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Antiviral peptides against *Coronaviridae* family: A review

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ABSTRACT

The *Coronaviridae* family comprises large enveloped single-stranded RNA viruses. The known human-infecting coronaviruses; severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), novel SARS-CoV-2, human coronavirus (HCoV)-NL63, HCoV-229E, HCoV-OC43 and HKU1 cause mild to severe respiratory infections. The viral diseases induced by mammalian and avian viruses from *Coronaviridae* family pose significant economic and public health burdens. Due to increasing reports of viral resistance, co-infections and the emergence of viral epidemics such as COVID-19, available antiviral drugs show low or no efficacy, and the production of new treatments or vaccines are also challenging. Therefore, demand for the development of novel antivirals has considerably increased. In recent years, antiviral peptides have generated increasing interest as they are from natural and computational sources, are highly specific and effective, and possess the broad-spectrum activity with minimum side effects. Here, we have made an effort to compile and review the antiviral peptides with activity against *Coronaviridae* family viruses. They were divided into different categories according to their action mechanisms, including binding/attachment inhibitors, fusion and entry inhibitors, viral enzyme inhibitors, replication inhibitors and the peptides with direct and indirect effects on the viruses. Reported studies suggest optimism with regard to the design and production of therapeutically promising antiviral drugs. This review aims to summarize data relating to antiviral peptides particularly with respect to their applicability for development as novel treatments.

1. Introduction

The *Coronaviridae* family comprises large enveloped single-stranded RNA viruses with genomes ranging from 25 to 32 kb. The International Committee on Taxonomy of Viruses (ICTV) divided this family into *Orthocoronavirinae* and *Letovirinae* sub-families and according to phylogenetic relationships *Orthocoronavirinae* consists of four genera including *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus* [1,2]. The alphacoronaviruses and betacoronaviruses infect mammals and usually cause respiratory disease in humans and gastroenteritis in animals. The gammacoronaviruses and deltacoronaviruses infect avian species; however, some of them have also been found in mammalian hosts [3,4].

The known human-infecting coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and novel SARS-CoV-2 responsible for the coronavirus disease 2019 (COVID-19) that cause severe lower respiratory tract infections with extra-pulmonary involvements are from betacoronavirus. The other human coronaviruses (HCoV-NL63, HCoV-229E, HCoV-OC43 and HKU1) which induce mild to severe respiratory tract illness belong to the alphacoronavirus and betacoronavirus genera [2,4].

Furthermore, the viral diseases caused by other members of *Coronaviridae* family pose significant economic and public health burdens. These viruses include porcine enteric diarrhea virus (PEDV), porcine transmissible gastroenteritis virus (TGEV), porcine hemagglutinating encephalomyelitis virus (PHEV), infectious bronchitis virus (IBV), feline infectious peritonitis virus (FIPV), Mouse hepatitis virus (MHV) and the recently emerged swine acute diarrhea syndrome coronavirus (SADS-CoV) [5–9].

Several potential drugs and vaccines against coronavirus infections have been reported in the literature [2,5,10]. However, these

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approaches are not effective against a broad range of viral infections. Moreover, due to increasing reports of viral resistances, co-infections, and the emergence of viral epidemics such as COVID-19, available antiviral drugs show low or no efficacy against several coronavirus infections and the production of new vaccines is also challenging and time consuming [11]. Therefore, demand for the development of novel antivirals has considerably increased that may serve as supplementary or alternative to the currently used drugs. Employing antimicrobial peptides is an alternative option to circumvent the issues mentioned earlier. The features that suggest them as potent candidates for pharmacological applications are their remarkable structural and functional diversity. The wide range of biological activities of antimicrobial peptides proposes that they could be incorporated in the treatment strategies against bacterial, viral, and fungal infections [12–15]. Antiviral peptides have generated an increasing interest as a promising therapeutic in recent years. They have been obtained from natural, biological, and computational sources and are highly specific and effective in low concentrations and possess broad-spectrum activities with minimum side effects and host toxicity [16,17].

Here, we have made an effort to compile and review the antiviral peptides with activity against the viruses of *Coronaviridae* family and their mechanisms of action, which may help the researchers to design and produce novel antiviral drugs.

2. Characteristics and mechanisms of action of antiviral peptides

Antiviral peptides affect viruses by inhibiting the essential stages of their life cycle or components such as inhibition of attachment, fusion, host cell entry, intracellular viral replication and transcription, maturation, and viral enzymes. Direct interaction with virus particles and its effect on viral pathogenesis has also been reported by some antiviral peptides [17–19].

2.1. Antiviral peptides against human-infecting coronaviruses

2.1.1. Binding/attachment inhibitor peptides

The interaction of attachment proteins expressed on the virion surface with the host cell receptors is a vital initial step in the viral infections [20]. The viral genome of coronaviruses encodes spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins. The S protein has extracellular, transmembrane and intracellular domains, and also, the extracellular domain is divided into two subunits: S1 and S2. The S1 subunit includes the receptor-binding domain (RBD). The RBD comprises the N-terminal domain (NTD) and the C-terminal domain (CTD). SARS-CoV and SARS-CoV-2 bind to the angiotensin-converting enzyme 2 (ACE2) (abundant on the lung and small intestine cells surfaces), and MERS-CoV binds to proteinaceous dipeptidyl peptidase 4 (DPP4) (expressed on the lung and kidney cells surfaces) by CTD [21,22]. The S protein is an attractive target for the development of antiviral agents against CoVs.

In a study conducted by Zheng et al., anti-SARS-CoV activity of two peptides (P2 and P6) was demonstrated using a cytopathic effect (CPE)-based assay. These peptides were designed and synthesized based on the variations of the S1 domain. They effectively protected cells from the CPEs and reduced viral titer compared to the untreated controls. Significant synergistic antiviral effects were also detected in the combination of two peptides. They suggested that P2 was not a competitive peptide for the ACE2 receptor binding but may hamper conformation changes of the binding site, and P6 binds to the S protein and interferes with the virus-cell interactions [23] (Fig. 1A). In the other research, ACE2-derived peptides (peptides representing critical regions of ACE2) could block ACE2-S protein interaction and inhibit virus binding to the host cell. The evaluated peptides (P4, P5 and P6) exhibited a notable antiviral activity with the relative low 50 % inhibitory concentrations (IC_{50}) [24]. The attachment blocker small peptides (SP-4, SP-8 and SP-10) were also synthesized in a previous study based on S protein's receptor-binding regions (Molecular weight ~ 1.3–1.4 KDa). Their efficient inhibitory activity against S protein binding to the ACE2 was detected using the enzyme-linked immunosorbent assay (ELISA) method in Vero E6 cells [25].

Moreover, Chang et al. indicated that two synthetic peptides, GA91 and GA101 (corresponding to SARS-CoV Spike protein) block the binding of the SARS-CoV S protein to the host cells, using Vero E6 cells in adhesion assay [26]. In a different study, a hexapeptide, which was derived from RBD of the S protein, showed antiviral activity against SARS-CoV and HCoV-NL63. This peptide blocks the binding sites essential for the initial viral attachment to the respective receptor (Fig. 1A) and effectually inhibits CoVs replication in cell culture [27].

In the recent *in-silico* studies, the inhibitor peptides targeting the interaction between SARS-CoV-2 spike protein and ACE2 were designed using computational approaches [28,29]. Baig and colleagues have

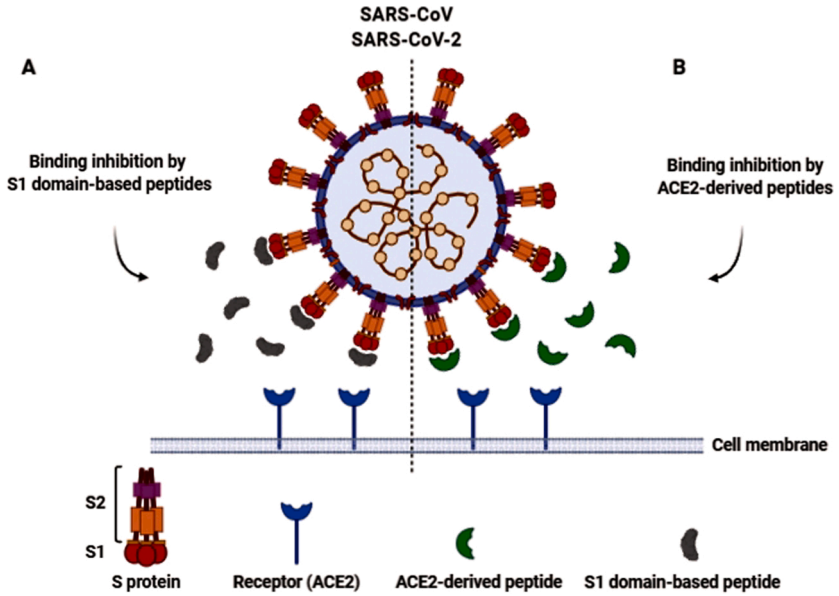

Fig. 1. Model of SARS-CoV/ SARS-CoV-2 binding inhibition by S1 domain-based inhibitor peptides (A) and ACE2-derived inhibitor peptides (B).

presented an ACE2-based peptide (18 aa peptide) that could block the viral attachment [28]. Furthermore, the S protein of SARS-CoV-2 was targeted by an inhibitor protein (ΔABP-D25Y) in Jaiswal et al., study. This protein includes two α-helical peptides which homologues to the ACE2 and also, its attachment to S protein is competitive [29] (Fig. 1B). Recent investigations showed that there are several potential heparin-binding sites located within the S1 domain of SARS-CoV-2. Heparin-binding peptides (HBPs) are capable of stopping SARS-CoV-2 infection. The interaction between HBPs and the S1 protein of SARS-CoV-2 was also described [30]. Another antimicrobial peptide with anti-SARS-CoV-2 activity is human defensin 5 (HD5). This alpha-defensin is secreted by intestinal Paneth cells and by neutrophils. It has high affinity to ACE2 and dramatically protects host cells from the adherence of the virus. HD5 is also capable of attaching SARS-CoV-2 S1 protein and affects its efficiency [31,32].

The characteristics of the antiviral peptides with binding inhibitory activity against human-infecting coronaviruses are expressed in Table 1.

2.1.2. Fusion and entry inhibitor peptides

As mentioned above, the extracellular domain of S protein contains two subunits; S1 and S2. The S2 mediates the membrane fusion. It contains various motifs: fusion peptide (FP), heptad repeat 1 (HR1) and HR2 regions, transmembrane (TM) domain and the cytoplasmic tail. FP is the functional fusogenic component and triggers the fusion event through insertion into the cell membrane. The HR1 and HR2 form a six-helix bundle or fusion core, and this conformational rearrangement is essential for the viral fusion and entry. There are two viral entry pathways for CoVs: plasma membrane (early) pathway and endosomal (late) pathway. The protease availability determines the route of entry. In the presence of exogenous or host membrane-bound proteases, most notably the transmembrane protease/serine sub-family member 2 (TMPRSS2), the virus can fuse via the plasma membrane pathway. TMPRSS2, cleaves the S protein at the S2' position which is located in upstream of the FP and activates plasma membrane fusion. Otherwise, the virus can fuse via an endosomal membrane pathway (clathrin- and non-clathrin-mediated endocytosis). Following endocytosis, the activated cysteine protease cathepsin L can cleave the S2' site in acidic conditions and trigger the subsequent fusion steps and CoVs genome releasing [21,22].

In a study conducted by Liu et al., the anti-SARS-CoV activity of the synthesized peptides based on HR1 and HR2 regions of S protein was investigated. One peptide derived from HR2 (CP-1) had inhibitory activity against SARS-CoV by binding to the HR1 and interfering with the conformational rearrangement (six-helix bundle formation) viral fusion. A HR1- derived peptide (NP-1) showed a marginal antiviral activity [33]. The inhibitory activity against membrane fusion and viral entry has also been described by a peptide derived from the SARS-CoV S2 protein HR segments in another research [34]. Moreover, Zheng and colleagues synthesized the peptides based on the sequence of SARS-CoV S2 domain. Significant antiviral effects were observed for two peptides (P8 and P10) through binding to the S2 protein and preventing virion–cell membrane fusion. They significantly protected cells from CPEs and reduced viral titer in the culture media. The peptide P8 had the highest antiviral potency and no virus was detected after P8 treatment. Furthermore, peptide combinations significantly improved their antiviral effects [23]. In the Sainz Jr et al., study, the peptides' ability analogous to the S2 subunit to inhibit SARS-CoV plaque formation has also been described [35]. Anti-SARS-CoV properties of HR-based antiviral peptides have been reported in more researches [36–38]. Fig. 2 shows the anti-fusion activity of the HR2-based peptides model against coronaviruses.

Although, activities of various S2 domain-based peptides against SARS-CoV were mentioned from several studies, the antiviral peptides with different origin have been characterized in the literature. The Griffithsin (GRFT) as an antiviral protein was originally obtained from a red alga. It can bind to the SARS-CoV spike glycoprotein specifically and inhibits viral entry. It prevents SARS-CoV infection both *in vitro* and *in vivo*. GRFT's activity against other human-infecting coronaviruses was also demonstrated [39].

Furthermore, a membrane fusion blocker peptide with basic amino acids in its composition was introduced by Zhao et al., in 2016. This mouse β-defensin-4 derived peptide (P9) efficiently attached to the SARS-CoV and MERS-CoV glycoproteins and entered the host cells with the viruses through endocytosis. P9 prevents endosomal acidification and thus, inhibits the subsequent fusion steps [40]. The inhibitory model of such peptides against coronaviruses is shown in Fig. 3.

Several researches have also been performed regarding antiviral activity of HR regions-based peptides against MERS-CoV. In the Lu et al., study, a MESR-CoV HR2 analogous peptide (HR2P) was synthesized and its antiviral activity was investigated against this virus. The peptide could inhibit MERS-CoV fusion through interaction with the viral HR1 domain and heterologous six-helix bundle formation [41]. A similar result was presented in the study by Channappanavar et al. [42]. A fusion inhibitor named MERS-five-helix bundle (MERS-5HB) was synthetized by Sun et al. MERS-5HB was derived from the MERS six-helix bundle and consisted of three copies of HR1 and two copies of HR2. Lack of one HR2 in 5HB led to its interaction with a native HR2 of the MERS-CoV and the fusion step interruption [43]. As mentioned above, six-helix bundle (hexameric structure) formation by HR1 and HR2 is necessary for viral fusion and entry. Wang et al., have designed the hydrocarbon-stapled peptide and lipopeptide with a hydrocarbon tail in the two separate studies that could effectively block MERS-CoV formation hexameric structure [44,45]. The MERS-CoV fusion inhibitory peptides derived from HR2 domain of HKU4 (bat coronavirus) have also been reported. The HKU4-HR2Ps binds to HR1 of MERS-CoV with high stability and blocks the fusion process [46] (Fig. 2). Recently, a gold nanorod-based HR1 peptide (PIH-AuNRs) was proposed as MERS-CoV fusion inhibitor. The HR1 peptide inhibitor (PIH) derived from viral HR2 region was conjugated by potential biocompatible and site-specific carriers (AuNRs). This complex could completely inhibit MERS-CoV fusion [47].

In an *in-silico* study conducted by Ling et al., an antiviral peptide targeting SARS-CoV-2 fusion was described. They predicted the HR1 and HR2 regions in S protein of SARS-CoV-2 using sequence alignment and simulated forming of the fusion core. Then, they designed HR2-baseed peptide that can competitively bind with HR1 to inhibit viral fusion core forming [48]. Furthermore, Xia and colleagues utilized a lipopeptide (EK1C4) derived from a pan-coronavirus fusion inhibitor, EK1 (targeting the HR1 domain), against SARS-CoV-2. This lipopeptide displayed potent inhibitory activity against the virus [49]. More lipopeptides with anti-fusion activity against SARS-CoV-2 were reported in the recent studies. A SARS-CoV-2 HR2 sequence-based lipopeptide (IPB02) was highly potent fusion inhibitor of SARS-CoV-2 and SARS-CoV [50] (Fig. 2). In another study, similar peptide activity corresponding to the C-terminal HR domain of SARS-CoV-2 conjugated by tetra-ethylene glycol-cholesterol was demonstrated against SARS-CoV-2 and MERS-CoV. The spread of SARS-CoV-2 in human airway epithelial (HAE) cultures was also blocked using this lipopeptide [51]. One derivative of aforementioned lipopeptide ($[SARS_{HRC}\text{-}PEG_4]_2$-chol) was able to block entry of SARS-CoV-2 in the host cells and the cells over-expressing the TMPRSS2 protease. Moreover, $[SARS_{HRC}\text{-}PEG_4]_2$-chol intranasal administration to ferrets, completely protected the animals from SARS-CoV-2 infection [52].

Zhao et al., studied the alkaline peptide P9R (a defensin-like peptide) as an antiviral activity against pH-dependent viruses such as SARS-CoV, SARS-CoV-2 and MERS-CoV that require endosomal acidification for their infection development. P9R is the modified form of P9 (weakly positively charged amino acids substitution), which was mentioned above, and its antiviral mechanism was similar to P9 [53] (Fig. 3). Anti-SARS-CoV-2 fusion activity of the peptides derived from food proteins such as lactoferrin, have also been proposed recently through inhibition of cathepsin L [54].

The HR-based antiviral peptides against other human-infecting

Table 1
The characteristics of the peptides with binding inhibitory activity against human-infecting coronaviruses.

Peptide name	Sequence	Peptide source	Target virus	Inhibitory concentration	Cell line	Ref.
P2	PTTFMLKYDENGTITDAVDC	S1 subunit of SARS-CoV	SARS-CoV	112.5 ± 26.3 μg/mL (IC50)	Fetal rhesus kidney (FRhK-4) cells	[23]
P6	YQDVNCTDVSTAIHADQLTP	S1 subunit of SARS-CoV	SARS-CoV	113.0 ± 27.6 μg/mL (IC50)	Fetal rhesus kidney (FRhK-4) cells	[23]
P4	EEQAKTFLDKFNHEAEDLFYQSS	ACE2	SARS-CoV	50 μM (IC50)	HeLa cells	[24]
P5	EEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEE	ACE2	SARS-CoV	6 μM (IC50)	HeLa cells	[24]
P6	EEQAKTFLDKFNHEAEDLFYQSSGLGKGDFR	ACE2	SARS-CoV	0.1 μM (IC50)	HeLa cells	[24]
SP-4	GFLYVYKGYQPI	S protein of SARS-CoV	SARS-CoV	4.3 ± 2.18 nmol (IC50)	–	[25]
SP-8	FYTTTGIGYQPY	S protein of SARS-CoV	SARS-CoV	6.99 ± 0.71 nmol (IC50)	–	[25]
SP-10	STSQKSIVAYTM	S protein of SARS-CoV	SARS-CoV	1.88 ± 0.52 nmol (IC50)	Vero E6 cells	[25]
GA91	–	S protein of SARS-CoV	SARS-CoV	–	Vero E6 cells	[26]
GA101	–	S protein of SARS-CoV	SARS-CoV	–	Vero E6 cells	[26]
BD-11b	YKYRYL	S protein of SARS-CoV	SARS-CoV	14 mM (IC90)	Vero E6 cells	[27]
BD-11b	YKYRYL	S protein	HCoV-NL63	7 mM (IC90)	CaCo2 cells	[27]
18 aa peptide	FLDKFNHEAEDLFYQSSL	ACE2	SARS-CoV-2	–	–	[28]
ΔABP-D25Y	GSHMGDAQDKLKYLVKQLERALRELKKSLDELERSLEELEKNPSEDALVENNRLNVENNKIIVEVLRIILELAKASAKLA	ACE2	SARS-CoV-2	–	–	[29]
P5 + 14	GGGYSKAQKAQAKQAKQAQKAQKAQAKQAKQAQKAQKAQAKQAKQ	S1 subunit of SARS-CoV-2	SARS-CoV-2	–	–	[30]
Human Defensin-5	–	Intestinal Paneth cells and neutrophils	SARS-CoV-2	10 μg/mL	Caco-2 cells	[31]

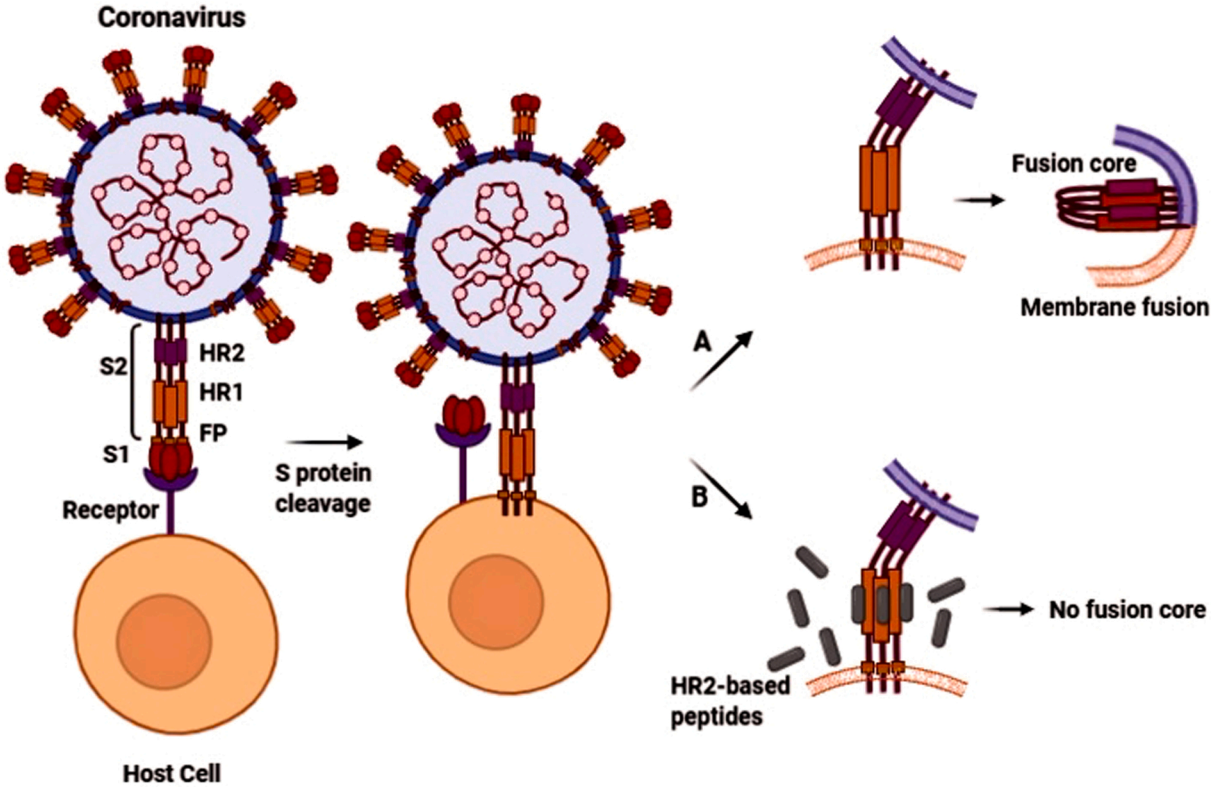

Fig. 2. Schematic presentation of anti-fusion activity of HR2-based peptides against coronaviruses. A) Fusion core (six-helix bundle) formation through HR1 and HR2 rearrangement and thus membrane fusion. B) Blocking fusion core formation and membrane fusion by HR2-based peptides.

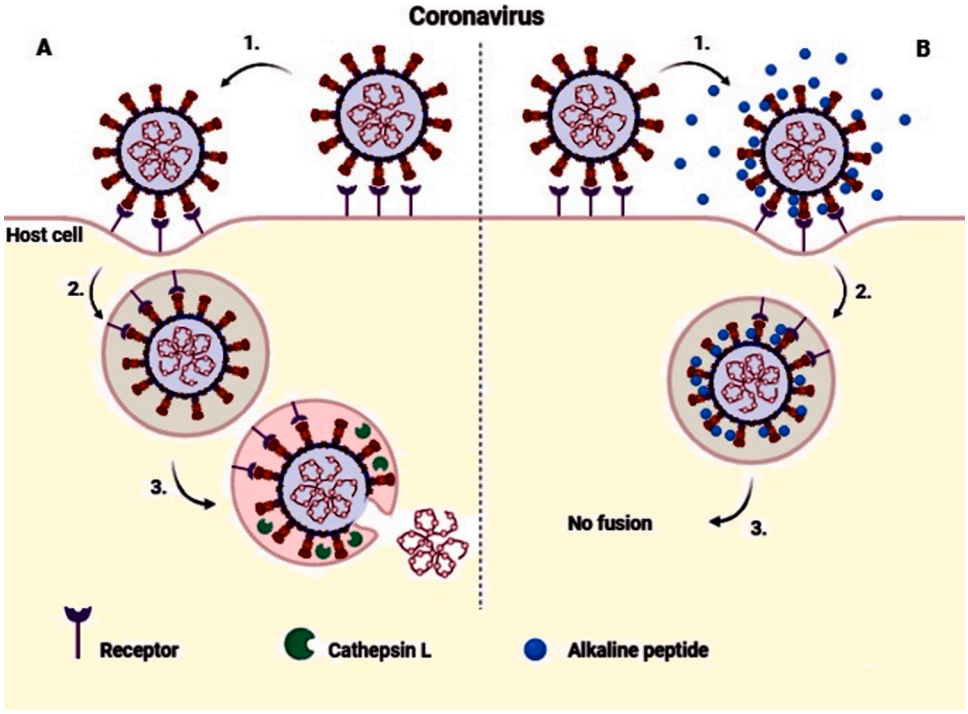

Fig. 3. Model of anti-fusion activity of alkaline peptides (peptides with basic amino acids) such as P9 and P9R against coronaviruses. This model depicts: A) Binding the virus with its receptor and endocytosis induction (1); Endocytosis (2); Endosome acidification, cathepsin L activation, fusion and viral genome release (3). B) Receptor binding and endocytosis induction in the presence of alkaline peptides (1); Endocytosis with attached alkaline peptides (2); Inhibition of endosome acidification, no cathepsin L activation and no fusion (3).

coronaviruses were suggested in the previous studies. The fusion inhibitor peptides targeting HCoV-229E via interaction with its six-helix bundle were characterized in a research. The peptides could efficiently inhibit CPEs of HCoV-229E infection in the host cells [55]. Meanwhile, the mentioned above pan-coronavirus fusion inhibitor (EK1) which was derived from HR2 domain of HCoV-OC43, possessed broad fusion inhibitory property against multiple human coronaviruses. This peptide and cholesterol-attached form exhibited effective inhibitory activities against HCoV-OC43, MERS-CoV, HCoV-229E and HCoV-NL63 [49,56] (Fig. 2). The characteristics of antiviral peptides with fusion or entry

Table 2
The characteristics of the peptides with fusion or entry inhibitory activity against human-infecting coronaviruses.

Peptide name	Sequence	Peptide source	Target virus	Effective / inhibitory concentration	Cell line / animal model	Ref.
CP-1	GINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYE	HR2 region of SARS-CoV	SARS-CoV	19 μmol/L (IC50)	Vero E6 cells	[33]
NP-1	GVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQ	HR1 region of SARS-CoV	SARS-CoV	50 μmol/L (IC50)	Vero E6 cells	[33]
–	SGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL	HR segment of SARS-CoV	SARS-CoV	–	–	[34]
P8	QYGSFCTQLNRALSGIAAEQ	S2 subunit of SARS-CoV	SARS-CoV	24.9 ± 6.2 μg/mL (IC90)	Fetal rhesus kidney (FRhK-4) cells	[23]
P10	IQKEIDRLNEVAKNLNESLI	HR2 region of SARS-CoV	SARS-CoV	73.5 ± 15.7 μg/mL (IC90)	Fetal rhesus kidney (FRhK-4) cells	[23]
$SARS_{WW-III}$	GYHLMSFPQAAPHGVVFLHVTW	S2 subunit of SARS-CoV	SARS-CoV	~ 2 μM (IC50)	Vero E6 cells	[35]
$SARS_{WW-IV}$	GVFVFNGTSWFITQRNFFS	S2 subunit of SARS-CoV	SARS-CoV	~ 2 μM (IC50)	Vero E6 cells	[35]
HR1-1	NGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTA	HR1 region of SARS-CoV	SARS-CoV	3.68 μM (EC50)	Vero E6 cells	[36]
HR2-18	IQKEIDRLNEVAKNLNESLIDLQELGK	HR2 region of SARS-CoV	SARS-CoV	5.22 μM (EC50)	Vero E6 cells	[36]
HR1-a	YENQKQIANQFNKAISQIQESLTTTSTA	S2 subunit of SARS-CoV	SARS-CoV	1.61 μM (EC50)	Vero E6 cells	[37]
GST-removed-HR2	DVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL	S2 subunit of SARS-CoV	SARS-CoV	2.18 μM (EC50)	Vero E6 cells	[37]
HR2	ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL	S2 subunit of SARS-CoV	SARS-CoV	0.34 μM (EC50)	Vero E6 cells	[37]
SR9	ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL	HR2 region of SARS-CoV	SARS-CoV	<100 nM (EC50)	Vero E6 cells	[38]
GRFT	SLTHRKFGGSGGSPFSGLSSIAVRSGSYLDAIIDGVHHG SGGNLSPTFTFGSGEYISNMTIRSGDYIDNISFETNMGR RFGPYGGSGGSANTLSNVKVIQINGSAGDYLDSLDIYYEQY	*Griffithsia* sp.	SARS-CoV	>100 μg/mL (IC50)	Vero 76 cells / BALB/c mice	[39]
P9	NGAICWGPCPTAFRQIGNCGHFKVRCCKIR	mouse β-defensin-4	SARS and MERS-CoVs	~ 5 μg/mL (IC50) 25 μg/mL (IC90)	FRhK-4 and Vero-E6 cells / BALB/c mice	[40]
HR2P	SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKEL	HR2 region of MERS-CoV	MERS-CoV	0.6 μM (IC50)	Calu-3 and Vero cells	[41]
HR2P-M2	SLTQINTTLLDLEYEMKKLEEVVKKLEESYIDLKEL	HR2 region of MERS-CoV	MERS-CoV	0.55 μmol/L (IC50)	Vero 81 cells	[42]
5HB	HR1-SGGRGG-HR2-GGSGGSGG-HR1-SGGRGG-HR2-GGSGGSGG-HR1	S2 subunit of MERS-CoV	MERS-CoV	1 μM (IC50)	Huh-7 cells	[43]
P21S10	LDLTYEMLSLQQVV KLNEY	S2 subunit of MERS-CoV	MERS-CoV	0.97–1.58 μM (EC50)	Huh-7 and Calu-3 cells	[44]
IIQ	IEEIQKKIEEIQKKIEEIQKKIEEIQKKIEEIQKK-β-alanine-K	–	MERS-CoV	0.13 μM (EC50)	Huh-7 cells	[45]
HKU4-HR2P2	EISKINTTLLDLSDEMAMLQQEVVKQLNDSYIDLKEL	HR2 domain of bat coronavirus HKU4	MERS-CoV	0.34 μM (IC50)	Huh-7 cells	[46]
HKU4-HR2P3	LDLSDEMAMLQQEVVKQLNDSYIDLKELGNYTYYNKW	HR2 domain of bat coronavirus HKU4	MERS-CoV	0.48 μM (IC50)	Huh-7 cells	[46]
PIH-AuNR	CGGGGGSLTEINTELLDLEYEMKKLEEVVKKLEESYIDLKEL-AuNR	HR2 domain of MERS-CoV	MERS-CoV	0.117 μM (IC90)	Huh-7 cells	[47]
HR2-anti-P	DISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL	HR2 domain of SARS-CoV-2	SARS-CoV-2	–	–	[48]
EK1C4	SLDQINVTFLDLEYEMKKLEEAIKKLEESYIDLKEL-PEG4-Chol	HR2 domain of HCoV-OC43	SARS-CoV-2	36.5 nM (IC50)	Vero E6 cells	[49]
IPB02	ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELK-Chol	HR2 domain of SARS-CoV-2	SARS-CoV-2 and SARS-CoV	0.08 and 0.251 μM (IC50)	293 T cells	[50]
–	DISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL-GSGSGC-TEG-Chol	C-terminal HR domain of SARS-CoV-2	SARS-CoV-2 and MERS-CoV	~ 6 and ~3 nM (IC50)	Vero E6 and human derived tracheo/bronchial epithelial cells	[51]
$[SARS_{HRC}\text{-}PEG_4]_2$-Chol	[DISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL-PEG_4]$_2$-Chol	C-terminal HR domain of SARS-CoV-2	SARS-CoV-2	~ 300 and ~ 5 nM (IC50)	Vero E6 and Vero E6 cells overexpressing the protease TMPRSS2 / Ferrets	[52]
P9R	NGAICWGPCPTAFRQIGNCGRFRVRCCRIR	Mouse β-defensin-4 like peptide	SARS-CoV-2, MERS-CoV and SARS-CoV	0.9, 2.2 and 4.2 μg/mL (IC50)	Vero E6 cells	[53]
GRFT	SLTHRKFGGSGGSPFSGLSSIAVRSGSYLDAIIDGVHHGGS GGNLSPTFTFGSGEYISNMTIRSGDYIDNISFETNMGRRFG PYGGSGGSANTLSNVKVIQINGSAGDYLDSLDIYYEQY	*Griffithsia* sp.	HCoV-OC43, HCoV-229E and HCoV-NL63	52, >10 and 10 μg/mL (IC50)	HCT-8, MRC-5, LLC-MK2 cells	[39]
229E-HR1P	AASFNKAMTNIVDAFTGVNDAITQTSQALQTVATALNK IQDVVNQQGNSLNHLTSQ	S2 subunit of HCoV-229E	HCoV-229E	13.2 μM (IC50)	Huh-7 and A549 cells	[55]
229E-HR2P	VVEQYNQTILNLTSEISTLENKSAELNYTVQKLQTLIDNINSTLVDLKWL	S2 subunit of HCoV-229E	HCoV-229E	1.96 μM (IC50)	Huh-7 and A549 cells / Balb/c mice	[55]
EK1	SLDQINVTFLDLEYEMKKLEEAIKKLEESYIDLKEL	HR2 domain of HCoV-OC43	HCoV-OC43, MERS-CoV, HCoV-229E and HCoV-NL63	0.62, 0.11, 0.69 and 0.48 μM (IC50)	HCT-8, Calu-3, A549 and LLC-MK2 cells / Balb/c mice	[56]
EK1C4	SLDQINVTFLDLEYEMKKLEEAIKKLEESYIDLKEL-PEG4-Chol	HR2 domain of HCoV-OC43	MERS-CoV, HCoV-OC43, HCoV-229E and HCoV-NL63	4.2, 24.8, 101.5 and 187.6 nM (IC50)	RD, Huh-7 and LLC-MK2 cells / Mice	[49]

PEG, poly-ethylene glycol; Chol, cholesterol; TEG, tetra-ethylene glycol.

inhibitory activity against human-infecting coronaviruses are illustrated in Table 2.

2.1.3. Viral enzymes inhibitor peptides

The coronavirus enzymes' pivotal roles, including proteases, helicases, RNA-dependent RNA polymerase (RdRp), and methyltransferase, have been well characterized in the viral life cycle. Coronaviruses genome contains at least 6 open reading frames (ORFs). The ORF1ab encodes the overlapping polyproteins which are cleaved into non-structural proteins (nsps) by the main protease (M^{pro} or $3CL^{pro}$) and the papain-like protease (PL^{pro}) [57]. Due to these cysteine proteases' critical role in viral gene expression and replication, they are favorable targets for the antiviral drug design.

In the study conducted by Gan et al., the designed octapeptide inhibited the SARS-CoV M^{pro} and blocked replication of the virus [58]. Similarly, the synthetized dipeptides (6a and EP128533) and tetrapeptide inhibited several CoVs by inhibiting their M^{pro} [59–61]. In the previous studies, SARS-CoV M^{pro} inhibitory activities of the peptide-based compounds (tetrapeptide, pentapeptides and octapeptides) were also proposed without biological experiments [62–64]. A recent study indicated that, *in-silico* hydrolysis of marine fish proteins, gastrointestinal enzymes generated active peptides. Some of them were identified as high-affinity oligopeptides binder to the M^{pro} of SARS-CoV-2. According to their results, the identified oligopeptides could be used as potential SARS-CoV-2 inhibitor drugs [65].

In addition, blocking other enzyme of SARS-CoV (Methyltransferase) was demonstrated previously by the synthesized peptides. The nsp16 acts as a methyltransferase and plays an essential role in the life cycle of coronaviruses. The designed peptides could markedly inhibit the activity of methyltransferase and thus viral replication [66]. Table 3 shows the characteristics of antiviral peptides with potential inhibitory activity against coronaviruses enzymes.

2.1.4. Viral replication inhibitor peptides

In the study performed by Lo et al., a synthesized peptide could inhibit replication of HCoV-229E in the host cells, significantly through the interference of the oligomerization of the viral nucleocapsid protein [67]. The application of such a strategy may assist the design and development of antiviral drugs against other human-infecting coronaviruses. Table 4 shows the characteristics of the viral replication inhibitor peptides.

2.1.5. Direct interaction of antiviral peptides with virus particles

The virucidal activity of a confirmed antimicrobial peptide against SARS-CoV was reported in the Li et al., study. The infectivity of virus decreased significantly via direct interaction of the peptide (mucroporin-M1) with the virus envelope [68]. The characteristics of mucroporin-M1 are shown in Table 5.

2.1.6. Effects of human antimicrobial peptides against coronaviruses pathogenesis

Indirect effects of human beta-defensin 2 (HBD 2) (an antimicrobial peptide produced by epithelial cells) against MERS-CoV were reported in the previous studies. HBD 2 enhanced primary antiviral innate immunity and effective adaptive immune responses [69,70]. Human cathelicidin (LL-37) also reduces the pathology of COVID-19 by affecting regulatory T cells. The probable role of LL-37 in the down-regulation of interleukin-17, which is involved in thrombosis and acute respiratory distress syndrome, was reported recently [71].

Table 3
The characteristics of the peptides with potential inhibitory activity against coronaviruses enzymes.

Peptide name	Sequence	Peptide source	Target virus	Effective / inhibitory concentration	Cell line	Ref.
Octapeptide	AVLQSGFR	Structure of SARS-CoV M^{pro}	SARS-CoV	0.027 μg/mL (EC50)	Vero cells	[58]
6a	LQQ-fmk	Cleavage site of SARS-CoV M^{pro}	SARS-CoV	2.5 μM (EC50)	Vero cells	[59]
EP128533	–	–	SARS-CoV	0.56−1.4 μg/mL (EC50)	Vero-76 cells	[60]
Protected tetrapeptide	Cbz-AVLQ	Cleavage site of the main proteases	SARS-CoV, HCoV-OC43, HCoV-229E, HCoV-NL63, HCoV-HKU1 and IBV	1.3–4.6 μM (IC50)	–	[61]
Pentapeptide aldehydes	ESTLQ, NSFSQ, DSFDQ and NSTSQ	Analyses of the SARS-CoV M^{pro}	SARS-CoV	–	–	[63]
Tetrapeptide aldehyde	TVFH	Analyses of the SARS-CoV 3CL protease	SARS-CoV	98 nM (IC50)	–	[64]
Oligopeptides	ITTIM, TVPIY, ICIY, PASQF, IITAM, TIIF, AIPAW, IVPIL, PVIDL, TVPIY, ICIY, PISQF, EQIVY, VISAW and PESW	Marine fish proteins	SARS-CoV-2	–	–	[65]
K 12	GGASCCLYCRCH	Sequence of nsp 10 of SARS-CoV	SARS-CoV	160 μM (IC50)	–	[66]
K 29	FGGASCCLYCRCHIDHPNPKGFCDLKGKY	Sequence of nsp 10 of SARS-CoV	SARS-CoV	160 μM (IC50)	–	[66]

Fmk, fluoromethyl ketones; Cbz, carboxybenzyl.

Table 4
The characteristics of the viral replication inhibitor peptides.

Peptide name	Sequence	Peptide source	Target virus	Effective / inhibitory concentration	Cell line / animal model	Ref.
C-terminal tail peptide	–	Nucleocapsid protein of HCoV-229E	HCoV-229E	300 μM	A549 cells	[67]
APB-13	ILPWKWPWWPWRR-NH_2	Cattle	TGEV	62.5 μg/mL	ST cells / Piglets	[88]
viperin	–	Porcine antiviral protein	PEDV	–	IPEC-J2 cells	[89]

Table 5
The characteristics of the peptides with direct effect on virus particles.

Peptide name	Sequence	Peptide source	Target virus	Effective / inhibitory concentration	Cell line	Ref.
Mucroporin-M1	LFRLIKSLIKRLVSAFK	Scorpion venom	SARS-CoV	7.12 μM	Hela-ACE2 cells	[68]
Caerin1.1	GLLSV LGSVA KHVLP HVVPV IAEHL-NH2	Skin of the Australian green tree frog	PEDV	10 μg/mL	Vero cells	[90]

Table 6
The characteristics of binding inhibitor peptides of the mammalian and avian viruses.

Peptide name	Sequence	Peptide source	Target virus	Effective concentration	Cell line	Ref.
F	FKPSSPPSITLW	S protein of TGEV	TGEV	20 μg/mL	Swine testis (ST) cells	[72]
H	HVTTTFAPPPPR	S protein of TGEV	TGEV	20 μg/mL	ST cells	[72]
S	SVVPSKATWGFA	S protein of TGEV	TGEV	20 μg/mL	ST cells	[72]
–	HDAISWTHYHPW	pAPN	TGEV	–	ST cells	[73]
TGEV-M7	HALTPIKYIPPG	M protein of TGEV	TGEV	62.5 μg/mL	ST cells	[74]
L	LMQINPTYYQIM	S1 protein of PEDV	PEDV	50 μg/mL	VeroE6 cells	[75]
W	WSFNPSTYTIAG	S1 protein of PEDV	PEDV	50 μg/mL	VeroE6 cells	[75]
I-S1-9	YGFWTIAYTNYTDVMVDVNG	S1 protein of FIPV	FIPV	100 μM	Felis catus whole fetus-4 (fcwf-4) cells	[76]
I-S1-16	YHWMNVTLHVVLNDTEKKYD	S1 protein of FIPV	FIPV	100 μM	fcwf-4 cells	[76]
Peptide 1	GSHHRHVHSPFV	–	IBV	8.3 μg/mL	Hela cells	[77]
SIAMP	–	Swine intestine	IBV	100 μg/mL	Chick embryos	[78]

2.2. Antiviral peptides against mammalian and avian viral strains

2.2.1. Binding/attachment inhibitor peptides

The antiviral peptides with binding inhibitory activities against mammalian and avian viral strains have also been reported. The TGEV is a porcine coronavirus and the causative agent of transmissible gastroenteritis (TGE). TGE is a highly contagious enteric disease of swine with up to 100 % mortality in suckling piglets and causes economic losses. The S protein of the virus interacts with its cellular receptor, porcine aminopeptidase N (pAPN). Also, APN is a cellular receptor for HCoV-229E and FIPV [72]. Phages displaying peptide sequences with protective effects against TGEV infection, have been described in the literature. The findings of Ren et al., study showed three chemically synthesized peptides (F, H and S) that had sequence homologies (same motifs identified in the S protein of TGEV) were able to inhibit TGEV infection through competition with binding the pAPN [72]. Similarly, an antiviral peptide's potential inhibitory effect was observed using a phage bearing the peptide with affinity to pAPN [73]. Furthermore, the inhibitory activity of a membrane (M) protein-derived peptide was demonstrated in the other study. Peptide TGEV-M7 was able to significantly reduce the virus's ability to infect host cells [74].

Cao et al., indicated the antiviral activity of two peptides (L and W) against porcine epidemic diarrhea virus (PEDV). The PEDV is another swine pathogen that causes severe diarrhea and dehydration. These peptides share a consensus motif with S1 protein of PEDV and inhibit the binding of the virus to the host cells [75].

FIPV is a type of feline coronavirus (FCoV) and belongs to the alphacoronaviruses. It can cause lethal disease in cats due to multiple organs involvement. The S1 domain of FIPV S protein also contains the RBD. Doki et al., synthesized peptides based on the S1 domain sequence

Table 7
The characteristics of the peptides with fusion or entry inhibitory activity against mammalian and avian viral strains.

Peptide name	Sequence	Peptide source	Target virus	Effective / inhibitory concentration	Cell line / animal model	Ref.
Surfactin	–	*Bacillus subtilis*	TGEV	0.002 mg/mL	Intestinal porcine epithelial cells (IPEC-J2)	[79]
Surfactin	–	*Bacillus subtilis*	TGEV and PEDV	15–50 μg/mL	ST cells/ Piglet	[80]
SLP 5	Palmityl-EVLDL	Surfactin	PEDV	16.5 ± 0.6 μg/mL (EC50)	Vero cells	[81]
HR2M, HR2L and HR2P	–	HR2 domain of PEDV	PEDV	4.97, 2.96 and 1.11 μM (IC50)	Huh-7 cells	[83]
H	HVTTTFAPPPPR	–	PEDV	1 μg/mL (EC50)	Vero cells	[82]
FP4	FNATYLNLTGEIDDLEFRSEKLHNTTVELAILIDNINNTLVNL	HR2 domain of FIPV	FIPV	1.8 μM (IC50)	Fcwf-4 cells	[84]
FP5	FNATYLNLTGEIDDLEFRSEKLHNTTVELAILIDNINNTLVNL EWLNRIE	HR2 domain of FIPV	FIPV	1.33 μM (IC50)	Fcwf-4 cells	[84]
MHV_{WW-IV}	GYFVQDDGEWKFTGSSYYY	S2 subunit of MHV	MHV	5 μM (IC50)	L2 cells	[35]
HR2	SLSLDFEKLNVTLLDLTYEMNRIQDAIKKLNESYINLKE	HR2 region of MHV	MHV	50 μM (IC90)	LR7 cells	[86]
NOVEL-1	NASDMEIKKVNKKIEEYIKKIEEVEKKLEEVNKK	HR2 domain of NDV and IBV	IBV	~5 μM (IC90)	Chicken embryo fibroblast (CEFs) cells	[85]
NOVEL-2	VNKKIEEIDKKIEELNKKLEELEKKLEEVNKK	HR2 domain of NDV and IBV	IBV	~5 μM (IC90)	CEFs cells	[35]
Alstotide As1	CRPYGYRCDGVINQCCDPYHCTPPLIGICL	*Alstonia scholaris* plant	IBV	~100 μM	Vero cells	[87]

and investigated their inhibitory effects. Two peptides (I-S1-9 and I-S1-16) inhibited the binding and infectivity of FIPV significantly. Moreover, significant reduction of viral adsorption to cells was observed using peptide I-S1-9 [76].

IBV that belongs to gammacoronavirus, is the etiologic agent of complex and highly contagious respiratory disease in chickens which remains an economic problem. Bo et al., showed that a phage-displayed peptide blocks binding the IBV S protein and inhibits virus infectivity and CPE occurrence in HeLa cells [77]. The other studies indicated that swine intestine antimicrobial peptide (SIAMP) inhibits IBV replication and decreases tissue injury caused by the virus. Interaction of SIAMP with IBV and blocking the virus binding to the embryos' epithelial cells were reported as the probable inhibitory mechanisms of the peptide [78]. Table 6 shows the characteristics of the peptides inhibiting the mammalian and avian coronaviruses binding.

2.2.2. Fusion and entry inhibitor peptides

The fusion or entry blocker peptides against other viruses from *Coronaviridae* family have been investigated in the literature. Wang et al., indicated that surfactin can effectively inhibit TGEV from entering the host cells [79]. Surfactin is a cyclic lipopeptide secreted by *Bacillus subtilis*, affects both the viruses and the cells as a membrane fusion inhibitor [79,80]. Likewise, it could inhibit the fusion between the TGEV envelope and the host cell membrane in another study. Surfactin inhibited the replication of TGEV and PEDV completely, and its oral administration also protected piglets from PEDV infection [80]. In another study, anti-PEDV properties of synthetic surfactin analogues were investigated. The SLP5 as a linear lipopeptide had lower cytotoxicity and similar antiviral activity, compared to the surfactin [81]. The PEDV entry was also blocked by a phage-displayed peptide through binding to the S protein, in Meng et al., study [82]. Anti-fusion and entry activities of S2 domain-based peptides against PEDV, FIPV, MHV and IBV have been described in the previous researches [35,83–86]. Moreover, the IBV infection in the host cells was inhibited by an antiviral peptide (As1) obtained from a medicinal plant. As1 binds to fusion and S protein of the virus and interferes with their function during IBV entry [87]. The characteristics of antiviral peptides with fusion or entry inhibitory activity against mammalian and avian viral strains are shown in Table 7.

2.2.3. Viral replication inhibitor peptides

In a recent study by Liang et al., indicated *in vivo* and *in vitro* anti-TGEV activities of Bovine antimicrobial peptide-13 (APB-13). The peptide had notable inhibitory effect on the expression of nucleocapsid protein of TGEV. Furthermore, viral shedding of animal rectum reduced significantly after treatment by APB-13 [88].

Wu et al., reported that the PEDV proliferation was regulated using an antiviral protein (viperin). The interaction of viperin with the N protein of PEDV led to interfering with viral replication or assembly [89]. Table 4 shows the characteristics of the viral replication inhibitor peptides.

2.2.4. Direct interaction of antiviral peptides with virus particles

The multiplication of PEDV was inhibited significantly by a cationic amphibian antimicrobial peptide in the host cells. The ability of Caerin1.1 to disrupt the integrity of the virus particles was mentioned as its inhibitory mechanism [90]. The characteristics of Caerin1.1 are shown in Table 5.

3. Conclusion

The emergence or re-emergence of *Coronaviridae* family viruses and enhanced cross-species dissemination due to potential viral mutations are still real threats to the worldwide population. Currently, there is no specific treatment for the majority of infections that caused by such viruses. This study was the first review regarding antiviral peptides with activity against all studied viruses from *Coronaviridae* family. However, several peptides were found with different antiviral mechanisms. This review mainly aimed to summarize data regarding the peptides that can be applicable and provide relevant information to develop novel treatments. Our study suggests that antiviral peptides are effective therapeutic options for these pathogens. The studies regarding fusion/entry inhibitors (notably HR-based) were more than the others and the HR2-based peptides possessed the most promising activity against investigated coronaviruses. The SR9 and PIH-AuNR were the most effective antiviral peptides against SARS-CoV and MERS-CoV, respectively. As mentioned in the text, the permeability and effectiveness of the peptides conjugated by the fatty acids (lipopeptides) were more than the pure peptides. For SARS-CoV-2, a lipopeptide (EK1C4) derived from a pan-coronavirus fusion inhibitor (EK1) displayed significant inhibitory activity against SARS-CoV-2 in Vero E6 cells. EK1C4 also inhibited other coronaviruses including MERS-CoV, HCoV-OC43, HCoV-229E and HCoV-NL63 in cell culture and mice. The spread of SARS-CoV-2 in human airway epithelial (HAE) cultures was also blocked using the peptide corresponding to C-terminal HR domain of SARS-CoV-2 conjugated by tetra-ethylene glycol-cholesterol. A derivative of this lipopeptide ([SARSHRC-PEG4]2-chol) was able to block the entry of SARS-CoV-2 in the Vero E6 cells. Its intranasal administration to ferrets completely protected the animals from SARS-CoV-2 infection. The antiviral activity and potency of antiviral peptides could be improved by combination with other peptides having different mechanisms of action, or with conventional antiviral drugs. Although, clinical trials and animal models testing have not yet been performed for the majority of the evaluated antiviral peptides, the obtained results from *in vivo* studies show the optimistic future to prepare the novel anti-CoVs drugs.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Not applicable.

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