS-CoV-2 M^{pro} IC_{50}.

Multitarget antiviral inhibitors can more effectively overcome the drug resistance and usually show more potent therapeutical effect. Scientists and researchers are trying to find broadspectrum antiviral drugs, which will help fight SARS-CoV-2 and future coronaviruses.

3.4. SARS-CoV-2 M^{pro} inhibitors obtained by drug design

To date, drug repurposing efforts have not produced safe and effective M^{pro} inhibitors for approved clinical use in humans. Therefore, scientists and researchers have optimized and modified the above structures, and designed and synthesized many new SARS-CoV-2 M^{pro} inhibitors. SARS-CoV-2 M^{pro} inhibitors can be divided into covalent and non-covalent inhibitors according to their inhibitory mechanisms.

3.4.1. Covalent SARS-CoV-2 M^{pro} inhibitors

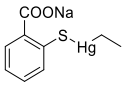
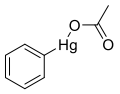
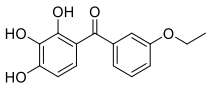
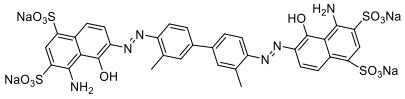
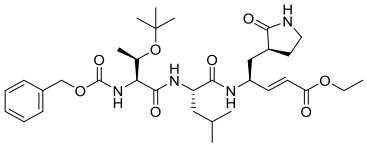
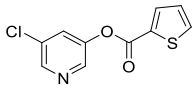
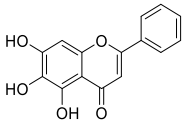
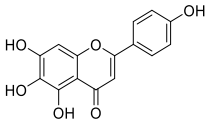
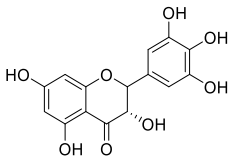
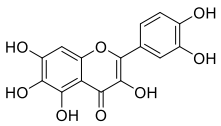
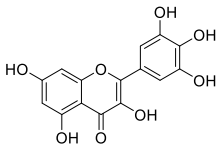
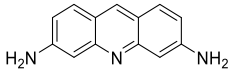
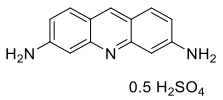
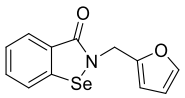
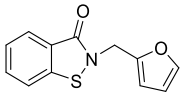
Covalent SARS-CoV-2 M^{pro} inhibitors, including peptidomimetic inhibitors and nonpeptidic small molecule inhibitors, bear different warhead groups and act through a two-step mechanism. First, these inhibitors bind to the active site and form non-covalent complexes with the target protease. Then, the warhead forms a covalent bond with nucleophilic residues, especially the catalytic Cys145 or other key cysteines (such as Cys300 and Cys44) [69,70], whose covalent bond modification may further cause the inactivation of SARS-CoV-2 M^{pro}.

Vankadara and his colleagues synthesized Nirmatrelvir and its 10 analogues with different electrophilic warheads, and determined its inhibitory activity against SARS-CoV-2 and HCoV-229E M^{pro} by using FRET based biochemical detection method [71]. Compound **55** partially substituted nitrile of Nirmatrelvir with a hydroxymethylketone moiety, and the IC_{50} values for SARS-CoV-2 and HCoV-229E M^{pro} were 0.008 and 0.013 μM, respectively (Fig. 4). Notably, these IC_{50} values were 4–11 fold higher than that of Nirmatrelvir (IC_{50} of 0.031 and 0.145 μM, respectively), indicating that the hydroxymethylketone warhead is more active than nitrile. Compound **56** replaced the nitrile of Nirmatrelvir with a ketobenzothiazole warhead, and the IC_{50} values for SARS-CoV-2 and HCoV-229E M^{pro} were 0.027 and 0.239 μM, respectively, similar to Nirmatrelvir. A subsequent HCoV-229E cell inhibition (EC_{50}) assay showed that compounds **55** and **56** had the same potency as Nirmatrelvir, indicating that both inhibitors may be candidates for further drug development [71].

Huff et al. designed a series of 2-phenyl-1,2-benzoselenzole-3-one derivatives targeting SARS-CoV-2 M^{pro}. Their substitutions are mainly concentrated in the N-phenyl moiety, including halogen (F, Cl and Br) and hydrophobic groups (CH_3, CF_3, SCH_3, OCH_3 and CH_2CH_3) as well as several lipophilic substituted phenyl moiety [72]. These compounds were determined to inhibit the replication of SARS-CoV-2 in infected Vero E6 and Calu-3 cells, and their potency was comparable to that of the clinical therapeutic drug Remdesivir. The most potent compound **57** demonstrated a nanomolar antiviral activity with EC_{50} value of 0.84 μM [72] (Fig. 4). This study provides a structural framework and mechanism of SARS-CoV-2 M^{pro} inhibitor, which will help to develop drugs for treating COVID-19.

Qiao and his colleagues designed and synthesized a series of **Ebselen** (**7**) derivatives, which replaced the critical groups of **Ebselen** (**7**) through non-covalent binding with SARS-CoV-2 M^{pro}, thus reducing the steric hindrance and ultimately improving the antiviral activity. Nine **Ebselen** (**7**) derivatives (EBs) had more potent inhibitory effects on SARS-CoV-2 M^{pro}, with IC_{50} values of 0.07–0.38 μM. Further evaluation of these derivatives showed that compound **58** exhibited the strongest

Table 4
Structures and biological activity data of compounds **40**–**54**.

Drug	Structure	Class	IC_{50}[a] (μM)
Thiomersal (40)		antiseptic and antifungal agent	0.60
Phenylmercury acetate (41)		fungicide and slimicide	0.40
42			10.60
Evans blue (43)		l-glutamate uptake inhibitor	0.20
44		SARS-CoV-1 M^{pro} inhibitor	0.15
MAC-5576 (45)		M^{pro} inhibitor	0.08
Baicalein (46)		Xanthine oxidase inhibitor	0.39
Scutellarein (47)		Anti-inflammatory	5.80
Dihydromyricetin (48)		Dihydropyrimidinase inhibitor	1.20
Quercetagetin (49)		Pim-1 kinase inhibitor	1.24
Myricetin (50)		Antioxidant	2.86
Acriflavine (51)		Antibacterial agent	5.60
Proflavine Hemisulfate (52)		Antibacterial agent	2.07
53		Metallo-β-lactamase inhibitor	0.07
54		Metallo-β-lactamase inhibitor	0.11

[a] SARS-CoV-2 M^{pro} IC_{50}.

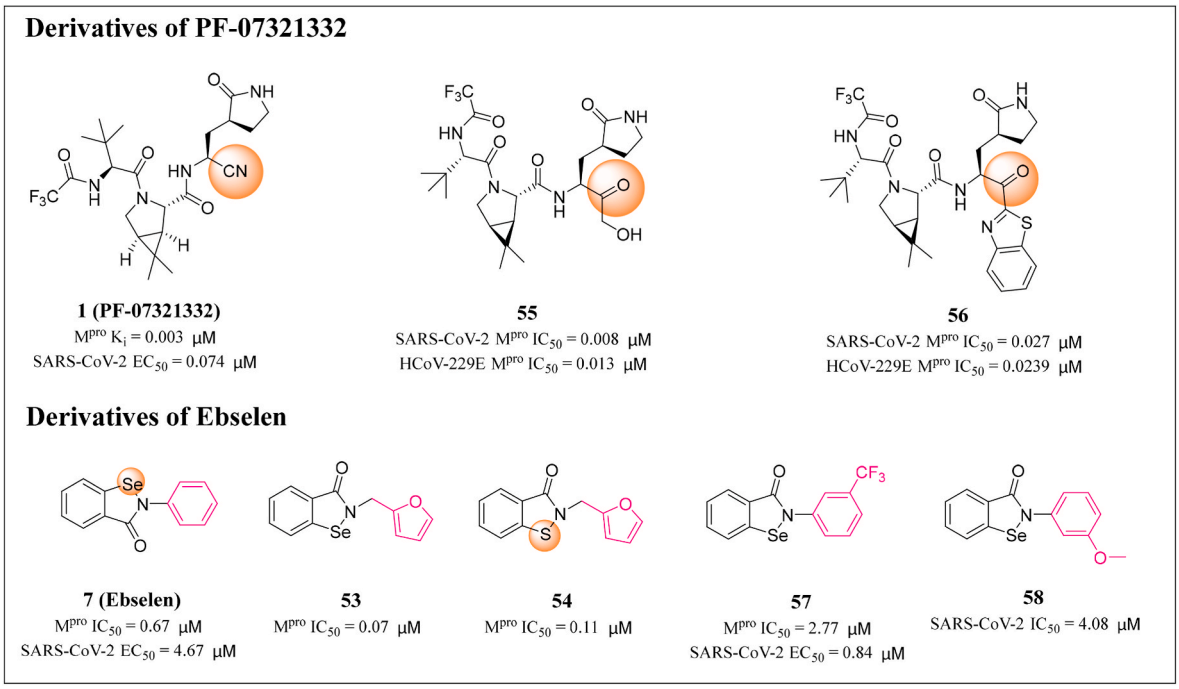


Fig. 4. Structures and biological activity data of derivatives **53**–**58** of **PF-07321332** and **Ebselen**.

inhibitory effect of SARS-CoV-2 virus replication, with an IC_{50} value of 4.08 μM in HPAepiC cells, as compared to the **Ebselen** (**7**) at 24.61 μM [73] (Fig. 4).

Dampalla and his colleagues synthesized a series of deuterated variants of an M^{pro} inhibitor **GC376** (**17**) and proved that the deuterated **GC376** (**17**) showed potent inhibitory activity against SARS-CoV-2 M^{pro}. In addition, the fatally infected mice were treated with deuterated derivatives of **GC376** (**17**). After 24h of infection, K18-hACE2 mice treated with compound **59** had an increased survival rate compared with vehicle-treated mice [74] (Fig. 5). The crystal structures of compound **59** and SARS-CoV-2 M^{pro} revealed that heavy deuteration did not change the interaction between **GC376** (**17**) and M^{pro} [44,75].

Sacco and his colleagues explored two series of SARS-CoV-2 inhibitors, one is the dual inhibitors targeting both M^{pro} and cathepsin L, such as calpain inhibitors **II** (**19**) and **XII** (**20**), and the other is the M^{pro} specific inhibitor, such as **GC-376** (**17**) analogues **UAWJ246** (**60**), **UAWJ247** (**61**), and **UAWJ248** (**62**) [76] (Fig. 5). In the plaque experiment, the EC_{50} values of **GC-376** (**17**), **UAWJ246** (**60**), **UAWJ247** (**61**), and **UAWJ248** (**62**) for inhibiting virus replication were 0.48, 4.61, 2.06, and 11.1 μM, respectively. Overall, these three **GC-376** (**17**) analogues had confirmed the antiviral activity in cell culture. Comparing the M^{pro} binding and antiviral efficacy of **GC-376** (**17**) analogues, it was found that the aldehyde warhead might be more suitable for cell activity than the α-ketoamide.

In 2021, Vederas and his colleagues designed and synthesized some new dipeptide derivatives with IC_{50} enzyme inhibition and EC_{50}

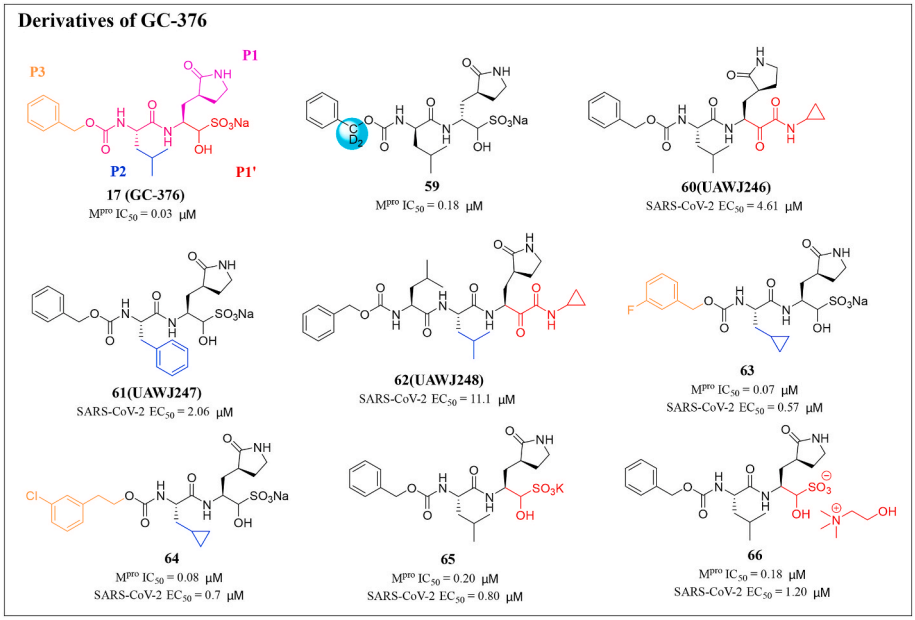


Fig. 5. Structures and biological activity data of derivatives **59**–**66** of **GC-376**.

antiviral value based on the antiviral peptide aldehyde GC373 and its bisulfite prodrug **GC-376** (**17**) [77]. Compared with **GC-376** (**17**), inhibitors **63** and **64** emerged as key compounds for M^{pro} inhibition with better IC_{50} and cellular EC_{50} values (Fig. 5). In addition, the newly designed and synthesized bisulfite adduct displayed a higher binding affinity for M^{pro}, which is 2.5–5.0 times higher, compared to **GC-376** (**17**). The Na^+ cation was replaced with K^+ cation (**65**) or choline (**66**), which showed very similar IC_{50} values to that of the parent Na^+ form, and demonstrated retained effectiveness combined with increased aqueous solubility [77]. The results of this study provide new insights for drug development based on cysteine protease inhibitors, whose significance is not limited to SARS-CoV-2.

Zhang et al. developed the lead compound **67** as a potent inhibitor of SARS-CoV-2 M^{pro} through structural optimization and transformation. To improve the half-life of compound **67** in plasma, they hid the amide bond in the pyridine ring. In addition, in order to increase the solubility of the compound in plasma, they replaced the hydrophobic cinnamoyl moiety with a less hydrophobic Boc group to obtain compound **68**. To enhance its antiviral activity on SARS-CoV-2, they replaced the cyclohexyl group in compound **68** with the smaller cyclopropyl group in compound **69** (Fig. 6). Compound **69** can effectively inhibit SARS-CoV-2 M^{pro} with IC_{50} value of 0.67 μM. In human Calu-3 cells infected with SARS-CoV-2, an EC_{50} of 4–5 μM was observed, whereas compound **70** lacking the Boc group was almost inactive [78]. This suggests that hydrophobic and bulky Boc groups are necessary for crossing the cell membrane, and here even more hydrophobic moieties may be advantageous.

Ojida et al. designed and synthesized a series of chlorofluoroacetamide (CFA) derivatives as potential SARS-CoV-2 M^{pro} covalent inhibitors by introducing a CFA unit into an azapeptide scaffold. The data showed that compound **71** strongly blocked SARS-CoV-2 replication in infected cells, and its effect is equivalent to that of Nimatralvir (Fig. 7). X-ray structural analysis revealed that compound **71** formed a covalent bond with Cys145 at the catalytic center of M^{pro} [79].

Dai et al. designed and synthesized two lead compounds **72** and **73** targeting SARS-CoV-2 M^{pro} based on common structural fragments of SARS-CoV-1 M^{pro} inhibitors and an aldehyde as a new warhead (Fig. 7). Compounds **72** and **73** exhibited high SARS-CoV-2 M^{pro} inhibitory activity with IC_{50} values of 0.053 and 0.040 μM, respectively. Besides, compounds **72** and **73** showed good anti-SARS-CoV-2 activity in cell culture, with EC_{50} values of 0.53 and 0.72 μM by plaque assay, respectively. Besides, they evaluated the pharmacokinetic properties of these two compounds. Compound **72** given to mice intraperitoneally (5 mg/kg) and intravenously (5 mg/kg) displayed a half-life ($T_{1/2}$) of 4.27 h and 4.41 h, respectively. They were also observed a high maximal concentration (C_{max} = 2394 ng/mL) and a good bioavailability of 87.8% when compound **72** was given intraperitoneally. The metabolic stability of compound **72** in mice was also good (clearance = 17.4 mL/min/kg). Compared with compound **72** administered intravenously to CD-1 mice, compound **73** displayed a shorter $T_{1/2}$ time (1.65 h) and a faster clearance rate (clearance = 20.6 mL/min/kg). The above pharmacokinetic results indicate that both compounds are promising candidate drugs [80].

In addition, Dai and his colleagues designed and synthesized a series of novel peptidomimetic aldehydes against the M^{pro} of enterovirus 71 (EV71). Among them, compound **74** demonstrated broad-spectrum antiviral activity and could effectively inhibit the activity of SARS-CoV-2 with EC_{50} value of 0.29 μM. Besides, it also exhibited good inhibitory activity against SARS-CoV-2 M^{pro} (IC_{50} = 0.034 μM) [81] (Fig. 7). Compound **74** may be a good starting point for the optimization of SARS-CoV-2 M^{pro} inhibitors.

Shang's research group has previously developed a series of covalent and noncovalent inhibitors of M^{pro}. Among those inhibitors, peptidomimetic aldehyde **75** showed certain inhibitory activity against SARS-CoV-2 M^{pro} with an IC_{50} value of 3.889 μM [82–85]. In 2022, Shang and his colleagues designed a series of peptide mimetic inhibitors based on compound **75**. Among them, compound **76** had an excellent inhibitory effect on SARS-CoV-2 M^{pro} with an IC_{50} value of 0.148 μM. Besides, compound **77** had significant antiviral activity against SARS-CoV-2 with EC_{50} value of 1.06 μM [86] (Fig. 7). These peptide mimetic inhibitors may be used as encouraging candidate drugs for further development of antiviral drugs.

In 2022, Pillaiya and his colleagues conducted a virtual screening of the Tübingen Kinase Inhibitor Collection (TüKIC). Then they docked the two screening hits, **IPA-3** (**78**) and **LN5535** (**79**), at the M^{pro} active site (PDB ID: 7RC0) and observed that they showed a similar orientation to the indole chloropyridyl ester derivatives. Finally, they designed a novel class of small molecule thioesters as SARS-CoV-2 M^{pro} inhibitors through inserting variable cyclic systems from **IPA-3** (**78**) and the (hetero)aryl

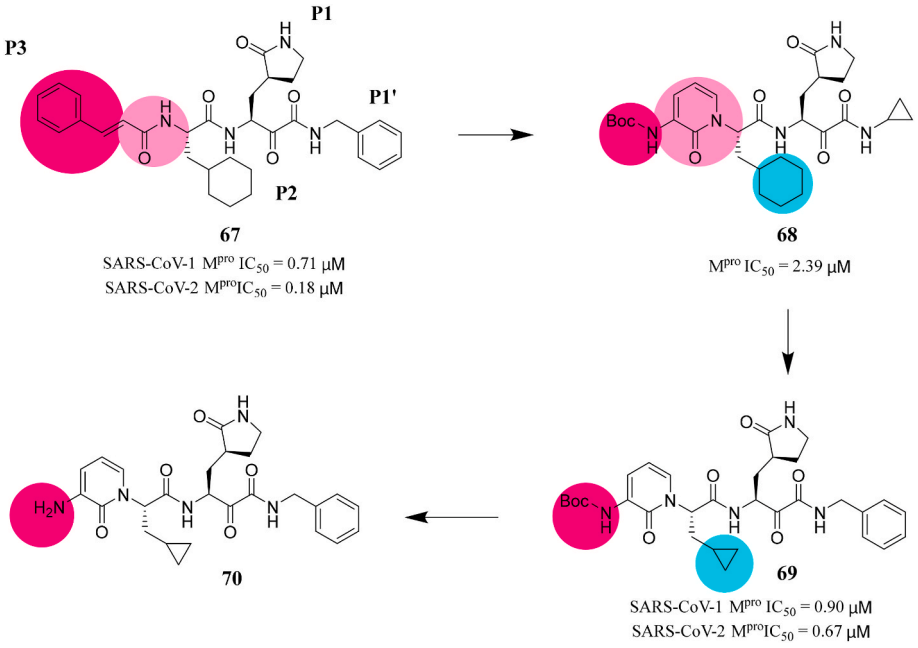


Fig. 6. Structures and biological activity data of compounds **67**–**70**.

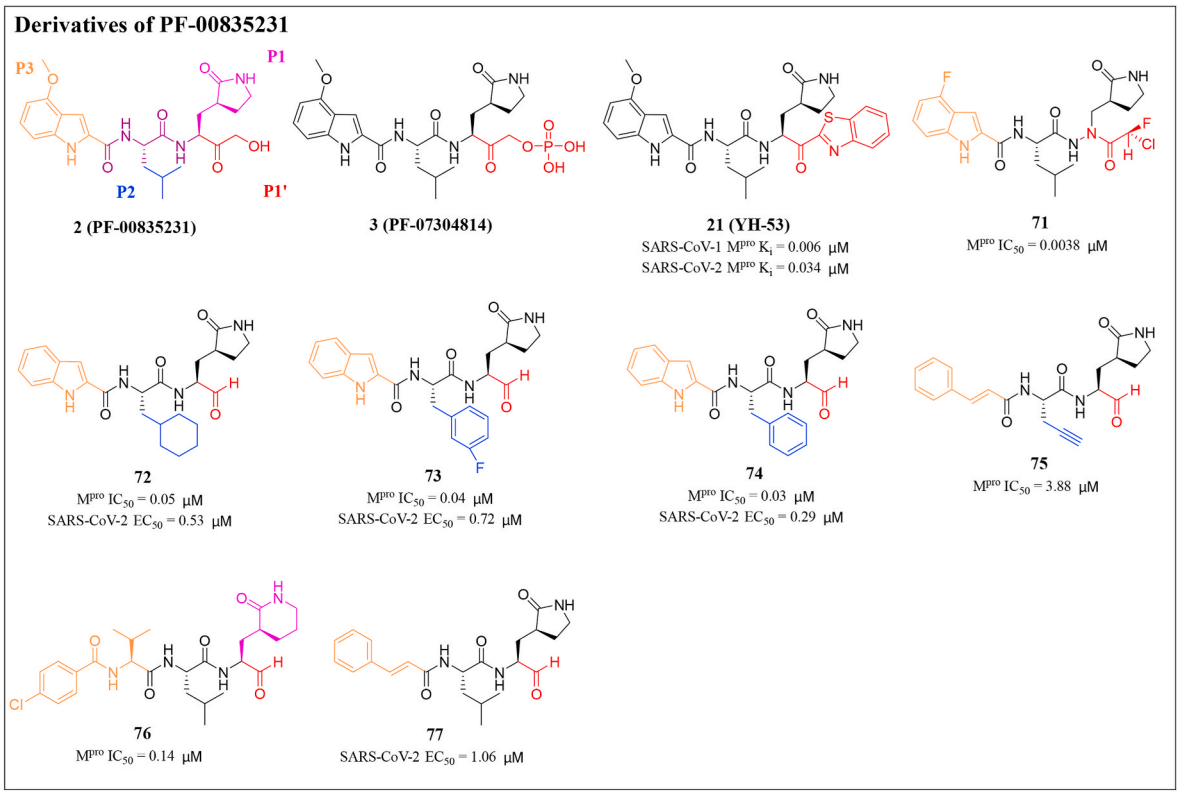


Fig. 7. Structures and biological activity data of derivatives **3**, **21** and **71**–**77** of **PF-00835231**.

thiols from **LN5535** (**79**) (Fig. 8). Compound **80** showed excellent SARS-CoV-2 M^{pro} inhibition, and its k_{inac}/K_i was 58700 $M^{-1}s^{-1}$ (K_i = 0.0141 μM). In Calu-3 and Vero E6 cells, several compounds exhibited antiviral activity in the nanomolar range, but were non-toxic to host cells [87]. The potent SARS-CoV-2 M^{pro} inhibitors also inhibited the M^{pro} of other β-coronaviruses, including SARS-CoV-1 and MERS-CoV, suggesting that they may contribute to the treatment of a wider range of coronavirus infections.

Liu's research group designed and synthesized 30 covalent inhibitors with a piperazine scaffold containing different warheads [88]. Among them, compound **81** showed the most potent inhibitory effect on SARS-CoV-2 M^{pro} with IC_{50} value of 0.18 μM, and displayed excellent antiviral potency against SARS-CoV-2 (EC_{50} = 2.64 μM) (Fig. 9). In addition, compound **81** presented favorable target selectivity for SARS-CoV-2 M^{pro} versus human cysteine proteases.

Qiao et al. designed and synthesized 32 new SARS-CoV-2 M^{pro} inhibitors containing bicyclic proline. First, an aldehyde was used in P1′ as a fighting part to form a covalent bond with the catalytic site Cys145, which is essential for antiviral activity [80]. Second, P2 fragment is from Boceprevir or Telaprevir, both of which are approved antiviral drugs. Finally, they decided to use a medium-sized P3 hydrophobic subgroups to enhance the potency and pharmacokinetic (PK) properties of the resulting inhibitors. Therefore, 32 compounds with different P3 fragments were designed and synthesized. Among them, **MI-23** (**82**) had the strongest inhibitory activity with IC_{50} of 0.007 μM. Both **MI-09** (**83**) and **MI-30** (**84**) showed excellent antiviral activity in cell-based assays (Fig. 9). In the transgenic mouse model of SARS-CoV-2 infection, oral or intraperitoneal injection of **MI-09** (**83**) or **MI-30** (**84**) significantly reduced the lung viral load and lung injury. They also showed good pharmacokinetic properties and safety in rats. After i.p. administration, **MI-09** (**83**) displayed a half-life ($T_{1/2}$) of 4.53 h, a bioavailability of 78.0%, and a clearance rate (CL) of 22.67 mL/min/kg. The corresponding values for **MI-30** (**84**) were $T_{1/2}$ = 3.88 h, bioavailability = 76.2%, and CL = 17.10 mL/min/kg [89].

In 2022, Jiao and his colleagues reported 2-(furan-2-ylmethylene) hydrazine-1- carbothioamide derivatives as novel inhibitors of SARS-CoV-2 M^{pro}. Through library screening and similarity search, they identified compound **85** as an inhibitor of SARS-CoV-2 M^{pro} with the enzymatic IC_{50} value of 10.76 μM. The 4-nitrophenyl group in compound **85** was located at the S1 site and formed a hydrogen bond with His163, and the furan ring was deeply buried in the S2 site and had a *π*–*π* stacking with the imidazole of His41 [90]. At the same time, the thiourea linker of compound **85** formed two hydrogen bonds with the main chain carbonyl of Cys44, therefore, the removal or replacement of the thiourea linker led to the loss of inhibitory ability. In addition, the right part of compound **85** was located at the solvent exposed region and forms a hydrogen bond with Ser46. Then, Jiao and his colleagues discovered compounds **86** and **87**, which are non-peptidomimetic inhibitors of M^{pro} with IC_{50} values of 1.57 and 1.55 μM, respectively, based on three rounds of optimization of drug structure design and synthetic modification (Fig. 9). Meanwhile, enzyme kinetics and mass spectrometry studies showed that compound **86** was a reversible covalent inhibitor of M^{pro}. Moreover, compound **86** had no obvious cytotoxicity in Vero and MDCK cells with CC_{50} values over 100 μM [90]. The SAR of the newly identified scaffolds was also discussed, which provides useful guidance for the further development of SARS-CoV-2 M^{pro} inhibitors.

Ghosh and his colleagues reported 5-chloropyridyl indole carboxylate derivatives as a class of potent SARS-CoV-2 M^{pro} inhibitors based on previous research. A number of compounds exhibited nanomolar M^{pro} inhibitory activity. The inhibition mode includes catalyzing cys145 nucleophilic attack on the ester carbonyl group of the inhibitor and forming a covalent bond between Cys145 and the carbonyl group of the active ester. Compound **88** inhibited SARS-CoV-2 M^{pro} with an IC_{50} of 0.25 μM and an antiviral EC_{50} of 2.8 μM in Vero E6 cells. Compound **89** with an N-allyl derivative demonstrated the most potent SARS-CoV-2 M^{pro} with IC_{50} value of 0.07 μM [91] (Fig. 9).

The 3,7-diazabicyclo[3.3.1]nonan (bispidine) framework belongs to the "privileged structures" in medicinal chemistry. For the first time,

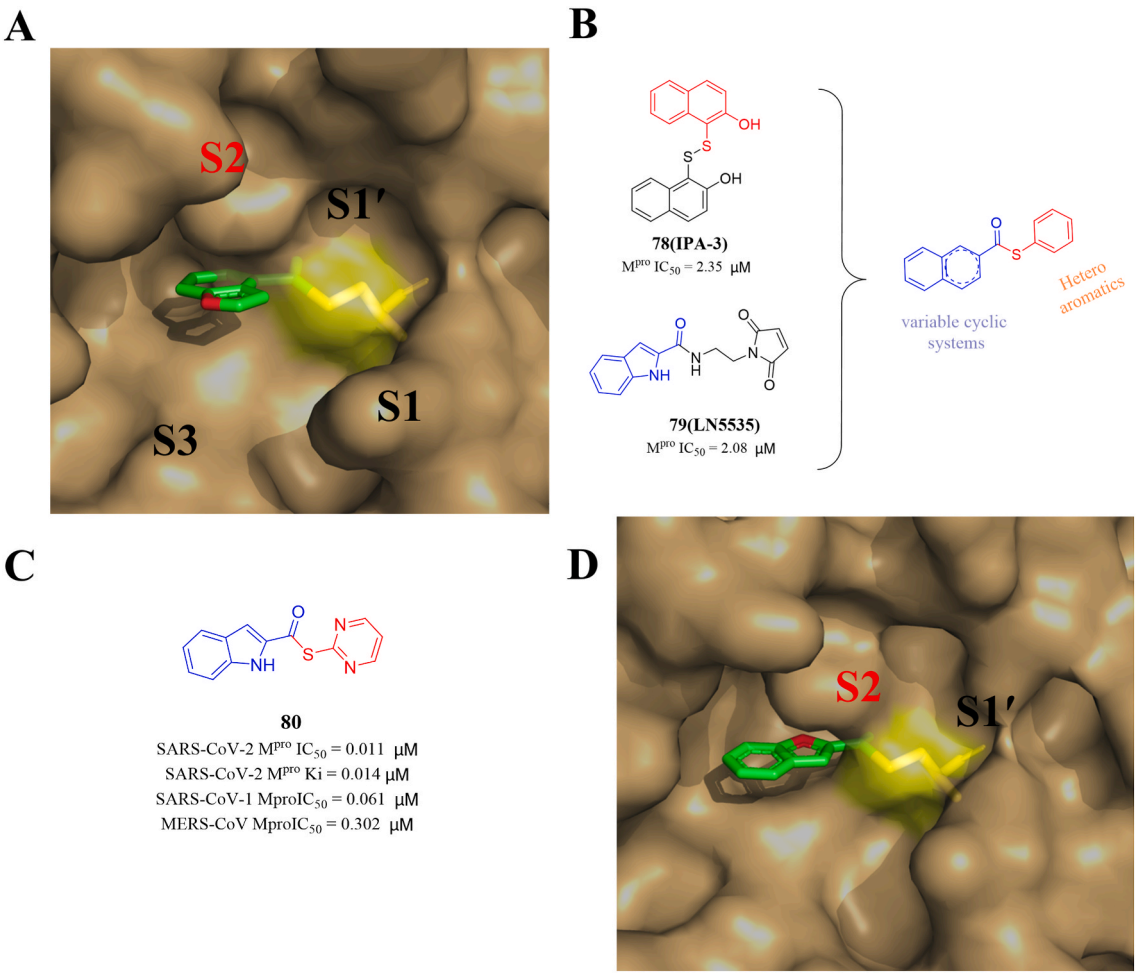


Fig. 8. The structures of compounds **78**–**80** and the crystal structure docking diagram with SARS-CoV-2 M^{pro} (PDB ID: 7RC0).

Shcherbakov et al. proposed the derivatives of 3,7-diazabicyclo- [3.3.1] nonane (bispidine) as potential SARS-CoV-2 M^{pro} inhibitors. The results of the experiments performed with bispidine compounds showed that 14 compounds exhibited activity in the concentration range 1–10 μM, and 3 samples exhibited submicromolar activity [92]. Compound **90** exhibited the strongest inhibitory activity against SARS-CoV-2 M^{pro} with an IC_{50} value of 0.75 μM (Fig. 9). The SAR studies exhibited that the molecule containing carbonyl group at the C-9 position of the bicycle had the greatest activity [92].

In order to identify Cys145 reactive electrophilic molecules with potent SARS-CoV-2 M^{pro} inhibition and high target selectivity, Ma and his colleagues systematically explored a series of new electrophilic reagents substituted by P1′ furan in compound **101**, which led to the discovery of several new cysteine reactive warheads. The most promising lead compounds **91** with the dichloroacetamide warhead and **92** with the tribromoacetamide inhibited SARS-CoV-2 M^{pro} with IC_{50} values of 0.43 and 0.08 μM, respectively [93] (Fig. 9). These two compounds showed potent inhibitory effects on SARS-CoV-2 in both Vero E6 and Caco2-hACE2 cells, with EC_{50} values ranging from micromolar to submicromolar. It is worth noting that both compounds **91** and **92** had high target specificity for M^{pro} and did not inhibit host proteases including calpain I, cathepsin B, cathepsin K, cathepsin L, caspase-3 and trypsin [93]. In contrast, **GC-376** (**17**) is not selective and inhibits calpain I, cathepsin B, cathepsin K, and cathepsin L with potency comparable to M^{pro}. The X-ray crystal structures of SARS-CoV-2 M^{pro} with compounds **91** and **92** showed that Cys145 forms covalent adducts with the reaction warhead.

In 2021, Malla et al. designed and synthesized penicillin derivatives, which were potent SARS-CoV-2 M^{pro} inhibitors. These derivatives form a stable acyl-enzyme complexes by reaction with the nucleophilic cysteine. Malla and his colleagues discovered the most potent compounds, which were the C6 dibromo-penicillin sulfones **93**, **94** and **95**, with IC_{50} values of 0.7, 0.6 and 0.5 μM, respectively [94] (Fig. 9). β-Lactams showed considerable potential as SARS-CoV-2 M^{pro} inhibitors.

In 2021, Moitessier research group transformed the non-covalent inhibitor **96** acting on SARS-CoV-1 M^{pro} into covalent inhibitors acting on SARS-CoV-2 M^{pro} [95]. The study of the crystal structure shows that compound **96** might be modified by adding a covalent warhead near the catalytic cysteine residue. As shown in Fig. 9, the sulfur atom of Cys145 is located at 3.2 Å from the imidazole part, which is the same position as the covalent warhead of **PF-00835231** (**2**). Therefore, they developed covalent inhibitors of SARS-CoV-2 M^{pro} by replacing imidazole with covalent warheads. They first used the docking program F_{ITTED}, which was specially modified to accommodate covalent inhibitors, and screened a group of covalent warheads. The docking poses confirmed that the reactive group substituting the imidazole ring should lead to effective covalent inhibition. It is satisfactory that the inhibitory efficacy of the lead compounds **97** and **98** developed by them is an order of magnitude higher than that of compound **96** [95].

The warhead groups of covalent SARS-CoV-2 M^{pro} inhibitors mainly include ketones, aldehydes and different types of Michael receptors and form a covalent bond with Cys145 residues in the M^{pro} S1′ pocket. Comparing the M^{pro} binding and antiviral efficacy of covalent inhibitors, it is found that the aldehyde warhead might be more effective to inhibit SARS-CoV-2 M^{pro}. The aldehyde compounds **72**, **73**, **83** and **84**, the

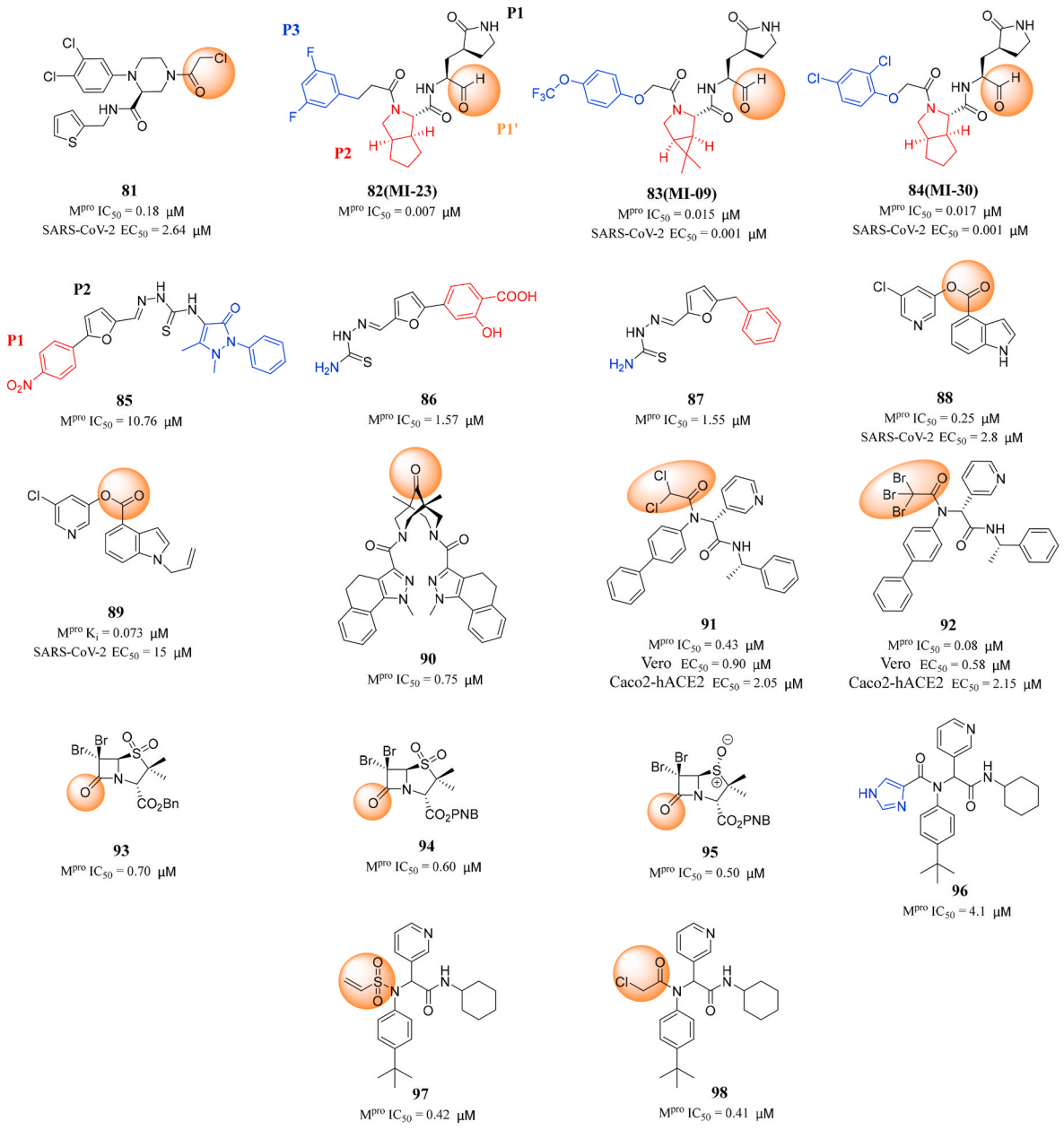


Fig. 9. Structures and biological activity data of compounds **81**–**98**.

peptidomimetic inhibitors designed and synthesized for SARS-CoV-2 M^{pro} by Dai and Qiao et al., exhibited good pharmacokinetic properties *in vivo*.

Covalent SARS-CoV-2 M^{pro} inhibitors generally include peptide drugs and small molecule drugs [96]. The main component of Pfizer's antiviral drug Paxlovid, **PF-07321332(1)**, is a peptidomimetic and covalent SARS-CoV-2 M^{pro} inhibitor [97]. Several significant peptidomimetic inhibitors of SARS-CoV-2 M^{pro} have been reported so far, including compounds **N3** (**9**), **69**, **72** and **GC-376** (**17**), which exhibited high SARS-CoV-2 M^{pro} inhibitory activity and SARS-CoV-2 inhibition at micromolar to submicromolar levels [37,44,78,80]. However, their peptide scaffolds are easily vulnerable to cell metabolism, resulting in low oral bioavailability. Therefore, **PF-07321332** (**1**) is taken orally in combination with the CYP3A inhibitor Ritonavir to avoid extensive metabolism. Nevertheless, the addition of Ritonavir increases the risk of drug interactions and side effects. Considering the shortcomings of the current covalent M^{pro} inhibitors, there is an urgent need for candidate drugs with better performance.

3.4.2. *Non-covalent SARS-CoV-2 M^{pro} inhibitors*

Covalent inhibitors possibly have off target problems, which may lead to unpredictable toxic and side effects in the clinic [98]. In addition, non-covalent inhibitors may have a higher selectivity for SARS-CoV-2 M^{pro}. Therefore, scientists and researchers have designed and synthesized some novel potent non-covalent SARS-CoV-2 M^{pro} inhibitors.

Previously, Liu et al. reported a series of N-substituted 5-carboxamide-isatin compounds as inhibitors of SARS-CoV-1 M^{pro}. The optimal compound has a submicromolar IC_{50} for SARS-CoV-1 M^{pro} [99]. In order to verify the inhibitory effect of isatin compounds on SARS-CoV-2 M^{pro}, they screened a series of isatin compounds from the internally synthesized compound library. Liu and his colleagues tested their inhibitory effects against SARS-CoV-2 M^{pro}, and the results showed that 29 N-substituted isatin derivatives showed inhibitory effect on SARS-CoV-2 M^{pro} [100]. Compound **99** was the most potent SARS-CoV-2 M^{pro} inhibitor with an IC_{50} value of 0.045 μM (Fig. 10). At the same time, these inhibitors have high cytotoxicity, which also hinders the quantitative determination of their anti-SARS-CoV-2 activity.

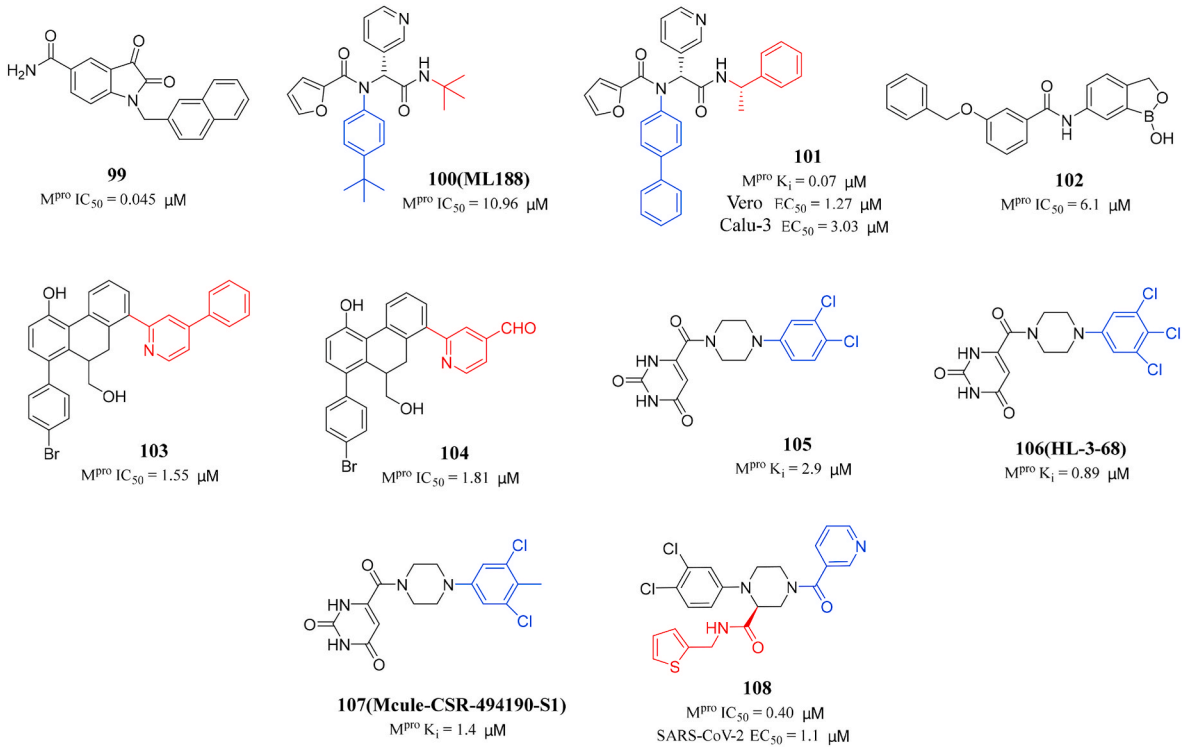


Fig. 10. Structures and biological activity data of compounds **99**–**108**.

The sequence and structure of SARS-CoV-1 and SARS-CoV-2 M^{pro} are similar, and the binding modes of **ML188** (**100**) and Calpain inhibitor **XII** (**20**) are similar. Therefore, based on the noncovalent SARS-CoV-1 M^{pro} inhibitor **ML188** (**100**), Kitamura and his colleagues found the noncovalent inhibitor **101** of SARS-CoV-2 M^{pro} [101,102] (Fig. 10). It was found that compound **101** was an active diastereomer with an IC_{50} value of 0.20 μM. The antiviral activity of compound **101** was tested against SARS-CoV-2 in Vero E6 cells by the immunofluorescence assay. It was found that the EC_{50} value of compound **101** was 1.27 μM. The second antiviral assay was carried out in human lung epithelial Calu-3 cell line, with an EC_{50} value of 3.03 μM. The X-ray crystal structure of SARS-CoV-2 M^{pro} with compound **101** complex revealed that there was a ligand-induced binding pocket between the S2 and S4 sites [102]. This study showed a promising noncovalent M^{pro} inhibitor **101**, which has potent cellular antiviral activity for further development.

Dengue virus (DENV) and SARS-CoV-2 pose a serious threat to global human health, and their proteases (NS2B/NS3 and M^{pro}) are considered as promising targets for drug development [103,104]. In 2022, KüHL et al. designed and synthesized several compounds with benzoxaborole motif and tested them against the DENV-2 protease and SARS-CoV-2 M^{pro}. Compound **102** has obvious inhibitory activity against SARS-CoV-2 M^{pro} with IC_{50} value of 6.1 μM. Besides, the EC_{50} values against DENV-2 protease was 2.4 μM in the cell-based DENVproHeLa assay [105] (Fig. 10). The majority of benzoxaboroles did not show relevant cytotoxicity or obvious off-target inhibition. These compounds provide an opportunity to develop drugs with anti-SARS-CoV-2 and DENV protease activities.

In 2021, Gao's research group reported 9,10-dihydrophenanthrene derivatives as non-peptidomimetic and non-covalent inhibitors of SARS-CoV-2 M^{pro} (Fig. 10). Among all tested 9,10-dihydrophenanthrene derivatives, compounds **103** and **104** had the strongest SARS-CoV-2 M^{pro} inhibition activity, with IC_{50} values of 1.55 and 1.81 μM, respectively. Besides, compound **103** exhibited excellent metabolic stability in the gastrointestinal tract, human plasma, and human liver microsomes [96]. This study can provide more structural references for the development of SARS-CoV-2 M^{pro} inhibitors.

In order to improve the affinity of compound **105** and explore the chemical determinants for ligand binding to S1 and S2, Kneller and his colleagues designed a series of derivatives of compound **105** (**HL-3** series). Isothermal titration calorimetry was used to evaluate the thermodynamic binding properties of compound **105** and the two most potent inhibitors **HL-3-68** (**106**) and **Mcule-CSR-494190-S1** (**107**) [106] (Fig. 10). **HL-3-68** (**106**) showed submicromolar affinity for SARS-CoV-2 M^{pro}, and its binding ability to the enzyme is about 2 times higher than the other two compounds. Notably, none of the compounds showed antiviral activity against SARS-CoV-2 in cell-based assays.

Liu's research group designed and synthesized potent non-covalent non-peptide SARS-CoV-2 M^{pro} inhibitors with 1,2,4-triasubstituted piperazine scaffold by modifying the piperazine nitrogen atoms and the carboxyl side chain of compound **105**, respectively. Compound **108** exhibited potent inhibitory effect on M^{pro} with IC_{50} values of 0.40 μM, and displayed excellent antiviral activity (EC_{50} = 1.1 μM) (Fig. 10). It is noteworthy that compound **108** showed low cytotoxicity (CC_{50} > 100 μM) and good target selectivity for SARS-CoV-2 M^{pro} (IC_{50} > 50 μM for cathepsins B, F, K, L, and caspase 3) [107].

Japan approved a non-covalent, non-peptidomimetic SARS-CoV-2 M^{pro} inhibitor **S-217622** (**6**) on November 22, 2022. **S-217622** (**6**) had significant inhibitory activity with IC_{50} value of 0.13 μM and also showed good drug metabolism and pharmacokinetic characteristics [34]. Compounds **101** and **103** are non-covalent inhibitors of SARS-CoV-2 M^{pro}, with IC_{50} values of 0.20 and 1.55 μM, respectively. Compound **103** displayed excellent metabolic stability in the gastrointestinal tract, human plasma and human liver microsomes [96,102]. Non-covalent drugs not only have potent biological activity, but also exhibit excellent pharmacokinetic characteristics and good cell permeability. So far, scientists and researchers have found relatively few non-covalent M^{pro} inhibitors of SARS-CoV-2, and further efforts are still needed.

4. Conclusion and future perspective

Coronavirus have caused three pandemics in the past 20 years,

including SARS, MERS and COVID-19. With the continuous epidemic of COVID-19, SARS-CoV-2 vaccines have been approved and used worldwide, but the emergence of virus mutation compromised the effectiveness of the vaccine [108]. In addition, the percentage of vaccinated people has remained at a moderate to low level in many countries. Therefore, the development of antiviral drugs targeting SARS-CoV-2 is still needed to reduce the incidence and symptom severity and fatality rates. M^{pro} plays an important role in the process of viral replication and transcription, and it is considered as a promising target for the development of antiviral drugs. Through drug repurposing and drug design, many SARS-CoV-2 M^{pro} inhibitors have been developed.

At present, the drug repurposing methods of SARS-CoV-2 M^{pro} inhibitors mainly include: (1) Reuse of homologous virus M^{pro} inhibitors: studying the potential of SARS-CoV-1 M^{pro} inhibitors as anti-SARS-CoV-2 drugs; (2) High throughput screening based on activity: using fluorescence technology to screen the activity of compounds from natural products, approved drugs, and clinical trial drugs libraries; (3) Virtual screening: using the reported M^{pro} crystal structure to reasonably narrow the range of active screening compounds. Through drug repurposing, a variety of scaffold structures can be provided for the design of SARS-CoV-2 M^{pro} inhibitors, and broad-spectrum antiviral agents that overcome drug resistance can also be obtained. In conclusion, drug repurposing can provide a reliable and efficient method to discover lead compounds (Fig. 11).

In the drug design section, relevant researchers designed and synthesized some covalent and non-covalent SARS-CoV-2 M^{pro} inhibitors by modifying and optimizing the structure of cysteine protease inhibitors. Through the analysis of SARS-CoV-2 M^{pro} peptidomimetic covalent inhibitors, we can summarize the most suitable molecular fragments (P1', P1, P2, P3) for interacting with M^{pro} active sites (S1', S1, S2, S3): (1) The P1' is the warhead group mainly consisting of ketones, aldehydes and different types of Michael receptors; (2) The most common fragment in the P1 is the γ-lactamic ring; (3) The P2 is usually aromatic or alkane groups, such as cyclopropyl, cyclohexyl, *etc*; (4) The P3 may be an aromatic group, such as indole and substituted phenyl (especially with halogens) (Fig. 12). The warhead groups form a covalent bond with the Cys145 residues in the M^{pro} S1' pocket, which determines the inhibitory activity of the M^{pro} inhibitors. The γ-lactamic ring in the P1 has the ability to mimic the natural amino acid glutamine and can penetrate into the S1 pocket to form a stablilizing interaction with key residues. Therefore, it can be maintained in the design of new M^{pro} inhibitors. The main component of Pfizer's antiviral drug Paxlovid, **PF-07321332**(**1**), is a peptidomimetic covalent SARS-CoV-2 M^{pro} inhibitor. On December 22, 2021, the United States Food and Drug Administration approved Paxlovid. Therefore, peptidomimetic covalent inhibitors can still be considered as the most promising antiviral drugs for the development of M^{pro} inhibitors. Notably, the covalent binding mode with M^{pro} may lead to the reduction of oral bioavailability and metabolic stability, which usually requires coadministration with pharmacokinetic enhancers such as Ritonavir. Non-peptidomimetic covalent inhibitor **Ebselen** (**7**) may be a potential lead compound as M^{pro} inhibitors.

Non-covalent drugs not only have potent biological activity, but also exhibit excellent pharmacokinetic characteristics and good cell permeability. In addition, **S-217622** (**6**) has been proved to be effective for most SARS-CoV-2 variants and to possess significant selectivity for M^{pro} versus host protease [34], indicating that non-covalent non-peptide inhibitors have great potential for the therapy of COVID-19. The mutation and recombination rate of SARS-CoV-2 is relatively high, thus multi-target antiviral inhibitors can more effectively overcome the drug resistance. Scientists and researchers are trying to find broadspectrum antiviral drugs for fighting SARS-CoV-2 and future coronaviruses. In addition, SARS-CoV-2 M^{pro} inhibitors with diverse chemical scaffolds and improved pharmaceutical profile need to be further explored and developed.

SARS-CoV-2 M^{pro} is currently targeted by small molecular inhibitors, which may become a potential target of proteolysis targeting chimeras (PROTACs). PROTACs are a newly developed technology that degrades target protein through ubiquitin-proteasome pathway [109]. After the PROTAC molecule enters the cell, it first binds to the target protein and recruits E3 ligase for ubiquitination labeling, and then degrades the target protein through the proteasome *in vivo* [110]. The feasibility of antiviral PROTACs was first established with the degradation of the hepatitis C virus (HCV) NS3/4A protease. We firmly believe that the PROTAC technique will be used in the research of COVID-19 drugs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

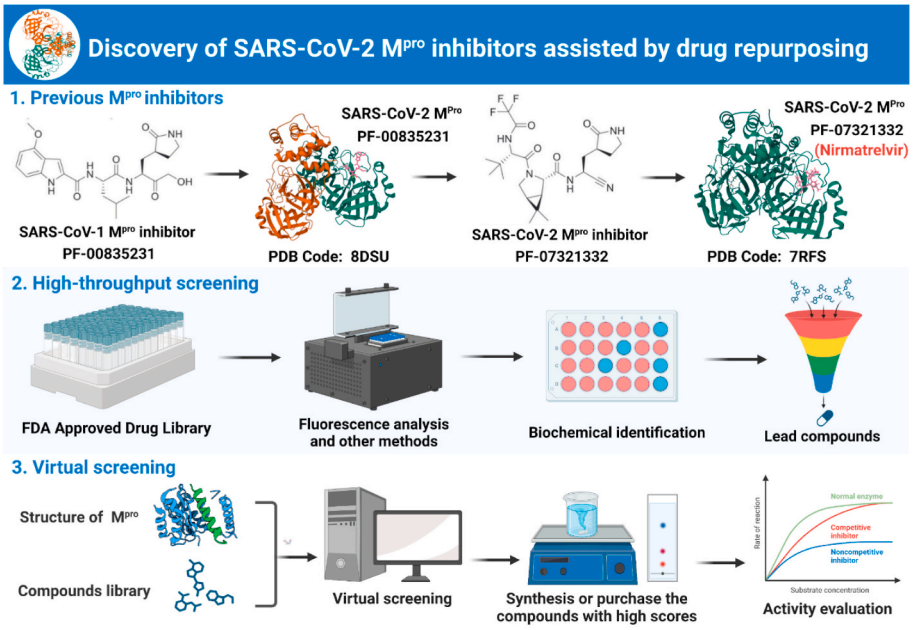


Fig. 11. Discovery of SARS-CoV-2 M^{pro} inhibitors assisted by drug repurposing.

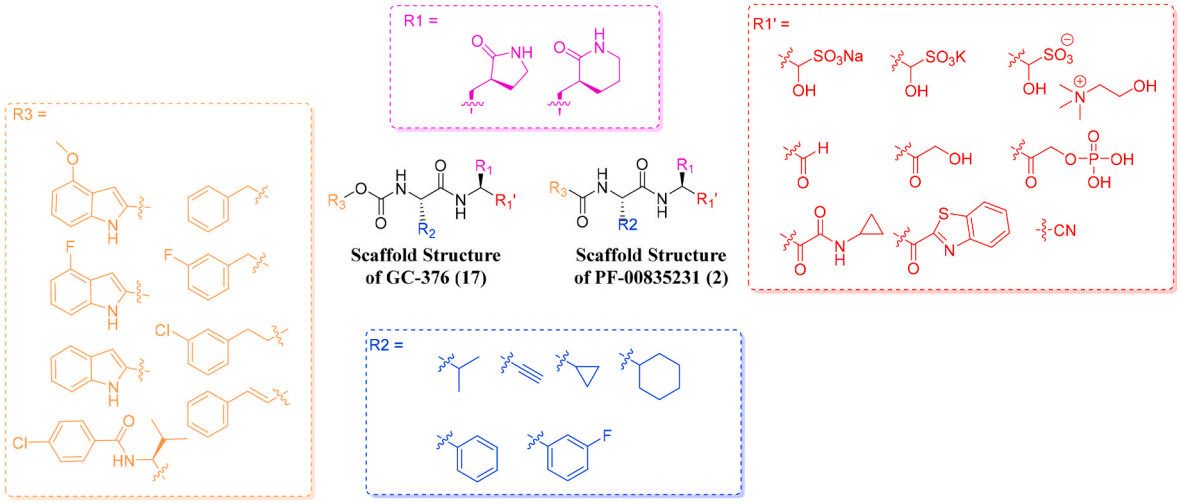


Fig. 12. The most common fragment in P1', P1, P2, P3.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank the National Natural Science Foundation of China (NSFC) (No. 82141216), Chunhui Program-Cooperative Research Project of the Ministry of Education, Liaoning Province Natural Science Foundation (No. 2022-MS-241), and Shenyang Young and Middle-aged Innovative Talents Support Program (No. RC210446) for financial supports. We acknowledged the support from Joint National Local Engineering Research Center of Fujian and Taiwan Chinese Medicine Molecular Biotechnology, Fujian Key Laboratory of Chinese Materia Medica, Fujian University Key Laboratory for Research and Development of TCM Resources, at Fujian University of Traditional Chinese Medicine.

References

[1] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (2020) 497–506.

[2] X. Xie, L. Hu, H. Xue, Y. Xiong, A.C. Panayi, Z. Lin, L. Chen, C. Yan, W. Zhou, B. Mi, G. Liu, Prognosis and treatment of complications associated with COVID-19: a systematic review and meta-analysis, Acta Materia Medica 1 (2022) 124–137.

[3] J.T. Wu, K. Leung, G.M. Leung, Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study, Lancet 395 (2020) 689–697.

[4] J.F.W. Chan, S. Yuan, K.H. Kok, K.K.W. To, H. Chu, J. Yang, F. Xing, J. Liu, C.C. Y. Yip, R.W.S. Poon, H.W. Tsoi, S.K.F. Lo, K.H. Chan, V.K.M. Poon, W.M. Chan, J. D. Ip, J.P. Cai, V.C.C. Cheng, H. Chen, C.K.M. Hui, K.Y. Yuen, A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster, Lancet 395 (2020) 514–523.

[5] S.A. Amin, T. Jha, Fight against novel coronavirus: a perspective of medicinal chemists, Eur. J. Med. Chem. 201 (2020), 112559.

[6] T. Pillaiyar, S. Meenakshisundaram, M. Manickam, Recent discovery and development of inhibitors targeting coronaviruses, Drug Discov. Today 25 (2020) 668–688.

[7] K. Nakagawa, K.G. Lokugamage, S. Makino, Viral and cellular mRNA translation in coronavirus-infected cells, Adv. Virus Res. 96 (2016) 165–192.

[8] J. de Groot Raoul, C. Baker Susan, S. Baric Ralph, S. Brown Caroline, C. Drosten, L. Enjuanes, A.M. Fouchier Ron, M. Galiano, E. Gorbalenya Alexander, A. Memish Ziad, S. Perlman, L.M. Poon Leo, J. Snijder Eric, M. Stephens Gwen, C.Y. Woo Patrick, M. Zaki Ali, M. Zambon, J. Ziebuhr, Commentary: Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the coronavirus study group, J. Virol. 87 (2013) 7790–7792.

[9] C.Y. Woo Patrick, K.P. Lau Susanna, C.m. Chu, K.h. Chan, H.w. Tsoi, Y. Huang, H. L. Wong Beatrice, W.S. Poon Rosana, J. Cai James, W.k. Luk, L.M. Poon Leo, S. Y. Wong Samson, Y. Guan, J.S.M. Peiris, K.y. Yuen, Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia, J. Virol. 79 (2005) 884–895.

[10] O. World Health, Clinical Management of Severe Acute Respiratory Infection when Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Infection Is Suspected: Interim Guidance, World Health Organization, 2019.

[11] H. Li, W. Wei, H. Xu, Drug discovery is an eternal challenge for the biomedical sciences, Acta Materia Medica 1 (2022) 1–3.

[12] X. Xu, P. Chen, J. Wang, J. Feng, H. Zhou, X. Li, W. Zhong, P. Hao, Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission, Sci. China Life Sci. 63 (2020) 457–460.

[13] Z. Ren, L. Yan, N. Zhang, Y. Guo, C. Yang, Z. Lou, Z. Rao, The newly emerged SARS-Like coronavirus HCoV-EMC also has an "Achilles' heel": current effective inhibitor targeting a 3C-like protease, Protein & Cell 4 (2013) 248–250.

[14] R. Ramajayam, K.P. Tan, P.H. Liang, Recent development of 3C and 3CL protease inhibitors for anti-coronavirus and anti-picornavirus drug discovery, Biochem. Soc. Trans. 39 (2011) 1371–1375.

[15] N. Dong, X. Yang, L. Ye, K. Chen, E.W.C. Chan, M. Yang, S. Chen, Genomic and Protein Structure Modelling Analysis Depicts the Origin and Infectivity of 2019-nCoV, a New Coronavirus Which Caused a Pneumonia Outbreak in Wuhan, bioRxiv, China, 2020.

[16] D. Forni, R. Cagliani, M. Clerici, M. Sironi, Molecular evolution of human coronavirus genomes, Trends Microbiol. 25 (2017) 35–48.

[17] M.A. Marra, S.J.M. Jones, C.R. Astell, R.A. Holt, A. Brooks-Wilson, Y.S. N. Butterfield, J. Khattra, J.K. Asano, S.A. Barber, S.Y. Chan, A. Cloutier, S. M. Coughlin, D. Freeman, N. Girn, O.L. Griffith, S.R. Leach, M. Mayo, H. McDonald, S.B. Montgomery, P.K. Pandoh, A.S. Petrescu, A.G. Robertson, J. E. Schein, A. Siddiqui, D.E. Smailus, J.M. Stott, G.S. Yang, F. Plummer, A. Andonov, H. Artsob, N. Bastien, K. Bernard, T.F. Booth, D. Bowness, M. Czub, M. Drebot, L. Fernando, R. Flick, M. Garbutt, M. Gray, A. Grolla, S. Jones, H. Feldmann, A. Meyers, A. Kabani, Y. Li, S. Normand, U. Stroher, G.A. Tipples, S. Tyler, R. Vogrig, D. Ward, B. Watson, R.C. Brunham, M. Krajden, M. Petric, D. M. Skowronski, C. Upton, R.L. Roper, The genome sequence of the SARS-associated coronavirus, Science 300 (2003) 1399–1404.

[18] Y. Wang, M. Grunewald, S. Perlman, Coronaviruses: an updated overview of their replication and pathogenesis, Methods Mol. Biol. 2203 (2020) 1–29.

[19] K. Anand, J. Ziebuhr, P. Wadhwani, J.R. Mesters, R. Hilgenfeld, Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs, Science 300 (2003) 1763–1767.

[20] H. Yang, M. Bartlam, Z. Rao, Drug design targeting the main protease, the achilles heel of coronaviruses, Curr. Pharmaceut. Des. 12 (2006) 4573–4590.

[21] U. Bacha, J. Barrila, A. Velazquez-Campoy, S.A. Leavitt, E. Freire, Identification of novel inhibitors of the SARS coronavirus main protease 3CLpro, Biochemistry 43 (2004) 4906–4912.

[22] K. Fan, L. Ma, X. Han, H. Liang, P. Wei, Y. Liu, L. Lai, The substrate specificity of SARS coronavirus 3C-like proteinase, Biochem. Biophys. Res. Commun. 329 (2005) 934–940.

[23] J. Ziebuhr, E.J. Snijder, A.E. Gorbalenya, Virus-encoded proteinases and proteolytic processing in the Nidovirales, J. Gen. Virol. 81 (2000) 853–879.

[24] S. Chen, F. Jonas, C. Shen, R. Higenfeld, Liberation of SARS-CoV main protease from the viral polyprotein: N-terminal autocleavage does not depend on the mature dimerization mode, Protein & Cell 1 (2010) 59–74.

[25] S. Tomar, M.L. Johnston, S.E. St John, H.L. Osswald, P.R. Nyalapatla, L.N. Paul, A.K. Ghosh, M.R. Denison, A.D. Mesecar, Ligand-induced dimerization of Middle East respiratory syndrome (MERS) coronavirus nsp5 protease (3CLpro): IMPLICATIONS for nsp5 regulation and the development of antivirals, J. Biol. Chem. 290 (2015) 19403–19422.

[26] M.F. Hsu, C.J. Kuo, K.T. Chang, H.C. Chang, C.C. Chou, T.P. Ko, H.L. Shr, G. G. Chang, A.H.J. Wang, P.H. Liang, Mechanism of the maturation process of SARS-CoV 3CL protease, J. Biol. Chem. 280 (2005) 31257–31266.
[27] C. Li, Y. Qi, X. Teng, Z. Yang, P. Wei, C. Zhang, L. Tan, L. Zhou, Y. Liu, L. Lai, Maturation mechanism of severe acute respiratory syndrome (SARS) coronavirus 3C-like proteinase, J. Biol. Chem. 285 (2010) 28134–28140.
[28] T. Pillaiyar, M. Manickam, V. Namasivayam, Y. Hayashi, S.H. Jung, An overview of severe acute respiratory syndrome–coronavirus (SARS-CoV) 3CL protease inhibitors: peptidomimetics and small molecule chemotherapy, J. Med. Chem. 59 (2016) 6595–6628.
[29] K. Anand, J. Ziebuhr, P. Wadhwani, J.R. Mesters, R. Hilgenfeld, Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs, Science 300 (2003) 1763–1767.
[30] L. Zhang, D. Lin, Y. Kusov, Y. Nian, Q. Ma, J. Wang, A. von Brunn, P. Leyssen, K. Lanko, J. Neyts, A. de Wilde, E.J. Snijder, H. Liu, R. Hilgenfeld, α-Ketoamides as broad-spectrum inhibitors of coronavirus and enterovirus replication: structure-based design, synthesis, and activity assessment, J. Med. Chem. 63 (2020) 4562–4578.
[31] R.L. Hoffman, R.S. Kania, M.A. Brothers, J.F. Davies, R.A. Ferre, K.S. Gajiwala, M. He, R.J. Hogan, K. Kozminski, L.Y. Li, J.W. Lockner, J. Lou, M.T. Marra, L. J. Mitchell, B.W. Murray, J.A. Nieman, S. Noell, S.P. Planken, T. Rowe, K. Ryan, G.J. Smith, J.E. Solowiej, C.M. Steppan, B. Taggart, Discovery of ketone-based covalent inhibitors of coronavirus 3CL proteases for the potential therapeutic treatment of COVID-19, J. Med. Chem. 63 (2020) 12725–12747.
[32] D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, Molecular properties that influence the oral bioavailability of drug candidates, J. Med. Chem. 45 (2002) 2615–2623.
[33] D.R. Owen, C.M.N. Allerton, A.S. Anderson, L. Aschenbrenner, M. Avery, S. Berritt, B. Boras, R.D. Cardin, A. Carlo, K.J. Coffman, A. Dantonio, L. Di, H. Eng, R. Ferre, K.S. Gajiwala, S.A. Gibson, S.E. Greasley, B.L. Hurst, E.P. Kadar, A.S. Kalgutkar, J.C. Lee, J. Lee, W. Liu, S.W. Mason, S. Noell, J.J. Novak, R. S. Obach, K. Ogilvie, N.C. Patel, M. Pettersson, D.K. Rai, M.R. Reese, M. F. Sammons, J.G. Sathish, R.S.P. Singh, C.M. Steppan, A.E. Stewart, J.B. Tuttle, L. Updyke, P.R. Verhoest, L. Wei, Q. Yang, Y. Zhu, An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19, Science 374 (2021) 1586–1593.
[34] Y. Unoh, S. Uehara, K. Nakahara, H. Nobori, Y. Yamatsu, S. Yamamoto, Y. Maruyama, Y. Taoda, K. Kasamatsu, T. Suto, K. Kouki, A. Nakahashi, S. Kawashima, T. Sanaki, S. Toba, K. Uemura, T. Mizutare, S. Ando, M. Sasaki, Y. Orba, H. Sawa, A. Sato, T. Sato, T. Kato, Y. Tachibana, Discovery of S-217622, a noncovalent oral SARS-CoV-2 3CL protease inhibitor clinical candidate for treating COVID-19, J. Med. Chem. 65 (2022) 6499–6512.
[35] J.D.A. Tyndall, S-217622, a 3CL protease inhibitor and clinical candidate for SARS-CoV-2, J. Med. Chem. 65 (2022) 6496–6498.
[36] B. Boras, R.M. Jones, B.J. Anson, D. Arenson, L. Aschenbrenner, M.A. Bakowski, N. Beutler, J. Binder, E. Chen, H. Eng, H. Hammond, J. Hammond, R.E. Haupt, R. Hoffman, E.P. Kadar, R. Kania, E. Kimoto, M.G. Kirkpatrick, L. Lanyon, E. K. Lendy, J.R. Lillis, J. Logue, S.A. Luthra, C. Ma, S.W. Mason, M.E. McGrath, S. Noell, R.S. Obach, M.N. O'Brien, R. O'Connor, K. Ogilvie, D. Owen, M. Pettersson, M.R. Reese, T.F. Rogers, M.I. Rossulek, J.G. Sathish, N. Shirai, C. Steppan, M. Ticehurst, L.W. Updyke, S. Weston, Y. Zhu, J. Wang, A. K. Chatterjee, A.D. Mesecar, M.B. Frieman, A.S. Anderson, C. Allerton, Discovery of a Novel Inhibitor of Coronavirus 3CL Protease for the Potential Treatment of COVID-19, bioRxiv, 2021.
[37] Z. Jin, X. Du, Y. Xu, Y. Deng, M. Liu, Y. Zhao, B. Zhang, X. Li, L. Zhang, C. Peng, Y. Duan, J. Yu, L. Wang, K. Yang, F. Liu, R. Jiang, X. Yang, T. You, X. Liu, X. Yang, F. Bai, H. Liu, X. Liu, L.W. Guddat, W. Xu, G. Xiao, C. Qin, Z. Shi, H. Jiang, Z. Rao, H. Yang, Structure of M(pro) from SARS-CoV-2 and discovery of its inhibitors, Nature 582 (2020) 289–293.
[38] E.D. Lynch, J. Kil, Development of ebselen, a glutathione peroxidase mimic, for the prevention and treatment of noise-induced hearing loss, Semin. Hear. 30 (2009) 47–55.
[39] J. Kil, E. Lobarinas, C. Spankovich, S.K. Griffiths, P.J. Antonelli, E.D. Lynch, C. G. Le Prell, Safety and efficacy of ebselen for the prevention of noise-induced hearing loss: a randomised, double-blind, placebo-controlled, phase 2 trial, Lancet 390 (2017) 969–979.
[40] C. Masaki, A.L. Sharpley, C.M. Cooper, B.R. Godlewska, N. Singh, S.R. Vasudevan, C.J. Harmer, G.C. Churchill, T. Sharp, R.D. Rogers, P.J. Cowen, Effects of the potential lithium-mimetic, ebselen, on impulsivity and emotional processing, Psychopharmacology 233 (2016) 2655–2661.
[41] C.V. Haritha, K. Sharun, B. Jose, Ebselen, a new candidate therapeutic against SARS-CoV-2, Int. J. Surg. 84 (2020) 53–56.
[42] N. Drayman, J.K. DeMarco, K.A. Jones, S.A. Azizi, H.M. Froggatt, K. Tan, N. I. Maltseva, S. Chen, V. Nicolaescu, S. Dvorkin, K. Furlong, R.S. Kathayat, M. R. Firpo, V. Mastrodomenico, E.A. Bruce, M.M. Schmidt, R. Jedrzejczak, M.Á. Muñoz-Alía, B. Schuster, V. Nair, K.y. Han, A. O'Brien, A. Tomatsidou, B. Meyer, M. Vignuzzi, D. Missiakas, J.W. Botten, C.B. Brooke, H. Lee, S.C. Baker, B.C. Mounce, N.S. Heaton, W.E. Severson, K.E. Palmer, B.C. Dickinson, A. Joachimiak, G. Randall, S. Tay, Masitinib is a broad coronavirus 3CL inhibitor that blocks replication of SARS-CoV-2, Science 373 (2021) 931–936.
[43] C.A. Menéndez, F. Byléhn, G.R. Perez-Lemus, W. Alvarado, J.J. de Pablo, Molecular characterization of ebselen binding activity to SARS-CoV-2 main protease, Sci. Adv. 6 (2020), eabd0345.
[44] C. Ma, M.D. Sacco, B. Hurst, J.A. Townsend, Y. Hu, T. Szeto, X. Zhang, B. Tarbet, M.T. Marty, Y. Chen, J. Wang, Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2 viral replication by targeting the viral main protease, Cell Res. 30 (2020) 678–692.
[45] L. Fu, F. Ye, Y. Feng, F. Yu, Q. Wang, Y. Wu, C. Zhao, H. Sun, B. Huang, P. Niu, H. Song, Y. Shi, X. Li, W. Tan, J. Qi, G.F. Gao, Both Boceprevir and GC376 efficaciously inhibit SARS-CoV-2 by targeting its main protease, Nat. Commun. 11 (2020) 4417.
[46] H.C. Hung, Y.Y. Ke, S.Y. Huang, P.N. Huang, Y.A. Kung, T.Y. Chang, K.J. Yen, T. T. Peng, S.E. Chang, C.T. Huang, Y.R. Tsai, S.H. Wu, S.J. Lee, J.H. Lin, B.S. Liu, W. C. Sung, S.R. Shih, C.T. Chen, J.T. Hsu, Discovery of M Protease inhibitors encoded by SARS-CoV-2, Antimicrob. Agents Chemother. 64 (2020).
[47] S. Iketani, F. Forouhar, H. Liu, S.J. Hong, F.Y. Lin, M.S. Nair, A. Zask, Y. Huang, L. Xing, B.R. Stockwell, A. Chavez, D.D. Ho, Lead compounds for the development of SARS-CoV-2 3CL protease inhibitors, Nat. Commun. 12 (2021), 2016.
[48] S. Konno, K. Kobayashi, M. Senda, Y. Funai, Y. Seki, I. Tamai, L. Schakel, K. Sakata, T. Pillaiyar, A. Taguchi, A. Taniguchi, M. Gutschow, C.E. Muller, K. Takeuchi, M. Hirohama, A. Kawaguchi, M. Kojima, T. Senda, Y. Shirasaka, W. Kamitani, Y. Hayashi, 3CL protease inhibitors with an electrophilic arylketone moiety as anti-SARS-CoV-2 agents, J. Med. Chem. 65 (2022) 2926–2939.
[49] P.K. Tripathi, S. Upadhyay, M. Singh, S. Raghavendhar, M. Bhardwaj, P. Sharma, A.K. Patel, Screening and evaluation of approved drugs as inhibitors of main protease of SARS-CoV-2, Int. J. Biol. Macromol. 164 (2020) 2622–2631.
[50] V. Ramos-Martín, A. Johnson, L. McEntee, N. Farrington, K. Padmore, P. Cojutti, F. Pea, M.N. Neely, W.W. Hope, Pharmacodynamics of teicoplanin against MRSA, J. Antimicrob. Chemother. 72 (2017) 3382–3389.
[51] S.A. Baron, C. Devaux, P. Colson, D. Raoult, J.-M. Rolain, Teicoplanin: an alternative drug for the treatment of COVID-19? Int. J. Antimicrob. Agents 55 (2020), 105944.
[52] N. Zhou, T. Pan, J. Zhang, Q. Li, X. Zhang, C. Bai, F. Huang, T. Peng, J. Zhang, C. Liu, L. Tao, H. Zhang, Glycopeptide antibiotics potently inhibit cathepsin L in the late endosome/lysosome and block the entry of ebola virus, Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus (SARS-CoV), J. Biol. Chem. 291 (2016) 9218–9232.
[53] F. Yu, T. Pan, F. Huang, R. Ying, J. Liu, H. Fan, J. Zhang, W. Liu, Y. Lin, Y. Yuan, T. Yang, R. Li, X. Zhang, X. Lv, Q. Chen, A. Liang, F. Zou, B. Liu, F. Hu, X. Tang, L. Li, K. Deng, X. He, H. Zhang, Y. Zhang, X. Ma, Glycopeptide Antibiotic Teicoplanin Inhibits Cell Entry of SARS-CoV-2 by Suppressing the Proteolytic Activity of Cathepsin L, bioRxiv, 2021.
[54] W. Zhu, M. Xu, C.Z. Chen, H. Guo, M. Shen, X. Hu, P. Shinn, C. Klumpp-Thomas, S.G. Michael, W. Zheng, Identification of SARS-CoV-2 3CL protease inhibitors by a quantitative high-throughput screening, ACS Pharmacol Transl Sci 3 (2020) 1008–1016.
[55] P. Wang, Y. Liu, G. Zhang, S. Wang, J. Guo, J. Cao, X. Jia, L. Zhang, G. Xiao, W. Wang, Screening and identification of lassa virus entry inhibitors from an FDA-approved drug library, J. Virol. 92 (2018), e00954-00918.
[56] Z. Jin, X. Du, Y. Xu, Y. Deng, M. Liu, Y. Zhao, B. Zhang, X. Li, L. Zhang, C. Peng, Y. Duan, J. Yu, L. Wang, K. Yang, F. Liu, R. Jiang, X. Yang, T. You, X. Liu, X. Yang, F. Bai, H. Liu, X. Liu, L.W. Guddat, W. Xu, G. Xiao, C. Qin, Z. Shi, H. Jiang, Z. Rao, H. Yang, Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors, Nature 582 (2020) 289–293.
[57] S. Afshar, A. Bahmani, M. Saidijam, Molecular docking and fragment-based QSAR modeling for in silico screening of approved drugs and candidate compounds against COVID-19, Avicenna Journal of Medical Biochemistry 8 (2020) 83–88.
[58] W.C. Chiou, M.S. Hsu, Y.T. Chen, J.M. Yang, Y.G. Tsay, H.C. Huang, C. Huang, Repurposing existing drugs: identification of SARS-CoV-2 3C-like protease inhibitors, J. Enzym. Inhib. Med. Chem. 36 (2021) 147–153.
[59] K. Steuten, H. Kim, J.C. Widen, B.M. Babin, O. Onguka, S. Lovell, O. Bolgi, B. Cerikan, C.J. Neufeldt, M. Cortese, R.K. Muir, J.M. Bennett, R. Geiss-Friedlander, C. Peters, R. Bartenschlager, M. Bogyo, Challenges for targeting SARS-CoV-2 proteases as a therapeutic strategy for COVID-19, ACS Infect. Dis. 7 (2021) 1457–1468.
[60] C. Coelho, G. Gallo, C.B. Campos, L. Hardy, M. Wurtele, Biochemical screening for SARS-CoV-2 main protease inhibitors, PLoS One 15 (2020), e0240079.
[61] H.X. Su, S. Yao, W.F. Zhao, M.J. Li, J. Liu, W.J. Shang, H. Xie, C.Q. Ke, H.C. Hu, M.N. Gao, K.Q. Yu, H. Liu, J.S. Shen, W. Tang, L.K. Zhang, G.F. Xiao, L. Ni, D. W. Wang, J.P. Zuo, H.L. Jiang, F. Bai, Y. Wu, Y. Ye, Y.C. Xu, Anti-SARS-CoV-2 activities in vitro of Shuanghuanglian preparations and bioactive ingredients, Acta Pharmacol. Sin. 41 (2020) 1167–1177.
[62] H. Liu, F. Ye, Q. Sun, H. Liang, C. Li, S. Li, R. Lu, B. Huang, W. Tan, L. Lai, Scutellaria baicalensis extract and baicalein inhibit replication of SARS-CoV-2 and its 3C-like protease in vitro, J. Enzym. Inhib. Med. Chem. 36 (2021) 497–503.
[63] J. Liang, M. Zheng, W. Xu, Y. Chen, P. Tang, G. Wu, P. Zou, H. Li, L. Chen, Acriflavine and proflavine hemisulfate as potential antivirals by targeting Mpro, Bioorg. Chem. 129 (2022), 106185.
[64] V. Napolitano, A. Dabrowska, K. Schorpp, A. Mourao, E. Barreto-Duran, M. Benedyk, P. Botwina, S. Brandner, M. Bostock, Y. Chykunova, A. Czarna, G. Dubin, T. Frohlich, M. Holscher, M. Jedrysik, A. Matsuda, K. Owczarek, M. Pachota, O. Plettenburg, J. Potempa, I. Rothenaigner, F. Schlauderer, K. Slysz, A. Szczepanski, K. Greve-Isdahl Mohn, B. Blomberg, M. Sattler, K. Hadian, G. M. Popowicz, K. Pyrc, Acriflavine, a clinically approved drug, inhibits SARS-CoV-2 and other betacoronaviruses, Cell Chem Biol 29 (2022) 774–784 e778.
[65] L.Y. Sun, C. Chen, J. Su, J.Q. Li, Z. Jiang, H. Gao, J.Z. Chigan, H.H. Ding, L. Zhai, K.W. Yang, Ebsulfur and Ebselen as highly potent scaffolds for the development of potential SARS-CoV-2 antivirals, Bioorg. Chem. 112 (2021), 104889.
[66] H. Lin, J. Zhou, K. Lin, H. Wang, Z. Liang, X. Ren, L. Huang, C. Xia, Efficacy of scutellaria baicalensis for the treatment of hand, foot, and mouth disease

associated with encephalitis in patients infected with EV71: a multicenter, retrospective analysis, BioMed Res. Int. (2016), 5697571, 2016.
[67] A.D. Rathnayake, J. Zheng, Y. Kim, K.D. Perera, S. Mackin, D.K. Meyerholz, M. M. Kashipathy, K.P. Battaile, S. Lovell, S. Perlman, W.C. Groutas, K.O. Chang, 3C-like protease inhibitors block coronavirus replication in vitro and improve survival in MERS-CoV-infected mice, Sci. Transl. Med. 12 (2020), eabc5332.
[68] K.D. Perera, A.C. Galasiti Kankanamalage, A.D. Rathnayake, A. Honeyfield, W. Groutas, K.O. Chang, Y. Kim, Protease inhibitors broadly effective against feline, ferret and mink coronaviruses, Antivir. Res. 160 (2018) 79–86.
[69] Y. Xiong, G.H. Zhu, Y.N. Zhang, Q. Hu, H.N. Wang, H.N. Yu, X.Y. Qin, X.Q. Guan, Y.W. Xiang, H. Tang, G.B. Ge, Flavonoids in Ampelopsis grossedentata as covalent inhibitors of SARS-CoV-2 3CLpro: inhibition potentials, covalent binding sites and inhibitory mechanisms, Int. J. Biol. Macromol. 187 (2021) 976–987.
[70] N. Verma, J.A. Henderson, J. Shen, Proton-coupled conformational activation of SARS coronavirus main proteases and opportunity for designing small-molecule broad-spectrum targeted covalent inhibitors, J. Am. Chem. Soc. 142 (2020) 21883–21890.
[71] S. Vankadara, M.D. Dawson, J.Y. Fong, Q.Y. Oh, Q.A. Ang, B. Liu, H.Y. Chang, J. Koh, X. Koh, Q.W. Tan, J. Joy, C.S.B. Chia, A warhead substitution study on the coronavirus main protease inhibitor Nirmatrelvir, ACS Med. Chem. Lett. 13 (2022) 1345–1350.
[72] S. Huff, I.R. Kummetha, S.K. Tiwari, M.B. Huante, A.E. Clark, S. Wang, W. Bray, D. Smith, A.F. Carlin, M. Endsley, T.M. Rana, Discovery and mechanism of SARS-CoV-2 main protease inhibitors, J. Med. Chem. 65 (2022) 2866–2879.
[73] Z. Qiao, N. Wei, L. Jin, H. Zhang, J. Luo, Y. Zhang, K. Wang, The Mpro structure-based modifications of ebselen derivatives for improved antiviral activity against SARS-CoV-2 virus, Bioorg. Chem. 117 (2021), 105455.
[74] C.S. Dampalla, J. Zheng, K.D. Perera, L.R. Wong, D.K. Meyerholz, H.N. Nguyen, M.M. Kashipathy, K.P. Battaile, S. Lovell, Y. Kim, S. Perlman, W.C. Groutas, K. O. Chang, Postinfection treatment with a protease inhibitor increases survival of mice with a fatal SARS-CoV-2 infection, Proc. Natl. Acad. Sci. U.S.A. 118 (2021).
[75] W. Vuong, M.B. Khan, C. Fischer, E. Arutyunova, T. Lamer, J. Shields, H. A. Saffran, R.T. McKay, M.J. van Belkum, M.A. Joyce, H.S. Young, D.L. Tyrrell, J. C. Vederas, M.J. Lemieux, Feline coronavirus drug inhibits the main protease of SARS-CoV-2 and blocks virus replication, Nat. Commun. 11 (2020) 4282.
[76] M.D. Sacco, C. Ma, P. Lagarias, A. Gao, J.A. Townsend, X. Meng, P. Dube, X. Zhang, Y. Hu, N. Kitamura, B. Hurst, B. Tarbet, M.T. Marty, A. Kolocouris, Y. Xiang, Y. Chen, J. Wang, Structure and inhibition of the SARS-CoV-2 main protease reveal strategy for developing dual inhibitors against Mpro and cathepsin L, Sci. Adv. 6 (2020), eabe0751.
[77] W. Vuong, C. Fischer, M.B. Khan, M.J. van Belkum, T. Lamer, K.D. Willoughby, J. Lu, E. Arutyunova, M.A. Joyce, H.A. Saffran, J.A. Shields, H.S. Young, J. A. Nieman, D.L. Tyrrell, M.J. Lemieux, J.C. Vederas, Improved SARS-CoV-2 M (pro) inhibitors based on feline antiviral drug GC376: structural enhancements, increased solubility, and micellar studies, Eur. J. Med. Chem. 222 (2021), 113584.
[78] L. Zhang, D. Lin, X. Sun, U. Curth, C. Drosten, L. Sauerhering, S. Becker, K. Rox, R. Hilgenfeld, Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors, Science 368 (2020) 409–412.
[79] Y. Hirose, N. Shindo, M. Mori, S. Onitsuka, H. Isogai, R. Hamada, T. Hiramoto, J. Ochi, D. Takahashi, T. Ueda, J.M.M. Caaveiro, Y. Yoshida, S. Ohdo, N. Matsunaga, S. Toba, M. Sasaki, Y. Orba, H. Sawa, A. Sato, E. Kawanishi, A. Ojida, Discovery of chlorofluoroacetamide-based covalent inhibitors for severe acute respiratory syndrome coronavirus 2 3CL protease, J. Med. Chem. 65 (2022) 13852–13865.
[80] W. Dai, B. Zhang, X.M. Jiang, H. Su, J. Li, Y. Zhao, X. Xie, Z. Jin, J. Peng, F. Liu, C. Li, Y. Li, F. Bai, H. Wang, X. Cheng, X. Cen, S. Hu, X. Yang, J. Wang, X. Liu, G. Xiao, H. Jiang, Z. Rao, L.K. Zhang, Y. Xu, H. Yang, H. Liu, Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease, Science 368 (2020) 1331–1335.
[81] W. Dai, D. Jochmans, H. Xie, H. Yang, J. Li, H. Su, D. Chang, J. Wang, J. Peng, L. Zhu, Y. Nian, R. Hilgenfeld, H. Jiang, K. Chen, L. Zhang, Y. Xu, J. Neyts, H. Liu, Design, synthesis, and biological evaluation of peptidomimetic aldehydes as broad-spectrum inhibitors against enterovirus and SARS-CoV-2, J. Med. Chem. 65 (2022) 2794–2808.
[82] Y. Ma, C. Shang, P. Yang, L. Li, Y. Zhai, Z. Yin, B. Wang, L. Shang, 4-Iminooxazolidin-2-one as a bioisostere of the cyanohydrin moiety: inhibitors of enterovirus 71 3C protease, J. Med. Chem. 61 (2018) 10333–10339.
[83] Y. Zhai, X. Zhao, Z. Cui, M. Wang, Y. Wang, L. Li, Q. Sun, X. Yang, D. Zeng, Y. Liu, Y. Sun, Z. Lou, L. Shang, Z. Yin, Cyanohydrin as an anchoring group for potent and selective inhibitors of enterovirus 71 3C protease, J. Med. Chem. 58 (2015) 9414–9420.
[84] Y. Ma, L. Li, S. He, C. Shang, Y. Sun, N. Liu, T.D. Meek, Y. Wang, L. Shang, Application of dually activated Michael acceptor to the rational design of reversible covalent inhibitor for enterovirus 71 3C protease, J. Med. Chem. 62 (2019) 6146–6162.
[85] Y. Zhai, Y. Ma, F. Ma, Q. Nie, X. Ren, Y. Wang, L. Shang, Z. Yin, Structure–activity relationship study of peptidomimetic aldehydes as enterovirus 71 3C protease inhibitors, Eur. J. Med. Chem. 124 (2016) 559–573.
[86] H. Wang, R. Pei, X. Li, W. Deng, S. Xing, Y. Zhang, C. Zhang, S. He, H. Sun, S. Xiao, J. Xiong, Y. Zhang, X. Chen, Y. Wang, Y. Guo, B. Zhang, L. Shang, The structure-based design of peptidomimetic inhibitors against SARS-CoV-2 3C like protease as Potent anti-viral drug candidate, Eur. J. Med. Chem. 238 (2022), 114458.
[87] T. Pillaiyar, P. Flury, N. Kruger, H. Su, L. Schakel, E. Barbosa Da Silva, O. Eppler, T. Kronenberger, T. Nie, S. Luedtke, C. Rocha, K. Sylvester, M.R.I. Petry, J. H. McKerrow, A. Poso, S. Pohlmann, M. Gutschow, A.J. O'Donoghue, Y. Xu, C. E. Muller, S.A. Laufer, Small-molecule thioesters as SARS-CoV-2 main protease inhibitors: enzyme inhibition, structure-activity relationships, antiviral activity, and X-ray structure determination, J. Med. Chem. 65 (2022) 9376–9395.
[88] S. Gao, L. Song, T. Claff, M. Woodson, K. Sylvester, L. Jing, R.H. Weisse, Y. Cheng, N. Strater, L. Schakel, M. Gutschow, B. Ye, M. Yang, T. Zhang, D. Kang, K. Toth, J. Tavis, A.E. Tollefson, C.E. Muller, P. Zhan, X. Liu, Discovery and crystallographic studies of nonpeptidic piperazine derivatives as covalent SARS-CoV-2 main protease inhibitors, J. Med. Chem. 65 (2022) 16902–16917.
[89] J. Qiao, Y.S. Li, R. Zeng, F.L. Liu, R.H. Luo, C. Huang, Y.F. Wang, J. Zhang, B. Quan, C. Shen, X. Mao, X. Liu, W. Sun, W. Yang, X. Ni, K. Wang, L. Xu, Z. L. Duan, Q.C. Zou, H.L. Zhang, W. Qu, Y.H.P. Long, M.H. Li, R.C. Yang, X. Liu, J. You, Y. Zhou, R. Yao, W.P. Li, J.M. Liu, P. Chen, Y. Liu, G.F. Lin, X. Yang, J. Zou, L. Li, Y. Hu, G.W. Lu, W.M. Li, Y.Q. Wei, Y.T. Zheng, J. Lei, S. Yang, SARS-CoV-2 Mpro inhibitors with antiviral activity in a transgenic mouse model, Science 371 (2021) 1374–1378.
[90] X. Dou, Q. Sun, G. Xu, Y. Liu, C. Zhang, B. Wang, Y. Lu, Z. Guo, L. Su, T. Huo, X. Zhao, C. Wang, Z. Yu, S. Song, L. Zhang, Z. Liu, L. Lai, N. Jiao, Discovery of 2-(furan-2-ylmethylene)hydrazine-1-carbothioamide derivatives as novel inhibitors of SARS-CoV-2 main protease, Eur. J. Med. Chem. 238 (2022), 114508.
[91] A.K. Ghosh, J. Raghavaiah, D. Shahabi, M. Yadav, B.J. Anson, E.K. Lendy, S. I. Hattori, N. Higashi-Kuwata, H. Mitsuya, A.D. Mesecar, Indole chloropyridinyl ester-derived SARS-CoV-2 3CLpro inhibitors: enzyme inhibition, antiviral efficacy, structure-activity relationship, and X-ray structural studies, J. Med. Chem. 64 (2021) 14702–14714.
[92] D. Shcherbakov, D. Baev, M. Kalinin, A. Dalinger, V. Chirkova, S. Belenkaya, A. Khvostov, D. Krut'ko, A. Medved'ko, E. Volosnikova, E. Sharlaeva, D. Shanshin, T. Tolstikova, O. Yarovaya, R. Maksyutov, N. Salakhutdinov, S. Vatsadze, Design and evaluation of bispidine-based SARS-CoV-2 main protease inhibitors, ACS Med. Chem. Lett. 13 (2022) 140–147.
[93] C. Ma, Z. Xia, M.D. Sacco, Y. Hu, J.A. Townsend, X. Meng, J. Choza, H. Tan, J. Jang, M.V. Gongora, X. Zhang, F. Zhang, Y. Xiang, M.T. Marty, Y. Chen, J. Wang, Discovery of di- and trihaloacetamides as covalent SARS-CoV-2 main protease inhibitors with high target specificity, J. Am. Chem. Soc. 143 (2021) 20697–20709.
[94] T.R. Malla, L. Brewitz, D.G. Muntean, H. Aslam, C.D. Owen, E. Salah, A. Tumber, P. Lukacik, C. Strain-Damerell, H. Mikolajek, M.A. Walsh, C.J. Schofield, Penicillin derivatives inhibit the SARS-CoV-2 main protease by reaction with its nucleophilic cysteine, J. Med. Chem. 65 (2022) 7682–7696.
[95] J.K. Stille, J. Tjutrins, G. Wang, F.A. Venegas, C. Hennecker, A.M. Rueda, I. Sharon, N. Blaine, C.E. Miron, S. Pinus, A. Labarre, J. Plescia, M. Burai Patrascu, X. Zhang, A.S. Wahba, D. Vlaho, M.J. Huot, T.M. Schmeing, A.K. Mittermaier, N. Moitessier, Design, synthesis and in vitro evaluation of novel SARS-CoV-2 3CL (pro) covalent inhibitors, Eur. J. Med. Chem. 229 (2022), 114046.
[96] J.W. Zhang, Y. Xiong, F. Wang, F.M. Zhang, X. Yang, G.Q. Lin, P. Tian, G. Ge, D. Gao, Discovery of 9,10-dihydrophenanthrene derivatives as SARS-CoV-2 3CL (pro) inhibitors for treating COVID-19, Eur. J. Med. Chem. 228 (2022), 114030.
[97] D.R. Owen, C.M.N. Allerton, A.S. Anderson, L. Aschenbrenner, M. Avery, S. Berritt, B. Boras, R.D. Cardin, A. Carlo, K.J. Coffman, A. Dantonio, L. Di, H. Eng, R. Ferre, K.S. Gajiwala, S.A. Gibson, S.E. Greasley, B.L. Hurst, E.P. Kadar, A.S. Kalgutkar, J.C. Lee, J. Lee, W. Liu, S.W. Mason, S. Noell, J.J. Novak, R. S. Obach, K. Ogilvie, N.C. Patel, M. Pettersson, D.K. Rai, M.R. Reese, M. F. Sammons, J.G. Sathish, R.S.P. Singh, C.M. Steppan, A.E. Stewart, J.B. Tuttle, L. Updyke, P.R. Verhoest, L. Wei, Q. Yang, Y. Zhu, An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19, Science 374 (2021) 1586–1593.
[98] M. Erak, K. Bellmann-Sickert, S. Els-Heindl, A.G. Beck-Sickinger, Peptide chemistry toolbox – transforming natural peptides into peptide therapeutics, Bioorg. Med. Chem. 26 (2018) 2759–2765.
[99] L. Zhou, Y. Liu, W. Zhang, P. Wei, C. Huang, J. Pei, Y. Yuan, L. Lai, Isatin compounds as noncovalent SARS coronavirus 3C-like protease inhibitors, J. Med. Chem. 49 (2006) 3440–3443.
[100] P. Liu, H. Liu, Q. Sun, H. Liang, C. Li, X. Deng, Y. Liu, L. Lai, Potent inhibitors of SARS-CoV-2 3C-like protease derived from N-substituted isatin compounds, Eur. J. Med. Chem. 206 (2020), 112702.
[101] J. Jacobs, V. Grum-Tokars, Y. Zhou, M. Turlington, S.A. Saldanha, P. Chase, A. Eggler, E.S. Dawson, Y.M. Baez-Santos, S. Tomar, A.M. Mielech, S.C. Baker, C. W. Lindsley, P. Hodder, A. Mesecar, S.R. Stauffer, 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. Med. Chem. 56 (2013) 534–546.
[102] N. Kitamura, M.D. Sacco, C. Ma, Y. Hu, J.A. Townsend, X. Meng, F. Zhang, X. Zhang, M. Ba, T. Szeto, A. Kukuljac, M.T. Marty, D. Schultz, S. Cherry, Y. Xiang, Y. Chen, J. Wang, Expedited approach toward the rational design of noncovalent SARS-CoV-2 main protease inhibitors, J. Med. Chem. 65 (2022) 2848–2865.
[103] S. Bhatt, P.W. Gething, O.J. Brady, J.P. Messina, A.W. Farlow, C.L. Moyes, J. M. Drake, J.S. Brownstein, A.G. Hoen, O. Sankoh, M.F. Myers, D.B. George, T. Jaenisch, G.R.W. Wint, C.P. Simmons, T.W. Scott, J.J. Farrar, S.I. Hay, The global distribution and burden of dengue, Nature 496 (2013) 504–507.
[104] C. Nitsche, S. Holloway, T. Schirmeister, C.D. Klein, Biochemistry and medicinal chemistry of the dengue virus protease, Chem. Rev. 114 (2014) 11348–11381.
[105] N. Kuhl, J. Lang, M.M. Leuthold, C.D. Klein, Discovery of potent benzoxaborole inhibitors against SARS-CoV-2 main and dengue virus proteases, Eur. J. Med. Chem. 240 (2022), 114585.

[106] D.W. Kneller, H. Li, S. Galanie, G. Phillips, A. Labbe, K.L. Weiss, Q. Zhang, M. A. Arnould, A. Clyde, H. Ma, A. Ramanathan, C.B. Jonsson, M.S. Head, L. Coates, J.M. Louis, P.V. Bonnesen, A. Kovalevsky, Structural, electronic, and electrostatic determinants for inhibitor binding to subsites S1 and S2 in SARS-CoV-2 main protease, J. Med. Chem. 64 (2021) 17366–17383.

[107] S. Gao, K. Sylvester, L. Song, T. Claff, L. Jing, M. Woodson, R.H. Weisse, Y. Cheng, L. Schakel, M. Petry, M. Gutschow, A.C. Schiedel, N. Strater, D. Kang, S. Xu, K. Toth, J. Tavis, A.E. Tollefson, C.E. Muller, X. Liu, P. Zhan, Discovery and crystallographic studies of trisubstituted piperazine derivatives as non-covalent SARS-CoV-2 main protease inhibitors with high target specificity and low toxicity, J. Med. Chem. 65 (2022) 13343–13364.

[108] J. Keehner, L.E. Horton, N.J. Binkin, L.C. Laurent, D. Pride, C.A. Longhurst, S. R. Abeles, F.J. Torriani, Resurgence of SARS-CoV-2 infection in a highly vaccinated health system workforce, N. Engl. J. Med. 385 (2021) 1330–1332.

[109] I. Churcher, Protac-induced protein degradation in drug discovery: breaking the rules or just making new ones? J. Med. Chem. 61 (2018) 444–452.

[110] D.P. Bondeson, A. Mares, I.E.D. Smith, E. Ko, S. Campos, A.H. Miah, K. E. Mulholland, N. Routly, D.L. Buckley, J.L. Gustafson, N. Zinn, P. Grandi, S. Shimamura, G. Bergamini, M. Faelth-Savitski, M. Bantscheff, C. Cox, D. A. Gordon, R.R. Willard, J.J. Flanagan, L.N. Casillas, B.J. Votta, W. den Besten, K. Famm, L. Kruidenier, P.S. Carter, J.D. Harling, I. Churcher, C.M. Crews, Catalytic in vivo protein knockdown by small-molecule PROTACs, Nat. Chem. Biol. 11 (2015) 611–617.