



## Review

# An overview of potential inhibitors targeting non-structural proteins 3 (PL<sup>PRO</sup> and Mac1) and 5 (3CL<sup>PRO</sup>/M<sup>PRO</sup>) of SARS-CoV-2

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## ABSTRACT

There is an urgent need to develop effective treatments for coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The rapid spread of SARS-CoV-2 has resulted in a global pandemic that has not only affected the daily lives of individuals but also had a significant impact on the global economy and public health. Although extensive research has been conducted to identify inhibitors targeting SARS-CoV-2, there are still no effective treatment strategies to combat COVID-19. SARS-CoV-2 comprises two important proteolytic enzymes, namely, the papain-like proteinase, located within non-structural protein 3 (nsp3), and nsp5, both of which cleave large replicase polypeptides into multiple fragments that are required for viral replication. Moreover, a domain within nsp3, known as the macrodomain (Mac1), also plays an important role in viral replication. Inhibition of their functions should be able to significantly interfere with the replication cycle of the virus, and therefore these key proteins may serve as potential therapeutic targets. The functions of the above viral targets and their corresponding inhibitors have been summarized in the current review. This review provides comprehensive updates of nsp3 and nsp5 inhibitor development and would help advance the discovery of novel anti-viral therapeutics against SARS-CoV-2.

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## 1. Introduction

Since the end of 2019, a novel respiratory disease that manifests as pneumonia has spread across many countries, and has garnered significant attention worldwide. Deep whole-genome sequencing of the patient samples revealed that the pathogen responsible for this respiratory disease is a novel coronavirus (nCoV), tentatively named 2019-nCoV by the World Health Organization (WHO). Similar to the severe acute respiratory syndrome coronavirus (SARS-CoV), 2019-nCoV belongs to the zoonotic  $\beta$ -coronaviruses family, and it shares a high degree of homology with SARS-CoV. On February 11, 2020, the International Committee on Taxonomy of Viruses (ICTV) renamed this coronavirus as SARS-CoV-2 [1]. The WHO termed the disease caused by SARS-CoV-2 as coronavirus disease 2019 (COVID-19) [2]. SARS-CoV-2 can achieve person-person transmission through a variety of ways, including prolonged close contact or via the inhalation of virus-containing aerosols [3,4]. People who are infected with SARS-CoV-2 typically present a fever, cough and chest discomfort, and severe patients develop acute respiratory distress syndrome (ARDS), leading to death in some cases [5,6]. SARS-CoV-2 has spread worldwide due to its high transmission rate, and the number of deaths caused by this disease continues to increase. The global COVID-19 pandemic not only brings panic or worry to people, but also poses a great threat to the global economy, public health and the daily lives of people all over the world [7,8]. Considering the current grave situation, the development of reliable inhibitors to combat SARS-CoV-2 has become a critical task. Currently, extensive efforts have been made to identify reliable inhibitors against SARS-CoV-2, and some inhibitors have even entered the stage of clinical trials. The results of some clinical trials have been briefly summarized in Table S1. Through the evaluation of possible inhibitors, it was found that some inhibitors failed to achieve the expected efficacy or showed obvious negative effects in clinical trials, which brings great difficulties to the treatment of COVID-19.

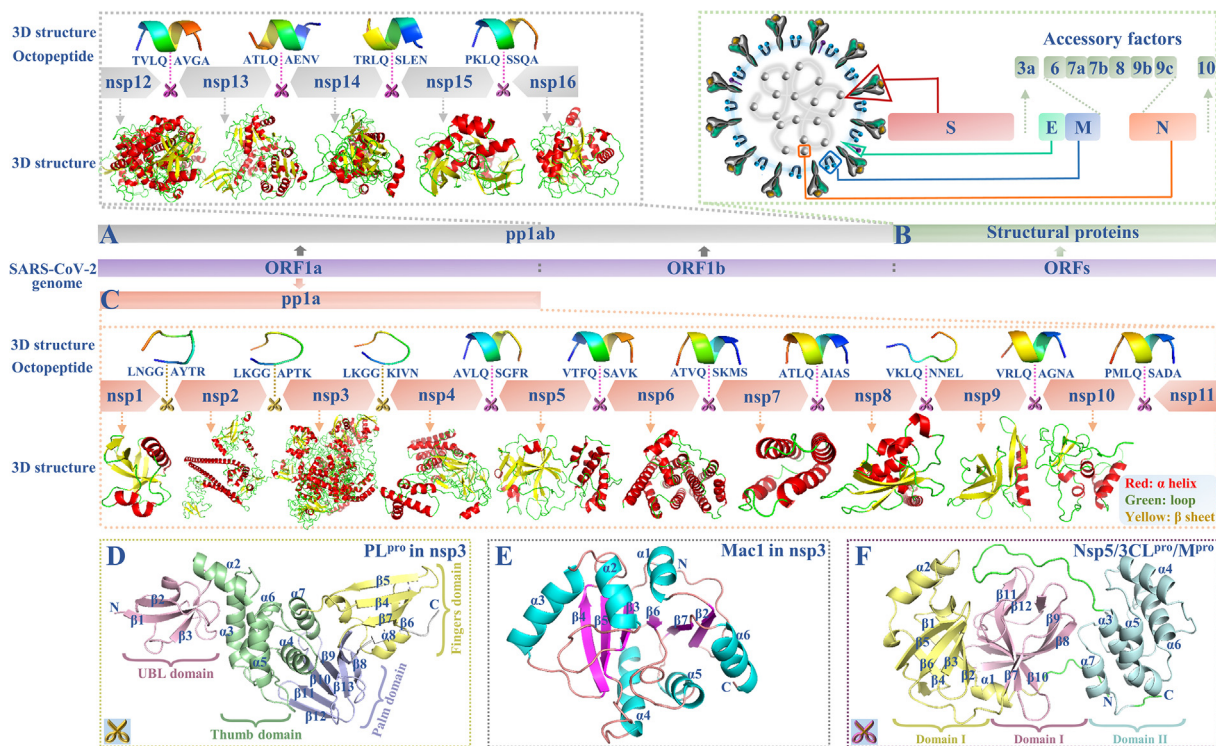
To identify effective viral inhibitors, it is necessary to have a comprehensive understanding of the potential therapeutic targets for SARS-CoV-2, and the study of the structural properties of SARS-CoV-2 can provide insights for the discovery of therapeutic targets and inhibitors. Recent developments in next-generation sequencing (NGS) technologies and related bioinformatics analysis methods can greatly assist in SARS-CoV-2 research. Within a month of the COVID-19 outbreak, the genome of SARS-CoV-2 was made available on public databases, such as the National Center for Biotechnology Information (NCBI) database [9]. SARS-CoV-2 has the largest genome among the known RNA viruses. The SARS-CoV-2 genome consists of 10 open reading frames (ORFs). Among them, ORF1a and ORF1b occupy approximately two-thirds of the whole SARS-CoV-2 genome, and they are used as templates to encode two large replicase polypeptides, pp1a and pp1ab, after the virus invades the host cell [10]. The pp1a and pp1ab can only initiate replication of their own genetic materials after they are cleaved into various fragments that perform different functions [11]; they can be separately cleaved into 11 non-structural proteins (nsp; nsp1-nsp11) and 16 nsps (nsp1-nsp16) by the virus-encoded 3-chymotrypsin-like proteinase (nsp5, also called 3CL<sup>pro</sup> or M<sup>pro</sup>) and the papain-like proteinase (PL<sup>pro</sup>) that is present in nsp3 (Fig. 1A and C). Detailed information on the cleavage sites,

cleavable octopeptides and the nsps of the replicase polypeptides in SARS-CoV-2 are shown in Fig. 1A and C, and they can be accurately predicted and included in our newly updated ZCURVE\_CoV database (<http://tubic.tju.edu.cn/CoVdb>) [12]. According to the predicted results in Table S2, it was found that three nsps (nsp1-nsp3) were produced via the cleavage by PL<sup>pro</sup>, while the others were produced by 3CL<sup>pro</sup>. Three-dimensional (3D) models of these nsps are shown in Fig. 1A and Fig. 1C and can be downloaded directly from <https://zhanglab.ccmb.med.umich.edu/C-I-TASSER/2019-nCoV> [13]. Some of the models are also available in the Protein Data Bank (PDB) database (<http://www.rcsb.org>) [14]. The 3D models of the cleavable octopeptides were also predicted using a suitable PEP-FOLD3 software tool with a lower limit of 5 amino acids (<http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3>) [15], as shown in Fig. 1. As described by Chou et al., Gan et al. and Du et al., the cleavable octapeptide of SARS-CoV should be able to bind to the active site of 3CL<sup>pro</sup>, and therefore, modified octapeptides could be used as ideal SARS-CoV inhibitors [16–18]. Similarly, modified octapeptides of SARS-CoV-2 are also expected to become candidate inhibitors for SARS-CoV-2, but there is a lack of relevant research in this respect. Apart from ORF1a and ORF1b, the remaining ORFs are distributed in the last third of the SARS-CoV-2 genome, and they encode at least four structural proteins: spike (S), envelope (E), membrane (M) and nucleocapsid (N), as well as some accessory proteins, including 3a, 6, 7a, 7b, 8, 9b, 9c, and 10 (Fig. 1B) [19].

S protein, as one of the four important structural proteins, plays a vital role in virus invasion and membrane fusion due to its two major regions (S1 and S2) [20]. S protein is regarded as an ideal target for vaccines and antibodies [20–22], and related studies have been well summarized by different groups [23–26]. Among all non-structural proteins, two viral proteases (nsp5 and nsp3) appear to be particularly important. Nsp5 and PL<sup>pro</sup> in nsp3 are responsible for cleaving the large replicase polypeptides into fragments that can perform multiple functions, thereby assisting the replication of the virus. Meanwhile, PL<sup>pro</sup> and nsp5 contain highly conserved and well-defined druggable binding sites, whereas no binding sites are found in some nsps, such as nsp2, nsp7 and nsp8 [27]. In addition to PL<sup>pro</sup>, nsp3 also contains another important domain (macrodomain) related to viral replication and to the host's innate immunity. Thus, nsp3 and nsp5 may potentially be good therapeutic targets for COVID-19. To promote a better understanding of the functions of nsp3 and nsp5 in SARS-CoV-2 infection and the current state of inhibitor discovery, we conducted a comprehensive review of the functions of these potential therapeutic targets and the development of corresponding inhibitors. This review is meant to provide a convenient resource of different viral targets and would assist with the development of specific inhibitors.

## 2. Structure, function and inhibitors of nsp3

SARS-CoV-2 nsp3 is a multi-domain enzyme with 1,945 amino acids, and it is the largest protein among all nsps. Nsp3 is remarkable because of the existence of two functional domains. One of the functional domains is a catalytically active PL<sup>pro</sup> domain. As shown in Fig. 1D, the PL<sup>pro</sup> can be divided into four distinct domains: the fingers ( $\beta$ 4– $\beta$ 7), thumb ( $\alpha$ 2– $\alpha$ 7), palm ( $\beta$ 8– $\beta$ 13) and ubiquitin-like



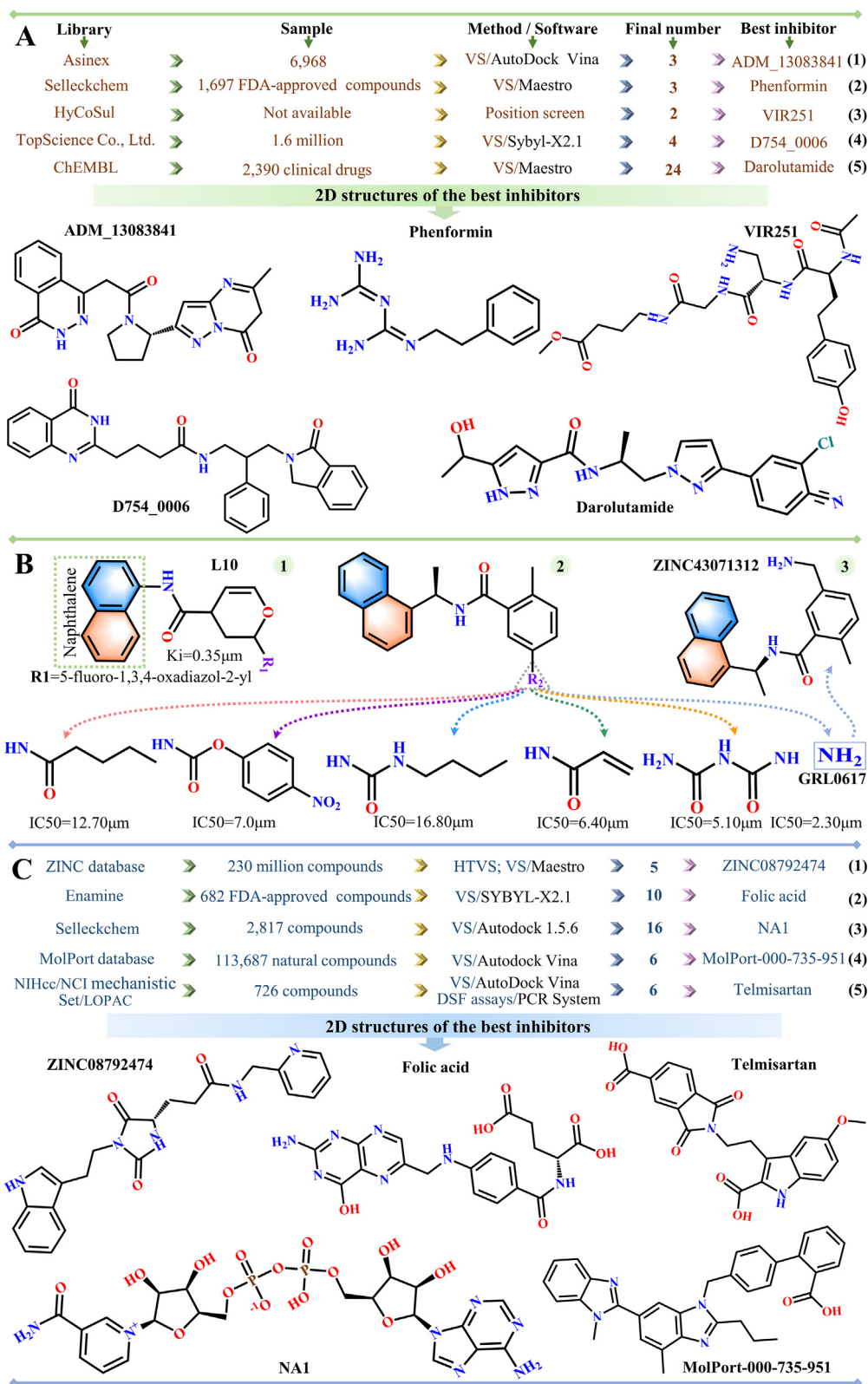
**Fig. 1.** Structure of SARS-CoV-2: (A) replicase polypeptides pp1ab and corresponding cleavable octopeptide, (B) structural proteins and (C) replicase polypeptides pp1a and corresponding cleavable octopeptide. Structures of non-structural proteins: (D) PL<sup>pro</sup> in nsp3, (E) Mac1 in nsp3 and (F) nsp5.

(UBL,  $\beta$ 1- $\beta$ 3), and the first three domains constitute the catalytic core of the protein [28]. The druggable substrate-binding pocket is mainly determined by nine residues (R166, L185, L199, V202, E203, M206-M208 and K232) [29]. PL<sup>pro</sup> participates in the efficient cleavage of the N-terminal replicase polyprotein to produce functional proteins; this process of functional protein production plays an essential role in maintaining the basic cellular processes of SARS-CoV-2, including viral replication [30]. The other important functional domain of nsp3 is the ADP-ribose phosphatase (ADRP) domain, also known as macrodomain (Mac1) or Macro X domain. Mac1 mainly consists of seven  $\beta$ -sheets ( $\beta$ 1- $\beta$ 7) and six  $\alpha$  helices ( $\alpha$ 1- $\alpha$ 6) (Fig. 1E). Among them, four  $\beta$ -sheets ( $\beta$ 3 and  $\beta$ 5- $\beta$ 7),  $\alpha$ 1, and two loops ( $\beta$ 3- $\alpha$ 2 and  $\beta$ 6- $\alpha$ 5) are involved in the formation of binding pocket [31]. Mac1 is a highly conserved and unique sequence in the genomes of many viruses and organisms, including human [32,33], and inhibitors targeting this enzyme may have broad-spectrum antiviral activity. Notably, both PL<sup>pro</sup> and Mac1 can help the virus escape the antiviral immune response of the host [34]. Consequently, inhibiting the activity of PL<sup>pro</sup> and Mac1 will interfere with the replication cycle of the virus, maintain the innate immune pathways of the host and reduce the infection rate. In view of the importance of these two functional domains of nsp3, we summarized the inhibitor-related research that has been performed regarding these domains; moreover, we selected the best inhibitors from each study, as shown in Fig. 2. To gain insight into the binding modes of inhibitors, the binding mechanisms of these selected inhibitors to proteins are summarized, as shown in Table S3.

### 2.1. Inhibitors of SARS-CoV-2 PL<sup>pro</sup>

In April 2020, Osipiuk et al. first reported the crystal structure of SARS-CoV-2 PL<sup>pro</sup>, which is available in PDB (PDB ID: 6W9C). Based on this structure, studies have been performed to explore potential inhibitors of PL<sup>pro</sup> enzymatic activity. Alamri and colleagues conducted virtual screening (VS) of 6,968 protease inhibitors from Asi-

nex library (<https://www.asinex.com/tease>) using AutoDock Vina software, and three compounds (ADM\_13083841, LMG\_15521745 and SYN\_15517940) were identified as potential inhibitors of PL<sup>pro</sup> (Fig. 2A1) [35]. Kandeel et al. used the Maestro software to virtually screen 1,697 FDA-approved inhibitors from Selleckchem Inc. database (<http://www.selleckchem.com>) and obtained 26 compounds with lower negative docking scores ( $< -7$  kcal/mol) [36]. After evaluating the binding free energies between compounds and PL<sup>pro</sup>, three compounds (phenformin, quercetin and ritonavir) were considered as potential inhibitors (Fig. 2A2). Rut et al. performed a comprehensive location screening of the LKGG motif based on the Hybrid Combinatorial Substrate Library (HyCoSul), and the results indicated that the tetrapeptides VIR250 and VIR251 are expected to behave as potent peptide inhibitors (Fig. 2A3) [37]. Using the structure of apo PL<sup>pro</sup> as a reference, they predicted the structures of VIR250 and VIR251 bound to PL<sup>pro</sup> (PDB IDs: 6WUU and 6WX4). Based on the interaction mode between VIR251 and SARS-CoV-2 PL<sup>pro</sup>, Pang et al. conducted virtual screening of 1.6 million compounds from TopScience Co., Ltd. (Shanghai, China) using the Sybyl-X2.1 software, verified their results through molecular dynamics (MD) simulations, and found that D754\_0006 was the best among the four shortlisted inhibitors (F403\_0159, F112\_0109, G805\_0497 and D754\_0006) (Fig. 2A4) [38]. Based on the same molecular structure, a virtual screening study was carried out by Delre et al. to search SARS-CoV-2 PL<sup>pro</sup> inhibitors from 2,390 inhibitors used in clinical trials, as reported in the ChEMBL bank (<https://www.ebi.ac.uk/chembl>). Two covalent inhibitors (curcumin and afatinib) and several different types of non-covalent inhibitors (protein kinase inhibitors [dasatinib, pexidartinib and copanlisib], protease inhibitors [amprenavir, indinavir, anagliptin, boceprevir and semagacestat], adrenergic receptor modulators [vilanterol, arformoterol and atenolol], ACE inhibitors and direct oral anticoagulants [cilazaprilat, edoxaban and rivaroxaban] and inhibitors belonging to other groups [acoti-



**Fig. 2.** Flowchart illustrating the screening of inhibitors against (A-B) PL<sup>PRO</sup> and (C) Mac1.

tidine, vilazodone and methotrexate) were obtained from this search [39]. Notably, covalent inhibitor binds irreversibly to the receptor through covalent bonds, while the binding of non-covalent inhibitor to the receptor is a reversible process. In general, the binding affinity of a covalent inhibitor to the target is stronger than that of the non-covalent inhibitor to the target [40,41].

In addition, naphthalene-based inhibitors of SARS-CoV PL<sup>PRO</sup> are highly effective in reducing the activity of SARS-CoV-2 PL<sup>PRO</sup> enzyme [42]. Based on the naphthalene scaffold structure, Bhati and Osipiuk et al. analyzed several SARS-CoV-2 PL<sup>PRO</sup>-related drugs through computational models and experiments, respectively. Among the 20 naphthalene-based compounds designed by Bhati, L10 (C<sub>18</sub>H<sub>14</sub>FN<sub>3</sub>-

O<sub>3</sub>) was considered to be the most potent anti-SARS-CoV-2 PL<sup>PRO</sup> inhibitor (Fig. 2B1) [43], with a docking score of  $-8.81$  kcal/mol. Osipiuk et al. tested the biochemical activity of their newly designed compounds, and six of them showed significant anti-PL<sup>PRO</sup> activity *in vitro*, particularly compound 1 (GRL0617) (PDB ID: 7CMD) [44]. The structures and corresponding half maximal inhibitory concentration (IC<sub>50</sub>) values for these six inhibitors are shown in Fig. 2B2. In view of the strong inhibitory activity of GRL0617 against SARS-CoV-2 PL<sup>PRO</sup>, a GRL0617-based virtual screening of chemical structures in the Binding database (BindingDB: <http://www.bindingdb.org/bind/index.jsp>) was conducted, five compounds (ZINC43063883, ZINC387735, ZINC78808978, ZINC43071312 and ZINC993539) were selected as potential inhibitors of SARS-CoV-2 PL<sup>PRO</sup> [45], and ZINC43071312 showed the strongest binding ability to SARS-CoV-2 PL<sup>PRO</sup> (Fig. 2B3).

## 2.2. Inhibitors of SARS-CoV-2 Mac1

The Mac1 of SARS-CoV-2 nsp3 has the characteristic of binding to ADP-ribose [46]; therefore, small molecules that can bind tightly to the binding sites of Mac1 and ADP-ribose are expected to be potential SARS-CoV-2 treatments. Based on an in-depth understanding of the interaction mechanism between Mac1 and ADP-ribose, investigations have been conducted on the inhibitors of this binding. Selvaraj et al. selected five inhibitors (ZINC08765069, ZINC08792474, ZINC08879336, ZINC08879971 and ZINC00897592) from 230 million compounds in the ZINC database (<http://zinc.docking.org>) via high-throughput virtual screening (HTVS), extra precision (XP) docking and quantum polarized ligand docking (QPLD) (Fig. 2C1) [46]. Based on the virtual screening method, 10 alternative inhibitors of Mac1 (folic acid, telmisartan, methotrexate, bosentan, lapatinib, gefitinib, ketoconazole, carvedilol, glyburide and avanafil) were identified from a screening of 682 FDA-approved compounds in the Enamine database by SYBYL-X2.1 (Fig. 2C2) [47], 16 inhibitors (ribostamycin sulfate, lactobionic acid, neohesperidin, lactitol, salivianolic acid, adenosine-5-diphosphoribose, folic acid, naringin, melibiose, maltose, rutin, nucleotide analogue 1 [NA1], NA2, NA3, nadide and citicholine) were identified from 2,682 FDA-approved compounds and 135 nucleoside analogues in the Selleckchem database by Autodock 1.5.6 (Fig. 2C3) [48], and 6 inhibitors (MolPort IDs: 000-735-951, 002-517-673, 021-745-738, 028-854-978, 028-856-111 and 035-700-887) were identified from 113,687 natural compounds in the MolPort database by Glide procedures (Fig. 2C4) [49]. After an in-depth assessment of the characteristics of inhibitors using various analyses, including MD analysis and absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis, Folic acid, NA1 and MolPort-000-735-951 were determined to be the best among the inhibitors screened by the aforementioned research groups (Fig. 2C2–C4). Notably, the MD method applied by these researchers is a method commonly used for exploring the binding ability of ligands to receptors, which plays an important role in drug design [50–52]. Additionally, the Eppendorf Mastercycler ep Realplex Quantitative Realtime PCR System and AutoDock Vina software were separately used by Viridi et al. to conduct differential scanning fluorimetry (DSF) assays and virtual screening of 726 compounds, and steroids (estradiol valerate and flunisolide),  $\beta$ -lactams (cefaclor and cefatrizine) and benzimidazoles (rabeprazole and telmisartan) were identified as suitable compounds (Fig. 2C5) [53].

## 3. Structure, function and inhibitors of nsp5

Nsp5 is also described as the main protease (M<sup>PRO</sup>) or 3CL<sup>PRO</sup>. It is a dimer structure composed of two monomers (residues 1–306) and each monomer has three domains (domains I, II and III), corresponding to residues 8–101, 102–184 and 201–303, respectively

(Fig. 1F) [54]. As reported by Jin et al., residues located between domains I and II form the binding pocket of 3CL<sup>PRO</sup>. Although the monomer state of 3CL<sup>PRO</sup> is inactive, the homodimer state formed by the dimerization of two monomers is active. Indeed, monomers and dimers exist simultaneously in the solution, and their equilibrium is affected by many aspects, including the binding of inhibitors and protein concentration [55–57]. 3CL<sup>PRO</sup> is essential for replication and transcription of the virus due to its function in cleaving replicase polypeptides (pp1a and pp1ab). Therefore, inhibiting the activity of this enzyme can significantly affect the replication and transcription of SARS-CoV-2. In addition, there are no homologues of 3CL<sup>PRO</sup> in human [58], 3CL<sup>PRO</sup> inhibitors are likely to cause fewer side effects on human. Therefore, 3CL<sup>PRO</sup> is a potential drug target against COVID-19. A literature retrieval revealed that investigators are looking for inhibitors of 3CL<sup>PRO</sup> from three main classes: natural compounds, approved or commercially available drugs and others.

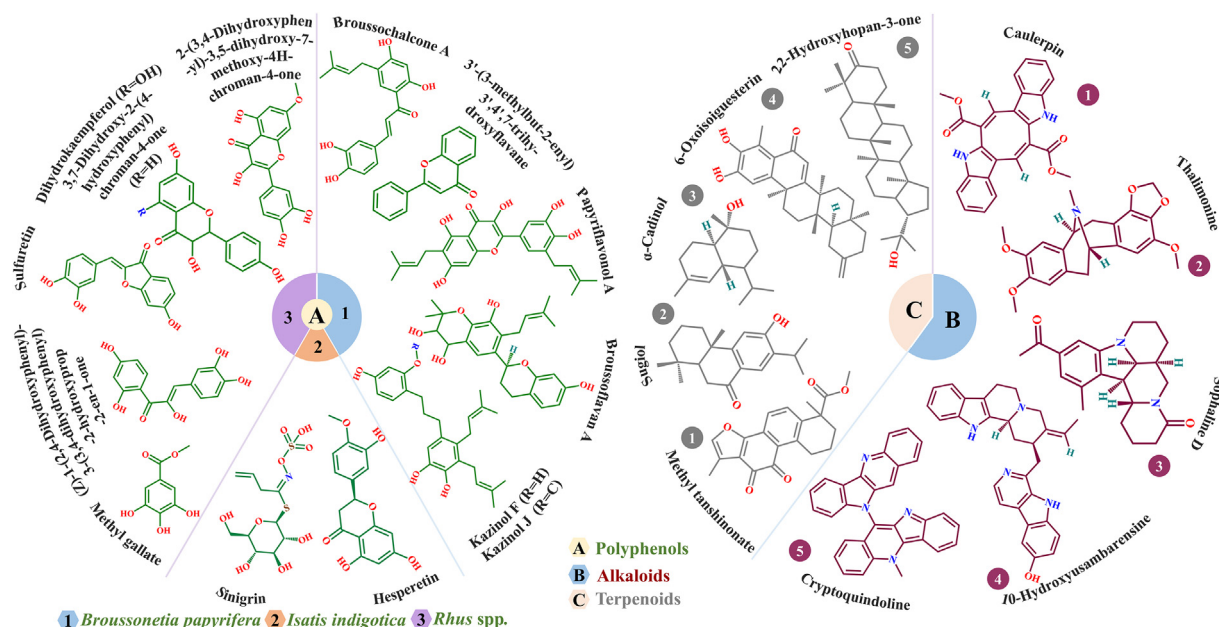
### 3.1. Inhibitors of SARS-CoV-2 3CL<sup>PRO</sup> screened from natural compounds

Natural compounds have the advantages of cost-effectiveness, high efficiency and low toxicity. Researchers are thus committed to mining inhibitors of COVID-19 from natural compounds. A literature retrieval revealed that the natural inhibitors currently under exploration are mainly derived from plants, marine organisms and microorganisms. The following is a summary of the natural inhibitors of SARS-CoV-2 3CL<sup>PRO</sup>, and the binding mechanisms of these natural inhibitors to 3CL<sup>PRO</sup> are listed in Table S4.

#### 3.1.1. Plant-based natural inhibitors

**3.1.1.1. Polyphenols.** Polyphenols are a class of chemically diverse natural metabolites, and their structures are characterized by multiple phenol groups. Polyphenols have received attention in the pharmaceutical field owing to their important properties including pathogen defense, anti-oxidation and cancer prevention [59]. Studies have shown that some plant-derived polyphenols can inactivate SARS-CoV-2 by inhibiting the activity of SARS-CoV-2 3CL<sup>PRO</sup>. Based on the structure of SARS-CoV-2 3CL<sup>PRO</sup>, Ghosh et al. evaluated the inhibitory ability of 18 polyphenolic compounds extracted from *Broussonetia papyrifera* (10 compounds) and *Isatis indigotica* root (8 compounds) using computational approaches. Notably, all the selected compounds have known anti-SARS-CoV activity. After evaluating the binding affinity, structural stability and physicochemical properties, six possible polyphenolic inhibitors of SARS-CoV-2 3CL<sup>PRO</sup> (broussonchalcone A, papyriflavonol A, 3'-(3-methyl but-2-enyl)-3',4',7-trihydroxyflavane, broussonflavan A, kazinol F and kazinol J) were screened from *Broussonetia papyrifera* and two inhibitors (sinigrin and hesperetin) were identified from *Isatis indigotica* (Fig. 3A1–A2) [60,61]. Another study found that six polyphenolic compounds extracted from *Rhus* spp. could also potentially assist in the treatment of COVID-19, and the structures of these compounds are shown in Fig. 3A3 [62].

**3.1.1.2. Alkaloids.** Alkaloids are a class of natural compounds found mainly in plants, particularly flowering plants. Alkaloids are considered to be one of the most pharmacologically active substances found in plants, including those that assist in the defense against pathogens [63]. Recently, computational analyses were performed on 10 public bioactive compounds and 20 alkaloid compounds, all of which have known antiviral activity [64,65]. After evaluating the compounds from various aspects including physicochemical properties, three alkaloid compounds (caulerpin, thalimonine and sophaline D) were identified as potential inhibitors of SARS-CoV-2 3CL<sup>PRO</sup> (Fig. 3B1–B3). Similarly, a computational approach was applied by Gyebi et al. to explore inhibitors of SARS-CoV-2 3CL<sup>PRO</sup> from 62 alkaloid compounds in African plants, which yielded two



**Fig. 3.** Structures of potential inhibitors targeting SARS-CoV-2 3CL<sup>pro</sup>: (A) polyphenolic inhibitors, (B) alkaloid inhibitors and (C) terpenoid inhibitors.

drug candidates, that is 10-hydroxyusambarensine and cryptoquinodoline (Fig. 3B4–B5) [66].

**3.1.1.3. Terpenoids and their derivatives.** Structurally, terpenoids contain one or more isoprene units, and their general structure is (C<sub>5</sub>H<sub>8</sub>)<sub>n</sub>. Terpenoids can be classified into different types according to the number of isoprene unit, such as monoterpenes, sesquiterpenes and diterpenes [67]. They are involved in metabolic pathways of all living organisms and have a variety of pharmacological applications [68]. Importantly, previous studies have shown that some subtypes of terpenoids have strong antiviral activity against coronaviruses, such as CoV-229E and SARS-CoV [69,70]. Thus, terpenoids and their derivatives may be helpful in the treatment of COVID-19.

In view of these findings, Diniz et al. reviewed 34 anti-coronavirus terpenoids from several studies, and identified three terpenoid compounds (methyl tanshinonate, sugiol and α-cadinol) that could bind tightly to SARS-CoV-2 3CL<sup>pro</sup> based on the binding free energies calculated using the molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) method (Fig. 3C1–C3) [71]. In addition, Gyebe et al. not only explored the antiviral properties of alkaloids, but also discovered two potential SARS-CoV-2 3CL<sup>pro</sup> inhibitors (6-oxoisoiguesterin and 22-hydroxyhopan-3-one) from a set of 100 terpenoids extracted from African plants (Fig. 3C4–C5) [66]. Therefore, the aforementioned studies also suggested that terpenoids and their derivatives can be potentially developed into drugs against SARS-CoV-2.

**3.1.1.4. Flavonoids.** Flavonoids are widely distributed in many parts of plants and are indispensable compounds for physiological processes in plants. Flavonoids, especially glycosylated flavonoids, are considered as potential inhibitors with the ability to inhibit the activities of multiple proteases, including SARS-CoV 3CL<sup>pro</sup> [72]. In addition, a study has shown that almost all the annotated flavonoids extracted from *Salvadora persica* could stably bind to SARS-CoV-2 3CL<sup>pro</sup> [73], suggesting that flavonoids may be suitable for the treatment of COVID-19.

Taking into account the sequence similarity between SARS-CoV and SARS-CoV-2 3CL<sup>pro</sup>, Jo et al. mined the flavonoid library using a proteolytic method, and three compounds (baicalin, pectolarin

and herbacetin) exhibited better inhibitory effects, with baicalin showing the greatest effect [74]. The measured IC<sub>50</sub> values for these three compounds were 34.71, 51.64 and 53.90 μM, respectively (Fig. 4A1–A3). However, baicalin, as one of the four main ingredients (baicalein, baicalin, wogonin, and wogonoside) of *Scutellaria baicalensis*, was not the compound with the strongest SARS-CoV-2 3CL<sup>pro</sup> inhibitory activity. Experimental investigation by Liu and colleagues found that the flavonoid baicalein was the most promising inhibitor of SARS-CoV-2 among the four, with a corresponding IC<sub>50</sub> of 0.39 μM. They then tested 10 analogues of baicalein obtained from suppliers, and 4 flavonoid compounds (scutellarein, dihydromyricetin, quercetagenin and myricetin) had the highest inhibitory activity against SARS-CoV-2 3CL<sup>pro</sup> (Fig. 4B1 and 4B2) [75]. It is worth noting that the high binding affinity between myricetin and SARS-CoV-2 3CL<sup>pro</sup> has been confirmed by other computational study [76]. Quercetin is also an analogue of baicalein, and it was recognized as the most promising inhibitor of SARS-CoV-2 3CL<sup>pro</sup> among 150 compounds, with an inhibition constant (K<sub>i</sub>) of 7 μM [77]. Other studies on SARS-CoV-2 3CL<sup>pro</sup> have also identified quercetin as potential inhibitor. Sen et al. performed molecular docking and dynamic studies on 1,040 natural compounds derived from an in-house database, and three (quercetin, aronadendin and leucopelargonidin) of the four identified inhibitors of SARS-CoV-2 3CL<sup>pro</sup> belong to the flavonoids group (Fig. 4B2 and B3) [78]. Furthermore, a large number of natural flavonoids obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov>) were evaluated, and three compounds (quercetin 3-rhamnoside, rutin and myricetin 3-rutinoside) were selected as drug candidates (Fig. 4C1–C3). Among them, quercetin 3-rhamnoside had the lowest negative docking score (−9.7 kcal/mol), whereas the rutin-3CL<sup>pro</sup> complex was the most stable [79]. Another drug study on SARS-CoV-2 3CL<sup>pro</sup> also confirmed that rutin had the strongest inhibitory activity among 18 compounds extracted from *Manilkara hexandra* (Roxb.) [80]. Hence, rutin and its derivatives are expected to be potential natural inhibitors of SARS-CoV-2 3CL<sup>pro</sup>. Huynh et al. optimized the structure of rutin and developed two hydrophobic analogues of rutin (M1 and M2), as shown in Fig. 4C4–C5 [81]. The binding of compounds M1 and M2 to SARS-CoV-2 3CL<sup>pro</sup> was significantly enhanced compared with the binding of rutin.

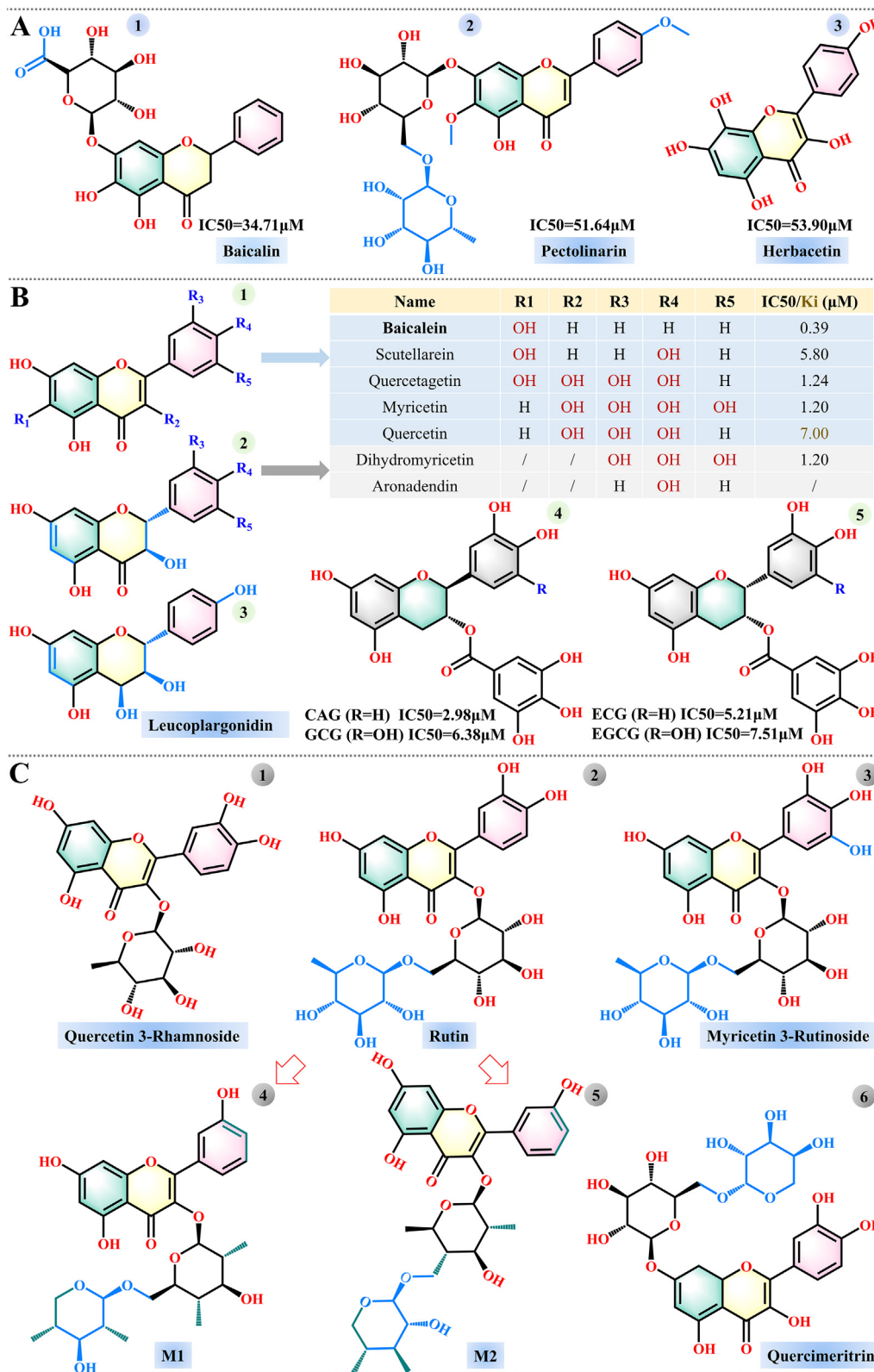
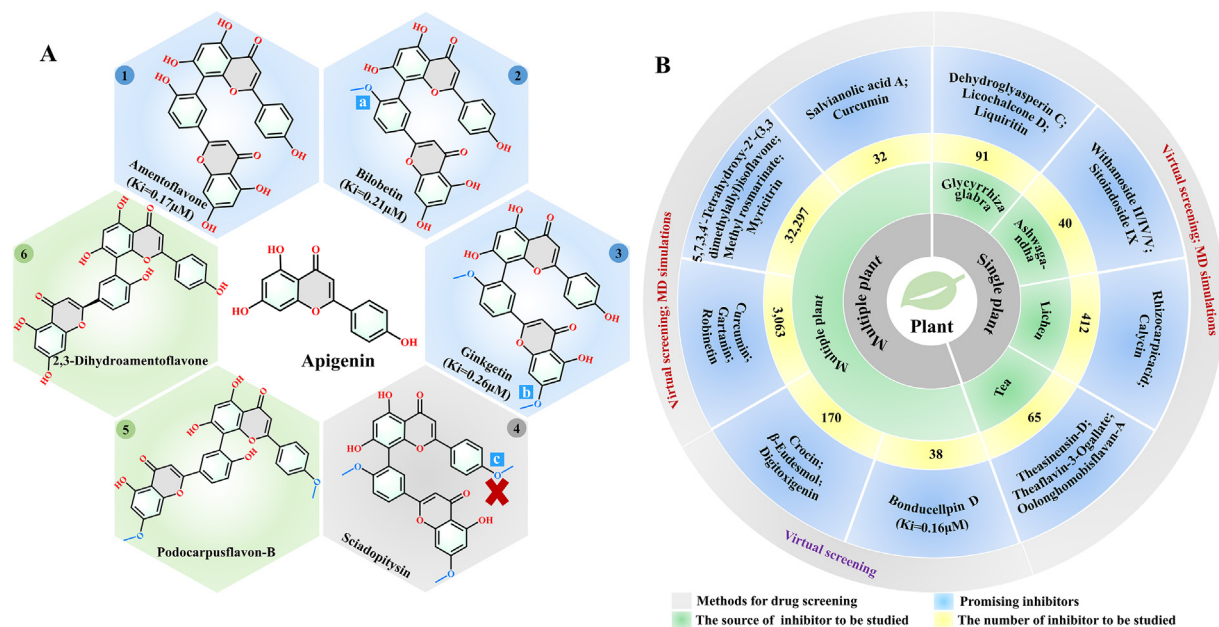


Fig. 4. Structures of flavonoid inhibitors of SARS-CoV-2 3CL<sup>pro</sup>.

Flavonoids can be divided into several sub-categories according to their chemical structure [82]. Anthocyanins represent one of the flavonoid groups, and have been extensively studied by Fakhar et al. [83]. They conducted a virtual screening of 3,435 anthocyanin-derived compounds from the PubChem database

and selected two optimal drug candidates (compound IDs: 44256921 and 131751762) based on their docking score, physico-chemical properties, structural stability and binding free energy. The basic structure of anthocyanins and these two compounds are shown in Fig. S1. Another class of flavonoids, flavan-3-ols, has



**Fig. 5.** Structures and sources of potential inhibitors targeting SARS-CoV-2 3CL<sup>pro</sup>: (A) structures of biflavonoid inhibitors of SARS-CoV-2 3CL<sup>pro</sup> and (B) potential inhibitors selected from different plants.

also attracted attention. Zhu et al. performed docking simulations and experimental investigations on 10 main Flavan-3-ols compounds, and found that four of them (catechin gallate [CAG], epicatechin gallate [ECG], gallic catechin gallate [GCG] and epigallocatechin-3-gallate [EGCG]) had the potential to be utilized as inhibitors of SARS-CoV-2 3CL<sup>pro</sup>, with IC<sub>50</sub> values of 2.98, 5.21, 6.38 and 7.51 μM, respectively (Fig. 4B4–B5) [84]. These four compounds are abundant in tea plants, particularly green tea [85,86]. The ingredients in green tea may be helpful in fighting SARS-CoV-2, owing to their multi-faceted functions [87]. In fact, a study of eight compounds with known antiviral activity in green tea also found that EGCG, ECG and GCG are the best drug candidates for inhibiting the activity of SARS-CoV-2 3CL<sup>pro</sup> [88]. Other related studies have also confirmed the inhibitory effect of EGCG against SARS-CoV-2 3CL<sup>pro</sup> [89–91].

**3.1.1.5. Biflavonoids.** Previous experiments confirmed that four biflavonoids (amentoflavone, bilobetin, ginkgetin, sciadopitysin) and eight diterpenoids isolated from *Torreya nucifera* showed inhibitory activity against SARS-CoV [92]. Recently, the inhibitory effect of these compounds against SARS-CoV-2 was evaluated by Ghosh et al. [93]. Their computation results show that three of the four bioflavonoids: amentoflavone, bilobetin and ginkgetin, can bind stably to the binding sites of 3CL<sup>pro</sup> (PDB ID: 6LU7). The binding free energies of these three inhibitors to the protein calculated using the molecular mechanics generalized Born surface area (MM-GBSA) method were −59.57, −66.31 and −63.62 kcal/mol, respectively, and the corresponding Ki values were 0.17, 0.21 and 0.26 μM, respectively (Fig. 5A1–A3). As shown in Fig. 5A1–A3, the screened compounds share structural similarity and the differences among them originate from the different substitutions at positions a and b of the apigenin motif. Notably, biflavonoid sciadopitysin was also derived from the replacement of the apigenin motif at positions a, b, and c (Fig. 5A4), but it was revealed to be a toxic molecule that is not suitable for use as an antiviral drug. Based on this result, substitutions on the structure of apigenin can be attempted to identify inhibitors with more favorable physical and chemical properties.

Flavonoids and biflavonoids are expected to behave as inhibitors of SARS-CoV-2 3CL<sup>pro</sup>. Bharadwaj et al. elucidated that four

best inhibitors screened from 653 natural compounds in the NP-lib database are flavonoids (rutin, quercimeritrin 6'-O-L-arabino pyranoside) (Fig. 4C2 and C6) and biflavonoids (2,3-dihydroamentoflavone and podocarpusflavon-B) (Fig. 5A5–A6) [94]. Therefore, there is an urgent need to design and optimize the structure of flavonoids and biflavonoids to develop powerful inhibitors of SARS-CoV-2.

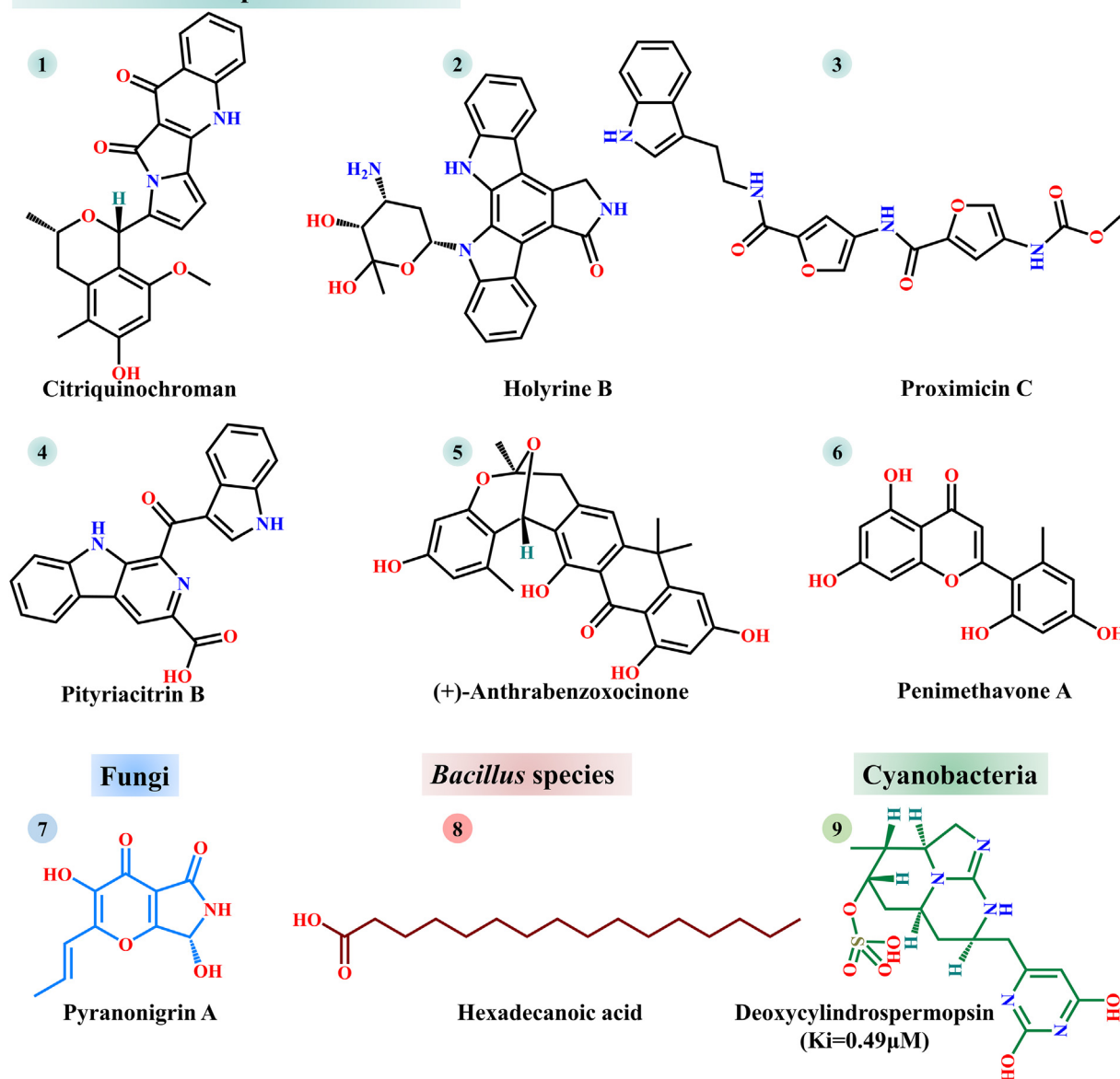
**3.1.1.6. Others.** Based on the above studies, it can be inferred that some medicinal plants may be good sources of compounds that could potentially behave as inhibitors targeting SARS-CoV-2. Due to the lack of effective drugs, some medicinal plants have been studied under emergency situations, and a few studies have attempted to extract antiviral medicinal ingredients from a single plant [95–98]. Potential inhibitors of SARS-CoV-2 3CL<sup>pro</sup>, including dehydroglyasperin C, licochalcone D and liquiritin, were found in *Glycyrrhiza glabra*, while other compounds including withanoside II, withanoside IV, withanoside V and sitiosindoside IX were extracted from *Withania somnifera* (Ashwagandha). Moreover, calycin and rhizocarpic acid have been found in *Lichen*. Efforts have also been made to identify the most effective inhibitors by screening compounds from multiple plants [99–103]. For example, Mahmud et al. identified three potential inhibitors (curcumin, gartnerin and robinetin) that can stably bind to SARS-CoV-2 3CL<sup>pro</sup> by screening 3,063 compounds from >200 plants. For ease of reference, all prospective SARS-CoV-2 3CL<sup>pro</sup> inhibitors and their corresponding sources are shown in Fig. 5B.

### 3.1.2. Natural inhibitors extracted from marine organisms

The marine ecosystem is rich in resources and comprises a wide variety of organisms. Recently, an increasing number of natural medicines for preventing or treating many diseases have been extracted from marine organisms. According to previous studies, some marine-derived natural compounds have been demonstrated to possess antiviral and antibacterial activities [104,105]. Moreover, some marine-derived natural compounds have activities that are applied in treating nervous and immune systems, while others are used as diagnostic tools. Therefore, marine natural compounds represent a favorable source for the development of pharmaceuticals that may be used to alleviate COVID-19 pandemic. The marine



## Microbial natural product database



**Fig. 6.** Microbe-derived natural inhibitors of SARS-CoV-2 3CL<sup>pro</sup>. Compounds 1–6, 7, 8 and 9 were selected from microbial natural product database, fungi, *Bacillus* species and cyanobacterial metabolites, respectively.

natural product library (<http://docking.umh.es/downloaddb>) is a valuable database for mining marine drugs, which contains >10,000 compounds. Structure-based and ligand-based virtual screening studies were performed separately using this database, and 17 of 14,064 marine natural compounds were selected as putative inhibitors of SARS-CoV-2 3CL<sup>pro</sup>. Among these, heptafuhalol A has the lowest binding free energy [106]. Other computational studies demonstrated that four marine drugs (eribulin mesylate, plitidepsin, trabectedin and fostularin 3) also had excellent affinity for binding to SARS-CoV-2 3CL<sup>pro</sup> [107,108].

### 3.1.3. Natural inhibitors of microbial origin

The earth is rich in microorganisms, and bioactive substances acquired from microorganisms are of great significance in the development of novel drugs. Therefore, natural inhibitors of microbial origin have attracted extensive attention of the pharmaceutical industry. Since the outbreak of SARS-CoV-2, researchers have

searched for inhibitors of microbial origin. There is currently a large microbial natural product database (<https://www.npatlas.org/joomla/index.php>) containing >20,000 compounds from bacteria and fungi [109]. To obtain high-efficiency inhibitors that target SARS-CoV-2 3CL<sup>pro</sup> activity, one research group conducted a layer-by-layer screening of 24,581 compounds from this database, and six of them (citriquinochroman, holyrine B, proximicin C, pityriacitrin B, (+)-anthrabenzoxocinone and penimethavone A) were identified to be high potential for inhibiting SARS-CoV-2 3CL<sup>pro</sup> (Fig. 6[1]–[6]) [110]. Another study conducted computational screening of 100 fungal metabolites from PubChem using molecular docking and MD simulations. Among these 100 selected metabolites, pyranonigrin A was regarded as a potent inhibitor of SARS-CoV-2 3CL<sup>pro</sup> (Fig. 6[7]) [111]. Based on similar computational approaches, hexadecanoic acid and deoxycylindrospermopsin were selected from the metabolites of *Bacillus* species and cyanobacteria, respectively (Fig. 6[8]–[9]) [112,113].

**Table 1**  
Potential inhibitors of SARS-CoV-2 3CL<sup>pro</sup> selected from approved or commercially available drugs.

Source	Number <sup>a</sup>	Method	Potential inhibitor
SuperDrug2	4,600 clinically approved drugs [125]	VS; Molecular docking; MD simulations	Binifibrate; Bamifylline
	3,987 FDA-approved drugs [126]	VS; Molecular docking; MD simulations;	Ivermectin (IC <sub>50</sub> = 21.53 μM); <b>Tipranavir</b> (IC <sub>50</sub> = 27.66 μM); Boceprevir (IC <sub>50</sub> = 31.36 μM); Miconazole (IC <sub>50</sub> = 47.63 μM); Paritaprevir (IC <sub>50</sub> = 73.38 μM); Ombitasvir (IC <sub>50</sub> = 75.49 μM); <b>Cobicistat</b> (IC <sub>50</sub> = 6.7 μM)
DrugBank	2,100 FDA-approved drugs [127]	Experimental evaluation	
	2,454 FDA-approved drugs [128]	VS; Molecular docking;	Hyaluronic acid; Acarbose; <b>Lopinavir</b>
	2,800 FDA-approved drugs [129]	MD simulations	Tipiracil; Aprepitant
	Commercially available drugs [130]		Leuprolide; Nelfinavir; <b>Ritonavir</b> ; <b>Teniposide</b> ; Valrubicin
Druglib	1,051 FDA-approved drugs [131]		R428; <b>Teniposide</b> ; VS-5584; Setileuton
ZINC	1,615 FDA-approved drugs and available in the market [132]		Dihydroergotamine (K <sub>d</sub> = 107.6 μM); Midostaurin (K <sub>d</sub> = 43.5 μM); Ziprasidone
	4,384 approved drugs [133]		Ergotamine; <b>Bromocriptine</b> ; Meclocycline; Amrubicin
	5,903 approved clinical drugs [134]		Viomycin; Capastat; Carfilzomib; <b>Saquinavir</b>
PubChem	77 FDA-approved drugs [135]		<b>Lopinavir-Ritonavir</b> ; <b>Tipranavir</b> ; Raltegravir
	400 commercially available curcumin analogues [136]		Cyclohexanone
DrugBank; PubChem Selleckchem	Approved drugs and bioactive compounds [137]		ChEMBL275592; Montelukast; ChEMBL288347; <b>Bromocriptine</b> ; <b>Saquinavir</b>
	3,809 conformations [138]	HTVS; Molecular docking; MD simulation	Amikacin (K <sub>d</sub> = 17.5 nM)
	487 FDA-approved drugs [139]	VS	Ribavirin; Telbivudine; Vitamin B12; Nicotinamide
Selleckchem; Targetmol	3,118 FDA-approved drugs [140]		Angiotensin II; GHRP-2; Indinavir; Polymyxin B; Fexofenadine; <b>Atazanavir</b> ;
e-Drug3D; Reaxys	6,466 approved drugs [141]		<b>Cobicistat</b> ; Caspofungin; <b>Lopinavir</b>
In-house database	2,000 approved drugs [142]	VS; Experimental evaluation	Peramppanel; Carprofen; Celecoxib; Alprazolam; Trovafloxacin; Sarafloxacin; Ethyl biscoumacetate
Screen-Well	774 FDA-approved drugs [143]	HTVS; Molecular docking	Manidipine (IC <sub>50</sub> = 4.8 μM); Boceprevir (IC <sub>50</sub> = 5.4 μM); Lercanidipine (IC <sub>50</sub> = 16.2 μM); Bedaquiline (IC <sub>50</sub> = 18.7 μM); Efonidipine (IC <sub>50</sub> = 38.5 μM)
DTC; BindingDB	3,410 FDA-approved drugs [144]	Deep learning	<b>Saquinavir</b> mesylate (IC <sub>50</sub> = 9.92 μM)
Literatures	100 clinically approved drugs [145]	Experimental evaluation	<b>Atazanavir</b> (K <sub>d</sub> = 94.94 nM); Remdesivir (K <sub>d</sub> = 113.13 nM); Efavirenz (K <sub>d</sub> = 199.17 nM); <b>Ritonavir</b> (K <sub>d</sub> = 204.05 nM); Dolutegravir (K <sub>d</sub> = 336.91 nM)
PubChem	22 FDA-approved glucocorticoids [146]	Molecular docking; MD simulations	Teicoplanin (IC <sub>50</sub> = 1.5 μM)
	40 FDA-approved non-steroidal anti-inflammatory drugs [147]		Ciclesonide; Dexamethasone; Betamethasone; Hydrocortisone; Fludrocortisone; Triamcinolone
	9 FDA-approved angiotensin receptor blocker drugs [148]		Sulfapyrazone; Indomethacin; Auranofin
Enamine	8960 commercially available compounds [149]	HTVS; Molecular docking; MD simulation; Experimental evaluation	Olmesartan (IC <sub>50</sub> = 1.808 μM)
			Z1244904919 (IC <sub>50</sub> = 0.73 μM); Z1759961356 (IC <sub>50</sub> = 0.69 μM)

**Note:** 1. The inhibitors in bold indicate that they have been confirmed through different studies.

2. The binding mechanisms of all inhibitors to 3CL<sup>pro</sup> are listed in Table S5.

<sup>a</sup> The number of all compounds used for drug screening.

### 3.2. Inhibitors of SARS-CoV-2 3CL<sup>pro</sup> screened from approved or commercially available drugs

Given the current critical public health situation, i.e. lack of effective drugs to control COVID-19, the repurposing of already approved or commercially available drugs is a quick and desirable

strategy to develop safe and effective treatments. Drug repurposing is a process of re-screening existing drugs for their new applications using related techniques, and therefore, it is also regarded as drug recycling, drug repositioning, etc [114]. Previously approved drugs have many undeniable advantages over newly developed drugs. For example, they have known safety, pharma-

**Table 2**  
Potential inhibitors of SARS-CoV-2 3CL<sup>pro</sup> selected from existing databases or literature.

Source	Number	Method	Potential inhibitor
ZINC	2,000 [150] 1,500 <sup>a</sup> [151] 5,811 <sup>b</sup> [152] 606 million [153] 1,000 <sup>c</sup> [154] > 8,722 [155] 360,000 [156]	VS  SCAR protocol VS; MD simulations	ZINC32960814/12006217/03231196/33173588 ZINC20291569/90403206/95480156 Telcagepant; Vidupiprant; Poziotinib; Fostamatinib. (–)-Taxifolin; Rhamnetin  ZINC000621278586/000621285995 BBB_26580140; SCHEMBL12616233/18616095/20148701 SN00017653 (SuperNatural II); Pseudostellarin C (in-house); Eledoisin (SuperDrug2); Naldemedine (SuperDrug2); Saralasin (Withdrawn); <b>Saquinavir</b> (Withdrawn)
Asinex; ChEMBL SuperNatural II; In-house database; SuperDrug2; Withdrawn			
Chemical Abstract Services Literatures; PubChem; Asinex	35,000 [157] > 10,584 <sup>d</sup> [158]		SKS-01; SKS-02; SKS-03; SKS-04; pq8; pq9; pq10; A12 <b>Note:</b> Structures of these inhibitors are shown in Fig. S2.
Protein Data Bank Literature PubChem Life Chemicals; Asinex Pharmit	2,692 [159] 49 <sup>e</sup> [160] 10,433 <sup>f</sup> [161] 21,207 <sup>g</sup> [162] 213.5 million [163]		PubChem IDs: 118098670; 104161460; 163632044 Ethaselen (IC50 = 4.51 μM) 6-Deaminosinefungin; UNII-09H5KY11SV F2679-0163(Life chemicals); F6355-0442 (Life chemicals); 8250 (Asinex) CSC057752019; PubChem-22029441/-11210821; MCULE-9349798441; MolPort-045-918-905 Acteoside; Chebulinic acid; Delphinidin-3,5-diglucoside; <b>Saquinavir</b> ; Lithospermic acid B; 11m_32045235
DrugBank; Literatures; In-house database	2,736 <sup>h</sup> [164]		
DrugBank	10,036 <sup>b</sup> [165] 10,246 [166] 13,227 [167]	VS	DB02388; <b>Cobicistat</b> Levothyroxine; Amobarbital; ABP-700 DB02128/01871/04502/02378/03063/04378/03648/04353/04692/047https://doi.org/ 10/08732/11938/03395/04653/04758/07571/07934/12955;DBMET00084/01550/ 01549/01548 ChEMBL1559003/2237553/1511674/3260476/1170272/1335000/2235580/3264032/ 1447105/589899/1539803/2216842/427787/1339675/3126648/1302388/3234783/ 1807774/2087984/2387487/2113271/476947/399042/2000247/3236740/1447944/ 1760165/2087965 Paritaprevir; Ciluprevir; Simeprevir; Deldeprevir; Indinavir; <b>Saquinavir</b> ; Faldaprevir; Brexanavir; Grazoprevir; <b>Lopinavir</b>
ChEMBL	1,485,144 [168]		
Protein Data Bank; SwissSimilarity Existing drug database	33 protease inhibitors [169] 133 <sup>i</sup> [170] > 2,500 [171]	VS; MD simulations; Experimental evaluation VS; Experimental evaluation	Melatonin  Dipyridamole (Ki = 0.04 μM); Hydroxychloroquine (Ki = 0.36 μM); Chloroquine (Ki = 0.56 μM) LASSBio-1945 (IC50 = 15.97 μM)
LASSBio	2,300 [172]		
DrugBank; PubChem Compound Library In-house database; TargetMol; Selleckchem; Antivirus Drug Library AVPdb database	5,016 <sup>j</sup> [173] 23,000 [174] ~ 10,000 [54] 88 [180]	HTVS; MD simulations HTVS HTVS; VS; Experimental evaluation Molecular docking; MD simulations	Isavuconazonium; α-KI; Pentagastrin Azanitrile (Ki = 24.0 nM); Pyridyl ester(Ki = 10.0 nM) Ebselen (IC50 = 0.67 μM); Disulfiram (IC50 = 9.35 μM); Tideglusib (IC50 = 1.55 μM); Carmofur (IC50 = 1.82 μM); Shikonin (IC50 = 15.75 μM); PX-12 (IC50 = 21.39 μM) <b>Note:</b> These inhibitors have been further confirmed [175–179]. P14, P39, P41, P74 <b>Note:</b> Sequences of these inhibitors are shown in Table S7.

Note: The binding mechanisms of all inhibitors to 3CL<sup>pro</sup> are listed in Table S6.

<sup>a</sup> These compounds were selected according to the pharmacophore features of inhibitor N3.

<sup>b</sup> Approved or investigational drugs.

<sup>c</sup> Protease-inhibitor-like compounds.

<sup>d</sup> Analogs of Chloroquine.

<sup>e</sup> Se-containing heterocyclic compounds.

<sup>f</sup> The compounds that have > 90% structural similarity with the 10 high-affinity molecules previously reported (PubChem IDs: 5281605, 16394003, 19323586, 44137675, 118737648, 787400, 8386889, 34755, 65482 and 145998233).

<sup>g</sup> 12,485 anti-SARS-CoV-2 or antiviral compounds in the Life Chemicals database and 8,722 antiviral compounds in Asinex database.

<sup>h</sup> 2,454 FDA approved drugs from DrugBank, 144 coronavirus M<sup>pro</sup> inhibitors from published literature and 138 natural compounds from an in-house database.

<sup>i</sup> 74 ligand-M<sup>pro</sup> complexes and 59 analogues.

<sup>j</sup> 6 analogs of SARS-CoV-2 3CL<sup>pro</sup> inhibitor (13b) [58] in DrugBank and 5,010 analogs of 11 antiviral agents (Atazanavir, Darunavir, Fosamprenavir, Indinavir, Lopinavir, Ritonavir, Saquinavir, Tipranavir, Delavirdine, Nevirapine and Remdesivir) in PubChem.

okinetics and toxicity profiles, which not only save time and investments, but also reduce the possibility of negative effects on the human body [115]. Additionally, there are infrastructures available for the large-scale production of approved or commercially available drugs, which greatly improves the efficiency of drug production [116]. It is therefore a good strategy to screen inhibitors that can control the activity of SARS-CoV-2 3CL<sup>pro</sup> among the approved or commercially available drugs. Of course, this strat-

egy will also encounter many challenges including patent application, investment and unexpected negative effects [117].

A literature retrieval revealed that the main sources of approved drugs are existing databases, including DrugBank (<http://www.drugbank.ca>), Druglib (<http://www.druglib.com>), eDrug3D (<http://cheminfo.ipmc.cnrs.fr/edrug3d>), Reaxys (<https://www.reaxys.com>), Selleckchem Inc., Targetmol (<https://www.targetmol.com>), SuperDrugs2 (<http://cheminfo.charite.de/superdrug2>),

**Table 3**  
Dual inhibitors against multiple SARS-CoV-2 targets including nsp3, nsp5 or both.

Promising dual inhibitor	Source	Target
Naloxone; Fluoxetine; Dopal; Thiamine Phenylephrine; Epinephrine; Aspirin Pseudoephedrine; Benzenebutyrate; Nelfinavir; Tipranavir	ZINC database [181,182]	Nsp5; PL <sup>pro</sup>
Bemcentinib; MFCD00832476	ZINC PubChem and NPASS <sup>a</sup> databases [183]	
Ginkgolic acid (IC50 = 1.79; 16.30 μM); Anacardic acid (IC50 = 2.07; 17.08 μM)	MedChemExpress [184]	
2001083-68-5; 2001083- 69-6; 833463-19-7	CAS Antiviral COVID19 database [185]	Nsp5; RdRp <sup>b</sup>
Nakinadine B; Amphimedoside C 20-hepacosenoic acid;	<i>Amphimedon sp</i> [186]	
Hydroxymatairesinol MCULE-3732245601-0 (IC50 = 0.45; 0.085; 0.29 μM) MCULE-7013373725-0 (IC50 = 0.11; 0.063; 0.29 μM)	Sesame [187] MCULE library [188]	Nsp5; PL <sup>pro</sup> ; RdRp Nsp5; PL <sup>pro</sup> ; furin protease
19 acetylsesterstatin 3 12β-acetoxy,16-epi- hyrtiolide	<i>Hyrtios erectus</i> [189]	Nsp5; nsp15
Cefiderocol; Plazomicin Vanganciclovir	PubChem database [190]	Nsp5; nsp16 Nsp5; N protein
Rutin	DrugBank [191]	Nsp5; S protein
Solanine, Acetoside; Rutin; Withanone	PubChem database [192,193]	

<sup>a</sup> NPASS: Natural Product Activity and Species Source.

<sup>b</sup> RdRp: RNA-dependent RNA polymerase.

ZINC, PubChem, in-house [118], Screen-Well<sup>®</sup> FDA v. 2.0 Approved Drug, Drug Target Common (DTC) (<https://drugtargetcommons.fimm.fi>), Enamine and BindingDB databases, and the commonly used methods for drug screening are VS, HTVS, molecular docking and MD simulations. The likely potential inhibitors, selected via studies on approved or commercially available drugs from different sources, are summarized in Table 1. Among these, inhibitors identified in multiple studies are shown in bold, i.e. tipranavir, cobicistat, lopinavir, ritonavir, teniposide, bromocriptine, saquinavir and atazanavir.

### 3.3. Others

Based on the aforementioned literature review, it is clear that existing databases play an important role in mining for inhibitors. In addition to the databases mentioned in this review, other databases have also attracted widespread attention, including Existing drug database (<https://www.pnas.org/content/suppl/2020/10/12/2010470117.DCSupplemental>), Asinex antiviral database (<https://www.asinex.com/antiviral>), ChEMBL, SuperNatural II (<http://bioinformatics.charite.de/supernatural>), WithDrawn (<http://cheminfo.charite.de/withdrawn>), Compound Library (<https://www.pharmchem1.uni-bonn.de/www-en/pharmchem1-en/mueller-laboratory/compound-library>), Chemical Abstract Services (CAS) (<https://www.cas.org/covid-19-antiviral-compounds-dataset>), LASSBio (<http://www.lassbio.icb.ufrj.br>), PDB database, SwissSimilarity (<http://www.swiss similarity.ch>), Life Chemicals database (<https://lifechemicals.com>) and Pharmit (<http://pharmit.csb.pitt.edu>). Following the sudden outbreak of SARS-CoV-2 infections, researchers have extensively screened the existing databases to investigate

effective inhibitors of SARS-CoV-2. Some studies were performed on a single database, while others were performed on multiple databases. In addition, existing literature also provides sets of potential compounds eligible for drug screening. The corresponding studies are summarized in Table 2. Meanwhile, some groups have carried out the redesign, modification, and re-synthesis of drugs for SARS-CoV-2 3CL<sup>pro</sup> based on known inhibitor, key pharmacophore or active sites of M<sup>pro</sup> [119–121], which may stimulate the discovery of novel drugs to alleviate the threat of COVID-19.

## 4. Dual inhibitors

The inhibitors summarized above are mainly for a single target (nsp3 or nsp5) of SARS-CoV-2. Of course, the development of dual inhibitors for different targets of SARS-CoV-2 is also a more promising direction that cannot be ignored, which may greatly reduce the combination of drugs and improve the efficiency of treatment. To better understand the current development status of dual inhibitors, we have briefly summarized the studies on dual inhibitors related to nsp3 and nsp5, which are listed in Table 3.

## 5. Summary and outlook

The paucity of relevant information on therapeutic targets and development of inhibitor against SARS-CoV-2 has hindered the treatment of COVID-19. In this review, we have summarized the functions and the most likely potential inhibitors of viral targets (nsp3 and nsp5) to promote the development of drugs against SARS-CoV-2. During the literature retrieval for this review, we deem that existing databases play an important role in drug screening. Based on the different domains of nsp3, the inhibitors of nsp3 can be divided into two categories: PL<sup>pro</sup> inhibitors and Mac1 inhibitors. Inhibitors of nsp5 can be divided into three categories according to their sources: natural compounds, approved or commercially available inhibitors and others. Natural compounds are derived mainly from plants, marine organisms and microorganisms. Plant-derived compounds can be further subdivided into six categories: polyphenols, alkaloids, terpenoids, flavonoids, biflavonoids and others. Meanwhile, the binding mechanisms of the aforementioned promising inhibitors to the targets are also summarized. From the perspective of methods used in drug screening, VS, HTVS, molecular docking and MD simulations are commonly used. However, there is still a need to develop faster and more accurate methods for drug screening.

Although extensive effort has been made to explore and develop SARS-CoV-2 inhibitors, most of the current research is focused on *in silico* analysis, and there is a lack of relevant experimental confirmation or *in vitro* verification. Future research should be conducted with more in-depth experimental investigations and *in vitro* verification based on the available computational data. Moreover, studies of dual inhibitors for multiple targets of SARS-CoV-2 are relatively lacking. In addition to the development of single inhibitors against COVID-19, more attention should also be paid to the exploration of dual inhibitors in the future. Another issue that cannot be ignored is that the proteases of SARS-CoV-2 may undergo unpredictable mutations at any time, and some mutations may enhance the structural stability and drug resistance of the proteases [122,123], which brings great challenge to the drug design targeting them. It is recommended to develop relevant technologies or software to predict high-risk or drug-resistant mutations, so as to find effective drugs to combat the mutant SARS-CoV-2 in advance. The last thing that needs to be pointed out is the importance of selective inhibitors, because some inhibitors of nsp3 and nsp5 may be related to the activity of the host protein, such as human cathepsins L and B [124].

## CRediT authorship contribution statement

**Fangfang Yan:** Investigation, Software, Visualization, Writing – original draft. **Feng Gao:** Conceptualization, Project administration, Funding acquisition, Writing – review & editing.

## 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.

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## Appendix A. Supplementary data

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