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Review

Development of SARS-CoV-2 entry antivirals

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ABSTRACT

The global outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) threatened human health and public safety. The development of anti-SARS-CoV-2 therapies have been essential to curb the spread of SARS-CoV-2. Particularly, antivirals targeting viral entry have become an attractive target for the development of anti-SARS-CoV-2 therapies. In this review, we elucidate the mechanism of SARS-CoV-2 viral entry and summarize the development of antiviral inhibitors targeting viral entry. Moreover, we speculate upon future directions toward more potent inhibitors of SARS-CoV-2 entry. This study is expected to provide novel insights for the efficient discovery of promising candidate drugs against the entry of SARS-CoV-2, and contribute to the development of broad-spectrum anti-coronavirus drugs.

1. Introduction

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in a worldwide health crisis associated with large socio-economic losses. Approximately 6.9 million deaths and more than 766 million cases have been confirmed worldwide, leading to the highest number of deaths caused by a viral pandemic.

This coronavirus (CoV) is an enveloped, positive-sense single stranded RNA virus with a large genome of roughly 30 kb. Characterized by clublike spikes emerging from its surface (Sivaraman, Er, Choong, Gavor, & Sivaraman, 2021). SARS-CoV-2 is a member of the Betacoronavirus (β-CoV) genus, belonging to the same branch of coronaviruses as SARS-CoV, HCoV-OC43, HCoV-HKU1, and MERS-CoV (Lan, Ge, Yu, Shan, Zhou, Fan, et al., 2020). Genome sequencing showed that the SARS-CoV-2 genome and the SARS-CoV genome share about 80% homology, while SARS-CoV-2 shares around 50% sequence homology with MERS-CoV. (J. Meng, Li, Zhang, Wang, Huang, Nie, et al., 2022). Both SARS-CoV and SARS-CoV-2 are composed of four main structural proteins: the spike glycoprotein (S), envelope (E), nucleocapsid (N), and membrane (M), as well as 16 nonstructural proteins, and 5–8 accessory proteins (Khailany, Safdar, & Ozaslan, 2020).

The SARS-CoV-2 life cycle can be generally divided into five steps, including cell entry, viral genome translation, subgenomic transcription, genome replication, and progeny virion formation (Hoffmann, Kleine--Weber, Schroeder, Kruger, Herrler, Erichsen, et al., 2020b). Cell entry is the first and major step in the initiation of the viral life cycle, representing an ideal target for antiviral interventions. The entry of coronaviruses depends on binding of the viral S proteins to cellular receptors. After host cell receptor binding, the S protein must be primed by host cell proteases. The host cell entry receptor needed for SARS-CoV-2 viral entry is the human ACE2 (hACE2) receptor (Letko, Marzi, & Munster, 2020; Wan, Shang, Graham, Baric, & Li, 2020). The receptor-binding domain (RBD) of the S protein binds to hACE2 receptors, followed by proteolytic activation, caused by human proteases, which initiate membrane fusion and viral entry (Matheson & Lehner, 2020; Shang et al., 2020). Specifically, the S protein can be proteolytically cleaved on the cell surface by the transmembrane serine protease, TMPRSS2. Alternatively, the S protein can be proteolytically cleaved in the cellular endosome by pH-dependent cysteine proteases, cathepsins B and L (cathepsin B/L), resulting

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in fusion of the viral membrane with the plasma membrane. The highly infectious nature of SARS-CoV-2 is partially due to a higher affinity of the S protein to ACE2 than that of SARS-CoV (Song, Li, Li, Huang, Liu, Fang, et al., 2022). Given its necessity for SARS-CoV-2 infection, the ACE2 receptor also represents a potential therapeutic target for COVID-19.

Prophylactic therapeutics, such as vaccinations are encouraged to prevent severe illness, hospitalization, and death. Nevertheless, persistent viral immune escape has resulted in waves of SARS-CoV-2 variants that are more virulent (Delta strain) or transmissible (Omicron strains), lowering the effectiveness of these vaccines. In the fight against SARS-CoV-2, three strategies for developing new drugs were proposed. The first one was to test existing broad-spectrum antivirals such as interferons, ribavirin, and cyclophilin inhibitors; the second strategy was to use existing molecular databases to screen for molecules that may have a therapeutic effect on SARS-CoV-2; the final strategy is to develop drugs targeting specific targets based on the genomic information and pathological characteristics of SARS-CoV-2. In addition to these strategies, it is also scientifically feasible to develop SARS-CoV-2 inhibitors using in silico virtual screening and molecular docking.

Previous studies have identified a variety of drugs and natural products that can be repurposed as potential therapeutic agents against SARS-CoV-2. In this comprehensive review, we discuss the entry mechanisms of SARS-CoV-2, and summarize the therapeutic approaches used to target SARS-CoV-2 cell entry.

2. Structural features and molecular mechanisms of SARS-CoV-2 cell entry

2.1. Structural features of SARS-CoV-2

SARS-CoV-2 is a positive-sense, single stranded RNA, and lipid enveloped virus. This coronavirus is known for having one of the largest RNA genomes, approximately 30 kb in size (Khailany et al., 2020). The 5′ end of the SARS-CoV-2 genome encodes two open reading frames ORF1a and ORF1b, whereas the 3′ terminal encodes four structural proteins, including the spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein.

The S protein, one of the four aforementioned structural proteins, is essential for viral entry into the host cells. The S protein is composed of two functional subunits, S1 and S2. The S1 subunit contains the receptor binding domain (RBD) and an N-terminal domain (NTD), while the S2 subunit contains the fusion peptide (FP), two heptad repeats (HR1 and HR2), and a C-terminal transmembrane region (Walls et al., 2020). Viral entry occurs when the S1 subunit engages with cellular hACE2 receptors, while the S2 subunit mediates viral and cellular membrane fusion. During biosynthesis and maturation in the infected cell, some S protein on the progeny virion is cleaved into the S1 and S2 subunits, by a host enzyme, furin. These two subunits will remain associated through non-covalent bonds (B. Hu, Guo, Zhou, & Shi, 2021; Madu, Roth, Belouzard, & Whittaker, 2009). A second cleavage occurs at the S2' site, located in the S2 subunit. This cleavage occurs during viral entry, mediated by host enzymes transmembrane serine protease 2 (TMPRSS2) or cathepsin L. The RBD and FP are the structures specifically linked to viral entry, a variety of strategies targeting RBD and FP for the treatment of SARS-CoV-2 have been proposed (Fig. 1).

2.2. Mechanism of SARS-CoV-2 cell entry

Two SARS-CoV-2 entry pathways have been described and are well understood. This is in part due to the close similarity of SARS-CoV-2 to SARS-CoV. These two pathways are known as the endosomal entry pathway and the cell surface entry pathway. Similar to SARS-CoV, the endosomal protease cathepsin L (CatL) and cell surface TMPRSS2 mediate these two SARS-CoV-2 entry pathways, respectively. (Ou, Liu, Lei, Li, Mi, Ren, et al., 2020). The S protein will first bind to the hACE2 receptor at the cell surface, this binding process leads the S protein to undergo a conformational change, resulting in S1 and S2 subunit dissociation. The broken bond causes the S1 to shed, consequently exposing the S2 subunit. SARS-CoV-2 S protein cleavage by either CatL or TMPRSS2 is obligatory for effective infection (Hoffmann, Kleine-Weber, & Pohlmann, 2020a; Kawase, Shirato, van der Hoek, Taguchi, & Matsuyama, 2012). Depending on the entry pathway, the S2' cleavage may occur at the plasma membrane by the serine protease TMPRSS2, or within the cell by CatL, leading to viral and cellular membrane fusion (Belouzard, Chu, & Whittaker, 2009; Shang et al., 2020) (Fig. 2).

3. Antiviral therapies against SARS-CoV-2 cell entry

Great advances in drug design were made due to the understanding of SARS-CoV-2 entry into the host cell. However, developing new drugs is a lengthy process. Repurposing existing drugs has successfully accelerated the development of direct antivirals, which are currently approved for treatment of SARS-CoV-2 infections. Furthermore, natural compounds possess tremendous structural range and unique chemical diversity, creating a good foundation for new drug discovery and development. In silico screening can be applied to identify druggable targets and to identify lead compounds in early research, accelerating the development of new drugs (Terstappen & Reggiani, 2001). Some of these therapies include inhibiting SARS-CoV-2 entry/fusion through neutralizing antibodies, small molecule inhibitors, protein or peptide inhibitors (Fig. 3).

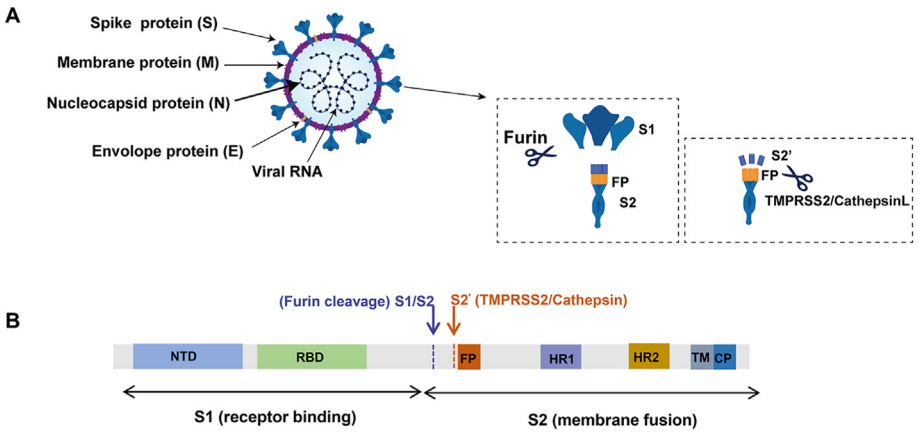


Fig. 1. Characteristics of SARS-CoV-2 Spike (S) protein. (A) Schematic representation of structure of SARS-CoV-2 virions. (B) Schematic drawing of the 1D structure of SARS-CoV-2 spike. FP (fusion peptide), HR1 (heptad repeat 1), and HR2 (heptad repeat 2) are structural units in coronavirus S2 that function in membrane fusion. SP, signal peptide; TM, transmembrane anchor; CP, cytoplasmic tail; S1/S2, cleavage site at S1/S2 boundary; S2', a second cleavage site within S2.

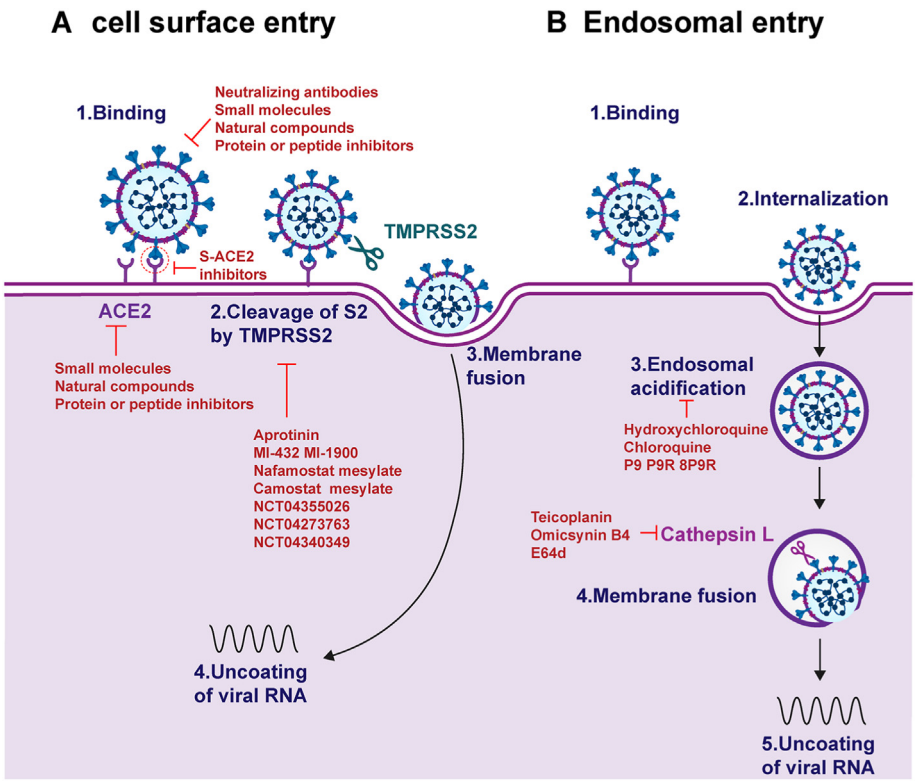


Fig. 2. SARS-CoV-2 replication cycle and promising drugs or small molecule compounds to inhibit viral infection. Upon binding to host receptor ACE2, the SARS-CoV-2 S protein can be activated by TMPRSS2 cleavage at the cell surface, resulting in fusion with plasma membrane (A). Alternatively, the virions enter cells via clathrin-dependent endocytosis, and the fusion process can be primed by endosomal cathepsin L (B).

3.1. Inhibitors targeting the SARS-CoV-2 S protein

Most of the ongoing research and development of anti-coronavirus agents have mainly focused on targeting the S protein. The SARS-CoV-2 S protein is efficiently processed into two subunits, S1 and S2, which mediate attachment and membrane fusion, respectively. This entry glycoprotein plays a crucial role in viral entry, making this an ideal target for drug, antibody, and vaccine development.

3.1.1. Neutralizing antibodies targeting the S protein

The RBD, responsible for ACE2 binding, and the NTD are both encoded in S1 subunit. These are a major target of neutralizing antibodies induced by natural infection. Due to this, the RBD is considered as an attractive target for the development of neutralizing antibodies, viral attachment inhibitors, and vaccines (Ju, Zhang, Ge, Wang, Sun, Ge, et al., 2020). This is largely due to the importance of the RBD during viral entry. A large number of antibodies that potently neutralize the ACE2-RBD binding interface, have been reported. It is important to consider that the SARS-CoV and SARS-CoV-2 RBD share ~70% sequence identity, suggesting that some monoclonal antibodies (mAbs) specific for the SARS-CoV RBD could cross-react with the SARS-CoV-2 RBD. Preliminary research mainly focused on finding cross-reactive neutralizing antibodies from SARS-CoV. Nevertheless, most of these antibodies are not cross--reactive with the SARS-CoV-2 RBD (Wec, Wrapp, Herbert, Maurer, Haslwanter, Sakharkar, et al., 2020; Xiu, Dick, Ju, Mirzaie, Abdi, Cocklin, et al., 2020), only a few mAbs such as CR3022 and S309 bind potently with SARS-CoV-2 RBD (Pinto, Park, Beltramello, Walls, Tortorici, Bianchi, et al., 2020; Tian, Li, Huang, Xia, Lu, Shi, et al., 2020). Additionally, Six SARS-CoV RBD-specific neutralizing mAbs including 46C1, 13B6, 29H4, S29, 7B11, and 18F3 could cross-react with SARS-CoV-2 RBD, two of which, 18F3 and 7B11, could neutralize SARS-CoV-2 infection (Tai, Zhang, He, Jiang, & Du, 2020). The administration of some neutralizing monoclonal antibodies, either as a single or a combination of antibodies as therapeutics in individuals with mild to moderate COVID-19 infection, had positive results in the early pandemic (Baum, Ajithdoss, Copin, Zhou, Lanza, Negron, et al., 2020; Gottlieb, Nirula, Chen, Boscia, Heller, Morris, et al., 2021; Jones, Brown-Augsburger, Corbett, Westendorf, Davies, Cujec, et al., 2021).

Nanobodies, also known as single-domain antibodies, are small, simple compact, and thermostable immunoglobulin elements capable of binding target epitopes with subnanomolar affinities (Czajka, Vance, & Mantis, 2021). A number of inhalable nanobodies targeting SARS-CoV-2 have also been developed. PiN-21 is an efficient trimeric nanobody that binds to the RBD of the SARS-CoV-2 S protein to prevent and treat SARS-CoV-2 infection (Nambulli, Xiang, Tilston-Lunel, Rennick, Sang, Klimstra, et al., 2021). Moreover, two closely related nanobodies named H11-D4 and H11–H4 were found to bind to the RBD, and block its interaction with ACE2 (Huo, Le Bas, Ruza, Duyvesteyn, Mikolajek, Malinauskas, et al., 2020).

In addition to RBD-targeting antibodies, The NTD is also a promising target for the development of mAbs against COVID-19 (L. Liu, Wang, Nair, Yu, Rapp, Wang, et al., 2020b). The 4A8 antibodies exhibit high

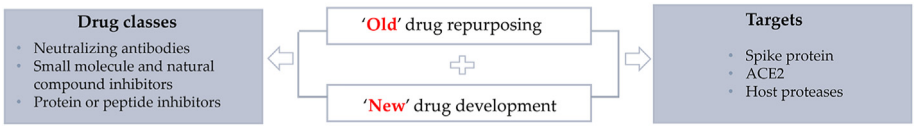


Fig. 3. Drug discovery strategies to target SARS-CoV-2 entry.

neutralization potency against the SARS-CoV-2 virus, stabilizing the NTD of the S protein, facilitating the construction of 5 NTD loops (Chi, Yan, Zhang, Zhang, Zhang, Hao, et al., 2020). Moreover, the combination of 4A8 with RBD-targeting antibodies may prevent the development of escape mutant viruses, and serve as promising "cocktail" therapeutic (Chi, et al., 2020). Moreover, three NTD-specific mAbs have been identified, and have been identified as NTD-1 through NTD-3. The CoVIC-247 (EMD-24355), CoVIC-245 (EMD-24360), and CoVIC020 (EMD-24356) antibodies belong to NTD-1, NTD-2, and NTD-3, respectively (Hastie, Li, Bedinger, Schendel, Dennison, Li, et al., 2021).

To date, Omicron subvariants have been identified as the variants of concern (VOCs) with the highest number of mutations on the S protein, with over 30-40 additional mutations compared to the initial SARS-CoV-2 strain, leading these to become the dominant variants worldwide (Fan, Wang, Liu, An, Liu, He, et al., 2020). Multiple studies have determined that new mutations present in the S protein of Omicron subvariants could lead to immune evasion, thus reducing the neutralization activity of neutralizing antibodies. (Ai, Wang, He, Zhao, Zhang, Jiang, et al., 2022; Q. Wang, Guo, Iketani, Nair, Li, Mohri, et al., 2022; Q. Wang, Iketani, Li, Liu, Guo, Huang, et al., 2023). Therefore, several single and combination mAb therapeutics are no longer authorized by the US Food and Drug Administration (FDA), due to the sustained emergence of resistant SARS-CoV-2 viral variants (Table 1). Some of these previously approved therapeutic mAbs include Bebtelovimab, Sotrovimab, Bamlanivimab alone or Bamlanivimab and Etesevimab, and Casirivimab with Imdevimab (REGN-COV2) (Bhimraj, Morgan, Shumaker, Baden, Cheng, Edwards, et al., 2022). In a study published in late 2022, a group examined the efficacy of FDA-approved monoclonal antibodies against multiple Omicron subvariants (Takashita, Yamayoshi, Simon, van Bakel, Sordillo, Pekosz, et al., 2022). Monoclonal antibodies Imdevimab, Casirivimab, Tixagevimab, Cilgavimab, Sotrovimab, and Bebtelovimab, and combinations of these were assayed against Omicron subvariants BA.1, BA.1.1, BA.2, BA.2.12.1, BA.4, and BA.5. Of these monoclonal antibodies, Bebtelovimab appeared to be the most effective neutralizing mAb, with neutralization capabilities against BA.2.12.2, BA.4, and BA.5. Unfortunately, as of November 2022, the FDA retired the emergency use authorization for this mAb due to the emergence of new Omicron subvariants BQ.1 and BQ.1.1 (U.S.Food & DRUG). Monoclonal antibodies Tixagevimab used in combination with Cilgavimab, also known as Evusheld (formerly known as AZD7442), had been previously approved for pre-exposure prophylaxis use against COVID-19. However, with the rise of new Omicron subvariants XBB and XBB.1.5, the FDA determined that Evusheld would not be effective at neutralizing these newer variants (U.S.Food & DRUG).

3.1.2. Small molecules and natural compound inhibitors targeting the S protein

Small molecules are the preferred viral therapeutic compared to proteins or peptides due to their improved pharmacokinetics, stability, and dosage logistics. The viral S protein remains as one of the ideal targets for small molecule drug development against SARS-CoV-2 entry.

The main strategies employed for antiviral drug development have mainly focused on repurposing licensed drugs, or large-scale compound screenings from compound libraries, or libraries with clinical-stage or FDA-approved small molecules. Currently, other than mAbs, there are no S-targeting small molecules approved for COVID-19 treatment in the USA. This poses a large gap in the drug development field, creating a need for effective S-specific small molecules. Due to this, many groups are currently working on the development of new S-specific antivirals. In a study, a group performed a high-throughput screen to evaluate a 15,000 small molecule unique compound library. Lead candidates were validated via infectious SARS-CoV-2 assays. Calpeptin was identified as a novel lead that appears to be a potent and specific inhibitor of both SARS-CoV-2 and highly virulent non-Omicron variants. In particular, calpeptin shows dual temporal activity, allowing the inhibition of both SARS-CoV-2 entry pathways (Mediouni, Mou, Otsuka, Jablonski, Adcock, Batra, et al., 2022). In addition to these findings, another study has shown that sertraline is an effective entry inhibitor against SARS-CoV-2 infection. It is speculated that sertraline binds to the S1 subunit of S protein, specifically the RBD, thereby blocking the interaction of S-hACE2 and interfering with the proteolytic process of S protein (Y. L. Chen, Wu, Chen, Zhan, Wu, Yang, et al., 2022). Notably, sertraline is also effective at preventing viral entry of pseudotyped SARS-CoV-2 variants, including Omicron BA.1. Arbidol, a small indole-derivative molecule, has a good inhibitory effect against SARS-CoV-2 *in vitro*. The mechanism of arbidol is to target the SARS-CoV-2 S protein and block its trimerization, inhibiting host cell adhesion and hijacking (Vankadari, 2020). Recently, clinical trials have demonstrated that Arbidol significantly increased the negative

Table 1
Monoclonal antibodies approved against SARS-CoV-2 and their authorization status (as of December 9, 2023).

Antibodies	Type of biologics	Binding epitopes	Indication	Against SARS-CoV-2 variants	Relevant progress
Bebtelovimab	IgG1-lamba mAb with unmodified Fc region	Binds to the RBD (class III) of S protein	Treatment of mild-to-moderate COVID-19	Alpha, Beta, Gamma, Delta and Omicron	Granted EUA by FDA in February 2022/withdrawn in November 2022
Tixagevimab and Cilgavimab (Evusheld, formerly AZD7442)	Recombinant human IgG1 -kappa mAbs with amino acid substitutions	Binds to different sites on the RBD (class I and II) of the S.	Pre-exposure prophylaxis of COVID-19 in certain adults and pediatric individuals	Alpha, Beta, Gamma, Delta and Omicron	Granted EUA by the FDA in December 2021/withdrawn in January 2023
Sotrovimab	IgG1-kappa mAb with a modified Fc region	Binds to the RBD (class III) of S protein	Treatment of mild-to-moderate COVID-19	Alpha, Beta, Gamma, Delta and Omicron	Granted EUA by the FDA in May 2021/withdrawn in August 2022
Bamlanivimab (LY-CoV555)	IgG1-kappa mAb with unmodified Fc region	Binds to the RBD (class II) of S protein	Treatment of mild-to-moderate COVID-19	Alpha	Granted EUA by the FDA in November 2020/withdrawn in April 2021
Bamlanivimab and etesevimab	Bamlanivimab: IgG1-kappa mAb with unmodified Fc region Etesevimab: IgG1-lambda mAb with a modified Fc region	Binds to different sites on the RBD (class I and II) of the S.	post-exposure prevention of COVID-19	Alpha, reduced susceptibility to Beta, Gamma, Delta	Granted EUA by the FDA in February 2021/withdrawn (May 2022)
Casirivimab and imdevimab (REGN-COV2)	IgG1 mAbs with unmodified Fc regions	Bind to different sites on the RBD (class I and III) of the S.	Treatment of mild-to-moderate COVID-19	Alpha, Beta, Gamma, Delta, Omicron	Granted EUA by the FDA in November 2020/withdrawn; approved for marketing in Japan, UK, EU, and Australia
Amubarvimab and romlusevimab	recombinant human IgG1 mAbs	Binds to the RBD (non-competing epitopes) of S protein	Treatment of mild-to-moderate COVID-19	Alpha, Beta, Gamma, Delta, Omicron	Approved for marketing in China in December 2021/withdrawn

Table 2
Potential preventive and therapeutic peptides against SARS-CoV-2 S protein.

Target	Name	Sequence	Mechanism of action
HR1 domain on S protein	EK-1	SLDQINVTFLDLEYEMKKLEEAIKKLEESYIDLKEL	Competitive combination with HR1 domain to inhibit virus entry
HR1 domain on S protein	EK1C4	SLDQINVTF LDLEYEMK KLEEAIKKL EESYIDLKEL GSGSG-PEG4-Cho	Competitive combination with HR1 domain to inhibit virus entry
HR1 domain on S protein	IPB02	IGNASVVNIQKEIDRLNEVANNESLILQELK(Chol)	Inhibits the S protein-mediated cell-cell fusion
HR1 domain on S protein	2019-nCoV-HR2P	DIGINASVVNIQKEIDRL NEVAKNLNESLIDLQEL	Inhibits the S protein-mediated cell-cell fusion
HR1 domain on S protein	$[SARS_{HRC}\text{-}PEG_4]_2$-chol	[DISGINASWNIQKEID RLNEVAKNLNESLIDLQEL -PEG4]2-chol	Inhibits the S protein-mediated cell-cell fusion
RBD on S protein	AHB1	MFVFLVLLPLVS	Inhibits RBD from binding to ACE2 receptors
RBD on S protein	AHB2	WYIWLGFIAGLIAIV MVTIMLCC	Inhibits RBD from binding to ACE2 receptors
RBD on S protein	LCB1	DKEWILQKIYEIMRLLD ELGHAEASMRVSDLIYEF MKKGDERLLEEAERLLEEVER	Inhibits RBD from binding to ACE2 receptors
RBD on S protein	LCB3	NDDELHMLMTDLVYEA LHFAKDEEIKKRVFQLFE LADKAYKNNDRQK LEKVVEELKELLERLLS	Inhibits RBD from binding to ACE2 receptors
RBD on S protein	ΔABP-D25Y	–	Inhibits RBD from binding to ACE2 receptors

conversion rate of the Omicron variant of SARS-CoV-2 within the first week and accelerate the recovery time (J. Zhao, Li, Chen, Xu, Yang, Zhang, et al., 2023).

Natural compounds have become an important resource for the development of new antivirals. Their rich sources, chemical diversity and biological activity, have made these natural compounds an attractive lead for the development of compounds against COVID-19. A computer-aided drug design technique combined with surface plasmon resonance (SPR) indicated that glycyrrhizic acid exerts inhibitory activity against the S protein of SARS-CoV-2 (S. Yu, Zhu, Xu, Yao, Zhang, Wang, et al., 2021). A recent study also revealed that 24 flavonoids exhibited antiviral entry activity by a SARS-CoV-2 pseudovirus model. Further research showed that kaempferol, quercetin, and myricetin dose-dependently bound to SARS-CoV-2 spike RBD (J. R. Meng, Liu, Fu, Shu, Yang, Zhang, et al., 2023). Angeloylgomisin O, schisandrin B, procyanidin, and oleanonic acid, were also found to be effective SARS-CoV-2 S protein entry inhibitors (Table 3). These were screened against pseudotyped SARS-CoV-2 and their EC_{50} values were found to be in the micromolar region. Further mechanistic studies revealed that these four agents inhibited pseudotyped SARS-CoV-2 viral entry by blocking S protein-mediated membrane fusion(J. Cao, Liu, Zhou, Dong, Hou, Jia, et al., 2022).

3.1.3. *Protein or peptide inhibitors targeting the S protein*

The HR1 and HR2 range are located in the S2 subunit. During viral entry, after S2' cleavage by a host protease, these two domains form the 6-helical bundle, necessary for viral and cellular membrane fusion. Generally, HR1/2-targeting peptides are specific to their corresponding viruses. However, the pan-coronavirus fusion inhibitor EK1 targets HR1 domains of several human coronaviruses, including SARS-CoV-1, MERS-CoV, SARS-CoV-2, and SARS-CoV-2 Omicron subvariants (Xia, Liu, Wang, Xu, Lan, Feng, et al., 2020a; Xia, Wang, Jiao, Yu, Xu, Huang, et al., 2023; Xia, Yan, Xu, Agrawal, Algaissi, Tseng, et al., 2019). Cholesterol was covalently attached to the C-terminus of EK1 linked by a PEG spacer to generate EK1C4. The resulting EK1C4 has a higher binding affinity to the HR1 domain and higher antiviral activity against SARS-CoV-2, compared to EK1 alone (Xia, Liu, et al., 2020b). Notably, both EK1 and EK1C4 could potently inhibit Omicron variant S-mediated cell to cell fusion (Xia, Chan, Wang, Jiao, Chik, Chu, et al., 2022), confirming that these peptides can be further developed as novel antiviral drugs for the treatment and prevention of SARS-CoV-2 infection and its variants, including Omicron, and possibly other emerging and reemerging HCoVs in the future.

Additionally, the lipopeptides IPB02 and 2019-nCoV-HR2P, developed based on the HR2 sequence, showed highly effective activity in inhibiting SARS-CoV-2 S protein-mediated cell-cell fusion (Xia, Zhu, Liu, Lan, Xu, Wu, et al., 2020b; Y. Zhu, Yu, Yan, Chong, & He, 2020). The optimization of the peptide, $[SARS_{HRC}\text{-}PEG_4]_2$-chol, modified by PEGylation, lipidation, and dimerization has demonstrated a long shelf life and high fusion inhibition activity. It is important to note that the lipopeptide fusion inhibitor $[SARS_{HRC}\text{-}PEG_4]_2$-chol is the first successful prophylactic that prevents SARS-CoV-2 transmission in a ferret infection model (de Vries, Schmitz, Bovier, Predella, Khao, Noack, et al., 2021). Griffithsin (GRFT), a lectin isolated from the red alga *Griffithsia* sp, can inhibit SARS-CoV-2 infection by binding to the glycosylation sites of the SARS-CoV-2 S protein S1 subunit, which is consistent with the mechanism of inhibition against SARS-CoV infection. Interestingly, the combination of GRFT and EK1 exhibited synergistic effects against SARS-CoV-2 pseudovirus viral entry (Cai, Xu, Gu, Cai, Qu, Lu, et al., 2020). Using ACE2 as a scaffold, AHB1 and AHB2 were designed to neutralize SARS-CoV-2, with IC_{50} values of 35 and 16 nM, respectively.

Additionally, LCB1 and LCB3 were identified by the de-novo approach, showing binding to the RBD with lower dissociation constants and neutralizing SARS-CoV-2 in the picomolar range (L. Cao, Goreshnik, Coventry, Case, Miller, Kozodoy, et al., 2020). ΔABPD25Y was designed based on the ACE2 α-helical region using molecular docking simulations. Additionally, it was found to inhibit SARS-CoV-2 infection by competitively blocking RBD interaction with ACE2 receptors on host cells (Table 2) (Jaiswal & Kumar, 2020).

3.2. *Inhibitors targeting ACE2 receptor*

ACE2 is the primary receptor of SARS-CoV-2, mediating viral entry into host cells. Besides inhibiting the S protein, blocking ACE2 may also be a potential target to inhibit SARS-CoV-2 infection; The likelihood of mutations in hACE2 receptors is lower compared to the mutation rate of the SARS-CoV-2 S protein (Lim, 2023).

3.2.1. *Small molecules and natural compound inhibitors targeting the ACE2 receptor*

Imatinib has been shown as a potent inhibitor of SARS-CoV and MERS-CoV fusion proteins (Han, Duan, Yang, Nilsson-Payant, Wang, Duan, et al., 2021). Recently, five clinical trials have been registered to treat COVID-19 patients with imatinib. Surface plasmon resonance (SPR) binding analysis suggested that imatinib binds to ACE2 to inhibit the entry of SARS-CoV-2 (Han, et al., 2021). SB27041 was identified as a novel small molecule that inhibited the interactions between SARS-CoV-2 RBD and ACE2. The mechanism of SB27041 involves modulating the ACE2 receptor without compromising the enzymatic activity necessary for its normal physiological functions (Shin, Jeong, Lee, Lee, Yim, Kim, et al., 2022). Amuvatinib, a receptor tyrosine kinase-specific inhibitor, has been found to have stronger activity against SARS-CoV-2.

Recently, Lv et al. used the ACE2/CMC-HPLC-IT-TOF-MS system to screen the active ingredients of the *Ephedra sinica*, a herb widely used in China to treat COVID-19. Three compounds were identified including

Table 3
Natural compounds inhibiting S protein, ACE2 and S-RBD/ACE2 interaction.

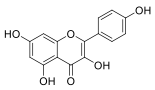
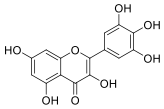
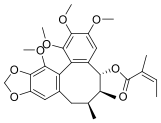
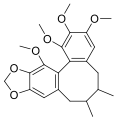
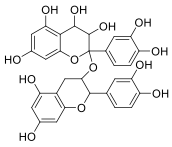
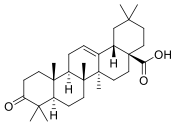
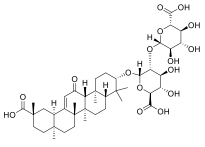
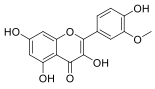
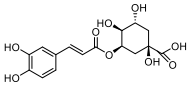
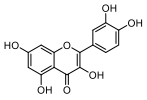
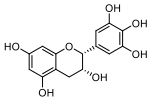
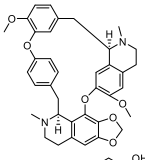
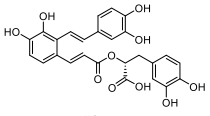
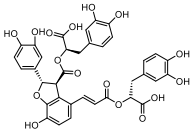
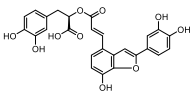
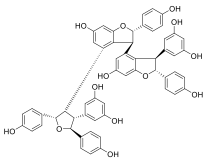
Compounds	Chemical class	Target	Structure
Kaempferol	Flavonoids	S-RBD	
Myricetin	Flavonoids	S-RBD	
Angeloylgomisin O	Lignans	S protein fusion machinery	
Schisandrin B	Lignans	S protein fusion machinery	
Procyanidin	Phenols	S protein fusion machinery	
Oleanonic acid	Terpenoids	S protein fusion machinery	
Glycyrrhizic acid	Terpenoids	S/S-RBD/ ACE2 interaction	
Isorhamnetin	Flavonoids	ACE2	
Neochlorogenic acid	Phenols	ACE2	
Quercetin	Flavonoids	S-RBD/ACE2 interaction	
Pigallocatechin-3-gallate	Flavonoids	S-RBD and ACE2	
Cepharanthine	alkaloid	S-RBD/ACE2 interaction	
Salvianolic acid A	Phenols	S-RBD and ACE2	
Salvianolic acid B	Phenols	S-RBD and ACE2	
Salvianolic acid C	Phenols		

Table 3 (*continued*)

Compounds	Chemical class	Target	Structure
		S-RBD and ACE2	
Ephedrine	Alkaloids	S-RBD and ACE2	
Pseudoephedrine	Alkaloids	S-RBD and ACE2	
Methylephedrine	Alkaloids	S-RBD and ACE2	
Kobophenol A	oligomeric stilbene	S-RBD/ACE2 interaction	

ephedrine, pseudoephedrine, and methylephedrine. All of these bind to ACE2, showing inhibitory activity against pseudotyped SARS-CoV-2 (Y. N. Lv, S. S. Wang, P. D. Liang, Y. M. Wang, X. Zhang, Q. Q. Jia et al., 2021b). Berbamine indirectly targets the ACE2 receptor, by compromising the endolysosomal trafficking of ACE2. This compound inhibits the transient receptor potential mucolipin channels, which leads to an increased excretion of ACE2 via exosomes and a concomitant decrease in the levels of ACE2 at the cell surface, thereby blocking SARS-CoV-2 from entering the host cells (Huang et al., 2021). The flavonoid compound isorhamnetin was shown to interact with ACE2, preventing pseudotyped SARS-CoV-2 viral entry (Y. Zhan et al., 2021). Oroxylin A was discovered to block the entrance of SARS-CoV-2 into HEK-293T-ACE2 cells, by specifically binding to the ACE2 receptor (Gao et al., 2021). Moreover, the comprehensive 2D ACE2 column/C18 column/TOFMS system was used to screen the active components in LHQW that may bind to ACE2. The neochlorogenic acid, one of the phenolic acids, was further confirmed to have good binding force with ACE2 through SPR, and could inhibit ACE2 *in vivo* (Table 3) (X. Chen, Wu, Chen, Gu, Zhu, Wang, et al., 2021).

3.2.2. *Protein or peptide inhibitors targeting the ACE2 receptor*

Human defensin 5 (HD5) is the most abundant α-defensin in the intestine. Due to its lectin-like activity, HD5 binds lipids and glycosylated proteins (C. Wang, Shen, Gohain, Tolbert, Chen, Zhang, et al., 2015; Wehkamp et al., 2006), making the ACE2 receptor and the SARS-CoV-2 S protein potential binding targets (Table 4). The binding of HD5 to ACE2 was confirmed using biolayer interferometry measurements. Through confocal microscopy studies, it was shown that pretreatment with HD5 reduced SARS-CoV-2 pseudoparticle infection (C. Wang, Wang, et al., 2020b), however, antiviral activity against infectious SARS-CoV-2 isolates remains to be demonstrated. A novel peptide, ATN-161 showed to reduce SARS-CoV-2 infection (IC_{50} = ~3 μM), suggesting that this integrin may play a role during SARS-CoV-2 entry (Beddingfield, Iwanaga, Chapagain, Zheng, Roy, Hu, et al., 2021).

A selective ACE2 inhibitor, DX600, may also help inhibit or decrease SARS-CoV-2 infections. Nevertheless, its clinical significance in COVID-19 has not been assessed (H. Zhu, Zhang, Zhou, Ding, Jiang, Liu, et al., 2021). The lipoglycopeptide antibiotic, dalbavancin, binds ACE2 and blocks its interaction with the SARS-CoV-2 S protein, showing potential as a promising anti-COVID-19 drug candidate (Table 4) (G. Wang, Yang, Duan, Liu, Jin, Long, et al., 2021). These aforementioned peptides are derived from the viral S protein and are optimized to target the ACE2 receptor. Unfortunately, this may limit their use as therapeutics as they might affect the normal physiological functions of ACE2.

Table 4
Potential preventive and therapeutic peptides against SARS-CoV-2 targeting ACE2 and host proteases.

Target	Name	Sequence	Mechanism of action
ACE2	HD5	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR	Inhibits viral attachment
ACE2	DX600	Ac-GDYSHCSPLRYYPWWKCTYPDPEGGG-NH2	Blocks SARS-CoV-2 S binding to ACE2
ACE2	Dalbavancin	–	Blocks SARS-CoV-2 S binding to ACE2
Furin	MI-1851	(S)–N-((S)-1-((4-Carbamimidoylbenzyl) amino)-4-(guanidinooxy)-1-oxobutan-2-yl)-2-((S)-2-(2-(4-(guanidinomethyl)phenyl) acetamido)-4-(guanidinooxy)butanamido)-3,3-dimethylbutanamide	Inhibits the cleavage of SARS-CoV-2 S protein
TMPRSS2	Aprotinin	RPDFCLEPPYTGPCK ARIIRYFYNAKAGLCQTFVY GGCRAKRNNFKSAED CMRTCGGA	Inhibits virus entry and membrane fusion
TMPRSS2	MI-432	(S)-3-(3-(4-(2-Aminoethyl)piperidin-1-yl)-2-((2′,4′-dichloro-[1,1′-biphenyl])-3-sulfonamido)-3-oxopropyl) benzimidamide	Inhibits virus entry and membrane fusion
TMPRSS2	MI-1900	(S)-4-(3-(3-Carbamimidoylphenyl)-2-((2′,4′-dimethoxy-[1,1′-biphenyl])-3-sulfonamido) propanoyl)-N-cyclohexylpiperazine-1-carboxamide	Inhibits virus entry and membrane fusion
Cathepsin L	Teicoplanin	–	Inhibits the proteolytic activity of Cathepsin L

3.3. Inhibitors of the S-protein-ACE2 interaction

The protein−protein interaction (PPI) between the SARS-CoV-2 S protein and ACE2, acts as a ligand−receptor pair that initiates viral attachment and cellular entry. This interaction is of considerable interest as a potential therapeutic target (Bojadzic et al., 2021).

Antibodies, small molecules or natural compounds can be designed as therapeutics that could disrupt the interaction between the S protein and the ACE2 receptor, thus inhibiting SARS-CoV-2 entry. According to a report, the B38 and H4 mAbs, isolated from convalescent COVID-19 plasma, can bind to the SARS-CoV-2 S-RBD. These two antibodies bind to different epitopes on the RBD, making them a potentially promising virus-targeting monoclonal antibody pair. Their synergistic function can also help prevent possible immune evasion, seen in clinical applications (Y. Wu, Wang, Shen, Peng, Li, Zhao, et al., 2020b). Notably, the ACE2--blocking monoclonal antibody of H11B11 exhibited potent inhibitory activity against SARS-CoV and SARS-CoV-2. This could serve as a potential therapeutic countermeasure against SARS-CoV, SARS-CoV-2, and escape variants (Du, Shi, Zhang, Duan, Li, Zhang, et al., 2021).

Protoporphyrin IX (PpIX) and verteporfin, two FDA-approved drugs for the treatment of conditions unrelated to COVID-19, were found to disrupt the S-RBD-ACE2 interaction by binding to ACE2 (Gu, Wu, Guo, Zhu, Xu, Wang, et al., 2021). A study found that Arbidol, is able to impede the PPI between the viral S-glycoprotein and the host cell ACE2, by binding to the recognition domain of the latter (Shuster et al., 2021).

Screening for small molecule inhibitors of the PPI between the S protein and ACE2 is gaining interest due to the challenges faced in the constantly mutating S protein. A previous study found that small molecule inhibitors of PPI with potential immunomodulatory effects can be identified from organic dyes as the starting reagent (Bojadzic & Buchwald, 2018; J. Chen et al., 2017). The development of a series of such compounds for their ability to inhibit the SARS-CoV-2 S-ACE2 PPI could be beneficial. Several dye-derived small-molecule compounds show promising broad-spectrum inhibition of the PPI between S proteins and their cognate ACE2 receptor. Such examples of these include Congo red and direct violet 1, Evans blue. In another study, the S protein-ACE2 complex was analyzed (Rodriguez et al., 2022). A structure-based molecular docking virtual screen of commercially available small molecules led to the identification of 10 lead candidates. Of these 10, only three displayed low-mid micromolar EC_{50} activity by testing these lead candidates against pseudotyped SARS-CoV-2. Although Omicron subvariants were not analyzed in this study, this method could serve as a basis for the development and discovery of Omicron-specific antivirals using computational biophysics. Furthermore, novel drug-like compounds including DRI-C23041, DRI-C91005 were able to inhibit the interaction of ACE2 with S proteins in a concentration-dependent manner (Bojadzic, et al., 2021). More importantly, these drug-like inhibitors were shown to also be effective against delta (B.1.617.2) and Omicron (B.1.1.529), as well as HCoV-NL63, showing potential broad-spectrum capabilities (Chuang & Buchwald, 2022).

In addition to the small molecules mentioned above, there are also many natural compounds that can block the binding of the S protein to ACE2. Among flavonoids, quercetin is the most widely studied S protein-ACE2 blocker, which has potential antiviral effects (Luo, Zhang, Luo, Liu, Zhao, Zhao, et al., 2020; Ye et al., 2021). Natural hesperidin from *Citrus aurantium* was found to interfere with the interactions between the S protein and ACE2. Specifically, it was predicted that this compound interacts with the middle shallow part of the RBD (C. R. Wu, Liu, Yang, Zhang, Zhong, Wang, et al., 2020a). Cepharanthine, a naturally occurring alkaloid, was found to inhibit SARS-CoV-2 infection in VeroE6-TMPRSS2 cells, with an IC_{50} value of 0.35 μM. In silico docking simulations showed that cepharanthine binds to the SARS-CoV-2 S protein and interferes with the interaction between SARS-CoV-2 S-RBD and the ACE2 receptor (Xiang, Wang, Chen, & Chen, 2021). A recent study showed that the ephedrine, pseudoephedrine, and methylephedrine had a double binding effect on the SARS-CoV-2 RBD and ACE2, and had low cytotoxicity effects against cells expressing ACE2 at a certain concentration (Y. Lv, S. Wang, P. Liang, Y. Wang, X. Zhang, Q. Jia et al., 2021a). The natural compounds mentioned above could be considered as multitarget inhibitors. Emodin (an anthraquinone compound) showed inhibition of viral entry when tested against pseudotyped SARS-CoV-2 in Vero E6 cells. It also significantly blocked S protein-ACE2 interactions in a dose-dependent manner (Ho, Wu, Chen, Li, & Hsiang, 2007). The active ingredient of licorice, glycyrrhizic acid, was found to effectively block S protein-RBD/ACE2 interactions. (S. Yu et al., 2021). In addition, phenolic compounds such as salvianolic acid A and salvianolic acid B could inhibit pseudotyped SARS-CoV-2 viropexis by binding to both the RBD and ACE2, suppressing the entry of SARS-CoV-2 pseudovirus into ACE2-expressing cells (S. Hu, Wang, et al., 2021). Epigallocatechin-3-gallate (EGCG) was reported to significantly inhibit SARS-CoV-2 and other coronavirus infections by blocking the S-protein-ACE2 interaction (Henss et al., 2021). A virtual screen and experimental verification led to the identification of Kobophenol A. This natural compound was found to inhibit the interaction between ACE2 and the RBD, suggesting that Kobophenol A could serve as a strong candidate compound against SARS-CoV-2 (Table 3) (Gangadevi, Badavath, Thakur, Yin, De Jonghe, Acevedo, et al., 2021).

3.4. Inhibitors of host proteases

The cell entry process of SARS-CoV-2 can be blocked using inhibitors that target the protease activators. Host proteases play an important role in SARS-CoV-2 viral entry and S protein maturation. During SARS-CoV-2 biosynthesis, a host enzyme, furin, cleaves the S protein into S1 and S2 subunits at the furin cleavage site (PRRA). During viral entry, the S protein must be cleaved at the S2' cleavage site by host proteases TMPRSS2 or CatB/L, to ensure membrane fusion (Simmons et al., 2005).

Targeting these proteases could serve as a potent mechanism of action against SARS-CoV-2 entry and development.

3.4.1. Furin inhibitors

As mentioned earlier, S protein cleavage by the host enzyme furin is necessary during viral biosynthesis to create a non-covalent bond between the S1 and S2 subunits in the SARS-CoV-2 S protein. This cleavage facilitates S2' cleavage for membrane fusion during viral entry for SARS-CoV-2. Thus, S protein activation by furin is a prerequisite for fusion of SARS-CoV-2, becoming a potential antiviral target. The furin inhibitor MI-1851 showed SARS-CoV-2 S protein cleavage inhibition and consequently produced significant antiviral effect on infected human airway epithelial cells (Table 4) (Bestle, Heindl, Limburg, Van, Pilgram, Moulton, et al., 2020a).

3.4.2. TMPRSS2 inhibitors

TMPRSS2 is a serine protease that cleaves the S protein at the cell surface. Prior to TMPRSS2 cleavage, the S1 subunit on the S protein must bind to the ACE2 receptor on the cell surface. ACE2 binding leads to the dissociation of the S1 subunit, allowing for S2 subunit cleavage by TMPRSS2, which begins a cascade of events that cause viral and cellular membranes to fuse. TMPRSS2 inhibitors could thus prove potential candidates to reduce SARS-CoV-2 infection.

Aprotinin is a broad range serine protease inhibitor and has long been known to inhibit the replication of influenza (Zhirnov, Klenk, & Wright, 2011) and Sendai virus (Hayashi, Hotta, Itoh, & Homma, 1991). Fortunately, it has also shown to be effective as a therapeutic treatment for COVID-19 (Bestle, Heindl, Limburg, Van, et al., 2020b). Two prospective peptidomimetic inhibitors of TMPRSS2, MI-432 and MI-1900, prevented the replication of the SARS-CoV-2 in infected Calu-3 cells in a dose-dependent manner, most-likely by inhibiting the membrane-binding host protease TMPRSS2 (Bestle, Heindl, Limburg, Van, et al., 2020a) (Table 4). Nafamostat mesylate, the most effective TMPRSS2 inhibitor, potently inhibits SARS-CoV-2 S protein-mediated fusion in a cell fusion assay system and also inhibits SARS-CoV-2 infection *in vitro* (Yamamoto, Kiso, Sakai-Tagawa, Iwatsuki-Horimoto, Imai, Takeda, et al., 2020). Camostat mesylate is a clinically proven serine inhibitor that partially prevents the virus from entering the cell. Camostat mesylate has been proven to inhibit SARS-CoV, SARS-CoV-2, and HCoV-NL63 (Amraei & Rahimi, 2020). Furthermore, camostat mesylate is able to potently inhibit viral entry of the Delta variant, however, it cannot inhibit Omicron variant viral entry. Another recent study revealed that bromhexine reduced TMPRSS2-activated cell-cell fusion in SARS-CoV and SARS-CoV-2 S proteins, and is currently being tested in at least three clinical trials for efficacy against COVID-19 (NCT04355026, NCT04273763, NCT04340349) (Hornich, Grosskopf, Schlagowski, Tenbusch, Kleine--Weber, Neipel, et al., 2021). Nevertheless, its metabolite ambroxol interacted weakly against TMPRSS2, and did not exhibit activating effects on cell-cell fusion.

3.4.3. Cathepsin L and endosomal acidification inhibitors

After the S1 subunit is dissociated from the S protein due to ACE2 binding, the S2' cleavage site must be cleaved by a host protease. Cathepsin L cleavage is most likely to occur in the absence of TMPRSS2, leading the SARS-CoV-2 virus to enter the cell via the endosomal pathway. When the virus is endocytosed, the endosome becomes acidic, activating cathepsin L proteases. Cathepsin activity is required for proper processing of the S protein to activate its fusogenic activity. Due to this, endosomal acidification and/or cathepsin L proteases can be targeted to inhibit SARS-CoV-2 entry.

As an early response against SARS-CoV-2 infections, the efficacy of endosomal acidification inhibitors chloroquine (CQ) and hydroxychloroquine (HCQ) were confirmed to function by blocking SARS-CoV-2 entry (M. Wang, Cao, Zhang, Yang, Liu, Xu, et al., 2020a; Yao, Ye, Zhang, Cui, Huang, Niu, et al., 2020). Subsequently, chloroquine could inhibit both Omicron and Delta viruses by inhibiting the endocytic pathway (Icho, Rujas, Muthuraman, Tam, Liang, Landreth, et al., 2022). However, HCQ and CQ have not been approved for treatment of COVID-19. Additionally, these are controversial therapeutics with potential adverse effects (X. Zhan, Dowell, Shen, & Lee, 2020). One agent that interferes with the activity of cathepsin L is peptide P9, which directly binds to SARS-CoV-2 particles and inhibits endosomal acidification, thereby indirectly interfering with the activity of cathepsin L (H. Zhao, Zhou, Zhang, Chu, Liu, Poon, et al., 2016). P9 was further optimized by increasing the number of proton-accepting amino acids to generate P9R, which showed antiviral activity against the coronaviruses MERS-CoV, SARS-CoV, and SARS-CoV-2 with IC_{50} values in the low μg/ml range (H. Zhao, To, Sze, Yung, Bian, Lam, et al., 2020). Recently, a dual-functional cross-linking peptide 8P9R was identified. This peptide was found to inhibit the two entry pathways of SARS-CoV-2 in cells (the endocytic pathway and the TMPRSS2-cell surface mediated pathway) (H. Zhao, To, Lam, Zhou, Chan, Peng, et al., 2021).

Teicoplanin, a commonly-used clinical glycopeptide antibiotic, significantly inhibited the viral entry of Ebola virus, SARS-CoV, and MERS-CoV by inhibiting the activity of cathepsin L (Table 4) (N. Zhou, Pan, Zhang, Li, Zhang, Bai, et al., 2016). Interestingly, teicoplanin could also inhibit the entry of SARS-CoV-2 in HEK293T-hACE2 high cells at a relatively low and safe dose, by suppressing the proteolytic activity of cathepsin L (F. Yu, Pan, Huang, Ying, Liu, Fan, et al., 2022). A recent study found that Omicsynin B4 is a potent inhibitor of both cathepsin L and TMPRSS2 at sub-nanomolar or sub-micromolar levels, respectively. These function by forming a covalent bond to the active site of these host proteases (Li, Wang, Sun, Wu, Wang, Shi, et al., 2023). The small molecule E64d reduces SARS-CoV-2 pseudoparticle viral entry in TMPRSS2 negative HEK293T and Vero cells by inhibiting cathepsin L. Notably, the Omicron variant is highly sensitive to the drug E64d, so inhibitors targeting cathepsin L, or endoplasmic acidification, are still expected to be effective for the Omicron variant (H. Zhou, Mohlenberg, Thakor, Tuli, Wang, Assaraf, et al., 2022).

3.4.4. Combination medications

Effective treatment strategies are still needed to tackle SARS-CoV-2, including those that can address drug resistance conferred by emerging variants, for which combination therapies could be explored.

Combinations of wide-spectrum antivirals, such as lopinavir with ritonavir, and peptides (EK1) with RNA synthesis inhibitors, have been used as antiviral treatments. The combination of furin inhibitor MI-1851 with TMPRSS2 inhibitors, such as aprotinin, MI-432, or MI-1900, generally increased antiviral efficacy compared to treatment with each inhibitor alone (Bestle, Heindl, Limburg, Van Lam van, Pilgram, Moulton, et al., 2020b). Therefore, the combination of furin and TMPRSS2 inhibitors provides a promising therapeutic strategy for treatment of SARS-CoV-2 infections. Moreover, the combination of these may not only enhance antiviral effects, but may also reduce drug toxicity and undesirable side effects, by allowing for a reduced dose of these inhibitors. Furthermore, TMPRSS2 and cathepsin L inhibitors often show synergism with each other. Protease inhibitor cocktail therapy using both TMPRSS2 and cathepsin L inhibitors has been suggested to combat COVID-19 (T. Liu, Luo, Libby, & Shi, 2020a; Song et al., 2022). Additionally, the combined effects of Cepharanthy (antiinflammatory), Semictin (anti--helminthic), and Mefloquine hydrochloride (anti-malaria), have shown that this synergistic effect is more effective than individual drugs for treating SARS-CoV-2 infection (Fan, et al., 2020).

4. Discussion

To prevent and treat COVID-19, the most potent strategy is to target the host or the virus to inhibit viral entry. Therefore, a better understanding of the structure–function relationships of drug targets provide an effective approach for novel antiviral drug discovery. Viral attachment and entry are of particular interest among possible therapeutic targets in the viral life cycle. However, there are very few approved antiviral drugs

targeting the virus entry/attachment, which requires effort to explore with the aid of the advanced technology in the future.

While vaccination and therapeutics have improved, SARS-CoV-2 infections remain a persistent threat as a result of continual antigenic drift. Furthermore, SARS-CoV-2 shares high sequence homology with SARS-CoV, the lack of advances in anti-SARS-CoV drug research and the high mutagenicity of SARS-CoV-2 have limited the number of candidate drugs available for screening anti-SARS-CoV-2 therapeutics. Despite incredible effort and a rapidly evolving understanding of SARS-CoV-2 infection, highly effective treatment options remain limited. Currently, one of the most effective methods is to directly block or indirectly interfere with the interaction between the SARS-CoV-2 S protein and the hACE2 receptor. In addition, inhibition of important proteases such as furin, TMPRSS2, and cathepsin L represents an interesting option for inhibiting the entry of SARS-CoV-2. Fortunately, TMPRSS2 and cathepsin L are both host proteins, which should not mutate after encountering viral mutants, thus having broad-spectrum antiviral effects against SARS-CoV-2 and its variants. Notably, host proteins involved in viral infection are conserved targets, thus being considered as promising targets for the development of highly broad-spectrum antiviral drugs. However, host proteins undertake crucial physiological functions, which still require more comprehensive safety and effectiveness evaluation, both *in vitro* and *in vivo* to prevent unwanted side effects.

A number of the therapeutic antibodies in later stage clinical trials are effective against earlier SARS-CoV-2 variants, however, they have been found to be less capable of fully neutralizing Omicron strains and its subvariants. Currently, available monoclonal antibodies most likely target the RBD and/or the NTD, which are prone to mutate. As more escape variants emerge, these antibodies are more likely to lose their neutralizing activities against newly emerging SARS-CoV-2 variants. In fact, due to this, there are very few mAbs approved for Omicron variant treatment. Many emergency use authorizations provided by the FDA have been revoked, furthermore, the NIH suggests against the use of mAbs for the treatment of COVID-19 infections. Hence, it is important to explore the possibility of developing broad-spectrum monoclonal antibodies that target conserved viral epitopes. Common challenges of using peptide inhibitors as drugs includes short half-life and poor oral absorption *in vivo*. Given that the combination of peptide and lipid therapeutics showed significantly improved antiviral efficacy and better pharmacokinetics, it shows promising targets to develop new combined peptide inhibitors.

Due to the time-consuming development process of new drugs, drug repurposing may be an effective solution to the epidemic of sudden infectious diseases. Large libraries of approved or clinical-stage drugs with diverse bioactivity might be useful for antiviral drug screening, however, access to such open-access libraries are currently lacking. Furthermore, the S protein undergos dynamic structural rearrangements, thus posing a challenge to developing small-molecule inhibitors. Lastly, lead compounds derived from natural compounds can also contribute to the generation of new drug therapies. Although a number of studies have shown that natural compounds have a significant inhibitory activity against SARS-CoV-2, these still require *in vivo* and clinical verification.

Given the tradeoff between infection control measures and socio-economic development, the research and production of vaccines will now take precedence over other therapeutic methods. The ongoing evolution of SARS-CoV-2 requires the design of efficient vaccines to keep the pace against emerging variants, to aid in the development of new monoclonal antibodies for the treatment of patients with severe cases of COVID-19, and to alleviate presence of this persistent pandemic. The development of antiviral small molecule inhibitors requires continuous research, especially when the goal is to reduce the constant emergence of new variants. Currently, the cases of a newly emerging viruses are few, however, there is always a possibility of reemergence. Therefore, effective strategies are needed to provide insightful data that will contribute to the efficient development of antivirals for future epidemics.

Declaration of competing interest

After deliberative consultation and discussion, the authors declare no conflict of interest.

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