Review

An overview of potential inhibitors targeting non-structural proteins 3 (PLpro and Mac1) and 5 (3CLpro/Mpro) of SARS-CoV-2

Fangfang Yan a, Feng Gao a,b,c,*

a Department of Physics, School of Science, Tianjin University, Tianjin 300072, China
b Frontiers Science Center for Synthetic Biology and Key Laboratory of Systems Bioengineering (Ministry of Education), Tianjin University, Tianjin 300072, China
c SynBio Research Platform, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, China

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 protease, 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.

Article history:
Received 25 April 2021
Received in revised form 2 August 2021
Accepted 21 August 2021
Available online 24 August 2021

Keywords:
SARS-CoV-2
COVID-19
Non-structural protein 3 (nsp3)
nsp5
Inhibitors

Contents

1. Introduction ......................................................... 4869
2. Structure, function and inhibitors of nsp3 ......................................................... 4869
   2.1. Inhibitors of SARS-CoV-2 PLpro ................................................................. 4870
   2.2. Inhibitors of SARS-CoV-2 Mac1 ................................................................. 4872
3. Structure, function and inhibitors of nsp5 ......................................................... 4872
   3.1. Inhibitors of SARS-CoV-2 3CLpro screened from natural compounds .......... 4872
      3.1.1. Plant-based natural inhibitors ............................................................... 4872
      3.1.2. Natural inhibitors extracted from marine organisms .......................... 4875
      3.1.3. Natural inhibitors of microbial origin ............................................... 4876
   3.2. Inhibitors of SARS-CoV-2 3CLpro screened from approved or commercially available drugs ................................................................. 4877
   3.3. Others ................................................................. 4879
4. Dual inhibitors ........................................................ 4879
5. Summary and outlook .......................................................... 4880
CRediT authorship contribution statement ....................................................... 4880
Declaration of Competing Interest ................................................................. 4880
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 β-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-to-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 replicate 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 (nsps; nsp1-nsp11) and 16 nsps (nsp1-nsp16) by the virus-encoded 3-chymotrypsin-like protease (nsp3, also called 3CLpro or Mpro) and the papain-like protease (PLpro) that is present in nsp3 (Fig. 1A and C). Detailed information on the cleavage sites, cleavable octapeptides and the nsps of the replicate 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://rubic.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 PLpro, while the others were produced by 3CLpro. 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 octapeptides 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 el al., the cleavable octapeptide of SARS-CoV should be able to bind to the active site of 3CLpro, 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].

5S 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 PLpro in nsp3 are responsible for cleaving the large replicate polypeptides into fragments that can perform multiple functions, thereby assisting the replication of the virus. Meanwhile, PLpro 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 PLpro, nsp3 also contains another important domain (macromdomain) 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 PLpro domain. As shown in Fig. 1D, the PLpro can be divided into four distinct domains: the fingers (β4-β7), thumb (α2-α7), palm (α8-α13) and ubiquitin-like...
(UBL, β1-β3), 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]. PLpro 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 β-sheets (β1-β7) and six α helices (α1-α6) (Fig. 1E). Among them, four β-sheets (β3 and β5-β7), α1, and two loops (β3-α2 and β6-α5) 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 PLpro and Mac1 can help the virus escape the antiviral immune response of the host [34]. Consequently, inhibiting the activity of PLpro 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 PLpro

In April 2020, Osipiuk et al. first reported the crystal structure of SARS-CoV-2 PLpro, which is available in PDB (PDB ID: 6W9C). Based on this structure, studies have been performed to explore potential inhibitors of PLpro enzymatic activity. Alamri and colleagues conducted virtual screening (VS) of 6,968 protease inhibitors from Asinex library (https://www.asinex.com/protease) using AutoDock Vina software, and three compounds (ADM_13083841, LMG_15521745 and SYN_15517940) were identified as potential inhibitors of PLpro (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 PLpro, 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 PLpro as a reference, they predicted the structures of VIR250 and VIR251 bound to PLpro (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 PLpro 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, pexidaritinib 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 [acetamide, bentriamide, lymecycline, canagliflozin, darolutamide, lafu-
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 PLpro are highly effective in reducing the activity of SARS-CoV-2 PLpro enzyme [42]. Based on the naphthalene scaffold structure, Bhati and Osipiuk et al. analyzed several SARS-CoV-2 PLpro-related drugs through computational models and experiments, respectively. Among the 20 naphthalene-based compounds designed by Bhati, L10 (C_{18}H_{14}FN_{3}O_2),

![Fig. 2. Flowchart illustrating the screening of inhibitors against (A-B) PLpro and (C) Mac1.](image-url)
O$_3$ was considered to be the most potent anti-SARS-CoV-2 PL$^{\text{pro}}$ 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$^{\text{pro}}$ activity in vitro, particularly compound 1 (GLR0617) (PDB ID: 7CMD) [44]. The structures and corresponding half maximal inhibitory concentration (IC50) values for these six inhibitors are shown in Fig. 2B2. In view of the strong inhibitory activity of GLR0617 against SARS-CoV-2 PL$^{\text{pro}}$, 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$^{\text{pro}}$ [45], and ZINC43071312 showed the strongest binding ability to SARS-CoV-2 PL$^{\text{pro}}$ (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 a 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, metronidazole, bosentan, lapatinib, gefitinib, ketoconazole, cardiolipin, 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, salvinorin acid, adenosine-5'-diphosphoribose, folic acid, naringin, melibiose, maltose, rutin, nucleotide analogue 1 [NA1], NA2, NA3, nadine 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 fluoxisoline), β-lactams (ceftriaxone 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$^{\text{pro}}$) or 3CL$^{\text{pro}}$. 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$^{\text{pro}}$. Although the monomer state of 3CL$^{\text{pro}}$ 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$^{\text{pro}}$ is essential for replication and transcription of the virus due to its function in cleaving replicase poly peptides (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$^{\text{pro}}$ in human [58], 3CL$^{\text{pro}}$ inhibitors are likely to cause fewer side effects on human. Therefore, 3CL$^{\text{pro}}$ is a potential drug target against COVID-19. A literature retrieval revealed that investigators are looking for inhibitors of 3CL$^{\text{pro}}$ from three main classes: natural compounds, approved or commercially available drugs and others.

3.1. Inhibitors of SARS-CoV-2 3CL$^{\text{pro}}$ 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$^{\text{pro}}$, and the binding mechanisms of these natural inhibitors to 3CL$^{\text{pro}}$ are listed in Table S4.

3.1.1. Plant-based natural inhibitors

3.1.1.1. Polysaccharides. Polysaccharides are a class of chemically diverse natural metabolites, and their structures are characterized by multiple phenol groups. Polysaccharides 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 polysaccharides can inactivate SARS-CoV-2 by inhibiting the activity of SARS-CoV-2 3CL$^{\text{pro}}$. Based on the structure of SARS-CoV-2 3CL$^{\text{pro}}$, Ghosh et al. evaluated the inhibitory ability of 18 polysaccharide 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 physico-chemical properties, six possible polysaccharide inhibitors of SARS-CoV-2 3CL$^{\text{pro}}$ 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 four polysaccharide 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 pharmaceutically 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 sophalone D) were identified as potential inhibitors of SARS-CoV-2 3CL$^{\text{pro}}$ (Fig. 3B1-B3). Similarly, a computational approach was applied by Gyebi et al. to explore inhibitors of SARS-CoV-2 3CL$^{\text{pro}}$ from 62 alkaloid compounds in African plants, which yielded two
drug candidates, that is 10-hydroxyusambarensine and cryptquindoline (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 (C₅H₈)n. 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-crownvirus terpenoids from several studies, and identified three terpenoid compounds (methyl tanshinonate, sugiol and α-coronavirus terpenoids from several studies, and identified three the treatment of COVID-19. [69,70]. Thus, terpenoids and their derivatives may be helpful in their 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-crownvirus terpenoids from several studies, and identified three terpenoid compounds (methyl tanshinonate, sugiol and α-coronavirus terpenoids from several studies, and identified three the treatment of COVID-19. [69,70]. Thus, terpenoids and their derivatives may be helpful in the treatment of COVID-19.

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\(^{pro}\) [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\(^{pro}\) [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\(^{pro}\), Jo et al. mined the flavonoid library using a proteolytic method, and three compounds (baicalin, pectolinarin and herbacetin) exhibited better inhibitory effects, with baicalin showing the greatest effect [74]. The measured IC50 values for these three compounds were 34.71, 51.64 and 53.90 \(\mu\)M, 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\(^{pro}\) 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 IC50 of 0.39 \(\mu\)M. They then tested 10 analogues of baicalein obtained from suppliers, and 4 flavonoid compounds (scutellarein, dihydromyricetin, quercetagatin and myricetin) had the highest inhibitory activity against SARS-CoV-2 3CL\(^{pro}\) (Fig. 4B1 and 4B2) [75]. It is worth noting that the high binding affinity between myricetin and SARS-CoV-2 3CL\(^{pro}\) 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\(^{pro}\) among 150 compounds, with an inhibition constant (Ki) of 7 \(\mu\)M [77]. Other studies on SARS-CoV-2 3CL\(^{pro}\) have also identified quercetin as a 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, aronadendrin and leucopelargonidin) of the four identified inhibitors of SARS-CoV-2 3CL\(^{pro}\) 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\(^{pro}\) complex was the most stable [79]. Another drug study on SARS-CoV-2 3CL\(^{pro}\) 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\(^{pro}\). 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\(^{pro}\) was significantly enhanced compared with the binding of rutin.

---

**Fig. 3.** Structures of potential inhibitors targeting SARS-CoV-2 3CL\(^{pro}\): (A) polyphenolic inhibitors, (B) alkaloid inhibitors and (C) terpenoid inhibitors.
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, physicochemical 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. 4. Structures of flavonoid inhibitors of SARS-CoV-2 3CLpro.
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], gallocatechin gallate [GGG] and epigallocatechin-3-gallate [EGCG]) had the potential to be utilized as inhibitors of SARS-CoV-2 3CL\textsuperscript{pro}, with IC\textsubscript{50} values of 2.98, 5.21, 6.38 and 7.51 \mu M, 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\textsuperscript{pro} [88]. Other related studies have also confirmed the inhibitory effect of EGG against SARS-CoV-2 3CL\textsuperscript{pro} [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 stably bind to SARS-CoV-2 3CL\textsuperscript{pro} (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 \mu M, 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\textsuperscript{pro}. Bharadwaj et al. elucidated that four best inhibitors screened from 653 natural compounds in the NPlib database are flavonoids (rutin, quercimeritrin 6’-O-L-arabinopyranoside) (Fig. 4C2 and C6) and biflavonoids (2,3-dihydroamentoflavone and podocarpsflavon-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\textsuperscript{pro}, including dehydroglyasperin C, licochalcone D and liquiritin, were found in Glycyrrhiza glabra, while other compounds including withanoside II, withanoside IV, withanoside V and sitoindoside 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 calcyons from multiple plants [99–103]. For example, Mahmud et al. identified three potential inhibitors (curcumin, gartanin and robinetin) that can stably bind to SARS-CoV-2 3CL\textsuperscript{pro} by screening 3,063 compounds from >200 plants. For ease of reference, all prospective SARS-CoV-2 3CL\textsuperscript{pro} 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.
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 3CLpro. 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 3CLpro [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 3CLpro 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 3CLpro (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 3CLpro (Fig. 6[7]) [111]. Based on similar computational approaches, hexadecanoic acid and deoxychlorndrospermopsin were selected from the metabolites of Bacillus species and cyanobacteria, respectively (Fig. 6[8]–[9]) [112,113].

Fig. 6. Microbe-derived natural inhibitors of SARS-CoV-2 3CLpro. Compounds 1–6, 7, 8 and 9 were selected from microbial natural product database, fungi, Bacillus species and cyanobacterial metabolites, respectively.
Table 1
Potential inhibitors of SARS-CoV-2 3CLpro selected from approved or commercially available drugs.

| Source                | Number† | Method          | Potential inhibitor                                                                 |
|-----------------------|---------|-----------------|-------------------------------------------------------------------------------------|
| SuperDrug2            | 4,600   | VS; Molecular docking; MD simulations | Binifibrate; Bamifiline                                                              |
| 3.987 FDA-approved drugs [125] | VS; Molecular docking; MD simulations | Ivermectin (IC50 = 21.53 μM); Tipranavir (IC50 = 27.66 μM); Boceprevir (IC50 = 31.36 μM); Micaflunin (IC50 = 47.83 μM); Paritaprevir (IC50 = 73.38 μM); Ombitasvir (IC50 = 75.49 μM); |
| DrugBank              | 2,100 FDA-approved drugs [127] | Experimental evaluation | Cobicistat (IC50 = 6.7 μM)                                                          |
| 2,454 FDA-approved drugs [128] | VS; Molecular docking; MD simulations | Hyaluronic acid; Acarbose; Lopinavir                                                 |
| 2,800 FDA-approved drugs [129] | Commercially available drugs [130] |                                             | Tipiracil; Aprepitant                                                                |
| Druglib               | 1,051 FDA-approved drugs [131] |                                             | Leuprolide; Nelfinavir; Ritonavir; Teniposide; Valrubicin                          |
| ZINC                  | 1,615 FDA-approved drugs and available in the market [132] |                                             | R428; Teniposide; VS-5584; Setileton                                                |
| 4,384 approved drugs [133] | 5,903 FDA-approved clinical drugs [134] |                                             | Dihydroergotamine (Kd = 107.6 μM); Midostaurin (Kd = 43.5 μM); Ziprasidone         |
| PubChem               | 77 FDA-approved drugs [135] |                                             | Ergotamine; Bromocriptine; Meclozycine; Amrubicin                                   |
| 400 commercially available curcumin analogues [136] |                                             | Viomycin; Capastat; Carfilzomib; Saquinavir                                         |
| PubChem; DrugBank; Selleckchem | 3,809 conformations [137] | HTVS; Molecular docking; MD simulation | Lopinavir-Ritonavir; Tipranavir; Raltegravir                                        |
| Selleckchem; Targetmol; e-Drug3D; Reaxys | 487 FDA-approved drugs [139] |                                             | Cyclohexanone                                                                       |
| In-house database     | 2,000 approved drugs [142] | VS; Experimental evaluation | ChEMBL275592; Montelukast; ChEMBL288347; Bromocriptine; Saquinavir                 |
| Screen-Well           | 774 FDA-approved drugs [143] | HTVS; Molecular docking | Amikacin (Kd = 17.5 μM)                                                              |
| DTC; BindingDB        | 3,410 FDA-approved drugs [144] | Deep learning | Ribavirin; Telmivudine; Vitamin B12; Nicotinamide                                   |
| Literatures           | 100 clinically approved drugs [145] | Experimental evaluation | Angiotensin II; GHRP-2; Indinavir; Polymyxin B; Fexofenadine; Atazanavir; Cobicistat; Cospfunglin; Lopinavir |
| PubChem               | 22 FDA-approved glucocorticoids [146] | Molecular docking; MD simulations | Perampanel; Carprofen; Celecoxib; Alprazolam; Truvaeloxin; Sarafloxaclin; Ethyl biscomuacetate |
| 40 FDA-approved non-steroidal anti-inflammatory drugs [147] | 9 FDA-approved angiotensin receptor blocker drugs [148] |                                             | Manidipine (IC50 = 4.8 μM); Boceprevir(IC50 = 5.4 μM); Lercanidpine (IC50 = 16.2 μM); Bedaquine (IC50 = 18.7 μM); Elodipine (IC50 = 38.5 μM) |
| Enamine               | 8960 commercially available compounds [149] | HTVS; Molecular docking; MD simulation | Ethachyric acid (IC50 = 1.11 μM); Naproxen (IC50 = 3.45 μM); Allopurinol (IC50 = 3.77 μM); Butenafine hydrochloride (IC50 = 5.40 μM); Ramifoxine hydrochloride (IC50 = 5.61 μM); Tranilcypromine hydrochloride (IC50 = 8.64 μM); Saquinavir mesylate (IC50 = 9.92 μM) |
|                      |                                             |                                             | Atazanavir (Kd = 94.94 nM); Remdesivir (Kd = 113.3 μM); Efavirenz (Kd = 199.17 nM); Ritonavir (Kd = 204.05 nM); Dolutegravir (Kd = 336.91 nM) Teicoplanin (IC50 = 1.5 μM) |
|                      |                                             |                                             | Ciclesonide; Dexamathasone; Betamathasone; Hydrocortisone; Fludrocortisone; Triamcinolone; Sulfinpyrazone; Indomethacin; Auranofin |
|                      |                                             |                                             | Olmesartan (IC50 = 1.808 μM)                                                        |
|                      |                                             |                                             | Z1244904919 (IC50 = 0.73 μM); Z1759961336(IC50 = 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 3CLpro are listed in Table S5.
† The number of all compounds used for drug screening.

3.2. Inhibitors of SARS-CoV-2 3CLpro 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. Drugrepurposing 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-
cokinetics 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 3CLpro among the approved or commercially available drugs. Of course, this strategy 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), and some other databases.

### Table 2

Potential inhibitors of SARS-CoV-2 3CLpro selected from existing databases or literature.

| Source | Number | Method | Potential inhibitor |
|--------|--------|--------|--------------------|
| ZINC   | 2,000  | VS     | ZINC32960814/12006217/03231196/33173588 |
|        | 1,500  | [151]  |                    |
|        | 5,811  | [152]  | Telcagepant; Vidipirapant; Pizotinib; Fostamatinib. |
|        | 606    | [153]  | (--)–Taxifolin; Rhamnetin |
|        | 1,000  | [154]  | ZINC000621278586/000621285995 |
| Asinex: CHEMBL | > 8,722 | [155]  | B88_26580140; SCEMBL12616233/18616095/20148701 |
| SuperNatural II; In-house database; SuperDrug2; WithDrawn | 360,000 | [156]  | SN00017653 (SuperNatural II); Pseudostellarin C (in-house); Eledoisin (SuperDrug2); Naldemedine (SuperDrug2); Saralenin (WithDrawn); Saquinavir (WithDrawn) |
| Chemical Abstract Services | 35,000 | [157]  | SKS-01; SKS-02; SKS-03; SKS-04; pq8; pq9; pq10; A12 |
| Literature; PubChem; Asinex | > 10,584 | [171]  | Note: Structures of these inhibitors are shown in Fig. 52. |
| Protein Data Bank | 2,892 | [159]  | PubChem IDs: 118098670; 104161460; 163632044 |
| Literature | 49 | [160]  | Ethaselen (IC50 = 4.51 μM) |
| PubChem | 10,433 | [161]  | 6-Deaminofungin; UNII-09H5KY11SV |
| Life Chemicals; Asinex | 21,207 | [162]  | F2679-0163 (Life chemicals); F6355-0442 (Life chemicals); 8250 (Asinex) |
| Pharmit | 213.5 million |  | CSC05775219; PubChem-22029441/-11210821; MCULE-934978441; MolPort-045-918-905 |
| DrugBank; Literature; In-house database | 2,736 | [164]  | Acetyloside; Chebulinic acid; Delphinidin-3,5-diglucoside; Saquinavir; Lithospermic acid B; 11m_32045235 |
| DrugBank | 10,038 | [165]  | DB02388; Cobicistat |
|         | 10,246 | [166]  | Levothyroxine; Anosobartal; ABP-700 |
|         | 13,227 | [167]  | Dipyridamole (Ki = 0.04 μM); Hydroxychloroquine (Ki = 0.36 μM); Chloroquine (Ki = 0.56 μM) |
| LASSBio | 2,300 | [172]  | LASSBio-1945 (IC50 = 15.97 μM) |
| DrugBank; PubChem | 5,016 | [173]  | Note: These inhibitors have been further confirmed [175–179]. |
| Compound Library | 23,000 | [174]  | Isavuconazonium; α-KI; Pentagastatin |
| In-house database; Targetmol; Selleckchem; Antivirus Drug Library | ~ 10,000 | [54]  | Note: Structures of these inhibitors are shown in Table S7. |
| AVPdb database | 88 | [180]  | Melatonin |
| Existing drug database | > 2,500 | [171]  | Dipyridamole (Ki = 0.04 μM); Hydroxychloroquine (Ki = 0.36 μM); Chloroquine (Ki = 0.56 μM) |
| LASSBio | 2,300 | [172]  | LASSBio-1945 (IC50 = 15.97 μM) |
| DrugBank; PubChem | 5,016 | [173]  | Isavuconazonium; α-KI; Pentagastatin |
| Compound Library | 23,000 | [174]  | Azanitrile (Ki = 24.0 nM); Pyridyl ester (Ki = 10.0 nM) |
| In-house database; Targetmol; Selleckchem; Antivirus Drug Library | ~ 10,000 | [54]  | Note: These inhibitors have been further confirmed [175–179]. |
| AVPdb database | 88 | [180]  | Note: Structures of these inhibitors are shown in Table S7. |

Note: The binding mechanisms of all inhibitors to 3CLpro are listed in Table S6.

a These compounds were selected according to the pharmacophore features of inhibitor N3.

b Approved or investigational drugs.

c Protease-inhibitor-like compounds.

d Analogs of Chloroquine.

e Se-containing heterocyclic compounds.

f The compounds that have > 90% structural similarity with the 10 high-affinity molecules previously reported (PubChem IDs: 5281605, 16394003, 19323586, 44137675, 118173648, 787440, 8368889, 34755, 65482 and 145998233).

g 12,485 anti-SARS-CoV-2 3CLpro inhibitors (13b) [58] in DrugBank and 5,010 analogs of 11 antiviral agents (Atazanavir, Darunavir, Fosamprenavir, Indinavir, Lopinavir, Ritonavir, Saquinavir, Tipranavir, Darvadione, Nevirapine and Remdesivir) in PubChem.
Table 3
Dual inhibitors against multiple SARS-CoV-2 targets including nsp3, nsp5 or both.

| Promising dual inhibitor | Source | Target |
|--------------------------|--------|--------|
| Nalonoxone; Fluoxetine; Dopal; Thiamine Phenylephrine; Epinephrine; Aspirin Pseudophedrine; Benzenebutyrate; Nelfinavir; Tipranavir | ZINC database [181,182] | Nsp5; PLpro* |
| Bemcentinib; MFCD00832476 | ZINC PubChem and NPASS® databases [183] | |
| Ginkgoic acid (IC50 = 1.79; 16.30 μM) | MedChemExpress [184] | |
| Anacardic acid (IC50 = 2.07; 17.08 μM) | CAS Antiviral COVID19 database [185] | Nsp5; RdRp* |
| 2001083-68-5; 2001083-69-6; 833463-19-7 | CAS Antiviral COVID19 database [185] | Nsp5; PLpro* |
| Nakinadine B; Amphilmedos sp | CAS Antiviral COVID19 database [185] | Nsp5; PLpro* |
| Hydroxymatairesinol | MCULE-3732245601–0 | Nsp5; PLpro*; RdRp* |
| (IC50 = 0.45; 0.085; 0.29 μM) | MCULE library [188] | Nsp5; PLpro*; RdRp* |
| MCULE-701337725–0 | CAS Antiviral COVID19 database [185] | Nsp5; fumarate protease |
| 19 acetylisteratin 3 | H. erectus [189] | Nsp5; ns15 |
| 12j-acetoxy-16-epi-hyrtiolide | PubChem database [190] | Nsp5; ns16 |
| Cefiderocol; Plazomicin | DrugBank [191] | Nsp5; N protein |
| Vanganciclovir | PubChem database [190] | Nsp5; S protein |
| Rutin | DrugBank [191] | |
| Solanone, Acetoside; Rutin; Withanolone | PubChem database [192,193] | |

* NPASS: Natural Product Activity and Species Source.
* RdRp: RNA-dependent RNA polymerase.

ZINC, PubChem, in-house [118], Screen-Well ® FDA v. 2.0 Approved Drug, Drug Target Common (DTC) (https://drugtargetcommons.fnim.nl), 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.DSupplemental), 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/wwn-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.ufpr.br), PDB database, SwissSimilarity (http://www.swisssimilarity.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 3CLpro based on known inhibitor, key pharmacophore or active sites of Mpro [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: PLpro 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 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.

Acknowledgements

This work was supported by the National Key Research and Development Program of China [Grant number 2018YFA0903700]; and the National Natural Science Foundation of China [Grant numbers 21621004 and 31571358]. The authors would like to thank Prof. Chun-Ting Zhang for the invaluable assistance and inspiring discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2021.08.036.

References

[1] Wu YT, Ho WZ, Huang WY, Jin DY, Li SY, et al. SARS-CoV-2 is a representative name for the new coronavirus. Lancet 2020;395:949–50.
[2] Organization WH. NGO Director-General's remarks at the media briefing on 2019-nCoV on 11 February 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020, 2020; Accessed 11 February 2020.
[3] Sia SF, Yan LM, Chin AWH, Fung K, Choy KT, Wong AYL, et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. Nature 2020;583:834–8.
[4] Klopman G, Baker MA, Rhee C. Airborne transmission of SARS-CoV-2: theoretical considerations and available evidence. JAMA 2020;324:441–2.
[5] Helms J, Kremer S, Mjerdij H, Cleeuw R, Schenk C, Muckemmer C, et al. Neurologic features in severe SARS-CoV-2 infection. New Engl J Med 2020;382:2268–70.
[6] Wu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol 2020;19:141–54.
[7] Poland GA. SARS-CoV-2: a time for clear and immediate action. Lancet Infect Dis 2020;20:231–2.
[8] Ivanov D. Predicting the impacts of epidemic outbreaks on global supply chains: a simulation-based analysis on the coronavirus outbreak (COVID-19/ SARS-CoV-2) case. Transport Res E-Log 2020;136:101592.
[9] Sayers EW, Agarwala R, Bolton EE, Brister JR, Guanse K, Clark K, et al. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 2019;47:D23–8.
[10] Chen Y, Liu QY, Guo DY. Emerging coronaviruses: genome structure, replication, and pathogenesis. J Med Virol 2020;92:418–23.
[11] Ziebuhr J, Snijder EJ, Gorbalenya AE. Virus-encoded proteinases and their implications to drug design against SARS-CoV-2. Signal Transduct Target Ther 2020;5:1949–60.
[12] Niu L, Wittrock KN, Cabagra GC, Siravastava V, Cho MW. A structural landscape of neutralizing antibodies against SARS-CoV-2 receptor binding domain. Front Immunol 2021;12:647934.
[13] Gavor E, Choong YK, Er SY, Siravaram H, Siravaram J. Structural basis of SARS-CoV-2 and SARS-CoV antibody interactions. Trends Immunol 2020;41:1006–22.
[14] Zhang CX, Zheng W, Huang XQ, Bell EW, Zhou XG, Zhang Y. Protein structure can predict the spike protein of SARS-CoV-2. Expert Opin Therar Ther 2021;25:413–24.
[15] Robson B. The use of knowledge management tools in viroinformatics. Example study of a highly conserved sequence motif in NSp5 of SARS-CoV-2 as a therapeutic target. Comput Biol Med 2020;125.
[16] Harcourt BH, Jukeliene D, Kanjanahaluethai A, Bechil J, Severson KM, Smith CM, et al. Identification of severe acute respiratory syndrome coronavirus replicate products and characterization of papain-like protease activity. J Virol 2004;78:13600–12.
[17] Michalska K, Kim Y, Jedrzejczak R, Maltese NI, Stols L, Endres M, et al. Crystal structures of SARS-CoV-2 ADP-ribose phosphatase: from the apo form to ligand complexes. JBC 2020;7:8142–23.
[18] Chen Y, Liu QY, Guo DY. Emerging coronaviruses: genome structure, replication, and pathogenesis. J Med Virol 2020;92:418–23.
[19] Ziebuhr J, Snijder EJ, Gorbalenya AE. Virus-encoded proteinases and their implications to drug design against SARS-CoV-2. Signal Transduct Target Ther 2020;5:1949–60.
[20] Niu L, Wittrock KN, Cabagra GC, Siravastava V, Cho MW. A structural landscape of neutralizing antibodies against SARS-CoV-2 receptor binding domain. Front Immunol 2021;12:647934.
[21] Gavor E, Choong YK, Er SY, Siravaram H, Siravaram J. Structural basis of SARS-CoV-2 and SARS-CoV antibody interactions. Trends Immunol 2020;41:1006–22.
[22] Zhang CX, Zheng W, Huang XQ, Bell EW, Zhou XG, Zhang Y. Protein structure can predict the spike protein of SARS-CoV-2. Expert Opin Therar Ther 2021;25:413–24.
[23] Robson B. The use of knowledge management tools in viroinformatics. Example study of a highly conserved sequence motif in NSp5 of SARS-CoV-2 as a therapeutic target. Comput Biol Med 2020;125.
[24] Harcourt BH, Jukeliene D, Kanjanahaluethai A, Bechil J, Severson KM, Smith CM, et al. Identification of severe acute respiratory syndrome coronavirus replicate products and characterization of papain-like protease activity. J Virol 2004;78:13600–12.
[25] Michalska K, Kim Y, Jedrzejczak R, Maltese NI, Stols L, Endres M, et al. Crystal structures of SARS-CoV-2 ADP-ribose phosphatase: from the apo form to ligand complexes. JBC 2020;7:8142–23.
[26] Chen Y, Liu QY, Guo DY. Emerging coronaviruses: genome structure, replication, and pathogenesis. J Med Virol 2020;92:418–23.
[27] Ziebuhr J, Snijder EJ, Gorbalenya AE. Virus-encoded proteinases and their implications to drug design against SARS-CoV-2. Signal Transduct Target Ther 2020;5:1949–60.
[28] Niu L, Wittrock KN, Cabagra GC, Siravastava V, Cho MW. A structural landscape of neutralizing antibodies against SARS-CoV-2 receptor binding domain. Front Immunol 2021;12:647934.
[29] Gavor E, Choong YK, Er SY, Siravaram H, Siravaram J. Structural basis of SARS-CoV-2 and SARS-CoV antibody interactions. Trends Immunol 2020;41:1006–22.
Selvaraj C, Dinesh DC, Panwar U, Boura E, Singh SK. High-throughput screening and quantum mechanics for identifying potent inhibitors against M^1 domain of SARS-CoV-2 Nsp3. IEEE T Comput Bi 2021;18:1362–70.

Jung LS, Gund TM, Napper M. Comparison of binding site of remdesivir and its metabolites with NSP12-NSP7-NSP6, and NSP3 of SARS-CoV-2 virus and alternative potential drugs for COVID-19 treatment. Protein J 2020;39:619–30.

Singh AK, Keshwaha PP, Prajapati KS, Shaiba M, Gupta S, Kumar S. Identification of FDA approved drugs and nucleotide analogues as potential SARS-CoV-2 A1 pp domain inhibitor: an in silico study. Comput Biol Med 2021;130:104185.

Debnath P, Debath B, Bhaumik S, Debath S. In silico identification of potential inhibitors of ADP-Ribose phosphate of SARS-CoV-2 nsp3 by combining E-pharmacophore- and receptor-based virtual screening of database. ChemNetSelect 2020;5:9388–96.

De Vito M, Masetti M, Botrugno G, Cavaoli A. Role of molecular dynamics and related methods in drug discovery. J Med Chem 2016;59:4035–61.

Yan FF, Gao. Comparison of the binding characteristics of SARS-CoV and SARS-CoV-2 RBDs to ACE2 at different temperatures by MD simulations. Brief Bioinform 2021;22:1122–36.

Chen JZ, Wang XY, Pang LX, Zhang JZH, Zhu T. Effect of mutations on binding affinity of SARS-CoV-2 spike protein. J Med Chem 2020;63:6343–53.

Jo S, Kim S, Kim DY, Kim KY, Shin DH. Flavonoids with inhibitory activity against SARS-CoV-2 3CLpro. J Biomol Struct Dyn 2020. https://doi.org/10.1080/07391102.2020.1796808.

Abian O, Ortega-Alarcon D, Jimenez-Alesanco A, Ceballos-Laita L, Vega S, Reyburn HT, et al. Structural stability of SARS-CoV-2 3CLpro and identification of quercetin as a potent inhibitor by experimental screening. Int J Biol Macromol 2020;164:1693–703.

Sen D, Debath P, Debath N, Bhaumik S, Debath S. Identification of potential inhibitors of SARS-CoV-2 main protease and spike receptor from 10 important spaces through structure-based virtual screening and molecular dynamics study. J Biomol Struct Dyn 2020;37:1805–16. https://doi.org/10.1080/07391102.2020.1811883.

Cherrak SA, Merzouk H, Mokhtari-Soulamine N. Potential bioactive glycosylated flavonoids as SARS-CoV-2 main protease inhibitors: a molecular docking and simulation studies. PLoS ONE 2020;15:e0240653.

Abd El-Mordy FM, El-Hamouly MM, Ibrahim MT, Abd El-Rahim M, Aly OM, Al-Barhan A, El-Merief AM, et al. Discovery of 48 novel inhibitors of SARS-CoV-2 main protease by phenolic compounds from Manilkara hirsuta (R. Jack) Dubb assisted by metabolite profiling and in silico virtual screening. RSC Adv 2020;10:32148–55.

Huyhn T, Wang H, Luan B. Structure-based lead optimization of herbal medicine rutin for inhibition of SARS-CoV-2’s main protease. Phytochem Phys Chem Phys 2020;22:2533–43.

Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci Food Technol 2016;5:47.

Ferriozza F, Faramarzi P, Patricio S, Faramarzi S. Anthocyanin derivatives as potent inhibitors of SARS-CoV-2 main protease: an in-silico perspective of therapeutic targets against COVID-19 pandemic. J Biomol Struct Dyn 2020. https://doi.org/10.1080/07391102.2020.1801510.

Zhu Y, Xie D. Docking characterization and in vitro inhibitory activity of flavan-3-ols and dimeric proanthocyanidins against the main protease activity of SARS-CoV-2. Front Plant Sci 2020;11:603136.

Dai H, Liu Y, Zhang JH, Yao SR, Liu J, Jiang X, et al. Discovery and characterization of tannins in green tea: in the design of hynarins. New Phytol 2020;226:1104–16.

Wang PQ, Liu Y, Zhang JL, Wang WZ, Hou H, Zhao Y, et al. Functional demonstration of plant flavonoid carbonations proposed to be involved in the biosynthesis of proanthocyanidins. Plant J 2020;101:18–36.

Upadhyay S, Tripathi PK, Singh M, Raghavendra S, Bhardwaj M, Patel AK. Evaluation of medicinal herbs as a potential therapeutic option against SARS-CoV-2: a comprehensive review. Phytother Res 2020;34:3411–9.

Ghosh R, Chakraborty A, Biswas A, Chowdhuri S. Identification of polyphenols from Broussonetia papyrifera as SARS-CoV-2 main protease inhibitors using in silico docking and molecular dynamics simulation approaches. J Biomol Struct Dyn 2020. https://doi.org/10.1080/07391102.2020.1803447.

Ghosh R, Chakraborty A, Biswas A, Chowdhuri S. Depicting the inhibitory potential of polyphenols from Isatis indigotica root against the main protease of SARS-CoV-2 using computational approaches. J Biomol Struct Dyn 2020. https://doi.org/10.1080/07391102.2020.1855184.

Shidham VN, Gabe S, Brown NM, Alghaith AH, Dossari R. Phytoc hemicals of rhus spp. as potential inhibitors of the SARS-Cov-2 main protease: molecular docking and drug-likeness study. Evid-Based Compl Alt 2021;2021:818490.

Roy A. A review on the alkaloids an important therapeutic compound from plant and its metabolites. JPR 2020;7:105.

Garg S, Roy A. In silico analysis of selected alkaloids against main protease (Mpro) of SARS-CoV-2. Chem-Biol Interact 2020;332:109309.

Abdelhame DA, Ahmed SA, Ab El-Maged HR, Mohamed HS, Rahman AA, Elsayed KNM, et al. The inhibitory effect of some natural bioactive compounds against SARS-COV-2 main protease: insights from molecular docking analysis and molecular dynamic simulation. J Environ Sci Heal A 2020;55:1793–806.

Cybici CA, Ogoanu OB, Adegunloye AP, Oguguehi OM, Afolabi SD. Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CLpro): an in silico screening of alkaloids and terpenoids from African medicinal plants. J Biomol Struct Dyn 2021;39:4362–74.

Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. Adv Nutr 2011;2:32–50.

Bergman ME, Davis B, Phillips MA. Medically useful plant terpenoids: biosynthesis, occurrence, and mechanism of action. Molecules 2021;26:3961.

Chang FR, Yen CT, El-Shazly M, Lin WF, Yen MH, Kim KH, et al. Anti-human coronavirus (anti-HCoV) Interperons from the leaves of euphorbia neriifolia. Nat Prod Commun 2021;15:1143–7.

Cnat J, Morgenstern B, Bauer G, Candra P, Rabenau H, Doerr HW, Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus, Lancet 2003;361:2045–6.

Debnath P, Debath B, Bhaumik S, Debath S. In silico identification of potential inhibitors of SARS-CoV-2 3CLpro inhibitors from molecular modeling studies. Biomolecules 2021;11:74.

Jo S, Kim S, Shin DH, Kim MS. Inhibition of SARS-CoV 3CL protease by flavonoids. J Enzym Inhib Med Chem 2020;35:145–51.
Abdusalam AAA, Murugaiyah V. Identification of potential inhibitors of 3CL protease of SARS-CoV-2 from ZINC database by molecular docking-based virtual screening. Front Mol Biosci 2020;7:100337.

Haider Z, Subhani MM, Farooq MA, Ishag M, Khalid M, Akram MN, et al. In-silico pharmacophoric and molecular docking-based drug discovery against the Main Protease (M pro) of SARS-CoV-2, a causative agent COVID-19. Pak J Pharm Sci 2020;33:2097–705.

Liu S, Zheng Q, Wang ZY. Potential covalent drugs targeting the main protease of the SARS-CoV-2 coronavirus. Bioinformatics 2020;36:3295–8.

Fischer A, Sellner M, Naranjan S, Snieuwo M, Lill MA. Potential inhibitors for novel coronavirus protease identified by virtual screening of 608 million compounds. Int J Mol Sci 2020;21:3626.

Kavitha K, Sivakumar S, Ramesh B. 1,2,4 triazolo 1,5-a pyrimidin-7-ones as potential inhibitors of 3CLpro of SARS-CoV-2: an in silico docking study. J Mol Sci 2021;33:115636.

Abel R, Paredes Ramos M, Chen Q, Pérez-Sánchez H, Coluzi F, Rocco M, et al. Computational prediction of potential inhibitors of the main protease of SARS-CoV-2. Front Chem 2020;8:590263.

Zia K, Khan SA, Ashraf S, Nur-e-Alam M, Ahmed S, Ul-Haq Z. Probing CAS database as prospective antiviral agents against SARS-CoV-2 main protease. J Mol Struct 2021;1213:129953.

Andrianov AM, Kornoushenko YV, Karpenko AD, Bosko IP, Tuzikov AV. Computational discovery of small drug-like compounds as potential inhibitors of SARS-CoV-2 main protease. J Biomol Struct Dyn 2020. https://doi.org/10.1080/07391102.2020.1799080.

Galahawat A, Kumar N, Kumar R, Sandhu H, Singh IP, Singh S, et al. Structure-based virtual screening to discover potential lead molecules for the SARS-CoV-2 main protease. J Biomol Struct Dyn 2020. https://doi.org/10.1080/07391102.2020.1799908.

Ibrahim MAA, Abdelrahman AHM, Hegazy M EF. In-silico drug repurposing and molecular dynamics puzzled out potential SARS-CoV-2 main protease inhibitors. J Biomol Struct Dyn 2020. https://doi.org/10.1080/07391102.2020.1799558.

Tjeera E, Munteans CR, López-Cortés A, Cabrera-Andrade A, Pérez-Castillo Y. Drugs repurposing using QSAR, docking and molecular dynamics for possible inhibitors of the SARS-CoV-2 Mpro protease. Molecules 2020;25:5172.

Pinzi L, Tuinwelja A, Caporuscio F, Rastelli G. Drug repurposing and polymopharmacology to fight SARS-CoV-2 through inhibition of the main protease. Front Pharmacol 2021;12:636989.

Tsui P. Potential anti-SARS-CoV-2 drug candidates identified through virtual screening of the ChEMBL database for compounds that target the main coronavirus protease. FEBS Open Bio 2020;10:995–1004.

Hakmi M, Bouricha EM, Kandossi I, Harti JE, Ibrahim A. Repurposing of known anti-virals as potential inhibitors for SARS-CoV-2 main protease using molecular docking analysis. Bioinformation 2020;16:301–6.

Feitosa EL, Júnior FTDS, Nery Neto JADO, Matos LFL, Moura MHDS, Rosales TO, et al. COVID-19: rational discovery of the therapeutic potential of Melatonin as a SARS-CoV-2 main protease inhibitor. Int J Med Sci 2020;17:2133–46.

Li Z, Li X, Huang YY, Wu Y, Liu R, Zhou L, et al. Identify potent SARS-CoV-2 main protease inhibitors via accelerated free energy perturbation-based virtual screening of existing drugs. P Natl Acad Sci 2020;117:23781–7.

Franco LS, Maia RC, Barreiro EJ. Identification of LASSBio-1945 as an inhibitor of SARS-CoV-2 main protease (M-PRO) through in silico screening supported by molecular docking and a fragment-based pharmacophore model. RSC Med Chem 2021;12:110–9.

Achililonu I, Iwuchukwu EA, Achililon OJ, Fernandes MA, Sayed Y. Targeting the SARS-CoV-2 main protease using FDA-approved isavuconazonium, a P2–P3 n-ketamine derivative and Pentagatrin: An in-silico drug discovery approach. J Mol Graph Model 2020:101:107730.

Breidenbach J, Lemke C, Pillayyar T, Schäkel I, Al Hamwi W, Diett M, et al. Targeting the main protease of SARS-CoV-2: from the establishment of high throughput screening to the design of tailored inhibitors. Angew Chem Int Ed 2021;60:2–9.

Jin ZM, Zhao Y, Sun Y, Zhang B, Wang HF, Wu Y, et al. Structural basis for the inhibition of SARS-CoV-2 main protease by antineoplasticdrug carmofur. Nat Struct Mol Biol 2020;27:529–32.

Menéndez CA, Bylén F, Perez-Lemus GR, Alvarado W, de Pablo JJ. Molecular characterization of ebselen binding activity to SARS-CoV-2 main protease. Sci Adv 2020;6:eabb00345.

Ma CL, Hu YM, Townsend JA, LagariasPJ, Marty MT, Kolocouris A, et al. Ebselen, disulfiram, carmofur, PX-12, tideglib, and shikonin are non-specific promiscuous SARS-CoV-2 main protease inhibitors. ACS Pharmacol Transl Sci 2021;4:1265–77.

Xu L, Tong J, Wu Y, Zhao S, Lin B-L. A computational evaluation of targeted optimization strategy (TOS) for potential inhibition of SARS-CoV-2 by disulfiram and analogues. Biopharm Chem 2021;276:106610.

Li J, Zhou X, Zhang Y, Zhong F, Lin C, McCormick PJ, et al. Crystal structure of SARS-CoV-2 main protease in complex with the natural product inhibitor shikonin illuminates a unique binding mode. Sci Bull 2021;66:661–3.

Mamudh S, Paul GK, Biswas S, Afrose S, Sita MA, Hasan MR, et al. Prospective role of peptide-based antiviral therapy against the pain Protease of SARS-CoV-2. J Biophys Chem 2021;18:628585.

Rajput S, Alagumuthu M, Baig MS. Dual targeting of 3CLPR and 3CLnP of SARS-CoV-2: a novel structure-based design approach to treat COVID-19. Curr Res Biol Sci 2021;3:9–18.

Mitra K, Ghanta P, Acharya S, Chakrapani G, Ramabia B, Doble M. Dual inhibitors of SARS-CoV-2 proteases: pharmacophore and molecular dynamics based drug repositioning and phytochemical leads. J Biomol Struct Dyn 2020. https://doi.org/10.1080/07391102.2020.1101092.

Jade D, Ayappaunnam S, Tallapaneni V, Poojamma N, Ramaiah B, Almeidi AM, et al. Virtual high throughput screening: Potential inhibitors for SARS-CoV-2 PRmP CLP and 3CLPrP proteases. Eur J Pharmacol 2021;901:174082.

Chen Z, Cui Q, Cooper L, Zhang P, Lee H, Chen Z, et al. Ginkgolic acid and anacardic acid are specific covalent inhibitors of SARS-CoV-2 cysteine proteases. Cell Biosci 2021;11:45.

Aouadate A, Ghaleb A, Chitita S, Aarjane M, Ousaa A, Maghat H, et al. Identification of a novel dual-target scaffold for 3CLpro and RdRp proteins of SARS-CoV-2 using 3D-similarity search, molecular docking, molecular dynamics and ADMET evaluation. J Biomol Struct Dyn 2021;39:4522–35.

Shady NH, Hayalah AM, Mohamed MFA, Ghoneim MM, Chilingaryan G, Al-Sawalha MA, et al. Targeting of SARS-CoV-2 activity of chloroquine and its analogs and in silico screening of main protease inhibitors. J Proteome Res 2021;20:100453.

Seligny SA, Fayed B, Hamdy R, Mahrous N, Mostafa A, Almeidi AM, et al. Promising anti-SARS-CoV-2 drugs by effective dual targeting against the viral and host proteases. Bioorg Med Chem Lett 2021;43:123899.

Elhady SS, Abdelhamed RFA, Mahalati RT, Alhald Al, Bogari HA, Almalki AJ, et al. Molecular docking and dynamics simulation study of tyrosin ecretis isolated scalarane sesterterpenes as potential SARS-CoV-2 dual target inhibitors. Bioinformatics 2021;10:385.

Yadav R, Parihar RD, Dhiman U, Dhajja P, Kumar S. Docking of fda approved drugs targeting nsp-16, n-protein and main protease of sars-cov-2 as dual inhibitors. Biointerface Res Appl Chem 2020;11:9846–81.

Kumar A, Rajput VS, Nagraal P, Koukreti H, Grover S, Grover A. Dual inhibition of SARS-CoV-2 spike and main protease through a repurposed drug, rutin. J Biomol Struct Dyn 2020. https://doi.org/10.1080/07391102.2020.1864476.

Teli DM, Shah MB, Chhabria MT. In silico screening of natural compounds as potential inhibitors of SARS-CoV-2 main protease and Spike RBD: targets for COVID-19. Front Mol Biosci 2021;7:990079.

Patil VS, Hupparare BB, Malgi AP, Deshpande SH, Patil SA, Mallapur SP. Dual inhibition of COVID-19 spike protein and main protease 3CLpro by Withanone from Withania somnifera. Chin Herb Med 2021;13:359–68.