Advances in COVID-19 Treatment

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COVID-19, which emerged in December 2019, was declared a global pandemic by the World Health Organization (WHO) in March 2020. The disease was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has caused millions of deaths worldwide and caused social and economic disruption. While clinical trials on therapeutic drugs are going on in an Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) public–private partnership collaboration, current therapeutic approaches and options to counter COVID-19 remain few. Therapeutic drugs include the FDA-approved antiviral drugs, Remdesivir, and an immune modulator, Baricitinib. Hence, therapeutic approaches and alternatives for COVID-19 treatment need to be broadened.

Keywords: COVID-19 ; peptides ; virus life cycle ; FDA-approved drugs ; drug development

1. Expanding the Therapeutic Approaches for COVID-19

Despite significant efforts to produce a successful COVID-19 treatment, there are now only a small number of FDA-approved therapeutic medicines for COVID-19 therapy. Remdesivir and baricitinib are the only available treatment medications. It would be advantageous to investigate additional possibilities, such as small compounds and peptides, for developing COVID-19 medicines. The therapeutic approaches also focus on boosting the host's immune system with monoclonal antibodies, inhibiting virus genome replication by inhibiting RdRp, and prescribing anti-inflammatory drugs and immune modulators to reduce the host's hyper inflammation response. Other strategies, such as preventing viral entry into host cells or inhibiting the formation of RTC through inhibiting the main protease, should also be considered.

1.1. Peptides Targeting S Protein RBD

Several strategies prevent viral entry into host cells, including blocking receptor-binding domain (RBD) and ACE2 binding, preventing S protein cleavage, and blocking the binding of HR1 and HR2 by small compounds and peptides. In 2020, Cao et al. designed several mini proteins, AHB1, AHB2, LCB1, and LCB3, which bind to the RBD of the SARS-CoV-2 S protein. These proteins inhibit infection of SARS-CoV-2 to Vero E6 cells with IC_{50} of 35 nM, 15.5 nM, 23.5 pM, and 48.1 pM, respectively [1]. However, these mini proteins are a few folds larger than standard peptides, and there is no information on in vivo models. Wolfe et al. generated a list of peptides using computer modeling and further tested their binding affinity using bio-layer interferometry (BLI). They highlighted four peptides, P89, P100, P168, and P180, which bind to subunit 1 of S protein with binding constants (KD) 124 nM, 185 nM, 143 nM, and 243 nM, respectively [2]. However, there is no information on additional analysis, such as IC_{50} determination or in vivo study.

The most promising stapled peptide created by Curelli et al. to target the S protein RBD was NYBSP-4. NYBSP-4 has a KD value of 2.2 µM using surface plasmon resonance (SPR). It expressed a calculated half-life (T1/2) of >289 min in human plasma, showing high proteolytic stability. It did not demonstrate toxicity even when tested with a maximum dose and has an IC_{50} of 1.97 µM and 2.8 µM in two cell types, HT1080/ACE2 and A549/ACE2 cells, upon infection with the SARS-CoV-2 pseudovirus [3]. However, there is no investigation using in vivo models. In addition, Karoyan et al. designed three interesting peptides, P8, P9, and P10, with IC_{50} values of 46 nM, 53 nM, and 42 nM on Calu-3 cells following SARS-CoV-2 infection with KD values of 24 nM, 0.09 nM, and 0.03 nM, respectively. These peptides did not cause any cytotoxicity in Vero-E5 and Calu-3 cells [4]. However, there is no testing of these peptides in vivo. Among the list of peptides developed by Chen et al. in 2021 is a peptide called AYn1, which has a KD value of 95.6 nM using localised surface plasmon resonance (LSPR) and an IC_{50} value of 4.9 µM when infecting HEK293T/hACE2 with SARS-CoV-2 pseudovirus and demonstrated minimal cytotoxicity to the cells. However, the result of AYn1 in in vivo models is not satisfactory [5].

1.2. Peptides Targeting S Protein HR1-HR2 Fusion

Several peptides have been reported to inhibit the fusion of HR1 and HR2 of SARS-CoV-2 [6]. One peptide, EK1C4, can inhibit infection of SARS-CoV-2 pseudotyped and live virus at IC_{50} of 15.8 nM and 36.5 nM, respectively. EK1C4 is found to exhibit little or no toxic effect in vitro. The modified EK1C4 peptide, EKL1C, has an inhibitory effect on SARS-CoV-2 pseudotyped and live virus infection at IC_{50} of 3–27 nM in various cell types. EKL1C also demonstrated the ability to reduce SARS-CoV-2 virus titer in the lungs of hACE2-Tg mice. It has stronger resistance to proteolytic enzymes and exhibits higher thermostability properties. A dimeric peptide candidate, [SARSHRC-PEG4]2-chol lipopeptide, has been reported to inhibit HR1 and HR2 fusion. It inhibits viral entry after 8 h on Vero-E6 and Vero-E6-TMPRSS2 cells with IC_{50} of ~300 nM and ~5 nM, respectively, and showed no toxicity in a cellular toxicity assay [7].

1.3. Small Molecules and Peptides Targeting Host Proteases and SARS-CoV-2 Main Protease

Several small molecules and peptides that target host proteases have been reported. The TMPRSS2 peptide mimetic inhibitors MI-432, MI-1900, the peptide aprotinin, and the furin inhibitor MI-1851 all suppress SARS-CoV-2 replication in Calu-3 [8]. Apart from this, furin/proprotein convertase (PC) inhibitors, decanoyl-RVKR-chloromethylketone (CMK), naphthofluorescein, and a TMPRSS2 inhibitor, camostat effectively decreases SARS-CoV-2 production in Vero-E6 cells [9]. Teicoplanin, a glycopeptide antibiotic that inhibits cathepsin L, effectively halted the infection of HEK293T and Huh7 cells by SARS-CoV-2 pseudotyped viruses [10]. However, further analyses such as cytotoxicity or in vivo model tests have not been carried out in these studies.

An in vitro fluorescence resonance energy transfer (FRET) assay identified five drugs that are inhibitors of the SARS-CoV-2 main protease and have IC_{50} values of 4.81 µM, 5.4 µM, 16.2 µM, 38.5 µM, and 18.7 µM, respectively. These drugs are manidipine, boceprevir, lercanidipine, efonidipine, and bedaquiline [11]. In another study, four peptides, p12, p13, p15, and p16, showed binding to the main protease and competitively inhibiting main protease activity. The peptides have an IC_{50} value of 5.36 µM, 3.11 µM, 5.31 µM, and 3.76 µM, respectively, as determined via solid-phase extraction coupled to mass spectrometry (SPE MS) [12]. In Vero cells infected with SARS-CoV-2, two other drugs, ebselen and a Michael acceptor inhibitor known as N3, showed antiviral effects [13]. This suggests that these two drugs may be able to permeate the host cell membrane and target the primary protease. Teicoplanin, also has inhibitory activity on the SARS-CoV-2 main protease [14]. However, further analyses, such as cytotoxicity or in vivo models, are also lacking.

2. A New Area That Can Be Considered for the Development of COVID-19 Drugs

There is an area that remained seemingly untouched in the therapeutic approach that prevents viral entry into host cells. Apart from inhibiting host cell proteases and the primary SARS-CoV-2 protease and preventing the interaction of the RBD with ACE2 and the HR1-HR2 fusion, studies on developing therapeutic drugs that can bind to the S protein's S1/S2 cleavage site and stop host proteases from breaking it down should be considered. The S protein has an N-terminal domain (residue 14–305), RBD (residue 319–541), S1/S2 cleavage site where host proteases cleave between residue 685 and 686, fusion peptide domain (residue 788–806), HR1 (residue 912–984), HR2 (residue 1163–1213), transmembrane domain (residue 1213–1237), and cytoplasm domain (residue 1237–1273) [15]. Compared to the RBD, the S protein's S1/S2 cleavage site is relatively conserved, as shown in **Table 1** [16]. For this reason, therapeutic drugs that target S1/S2 cleavage site might face fewer challenges caused by mutation of the targeted binding site.

Table 1. Mutations in the variants of SARS-CoV-2 [16].

SARS-CoV-2 Variant	Mutation on RBD	Mutation near S1/S2 Cleavage Site	Mutation on Other Sites
Alpha	**N501Y**	**P681H**	**D614G**
Beta	**K417N, E484K, N501Y**	**-**	**D614G, A701V**
Gamma	**K417T, E484K, N501Y**	**-**	**D614G, H655Y**
Delta	**L452R, T478K**	**P681R**	**D614G**
Omicron	**G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H**	**P681H**	**A67V, Δ69-70, T95I, G142D, Δ143-145, N211I, Δ212, ins215EPE, T547K, D614G, H655Y, N679K, N764K, D796Y, N856K, Q954H, N969K, L981F**

Developing therapeutic drugs targeting the S1/S2 cleavage site instead of host proteases seems more applicable, as the host proteases are involved in important physiological processes. Consider the PC family member furin, which is a key player in embryogenesis. Experimenting on furin knockout mice results in embryonic fatality. Furin also aids in synaptic innervation, which is another crucial role. It is accomplished by the pro-nerve growth factor's pro-NGF neurotrophin, created by furin cleaving it and binding to the Trk receptors (NGF). Pro-NGF is secreted when furin is inhibited, and it binds to the neurotrophin receptor (p75NTR) and triggers apoptosis [17]. In addition, Cathepsin L is involved in proteolytic activities and generates active enzymes, receptors, and biologically active peptides [18].

Furthermore, furin belongs to the PC family, TMPRSS2, the transmembrane-bound serine proteases family, and cathepsin L, the cathepsin family. Proteases within a common family possess similar structures and mechanisms. Hence, the inhibitors targeting these proteases might also end up targeting proteases of the same family, disrupting important physiological functions with unforeseen consequences. As a result, targeting the S protein's S1/S2 cleavage site might be a better course of action than suppressing the host proteases.

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