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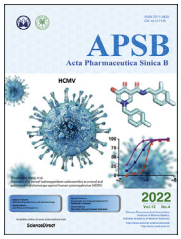

REVIEW

Recent advances in developing small-molecule inhibitors against SARS-CoV-2

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Abstract The COVID-19 pandemic caused by the novel SARS-CoV-2 virus has caused havoc across the entire world. Even though several COVID-19 vaccines are currently in distribution worldwide, with others in the pipeline, treatment modalities lag behind. Accordingly, researchers have been working hard to understand the nature of the virus, its mutant strains, and the pathogenesis of the disease in order to uncover possible drug targets and effective therapeutic agents. As the research continues, we now know the genome structure, epidemiological and clinical features, and pathogenic mechanism of SARS-CoV-2. Here, we summarized the potential therapeutic targets involved in the life cycle of the virus. On the basis of these targets, small-molecule prophylactic and therapeutic agents have been or are being developed for prevention and treatment of SARS-CoV-2 infection.

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1. Introduction

In 2019, a new infectious respiratory disease emerged. A novel coronavirus was identified as the pathogen causing the outbreak of atypical pneumonia[1,2] and given a nomenclature of 2019 novel coronavirus (2019-nCoV) by the World Health Organization (WHO). The International Committee on Taxonomy of Viruses (ICTV) renamed the virus as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the disease as coronavirus disease 2019 (COVID-19)[7]. Some virologists suggested changing the name to human coronavirus 2019 (HCoV-19) to avoid confusion with SARS-CoV that emerged in 2002[3].

SARS-CoV-2 belongs to the genus betacoronavirus, together with SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV, with 82% and 50% homology, respectively)[4–6]. The main symptoms of COVID-19 include fever, fatigue, dry cough, upper chest discomfort and dyspnea. Severe cases are reported to show sepsis, secondary infections and organ failure. By 22 June 2021, COVID-19 had spread to more than 223 countries with more than 178,360,849 confirmed cases reported globally, including more than 3,869,384 deaths (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). On 30 January 2020, WHO declared the COVID-19 outbreak to be a global public health emergency, and on 11 March 2020, WHO characterized COVID-19 as a pandemic[1]. While several COVID-19 vaccines have been approved for general or emergency use, no specific antiviral drugs are now available for prophylaxis or treatment of SARS-CoV-2[19,244]. Therefore, it is still urgently needed to develop effective prophylactics and therapeutics against SARS-CoV-2 infection. Here, we summarized the potential therapeutic targets involved in the life cycle of the virus, treatments now in clinical use, the progress of candidate drugs, and potential prophylactic and therapeutic drugs based on these targets to treat COVID-19 in the future.

2. Viral structure

SARS-CoV-2 is an enveloped virus with a positive-sense, single-stranded RNA [(+) ssRNA] genome of ~30 kb[8] (Fig. 1). Upon cell entry, genomic RNA is translated as either ORF1a or ORF1ab owing to a frame shift, which is then cleaved into nonstructural proteins (nsps) by viral proteinases. These nsps are mainly responsible for the replication and transcription of genomic RNA. A series of subgenomic RNAs (sgRNAs) are discontinuously transcribed and finally translated into structural proteins [spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N)], and several accessory proteins (3a, 3b, 6, 7a, 7 b, 8, 9b, 9c and 10)[8,9]. A lipid bilayer comprising the S protein, the M protein and the E protein cloaks the helical nucleocapsids, consisting of the N protein that is associated with the viral RNA (Fig. 1).

3. SARS-CoV-2 life cycle and potential targets for the development of small-molecule inhibitors against SARS-CoV-2 infection

SARS-CoV-2 enters the target cell through two different ways, either plasma or endosomal membrane fusion (Fig. 2). The S protein of SARS-CoV-2 mediates the attachment of virus to the membrane of the host cell through its interaction with angiotensin-converting enzyme 2 (ACE2) and cellular heparan sulfate as the entry receptors, respectively[10,11]. For plasma membrane fusion, the S protein can be activated by transmembrane protease serine 2 (TMPRSS2) in close proximity to the ACE2 receptor, which initiates fusion between the viral membrane and the plasma membrane. In the absence of TMPRSS2, SARS-CoV-2 can be internalized *via* endocytosis[10]. After the virus enters the cell *via* the endocytic pathway, a lysosome-mediated drop in pH occurs in the endosome. The low pH environment activates endosomal proteinases, such as cathepsin B/L, which activate the S protein and prepares the virus for subsequent steps of fusion[10,12].

After membrane fusion, either with the host cell membrane or the endosome membrane, the viral (+)-sense genomic RNA [(+) gRNA] is released into the cytoplasm to allow translation of the two polyproteins, pp1a and 1 ab. Autoproteolytic cleavage of polyproteins produces more than a dozen nsps[13], including papain-like protease (PLpro), 3C-like protease (3CLpro), RNA-dependent RNA polymerase (RdRp) and helicase. Of these, PLpro and 3CLpro are responsible for polyprotein cleavage. And some nsps use (+) gRNA as a template to form a replicase−transcriptase complex.

After generating (−)-sense genomic RNA [(−) gRNA], it can serve as the template for the synthesis of (+) gRNA, which becomes the genome of the new virus particle[13,14]. Transmembrane structural proteins (S, M and E) undergo a series of steps in the endoplasmic reticulum (ER) including being synthesized, inserted and folded, and finally transported to the ER-Golgi intermediate compartment (ERGIC)[15]. The N proteins are translated in the cytoplasm and encapsulate (+) gRNA to form nucleocapsids[16]. Virion assembly occurs in the ERGIC, and virions are then transported from the ERGIC through the Golgi apparatus to the cell surface *via* small vesicles[16].

Potential small-molecule anti-SARS-CoV-2 drugs can be divided into two categories, depending on the targets. The first class of inhibitors target the viral proteins, such as S protein, viral enzymes (PLpro, 3CLpro, RdRp and helicase)[17–20], and some structural proteins[21,22] (Fig. 2 and Table 2). The second class of inhibitors can interact with the host proteins, such as the receptor ACE2 or heparan sulfate, the serine protease TMPRSS2, or the endosomal acid protease cathepsin L to prevent viral entry[10,23,24], as well as some regulators of the human immune system signaling pathways required for virus replication and infection[25,26] (Fig. 2 and Table 3).

4. Strategies for developing small-molecule SARS-CoV-2 inhibitors

Based on individual approaches used for the discovery of anti-SARS-CoV-2 agents, strategies can be divided into three major categories. The first one is virtual screening. Cryo-electron microscopy, X-ray crystallography, as well as homology modeling, could provide various efficacious protein structures, promoting molecular docking for rapid identification of hit or lead compounds through screening free or commercially accessible databases, such as ZINC, DrugBank, or ChemDiv. This approach is effective, economical, and time-saving. The second approach is the experiment-based high-throughput screening (HTS). Similar to virtual screening, the first objective of HTS is to identify active small molecules within compound libraries, including approved drugs, clinical trial drug candidates and even in-house compound databases. This method, which screens a large number of compounds against a known drug target, is also a time-consuming and expensive process with no guarantee of success. The third strategy

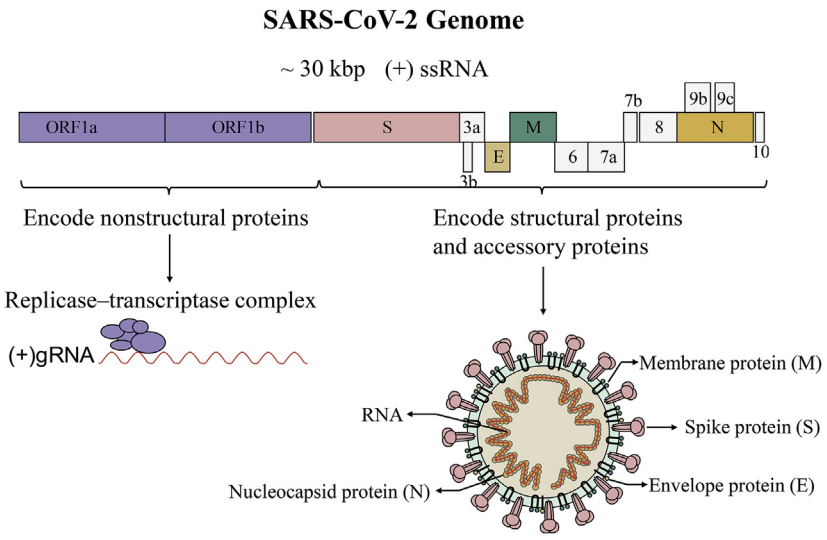

Figure 1 Schematic diagram of genome composition and particle structure of SARS-CoV-2. SARS-CoV-2 genome consists of open reading frames (ORFs) expressing structural proteins including spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N), and ORFs1a, 1 b, etc. Expressing non-structural and accessory proteins. Among them, N protein and (+) ssRNA form nucleocapsids with three other structural proteins to form mature viral particles.

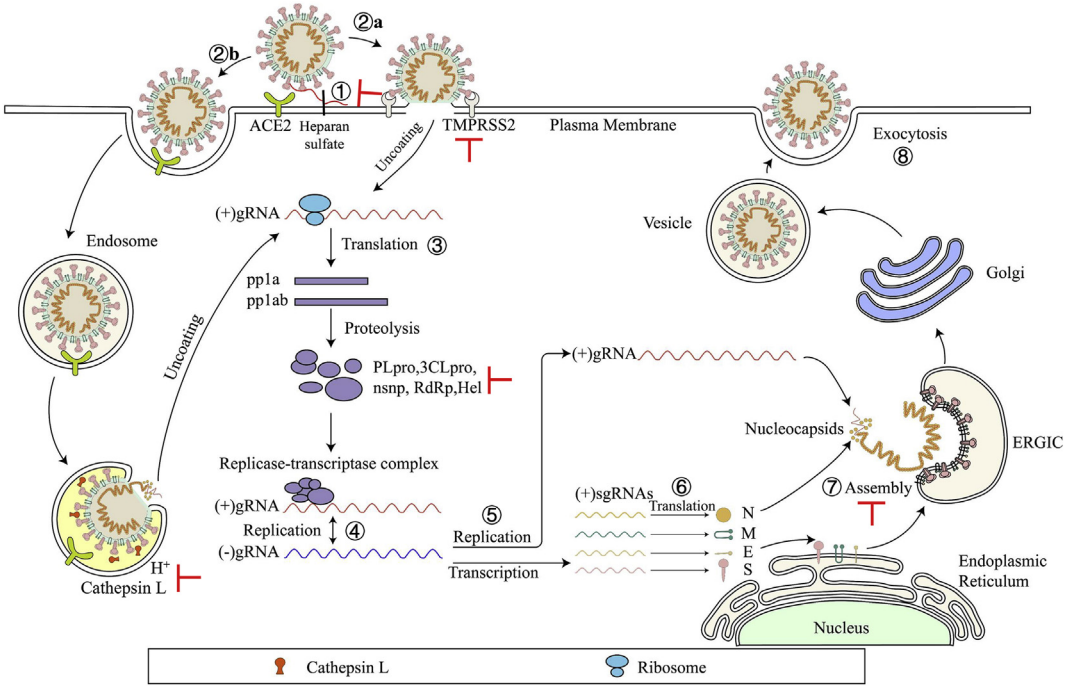

Figure 2 Life cycle of SARS-CoV-2. SARS-CoV-2 first binds, *via* its S protein, to the receptor ACE2 on the target cell (①). Then, the virus must gain access to the host cell cytosol through plasma (②a) or endosomal membrane fusion (②b). This is assisted by activation of S protein by TMPRRS2 (②a) or cathepsin B/L (②b), followed by fusion of the viral and cellular membranes. The viral genome is released, uncoated and translated into viral replicase polyproteins pp1a and 1 ab (③), which are then cleaved into nonstructural proteins (nsps) by viral proteinases as papain-like protease (PLpro) and 3C-like protease (3CLpro). Many of these nsps as RNA-dependent RNA polymerase (RdRp) or Helicase (Hel) then assemble into the replicase−transcriptase complex which replicates the (+)-sense genomic RNA ((+) gRNA). (−)-sense genomic RNA ((−) gRNA) is synthesized and used as a template to form (+) gRNA) and subgenomic RNAs (sgRNAs) (⑤). The viral structural proteins, S, E, and M are translated from sgRNAs (⑥) and inserted into the endoplasmic reticulum (ER), from where they are transported to the ER−Golgi intermediate compartment (ERGIC) to interact with the (+) gRNA-encapsidated N proteins and assemble into viral particles (⑦). The budded vesicles containing mature viral particles are then transported to the cell surface for release after maturation in the Golgi bodies (⑧). Possible targets for inhibitors are marked in red.

Table 1 Summary of peptide-based SARS-CoV-2 inhibitors.

Peptide	Sequence	Testing model	Activity IC_{50} (μmol/L)	Toxicity CC_{50} (μmol/L)	Clinical information	Ref.
EK1	SLDQINVTFLDLEYEMKKLEEA IKKLEESYIDLKEL	*In vitro*	2.468	–	Preclinical	45
EK1C4	SLDQINVTFLDLEYEMKKLEEAIKK LEESYIDLKELGSGSG-PEG4 (cholesterol)	*In vitro*	0.0365	≥5	Preclinical	45
IPB02	ISGINASVVNIQKEIDRLNEVAKNLNE SLIDLQELK (cholesterol)	*In vitro* (pseudovirus)	0.08 ± 0.017	–	Preclinical	49
SARS-CoV-2-HR2P (aa 1168−1203)	DISGINASVVNIQKEIDRLNEVAKNLN ESLIDLQEL	*In vitro* (pseudovirus)	0.98	–	Preclinical	50
$[SARS_{HRC}\text{-}PEG_4]_2$-chol	[DISGINASWNIQKEIDRLNEVAKNLNE SLIDLQEL-PEG4]2-chol	*In vivo*	~0.005	>100	Preclinical	51
SBP1	IEEQAKTFLDKFNHEAEDLFYQS	–	–	–	Preclinical	52
AHB1	DEDLEELERLYRKAEEVAKEAKDASRR GDDERAKEQMERAMRLFDQVFEL AQELQEKQTDGNRQKATHLDKAVKE AADELYQRVRELEEQVMHVLDQVSELAH ELLHKLTGEELERAAYFNWWATEMMLEL IKSDDEREIREIEEEARRILEHLEELARK	*In vitro*	0.035	–	Preclinical	53
AHB2	ELEEQVMHVLDQVSELAHELLHKLTGEELE RAAYFNWWATEMMLELIKSDDEREIR EIEEEARRILEHLEELARK	*In vitro*	0.016	–	Preclinical	53
LCB1	DKEWILQKIYEIMRLLDELGHAEASMRVSD LIYEFMKKGDERLLEEAERLLEEVER	*In vitro*	0.000024	–	Preclinical	53
LCB3	NDDELHMLMTDLVYEALHFAKDEEIKKRVF QLFELADKAYKNNDRQKLEKVVEEL KELLERLLS	*In vitro*	0.000048	–	Preclinical	53
ATN-161	Ac-PHSCN-NH2	*In vitro*	3.16	>1000	Preclinical	54
SARS-BLOCK Peptide **5**	Unknown	*In vitro* (pseudovirus)	Sub-micromolar	<20	Preclinical	57
P9	NGAICWGPCPTAFRQIGNCGHFKVRCCKIR	*In vitro*	2.4 μg/mL	–	Preclinical	184
P9R	NGAICWGPCPTAFRQIGNCGRFRVRCCRIR	*In vitro*	0.9 μg/mL	≥300 μg/mL	Preclinical	184
8P9R	(NGAICWGPCPTAFRQIGNCGRFRVRCCRIR)*8	*In vivo*	0.3 μg/mL	≥200 μg/mL	Preclinical	185

Table 2 Small-molecule SARS-CoV-2 inhibitors targeting viral proteins.

Number of chemical structures in Fig.	Inhibitor	Testing model	Activity IC_{50} (μmol/L)	Toxicity CC_{50} (μmol/L)	Clinical information	Ref.
Entry inhibitor						
Fig. 4 (**1**)	Salvianolic acid C (Sal-C)	*In vitro*	3.41	≥100	Preclinical	58
Fig. 4 (**2**)	Arbidol	*In vitro*	4.11	31.79	ChiCTR2000029573 NCT04252885	60,64
Fig. 4 (**3**)	DRI-C23041	*In vitro* (pseudovirus)	5.6	≥135	Preclinical	65
Fig. 4 (**4**)	Cepharanthine	*In vitro*	1.41	11.22	Preclinical	66
		In vitro	0.13	–	Preclinical	78
		In vitro	2.8	12.9	Preclinical	94
Fig. 4 (**5**)	Abemaciclib	*In vitro*	3.16	7.08	Preclinical	66
Fig. 4 (**6**)	Osimertinib	*In vitro*	3.98	10.00	Preclinical	66
Fig. 4 (**7**)	Trimipramine	*In vitro*	20.52	≥20	Preclinical	66
		In vitro	1.5	–	Preclinical	78
Fig. 4 (**8**)	Colforsin	*In vitro*	23.06	25.2	Preclinical	66
Fig. 4 (**9**)	Ingenol	*In vitro*	0.06	≥20	Preclinical	66
Fig. 4 (**10**)	Clofazimine	*In vitro*	0.31	–	Preclinical	68
Replication inhibitors Target 3CLpro						
Fig. 5 (**11**)	Tafenoquine	*In vitro*	2.5	–	Preclinical	72
		In vitro	~ 2.6	≥50	Preclinical	73
Fig. 5 (**12**)	**12**	*In vitro*	0.25 ± 0.15	≥100	Preclinical	74
Fig. 5 (**13**)	**13**	*In vitro*	0.15 ± 0.14	63.3 ± 2.3	Preclinical	74
Fig. 5 (**14**)	**14**	*In vitro*	0.9 ± 0.8	≥100	Preclinical	74
Fig. 5 (**15**)	**15**	*In vitro*	0.8 ± 0.7	≥100	Preclinical	74
Fig. 5 (**16**)	Baicalin	*In vitro*	10.27	≥200	Preclinical	75
Fig. 5 (**17**)	Baicalein	*In vitro*	1.69	≥200	Preclinical	75
		In vitro	10	≥100	Preclinical	76
		In vitro	2.9	>500	Preclinical	77
Fig. 5 (**18**)	Masitinib	*In vitro*	3.2	–	Preclinical	78
Fig. 5 (**19**)	Ebselen	*In vitro*	4.67 ± 0.80	–	Preclinical	79
Fig. 5 (**20**)	N3	*In vitro*	16.77 ± 1.70	–	Preclinical	79
Fig. 5 (**21**)	Cinanserin	*In vitro*	20.61 ± 0.97	≥200	Preclinical	79
Fig. 5 (**22**)	**22**	*In vitro*	0.53 ± 0.01	≥100	Preclinical	82
Fig. 5 (**23**)	**23**	*In vitro*	0.72 ± 0.09	≥100	Preclinical	82
Fig. 5 (**24**)	**24**	*In vitro*	4–5	–	Preclinical	83
Fig. 5 (**25**)	GC373	*In vitro*	1.5	≥200	Preclinical	84
Fig. 5 (**26**)	GC376	*In vitro*	0.92	≥200	Preclinical	84
		In vitro	3.37 ± 1.68	≥100	Preclinical	86
		In vitro	0.70	≥200	Preclinical	87
		In vitro	2.189 ± 0.092	≥100	Preclinical	85
Fig. 5 (**27**)	**27**	*In vitro*	2.883 ± 0.227	≥100	Preclinical	85
Fig. 5 (**28**)	Boceprevir	*In vitro*	1.31 ± 0.58	≥100	Preclinical	86
		In vitro	15.57	≥200	Preclinical	87
		In vitro	50.1	>10	Preclinical	94
Fig. 5 (**29**)	Calpain inhibitors II	*In vitro*	2.07 ± 0.76	≥100	Preclinical	86
Fig. 5 (**30**)	Calpain inhibitors XII	*In vitro*	0.49 ± 0.18	≥100	Preclinical	86
Fig. 5 (**31**)	Bepridil	*In vitro*	0.86	>25	Preclinical	92
Fig. 5 (**32**)	MPI5	*In vitro*	2.5–5	–	Preclinical	93
Fig. 5 (**33**)	MPI8	*In vitro*	1.25–2.5	–	Preclinical	93
Fig. 5 (**34**)	Nelfinavir mesylate	*In vivo*	3.3	12.3	Preclinical	94
		In vitro	1.13	24.32	Preclinical	152
Fig. 5 (**35**)	Z-FA-FMK	*In vitro*	0.13	≥50	Preclinical	95
Fig. 5 (**36**)	DA-3003-1	*In vitro*	4.47	7.74	Preclinical	95
Fig. 5 (**37**)	MG-115	*In vitro*	0.023	1.13	Preclinical	95
Fig. 5 (**38**)	MK0893	*In vitro*	3.16	12.59	Preclinical	95
Fig. 5 (**39**)	Suramin	*In vitro*	~20	≥5000	Preclinical	96
Fig. 5 (**40**)	Lopinavir	*In vitro*	26.63	49.75	Phase 2 (NCT04276688, NCT04315948)	99,104,124
Fig. 5 (**41**)	Ritonavir	*In vitro*	≥100	48.91	Phase 2 NCT04276688	99,104

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Table 2 (*continued*)

Number of chemical structures in Fig.	Inhibitor	Testing model	Activity IC_{50} (μmol/L)	Toxicity CC_{50} (μmol/L)	Clinical information	Ref.
		In vitro	8.63	74.11		152
Fig. 5 (**42**)	Dipyridamole (DIP)	*In vitro*	0.1	–	Preclinical	106
Fig. 5 (**43**)	GRL-0920	*In vitro*	2.8	≥100	Preclinical	107
Fig. 5 (**44**)	MI-09	*In vivo*	0.86	–	Preclinical	108
Fig. 5 (**45**)	MI-30	*In vivo*	0.54	–	Preclinical	108
Target PLpro						
Fig. 5 (**46**)	Dasatinib	–	–	–	Clinical cases	114
Fig. 5 (**47**)	Dronedarone	*In vitro*	4.5	12.1	Preclinical	94
Fig. 5 (**48**)	**48**	*In vitro*	21.0	≥80	Preclinical	109
Fig. 5 (**49**)	GRL-0617	*In vitro*	2.1	≥100	Preclinical	117
Target RdRp						
Fig. 5 (**50**)	Remdesivir	*In vivo*	0.77	≥100	Phase 4 (NCT04252664, NCT04257656, NCT04315948,	19,124,119,125
Fig. 5 (**51**)	Favipiravir	*In vitro*	61.88	>400	ChiCTR 2000029600 ChiCTR 2000030254	19
Fig. 5 (**52**)	β-D-*N*4-Hydroxycytidine (EIDD-1931)	*In vitro*	0.3	>10	Preclinical	120
Fig. 5 (**53**)	Dolutegravir	*In vitro*	22.04	≥40	Preclinical	146
Other small-molecule inhibitor						
Fig. 6 (**54**)	Tipranavir	*In vitro*	13.34	76.80	Preclinical	152
Fig. 6 (**55**)	Saquinavir	*In vitro*	8.83	44.43	Preclinical	152
Fig. 6 (**56**)	Atazanavir	*In vitro*	9.36	>81	Preclinical	152
Fig. 6 (**57**)	Azithromycin	*In vitro*	2.12	>40	Phase 2 NCT04329832	146
Fig. 6 (**58**)	Spiperone	*In vitro*	2.49	>40	Preclinical	146
Fig. 6 (**59**)	Opipramol dihydrochloride	*In vitro*	5.05	>40	Preclinical	146
Fig. 6 (**60**)	Quinidine hydrochloride	*In vitro*	5.11	>40	Preclinical	146
Fig. 6 (**61**)	Alprostadil	*In vitro*	5.39	>40	Preclinical	146
Fig. 6 (**62**)	Ivermectin	*In vitro*	~2	–	Preclinical	157
		In vitro	4.1	13.2	Preclinical	94
Fig. 6 (**63**)	Penciclovir	*In vitro*	95.96	>400	Preclinical	19

is to repurpose the application of clinical and preclinical drugs. Given the available knowledge on their safety profiles, these methods could be easily implemented to rapidly identify effective drugs for use in clinics or clinical trials to treat patients based on emergency use authorization.

Other methods of computer-aided drug design are used much less frequently than virtual screening. Structure- and fragment-based drug designs are iterative processes for designing new small-molecule inhibitors. For example, molecular docking, *de novo* drug design, pharmacophore modeling and quantitative structure−activity relationship models are commonly used for guiding inhibitor screening and optimization. Compared with repurposing old drugs for new indications, other strategies mentioned above are generally limited by long processing time and high cost, making them unsuited for the development of drugs for emergency use, such as COVID-19. However, we propose that computer-aided, small-molecule drug discovery will be one of the most important strategies for research and development of COVD-19 therapeutics and prophylactics in the future.

The development of antiviral agents against SARS-CoV-2 infection calls for the use of human disease-related cells to create novel models to study the biological characteristics of SARS-CoV-2 and to promote drug screening. Given that SARS-CoV-2 mainly infects the respiratory tract, researchers have used human pluripotent stem cells (hPSCs) to develop a lung organoid model (hPSC-LO) for SARS-CoV-2 infection[27]. hPSC-LOs, especially alveolar type II-like cells, could be easily infected by SARS-CoV-2, displaying strong induction of chemokines upon SARS-CoV-2 infection, consistent with the phenomenon observed in COVID-19 patients. At the same time, as a supplement to hPSC-LOs, these researchers also used hPSCs to construct colon organoids (hPSC-COs) to explore the response of colon cells to SARS-CoV-2 infection. Using hPSC-LOs, they conducted HTS of drugs approved by FDA and identified several SARS-CoV-2 entry inhibitors, including imatinib mesylate [Fig. 10B (**127**)], mycophenolic acid and quinacrine dihydrochloride [Fig. 10C (**159**, **160**). These three drugs were proven to be blockers of SARS-CoV-2 infection in a toxicity-independent manner in Vero E6 cells with

Table 3 Small-molecule SARS-CoV-2 inhibitors targeting host cell proteins.

Number of chemical structures in Fig.	Inhibitor	Testing model	Activity IC_{50} (μmol/L)	Toxicity CC_{50} (μmol/L)	Clinical information	Ref.
Target ACE2						
Fig. 7 (**64**)	Candesartan	*In vitro*	–	–	Preclinical	160
Fig. 7 (**65**)	Olmesartan	*In vitro*	–	–	Preclinical	159
Fig. 7 (**66**)	Telmisartan	–	–	–	Phase 3 NCT04356495	
Fig. 7 (**67**)	Losartan	–	–	–	Phase 1 (NCT04312009, NCT04311177, NCT04328012, NCT04335123)	162–164
Target TMPRSS2						
Fig. 8 (**68**)	Camostat mesylate	*In vitro* (pseudovirus)	~1	≥500	Phase 2a/4 (NCT04321096, NCT04338906)	10
Fig. 8 (**69**)	Nafamostat	*In vitro*	22.50	≥100	NCT04352400	19
			0.01	–		177
Fig. 8 (**70**)	MI-432	*In vitro*	≤10	≥50	Preclinical	180
Fig. 8 (**71**)	MI-1900	*In vitro*	≤50	≥50	Preclinical	180
Target cathepsin B/L						
Fig. 9 (**72**)	Amantadine	*In vitro*	≥100	≥100	Preclinical	187
Fig. 9 (**73**)	Chlorpromazine	*In vitro*	≥100	≥100	Preclinical	187
Fig. 9 (**74**)	E64-d	*In vitro*	~4.487	≥0	Preclinical	187
Fig. 9 (**75**)	Chloroquine	*In vitro*	2.71	273.20	Several clinical trials (ChiCTR2000029609 et al.)	97,188,189
		In vitro	2.01	>30		212
Fig. 9 (**76**)	Hydroxychloroquine	*In vivo* (macaques)	4.51	249.50	Several clinical trials (ChiCTR2000029803, ChiCTR2000029868, ChiCTR2000029898, ChiCTR2000029899, ChiCTR2000029992, ChiCTR2000030054, NCT04315948)	124,146,188,189,191
		In vitro	4.47	>30		212
Fig. 9 (**77**)	Apilimod	*In vitro*	0.023	–	Preclinical	68
		In vitro	~0.01	–	Preclinical	197
Fig. 9 (**78**)	MDL-28170	*In vitro*	0.22	–	Preclinical	68
Fig. 9 (**79**)	Z LVG CHN2	*In vitro*	0.19	–	Preclinical	68
Fig. 9 (**80**)	VBY-825	*In vitro*	0.3	–	Preclinical	68
Fig. 9 (**81**)	ONO 5334	*In vitro*	0.41	–	Preclinical	68
Fig. 9 (**82**)	Teicoplanin	*In vitro*	1.66	–	Preclinical	201
Fig. 9 (**83**)	Omeprazole	*In vitro*	17.06	≥40	Preclinical	146

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Table 3 (*continued*)

Number of chemical structures in Fig.	Inhibitor	Testing model	Activity IC_{50} (μmol/L)	Toxicity CC_{50} (μmol/L)	Clinical information	Ref.
Dual inhibitors: Target cathepsin L and 3CLpro						
Fig. 5 (**29**)	Calpain inhibitors II	*In vitro*	2.07 ± 0.76	≥100	Preclinical	86,88
Fig. 5 (**30**)	Calpain inhibitors XII	*In vitro*	0.49 ± 0.18	≥100	Preclinical	86,88
Other small-molecule inhibitor						
Fig. 10 (**84**)	Thalidomide	–	–	–	Phase 2 (NCT04273581, NCT04273529)	205
Fig. 10 (**85**)	Fingolimod	–	–	–	NCT04280588	205,207
Fig. 10 (**86**)	Teriflunomide	*In vitro*	6	–	Small patient cohort	208
		In vitro	26.06	850.5		210
Fig. 10 (**87**)	Leflunomide	*In vitro*	41.49	879.0	Preclinical	210
Fig. 10 (**88**)	Brequinar	*In vitro*	0.123	231.3	Preclinical	210
Fig. 10 (**89**)	S312	*In vitro*	1.56	158.2	Preclinical	210
Fig. 10 (**90**)	S416	*In vitro*	0.017	178.6	Preclinical	210
Fig. 10 (**91**)	ROC-325	*In vitro*	3.28 ± 0.57	>30	Preclinical	212
Fig. 10 (**92**)	Clomipramine	*In vitro*	13.6 ± 2.96	>30	Preclinical	212
Fig. 10 (**93**)	Hycanthone	*In vitro*	5.79 ± 0.26	14.2	Preclinical	212
Fig. 10 (**94**)	Mefloquine	*In vitro*	3.85 ± 0.24	8.78	Preclinical	212
		In vitro	8.06	18.53	Preclinical	244
		In vivo	3.2	≥10	Preclinical	94
Fig. 10 (**95**)	Dithioerythritol thiosulfonate 16	*In vitro*	50	≥500	Preclinical	214
	GNS561	*In vitro*	0.006 for USA-WA1/2020; 0.03 for IHU MI6	2.0 for USA-WA1/2020; 6.7 for IHU MI6	Preclinical	215
Fig. 10 (**96**)	VPS34-IN1	*In vitro*	0.55	≥50	Preclinical	218
Fig. 10 (**97**)	PIK-III	*In vitro*	0.12	≥50	Preclinical	218
Fig. 10 (**98**)	Orlistat	*In vitro*	21.25	≥1000	Preclinical	218
Fig. 10 (**99**)	Triacsin C	*In vitro*	0.04	≥50	Preclinical	218
Fig. 10 (**100**)	MI-1851	*In vitro*	≤10	≥50	Preclinical	180
Fig. 10 (**101**)	Decanoyl-RVKR-chloromethylketone (dec-RVKR-cmk)	*In vitro*	0.057	318.2	Preclinical	221
Fig. 10 (**102**)	Homoharringtonine	*In vitro*	2.55	59.75	Preclinical	99
Fig. 10 (**103**)	Emetine	*In vitro*	0.46	56.46	Preclinical	99
		In vitro	0.0004	>10	Preclinical	94
Fig. 10 (**104**)	2-Deoxy-D-glucose (2-DG)	*In vitro*	9090	–	Preclinical	228,229
Fig. 10 (**105**)	Pladienolide B	*In vitro*	0.007	–	Preclinical	228
Fig. 10 (**106**)	Ribavirin	*In vitro*	70	–	NCT04356677	228
Fig. 10 (**107**)	NMS-873	*In vitro*	0.025	–	Preclinical	140,228
Fig. 10 (**108**)	Cycloheximide	*In vitro*	0.17	–	Preclinical	228
Fig. 10 (**109**)	Baricitinib	–	–	–	Phase 2,3,4 (2020-001854-23, 2020-001354-22, NCT04358614)	232

Fig. 10 (**110**)	Nitazoxanide	*In vitro*	2.12	>35.53	Phase 2, 3 (NCT04341493, NCT01056380, NCT04348409)	19,238
		In vitro	4.90	>300		237
Fig. 10 (**111**)	JIB-04	*In vitro*	0.695	>300	Preclinical	237
Fig. 10 (**112**)	Fenofibrate	*In vitro*	20	>100	Preclinical	187
Fig. 10 (**113**)	Plitidepsin	*In vitro*	0.70 nmol/L for Vero E6; 0.73 nmol/L for hACE2-293 T; 1.62 nmol/L for human lung cells	1.99 nmol/L in Vero E6; ≥200 nmol/L for hACE2-293 T; 65.43 nmol/L for human lung cells	Phase 1/2 (NCT04382066)	187,239
Fig. 10 (**114**)	Clemizole hydrochloride	*In vitro*	23.94	≥40	Preclinical	146
Fig. 10 (**115**)	Benztropine mesylate	*In vitro*	17.79	>>50	Preclinical	244
		In vitro	1.8	–	Preclinical	78
Fig. 10 (**116**)	Fluphenazine dihydrochloride	*In vitro*	8.98	20.02	Preclinical	244
Fig. 10 (**117**)	Amodiaquine hydrochloride	*In vitro*	5.64	>38.63	Preclinical	244
Fig. 10 (**118**)	Amodiaquine dihydrochloride dihydrate	*In vitro*	4.94	34.42	Preclinical	244
Fig. 10 (**119**)	Thiethylperazine maleate	*In vitro*	8.02	18.37	Preclinical	244
Fig. 10 (**120**)	Triparanol	*In vitro*	6.41	21.21	Preclinical	244
Fig. 10 (**121**)	Terconazole	*In vitro*	16.14	41.46	Preclinical	244
Fig. 10 (**122**)	Fluspirilene	*In vitro*	5.32	30.33	Preclinical	244
Fig. 10 (**123**)	Clomipramine hydrochloride	*In vitro*	7.59	>29.68	Preclinical	244
Fig. 10 (**124**)	Promethazine hydrochloride	*In vitro*	10.44	>42.59	Preclinical	244
Fig. 10 (**125**)	Toremifene citrate	*In vitro*	11.3	20.51	Preclinical	244
Fig. 10 (**126**)	Tamoxifen citrate	*In vitro*	8.98	37.96	Preclinical	244
Fig. 10 (**127**)	Imatinib mesylate	*In vitro*	5.32	>30.86	Preclinical	244
		In vivo (humanized mice carrying hPSC-derived lung xenografts)	2.15	–	Preclinical	27
Fig. 10 (**128**)	Bruceine A	*In vitro*	0.011	31.4	Preclinical	246
Fig. 10 (**129**)	Bufalin	*In vitro*	0.018	>40	Preclinical	246
Fig. 10 (**130**)	Cinobufagin	*In vitro*	0.018	>40	Preclinical	246
Fig. 10 (**131**)	Bufotaline	*In vitro*	0.0259	>40	Preclinical	246
Fig. 10 (**132**)	Periplocoside	*In vitro*	0.0657	>40	Preclinical	246
Fig. 10 (**133**)	Brusatol	*In vitro*	0.0492	19	Preclinical	246
Fig. 10 (**134**)	Digoxin	*In vitro*	0.1541	>40	Preclinical	246
Fig. 10 (**135**)	Veratridine	*In vitro*	2.376	>100	Preclinical	246
Fig. 10 (**136**)	Oridonin	*In vitro*	1.462	>40	Preclinical	246
Fig. 10 (**137**)	Isoalantolactone	*In vitro*	1.483	>40	Preclinical	246
Fig. 10 (**138**)	Isoliensinine	*In vitro*	1.615	40	Preclinical	246
Fig. 10 (**139**)	Alantolactone	*In vitro*	1.724	36.7	Preclinical	246
Fig. 10 (**140**)	Cryptotanshinone	*In vitro*	5.024	>100	Preclinical	246
Fig. 10 (**141**)	Dehydrocostus lactone	*In vitro*	2.322	36.2	Preclinical	246
Fig. 10 (**142**)	Momordinic	*In vitro*	3.529	>40	Preclinical	246

(*continued on next page*)

Table 3 (*continued*)

Number of chemical structures in Fig.	Inhibitor	Testing model	Activity IC_{50} (μmol/L)	Toxicity CC_{50} (μmol/L)	Clinical information	Ref.
Fig. 10 (**143**)	Liensinine	*In vitro*	2.537	25.4	Preclinical	246
Fig. 10 (**144**)	Dehydrodiisoeugenol	*In vitro*	10.29	≥100	Preclinical	246
Fig. 10 (**145**)	Cornuside	*In vitro*	5.262	≥40	Preclinical	246
Fig. 10 (**146**)	Roburicacid	*In vitro*	5.267	≥40	Preclinical	246
Fig. 10 (**147**)	Coniferylaldehyde	*In vitro*	11.03	≥40	Preclinical	246
Fig. 10 (**148**)	Panduratin A	*In vitro*	0.81	14.71	Preclinical	247
Fig. 10 (**149**)	Thioguanine	*In vitro*	1.7	25.4	Preclinical	94
Fig. 10 (**150**)	Moxidectin	*In vitro*	3.1	6.9	Preclinical	94
Fig. 10 (**151**)	Ivacaftor	*In vitro*	3.7	12.9	Preclinical	94
Fig. 10 (**152**)	Azelnidipine	*In vitro*	5.3	12.9	Preclinical	94
Fig. 10 (**153**)	Penfluridol	*In vitro*	2.4	12.9	Preclinical	94
Fig. 10 (**154**)	Salinomycin	*In vitro*	0.00048	13.1	Preclinical	94
Fig. 10 (**155**)	Monensin	*In vitro*	6.4	6.6	Preclinical	94
Fig. 10 (**156**)	Maduramicin	*In vitro*	1.3	3.4	Preclinical	94
Fig. 10 (**157**)	Tilorone	*In vitro*	0.18	–	Preclinical	248
Fig. 10 (**158**)	Pyronaridine	*In vitro*	0.198	–	Preclinical	248
Fig. 10 (**159**)	Mycophenolic acid	*In vivo* (humanized mice carrying hPSC-derived lung xenografts)	0.9	–	Preclinical	27
Fig. 10 (**160**)	Quinacrine dihydrochloride	*In vivo* (humanized mice carrying hPSC-derived lung xenografts)	0.84	–	Preclinical	27

half maximal inhibitory concentrations (IC_{50}s) of 2.15, 0.9 and 0.84 μmol/L, respectively (Table 3)[27]. Taken together, these data demonstrate that cell disease models provide valuable tools for drug screening to identify COVID-19 therapeutic candidates.

Traditional Chinese medicine (TCM) has been widely applied in clinics in China for COVID-19 patients. Ni et al.[28] have reported that after three patients with COVID-19 treated with Shuanghuanglian Oral Liquid, their symptoms improved, and the patients finally recovered without any adverse reactions. In addition, Lianhuaqingwen was proven to be effective in inhibiting SARS-CoV-2 infection in Vero E6 cells and attenuating the production of proinflammatory cytokines, suggesting that Lianhuaqingwen may have a potential inhibitory effect on the cytokine storm induced by SARS-CoV-2 infection[29]. From the above experience, researchers should also make some headway to screen and develop promising TCM compounds or extracts as efficacious COVID-19 therapeutics.

Some researchers have provided a novel drug discovery strategy to manage COVID-19 by systematically studying the molecular details of SARS-CoV-2[30]. They first successfully cloned, labeled, and expressed 26 of 29 viral proteins in human cells, and then applied affinity purification mass spectrometry (AP-MS) for identification of human proteins, which could physically interact with each viral protein. In the end, they identified 332 high-confidence SARS-CoV-2-human protein−protein interactions. Sixty-six druggable human proteins, or host factors targeted by 69 existing FDA-approved drugs, are reported. The efficacy in live SARS-CoV-2 infection assays of 69 compounds is currently being evaluated[30]. Host-dependent factors that mediate viral infection may become effective molecular targets for the development of a broadly effective antiviral therapy against SARS-CoV-2 infection.

5. Small-molecule SARS-CoV-2 inhibitors targeting viral proteins

5.1. *Entry inhibitors*

Like SARS-CoV, SARS-CoV-2 uses a glycosylated, homotrimeric class I fusion S protein to gain entry into host cells[31−33]. The SARS-CoV-2 S gene denotes the functional components: signal

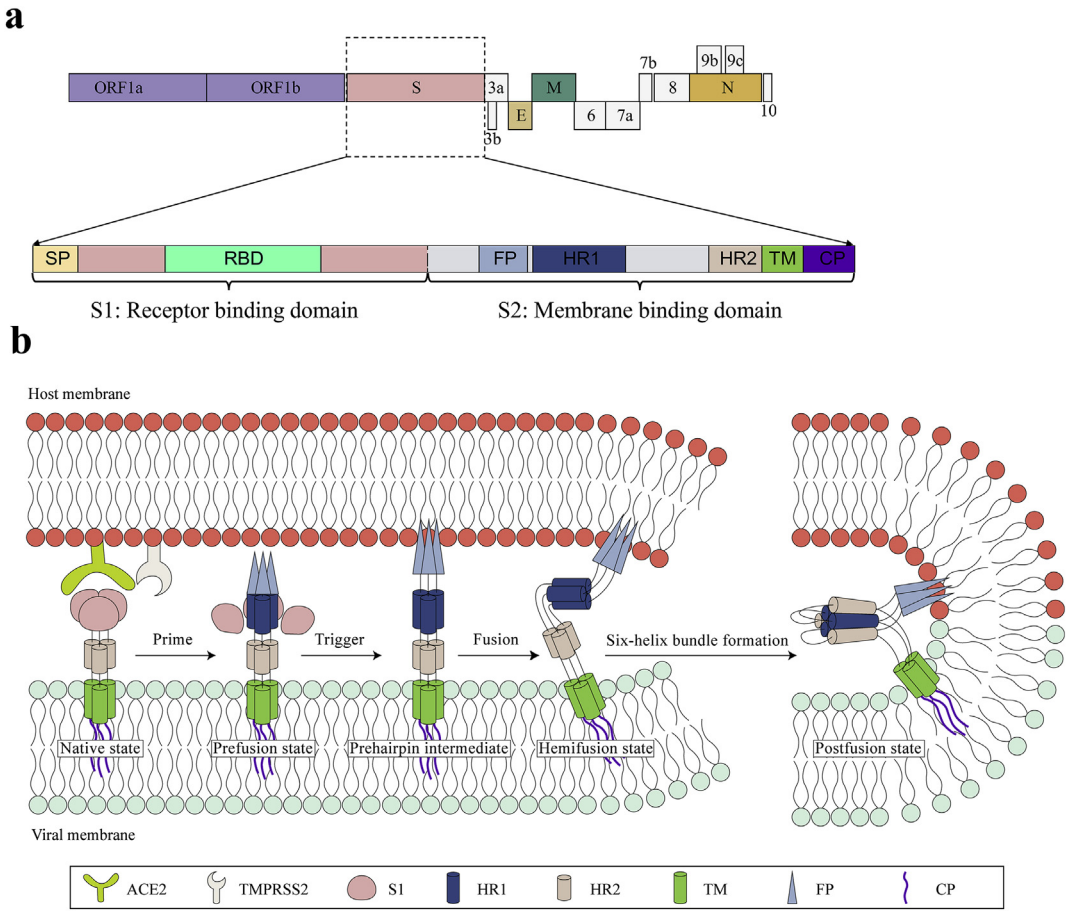

Figure 3 Structural regions and fusion mechanism of SARS-CoV-2 S protein. (A) The functional regions in SARS-CoV-2 S protein include SP (signal peptide, light yellow), RBD (receptor-binding domain; light green), FP (fusion peptide; light blue), HR1 (heptad repeat 1; gray-blue), HR2 (heptad repeat 2; flesh), TM (transmembrane; grass green), and CP (cytoplasmic; purple). (B) SARS-CoV-2 S protein fusion pathway base on class I fusion protein. The S protein starts in the native state and undergoes priming of the S1 subunit by relevant proteases to achieve the prefusion state. Subsequent triggering by relevant proteases will enable the FP to insert in the host membrane and allow the S protein to form the prehairpin intermediate. The prehairpin begins to fold back on itself due to HR1 and HR2 interactions forming the 6-HB, and eventual postfusion stable states. During the S protein foldback, the two membranes will approach each other until the outer leaflets merge (hemifusion) and eventually the inner leaflets merge.

peptide (SP) and receptor-binding domain (RBD) in the S1 subunit and fusion peptide (FP), heptad repeat 1 (HR1), heptad repeat 2 (HR2), transmembrane (TM) and cytoplasm (CP) in the S2 subunit (Fig. 3A). Class I fusion proteins catalyze membrane fusion reaction through a sequence of states: (1) native state, (2) prefusion state, (3) prehairpin intermediate state, (4) hemifusion state, and (5) postfusion state (Fig. 3B). The S protein is comprised of S1 and S2 subunits and exists in a metastable prefusion conformation. Binding between the RBD of S1 and the receptor ACE2 triggers a conformational change of the S2 subunit, which destabilizes the prefusion trimer, and this results in shedding of the S1 subunit and activating the fusogenic activity of the S2 subunit[34−36]. During the fusion process, the FP is exposed and inserts into the host cell membrane, triggering the transient formation of a prehairpin intermediate that bridges the viral and cell membranes. Then, the HR1 and HR2 associate with each other to form a six-helix bundle (6-HB), drawing both viral and target cell membranes into close proximity in a manner that results in fusion between the viral and host cell membranes[15].

5.1.1. *Peptides*

Peptides derived from the HR1 and HR2 domains in the class I viral fusion protein block the viral 6-HB formation by binding to the pre-hairpin intermediate, thus showing antiviral activity[37]. This activity has been reported for emerging CoVs, including SARS-CoV and MERS-CoV[35,38−40]. In response to the outbreak of SARS-CoV, a group of HR2-based peptides that could effectively inhibit viral infection were developed[35,40−43]. A pan-CoV fusion inhibitor, designated EK1, was designed, which could inhibit the fusion of diverse HCoVs, including SARS-CoV, MERS-CoV, HCoV-229 E, HCoV-NL63, and HCoV-OC43[44]. More recent studies showed that EK1 is an effective peptide inhibitor against SARS-CoV-2 S protein-mediated membrane fusion and pseudovirus infection in a dose-dependent manner (Table 1)[45]. Recent studies have shown that conjugation of a lipid group to a peptide is a feasible strategy to enhance the antiviral activity and *in vivo* stability of the lipopeptide viral fusion inhibitor[46−48]. The lipopeptide EK1C4 derived from EK1 was found to be an effective inhibitor against S protein-mediated membrane fusion and pseudotyped SARS-CoV-2 infection, with IC_{50}s of 1.3 and 15.8 nmol/L, respectively, which are about 241- and 149-fold more potent than that of the unmodified peptide EK1, respectively (Table 1)[45]. Similarly, Zhu et al.[49] designed an HR2 sequence-based lipopeptide fusion inhibitor, termed IPB02, which exhibited highly potent activity in inhibiting SARS-CoV-2 S protein-mediated cell−cell fusion and pseudovirus infection (Table 1).

The sequence alignment has shown that the S2 subunits of SARS-CoV-2 and SARS-CoV are highly conserved, and the overall identity of the HR1 and HR2 domains is 92.6% and 100%, respectively, while in the HR1 core region, eight of 21 residues showed mutations (about 38% difference)[50]. Therefore, it is necessary to design fusion inhibitory peptides based on the amino acid sequences of SARS-CoV-2 HR1 and HR2. Unlike EK1, SARS-CoV-2-HR1P (aa924–965) and SARS-CoV-2-HR2P (aa1168–1203) are derived from the HR1 and HR2 of SARS-CoV-2[50]. Results reveal that SARS-CoV-2-HR2P showed potent fusion−inhibitory activity with an IC_{50} of 0.18 μmol/L, whereas SARS-CoV-2-HR1P exhibited no significant inhibition at concentrations up to 40 μmol/L (Table 1)[50].

de Vries et al.[51] designed a dimeric lipopeptide fusion inhibitor, $[SARS_{HRC}\text{-}PEG_4]_2$-chol, varying from SARS-CoV-2-HRP2 only in a single amino acid[50]. $[SARS_{HRC}\text{-}PEG_4]_2$-chol inhibited SARS-CoV-2 entry with an IC_{50} of ~5 nmol/L in TMPRSS2-positive Vero E6 cells. While toxicity of $[SARS_{HRC}\text{-}PEG_4]_2$-chol in human airway epithelium was minimal, even at the high concentrations tested (< 20% at 100 μmol/L, Table 1). It is worth noting that daily intranasal administration to SARS-CoV-2 ferrets can completely prevent SARS-CoV-2 direct-contact transmission[51].

Peptides to disrupt SARS-CoV-2-RBD binding to ACE2 can also inhibit the virus which target the stage of viral attachment to prevent entry to host cells, a new modality for COVID-19 therapeutic intervention. For example, SBP1 (a 23-mer peptide fragment) consisting of amino acids in the α1 helix of the ACE2 peptidase domain (PD) was synthesized. The results of bio-layer interferometry revealed that SBP1 could specifically bind with SARS-CoV-2-RBD in low nanomolar concentration (Table 1)[52], and block the interaction between SARS-CoV-2 S protein and ACE2, thereby preventing the virus from entering host cells and providing a new treatment and diagnostic strategy against COVID-19[52].

To inhibit the viral attachment between S protein and ACE2, the peptides designed by Cao et al.[53] using two *de novo* design approaches, also known as minibinders, were either built around an ACE2 helix or based on RBD-binding motifs. AHB1 and AHB2 (Table 1), followed the first approach, exhibited strongly neutralization SARS-CoV-2 with IC_{50}s of 35 and 15.5 nmol/L, respectively. Using the second approach, LCB1 and LCB3 neutralized SARS-CoV-2 with IC_{50}s of 23.54 and 48.1 pmol/L, respectively (Table 1). These hyperstable minibinders provide new approaches for SARS-CoV-2 therapeutics.

Beddingfield and colleagues identified ATN-161, the fibronectin-derived anticancer peptide, that inhibited SARS-CoV-2 attachment through a hypothesized $\alpha5\beta1$ integrin-based mechanism and indicated that ATN-161 could reduce SARS-CoV-2 infection with an IC_{50} of 3.16 μmol/L[54] (Table 1). Integrins have been shown to bind to ACE2 and SARS-CoV-2 S protein[55,56]. The results of Beddingfield et al.[54] suggest that inhibiting S protein interaction with $\alpha5\beta1$ integrin and the interaction between $\alpha5\beta1$ integrin and ACE2 using ATN-161 represents a promising approach to treat COVID-19.

Watson et al.[57] designed peptides, SARS-BLOCK™, by mimicking the SARS-CoV-2 RBD that target the stage of viral attachment. They designed, simulated, synthesized, modeled epitopes, predicted peptide folding, and characterized behavior of synthetic peptides. Among of them, peptides **1**, **4**, **5** and **6** blocked SARS-CoV-2 pseudotyped virus infection in ACE2-HEK293 cells. And peptide **5** showed inhibitory activity with an IC_{50} in the sub-micromolar concentrations and an IC_{95} of ~2.22 μmol/L (Table 1).

5.1.2. *Small-molecule compounds*

The SARS-CoV-2 S protein plays a key role in recognizing receptor and mediating virus-cell membrane fusion showing itself to be an efficient mediator of viral entry. The S protein is not only an important binding site for neutralizing antibodies, but it is also a major target for therapeutic drug development. Yang et al.[58] report that salvianolic acid C [Sal-C, Fig. 4 (**1**)], a hydrophilic compound from Danshen, a TCM, potent to inhibit SARS-CoV-2 infection in blocking the formation of 6-HB core of S protein. And Sal-C exhibited potent antiviral activity against authentic SARS-CoV-2 with an IC_{50} of 3.41 μmol/L (Table 2)[58]. Their study advances a potential use of Sal-C for COVID-19 therapy or prophylaxis and

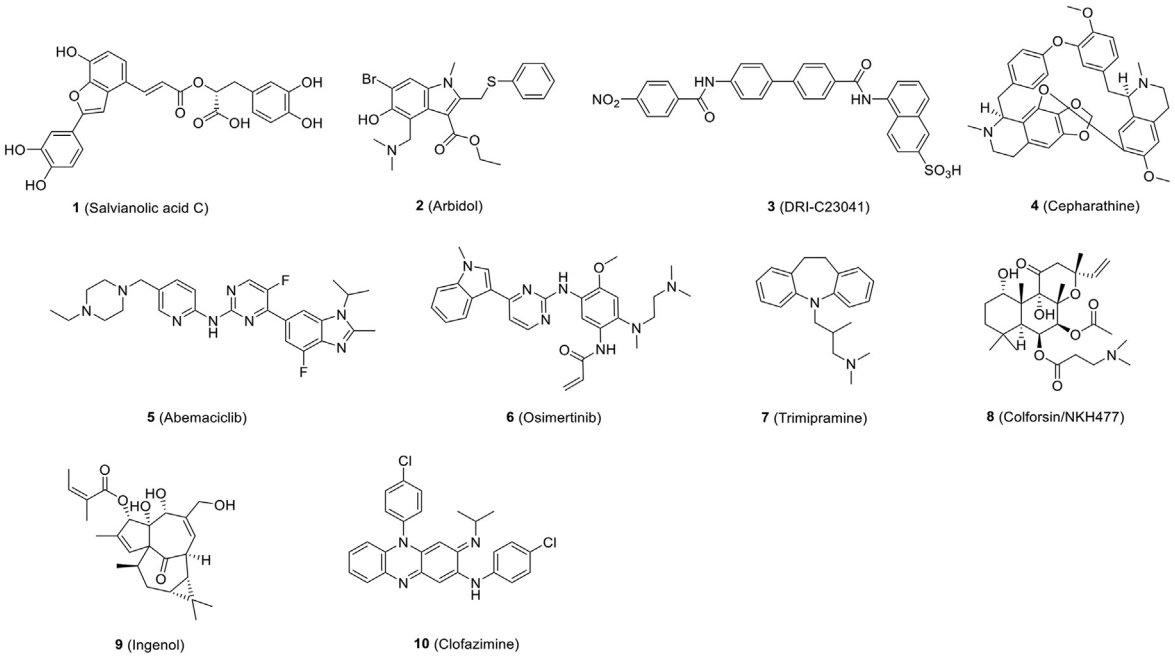

Figure 4 Chemical structures of small-molecule inhibitors that inhibit SARS-CoV-2 entry.

provides a basis for the development of fusion inhibitors against SARS-CoV-2 infection.

Arbidol [umifenpvor, Fig. 4 (**2**)], an anti-influenza drug approved in China and Russia, targets the SARS-CoV-2 S protein to impede S protein-mediated membrane fusion and, hence, the entry of virus into host cells[59]. It showed satisfactory activity against SARS-CoV-2 *in vitro*[60]. IC_{50} and the 50% cytotoxic concentration (CC_{50}) of Arbidol in cell-based assays were 4.11 and 31.79 μmol/L, respectively, and the selectivity index (SI = CC_{50}/IC_{50}) was 7.73 (Table 2)[60]. Several clinical trials were evaluated for the treatment of COVID-19, including arbidol monotherapy and arbidol combined with lopinavir/ritonavir[61,62]. According to clinical trials, post-exposure prophylaxis using arbidol could reduce infection exposed to confirmed cases of COVID-19[63]. Compared to a supportive care group, arbidol monotherapy presented little effect for patients hospitalized with mild and moderate COVID-19[64].

Novel drug-like compounds, DRI-C23041, DRI-C91005, which targeted the viral attachment stage, inhibited the interaction of hACE2 with SARS-CoV-2 S protein in cell-free ELISA-type assays[65]. DRI-C23041 [Fig. 4 (**3**)] inhibited SARS-CoV-2-S pseudovirus with IC_{50} of 5.6 μmol/L (Table 2)[65].

Using the SARS-S and MERS-S pseudovirus infection assays, six compounds (cepharanthine, abemaciclib, osimertinib, trimipramine, colforsin, and ingenol) [Fig. 4 (**4**–**9**)], were identified as cell entry inhibitors from a HTS in approved drug libraries. The molecular mechanism of action of these small-molecule entry inhibitors has not been fully characterized. These inhibitors have been further confirmed to reduce (> 30%) cytopathic effect (CPE) caused by SARS-CoV-2 infection in Vero E6 cells with IC_{50}s of 1.41, 3.16, 3.98, 20.52, 23.06 and 0.06 μmol/L, respectively (Table 2)[66].

Clofazimine [Fig. 4 (**10**)], an FDA-approved molecule, was found to be an anti-tuberculosis drug, which was later used to treat leprosy[67] and then showed antiviral activity against SARS-CoV-2 with an IC_{50} of 310 nmol/L *in vitro*[68]. Clofazimine, which has recently been identified as a broad-spectrum inhibitor of coronaviruses, may be a promising candidate for coronaviruses that have emerged and may emerge in the future. Because of its comparatively low manufacturing cost, clofazimine could significantly reduce the health burden, particularly in developing countries. In antiviral assays, clofazimine exhibited excellent antiviral activity against SARS-CoV-2 *in vitro* and *in vivo*. In addition, when combined with remdesivir, antiviral synergy was demonstrated against SARS-CoV-2 *in vitro* and *in vivo*. In mechanistic studies, clofazimine was shown to inhibit cell fusion between effector cells expressing SARS-CoV-2 S protein and Vero cells[69].

5.2. *Replication inhibitors*

5.2.1. *3C-like protease inhibitors*

Two large polyproteins, pp1a and pp1ab, are inactive until cleavage by virally encoded cysteine proteases, namely, 3CLpro and PLpro[70], into different numbers of nsps in replication of SARS-CoV-2. The processing of pp1a and pp1ab is indispensable for viral life cycles. Thus, inhibition of cysteine proteases, is an effective therapeutic strategy for COVID-19.

3CLpro represents the most attractive target for the discovery of SARS-CoV-2 inhibitors. Previous studies demonstrate that amino residue Cys145 in the catalytic pocket of 3CLpro is an effective site for the development of covalent inhibitors against SARS-CoV and other coronaviruses[71].

Chen and his colleagues[72] established a HTS platform based on fluorescence resonance energy transfer (FRET) to identify drugs targeting the SARS-CoV-2 3CLpro from compound libraries, especially FDA-approved drugs, to be used immediately to treat patients with COVID-19. Their findings indicate that the 8-aminoquinoline antimalarial drug tafenoquine [Fig. 5A-1 (**11**)] induced significant conformational change in SARS-CoV-2 3CLpro, exposing some hydrophobic residues and ultimately

leading to protein aggregation, diminishing its protease activity. Moreover, tafenoquine significantly repressed the yield of SARS-CoV-2 RNA in a cell culture system with an IC_{50} of around 2.5 μmol/L (Table 2). In Vero E6 cells, another research team reported that tafenoquine has an IC_{50} of ~2.6 μmol/L for SARS-CoV-2 (Table 2), which is four times more potent than hydroxychloroquine[73]. Time-of-addition experiment is consistent with the different mechanism for tafenoquine *versus* hydroxychloroquine. Physiologically based pharmacokinetic models indicate that the unbound concentration of tafenoquine may exceed EC_{90} for at least 8 weeks after administration in the lungs of COVID-19 patients.

Some 3CLpro inhibitors synthesized by Rathnayake et al.[74] show activity against multiple coronaviruses in enzyme- and cell-based assays. Among compounds **12**−**15** (**6c, 6e, 6h** and **6j** in Ref. 74) [Fig. 5A-1 (**12**−**15**)], **13** showed the most potent antiviral activity against SARS-CoV-2 3CLpro in both fluorescence resonance energy transfer enzyme assay (IC_{50}, 0.17 μmol/L) and cell-based assay (IC_{50}, 0.15 μmol/L, Table 2).

Baicalin and baicalein [Fig. 5A-1 (**16**, **17**)], key components in TCM *Scutellaria* B., were reported as the first noncovalent, non-peptidomimetic inhibitors of SARS-CoV-2 3CLpro, which exhibited potent antiviral activity with IC_{50}s of 10.27 and 1.69 μmol/L, respectively, in a cell-based system (Table 2)[75]. Crystal structure of 3CLpro in complex with baicalein shows that baicalein occupies the core of the substrate-binding pocket through interacting with the crucial S1/S2 subsites and the oxyanion loop, blocking substrates from approaching the active site[75]. This unique mode of action can be regarded as a completely different type of 3CLpro inhibitor. Another team reported that infection of SARS-CoV-2 and VSV were potently inhibited by baicalein with IC_{50}s around 10 and 15 μmol/L, respectively[76]. Mechanistically, baicalein inhibits mitochondrial OXPHOS, which is reversibly related to mPTP activity in host cells. The virus changes mitochondrial metabolism by inactivating mPTP to promote its production, and the inhibition of OXPHOS attenuates viral replication. A recent research team also reported that the ethanol extract of *Scutellaria baicalensis* and baicalein, the major component, showed inhibition of SARS-CoV-2 replication in Vero cells with IC_{50}s of 0.74 and 2.9 μmol/L, respectively[77]. Ethanol extract inhibits virus entry, whereas baicalein mainly acts on the post-entry stage of the virus.

To inhibit the replication of HCoV-OC43, Drayman's team[78] screened a library of 1900 clinically safe drugs, identified 26 top hits and further tested their antiviral activity against SARS-CoV-2. Of the 26 drugs tested, the compounds with the best antiviral activity against SARS-CoV-2 were cepharanthine (IC_{50} = 0.13 μmol/L), flupenthixol (IC_{50} = 0.56 μmol/L), desloratadine (IC_{50} = 0.9 μmol/L), trimipramine (IC_{50} = 1.5 μmol/L), lapatinib (IC_{50} = 1.6 μmol/L), benztropine (IC_{50} = 1.8 μmol/L), bafetinib (IC_{50} = 2.2 μmol/L), azelastine (IC_{50} = 2.4 μmol/L) and masitinib [Fig. 5A-1 (**18**)] (IC_{50} = 3.2 μmol/L, Table 2). By studying the mechanism of action, they found that masitinib, a cancer treatment drug developed as a tyrosine-kinase inhibitor, inhibited the activity of the SARS-CoV-2 3CLpro.

To identify drug candidates for clinical trials, Jin and coworkers[79] initiated multiple strategies that combining structure-assisted drug design, virtual drug screening and HTS could rapidly discover novel lead compounds. This strategy resulted in the development of a FRET assay to test more than 10,000 compounds as inhibitors of 3CLpro. First, they identified a mechanism-based inhibitor, N3, by computer-aided drug design and then determined that N3 bound with 3CLpro of SARS-CoV-2 at a resolution of 2.1 Å (PDB code 7BQY) by measuring the crystal structure of 3CLpro in complex with N3. Finally, they found that seven FDA-approved or clinical drugs (ebselen, disulfiram, TDZD-8, tideglusib, carmofur, shikonin and PX-12) could inhibit 3CLpro using an enzymatic inhibition assay. However, it should be pointed out that the mechanism of action of six of them (ebselen, disulfiram, tideglusib, carmofur, shikonin and PX-12) remains to be elucidated. Ma et al.[80] proved that these six inhibitors were nonspecific inhibitors of 3CLpro. Among these compounds, ebselen and N3 [Fig. 5A-1 (**19**, **20**)] showed the strongest inhibition against SARS-CoV-2 in a plaque-reduction assay with IC_{50}s of 4.67 and 16.77 μmol/L, respectively (Table 2). They also identified cinanserin [Fig. 5A-1 (**21**)], a well-characterized serotonin antagonist, which displayed moderate inhibition against SARS-CoV-2 with an IC_{50} value of 20.61 μmol/L (Table 2)[79]. Later, Yang and coworkers[81] presented the X-ray crystal structure of SARS-CoV-2 3CLpro in complex with carmofur at a resolution of 1.6 Å (PDB code 7BUY). The Yang and Liu groups[82] also co-published X-ray crystal structures of SARS-CoV-2 3CLpro in complex with peptidomimetic aldehyde compounds **22** and **23** (**11a** and **11b** in Ref. 82) [Fig. 5A-1 (**22**, **23**)]. Both **22** and **23** exhibited good anti-SARS-CoV-2-infection activities in cells with IC_{50}s of 0.53 ± 0.01 and 0.72 ± 0.09 μmol/L, respectively, using a plaque-reduction assay (Table 2). Cytotoxicity and pharmacokinetic experiments were carried out later, suggesting that the two compounds were promising drug candidates for further clinical studies[82].

Zhang et al.[83] synthesized a series of peptidomimetic α-ketoamides as broad-spectrum inhibitors of 3CLpro of alphacoronaviruses, betacoronaviruses and enteroviruses. Recently, they determined the crystal structure of 3CLpro of SARS-CoV-2 at 1.75 Å resolution (PDB code 6Y2E) and co-crystal structures bound with compound **24** (**13b** in Ref. 83) [PDB code 6Y2F, Fig. 5A-1 (**24**)] with α-ketoamide as the warhead. **24** exhibited inhibition against SARS-CoV-2 infection in human Calu 3 cells with an IC_{50} of 4−5 μmol/L (Table 2). The pharmacokinetic studies of **24**, given these favorable results, provide a promising framework for the development of new 3CLpro inhibitors for COVID-19.

Similarly, the dipeptide-based protease inhibitors GC373 and GC376 [Fig. 5A-1 (**25**, **26**)] were effective inhibitors against 3CLpro of both SARS-CoV and SARS-CoV-2. GC373 is a peptide aldehyde metabolite of GC376.The binding mode of the inhibitors on SARS-CoV-2 3CLpro showed a covalent modification of the amino residue Cys145 in the catalytic site (PDB code 6WTK and 6WTJ). More importantly, both GC373 and GC376 were found to be potent inhibitors of SARS-CoV-2 replication in cells with IC_{50}s near 1 μmol/L with little to no toxicity (Table 2), making them potent drug candidates for the treatment of COVID-19[84]. Structural comparison of reported 3CLpro–inhibitor complex reveals that all of the covalent inhibitor connected to the sulphur atom of amino residue Cys145 (Fig. 5B).

Iketani et al.[85] described three structurally diverse compounds−**27** (**4** in Ref. 85) [Fig. 5A-1 (**27**)], GC376, and MAC-5576−with inhibitory activity against the SARS-CoV-2 3CLpro. Next, they tested these compounds for inhibition of SARS-CoV-2 viral replication. They found that **27** and GC376 could block SARS-CoV-2 infection with IC_{50} values of 2.883 ± 0.227 and 2.189 ± 0.092 μmol/L (Table 2), respectively, whereas MAC-5576 did not.

Recently, four inhibitors targeting the 3CLpro of SARS-CoV-2, namely GC376, boceprevir, and calpain inhibitors II and XII [Fig. 5A-1 (**26**, **28**−**30**)], were identified with IC_{50}s ranging from

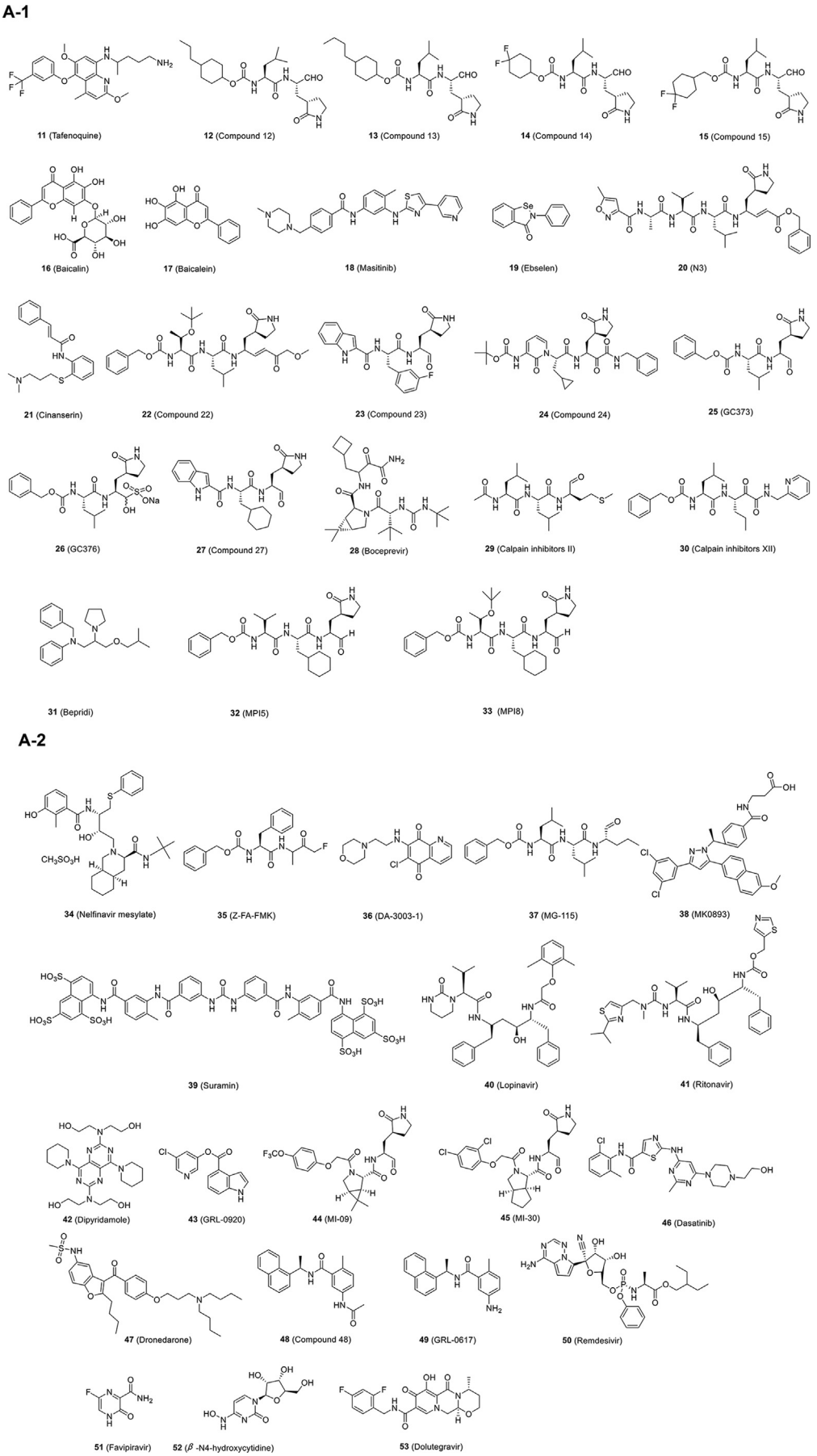

Figure 5 SARS-CoV-2 replication inhibitors. (A) Chemical structures of SARS-CoV-2 replication inhibitors. (B) Overall views of the 3CLpro-N3 (yellow) complex overlapped with carmofur (blue), 13 b (silver), GC373 (red) and GC376 (green) (PDB ID: 7BQY, 7BUY, 6Y2F, 6WTJ and 6WTK), and amino residue Cys145 was shown as cyan. (C) Overall views of the RdRp-suramin (cyan) complex overlapped with the remdesivir (magenta)-bound RdRp structure (PDB ID: 7D4F and 7BV2). nsp 12 was shown as yellow and accessary subunits nsp 7 and nsp 8 were shown as blue and pink.

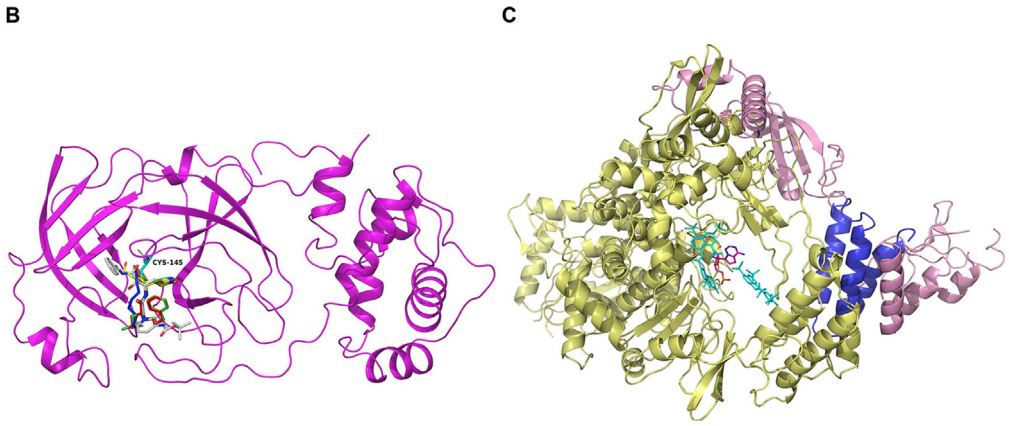

Figure 5 (*continued*)

0.45 to 4.13 μmol/L in the enzymatic assay[86]. Significantly, the four compounds exhibited inhibition against SARS-CoV-2 replication in cells with IC_{50}s ranging from 0.49 to 3.37 μmol/L (Table 2). Especially, boceprevir and calpain inhibitors II and XII represent novel chemotypes, providing a starting point for the development of novel SARS-CoV-2 inhibitors[86]. Another article also reported that boceprevir and GC376 showed inhibitory effects against SARS-CoV-2 in Vero cells [multiplicity of infection (MOI) = 0.01] with IC_{50} values of 15.57 and 0.70 μmol/L, respectively (Table 2)[87]. Moreover, combination of GC376 with remdesivir had a sterilizing additive effect[87]. In addition, some researchers found that the anti-3CLpro activity of calpain inhibitors II and XII was weaker than that of another 3CLpro inhibitor, GC376, in the SARS-CoV-2 3CLpro enzyme inhibition assay[88]. However, calpain inhibitors II and XII actually performed better than GC376 in reducing the replication of SARS-CoV-2 in cell culture[86]. They recently discovered that calpain inhibitors II and XII are also have inhibitory activity against human cathepsin L, which is a host-protease responsible for viral entry[88]. Calpain inhibitors II and XII are dual inhibitors that efficiently target viral protease 3CLpro and human protease cathepsin L, which may explain the excellent antiviral activity of them, despite having inferior affinity for 3CLpro when compared to the specific inhibitor GC376. Dual inhibitors can potentially inhibit drug resistance. Even if the viral protein changes, this type of inhibitor remains effective against unchanged human host proteins. In addition, Hu et al.[89] found that boceprevir, calpain inhibitors II and XII, and GC-376 showed broad-spectrum antiviral activity against SARS-CoV-2, SARS-CoV and MERS-CoV infection, as well as human coronaviruses (CoVs) 229 E, OC43, and NL63. In addition, Cáceres et al.[90] has reported that GC376 is effective in inhibiting SARS-CoV-2 infection *in vivo*. Treatment of SARS-CoV-2-infected K18-hACE2 mice with GC376 resulted in decreased viral loads and reduced inflammation. Recently, Shi et al.[91] reported that application of low-dose GC376 in combination with GS441524, a parent nucleotide analog of remdesivir, that targets the coronavirus RdRp, *via* intranasal or intranasal and intramuscular administration could effectively protect mice against challenge of mouse-adapted SARS-CoV-2.

Vatansever et al.[92] have reported that 6 small-molecule drugs (pimozide, ebastine, rupintrivir, bepridil, sertaconazole, and rimonabant) exhibited 50% inhibition of 3CLpro activity at concentration below 100 μmol/L and that bepridil [Fig. 5A-1 (**31**)] was the basic molecule that potentiates dual functions by raising endosomal pH to interfere with SARS-CoV-2 entry into the host cell, thereby inhibiting 3CLpro activity in infected cells. Their results revealed that bepridil inhibited CPE induced by SARS-CoV-2 infection in Vero E6 and A549/ACE2 cells with IC_{50} value of 0.86 and 0.46 μmol/L, respectively.

Based on the previous medicinal chemistry studies about 3CLpro of SARS-CoV, Yang et al.[93] have designed and synthesized a series of SARS-CoV-2 3CLpro inhibitors that contain *β*-(*S*-2-oxopyrrolidin-3-yl)-alaninal (Opal) for the formation of a reversible covalent bond with the cysteine C145 in SARS-CoV-2 3CLpro active site. Among them, MPI5 and MPI8 [Fig. 5A-1 (**32**, **33**)] completely inhibited CPE induced by SARS-CoV-2 infection in Vero E6 cells at 2.5−5 μmol/L and A549 cells at 0.16−0.31 μmol/L. In preclinical and clinical studies on COVID-19 treatment, their inhibitory potency was remarkably higher than that of some existing molecules.

Jan et al.[94] identified that boceprevir and nelfinavir mesylate [Fig. 5A (**28**, **34**)] can inhibit SARS-CoV-2 infection and replication with IC_{50}s of 50.1 and 3.3 μmol/L, respectively, by measuring viral-induced CPE (Table 2). Through target-based assay, they found that nelfinavir mesylate and boceprevir showed inhibitory activity against 3CL pro. Also, nelfinavir mesylate was selected to evaluate its anti-infective efficacy in female golden Syrian hamsters. Nelfinavir mesylate showed good antiviral effects *in vivo*, and the viral load in hamster lungs was significantly reduced.

Walrycin B, hydroxocobalamin, suramin sodium, Z-DEVD-FMK, LLL-12, and Z-FA-FMK were identified as the most potent 3CLpro inhibitors among 23 hits in a SARS-CoV-2 3CLpro enzyme assay[95]. The protease inhibitor Z-FA-FMK [Fig. 5A-2 (**35**)] inhibited CPE induced by SARS-CoV-2 infection with an IC_{50} of 0.13 μmol/L with no apparent cytotoxicity (Table 2). However, hydroxocobalamin, suramin sodium, and Z-DEVP-FMK were invalidated in the CPE assay. Walrycin B and LLL-12 showed apparent toxicity to Vero E6 cells. Other compounds identified, including DA-3003-1, MG-115 and MK0893 [Fig. 5A-2 (**36**−**38**)], all exhibited antiviral activity, as well, with more or less cytotoxicity to Vero E6 cells (Table 2). Another research team reported that an antiparasitic drug suramin [Fig. 5A-2 (**39**)] inhibits SARS-CoV-2 replication and SARS-CoV-2 infection in Vero E6 cells with an IC_{50} of ~20 μmol/L[96] (Table 2). And suramin could reduce the viral load by two−three logs

in Vero E6 cells or Calu-3 2B4 cells (the human lung epithelial cell line). Analysis of time-of-addition and plaque reduction assays performed on Vero E6 cells indicated that suramin acts in the early stages of the replication cycle and may prevent virus binding or entry.

Lopinavir/ritonavir [LPV/r, Fig. 5A-2 (**40**, **41**)], a protease inhibitor used for HIV infection, showed inhibitory activity against the replication of SARS-CoV, MERS-CoV and SARS-CoV-2 *in vitro* (Table 2)[97–99]. Although these drugs were initially thought to inhibit SARS-CoV and MERS-CoV 3CLpro[18,100,101], it should be pointed out that lopinavir and ritonavir failed to inhibit the activity of SARS-CoV-2 3CLpro[86]. In Korea, clinical administration of LPV/r reduced SARS-CoV-2 viral load rapidly, but no placebo-controlled trial was carried out, and the sample of patients was limited[102]. On the other hand, Cao et al.[103] carried out a randomized, controlled, open-label clinical trial for the treatment of severe COVID-19 with LPV/r (400 and 100 mg, respectively). The two sets of clinical results showed that LPV/r offered little clinical improvement beyond the standard of care. Further, Hung et al.[104] carried out a multicenter, prospective, open-label, randomized phase two trial in COVID-19 adult patients admitted to six hospitals in Hong Kong. Early triple antiviral therapy (combination of lopinavir 400 mg and ritonavir 100 mg every 12 h, ribavirin 400 mg every 12 h, and three doses of eight million international units of interferon β-1b on alternate days) was safe and superior to lopinavir−ritonavir alone (lopinavir 400 mg and ritonavir 100 mg every 12 h) in alleviating symptoms and shortening the time of viral elimination and length of hospitalization in patients with mild to moderate COVID-19[104]. Dual antiviral therapy with interferon β-1b as the backbone will be conducted in clinical studies in the future. Most recently, WHO reported the latest solidarity trial interim results of lopinavir, which, unlike the previously reported results, appeared to have little or no effect on hospitalized COVID-19 patients[105].

The Luo group[106] screened the U.S. FDA-approved drug library and found that the anticoagulation agent dipyridamole [DIP, Fig. 5A-2 (**42**)] might bind to the SARS-CoV-2 protease 3CLpro, suppressing more than 50% of SARS-CoV-2 replication at a concentration of 100 nmol/L in Vero E6 cells (Table 2). Indeed, after two weeks of DIP adjunctive treatment, all eight severe patients showed remarkably positive outcomes.

Hattori et al.[107] reported that one indole-chloropyridinyl-ester derivative, GRL-0920 [Fig. 5A-2 (**43**)], targeting 3CLpro of SARS-CoV-2, exerted potent activity against SARS-CoV-2 (IC_{50} = 2.8 μmol/L) in cell-based assays performed using Vero E6 cells without significant toxicity, as examined with immunocytochemistry (Table 2).

As mentioned above, although some SARS-CoV-2 3CLpro inhibitors have been reported, the previous literature on SARS-CoV-2 3CLpro inhibitors has not included data from the experiments using SARS-CoV-2-infected animal models. Qiao et al.[108] designed and synthesized 32 new bicycloproline-containing 3CLpro inhibitors, all of which derived from boceprevir or telaprevir. All compounds inhibited the activity of SARS-CoV-2 3CLpro with IC_{50}s of 7.6−748.5 nmol/L *in vitro*. Two compounds, MI-09 and MI-30, [Fig. 5A-2 (**44**, **45**)] showed excellent antiviral activity against SARS-CoV-2 with IC_{50}s of 0.86 and 0.54 μmol/L (MOI of 0.1, Vero E6 cells), respectively in cell-based assays (Table 2). In a transgenic mouse model, MI-09 or MI-30 could significantly reduce lung viral load and lung lesions. Also, MI-09 or MI-30 showed good pharmacokinetic properties and safety in rats.

5.2.2. *Papain-like protease inhibitors*

PLpro is the other crucial viral protease spurring the discovery of anti-SARS-CoV-2 drugs, and its crystal structure has recently been resolved (PDB code 6W9C). PLpro is also reported to drive virus evasion of host innate immune defenses by reversing host ubiquitination and ISGylation events[109]. Thus, PLpro inhibitors may not only directly inhibit SARS-CoV-2 replication, but also perform a complementary function by normalizing the body's immune response against virus invasion. Previous studies identified that thiopurine (6-mercaptopurine and 6-thioguanine, antitumor drugs) showed inhibitory activity against SARS-CoV and MERS-CoV PLpro[110], so repurposing these candidates for treating COVID-19 seems to be a reasonable approach.

Some noncovalent small-molecule inhibitors, rac3j, rac3k and rac5c, against SARS-CoV PLpro[111] also target SARS-CoV-2 PLpro, preventing the self-processing of nsp 3 in cells and reducing SARS-CoV-2-induced CPE under high (33 μmol/L) concentrations. For rac5c, it is worth mentioning that treatment at 11 μmol/L in 0.1% DMSO continued to show a clear reduction of CPE, indicating effective antiviral activity. For rac3j and rac3k, CPE reduction diminished at lower concentrations[112].

Referring to the crystal structure of SARS-CoV-2 PLpro, Kouznetsova et al.[113] performed useful data mining of the conformation of FDA-approved drugs. 147 compounds were identified as potential inhibitors of SARS-CoV-2 PLpro. Among them, dasatinib [Fig. 5A-2 (**46**), Table 2] showed antiviral activity against SARS-CoV-2 in clinical cases[114], but it was unclear whether dasatinib directly interacts with PLpro. A patient with chronic myeloid leukemia and COVID-19 was treated with dasatinib (100 mg/day) in combination with antibiotics for 11 days, resulting in the disappearance of fever. Two weeks later, two consecutive swab tests were negative for SARS-CoV-2 RNA. Dronedarone [Fig. 5A-2 (**47**)], which was reported by Jan et al.[94], is effective in inhibiting the activity of PLpro. And dronedarone showed antiviral activity against CPE induced by SARS-CoV-2 infection with an IC_{50} of 4.5 μmol/L (CC_{50} of 12.1 μmol/L) in Vero E6 cells (Table 2), which is an ion channel modulator.

Mirza et al.[115] reported compound **Z93** as a potential human ubiquitin-specific protease 2 (USP2) inhibitor through integrated *in silico* efforts. USP2 inhibitors, such as thiopurine analogs, have been reported to inhibit SARS-CoV PLpro. However, based on the above results, it can only be speculated that **Z93** might be a potential chemical lead targeting SARS-CoV-2 PLpro, thus warranting further evaluation *in vitro*. Additionally, the Pegan group[109] declared that naphthalene-based inhibitors [**48** (**6** in Ref. 109) and GRL-0617, Fig. 5A-2 (**48**, **49**)] showed inhibitory activity against SARS-CoV-2 PLpro and antiviral activity with IC_{50}s of 21.0 and 27.6 μmol/L, respectively (Table 2). Gao et al.[116] showed that GRL-0617 was effective in inhibiting SARS-CoV-2 PLpro activity with an IC_{50} of 2.2 ± 0.3 μmol/L and that its mechanism of action was not limited to occupying the substrate pockets, but rather extended to sealing the entrance to the substrate binding cleft, thereby preventing the binding of the substrate. Another team reported that GRL-0617 showed a promising inhibitory activity against SARS-CoV-2 PLpro *in vitro* with an IC_{50} of 2.1 μmol/L and effective antiviral inhibition of SARS-CoV-2 in cell-based assays. No apparent cytotoxicity of GRL-

0617 on Vero E6 cells was observed with concentrations up to 100 μmol/L[117].

5.2.3. RNA-dependent RNA polymerase (RdRp) inhibitors

RdRp, a nsp, also known as nsp 12, catalyzes the synthesis of viral genome, which plays a central role in coronaviral replication. Thus, it is considered as an excellent drug target for antiviral inhibitors. The Rao group[118] solved a cryo-electron microscopy structure of full-length RdRp in complex with nsp 7 and nsp 8 at a resolution of 2.9 Å and speculated the binding mode of nucleotide analogs remdesivir and favipiravir to explain the mechanisms of inhibition. The elegant results provide novel insight and lay a solid foundation for structure-based antiviral inhibitor design.

Remdesivir [GS-5734, Fig. 5A-2 (**50**)], a nucleotide analogue, was originally developed for Ebola treatment[119]. Since its triphosphate form resembles adenosine triphosphate (ATP), it was used as a substrate for viral RdRp, and it performed broad-spectrum activity against coronaviruses[120,121]. Remdesivir was first suggested by Morse et al.[6] as a COVID-19 therapeutic for treatment of patients infected by SARS-CoV-2. Remdesivir (IC_{50} = 0.77 μmol/L; CC_{50} > 100 μmol/L; SI > 129.87) potently blocked SARS-CoV-2 infection in Vero E6 cells at a MOI of 0.05 and showed high SI (Table 2)[19]. Recently, Beigel et al.[122] reported the results of the ACTT-1 clinical trial of remdesivir. The ACTT-1 clinical trial is a double-blind, randomized, placebo-controlled global phase III clinical trial. Remdesivir was superior to placebo in shortening recovery time and reducing lower respiratory tract infections in adult hospitalized patients with COVID-19[122]. On 1 May 2020, remdesivir was made available under the U.S. FDA Emergency Use Authorization (EUA) for the treatment of severely ill hospitalized patients with COVID-19. The research group also pointed out that combining remdesivir with other treatments or antiviral drugs to improve the prognosis of COVID-19 patients should be evaluated in the future[122]. Despite this, a randomized, double-blind, placebo-controlled study showed that 237 enrolled patients showed no association between remdesivir use and statistically significant clinical benefits[123]. This study had to be terminated early owing to the adverse events observed in patients. WHO published the mid-term results of the Solidarity Trial of Remdesivir using for COVID-19 patients on 15 October 2020. Unlike earlier expectations, remdesivir appeared to have little, or no, effect on hospitalized COVID-19 patients in terms of overall mortality, initiation of ventilation and duration of hospital stay[124]. Yin et al.[126] have reported that suramin, a 100-year-old drug, and its derivatives are at least 20-fold more potent than remdesivir in inhibiting SARS-CoV-2 infection by targeting RdRp. The crystal structure of RdRp in complex with suramin has revealed two binding sites in RdRp (Fig. 5C). As a non-nucleotide inhibitor, suramin could ultimately aid in structure-based drug development for COVID-19[126].

Favipiravir [Fig. 5A-2 (**51**), Table 2, IC_{50} = 61.88 μmol/L, CC_{50} > 400 μmol/L, SI > 6.46] had low anti-SARS-CoV-2 activity in cell culture assays[19]. However, favipiravir has been shown to completely protect mice against Ebola virus challenge and has an IC_{50} value of 67 μmol/L in Vero E6 cells[127], suggesting that further *in vivo* studies should be undertaken to assess the efficacy of this antiviral nucleoside in the treatment of COVID-19. In Shenzhen, a clinical trial of favipiravir on COVID-19 patients was conducted (ChiCTR2000029600), and it showed that 35 patients in the favipiravir arm had significantly shorter viral clearance duration in contrast with the control arm containing 45 patients[128]. In another multi-centric randomized study (ChiCTR200030254), treatment with favipiravir of COVID-19 patients led to an improved recovery at the 7th day[129].

β-D-*N*4-Hydroxycytidine [EIDD-1931, Fig. 5A-2 (**52**)] is an orally available ribonucleoside analogue with broad-spectrum antiviral activity against RNA viruses, including influenza, Ebola, CoV, and Venezuelan equine encephalitis (VEE) virus[130–133]. Sheahan et al.[120] reported that EIDD-1931 showed antiviral activity against SARS-CoV-2 in Vero cells with an IC_{50} of 0.3 μmol/L (Table 2). Both prophylactic and therapeutic administration of EIDD-2801, an orally available EIDD-1931-prodrug, in mice infected with SARS-CoV or MERS-CoV, could improve pulmonary function and reduce virus titer and body weight loss[120]. The mechanism of action of EIDD-2801 is different from that of remdesivir. Remdesivir is a chain terminator[134], while EIDD-2801 causes mutagenesis in the viral RNA. In addition, EIDD-2801 is active against remdesivir-resistant mutants and has a higher genetic barrier to drug resistance than remdesivir[120]. For VEE and influenza viruses, compound EIDD-2801 inhibited RdRp to exert its antiviral functions, but the mechanism of action against coronaviruses was not well documented[135].

5.2.4. Helicase inhibitors

Helicase, a motor protein, is responsible for separation and/or rearrangement of viral nucleic acid duplexes before transcription or replication[136]. The helicase known as nsp 13 consists of three major domains—a putative N-terminal metal-binding domain (MBD), a hinge domain, and a helicase domain. And the N-terminal forms a Zn-binding domain, while the C-terminal forms a helicase domain with a conserved motif and participates in both unravelling double-stranded (ds) DNA and capping of viral RNA. Studies have shown that nsp13-dependent disintegration was an essential process for the replication, transcription, and translation of SARS-CoV-2 genome[137]. Therefore, helicases are potential targets for antiviral therapies of COVID-19 and inhibitors of helicases, such as bananins, 5-hydroxychromone derivatives, ADKs, and SSYA10-001, and are expected to be used in the treatment of COVID-19[138–141]. In addition, clofazimine was shown to inhibit SARS-CoV-2 replication by interfering with the function of helicase[69]. The biggest challenge in targeting helicase is the relatively low selectivity of helicase inhibitors. Right now, no antiviral targeting helicase has moved beyond preclinical development.

5.2.5. 2′-*O*-Ribose methyltransferase (2′-*O*-MTase) inhibitors

The nsp 16, or 2′-*O*-MTase, is another crucial protein responsible for SARS-CoV replication[142–144] by catalyzing 5′-terminal caps structures (m7GpppN) of mRNA for methylation, thereby preventing recognition and activation of host immune responses[144,145].

By using model docking and molecular dynamics simulation, Khan et al.[144] established dolutegravir [Fig. 5A-2 (**53**), detaG = −9.4 kcal/mol] as an excellent lead candidate for the crucial protein 2′-*O*-Mtase. Dolutegravir, an integrase strand-transfer inhibitor, with inhibitory activity against human immunodeficiency virus type 1 (HIV-1) infection, could suppress SARS-CoV-2 replication with IC_{50} of 22.04 μmol/L and CC_{50} > 40 μmol/L in Vero E6 cells (Table 2)[146].

5.2.6. RNA-binding N-terminal domain inhibitors

N protein, usually located inside the virions, is an abundant coronavirus protein that binds with the viral genome to form the ribonucleoprotein. It plays a critical role in viral RNA

transcription and replication[147], making it a potential antiviral drug target. Recent studies showed that N protein is a multifunctional protein responsible for binding to the viral RNA genome and packing it into a long helical nucleocapsid structure[148]. It is also reported to regulate host−pathogen interaction and induce protective immune responses[149].

The Medhi group[22] identified two potential hit compounds, ZINC00003118440 and ZINC0000146942, both of which might bind the RNA-binding N-terminal domain of SARS-CoV-2 N protein, which were theophylline and pyrimidone derivatives, respectively. Thereafter, Kang et al.[150], for the first time, resolved the X-ray crystal structure of SARS-CoV-2 N protein at a resolution of 2.7 Å, revealing the specific surface charge distributions that facilitates the drug discovery specific to ribonucleotide binding domain of SARS-CoV-2 N protein.

5.3. *Others*

Studies have shown that the genome sequence of SARS-CoV-2 is very similar to that of SARS-CoV. Several SARS-CoV-2 proteins with > 90% sequence similarity, such as S protein, 3CLpro, PLpro, RdRp and 2′-*O*-MTase, could be used as drug targets. Meanwhile, many small molecules have been described as potential drug candidates for treatment of COVID-19, but their targets have not been identified. Some of these small-molecule drugs, are summarized below.

5.3.1. *Broad-spectrum antiviral compounds*

Early in the COVID-19 pandemic, some anti-flu drugs (for example, oseltamivir) were applied to treat COVID-19 patients[151]. Yamamoto et al.[152] reproted that nine approved HIV-1 protease inhibitors exhibited antiviral activity against SARS-CoV-2 *in vitro*. Among these inhibitors, tipranavir [Fig. 6 (**54**), IC_{50} = 13.34 μmol/L, CC_{50} = 76.80 μmol/L, SI = 5.76], ritonavir [Fig. 5A-2 (**41**), IC_{50} = 8.63 μmol/L, CC_{50} = 74.11 μmol/L, SI = 8.59], saquinavir [Fig. 6 (**55**), IC_{50} = 8.83 μmol/L, CC_{50} = 44.43 μmol/L, SI = 5.03], atazanavir [Fig. 6 (**56**), IC_{50} = 9.36 μmol/L, CC_{50} > 81 μmol/L, SI > 8.65] and nelfinavir [Fig. 5A-2 (**34**), IC_{50} = 1.13 μmol/L, CC_{50} = 24.32 μmol/L, SI = 21.52] all exhibited antiviral activity (Table 2). Notably, nelfinavir effectively inhibited SARS-CoV-2 replication at a low concentration and exhibited the high SI among of them[152], Indicated that nelfinavir is a potential drug candidate for the treatment of COVID-19 and should therefore be evaluated in patients with SARS-CoV-2 infection.

5.3.2. *Screening of FDA-approved drugs for the prevention of SARS-CoV-2*

Repurposing of approved drugs is a time-saving strategy for drug development. In this way, Touret et al.[146] screened the Prestwick Chemical Library composed of 1520 approved drugs in an SARS-CoV-2-infected cell-based assay. The results showed that 15 molecules exhibited inhibition of SARS-CoV-2 replication *in vitro*. Among of them, 11 compounds exhibited antiviral potency with 2 < IC_{50} ≤ 20 μmol/L. Two of them with the highest antiviral activity were obtained from azithromycin [Fig. 6 (**57**), IC_{50} = 2.12 μmol/L, SI > 19] and hydroxychloroquine [Fig. 9 (**76**), IC_{50} = 4.17 μmol/L, SI > 10] and were therefore selected for clinical trials (Table 2)[153]. Among of them, spiperone [Fig. 6 (**58**)], a dopaminergic D2 antagonist, which was already identified as an antiviral molecule against the human pathogenic polyomaviruses[154], showed most potent antiviral activity against SARS-CoV-2 infection with an IC_{50} of 2.49 μmol/L and SI value of 16 (Table 2). The next three most efficient drugs were opipramol dihydrochloride [Fig. 6 (**59**), IC_{50} = 5.05 μmol/L, SI > 7.9], a tricyclic antidepressant, quinidine hydrochloride [Fig. 6 (**60**), IC_{50} = 5.11 μmol/L, SI > 7.8], an antiarrhythmic drug, and alprostadil [Fig. 6 (**61**), IC_{50} = 5.39 μmol/L, SI > 7.4], a prostaglandin known as a cardiovascular drug. The remaining nine drugs out of 15 showed 1.0 < SI < 5.3 (Table 2)[146]. Some of these candidates may provide information to guide downstream experiments in small animal models, discover more effective derivatives, or evaluate drug combinations *in vitro* with potential enhancement of efficacy.

Ivermectin [Fig. 6 (**62**)], an FDA-approved antiparasitic agent with a broad spectrum of activity, high efficacy and a broad safety profile, is reported to have potent antiviral activity against HIV-1 and dengue virus by inhibiting protein nuclear import[155,156]. Caly et al.[157] found that ivermectin could inhibit SARS-CoV-2 replication with an IC_{50} of ~2 μmol/L *in vitro* (Table 2), demonstrating that it is worthy of further research to treat COVID-19.

The repurposed drug anakinra in a phase III randomized clinical trial was able to reduce the requirement of invasive mechanical ventilation and mortality rate in severe COVID-19 cases without serious side effects[158].

Five FDA-approved drugs, including penciclovir [Fig. 6 (**63**)], were reevaluated for their effects on cytotoxicity, viral production,

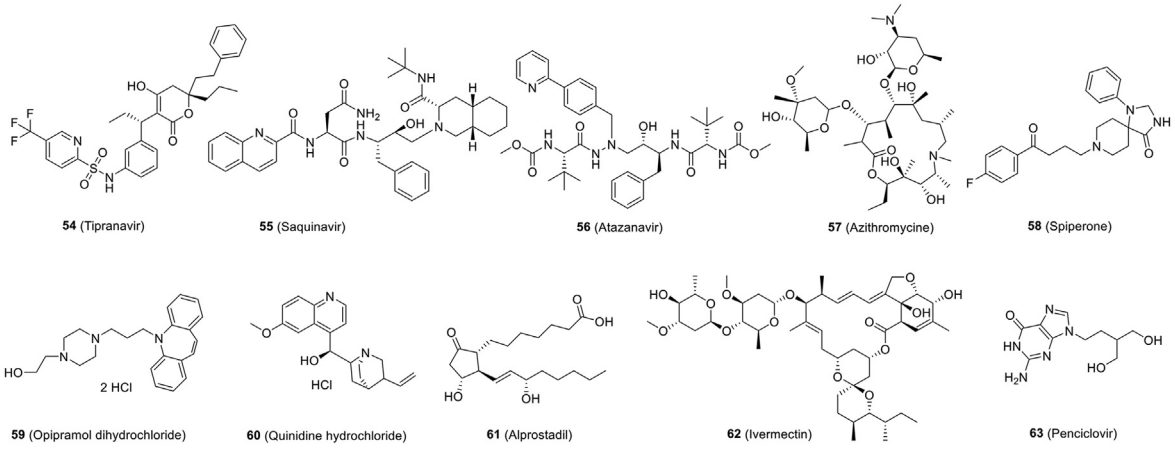

Figure 6 Chemical structures of other small-molecule inhibitors against SARS-CoV-2.

and infection rates of SARS-CoV-2 by using standard methods[19]. The results showed that penciclovir inhibited viral infection with an IC_{50} value of 95.96 μmol/L (Table 2), which was far from satisfactory compared to other compounds tested at the same time. However, some data indicated that compounds with high IC_{50} values may have surprising antiviral activity *in vivo*[127]. Therefore, it is necessary to detect the antiviral activity of these compounds *in vivo*.

6. Small-molecule SARS-CoV-2 inhibitors targeting host proteins

Based on existing treatments, new SARS-CoV-2 mutations are likely to be resistant to drugs. Therefore, an initial suggestion is to use host-targeting therapeutic approach to reduce the aggressiveness and mortality resulted from SARS-CoV-2 infections.

6.1. *Inhibitors targeting ACE2 to block the interaction between S protein and ACE2*

Many studies have shown that host ACE2 is the specific receptor for SARS-CoV-2 S protein[5,10]. Inhibitors that block the binding between S protein and ACE2 have been considered to use for treatment of COVID-19 by preventing virus entry into the host cells. Candesartan and olmesartan [Fig. 7 (**64**, **65**)], which are angiotensin II receptor blockers (ARBs), as reported, play a key role in virus entry[23,159,160]. Clinical trials will also be conducted on another ARB derivative, telmisartan [Fig. 7 (**66**), Clinical Trials gov, NCT04356495][161]. In addition, blockers of angiotensin receptor one, such as losartan [Fig. 7 (**67**)], as inhibitors of the renin-angiotensin system, could be a useful therapeutic option in reducing lung inflammation and pneumonia in COVID-19 patients[162,163]. Currently on the clinical trial website, losartan is being used to study its effect on suppress the pathologic damage in inpatients and outpatients with COVID-19 and its safety to the COVID-19 patients with respiratory failure (Table 3)[164,165].

An anthraquinone compound, emodin, which derived from genus *Rheum* and *Polygonum*, can interfere with the interactions between S protein and ACE2 by competing with ACE2 and inhibiting the 3a ion channel of coronavirus[166,167]. Promazine is an anti-psychotic drug that shares a similar structure with emodin and presents comparable inhibitory effect on the replication of SARS-CoV with a similar mechanism of action[167]. Based on the similarity of S protein sequences between SARS-CoV-2 and SARS-CoV, we could conclude that emodin and promazine may inhibit SARS-CoV-2 infection by blocking the binding between S protein and ACE2, and both of them are considered as potential drugs for the treatment of COVID-19[168–170].

Nicotianamine, a unique secondary metabolite in soybean, is a potent inhibitor of ACE2[171], and it is considered as a potential drug candidate for the treatment of COVID-19[172,173]. Similarly, flavonoids were also a class of natural products which possess antioxidant, anti-inflammatory and antiviral functions. Lead flavonoids (*e.g.*, hesperidin, naringin, ECGC and quercetin) screened out by molecular docking might serve as cell entry inhibitors by targeting S protein or ACE2[174].

6.2. *TMPRSS2 inhibitors*

SARS-CoV-2 attaches to the ACE2 receptors *via* S protein, which is subsequently cleaved by TMPRSS2, a host serine protease that has been exploited as therapeutic targets. Hoffmann and colleagues demonstrated that the TMPRSS2 inhibitors proved useful in blocking virus entry[10].

Camostat mesylate [Fig. 8 (**68**)], a TMPRSS2 inhibitor in clinical, significantly reduced SARS-CoV-2 pseudovirus entry into Calu-3 cells with an IC_{50} of ~1 μmol/L with no cytotoxic effects (CC_{50} > 500 μmol/L, Table 3)[10]. Similarly, camostat mesylate was effective significantly in reducing Calu-3 authentic SARS-CoV-2 infection in Calu-3 cells and SARS-CoV-2 pseudovirus infection in primary human lung cells[10]. A randomized phase 2a clinical trial (Clinical Trials gov, NCT04321096) and double-blinded, randomized, placebo-controlled phase four trials in (Clinical Trials gov, NCT04338906) were performed to evaluate the activity of camostat mesylate as a treatment for SARS-CoV-2 infection. Bromhexine, a generic mucolytic, is a TMPRSS2 inhibitor[175], which is currently being evaluated clinically as a treatment for COVID-19 (Clinical Trials gov, NCT04273763). In order to evaluate the impact of bromhexine on COVID-19 treatment, a larger phase one clinical trial with 140 participants (Clinical Trials gov, NCT04340349) was performed, including treatment with bromhexine alone or in combination with hydroxychloroquine sulfate. A broad-spectrum serine protease

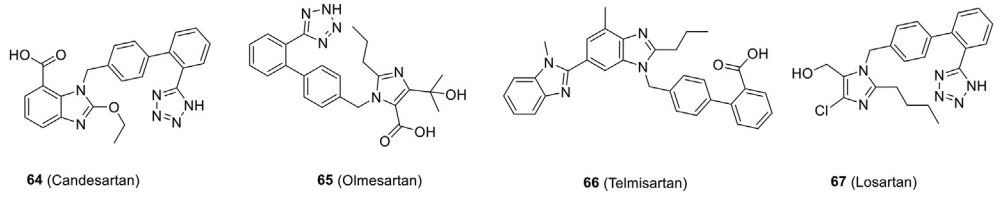

Figure 7 Chemical structures of small-molecule inhibitors targeting ACE2.

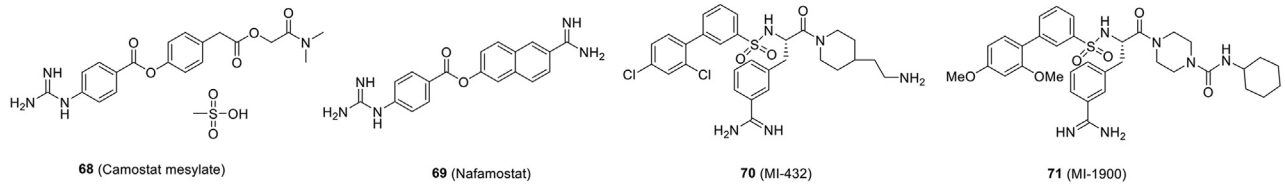

Figure 8 Chemical structures of small-molecule inhibitors targeting TMPRSS2.

inhibitor, nafamostat [Fig. 8 (**69**)], could inhibit the activity of TMPRSS2. Experiments have shown that nafamostat mesylate can inhibit SARS-CoV-2 infection to human lung cells[176]. Standard assays were also carried out by Wang et al.[19] to test the effects of nafamostat on the cytotoxicity, virus yield and infection rates of SARS-CoV-2, and the results demonstrate that the IC_{50} value of nafamostat is 22.50 μmol/L with cytotoxicity $CC_{50} > 100$ mmol/L (Table 3). In another article, nafamostat mesylate inhibited SARS-CoV-2 infection CPE in Calu-3 cells with an IC_{50} of around 10 nmol/L[177]. Furthermore, Asakura and Ogawa[178] claimed that the combination of nafamostat and heparin may be effective against COVID-19 from the perspectives of antiviral and anti-DIC (disseminated intravascular coagulation) with enhanced fibrinolysis symptoms for COVID-19 patients. In addition, a randomized clinical trial was conducted to the evaluated ability of nafamostat to slow down lung disease for adult COVID-19 patients (Clinical Trials gov, NCT04352400).

MI-432[179] and MI-1900 [Fig. 8 (**70**, **71**)] are two TMPRSS2 inhibitors, which are prospective peptide mimetic inhibitors[180]. The inhibitor MI-1900 is the less polar analog of MI-432. Recently, MI-432 showed inhibition against SARS-CoV-2 infection in Calu-3 cells, which reduced by 75-fold virus titer at a concentration of 10 μmol/L, and MI-1900 exhibited strong inhibition of SARS-CoV-2 replication at 50 μmol/L and reduced by 35- to 280-fold viral titers (Table 3)[180].

6.3. *Cathepsin B/L*

In addition to TMPRSS2, cathepsin B and cathepsin L, being active in the early and late endosome, respectively, can also trigger viral S protein cleavage and promote viral fusion[181,182].

P9, derived from mouse β-defensin-4, is a peptide that interfere cathepsin L activity, which showed antiviral activity against SARS-CoV, MERS-CoV, and influenza viruses through the inhibition of endosomal acidification thus indirectly interfering cathepsin L activity[183]. The researchers optimized P9 by replacing arginine with the weakly positively charged amino acids (histidine and lysine) to generate P9R, which showed significantly higher potency against SARS-CoV-2 infection than P9, as determined by a plaque reduction assay in Vero E6 cells (0.9 *vs*. 2.4 μg/mL, Table 1). And the CC_{50} of P9R was >300 μg/mL in MDCK, Vero E6 and A549 cells[184]. Further, the authors describe an eight-branched derivative, 8P9R, that showed more potent antiviral activity (IC_{50} = 0.3 μg/mL) in high salt condition (PBS) than that of P9R in Vero E6 cells. The cytotoxicity assay indicated that CC_{50} of 8P9R was higher than 200 μg/mL in Vero E6 cells (Table 1). The 8P9R can inhibit the two entry pathways of SARS-CoV-2 in cells including endocytic pathway and TMPRSS2-mediated surface pathway by aggregating viral particles. In addition, 8P9R, or the combination of repurposed drugs, arbidol, chloroquine and camostat could significantly suppress SARS-CoV-2 replication in hamsters and SARS-CoV in mice[185].

Amantadine and chlorpromazine [Fig. 9 (**72**, **73**)], which were previously used to abrogate viral entry *via* clathrin-mediated endocytosis[24,186], proved to have no prominent antiviral efficacy against SARS-CoV-2 and only a partial inhibition at 100 μmol/L for amantadine in Vero E6 cells (Table 3)[187]. E64-d [Fig. 9 (**74**)], a broad cathepsin B/L inhibitor, showed inhibitory activity against SARS-CoV-2 pseudovirus infection with an IC_{50} of ~4.487 μmol/L in Vero E6 cells (Table 3).

Chloroquine [Fig. 9 (**75**)] prevents viral infection by increasing the endosomal pH, which, in turn, inhibits hydrolytic activity of cathepsin L[19]. In *in vitro* experiments, chloroquine showed strong inhibitory activity against SARS-CoV-2 with an EC_{50} of 1.13 μmol/L, $CC_{50} > 100$ μmol/L and SI > 88.50 (Table 3) in Vero E6 cells (MOI = 0.05)[19]. Hydroxychloroquine [Fig. 9 (**76**)] is a

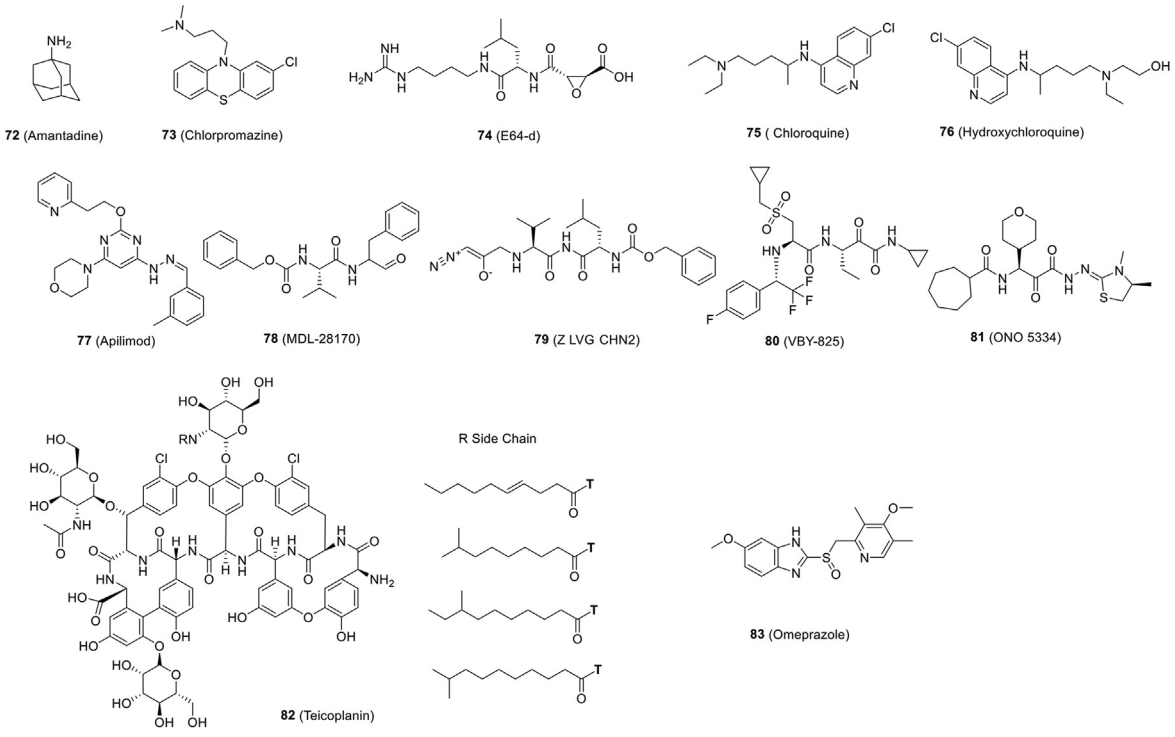

Figure 9 Chemical structures of small-molecule inhibitors targeting cathepsin B/L.

derivative of chloroquine and is used to treat autoimmune diseases. The Chinese Clinical Trials Registry has documented several clinical trials using chloroquine or hydroxychloroquine for COVID-19[188,189]. Consequently, some research groups compared hydroxychloroquine with chloroquine for inhibitory against SARS-CoV-2 *in vitro*. The results showed that hydroxychloroquine had lower antiviral activity compared to chloroquine, at least at certain MOIs (Table 3)[189]. Although it was proven that chloroquine could inhibit SARS-CoV-2 infection in Vero cells, it does not block SARS-CoV-2 infection in the TMPRSS2-expressing Calu-3 cells[190]. The Maisonnasse group[191] tested different treatment strategies for hydroxychloroquine in rhesus macaques, including comparison with placebo treatment alone or in combination with azithromycin in macaques. Neither hydroxychloroquine nor the combination of hydroxychloroquine and azithromycin showed a significant effect on viral load in any of the analyzed tissues. The U.S. FDA has withdrawn its EUA status given to chloroquine and hydroxychloroquine in view of these recent developments. The latest clinical results of hydroxychloroquine were also published by WHO in the interim results of the Solidarity Trial of antiviral drugs for the treatment of COVID-19. The results showed that hydroxychloroquine cannot significantly reduce mortality of COVID-19 hospitalized patients. At the same time, it cannot reduce the rate of mechanical ventilation (intubation) and duration of hospital stay[124].

To identify candidate therapeutics for COVID-19, Riva et al.[68] screened a drugs library consisting of approximately 12,000 clinical-stage or FDA-approved small molecules. They reported the identification of 21 molecules that inhibit SARS-CoV-2 infection in a dose-dependent manner. Five of the most potent compounds, apilimod [Fig. 9 (**77**)] and the cysteine protease inhibitors MDL-28170, Z LVG CHN2, VBY-825 and ONO 5334 [Fig. 9 (**78**−**81**)], were effective against SARS-CoV-2 infection with IC_{50}s of 0.023, 0.22, 0.19, 0.3 and 0.41 μmol/L, respectively, in Vero E6 cells (Table 3). As reported, MDL-28170, a cathepsin B inhibitor, could impairs infection by SARS-CoV and Ebola virus[192,193], ONO 5334 is a cathepsin K inhibitor[194], and VBY-825 acts as a reversible cathepsin protease inhibitor[195]. Apilimod, a specific PIKfyve kinase inhibitor, was shown to be effective in inhibiting virus entry, which is in agreement with the report that PIKfyve is predominately presented in early endosomes and plays an important role in maintaining endomembrane homeostasis[196]. Another study reported that apilimod inhibits infection with authentic SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020 virus in Vero E6 cells with an IC_{50} of ~10 nmol/L (Table 3)[197]. Z LVG CHN2 probably acts as inhibitor of an endosomal protease[198]. Notably, MDL-28170, ONO 5334 and apilimod exhibited inhibitory activity against viral replication in human pneumocyte-like cells that derived from induced pluripotent stem cells, and apilimod displayed antiviral activity in a primary human lung explant model[68].

Teicoplanin [Fig. 9 (**82**)], a currently used antibiotic in the treatment of Gram-positive bacterial infection, showed to be active against SARS-CoV, MERS-CoV and Ebola virus *in vitro*[199,200]. In the reports of Zhang et al.[201], teicoplanin acts on the early step of coronavirus viral life cycle through directly inhibiting the enzymatic activity of cathepsin L. The concentration of teicoplanin required to inhibit 50% of SARS-CoV-2 pseudoviruses into the cytoplasm was only 1.66 μmol/L (Table 3) in HEK293T cells, which is much lower than the commonly used 8.78 μmol/L dose to inhibit Gram-positive bacteria[201]. Baron and coworkers encourage further investigation of teicoplanin for the treatment of COVID-19[199]. Dalbavancin, a homolog of teicoplanin, also showed inhibitory activity against SARS-CoV-2 entry in a dose-dependent manner[201].

Touret et al.[146] found that omeprazole [Fig. 9 (**83**)] can inhibit SARS-CoV-2 with IC_{50} of 17.06 μmol/L and $CC_{50} > 40$ μmol/L in Vero E6 cells (Table 3). Omeprazole, a proton pump inhibitor used as an antiulcer agent, has been demonstrated to increase the pH of endosomal/Golgi pathway either by inhibiting ATPase proton pomp, or by buffering the pH. This endosomal pH modification will limit the processing of S protein by endosomal proteases, thereby blocking the entry of viruses mediated by the membrane fusion process[146].

The 3CLpro calpain inhibitors II/XII are also reported to be active against human cathepsin L[88]. Calpain inhibitors II/XII inhibit the activity of cathepsin L with IC_{50}s of 0.41 and 1.62 nmol/L, respectively[88]. One of the advantages of calpain inhibitors II and XII, as dual inhibitors, is their ability to target the viral protease 3CLpro and the human protease cathepsin L, thus showing better drug effects at lower doses. Another advantage is that they can inhibit drug resistance.

Cathepsin B/L are crucial elements of the lysosomal pathway, and disruption of these host cell proteases offers potential for COVID-19 therapies. Smieszek et al.[202] conducted a HTS to identify compounds that could downregulate the expression of cathepsin L/cathepsin B. Amantadine (10 μmol/L) can downregulate the expression of the cathepsin L gene, which appears to further disrupt the lysosomal pathway. Based on this, researchers believe that amantadine can reduce the viral load in SARS-CoV-2-positive patients and that it may be used as an effective treatment to reduce virus replication and infectivity, which may lead to better clinical outcomes.

6.4. *Others*

Innate immune response plays roles in combating coronavirus infection, while interferon can enhance the immune responses[25]. In human cells, blockage of the signal pathway required for viral replication is expected to exhibit some antiviral effect.

In patients infected with SARS-CoV-2, histological examination showed a strong cytokine storm and inflammatory response, and with the release of large amounts of interleukin (IL)-6, excessive inflammation and further lung damage resulted[26,203]. Thalidomide [Fig. 10A (**84**)] has anti-inflammatory activity owing to its ability to accelerate the degradation of messenger RNA in blood cells, thereby reducing reduce tumor necrosis factor-α. In addition, thalidomide can increase the secretion of interleukins, such as IL-12, and activate natural killer cells[204]. Thalidomide was one of many drugs redirected after the COVID-19 outbreak and used in clinical trials to treat COVID-19 patients (Table 3)[205]. It is a treatment for cancer and inflammatory diseases, but it is currently being used in two phase two clinical trials in combination with low-dose hormone therapy and adjuvant therapy for COVID-19.

Multiple sclerosis (MS) is an immune-mediated neurological disease that requires long-term immunotherapy and has been shown to increase the risk of SARS-CoV-2 infection[206]. Fingolimod [Fig. 10A (**85**)], a sphingosine-1-phosphate receptor immunomodulator, which is effective in the treatment of MS and is currently being tested as a treatment for COVID-19-associated acute respiratory distress syndrome (Table 3)[205,207]. A patient with MS infected with SARS-CoV-2 was treated with fingolimod and had a favorable outcome[206].

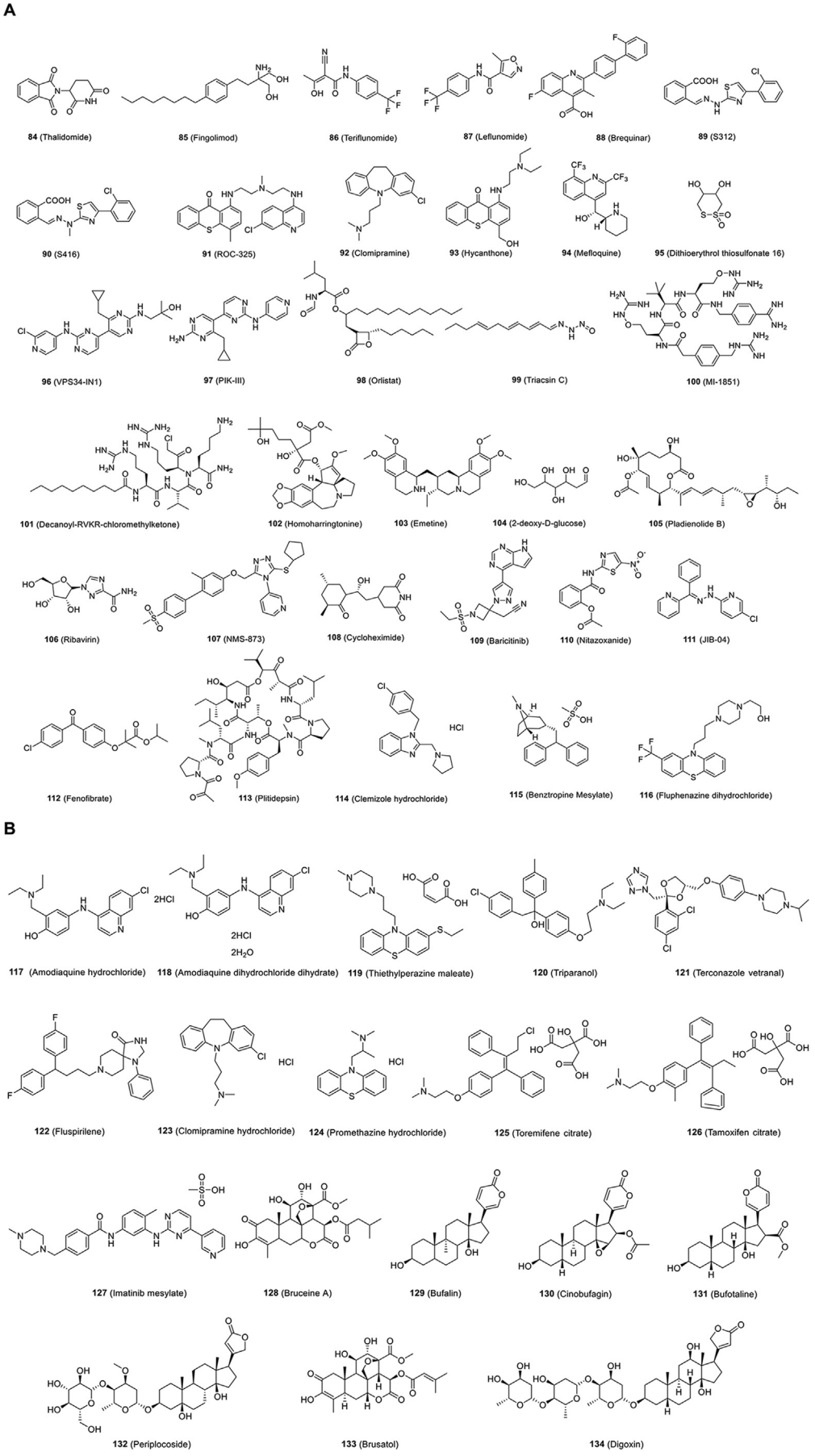

Figure 10 Chemical structures of other small-molecule inhibitors targeting host.

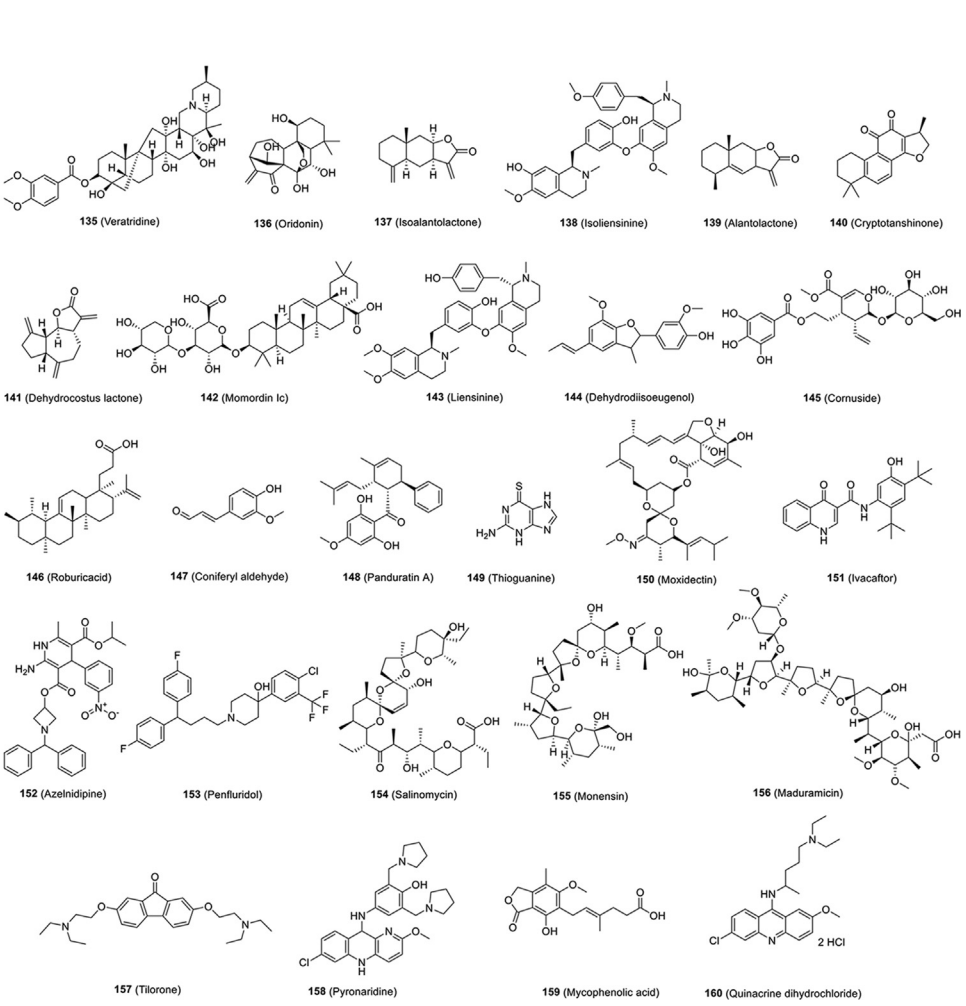

Figure 10 (*continued*)

In one study, Hu et al.[208] evaluated leflunomide for COVID-19 treatment with a small cohort of patients, while the active metabolite of leflunomide, teriflunomide [Fig. 10A (**86**)], as an approved DHODH inhibitor, has been approved for treating autoimmune diseases[209]. They also proved that teriflunomide conferred a profound antiviral efficacy of IC_{50} = 6 μmol/L in SARS-CoV-2-infected cells at MOI of 0.03 (Table 3). Clinical data show that patients treated with leflunomide had shorter viral shedding time (median of 5 days) than that of the controls (median of 11 days). The C-reactive protein levels of patients given leflunomide also decreased significantly, indicating that immunopathological inflammation was well controlled. In leflunomide-treated patients, no obvious adverse reactions were observed[208]. This small-scale preliminary study on the compassionate use of leflunomide provides data basis for leflunomide as a potential therapeutic for COVID-19 in the future.

By virtual screening, Xiong et al.[210] identified two potent human DHODH inhibitors with the same novel scaffold, S312 and S416, which is obviously different from those of leflunomide/teriflunomide[208] or brequinar[211]. All compounds of teriflunomide, leflunomide, brequinar, S312, and S416 [Fig. 10A (**86**–**90**)] showed inhibitory effects against SARS-CoV-2 (at MOI = 0.05), with IC_{50}s of 26.06, 41.49, 0.123, 1.56 and 0.017 μmol/L and CC_{50}s of 850.5, 879.0, 231.3, 158.2 and 178.6 μmol/L, respectively[210] (Table 3). These results indicate that both S312/S416 and leflunomide/teriflunomide, which have dual actions of antiviral and immunoregulation, may have the clinical potential to cure SARS-CoV-2.

Chloroquine and hydroxychloroquine, which are widely studied as clinical drugs for COVID-19, have a variety of cellular effects, including alkalizing lysosomes and blocking autophagy. Therefore, Gorshkov et al.[212] evaluated additional lysosomotropic compounds to identify drugs that inhibit SARS-CoV-2. Six compounds (ROC-325, clomipramine, hycanthone, chloroquine, hydroxychloroquine and mefloquine) [Fig. 10A (**91**–**93**), Fig. 9 (**75**, **76**), Fig. 10A (**94**)] showed inhibition of SARS-CoV-2 infection in Vero E6 cells with IC_{50}s ranging from 2.0 to 13 μmol/L (Table 3) and SIs ranging from 1.5- to > 10-fold. In the EpiAirway 3D tissue model, these compounds exhibited a variety of functions, including prevention of lysosome function and autophagy and the entry of pseudotyped particles, increase of the pH of the lysosome, and reduction of (ROC-325) viral titers.

In order to enter and infect cells, viruses use a series of strategies to take advantage of the cellular and biochemical properties of cell membranes. As demonstrated, one of the entry mechanisms used by HIV was thiol-mediated uptake[213]. Cheng et al.[214] systematically screened many effective inhibitors using fluorescent cyclic oligochalcogenides that enter cells by thiol-mediated uptake. Preliminary results on SARS-CoV-2 pseudovirus infection

showed that the most potent activities were found for dithioerythritol thiosulfonate 16 [Fig. 10A (**95**)] with an IC_{50} of around 50 μmol/L, while toxicity was detected only at 500 μmol/L[214]. This study revealed that thiol-mediated uptake may be an interesting research direction for the development of future anti-SARS-CoV-2 drugs.

Halfon et al.[215] reported that GNS561 showed most potent antiviral effect against two SARS-CoV-2 strains (IC_{50} = 0.006 μmol/L for USA-WA1/2020 and IC_{50} = 0.03 μmol/L for IHU MI6; MOI = 0.1) compared to chloroquine and remdesivir (Table 3). GNS561, located in LAMP2-positive lysosomes, together with SARS-CoV-2, blocked autophagy by increasing the size of LC3-II spots and increasing the volume of autophagic vacuoles in the cytoplasm with the presence of multilamellar bodies characteristic of a complexed autophagy[215].

VPS34 is a multifunctional protein involved in autophagy, endocytosis and other processes. Two well-characterized VPS34 inhibitors, VPS34-IN1 [Fig. 10A (**96**)][216] and PIK-III [Fig. 10A (**97**)][217], showed anti-SARS-CoV-2 effects at a MOI of 0.01 with IC_{50}s of 0.29 and 0.202 μmol/L in Vero E6 cells, respectively. However, both VPS34-IN1 and PIK-III exhibited strong cytotoxicity at 50 and 16.67 μmol/L in Vero E6 cells, respectively (Table 3)[218]. Orlistat and triacsin C [Fig. 10A (**98**, **99**)] inhibit fatty acid metabolism, which have exhibited antiviral activity[219,220]. Both compounds also exhibited antiviral activity against SARS-CoV-2 with IC_{50}s of 422.3 and 19.5 μmol/L in Vero E6 cells, respectively (Table 3) and did not induce obvious cytotoxicity, even at the high concentrations of 50 and 500 μmol/L, respectively. Time-of-addition studies show that these four inhibitors act at the post-entry step in the virus life cycle. Next, the researchers explored if these inhibitors were effective in Calu-3 by directly measuring production of infectious SARS-CoV-2 and cytotoxicity. IC_{50}s of 0.55 μmol/L (VPS34-IN1), 0.12 μmol/L (PIK-III), 21.25 μmol/L (orlistat), and 0.04 μmol/L (triacsin C), and CC_{50}s of > 50 μmol/L (VPS34-IN1), > 50 μmol/L (PIK-III), > 1 mmol/L (orlistat), and > 50 μmol/L (oriacsin C) were reported at the same time (Table 3)[218].

MI-1851 [Fig. 10A (**100**)], a furin inhibitor, efficiently inhibited SARS-CoV-2 multiplication in Calu-3 cells, which produced a 30- to 190-fold reduction in virus titers at 10 μmol/L (Table 3). Combining various TMPRSS2 inhibitors (MI-432 or MI-1900) with furin inhibitor MI-1851 exhibited more potent antiviral activity against SARS-CoV-2 than any TMPRSS2 or furin inhibitor in an equimolar amount[180]. In addition, decanoyl-RVKR-chloromethylketone, a peptidomimetic furin inhibitor [Fig. 10A (**101**)] was shown to block SARS-CoV-2 S processing and inhibit SARS-CoV-2 infection with an IC_{50} of 0.057 μmol/L by plaque reduction assay[221] (Table 3).

Homoharringtonine and emetine [Fig. 10A (**102**, **103**)] showed antiviral activity against SARS-CoV-2 with IC_{50}s of 2.55 and 0.46 μmol/L in Vero E6 cells, respectively (Table 3)[99]. Homoharringtonine has been reported to have antitumor activity by inhibiting protein transcription *via* its binding the site for ribosomal A[222,223]. Homoharringtonine has shown effective antiviral activity against herpes virus, coronavirus, rhabdoviruses and other viruses[222,224,225]. Emetine is a protein synthesis inhibitor, inhibits malaria through binding to the ribosomal E site of *Plasmodium falciparum*[226,227]. However, potential cardiotoxicity has limited its clinical use in recent years.

Bojkova et al.[228] have identified SARS-CoV-2-modulated host cell pathways. Inhibition of these pathways could suppress SARS-CoV infection in human cells. A human cell-culture model infected with a clinical isolate of SARS-CoV-2 was established to analyze the infection profile of SARS-CoV-2 by translatome and proteome proteomics. Their results showed that SARS-CoV-2 reshaped the central cellular pathways, such as translation, splicing, carbon metabolism, protein homeostasis and nucleic acid metabolism. Small-molecule inhibitors that target these pathways prevent the virus from replicating in cells. Indeed, inhibition of glycolysis by 2-deoxy-D-glucose [2-DG, Fig. 10A (**104**)], an inhibitor of hexokinase (*i.e.*, glycolysis), was previously shown to suppress rhinovirus infection in mice[229]. Blocking glycolysis with nontoxic concentrations of 2-DG inhibited SARS-CoV-2 replication in Caco-2 cells (Table 3)[228]. Pladienolide B [Fig. 10A (**105**)], a spliceosome inhibitor, targets the splicing factor SF3B1, which inhibited SARS-CoV-2 replication with an IC_{50} of 0.007 μmol/L that were not toxic to human Caco-2 cells (Table 3)[228]. Next, the team performed gene ontology analysis and identified a major cluster of metabolic pathways consisting of diverse nucleic acid metabolism sub-pathways upon SARS-CoV-2 infection. The researchers further confirmed that the replication of coronavirus depends on availability of cellular nucleotide pools[230]. Compounds interfering with nucleic acid metabolism, such as ribavirin [Fig. 10A (**106**)], could inhibit SARS-CoV-2 replication at low micromolar (IC_{50} = 0.07 mmol/L) and clinically achievable concentrations (Table 3)[228,231]. A clinical trial for ribavirin was recently initiated (Clinical Trials gov; NCT04356677). At the same time, NMS-873 [Fig. 10A (**107**)], a small-molecule inhibitor of ATP P97, could effectively inhibit SARS-CoV-2 replication at low nanomolar concentrations (IC_{50} = 0.025 μmol/L, Table 3)[140,228], revealing that these two types of inhibitors can be used as potential treatment options for SARS-CoV-2. Cycloheximide [Fig. 10A (**108**)], an inhibitor of translation elongation, significantly inhibited SARS-CoV-2 replication with an IC_{50} of 0.17 μmol/L that are not toxic to human Caco-2 cells (Table 3)[228].

AP-2-associated protein kinase 1 (AAK1) is a host kinase that regulates clathrin-mediated endocytosis[232]. Baricitinib [Fig. 10A (**109**)], a janus kinase inhibitor, is an AAK1-binding drug, which was supposed as a suitable drug candidate for COVID-19 because it can inhibit viral assembly by preventing AAK1-mediated endocytosis[232]. According to the EU Clinical Trials Register phases two and 3 (2020-001854-23), as well as phase-4 (2020-001354-22), clinical trials are now using baricitinib on COVID-19 patients (Table 3).

Nitazoxanide [Fig. 10A (**110**)] is an FDA-approved antibiotic for the treatment of diarrhea caused by *Giardia parvum* and *Giardia lamblia* and has broad-spectrum antiviral activity[233]. Nitazoxanide, which targets host-regulated processes involved in viral replication[233], has shown *in vitro* activity against MERS-CoV and other animal coronaviruses[234,235]. The recent experimental result showed that nitazoxanide also had inhibitory activity against SARS-CoV-2 *in vitro* (IC_{50} = 2.12 μmol/L; CC_{50} > 35.53 μmol/L; SI > 16.76) (Table 3)[19]. In another study, nitazoxanide and JIB-04 [Fig. 10A (**111**)] had IC_{50} values of 4.90 μmol/L and 695 nmol/L in Vero E6 cells (Table 3), respectively. JIB-04 is a pan-Jumonji histone demethylase inhibitor[236]. Neither drug induced cytotoxicity at 300 μmol/L and thus had excellent selectivity index (SI > 150)[237]. A recent review evaluated nine clinical trials of nitazoxanide for assessing the safety, cost and potential use of this drug for COVID-19[238].

Rodon et al.[187] screened drugs that can combat SARS-CoV-2-induced CPE and virus replication *in vitro* from existing drugs approved for use in humans. Among of them, Fenofibrate [Fig. 10A (**112**)] is clinically used to treat dyslipidemia *via* activation

of PPARa, and it also inhibited the CPE exerted by SARS-CoV-2 on Vero E6 cells at 20 μmol/L (Table 3). Plitidepsin [Fig. 10A (**113**)], the most potent one, targets eukaryotic elongation factor 1A2 and has been previously used for the treatment of multiple myeloma[187]. White and colleagues also reported that plitidepsin exhibits antiviral activity against SARS-CoV-2[239]. Based on immunofluorescence technology, they tested the inhibitory effects of plitidepsin on SARS-CoV-2 replication in Vero E6 cells, hACE2-293 T cells and human lung cells with IC_{50}s of 0.70, 0.73 and 1.62 nmol/L with limited toxicity in cell culture (Table 3). Meanwhile, they demonstrated the *in vivo* antiviral activity of plitidepsin in two SARS-CoV-2 infected mouse models with reduced viral replication in the lungs. Plitidepsin has also successfully completed a phase 1/2 clinical study for the treatment of COVID-19 (Clinical Trials gov, NCT04382066).

Nitric oxide (NO) is a gas with various biological activities produced by arginine through NO synthase. NO inhalation is beneficial for most patients with severe ARDS[240]. Inhalation of NO triggers the relaxation of smooth muscles in pulmonary blood vessels, leading to increased blood flow and adequate ventilation in the lungs[241]. NO showed inhibition of the synthesis of viral RNA and proteins[242]. Therefore, NO inhalation may be an effective method for the treatment of patients with severe COVID-19[169,241].

Clemizole hydrochloride [Fig. 10A (**114**)], a potent inhibitor of transient receptor potential channel $TRPC_5$ and an orally bioavailable histamine H1 antagonist with potential antitumor and anti-allergic activities[243], could prevent SARS-CoV-2 replication with an IC_{50} of 23.94 μmol/L and $CC_{50} > 40$ μmol/L in Vero E6 cells (Table 3)[146].

Benztropine Mesylate [Fig. 10A (**115**)], an anticholinergic that works by blocking a certain natural substance (acetylcholine) is used to treat symptoms of Parkinson's disease or involuntary movement. It could inhibit SARS-CoV-2 replication with an IC_{50} of 13.8 μmol/L (MOI = 0.004) or 17.79 μmol/L (MOI = 0.01) and CC_{50} of >> 50 μmol/L in Vero E6 cells by CellTiter-Glo assays (Table 3)[244].

The Weston group presented data on the antiviral activity of several FDA-approved drugs against SARS-CoV-2, including mefloquine hydrochloride [Fig. 10A (**94**)], fluphenazine dihydrochloride, amodiaquine hydrochloride, amodiaquine dihydrochloride dihydrate, thiethylperazine maleate, triparanol, terconazole vetranal, fluspirilene, clomipramine hydrochloride, promethazine hydrochloride, toremifene citrate, tamoxifen citrate and imatinib mesylate [Fig. 10A and B (**116**−**127**)], and the IC_{50} values of these drugs are at non-cytotoxic concentrations[244]. The IC_{50}s at MOI = 0.01 and CC_{50}s in Vero E6 cells of these drugs are shown in Table 3. Also, another team reported that toremifene and tamoxifen, as selective estrogen receptor modulators (SERMs), can affect ACE2 expression and offer a potential therapeutic approach for SARS-CoV-2[170,245].

Zhang et al.[246] established a CPE-based HTS assay in Vero-E6 cells that are permissive to SARS-CoV-2 infection to screen for inhibitors aiming for the entire viral life cycle. They screened a library collection of natural compounds containing 1058 compounds to identify potential inhibitors of SARS-CoV-2 in cell culture. After the primary screening, 30 hits with > 50% protection from CPE were identified. Among them, 17 drugs are newly discovered inhibitors of SARS-CoV-2. They next evaluated the effects of 17 newly discovered anti-SARS-CoV-2 compounds (bruceine A, cinobufagin, bufotaline, periplocoside, brusatol, veratridine, oridonin, isoalantolactone, isoliensinine, alantolactone, dehydrocostus lactone, momordinic, liensinine, dehydrodiisoeugenol, cornuside, roburicacid, coniferylaldehyde) and three previously reported coronaviruses inhibitors (bufalin, digoxin, and cryptotanshinone) [Fig. 10B (**128**−**147**)] by measuring the alterations of viral genome levels. All tested compounds showed inhibitory effects on virus propagation in a dose-dependent manner with the IC_{50} values ranging from 0.011 to 11.03 μmol/L (Table 3). According to the results of CCK-8 assay, the CC_{50} values of these compounds were also calculated (Table 3).

Kanjanasirirat et al.[247] have used fluorescence-based SARS-CoV-2 nucleoprotein detection and plaque reduction assays to screen for antiviral candidates. Among 122 Thai natural products, they found that panduratin A [Fig. 10B (**148**)] could significantly inhibit SARS-CoV-2 infection in Vero E6 cells with IC_{50} of 0.81 μmol/L (CC_{50} = 14.71 μmol/L, Table 3). Their study also reported that treatment with panduratin A was able to inhibit viral infectivity in human airway epithelial cells.

Using cell-based infection assays, Jan et al.[94] have screened more than 3000 agents that have been applied in humans and animals, including 2855 small molecules and 190 traditional herbal medicines. They have identified 15 active small molecules in concentrations ranging from 0.1 nmol/L to 50 μmol/L (Table 3), including nelfinavir [Fig. 5A-2 (**34**)], boceprevir [Fig. 5A-1 (**28**)], thioguanine [Fig. 10B (**149**)], cepharanthine [Fig. 4 (**4**)], emetine [Fig. 10A (**103**)], ivermectin [Fig. 6 (**62**)], moxidectin [Fig. 10B (**150**)], mefloquine [Fig. 10A (**94**)], ivacaftor, azelnidipine, penfluridol [Fig. 10B (**151**−**153**)], dronedarone [Fig. 5A-2 (**44**)], salinomycin, monensin and maduramicin [Fig. 10B (**154**−**156**)]. Mefloquine identified protected hamster disease models against challenge with SARS-CoV-2.

Puhl et al.[248] found that some drugs shown to have *in vitro* activity against Ebola also showed activity against SARS-CoV-2. Therefore, they tested three small-molecule drugs active against Ebola virus in various cell lines (VeroE6, Vero 76, Caco-2, Calu-3, A549-ACE2, HUH-7 and monocytes) infected with SARS-CoV-2[248]. The compilation of these results suggests that there is considerable variability in antiviral activity observed in different cell lines. They found that tilorone and pyronaridine [Fig. 10B (**157**, **158**)] inhibited virus replication with IC_{50}s of 180 and 198 nmol/L in A549-ACE2 cells, respectively (Table 3).

Hopefully, clofazimine was verified to inhibit SARS-CoV-2 by its interference with viral membrane fusion and the function of helicase, as well as upregulated gene expression of innate immune-related pathways in cells[69].

7. Conclusions and perspectives

Despite tremendous global efforts, COVID-19 remains a serious concern. Although many clinical trials of the repurposed drugs, immune-based therapies and investigational antivirals have been conducted, there is still no highly effective therapeutic for treatment of COVID-19 available. The mutation and pandemic of SARS-CoV-2 make vaccine and drug discovery more uncertain. Accordingly, developing specific or broad-spectrum inhibitors for SARS-CoV-2 virus entry, replication or prevention is urgently needed.

Continuous worldwide surveillance of the SARS-CoV-2 genome and continued efforts for quick screening of small-molecule databases will allow us to produce effective lead compounds or drug candidates, facilitating further *in vitro*, *in vivo* and clinical trials with which to determine their efficacy in the management of COVID-19. Computer-based drug design can

accelerate the drug development process, but new interventions are still likely to require months to years to develop. Considering the case fatality rate of COVID-19, the quick screening of therapeutic agents for the repurposing of FDA-approved and clinical trial drugs may be a more practical approach. Repurposing old drugs for new indications may potentially lower development costs and shorten development timelines. Indeed, several clinical drugs and drug combinations are under repositioning in the treatment of COVID-19 patients. However, none of the clinical trials has resulted in completely satisfactory results, except for experiments still ongoing without final results.

In the long-term perspective, much basic work needs to be done in terms of generating effective small-molecule drugs. Like SARS-CoV and MERS-CoV, SARS-CoV-2 genome encodes nsps (3CLpro, PLpro, helicase and RdRp), structural proteins (S glycoprotein) and accessory proteins. S proteins and nsps described above are identified as attractive targets for antiviral agent development. So far, hundreds of active compounds have been screened for inhibitors against SARS-CoV-2 infection; however, many of these studies have not been rigorously conducted. Thus, it is essential for researchers to fully assess promiscuous compounds, avoiding the false positive activity readouts. In many cases, hit compounds containing Michael receptor, phenolic and quinone substructures or other unstable chemical bonds are actually nondruggable, now recognized as pan assay interference compounds (PAINS). Furthermore, protein structural analyses suggest that active sites in viral proteins are probably conserved among SARS-CoV, MERS-CoV and SARS-CoV-2. Therefore, we have enough relevant experience to design coronavirus inhibitors. Continuous efforts toward the accurate crystal structure of verified drug targets, preclinical evaluation of drug candidates, gathering of clinical evidence, as well as artificial intelligence, are all necessary for the successful identification of anti-SARS-CoV-2 drugs. Furthermore, host-targeted agents able to stimulate innate antiviral responses, or modulate virus−host interactions, are also options for COVID-19 treatment. With the ongoing efforts to prevent gradually increasing cases around the globe, the outbreak of COVID-19 has highlighted the importance for the development of broad-spectrum antiviral agents to combat future coronaviruses.

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Author contributions

Shibo Jiang and Fei Yu designed the work. Fei Yu, Yaning Gao, Rong Xiang, Zhengsen Yu, Yang Wang, and Lili Wang collected data and wrote the manuscript. Shanshan Huo, Yanbai Li, Ruiying Liang and Qinghong Hao designed and regenerated the conceptual pictures. Shibo Jiang, Fei Yu and Tianlei Ying revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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