

REVIEW



Antimicrobial peptides: A plausible approach for COVID-19 treatment

Pooja Rani ^a, Bhupinder Kapoor ^a, Monica Gulati ^a, Atanas G. Atanasov ^{b,c,d}, Qushmua Alzahrani ^e
and Reena Gupta ^a

^aSchool of Pharmaceutical Sciences, Lovely Professional University, Phagwara, India; ^bLudwig Boltzmann Institute for Digital Health and Patient Safety, Medical University of Vienna, Vienna, Austria; ^cInstitute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Magdalenka, Poland; ^dDepartment of Pharmaceutical Sciences, University of Vienna, Vienna, Austria; ^eDepartment of Pharmacy/Nursing/Medicine Health and Environment, University of the Region of Joinville (UNIVILLE) volunteer researcher, Joinville, Brazil

ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19), which emerged as a major public health threat, has affected >400 million people globally leading to >5 million mortalities to date. Treatments of COVID-19 are still to be developed as the available therapeutic approaches are not able to combat the virus causing the disease (severe acute respiratory syndrome coronavirus-2; SARS-CoV-2) satisfactorily. However, antiviral peptides (AVPs) have demonstrated prophylactic and therapeutic effects against many coronaviruses (CoVs).

Areas covered: This review critically discusses various types of AVPs evaluated for the treatment of COVID-19 along with their mechanisms of action. Furthermore, the peptides inhibiting the entry of the virus by targeting its binding to angiotensin-converting enzyme 2 (ACE2) or integrins, fusion mechanism as well as activation of proteolytic enzymes (cathepsin L, transmembrane serine protease 2 (TMPRSS2), or furin) are also discussed.

Expert opinion: Although extensively investigated, successful treatment of COVID-19 is still a challenge due to emergence of virus mutants. Antiviral peptides are anticipated to be blockbuster drugs for the management of this serious infection because of their formulation and therapeutic advantages. Although they may act on different pathways, AVPs having a multi-targeted approach are considered to have the upper hand in the management of this infection.

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1. Introduction

Coronaviruses (CoVs) form the largest cluster of viruses that cause respiratory and gastrointestinal infections [1,2]. The seven CoVs that infect humans are HCoV-HKU1, HCoV-OC43, HCoV-NL63, HCoV-229E, MERS-CoV (Middle east respiratory syndrome-corona virus), SARS-CoV (severe acute respiratory syndrome-corona virus), and SARS-CoV-2 (Figure 1) [3].

In December 2019, a mysterious pneumonia, characterized by fever, cough, chest discomfort, dyspnea, and bilateral lung infiltration was detected in China [6]. The causative agent of the disease was identified as the novel CoV, named later as 'SARS-CoV-2,' while the disease was given the name 'COVID-19' [7–9]. It was declared as a pandemic by WHO in March, 2020 [7].

Numerous clinical trials have either been conducted or are underway for repositioning the existing drugs to prevent and treat this disease. Some of these drugs are remdesivir, ivermectin, nelfinavir, cepharanthine, hydroxychloroquine, and dexamethasone [10].

Despite all the efforts, treatment of COVID-19 still remains elusive. Antimicrobial peptides (AMPs) have been reported to treat various viral infections such as AIDS, hepatitis, dengue,

and influenza. AMPs, therefore, offer a potential treatment option of COVID-19 [11–13].

1.1. Search strategy and selection criteria

Articles for this review were identified through searches of PubMed for the period from January 2020, to July 2021, by use of the terms 'COVID-19,' and 'SARS-CoV-2.' Articles published in English only were included.

2. Structure assembly and replication of SARS-CoV-2 virus

SARS-CoV-2 virus is a large spherical albeit pleomorphic, enveloped virus, having positive sense RNA (with a length of 29.9 kb), encoding 9860 amino acids [14]. Proteins of SARS-CoV-2 include two polyproteins, i.e. ORF1a and ORF1b, which undergo proteolytic cleavage to form 16 nonstructural proteins, Nsp1–16, four structural proteins: nucleocapsid (N), membrane (M), envelope (E), and spike (S), and nine accessory proteins: Orf_{3a}, Orf_{3b}, Orf₆, Orf_{7a}, Orf_{7b}, Orf₈, Orf_{9b}, Orf_{9c}, and Orf₁₀ [15,16,17]. The N protein forms the capsid outside the genome, while the remaining structural proteins participate in

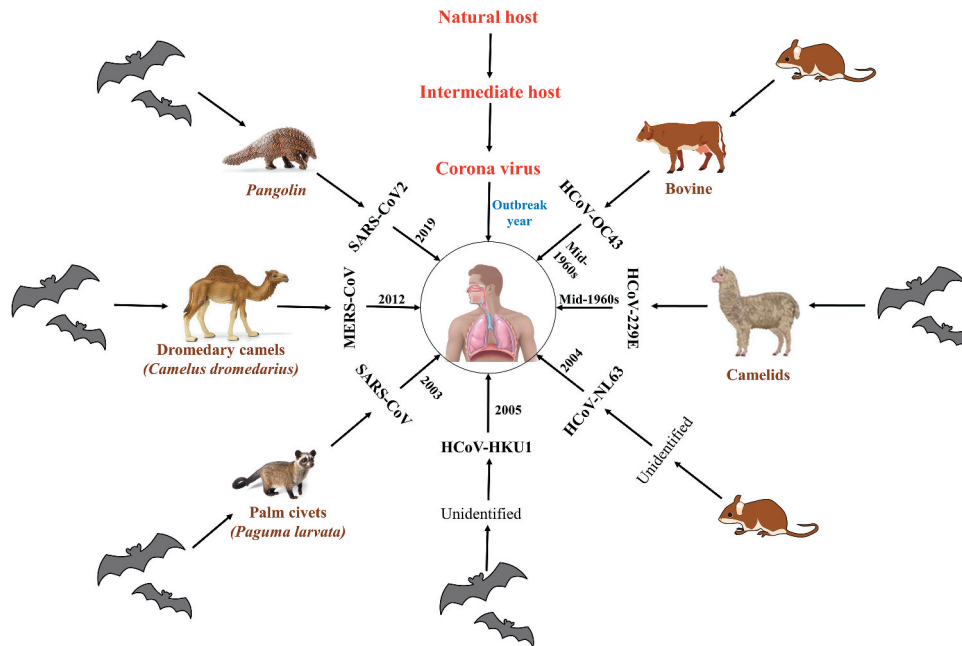


Figure 1. Classification of coronavirus diseases based on natural and intermediate hosts [4,5,18].

host cell infection and its replication [18]. Nsp1-16 facilitates viral replication and transcription (Figure 2) [19].

Virus entry to the host cells is facilitated by transmembrane S glycoprotein, consisting of S1 and S2 subunits, which are responsible for binding to host cell and fusion between the viral envelop and cellular membranes of host [21]. Receptor-binding domain (RBD), present in S1 subunit, recognizes the angiotensin-converting enzyme 2 (ACE2) peptidase domain of host cell and initiates direct binding with it, while membrane fusion is attributed to the basic elements present in S2 subunit [22]. ACE2 are considered as the target receptors of SARS-CoV-2 [23]. After binding, the host protease cleaves and activates S proteins to allow the insertion of viral fusion peptide into the host cell membrane. After that, the three pairs of heptad repeat region (HR1 and HR2) interact leading to formation of a six-helix coiled bundle, which pulls the viral membrane towards that of the host cell, leading to their fusion. This results in the entry of viral genome into the host cell [24]. Presence of 5' methylated cap and a 3' polyadenylated tail in viral genome permits the attachment of RNA to the ribosome of host cells for translation. Replication of viral genome and transcription of the negative-sense sub-genomic mRNA are mediated by RNA polymerase [25]. Host ribosomes translate these RNAs into structural and accessory proteins in the endoplasmic reticulum. Protein-protein interactions required for virus assembly are initiated by M proteins, leading to the formation of progeny viruses which are released into the extracellular space through exocytosis [26].

3. Peptides as drugs against COVID-19

Therapeutic peptides could not gain much popularity due to their limitations like susceptibility to degradation by enzymes, limitation of effective delivery methods, low membrane permeability, limited bioavailability on oral administration, rapid

clearance from body, and low target specificity [27]. Recent advancement in processing technologies, however, has again generated interest in these therapeutic peptides. Properties of peptides, now, can be modulated to overcome the above-stated challenges by modification of amino acid sequences or addition of certain moieties. These modifications improve stability, transport, affinity profiles, and oral availability of peptides [28,29]. Peptides meant for treatment of COVID-19 are classified into the following categories:

3.1. Peptides preventing SARS-CoV-2 spike protein binding to ACE2 receptors

A crucial phase during the interaction of SARS-CoV-2 with the host cell involves the attachment of virus to ACE2 receptors, mediated by S glycoproteins present on virus cell surface. A plausible strategy for the prevention of infection is the inhibition of this interaction (Figure 3).

Computational tools have been utilized to design novel peptides capable of inhibiting the interaction between ACE2 receptors and SARS-CoV-2 spike proteins, and thus preventing the virus entry into host cells. A portion consisting of 23 amino acids between Glu23 and Leu45 of the ACE2 PD N-terminal region is mainly involved in their interaction with spike protein. Computation alanine scanning further shortened the peptide chain containing 23 amino acids to 18 amino acids peptide (18aa peptide) that was found to be non-toxic in comparison with mutated peptides in the *in silico* toxicity evaluation [22]. In another study, protein-peptide docking revealed a stronger affinity of peptides toward ACE2 over RBD. Peptides generally associate with the residue binding motif (RBM), which, in turn, facilitates their binding to ACE2. On the other hand, peptides preferentially bind the core for ACE2. Among various peptides, AVP1244 and AVP0671 at ACE2 and RBD interfaces, respectively, decrease the binding

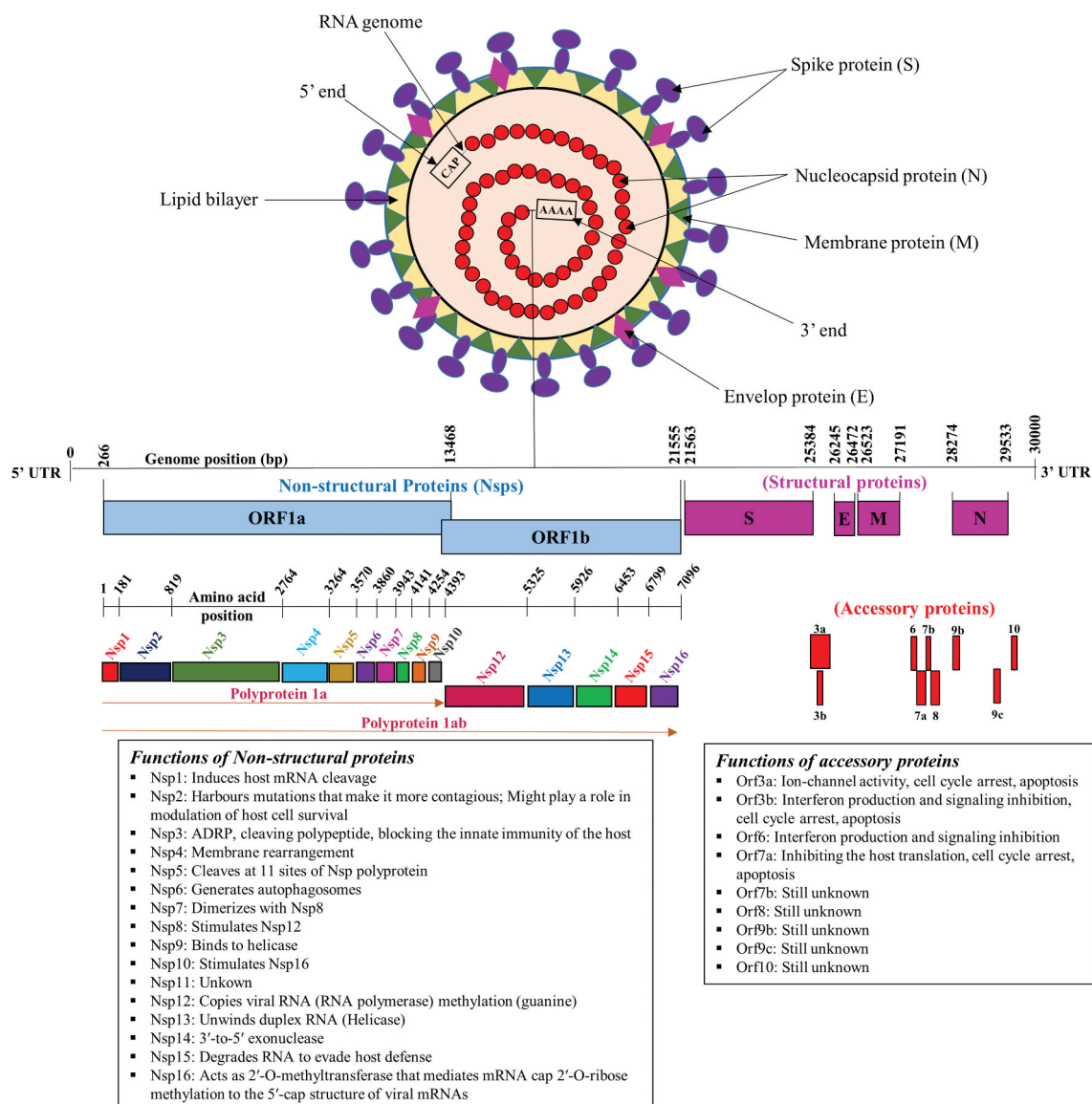


Figure 2. Genome organization of the SARS-CoV-2 and its genomic organization [16,20].

affinity to a significant extent. They are also reported to alter the orientation of binding by RBD and ACE2 binding [30]. Chimeric peptide design strategy has been utilized for the designing of novel peptides, based on the analysis of key residues interacting with SARS-CoV-2-RBD and human ACE2, as well as screening of antibacterial peptides against SARS-CoV-2 spike RBD. Among them, peptides DBP6, AC23, AC20, and cnCoV-1-cnCoV-7 are expected to show potent activity against SARS-CoV-2 [31]. Badhe et al. created a series of peptides with a tendency to bind RBD of virus S protein and inhibit its binding on the ACE2 receptors. Two of the designed peptides exhibited both good RBD affinity as well as aqueous stability. However, for all the above studies, the peptides that have been designed by the *in silico* approach need to be synthesized and evaluated in *in vitro* and *in vivo* studies, before their use in COVID-19 therapy is proposed [32].

In addition to ACE2, glucose-regulating protein 78 (GRP78) receptor is another target assisting SARS-CoV-2 for its entry into the host cells. Allam *et al.* used *in silico* approach to

identify bioactive peptides (satpdb12488, satpdb14438, satpdb18446, satpdb18674, and satpdb28899) which were found to act on regions III and IV of the viral S proteins and on its binding sites in GRP78. Bioassays, however, must be performed to ensure their inhibitory activity [33]. In another study, a protein inhibitor (Δ ABP-D25Y) designed by computational approach has been found to exhibit exclusive binding at the ACE2 binding site of S protein in docking as well as molecular dynamic simulation studies. Higher binding affinity of the inhibitor as compared to that of ACE2 indicates its potential role in SARS-CoV-2 infection [34].

Han et al. prepared a number of peptide inhibitors after analyzing the interactions of amino acids at the ACE2 and RBD interface. The inhibitors consist of two sequential, self-supporting α -helices (bundle) from the protease domain (PD) of ACE2, which bind to the SARS-CoV-2 RBD. α -helical peptides are responsible for maintaining their secondary structure and specificity, as well as stable binding (blocking) to SARS-CoV-2. Enhancement of binding affinity can be achieved by

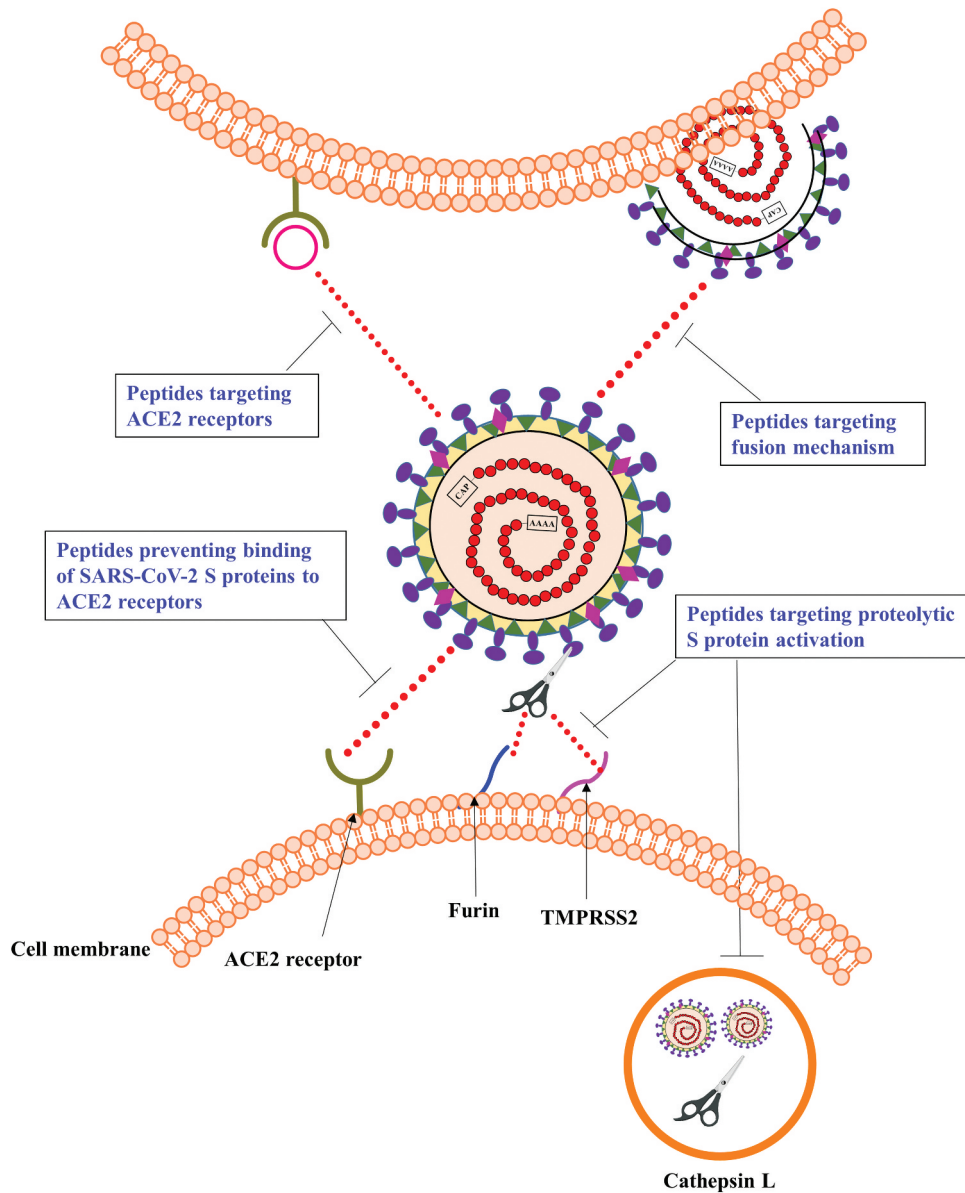


Figure 3. Targets of antiviral peptides against COVID-19.

multivalent binding of a number of peptides that are anchored onto the surfaces of various novel particulate delivery systems like dendrimers, nanoparticles, and clusters [35].

Scientists have also explored the use of hrsACE2, a human recombinant soluble ACE2. As ACE2 regulates many cellular functions including blood pressure and other cardiovascular parameters, its modulation with hrsACE2 could result in dysregulation of these key functions, leading eventually to serious side effects in patients. To overcome this, a series of peptides (22–44, 22–57, 22–44-g-351–357 and 351–357) were prepared and evaluated for their binding inhibition properties. All the synthesized peptides exhibited IC_{50} values in the μM range (1–10 μM). Peptide [22–44] was able to inhibit the formation of S1–ACE2 to the maximum extent (~76% reduction in binding probability) followed by [22–57] peptide (~74% reduction) and ~60% for the remaining peptides, pointing at their high therapeutic potential [21]. Three short peptides (pep1c, pep1d and

pep1e), based on the ACE2 sequence/structure fragments, were designed and evaluated for their affinity to RBD of S protein. Two of these peptides (pep1c, pep1d) associate with the RBD of S protein. Dissociation constant for this binding was reported to be in nM range (<300 nM). In *in vitro* RBD–ACE2 interaction inhibition assay, IC_{50} value of $3 \cdot 3 \pm 0 \cdot 8$ mM was exhibited by pep1c. This is considered to be too high for any pharmacological application. Nevertheless, this may be taken as a lead molecule for further preparation of similar agents [36].

Cao et al. designed miniproteins by *de novo* sequencing utilizing ACE2 as scaffold, based on interaction motifs of RBD-binding. Out of these, two miniproteins i.e. AHB1 and AHB2 were found to exhibit IC_{50} of 35 and 15 · 5 nM, respectively, against SARS-CoV-2. Neutralization of SARS-CoV-2 of a much higher magnitude was observed with LCB1 and LCB3 (IC_{50} of 23 · 54 and 48 · 1 pM respectively) miniproteins designed by

de novo approach. The designed miniproteins have many potential advantages over antibodies, such as a range of high-affinity binding modes, long term retention of activity even at high temperature, less molecular weight (5% of full antibody molecule), less scale up cost and amenability to formulation in the form of nasal gel or inhalational spray for respiratory tract [37]. Karoyan et al. designed a series of peptides resembling the N-terminal helix of human ACE2 protein and exhibiting high helical folding tendency in aqueous media, which is responsible for enhanced interactions with spike RBD and thereby, blocks SARS-CoV-2 pulmonary cell infection. These peptides were able to decrease SARS-CoV-2 viral titers while exhibiting complete efficacy at 1 μ M in pulmonary cells. Also, these peptides were devoid of cell toxicity at effective concentrations, highlighting their safety [38].

A series of conformationally constrained peptidomimetics of ACE2 PD α 1 helix were synthesized by solid-phase peptide synthesis approach and evaluated in a number of assays for antiviral activity. Interestingly, all of these failed to show any activity. This indicates the requirement of an enhanced binding interface in these peptides to display higher affinity for SARS-CoV-2 S-protein RBD binding than the membrane bound ACE2, thereby preventing virus entry [39].

3.2. Peptides targeting ACE2 and integrins

Blockage of the ACE2 receptors that act as entry point for the virus is another promising strategy for the management of COVID-19 (Figure 3) [40,41]. This proof-of-concept is supported by a number of clinically available molecules such as maraviroc (targeting HIV-1 co-receptor CCR5) [42], AMD3100 and EPI-X4 (HIV-1 co-receptor CXCR4 inhibitors) [43,44] and myrlcludex B (that not only targets sodium taurocholate co-transporting polypeptide, but also the receptors for viral entry of hepatitis B and D) [45].

Defensins, a group of cytotoxic peptides, play a significant role in host defense mechanisms. Human defensin 5 (HD5) has much higher affinity to ACE2 receptors (39 \cdot 3 nM) in comparison with that of SARS-CoV-2 (14 \cdot 7 nM). It is also known to bind glycosylated Corona S1 protein, thus preventing the virus invasion due to its dual action [46]. Although pretreatment with HD5 has been shown to decrease the titers of SARS-CoV-2 pseudoparticles, its effect on SARS-CoV-2 isolates has not been established yet [47]. Moreover, blockage of ACE2 receptors may lead to deleterious consequences, as these receptors regulate some of the important cellular functions in the body. Further development of MLN-4760 (ACE2 blocker) was, therefore, stopped after phase Ib/IIa clinical trials due to toxicity concerns [48].

In addition to the ACE2 receptors, involvement of an interaction between the RGD (arginine-glycine-aspartate) motif in the spike protein and integrin $\alpha_5\beta_1$ is also reported in the viral cell entry mechanism [49]. ATN-161, a non-RGD peptide obtained from fibronectin, has been reported to bind certain integrins, leading to inhibition of their activity [50,51]. ATN-161 was reported to reduce SARS-CoV-2 infection in renal cell line (VeroE6) of *Chlorocebus atheiops*. Pretreatment with ATN-161 increased cell viability, demonstrated by increased

production of adenosine triphosphate [52]. Moreover, therapeutic efficacy of ATN-161 in *in vivo* study against closely related betacoronavirus (porcine hemagglutinating encephalomyelitis virus) [53] as well as in phase 2 clinical trial (NCT00352313) supported the further exploration of ATN-161 as a promising treatment for COVID-19.

3.3. Peptides targeting the fusion mechanism

The six-helix bundle created by interaction of HR1 with HR2, responsible for getting both viral and host cell membranes in each other's vicinity for fusion, is another significant target for the development of antiviral drugs [54]. Certain lipopeptides obtained from EK1, a pan-coronavirus fusion inhibitor effective against five HCoVs, including SARS-CoV, MERS-CoV, and three SARS-related CoVs, were synthesized employing covalent attachment to cholesterol or palmitic acid using polyethylene glycol as a spacer to give corresponding lipopeptides EK1C and EK1P, respectively. These two peptides inhibited SARS-CoV2 mediated cell-cell fusion indicating that lipidation, especially cholesterol-modification is an attractive approach. To explore further, Xia et al. synthesized a number of derivatives of EK1C using multiple linkers containing 3–5 glycine/serine amino acid residues and polyethylene glycol-based spacer with variable arm length. Among the synthesized derivatives, EK1C4 containing GSGSG linker and PEG4 spacer was reported to be the most potent fusion inhibitor. The *in vivo* study in murine model also indicated the protective effect of EK1C4 against HCoV-OC43 infection, indicating its potential in prophylaxis as well as treatment of SARS-CoV-2 infection [55].

The S2 subunits of SARS-CoV and SARS-CoV-2 are quite strongly conserved in terms of their sequence alignment as they were found to be homologous to the extent of 92 \cdot 6% and 100% in HR1 and HR2 domains, respectively [56]. Therefore, peptides obtained from SARS-CoV HR are anticipated to prevent infection. Working in this direction, Xia et al. prepared HR1- and HR2-derived peptides, named as 2019-nCoV-HR1P (aa924–965) and 2019-nCoV-HR2P (aa1168–1203). In cell-cell fusion assay, 2019-nCoV-HR2P demonstrated strong inhibition of fusion activity (IC_{50} : 0 \cdot 18 μ M), pointing the possibility of 2019-nCoV HR1 region as a potential target site. 2019-nCoV-HR1P, however, failed to exhibit any considerable inhibitory effect (up to a strength of 40 μ M), similar to the lack of activity of other coronavirus HR1-derived peptides, such as SARS-HR1P and MERS-HR1P [57]. 2019-nCoV-HR2P also inhibited the pseudovirus infection in a dose-dependent manner in ACE2-expressing 293 T cells [56]. Zhu et al. synthesized IPB01 peptide containing the HR2 sequence, which exhibited strong inhibition of SARS-CoV-2 S protein-mediated cell-cell fusion and pseudovirus transduction. For enhancing the antiviral activity as well as *in vivo* stability, IPB01 was conjugated with cholesterol group at its C terminus, leading to the formation of lipopeptide IPB02. Although IPB02 exhibited similar cell-cell fusion inhibitory activity (IC_{50} : 0 \cdot 25 μ M), there was significant improvement in anti-SARS-CoV-2 pseudovirus activity (IC_{50} : 0 \cdot 08 μ M) [58]. To further improve antiviral potency, de Varies et al. designed a series of lipophilic peptides by PEGylation, lipidation, and dimerization.

Dimerization of both nonlipidated as well as lipidated peptides was reported to potentiate their effect. Among various peptides, SARS_{HRC}-PEG₂₄-chol (monomeric peptide) and [SARS_{HRC}-PEG₄]₂-chol (dimeric peptide) were found to be the most potent lipopeptides. In biodistribution studies in humanized K18 hACE2 mice, both the peptides achieved similar lung concentration after intranasal administration in the first hour, followed by longer retention of dimeric peptide in lungs after 24 h as compared to that of monomeric peptide. On intranasal administration, this dimeric peptide was found to show thorough distribution throughout the lungs. Cellular toxicity assay in primary human airway epithelial cells, exhibited minimal toxicity even after 6 days at the highest concentrations tested while at its 90% maximal inhibitory concentration any kind of toxicity was absent. The dimeric peptide inhibited SARS-CoV-2 entry into VeroE6 cells or VeroE6 cells overexpressing transmembrane serine protease 2 (TMPRSS2). Intranasal administration of the dipeptide to ferrets for prophylactic use was able to protect them from transmission when they were co-housed with SARS-CoV-2-infected counterparts [59].

A series of short- and long-chain peptides, comprising 24 or 36 amino acids, were designed as SARS-CoV-2 fusion inhibitors. Although short chain peptide showed no effect on fusion activity, long-chain peptide exhibited potent inhibitory activity (IC₅₀: 1 μM). It also suppressed the infectious activity of the pseudotyped viral particles by prevention of viral-cell fusion, through its association with the SARS-CoV-2 spike [60]. Sun and coworkers prepared four peptides from S protein of the virus. One of these peptides exhibited substantial inhibitory effects on cell-cell fusion and excellent neutralization activity against pseudotype SARS-CoV-2, and was able to inhibit the authentic viral infection to Vero E6 [61].

3.4. Peptides targeting proteolytic S protein activation

The steps of SARS-CoV-2 viral propagation including viral cell entry, its replication, polyprotein maturation as well as assemblage of the virions for further diffusion are regulated by various proteolytic enzymes of either host (serine-proteases) or virus (cysteine-proteases) origin. Binding and fusion of CoV S protein are initiated by cleavage events occurring at different sites, facilitated by a number of proteases at a number of sites in various cell compartments. Depending upon the viral strain and host cell type, S protein gets activated by one or more of the host protease enzymes, e.g. furin, trypsin, cathepsin L, transmembrane protease serine protease 2 (TMPRSS2), TMPRSS4, or human airway trypsin-like protease [62].

Furin protease is the most abundant host protease, which is expressed in a number of cells. Despite the role of furin to SARS-CoV-2 S activation still being ambiguous, it has been widely reported to be involved in other CoV infections as well. Furin is responsible for the cleavage of S protein at polybasic S1/S2 site (PRRAR), leading to two non-covalently linked distinct sub-units [63]. The second cleavage needed for SARS-CoV-2 activation is processed by TMPRSS2, leading to release of the fusion protein of S2 [64]. Cysteine protease, cathepsin L in lysosomes has also been reported to mediate activation of S protein without any involvement of furin-

mediated priming [65]. Therefore, targeting these proteases is another promising approach for treatment of COVID-19 infection.

3.4.1. Peptides targeting furin

Two synthetic inhibitors targeting furin i.e. decanoyl-Arg-Val-Lys-Arg-chloromethylketone (dec-RVKK-cmk) and hexa-D-arginine (D6R) demonstrated antiviral activity against many viruses. They inhibit cleavage mediated by furin and the subsequent fusion of viral glycoproteins, thus exerting antiviral activity against viruses responsible for human immunodeficiency syndrome, Chikungunya, hepatitis B, influenza A, Ebola, and papilloma [66]. Recently, the role of dec-RVKK-cmk in COVID-19 has been explored. It has been reported to suppress the concentration of processed S protein fragments, cytopathic effects, and viral production in SARS-CoV-2-infected VeroE6 cells, indicating furin inhibitor activity [67,68]. D6R, another furin inhibitor, is safer and therapeutically more effective than the other members of this class, but its effectiveness in SARS-CoV-2 infection is yet to be explored [69]. MI-1851 is another synthetic furin inhibitor that has been reported to suppress SARS-CoV-2 replication in Calu-3 human airway cells, leading to a 30- to 190-fold reduction in virus titers at a dose of 10 μM [70,71]. All these studies suggest that furin inhibitors may lead to a plausible approach for the treatment of SARS-CoV-2 infection.

3.4.2. Peptides targeting TMPRSS2

An androgen-responsive enzyme, TMPRSS2, which is encoded by TMPRSS2 gene, is involved in various processes such as digestion, tissue remodeling, blood coagulation, fertility, inflammatory responses, tumour cell invasion, apoptosis, and pain [72]. It is expressed widely in epithelial cells of gastrointestinal, respiratory, and urogenital tracts [73]. This enzyme plays a significant role in cleavage of SARS-CoV-2 S glycoprotein, thus mediating entry of virus into the cell and its activation [74]. Inhibition of TMPRSS2 by camostat mesylate and nafamostat mesylate led to a reduction of SARS-CoV-2 infection in human pulmonary tissue in an *ex vivo* evaluation [75].

A protein-based drug, aprotinin, popularly known as bovine pancreatic trypsin inhibitor, is a naturally occurring polypeptide (58 amino acids), which inhibits the activity of some serine proteases. Inhibition of SARS-CoV-2 replication by aprotinin, probably via inhibition of TMPRSS2 has also been reported. Aprotinin was reported to exert anti-SARS-CoV-2 effects in three models of cell culture [Caco2, Calu-3, and air-liquid interface (ALI) from primary bronchial epithelial cells] and against three SARS-CoV-2 strains (FFM1, FFM2, and FFM6). The IC₅₀ values of aprotinin that reduce cytopathogenic effects formation, SARS-CoV-2 S levels, and SARS-CoV-2-induced caspase 3/7 activation were in the range of 0.8–1.0 μM, 0.8–1.65 μM, and 0.3–0.7 μM, respectively, for all the three tested SARS-strains. A targeted proteomics assay indicated the suppression of N and M proteins expression in SARS-CoV-2-infected ALI cultures by aprotinin. SARS-CoV-2 S expression in SARS-CoV-2/FFM7-infected Calu-3 lung adenocarcinoma cells were also found to be suppressed by aprotinin

[76]. Bestle et al. also documented the suppression of SARS-CoV-2 multiplication in Calu-3 human airway cells by aprotinin treatment in a dose-dependent manner [70]. When aprotinin was added to broncho-alveolar lavage of COVID-19 patients, it led to inhibition of the viral entry into the host cells. The thrombo-inflammatory response was also found to decrease [77]. Aprotinin aerosol, approved for local treatment of influenza in Russia [78], may be particularly useful for the suppression of the viral replication cycle.

MI-432 and MI-1900 are synthetic peptidomimetic inhibitors of TMPRSS2 that prevent SARS-CoV-2 replication as well as cytopathogenic effects in Calu-3 cells in a dose-dependent manner, but with lower efficiency as compared to aprotinin. Treatment with MI-1900 were able to inhibit the multiplication of SARS-CoV-2 more effectively than MI-432. A combination of MI-1851 (furin inhibitor) with any of these i.e. aprotinin, MI-432 or MI-1900 was found to act synergistically leading to inhibition of both activation as well as replication of SARS-CoV-2. MI-432 and MI-1851 combination decreased the virus titers 10- to -32 folds in comparison with that by individual inhibitor. Low dose combination of MI-1900 and MI-1851 (10 μ M each) failed to exhibit any increase in the antiviral activity as compared to that by individual drug treatments at 10 μ M. There was, in fact, a fivefold decrease in viral titers in cells treated with 50 μ M each of MI-1900 and MI-1851 in comparison to cells exposed to 50 μ M of each inhibitor singly [70]. Thus, treatment modalities acting at different steps processing and activation of S protein by combining furin and TMPRSS2 inhibitors seems to be a plausible treatment strategy for this viral infection.

α 1-Antitrypsin, the most widely present serine protease inhibitor in plasma, is reported to hinder SARS-CoV-2 viral infection by inhibition of TMPRSS2, disintegrin, and metalloproteinase 17 (ADAM17) activity [79,80]. Reduced ADAM17 activity helps to control the release of inflammatory mediators, and consequently 'cytokine storm,' a characteristic feature of COVID-19 that enhances the probability of vascular hyperpermeability, multi-organ failure, as well as mortality [81]. Currently, α 1-antitrypsin along with other serine protease inhibitors is undergoing clinical trials for the treatment of COVID-19 (NCT04385836, NCT04547140, NCT04495101, EudraCT 2020-001391-15) [82-84].

3.4.3. Peptides targeting cathepsin L

Cathepsins are lysosomal enzymes that can be subdivided into three groups based on active site amino acid residue i.e. serine (cathepsins A and G), aspartyl (cathepsins D and E), and cysteine (cathepsins B, C/DPP1, F, H, K, L, O, S, W, V, and Z/X) [85]. Among them, cathepsin L is reported to be critical for entry of virus into the host cells by activating the viral S protein in the endosome or lysosome [86,87]. For optimum activity, cathepsins require acidic pH (between 4.5-5), such as found in the lysosome [88]. Inhibition of cathepsins L is expected to be a potential approach for the prevention of infection. A number of drugs already in the market for different indications such as clofazimine, rifampicin, saquinavir, chloroquine, astaxanthin, dexamethasone, clenbuterol, and heparin are reported to exhibit cathepsin inhibitory activity [89].

P9, a short peptide (30 amino acids) obtained from mouse β -defensin-4, showed potent and broad-spectrum activity against a number of respiratory viruses including influenza A virus, SARS-CoV and MERS-CoV. This is attributed to high-affinity binding to S protein along with the presence of abundant basic amino acids in its composition, which do not allow acidification in endosomes and thus, inhibit release of viral RNA [10,90]. In order to further improve the potency of P9, Zhao et al. developed P9R by substituting histidine and lysine that possess weak positive charge, with arginine at three positions i.e. 21, 23, and 28. This increases the net positive charge on the molecule. The developed peptide showed strong antiviral activity against pH-dependent viruses, including enveloped pandemic A(H1N1)pdm09 virus, avian influenza A(H7N9) virus, coronaviruses (SARS-CoV-2, MERS-CoV and SARS-CoV), and the non-enveloped rhinovirus. Mechanistic studies revealed that P9R shows potent antiviral activity via dual mechanisms i.e. direct binding to viruses and inhibition of virus-host endosomal acidification in contrast to PA1 (only binding to viruses) and P9RS (only inhibiting endosomal acidification) [91]. Recently, dual-functional cross-linking peptide 8P9R developed from P9 and P9R has been reported to inhibit virus entry mediated by TMPRSS2 surface pathway and endocytic pathway. Moreover, 8P9R is also demonstrated to enhance the antiviral efficacy of arbidol (a spike-ACE2 fusion inhibitor) at a strength lower than its normal IC₅₀ [92]. However, further clinical trials are required for the assessment of the therapeutic efficacy of this combination therapy in COVID patients.

Teicoplanin, a glycopeptide antibiotic, has been reported to be effective against infections by Gram-positive bacteria. It has also exhibited antiviral activity against SARS-CoV and MERS-CoV [93]. Teicoplanin inhibits the activation of viral S protein by cathepsin L, thereby interfering with the virus replication cycle. The dual antibacterial and antiviral potential of teicoplanin has been explored in the treatment of *S. aureus* superinfection in highly morbid patients suffering from severe SARS-CoV-2 pneumonia [94].

Table 1

4. Conclusions

Despite the extensive investigations on SARS-CoV-2, the prevention and treatment of COVID-19 still remain a challenge. Bioactive peptides, because of their satisfactory bioavailability, therapeutic efficacy, and negligible off-target activity seem to be strong candidates for mitigation of SARS-CoV-2 etiopathology at different steps of viral infection. Moreover, rapid and large-scale cost-effective synthesis (depending on peptide composition), high target specificity and better tolerability makes them an attractive alternative to small synthetic molecules. A number of peptide-based drugs have been rationally designed and few to them have been synthesized and tested in *in vitro* and *in vivo* studies. These peptides act on various processes from virus entry to replication in host such as binding to ACE2, fusion mechanism, and proteolytic activation of S protein by targeting furin, TMPRSS2, and cathepsins L. However, emergence of virus mutants and reduced antibodies titers after recovery,

Table 1. Characteristic features of anti-viral peptides effective against SARS-CoV-2.

Target protein	Target domain	Name	Sequence derived from	Sequence/IUPAC name	IC50	Reference	
Spike protein	RBD	Inhibitor 1	ACE2 (21–55)	IEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNIT	-	[35]	
		Inhibitor 2	ACE2 (21–88 and 349–357)	a. IEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITWWNVQ NMNAGDKWSAFLKEQSTLAQMYPLEIQALTYKQLQAL b. WDLGKGDFFR	-		
Spike protein	RBD	Inhibitor 3	ACE2 (21–105 and 323–362)	a. IEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITWWNVQ NMNAGDKWSAFLKEQSTLAQMYPLEIQALTYKQLQAL QQNGS	-	-	
				b. MTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFFRILMCT			
Spike protein	RBD	Inhibitor 4	ACE2 (21–95 and 335–400)	a. IEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITWWNVQ NMNAGDKWSAFLKEQSTLAQMYPLEIQALTYKQL	-	-	
				b. DPGNVQKAVCHPTAWDLGKGDFFRILMCTKVTMDDFL TAHHEMGIHQYDMAYAAQPFLLRNGANEFG			
Spike protein	RBD	AVP0671	Peptide library	TWLATRGLLRSPGRVYVYFSPASTWPVGIWTTGELVLCDAAL	-	[30]	
ACE2	RBD-binding site	AVP1244	Peptide library	GCASRCKAKACARRCKGWASAFRGRCYCKCFRC	-	[30]	
Spike protein	RBD	18aa	ACE (28–45)	FLDKFNHEAEDLFYQSSL	-	[22]	
		23aa	ACE (23–45)	EQAKTFLDKFNHEAEDLFYQSSL			
Spike protein	RBD	Peptide 1		EQEERIQQDKRKNEDQEDKRYQRYGRGKGHQ	-	[32]	
		Peptide 2		EQQRRIQEDQYRNDWEDEEYQRKGRGKGHQ			
		Peptide 3		EQQRRIQQDQSDNDREDKEYQKRGKGGKHNH			
		Peptide 4		QEEQIQEDQRRNDKEHKRQRYGRGCGKQN			
		Peptide 5		EQEERIQRDKRKNEDKRYQRYGRGKGHQ			
		Peptide 6		EQEERIQQDKRKNEDQEDKRYQRYGRGKGHQ			
		Peptide 7		EQEERIQQDKRKNEDQEDKRYQRYGRGKGHQ			
		Peptide 8		EQEERIQRDKRKNEDQEDKRYQRYGRGKGHQ			
		Peptide 9		EQEERIQQDKRKNEDQEDKRYQRYGRGKGHQ			
		Peptide 10		EQEERIQQDKRKNEDQEDKRYQRYGRGKGHQ			
		Peptide 11	ACE (22–53)		EEQAKTFLDKFNHEAEDLFYQSSGLGKGDFFR		-
		Peptide 12	ACE (21–43)		IEEQAKTFLDKFNHEAEDLFYQS		-
Spike protein	RBD	satpdb12488		PTTFMLKYDENGITITDAVDC	-	[33]	
Spike protein	RBD	satpdb14438		SNNTAIPTNFSISITTEVM	-	-	
		satpdb28899		RDVSDFTDSVRDPKTEILD			
		satpdb18674		QYGSFCTQLNRAALSGIAAEQ			
		satpdb18446		VLYNSTFFSTFKCYGVSAIK			
		[22–44]	ACE (22–44)		EEQAKTFLDKFNHEAEDLFYQSS		
Spike protein	RBD	[22–57]	ACE (22–57)	EEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEE	-	-	
		[22–44-g-351-357]	ACE (22–44 and 351–357)	EEQAKTFLDKFNHEAEDLFYQSS G LGKGDFFR			
		[351–357]	ACE (351–357)	LGKGDFFR			

(Continued)

Table 1. (Continued).

Target protein	Target domain	Name	Sequence derived from	Sequence/IUPAC name	IC50	Reference
Spike protein	RBD	pep1c	ACE (30–38)	DKFNHEAED	3 · 3 ± 0 · 8 mM	[36]
		pep1d	ACE (30–35)	DKFNHE	-	-
		pep1e	ACE (37–42)	EDLFYQ	-	-
		AHB1	ACE2	DEDLEELERLYRKAEEVAKEAKDASRRGDERAKEQMERAMRLFDQVFELAQLQEQTDGNRQKATHLDKAVKEAADELYQRVR	35 nM	[37]
		AHB2	ACE2	ELEEQVMHVLQDVSELAHELLHKL TGEELERAAYFNWWTMMLELIKSDDEREIREIEEEARRILLELEELARK	15 · 5 nM	-
		LCB1	de novo design	DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKKGGDERLLEEAERLLEEVER	23 · 54 pM	-
		LCB2	de novo design	SDDSDSVRYLLYMAELRYEQGNPEKAKILEMAEFAKRNNEELERLVREVKRRL	-	-
		LCB3	de novo design	NDDELHMLMTDLVYEALHFADKEEIKRVFQLFELADKAYKNNDRQKLEKVVVEELKELLERLLS	48 · 1 pM	-
		LCB4	de novo design	QREKRLKQLEMILLEAIERNDPVL MFDVAVEMLR LAEENNDERIERAKRILEEYE	-	-
		LCB5	de novo design	SLEELKEQVKELKELSPEMRRRIIEALRFLEEGNPAMAMMWLSDLVYQLGDPVIDLYMLYTKT	-	-
		LCB6	de novo design	DREQRLVRFVLRLASKFNLSPEIQILQFVLEELLELRGVSSEIEIRKQLEEVAKELG	-	-
		LCB7	de novo design	DDDIRLYIMAKLRLEQGNPEAEKVL EMARFLAERLGMEEELKEVRELLRMEELR	-	-
LCB8	de novo design	PIELLREAKEINDEF AISDALYLVNELLQRTGDPRLLEEVLYLIWRALKKEDPRLLDRAIELFER	-	[38]		
Spike protein	RBD	P1	ACE	STIEE QAKTF LDKFN HEAED LFYQS SL-NH ₂	-	-
		P1scr		AHLFS YLTTK EEQDN DAIFL QEFK ES-NH ₂	-	-
		Ppen		IEE QAKTF LDKFN HEAED LFYQS-NH ₂	-	-
		P2		SALEE QIKTF LDKFL HELED LLYQL SL-NH ₂	-	-
		P3		SALEE QIKTF LDKFL HELED LLYQL AL-NH ₂	-	-
		P4		Ac-SALEE QIKTF LDKFL HELED LLYQL AL-NH ₂	-	-
		P5		SALEE QIKTF LDKFL HELED LLYQL AL-NH ₂	-	-
		P6		SALEE QIKTF LDKFL HELED LLYQL ALAL-NH ₂	-	-
		P7		SALEE QYKTF LDKFM HELED LLYQL AL-NH ₂	>1 μM	-
		P8		SALEE QIKTF LDKFM HELED LLYQL AL-NH ₂	46 nM	-
		P9		SALEE QYKTF LDKFM HELED LLYQL SL-NH ₂	53 nM	-
		P10		SALEE QYKTF LDKFM HELED LLYQL AL-NH ₂	42 nM	-
Spike protein	RBD	Native (1)	ACE	IEEQAKTFLDKFNHEAEDLFYQS	-	-
		2		IR ₈ EQAKTF ₅ DKFNHEAEDLFYQS	-	-
		3		IEEQR ₈ KTFLDK ₃ NHEAEDLFYQS	-	-
		4		IEEQAR ₈ TFLDKF ₅ HEAEDLFYQS	-	-
		5		IEEQAKTFR ₈ DKFNHE ₅ EDLFYQS	-	-
		6		IEEQAKTFLDKR ₈ NHEAED ₅ FYQS	-	-
		7		IEEQAKTFLDKFR ₈ HEAED ₅ YQS	-	-
		8		IEEQAKTFLDKFNHER ₈ EDLFYQS ₅	-	-
		9		HEAEDLFYQS	-	-
		10		HE ₃ EDL ₅ YQS	-	-
		11		IEEQAKTFLDKFNHE	-	-
		12		IEEQR ₈ KTFLDK ₃ NHE	-	-

(Continued)

Table 1. (Continued).

Target protein	Target domain	Name	Sequence derived from	Sequence/IUPAC name	IC50	Reference	
$\alpha_5\beta_1$ integrin Spike S2	ACE2 and RBD-binding domain HR1	13	Fibronectin	TFLDKFNHEAEDL	-	[52]	
		14		TFR ₈ DKFNHES ₅ EDL	-		
		15		TS ₃ LDKS ₅ NHEAEDL	-		
		ATN-161		Ac-PHSCN-NH ₂	3 · 16 nM		
		EK1		HR2 (OC43)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL		286 · 7 nM
		EK1P		HR2 (OC43)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL-PEG4-Palm		69 · 2 nM
		EK1C		HR2 (OC43)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL-PEG4-Chol		48 · 1 nM
		EK1C1		HR2 (OC43)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL-PEG4-Chol		56 · 8 nM
		EK1C2		HR2 (OC43)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL-GSG-Chol		48 · 2 nM
		EK1C3		HR2 (OC43)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL-GSG-PEG4-Chol		10 · 6 nM
Spike S2	HR1	EK1C4	HR2 (1168-1203)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL-GSG-PEG4-Chol	1 · 3 nM	[55]	
		EK1C5	HR2 (1168-1203)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL-GSG-PEG4-Chol	3 · 1 nM		
		EK1C6	HR2 (1168-1203)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL-GSG-PEG4-Chol	3 · 9 nM		
		EK1C7	HR2 (1168-1203)	SLDQINVTFLDLE YEMMKLEEAIAIKKLEESYIDLKEL-GSG-PEG4-Chol	3 · 9 nM		
		2019-nCoV-HR2P	HR2 (1168-1203)	DISGINASVWNIQKIDRLNEVAKNLESIDLQEL	0 · 18 μ M		
		IPB01	HR2 (1162-1205)	ISGINASVWNIQKIDRLNEVAKNLESIDLQEL	0 · 022 μ M		
		IPB02	HR2 (1162-1205)	ISGINASVWNIQKIDRLNEVAKNLESIDLQELK (Chol)	0 · 025 μ M		
		IPB03	HR2 (1162-1205)	INASVWNIQKIDRLNEVAKNLESIDLQELGK (Chol)	0 · 015 μ M		
		IPB04	HR2 (1162-1205)	SVWNIQKIDRLNEVAKNLESIDLQELGK (Chol)	0 · 033 μ M		
		IPB05	HR2 (1162-1205)	IQKIDRLNEVAKNLESIDLQELGK (Chol)	>5 μ M		
Spike S2	HR1	IPB06	HR2 (1162-1205)	IDRLNEVAKNLESIDLQELGK (Chol)	>5 μ M	[56]	
		IPB07	HR2 (1162-1205)	IQKIDRLNEVAKNLESIDLQELGKYEYIK (Chol)	0 · 017 μ M		
		IPB08	HR2 (1162-1205)	ISGINASVWNIQKIDRLNEVAKNLESLIK (Chol)	4 · 66 μ M		
		IPB09	HR2 (1162-1205)	SVWNIQKIDRLNEVAKNLESLIK (Chol)	>5 μ M		
		SARS _{HRC}	HR2 (1168-1203)	DISGINASVWNIQKIDRLNEVAKNLESIDLQEL	-		
		SARS _{HRC} -chol	HR2 (1168-1203)	DISGINASVWNIQKIDRLNEVAKNLESIDLQEL-PEG ₂₄ -chol	-		
		SARS _{HRC} -PEG ₄ -chol	HR2 (1168-1203)	DISGINASVWNIQKIDRLNEVAKNLESIDLQEL-PEG ₄ -chol	-		
		SARS _{HRC} -PEG ₁₁ -chol	HR2 (1168-1203)	DISGINASVWNIQKIDRLNEVAKNLESIDLQEL-PEG ₁₁ -chol	-		
		SARS _{HRC} -PEG ₂₄ -chol	HR2 (1168-1203)	DISGINASVWNIQKIDRLNEVAKNLESIDLQEL-PEG ₂₄ -chol	-		
		[SARS _{HRC}]-PEG ₁₁ -chol	HR2 (1168-1203)	[DISGINASVWNIQKIDRLNEVAKNLESIDLQEL] ₂ -PEG ₁₁	-		
Spike S2	HR1	[SARS _{HRC}]-PEG ₄₂ -chol	HR2 (1164-1202)	[DISGINASVWNIQKIDRLNEVAKNLESIDLQEL-PEG ₄₂]-chol	-	[60]	
		Peptide #1	HR2 (1164-1202)	VWNIQKIDRLNEVAKNLESIDLQEL	1 μ M		
		Peptide #2	HR2 (1164-1202)	DISGINASVWNIQKIDRLNEVAKNLESIDLQEL	7 · 2 μ M		
		Peptide #3	HR2 (1164-1202)	DISGINASVWNIQKIDRLNEVAKNLESIELQEL	-		
		Peptide #4	HR2 (1164-1202)	DISGINASVWNIQKIDRLNEVAKNLESIKLQEL	-		
		Peptide #5	HR2 (1164-1202)	DISGINASVWNIQKIDRLNEVAKNLESIGLQEL	4 · 7 μ M		
Peptide #6	HR2 (1164-1202)	DISGINASVWNIQKIDRLNEVAKNLESIGLQEL	-				

(Continued)

Table 1. (Continued).

Target protein	Target domain	Name	Sequence derived from	Sequence/IUPAC name	IC50	Reference
		Peptide #7		DISGINASVWNIQKEIDRLNEVAKNLPESLIGLQEL	-	
		Peptide #8		DISGINASVWNIQKEIDRLDEVAKNLESIDLQEL	-	
		Peptide #9		DISGINASVWNIQKEIDRLGEVAKNLESIDLQEL	-	
		Peptide #10		DISGINASVENIQKEIDRLNEVAKNLESIDLQEL	8 · 9 µM	
		Peptide #11		DISGINASVWNIQKEIDRLNEVAKNLSGESLIEIQEL	-	
		Peptide #12		DISGINASVWNIQKEIDRLDEVAKNLPESLIEIQEL	-	
Spike S2	HR1	Peptide 1	HR2 (1163–1203)	GYHLMSPFQSPHGWVFLHVTW	-	[61]
		Peptide 2		GVFVSNQTHWFVTQRNFYE	-	
		Peptide 3		ISGINASVWNIQKEIDRLNEVAKNLESIDLQEL	0 · 72 µM	
		Peptide 4		IQKEIDRLNEVAKNLESIDLQELGK	-	
Furin	Catalytic domain	dec-RVKR-cmk	PCSK target motif	N-(1-(2-diamino-9-(4-aminobutyl)-6-(2-chloroacetyl)-1,20-dimino-12-isopropyl-8,11,14-trioxo-2,7,10,13,19-pentaazaicosan-15-yl)decanamide)	0 · 057 µM	[67]
	Probably catalytic domain	MI-1851	Peptidomimetic	N-(1-(4-carbamimidoyl(benzyl)amino)-4-(guanidinooxy)-1-oxobutan-2-yl)-2-(2-(4-(guanidinomethyl)phenyl)acetamido)-3-guanidinooxy)propanamide)	-	[70]
TMPR52	Catalytic site	Aprotinin	Natural polypeptide	RPDFC LEPPY TGPKC ARIR YFYNA KAGLC QTFVY GGCRA KRNNF KSAED CMRTC GGA	-	[70,76]
		MI-432	Synthetic peptidomimetic	3-(3-(4-(2-Aminoethyl)piperidin-1-yl)-2-(2',4'-dichloro-[1,1'-biphenyl])-3-sulfonamido)-3-oxopropyl)benzimidamide	-	[70]
		MI-1900	Synthetic peptidomimetic	4-(3-(3-Carbamidoylphenyl)-2-(2',4'-dimethoxy-[1,1'-biphenyl])-3-sulfonamido)propanoyl)-N-cyclohexylpiperazine-1-carboxamide	-	[70]
		α1-Antitrypsin	Natural glycoprotein	Polypeptide (394 amino acids with three carbohydrate side chains)	10–20 µM	[79,95]
Cathepsin L	Acidification	P9	Mouse-β-defensin -4 derived	NGAICWGPCPTAFRQIGNCGHFVKVRCCKIR	2 · 4 µg/mL	[91]
		P9R	Mouse-β-defensin -4 derived	NGAICWGPCPTAFRQIGNCGFRFRVRCRRIR	0 · 9 µg/mL	[91]
		PA1	Mouse-β-defensin -4 derived	NGAICWGPCPTAFRQIGNCGHFVKVRCCKIRDED	-	[91]
		P9RS	Mouse-β-defensin -4 derived	NGAHSWHPNETHFRQIHNSGRHVRSHRIR	-	[91]
		8P9R	Mouse-β-defensin -4 derived	NGAICWGPCPTAFRQIGNCGFRFRVRCRRIR	0 · 3 µg/mL	[92]

PCSK: Pro-protein convertase subtilisin/kexin

enhance the probability of COVID-19 infection in healthy individuals as well as patients already recovered from this infection. Antiviral peptides having multi-target approach is considered to have upper-hand over the drugs acting on single target.

5. Expert opinion

Since the emergence of COVID-19 pandemic, extensive studies have been conducted to understand its etiopathogenesis, viral protein structure and composition, and the viral-host interaction to develop effective direct-acting antiviral and host-directed agents. However, none of the treatment strategies developed hitherto has been able to exhibit the optimum clinical efficacy. This has been attributed to a number of factors like development of viral resistance, co-infections, and the emergence of new viral strains [96]. Development of novel antiviral agents that are effective either in combination with the currently used drugs or in a stand-alone manner is, therefore, the need of the hour.

A number of peptide-based therapeutics have been developed and evaluated for the management of different diseases. Along with more than 100 peptide-based drugs already in the global market, a number of peptide drugs are undergoing clinical trials [11,97]. Approval of peptide-based antiviral therapeutics for the Human immunodeficiency virus (Enfuvirtide and Maraviroc) [98], and Hepatitis virus (Myrcludex b) [99] indicates their potential in the treatment of life-threatening viral infections. The extensive investigation of SARS-CoV-2 structural proteins and their cellular targets resulted in rational designing and optimizing of AVP against this deadly virus. These peptides inhibit SARS-CoV-2 entry to the host cells virus by targeting its binding to ACE2 or integrins, fusion mechanism as well as activation of proteolytic enzymes (cathepsin L, TMPRSS2, or furin). It is pertinent to mention here that targeting the virus entry is the most pragmatic approach for combating the infection as it does not require cell-penetration property. Moreover, it minimizes the detrimental effects of infection because it is able to inhibit the viral replication cycle in its early stages. Notably, the S-protein-targeting peptides EK1C4, SARS_{HRC}-PEG₂₄-chol (monomeric peptide), [SARS_{HRC}-PEG₄]₂-chol (dimeric peptide), LCB1, and LCB3 exhibited promising antiviral potency against SARS-CoV-2 without affecting the function of the host protein. Therefore, application of these peptides via oral, intranasal, or inhalational routes directly at the site of replication may obviate the systemic administration and therefore, the need for optimization of their properties such as tissue-penetration, plasma stability, or half-life.

Despite their strong potential, the use of peptide-based drugs is limited by bottlenecks like instability, short half-life, less potency, inability to cross membrane barriers and poor bioavailability due to protease degradation. For the successful development of peptide-based drugs, these challenges need to be overcome. For this, a number of approaches have been utilized which include their conjugation with polymers like polyethylene glycol to enhance

chemical stability [100], replacement of redundant hydrophobic amino acids to charged/polar residues for improving oral bioavailability [101,102], linkage of peptides to the cell penetrating peptides to enhance their cell permeability [100], and encapsulation in nanoparticles for efficient delivery [103]. Although these modifications help to address the challenges of poor pharmacokinetic properties of non-modified peptides, the systematic designing and appropriate formulation development need to be focused for the successful commercialization of peptide-based therapeutics.

Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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ORCID

Pooja Rani  <http://orcid.org/0000-0003-1363-9295>
 Bhupinder Kapoor  <http://orcid.org/0000-0001-8057-0385>
 Monica Gulati  <http://orcid.org/0000-0002-3644-5162>
 Atanas G. Atanasov  <http://orcid.org/0000-0003-2545-0967>
 Qushmua Alzahrani  <http://orcid.org/0000-0001-5305-799X>
 Reena Gupta  <http://orcid.org/0000-0002-4913-1920>

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