Review

Exploring peptide studies related to SARS-CoV to accelerate the development of novel therapeutic and prophylactic solutions against COVID-19

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A B S T R A C T

Recent advances in peptide research revolutionized therapeutic discoveries for various infectious diseases. In view of the ongoing threat of the COVID-19 pandemic, there is an urgent need to develop potential therapeutic options. Intense and accomplishing research is being carried out to develop broad-spectrum vaccines and treatment options for corona viruses, due to the risk of recurrent infection by the existing strains or pandemic outbreaks by new mutant strains. Developing a novel medicine is costly and time consuming, which increases the value of repurposing existing therapies. Since, SARS-CoV-2 shares significant genomic homology with SARS-CoV, we have summarized various peptides identified against SARS-CoV using *in silico* and molecular studies and also the peptides effective against SARS-CoV-2. Dissecting the molecular mechanisms underlying viral infection could yield fundamental insights in the discovery of new antiviral agents, targeting viral proteins or host factors. We postulate that these peptides can serve as effective components for therapeutic options against SARS-CoV-2, supporting clinical scientists globally in selectively identifying and testing the therapeutic and prophylactic agents for COVID-19 treatment. In addition, we also summarized the latest updates on peptide therapeutics against SARS-CoV-2.

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Contents

Introduction

Human coronaviruses, causing respiratory tract infections in humans, belong to the family Coronaviridae in the order Nidovirales. They are enveloped viruses with a single stranded positive sense RNA genome. Of the seven known corona viruses causing human infection, 229E, NL63, OC43 and HKU1 are known to cause mild common cold like disease [1]. The other three, including Severe Acute Respiratory Syndrome (SARS-CoV), Middle East Respiratory Syndrome (MERS-CoV) and the novel corona virus (SARS-CoV-2) cause severe respiratory infections and deadly pneumonia outbreaks. The disease SARS, the first known epidemic caused by a coronavirus, appeared in 2002 in China [2]. In 2012, MERS-CoV, responsible for the acute human respiratory syndrome, was identified in Jeddah, Saudi Arabia [3]. SARS-CoV and SARS-CoV-2 are Beta coronaviruses [4], which show extremely high homology at the nucleotide level, upon comparative genomic analysis [150]. They also share homology at the proteomic level, in the major viral proteins such as spike protein [5], RdRp, 3CLpro etc. [6] which are all key drug targets.

The outbreak of SARS-CoV-2 and the disease COVID-19 is the major public health emergency of this century. There have been many studies published about its clinical presentation, pathophysiology and animal models [7,8]. Though we have been able to develop some successful vaccines, there is an urgent need to develop effective anti-viral regimens and strategies. SARS-CoV-2 is more infectious and transmissible than previous coronaviruses. As of June 15, 2021, over 177,057,900 cases and 3,828,400 deaths have been reported globally. COVID-19 began as an outbreak of a mysterious illness, characterized by pneumonia, came from Wuhan, in the Chinese province of Hubei. The cause of the illness was later attributed to a new coronavirus [9,10], initially called the 2019-novel coronavirus (2019-nCoV). It is currently designated as the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses [11], due to its close resemblance with SARS-CoV, which appeared in 2002.

Characteristic features of SARS-CoV and SARS-CoV-2 proteins and the importance in drug discovery

There are four major structural proteins in SARS-CoV-2 such as the spike (S) protein, the envelope (E) protein, the membrane (M) protein, and the nucleocapsid (N) protein [12]. In addition, there are 16 non-structural proteins (NSPs), which may also be considered for drug development [13]. S protein consists of two units namely, S1 and S2. S1 region of S protein consists of a receptor-binding domain (RBD) involved in the entry and binding to the host receptor protein ACE2 and S2 involves in viral fusion [14,15]. M and E proteins are primarily involved in virus assembly. N protein is a multivalent RNA-binding protein critical for viral replication and genome packaging. Mutational changes in the structural and non-structural proteins being highly significant in virulence and virus spread, should also be considered while designing therapeutics and vaccines [16].

SARS-CoV-2 harbors significant genetic mutations which are completely new to the innate immune system of the human population. Studies show that, this virus is capable of affecting a large section of the human populations. Ganesh et al. have compiled the findings which can be made use of while therapeutics are being designed, on comparing the epidemiology and pathobiology of SARS-CoV-2 with SARS and MERS [17]

In the fight against coronaviruses, scientists developed three strategies for developing new drugs [18]. The first strategy is to test existing broad-spectrum antivirals [19]. Though such inhibitors have certain advantages such as clarity in terms of their metabolic characteristics, dosages used, potential efficacy, and side effects, their spectrum is too broad. The second strategy is high-throughput screening of existing molecular databases to screen for molecules that may have a therapeutic effect on coronaviruses [20,21]. The third strategy is to develop new targeted drugs, directly based on the genomic information and pathological characteristics of different coronaviruses. Based on these observations, we accumulated research findings related to a specific class of therapeutic options, the peptide therapeutics and their molecular mechanisms. The compilation of publications described in this review provides an overview of the use of peptide therapeutics developed against SARS-CoV, which could be repurposed for SARS-CoV-2, as well as an update of the peptides already developed against SARS-CoV-2. However, wet lab and clinical studies are necessary to completely understand SARS-CoV-2 infection and to improve the development of these potential therapeutic options.

Literature search

We performed a literature search on the Web of Science (ISI), PubMed, Embase, Medline, and Scopus databases with keywords such as SARS-CoV, 2019-nCoV, SARS-CoV-2, peptides, vaccines, and related words, for example, peptide sequences, entry peptides, and fusion peptides. All peer reviewed research and review publications, and patent studies published in the English language from 2003 to 2021 were considered. The titles and abstracts of the publications were reviewed by two authors.

Included studies

Publications focusing on the peptides useful in terms of SARS-CoV and SARS-CoV-2 were included. Priority was given to publications related to three categories such as peptides identified

in silico, evaluated with wet lab techniques, patented or in clinical trials.

Excluded studies

Publications with only an abstract available were excluded from this review. Some publications were also excluded because their focus was SARS and SARS-CoV-2, without any development of therapy or peptides studies.

Data extraction and review

Data from the publications were extracted independently by two authors and entered in a table. The title and abstract of the publications which included domains such as mechanism of action, source and sequence of peptides, proposed use were reviewed. The Mendeley library was used to import citations from all the databases. Duplicate citations were removed.

Peptide therapeutics and their importance in SARS-CoV-2 research

Existing antiviral drugs have specific limitations, including insufficient activity, the development of resistance, safety and efficacy issues and side effects [22]. Small-molecule inhibitors are often less effective at disrupting extended protein binding interfaces [23]. There is an increased interest in peptides in the pharmaceutical industry. Peptide-based drugs are a superior choice compared to small molecule drugs due to their specificity, efficiency, low molecular weight, lower toxicity and low side effects. These are synthetically accessible and can be stabilized by chemical modifications. Peptides and peptidomimetics are widely accepted because of their applications in chemical biology [24]. Peptides inhibit protein-protein interactions and mimic a protein surface to effectively compete for binding [25]. Peptide drugs have several disadvantages, including instability, susceptibility to hydrolysis and oxidation, a short half-life, and low membrane permeability [26]. There are currently more than 400 peptide based drugs in clinical development, with over 60 approved for clinical use [27]. Literature also suggests possibilities for epitope-based vaccine screening, based on the protein motif regions, making use of peptide studies [28]. However, no effective peptide-based drugs are currently available against SARS-CoV-2.

Antiviral peptides (AVPs) could represent a potential class of antiviral agents against SARS-CoV-2 [29]. AVPs are of special interest due to their higher efficacy in inhibiting viral infection by targeting various stages of the viral life cycle [30]. AVPs inhibit viral replication at the stages of adsorption, viral penetration, endosomal escape, viral uncoating, viral genome replication and release of mature virions. [31]. Studies related to natural and biological sources and computational approaches, such as high-throughput screening, are supportive in identifying a promising AVP [32]. In our previous studies, we identified seven antimicrobial peptides (AMPs) that could serve as therapeutic components for MERS [151]. The various strategies and opportunities available for peptide therapeutics have been reviewed in detail by Fosgerau et al. [33]. The feasibility of developing effective therapeutic agents against MERS-CoV by repurposing existing and clinically approved antiviral peptide drugs has been proposed [34]. The existing antivirals and knowledge gained from the SARS and MERS outbreaks offer the easiest and fastest route to manage the current coronavirus epidemic [35,36].

The relevance of using peptides as therapeutic options for COVID-19 have been reviewed by several authors [37,38]. Virus-based and host-based drug repurposing perspectives for coronaviruses, in general and specifically for SARS-CoV-2, have been reviewed by Cherian et al. [39]. The significance of repurposing drugs for SARS-CoV-2 are highlighted by many authors [40–43]. As it is evident that drug repurposing is a valid alternative for finding therapeutic solutions for COVID-19, we emphasize the importance of using peptides as anti-SARS-CoV-2 components. We present a comprehensive review of the peptides with therapeutic and prophylactic potential in SARS-CoV that could be repurposed for SARS-CoV-2, in addition to the peptides identified as effective against SARS-CoV-2. As we discuss in Tables 1–3, it is evident that significant progress has been made in this sector, since the discovery of SARS-CoV.

Classification of peptide therapeutics for COVID-19 based on the mechanism of action

Potential anti-coronavirus therapies can be divided in two categories depending on the target, acting on the viral factors or on the host factors or immune system. Based on the mechanism of action, we classified potential peptide therapeutics to combat SARS-CoV-2 in three categories, including virus entry/fusion blockers, virus replication blockers and immune modulators.

Peptides affecting viral entry and fusion

The SARS-CoV spike (S) protein, a surface glycoprotein, mediates coronavirus entry into receptor-bearing cells by binding to the human angiotensin-converting enzyme 2 (hACE2) receptor [52]. Lu et al. performed bioinformatics analysis to identify peptides containing relevant B cell epitopes within the S protein of SARS-CoV which has the potential for application in the diagnosis [49]. RBD in the S1 subunit of the spike protein is a target for the development of virus attachment inhibitors, neutralizing antibodies, and vaccines [59]. Barh et al. have identified certain potential peptides to block the SARS-CoV-2 spike RBD [60]. They designed ACE2 sequence based peptides after identifying the key residues interacting with the spike RBD using bioinformatics approaches. The α-helices extracted from the protease domain of ACE2 were used to design peptide inhibitors against SARS-CoV-2 [61]. S1 of RBD directly binds to the peptidase domain (PD) of ACE2. A 23-mer peptide fragment of the ACE2 PD α1 helix has been synthesized and named as SBP1, which is capable of blocking the SARS-CoV-2 spike protein interacting with ACE2 [62].

The coronavirus spike proteins belong to class I fusion proteins, and are characterized by the existence of two heptad repeat (HR) regions, HR1 and HR2 [63]. As soon as the S protein binds to the ACE2 receptor on the target cell, the heptad repeats interact to form a six helical bundle which is a critical and conserved mechanism for viral fusion and entry, and is shared by all coronaviruses. Hence so many attempts have been made at discovering fusion inhibitors that could act as therapeutics addressing CoV infections. A look at the sequence alignment of the HR1 and HR2 regions between SARS-CoV and SARS-CoV-2 show 92.6% and 100% sequence homology respectively, suggesting that HR2 peptides, identified against SARS-CoV, may also inhibit SARS-CoV-2 [64]. HR2 based inhibitory peptides acting on SARS-CoV and MERS-CoV were reviewed by Tang et al. [65], creating possibilities for peptide or small molecule based anti-CoV fusogenics, which can arrest membrane fusion in SARS-CoV-2. Recently, a few HR2 sequence-based fusion inhibitors have been reported to potentially inhibit cell fusion in a dual-split protein (DSP) based cell fusion assay on SARS-CoV-2 [66]. Xia et al. developed a pan-coronavirus inhibitor EK1 that targets the HR1 domain and inhibits coronavirus infection [52]. They also generated a highly potent lipopeptide EK1C4, which acts as a pan-coronavirus fusion inhibitor, targeting the spike protein [67]. EK1C4 was developed by adding cholesterol residue to the EK1 peptide to improve

Table 1
List of peptide drugs identified as entry/fusion inhibitors and recommended for the treatment of SARS-CoV infection.

Peptide	Peptide sequence	Source (natural/synthetic)	Target/mechanism of action	Cell line/animal model/assay	$IC_{50\,Value}/EC_{50}$	Proposed use	Reference
P6	EEQAKTFLDKFNHEAEDLFYQSSGLGKGDFR	Synthetic - comprised of two discontinuous segments of ACE2 (a.a. 22–44 and 351–357) artificially linked together by glycine	Interaction with S glycoprotein- entry inhibitor	VeroE6 cells or HeLa cells	˜0.1 μM	Therapeutic candidate that would not only inhibit SARS-CoV infection but also prevent severe lethal lung failure	[44]
EPL5 (DP)	KKKKYRNIRRPG	Antisense peptide based combinatorial peptide library	Prevents virus entry	ELISA assay	~45 μg/mL	Lead compound of potent inhibitor of SARS-CoV	[45]
Peptide 9626	TLKPIFKLPLGINITNFR	SARS-CoV spike protein S residues 217–234	Inhibit S- mediated viral entry	293 T cells -Luciferase assay	~ 11 μM	For design of novel therapeutic treatments against SARS-CoV	[46]
HR1-a	YENQKQIANQFNKAISQIQESLTTTSTA	Synthetic SARS-CoV spike protein	Inhibit viral entry	Vero E3-Luciferase assay	~ 1.16 μM	SARS-CoV entry inhibitors	[47]
HR2	ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL	Synthetic SARS-CoV spike protein	Inhibit viral entry	Vero E3-Luciferase assay	~ 0.34 μM	SARS-CoV entry inhibitors	[47]
GST-removed HR2	DVDLGDISGINASVVNIQKEIDRLNEV-AKNLNESLIDLQELGKYEQYI	Synthetic SARS-CoV spike protein	Inhibit viral entry	Vero E3-Luciferase assay	~2.15 μM	SARS-CoV entry inhibitors	[47]
RBD-11b	YKYRYL	Synthetic-Receptor binding domain of SARS-CoV-Y438-L443	Block SARS-CoV viral entry & also inhibits proliferation of coronavirus NL63.	Vero E6 - RT PCR	High	Can be used as lead structure to design potential entry inhibitors against SARS-CoV and related viruses	[48]
P8	PSSKRFQPFQQFGRDVSDFT	Synthetic SARS-CoV spike protein S1, aa 540–559	Inhibit virus entry-inhibited syncytia formation	HEK293T-Syncytia assay	50%	Target for anti-SARS-CoV drug design	[49]
P9	CANLLLQYGSFCTQLNRALSGIA	Synthetic SARS-CoV spike protein S2, aa 731–753	Inhibit virus entry-inhibited syncytia formation	HEK293T-Syncytia assay	50%	Target for anti-SARS-CoV drug design	[49]
HR1-1	NGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTA	Derived from HR1 regions in the S2 protein, aa 889–926	Inhibit viral entry	293T- Luciferase assay	˜0.14 μM	Can serve as functional probes for dissecting the fusion mechanism of SARS-CoV to find potent inhibitor peptides for SARS-CoV.	[50]
HR2-18	IQKEIDRLNEVAKNLNESLIDLQELGK	Derived from HR2 regions in the S2 protein, aa 1161–1187	Inhibit viral entry	293T- Luciferase assay	˜1.19 μM	Can serve as functional probes for dissecting the fusion mechanism of SARS-CoV to find potent inhibitor peptides for SARS-CoV	[50]
HR2-8	ELDSFKEELDKYFKNHTSPDVDLGDISGINASVVN-IQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK	Synthetic-SARS-CoV spike protein	Competitive binding to the HR1 region of the SARS-CoV spike Protein, thus blocking membrane fusion	Vero cells-Immuno fluorescence assay	17 μM	Can be used as a lead for the further development of more effective SARS-CoV peptide inhibitors	[51]

Table 1 (*Continued*)

Peptide	Peptide sequence	Source (natural/synthetic)	Target/mechanism of action	Cell line/animal model/assay	IC$_{50\ Value}$/EC$_{50}$	Proposed use	Reference
EK1	SLDQINVTFLDLEYEMKKLEEAIKKLEESYIDLKEL	Synthetic- modified OC43-HR2P peptide	Binds to HR1s of different HCoVs and form stable complexes, thereby blocking S protein mediated fusion.	Cell lines & Mouse model	0.19 to 0.62 μM	Anti-viral agent against multiple HCoVs	[52]
SARS$_{WW-I}$	MWKTPTLKYFGGFNFSQIL	Synthetic-S2 subunit of SARS-CoV N-terminal aa 770–788	Fusion inhibition	Vero E6 cells -plaque assay	–	Potential therapeutics	[53]
SARSWW-II	ATAGWTFGAGAALQIPFAMQMAY	Synthetic- S2 subunit of SARS-CoV N-terminal, 864–886	Fusion inhibition	Vero E6 cells plaque assay	–	Potential therapeutics	[53]
SARSWW-III	GYHLMSFPQAAPHGVVFLHVTW	Synthetic- S2 subunit of SARS-CoV Loop aa 1028–1049	Fusion inhibition	Vero E6 cells plaque assay	~2 μM	Potential therapeutics	[53]
SARSWW-IV	GVFVFNGTSWFITQRNFFS	Synthetic-S2 subunit of SARS-CoV Loop aa 1075–1093	Fusion inhibition	Vero E6 cells plaque assay	~2–4 μM	Potential therapeutics	[53]
SARSWW-Va	NEVAKNLNESLIDLQELGKYEQYIKWPWYVW	Synthetic-S2 subunit of SARS-CoV HR2-Aromatic aa 1169–1199	Fusion inhibition	Vero E6 cells plaque assay	–	Potential therapeutics	[53]
SARSWW-Vb	AACEVAKNLNESLIDLQELGKYEQYIKW	Synthetic-S2 subunit of SARS-CoV HR2-ΔAromaticaa 1169–1194	Fusion inhibition	Vero E6 cells plaque assay	–	Potential therapeutics	[53]
P6	GINASVVNIQKEIDRLNEVAKNL	SARS-CoV spike protein, HR2 peptide	Block the fusion of SARS-CoV	HeLa cells-cell fusion inhibition assay	2.28 ± 0.81 μM	Potential lead peptide for future drug development	[54]
N46	QKQIANQFNKAISQIQESLTTTSTALGK-LQDVVNQNAQALNTLVKQ	SARS-CoV spike protein, HR1 peptide	Block the fusion of SARS-CoV	HeLa cells-cell fusion inhibition assay	3.97 ± 1.40 μM	Strategy to achieve promising inhibition by HR1 peptide for class I envelope viruses	[54]
N46eg	QNQSANQFQKEISQINEVLTTTNTSLGKLQD-DVNQNNQSLNTLQKE	SARS-CoV spike protein- HR1 peptide (mutated version of N46)	Block the fusion of SARS-cov	HeLa cells-cell fusion inhibition assay	5.07 μM	Strategy to achieve promising inhibition by HR1 peptide for class I envelope viruses	[54]

Table 2
List of peptide drugs identified as replication/maturation inhibitors and recommended for the treatment of SARS-CoV infection.

Peptide	Peptide sequence	Source (natural/synthetic)	Target/mechanism of action	Cell line/animal model/assay	$IC_{50\,Value}/EC_{50}$	Proposed use	Reference
K12	GGASCCLYCRCH	Synthetic-nsp10 of SARS-CoV, aa 69–80	Replication- inhibiting 2′-O-methyltransferase activity of SARS-CoV nsp16/10 complex	*E. coli*-MTase Activity Assay	169 μM	May be further developed into potential specific antivirals against coronavirus infection	[148]
K29	FGGASCCLYCRCHIDHPNPKGFCDLKGKY	Synthetic-nsp10 of SARS-CoV, aa 68–96	Replication- inhibiting 2′-O-methyltransferase activity of SARS-CoV nsp16/10 complex	*E. coli* -MTase Activity Assay	160 μM	May be further developed into potential specific antivirals against coronavirus infection	[148]
Mucroporin-M1	LFRLIKSLIKRLVSAFK	Synthetic-Scorpion venom	Replication- direct interaction with the virus envelope, thereby decreasing the infectivity of virus	MDCK cells-Plaque assay	14.46 μg/mL	For developing broad-spectrum antiviral agents against RNA viruses	[149]
Nitrile-based peptidomimetic inhibitor	Cbz-AVLQ-CN	Synthetic-Cbz: carboxybenzyl, CN-Nitrile	Replication-inhibitory effect on 3CL^{pro} from a broad range of coronaviruses	Protease activity assay	4.6 ± 0.2 μM	For drug designing against various coronaviruses	[147]
Peptide-aldehyde	Ac-Val-Leu-NHCH(CH$_2$CH$_2$CON(CH$_3$)$_2$)-CHO	Synthetic	Virus maturation	Protease activity assay	~6 mM	A promising reversible SARS 3CL protease inhibitor	[145]
		Modification: Ac: Acetyl, -CHO: Aldehyde moiety					
Peptide-aldehyde	Ac-Ala-Val-Leu-NHCH(CH$_2$CH$_2$CON(CH$_3$)$_2$)-CHO	Synthetic	Virus maturation	Protease activity assay	155 μM	A promising reversible SARS 3CL protease inhibitor	[145]
		Modification: Ac: Acetyl, -CHO: Aldehyde moiety					
Peptide-aldehyde	Ac-Ser-Ala-Val-Leu-NHCH(CH$_2$CH$_2$CON(CH$_3$)$_2$)-CHO	Synthetic	Virus maturation	-protease activity assay	37 μM	A promising reversible SARS 3CL protease inhibitor	[145]
		Modification: Ac: Acetyl, -CHO: Aldehyde moiety					
Peptide-aldehyde	Ac-Thr-Ser-Ala-Val-Leu-NHCH(CH$_2$CH$_2$CON(CH$_3$)$_2$)-CHO	Synthetic	Virus maturation	-protease activity assay	26 μM	A promising reversible SARS 3CL protease inhibitor	[145]
		Modification: Ac: Acetyl, -CHO: Aldehyde moiety					
Proprotein convertase inhibitor peptide	dec-RVKR-cmk	Synthetic	Virus release- blocks a PC-mediated processing step required for extensive virus spread and ensuing CPE	Vero E6- Immuno precipitation assay	–	PC-inhibitor could be used along with vaccination and/or in conjunction with interferon treatment or with the novel compounds targeting the SARS-CoV proteins	[146]
		Modification: dec: decanoyl, cmk: chloro methyl ketone					

Table 3
List of peptide drugs identified as immunological modulators and recommended for the treatment of SARS-CoV infection.

Peptide	Peptide sequence	Source (natural/synthetic)	Target/mechanism of action	Cell line/animal model/assay	Proposed use	Reference
P15	KLPDDFMGCV	S411–420- derived from S protein	Binding mechanism with HLA molecules. Elicited specific IFN-γ-producing CTLs from SARS-CoV S gene-based DNA-vaccinated Tg mice	Transgenic mice-ELISPOT assay and tetramer staining	SARS associated corona virus-specific CTL epitope. Potential target for characterization of virus control mechanisms and evaluation of candidate SARS vaccines	[55]
M1-31	MADNGTITVEELKQLLEQWNLVIGFLFLAWI	Synthetic-derived from the N-terminal epitope	Epitope and binding mechanism of M protein- induce potent antibody responses	BALB/c mice and New Zealand White rabbits-ELISA	For developing SARS diagnostics and vaccines	[56]
M132-161	LMESELVIGAVIIRGHLRMAGHPLGRCDIK	Synthetic-derived from the C-terminal epitope	Epitope and binding mechanism of M protein- induce potent antibody responses	BALB/c mice and New Zealand White rabbits-ELISA	For developing SARS diagnostics and vaccines	[56]
S5	LPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYG	Synthetic- residues 788-820	Block viral invasion by eliciting an immune response specific to SARS-CoV S protein	rabbits and monkeys-ELISA	Development of synthetic peptide SARS vaccine	[57]
S6	ASANLAATKMSECVLGQSKRVDFCGKGYH	Synthetic- residues 1002-1030	Block viral invasion by eliciting an immune response specific to SARS-CoV S protein	rabbits and monkeys-ELISA	Development of synthetic peptide SARS vaccine	[57]
S471-503	ALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFEL	Synthetic- SARS-CoV receptor binding domain (RBD)	Block S1-RBD: ACE-2 binding and thus prevent entry of SARS-CoV	Vero cells-Plaque assay	Immune antigen for the development of peptide-based vaccine or for further drug evaluation against the SARS-CoV virus cell fusion	[58]

the inhibitory activity against SARS-CoV-2 [68]. EKIC4 was found to have remarkable inhibitory activity against membrane fusion mediated by SARS-CoV-2 spike protein and also against entry of pseudotyped human coronaviruses including SARS-CoV and MERS-CoV. EK1C4 potently inhibits SARS-CoV-2 replication, with an EC_{50} value of 36.5 nM, which is more potent than the EK1 peptide with an EC_{50} value of 2.47 μM [66]. Another HR2 sequence-based lipopeptide fusion inhibitor, termed IPB02, showed highly potent activities in inhibiting SARS-CoV-2 spike protein-mediated cell-cell fusion and pseudovirus transduction [9,10].

EK1C4 has many advantages over other peptide drugs, as it can be inhaled as an aerosol formulation, reducing the viral load in the lung, and alleviating the pulmonary inflammatory reaction [69]. The intranasal application of EK1C4 indicates that it could be used as an effective therapeutic against infection by SARS-CoV-2 [67]. The sequence of its target, the HR1 domain in the S2 subunit of the S protein, is highly conserved, which means EK1C4 cannot easily induce drug-resistant mutations.

Fig. 1 displays a graphical representation of the mechanism of entry and fusion of SARS-CoV-2, highlighting peptide therapeutics.

Peptides inhibiting viral replication and release

Polyprotein processing

The coronavirus alters the host transcription machinery in its favor to synthesize its proteins once the virus enters the host cell. The SARS-CoV positive stranded RNA genome encodes two open reading frames (ORF), ORF1a and ORF1b, which on translation produces two polyproteins (pp), pp1a and pp1ab. These polyproteins are proteolytically cleaved to generate 16 non-structural proteins, required for viral replication [70], which include two proteases, 3-chymotrypsin-like protease (3CL^{pro}/Nsp5) and papain-like protease (PL^{pro}/Nsp3) that mediate the proteolytic processing of the

polyprotein. The 3CL^{pro} of SARS-CoV-2 has a 99.02% sequence identity with the SARS-CoV [71]. As the active sites of 3CL^{pro} are highly conserved in multiple coronaviruses [72], the possibility of designing peptide inhibitors targeting the substrate binding pocket of SARS-CoV-2 3CL^{pro} has been explored and two peptidomimetic aldehydes 11a and 11b were identified. Both exhibited excellent inhibitory activity and potent anti-SARS-CoV-2 infection activity [73] (Fig. 2). The another protease, PLPro (Papain like protease) involved in the proteolytic processing of the replicase polyprotein, which also plays a significant role in antagonizing the host's innate immunity, is an attractive antiviral drug target as well [74]. Target based virtual ligand screening against the ZINC drug database identified Glutathione, currently used for the treatment of liver diseases [75], as effective against PLPro with a docking score of 20.

Fig. 2 displays a graphical representation of the mechanism of replication and maturation of SARS-CoV-2, highlighting peptide therapeutics.

Viral replication and transcription

Coronavirus genome replication and transcription involve coordinated processes of both continuous and discontinuous RNA synthesis mediated by the viral replicase, a huge protein complex encoded by the 20-kb replicase gene [76]. The replicase-transcriptase proteins, together with other viral proteins and possibly cellular proteins, assemble into membrane-bound replication-transcription complexes (RTC). The central component of coronoviral replication/transcription is Nsp12 (RNA dependent RNA polymerase/RdRp) [77], which is the primary target for remdesivir, a nucleoside analog antiviral inhibitor, currently used for SARS-CoV-2 [78,79]. Remdesivir exhibits a broad spectrum of antiviral activity against many viruses, including Ebola, Nipah and the respiratory syncytial virus (RSV), SARS-CoV and MERS-CoV [80]. This molecule is assumed to affect the viral RNA synthesis by
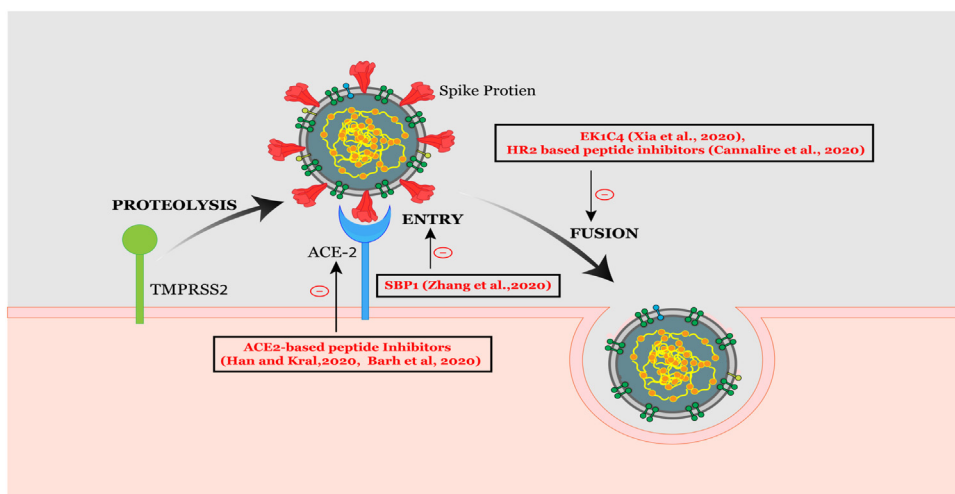
Fig. 1. Graphical representation of the mechanism of entry and fusion of SARS-CoV-2. Coronavirus entry and fusion are represented along with the identified peptide compounds.
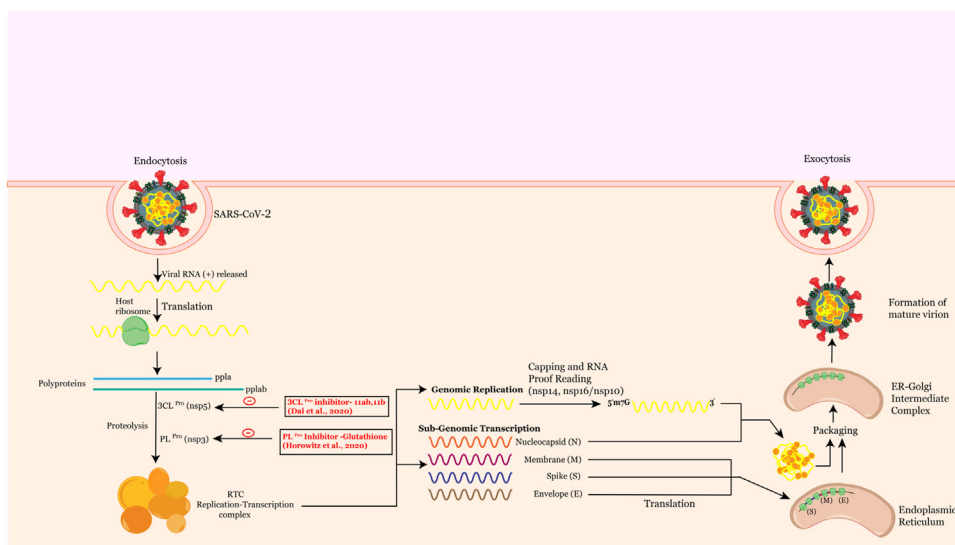

Fig. 2. Shows a graphical representation of the mechanism of replication and maturation of SARS-CoV-2 highlighting peptide therapeutics.

a delayed chain termination process in the three coronaviruses (MERS-CoV, SARS-CoV and SARS-CoV-2). Remdesivir is currently being tested in the clinic in many countries and approved for emergency treatment of patients with COVID-19 [81]. A recent study indicated that remdesivir was effective in lowering the respiratory tract infection of COVID-19 adult patients in a double blind, randomized placebo controlled trial [82].

The high homology exhibited by the SARS-CoV-2 Nsp12 with SARS-CoV, indicating a conserved mechanism of action confirmed by cryo-EM structural studies [83] should be noted. The structure of Nsp12 contains a right hand RdRp domain and a nidovirus-unique N-terminal extension domain that adopts a nidovirus RdRp-associated nucleotidyl transferase architecture. The synthesis of viral RNA by Nsp12 possibly occurs with the assistance of Nsp7 and Nsp8 as cofactors [79]. Nsp8 synthesizes a primer of 6 nucleotide lengths for RdRp RNA synthesis, and the Nsp7-Nsp8 complex increases the binding of Nsp12 to RNA and enhances the RdRp enzymatic activity of Nsp12. The interactions of these non-structural proteins also offers great therapeutic possibilities, specifically investigating the Nsp12-Nsp8 interaction [84].

Nsp9, highly conserved in beta coronaviruses, is assumed to mediate viral replication, overall virulence, and viral genomic RNA reproduction. Selinexor, a synthetic peptide, an inhibitor of CRM1 (Chromosome region maintenance protein), also known as exportin 1 (XPO1) involved in nuclear export, could be effective in inhibiting the interactions between Nsp9 and nuclear pore proteins [85,86]. Low oral doses of this peptide is being tested in ongoing clinical trials against COVID-19 [87]. Ternatin 4-N-methylated cyclic hexapeptide, derived from mushrooms, inhibits adipogenesis at low concentrations ($EC_{50} = 20$ nm) [88] and becomes cytotoxic at a 10-fold higher concentration [89] and known to kill cancer cells [90], also inhibits the translation machinery. Since ternatin 4 can affect the host translation process, it is included as a preclinical compound that could be repurposed against SARS-CoV-2.

Epigenetic changes that regulate chromatin rearrangement have been implicated in the pathophysiology of viral infection [91]. Several studies have highlighted the importance of epigenetic regulators in SARS-CoV studies [92,93]. Recently, DNA methylation analysis of ACE2 expression has been shown to throw light on the epidemiology of SARS-CoV-2 infection [94]. ACE2 expression is also found to be regulated by Bromodomain and extratermi-

nal (BET) proteins which are epigenetic readers [95]. Gordon etal has identified an interaction of BRD4 with the E protein of SARS-CoV-2, which highlights possibilities of exploring peptide based bromodomain inhibitors as SARS-CoV-2 therapeutics. In the same SARS-CoV-2 interactome study, HDAC2 was found to have a high confidence interaction with the viral protein Nsp5 [85]. Nsp5 is thought to inhibit HDAC2 transport into the nucleus, thereby enhancing inflammation and interferon response. We suggest looking for peptide inhibitors of Nsp5, which seems to be the switch to start inflammation in COVID-19. Also, HDAC inhibitors have been proposed to serve as preventive drug against COVID-19 [96]. These suggest epigenetic interventions such as epidrugs could serve as promising therapeutic or prophylactic options [97]. The approach used for the discovery of Apicidin, a natural tetrapeptide inhibitor of HDAC via a cyclic $\alpha_3\beta$-tetrapeptide scaffold [98] is interesting and can be used to identify more anti-coronaviral peptide inhibitors.

RNA processing

Besides RNA-dependent RNA polymerase, RNA helicase and protease activities, common to RNA viruses, the coronavirus replicase also employs a variety of RNA processing enzymes that are not (or extremely rarely) found in other RNA viruses [99]. Several enzymatic activities are required for the formation of a cap structure in the viral RNA, followed by Ribose 2′-O-methylation of the cap that provides a mechanism for viruses to escape host immune recognition [100]. RNA capping in CoVs involves the activities of several nonstructural proteins (nsps): Nsp13, a bifunctional RNA/NTP triphosphatase (TPase) and helicase; Nsp14, a bifunctional 3′→5′ mismatch exonuclease, and mRNA cap guanine-N7 methyltransferase, Nsp16, a cap ribose 2′-O methyltransferase, and an elusive guanylyl transferase [101]. Of the enzymes involved in capping, the N7-Methyltransferase of Nsp14 is an attractive antiviral target as this domain exhibits a non-canonical methyltransferase fold, different from cellular methyltransferases [102]. A peptide inhibitor targeting the 2′-O-methyltransferase efficiently suppresses coronavirus replication, suggesting that the 2′-O-methyltransferases of coronaviruses are ideal and potential antiviral drug targets [103].

Virus maturation

The RTC uses the genome to synthesize progeny genomes and a series of subgenomic mRNAs, using negative-stranded intermediates. The structural proteins S, M, E and N are generated by the translation of these mRNAs, which participate in the formation of mature virions. The E (envelope) protein of SARS-CoV-2 plays key roles in various phases of the viral life cycle, such as envelope formation, pathogenesis, budding and assembly. Unlike the other major structural proteins, N is the only protein that functions primarily to bind to the CoV RNA genome, constituting the nucleocapsid to make new virons [104]. The N protein of SARS-CoV-2 is highly homologous to that of SARS-CoV [105]. A recent study by Cascarina et al. reports that the N protein of SARS-CoV-2 is capable of regulating the biomolecular interaction with RNA and key host cell proteins [106]. The N protein also binds to the stress granule proteins, which induce the host's immune response [107]. The manipulation of stress granules could be promising in inhibiting the replication of coronaviruses [108,109]. N protein is also shown to interact with the Ras-GTPase activating SH3-domain-binding-protein 1 (G3BP1) [85], which inhibits RNA virus replication [110].

Peptides that act as immune modulators

After the virus gains access to the target cell, the host immune system recognizes the whole virus or its surface epitopes, and elicits an innate or adaptive immune response. The SARS-CoV-2 virus infects through the naso-oral route, followed by infection in cells expressing the ACE2 receptor in the lung. These viruses dampen the anti-viral IFN responses by evading the innate immune cells, resulting in unrestrained virus replication. At the same time, the activation of macrophages and other immune cells, cause the production of cytokines.

Innate immune response

When SARS-CoV-2 infects the upper and lower respiratory tract, it can cause a mild or highly acute respiratory syndrome with consequent release of pro-inflammatory cytokines, such as the interleukins [111]. A case report shows that the compstatin-based complement C3 peptidic inhibitor AMY-101 was successful in treating a patient with severe ARDS due to COVID-19 pneumonia [112]. Anakinra, an interleukin-1 receptor antagonist, currently used for the treatment of rheumatoid arthritis [113], is beneficial in association with remdesivir in controlling the pathological immune response to SARS-CoV-2 [114].

NK cells lyse viral infected cells and hence play an important role in anti-viral immunity. A reduced number of NK cells have been found in the peripheral blood of COVID-19 patients [115]. In a murine model of SARS-CoV infection, increased production of chemokines was correlated with increased migration of NK cells into the lungs [116]. The high expression of chemokines in the lungs of COVID-19 patients also seem to facilitate NK cell migration from the peripheral blood to the lungs [117].A transcriptomic signature analysis also revealed an infiltration of the NK cells into lungs [118], possibly mediated by the CXCR3 pathway [119]. Since chemokines are vital in attracting NK cells to clear the virus, peptide inhibitors blocking such chemokine receptors are promising drugs for SARS-CoV-2. Moreover, peptide antagonism is an established mechanism for NK cell activation, and there is a major scope for more peptide therapeutics in this aspect [120].

Adaptive immune response

Adaptive immune response and immunological memory are crucial for the success of a vaccine. An effective vaccine should induce a specific immune response against the specific antigen by selectively stimulating antigen-specific B-cells or CTLs or T helper cells. In response to a SARS-CoV-2 infection, antibodies, CD4+ T cells and CD8+ T cells are synthesized in the body. During viral infection, T cells recognize the viral antigens presented by the MHC class I [MHC; Human Leukocyte Antigen (HLA) in humans], which promotes the cytokine release [121]. In other cases, MHC class II also present SARS-CoV peptides to CD4+ T cells. This should be the basis of designing effective vaccines, to stimulate immune responses against the target pathogen. Kiyotani et al. have identified 781 HLA-Class I and 418 Class II peptide epitopes for SARS-CoV-2 through the computational screening of potential epitopes present on the HLA class I molecules, frequently observed in the Japanese population [122]. Immunoinformatic tools were used to analyse the proteome of SARS-CoV-2 and an epitope with HLA allele binding affinity, ITLCFTLKR has been identified as a vaccine candidate [123] (Fig. 3). Similarly, peptides binding to the MHC class I and MHC class II were promising epitope based peptide vaccine candidates, when envelope protein was used as an immunogenic target [124]. A total of 10 epitopes based on the structural proteins designed by molecular docking analysis against MHC-I were identified which have the potential to be developed as peptide vaccine against SARS-CoV-2 [125]. A computational analysis identified the sequence KRSFIEDLLFNKV as particularly conserved in many coronaviruses, ranging from the common cold coronavirus to SARS-CoV-2. This sequence motif can form the basis for proposing a specific synthetic vaccine epitope and peptidomimetic agent [126].

Epitopes are immunogenic components, which can also be used in vaccine discovery studies [127]. Since discovering epitopes by traditional experimental methods is often expensive and time-consuming, in silico approaches become the preferred strategy.
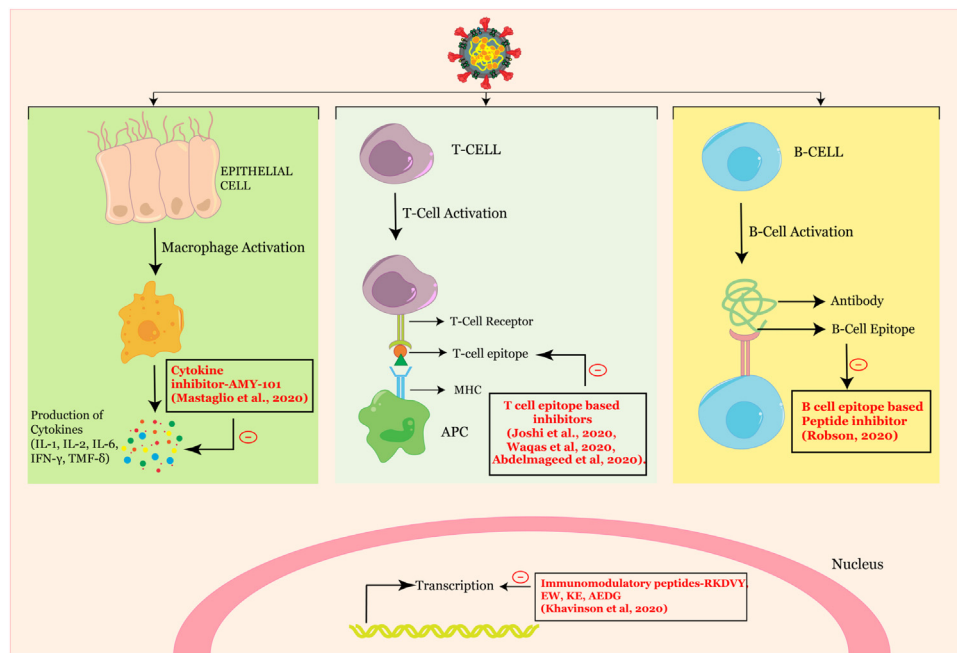
Fig. 3. Graphical representation of the mechanism of immune modulation of SARS-CoV-2 highlighting peptide therapeutics.

Thus, it can be rightly said that computational immunoinformatics is the basis of epitope vaccine development [128]. Many epitope studies have been conducted with SARS-CoV proteins [129,130]. The Immune Epitope Database and Analysis Resource (IEDB) that catalogs available data related to other coronaviruses, can be used effectively to predict and identify candidate targets for immune responses to SARS-CoV-2 [131]. A study carried out by comparing the SARS-CoV-2 and the human proteomes identified certain epitopes embedded in the S protein that could serve as possible vaccine candidates [132]. In another study, two immunodominant linear B-cell epitopes on the S glycoprotein of SARS-CoV-2 that could elicit neutralizing antibodies, have been identified [133]. Nucleocapsid proteins within SARS-CoV-2 contain multiple Class I epitopes with predicted HLA restrictions, consistent with a broad population coverage, which can be used to develop a CTL peptide vaccine against COVID-19 [134]. A multiepitope vaccine construct to combat SARS-CoV-2 infection was made from spike S protein and orf1ab polyprotein against B and T lymphocytes [135]. Recently, a comprehensive reverse-vaccinology workflow that can be used to identify and shortlist potential T- and B-cell epitopes has also been proposed [136].

Possibilities of harnessing Omics approaches to combat COVID-19

Various disciplines, such as genomics and proteomics, have a major role in combating COVID-19 [137]. It is important to note that intensive research with the SARS-CoV-2 virus is ongoing to understand its pathogenesis and mechanism of virulence. Genomics is employed in identifying genomic variants of SARS-CoV-2, which is highly important, especially when the penetrance of host genetic variants is high [138]. The information related to the genetic variants of SARS-CoV-2 is a decision making tool, in terms of patient care, prioritization for therapies, clinical research, and public health containment practices. In addition, genomics studies are significant in delineating the host factors associated with the variability in susceptibility, infectivity, and also the severity of the disease [138,139]. The progress of these Omics studies can be extrapolated to applications in many fields, including immunology. This supports a deeper

level of knowledge and the provision of substantial data used in computational studies, namely immunoinformatics [140].

Proteomics facilitates the deciphering of the knowledge related to structure–function relationships of protein targets. This information would provide novel insights into drug development to prevent infection and as well as to control transmission [141]. Several molecular docking, dynamics and pharmacology approaches have been investigated based on the binding affinity of drug components against COVID-19 [142–144]. Proteomics based directed drug repurposing might be a rapid response solution for the COVID-19 pandemic, based on the experience with SARS-CoV, MERS-CoV, or other coronaviruses. Although many countries are vaccinating their citizens, there is no effective drug to treat COVID-19. We urge the research community to exploit the omics approaches to rapidly identify new potential drug molecules.

Conclusion

The development of peptide therapeutics is considered risky, due to the poor performance in ADME (absorption, distribution, metabolism, elimination) properties. However, pharmaceutical companies are considering this strategy due to its many advantages. The data presented in this review reinforces the need for further studies and new therapeutic proposals to combat the threat of COVID-19. The knowledge achieved in the treatment of SARS-CoV may be used to enhance this process. It should be noted that the accuracy and coverage of this review will be short-lived, due to the rapid advances being made in this area; however, this review will be significant in accelerating the development of novel solutions for COVID-19.

Competing interests

None declared.

Ethical approval

Not required.

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