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A review of the antiviral activity of cationic antimicrobial peptides

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ABSTRACT

Viral epidemics are occurring frequently, and the COVID-19 viral pandemic has resulted in at least 6.5 million deaths worldwide. Although antiviral therapeutics are available, these may not have sufficient effect. The emergence of resistant or novel viruses requires new therapies. Cationic antimicrobial peptides are agents of the innate immune system that may offer a promising solution to viral infections. These peptides are gaining attention as possible therapies for viral infections or for use as prophylactic agents to prevent viral spread. This narrative review examines antiviral peptides, their structural features, and mechanism of activity. A total of 156 cationic antiviral peptides were examined for information of their mechanism of action against both enveloped and non-enveloped viruses. Antiviral peptides can be isolated from various natural sources or can be generated synthetically. The latter tend to be more specific and effective and can be made to have a broad spectrum of activity with minimal side effects. Their unique properties of being positively charged and amphipathic enable their main mode of action which is to target and disrupt viral lipid envelopes, thereby inhibiting viral entry and replication. This review offers a comprehensive summary of the current understanding of antiviral peptides, which could potentially aid in the design and creation of novel antiviral medications.

1. Background

Emerging and reemerging viruses are a serious concern worldwide. The investigation of the origins of antimicrobial peptides as potential agents against human viruses is a key area of research in tackling this problem [1]. A recent in-depth review focused on approved antiviral peptides (AVPs), providing a comprehensive analysis of important patents and their applications spanning from 2000 to 2020. This review also explored the advancements made in the development of antiviral drugs utilizing peptides and related compounds. However, despite the FDA's approval of specific peptides for antiviral treatment, there is still a lack of effective therapeutics specifically designed to target viral infections [1,2]. It is challenging to design antivirals that do not impact the host as viruses use the host cell machinery for replication. Moreover, the continuous evolution and mutation of viral genomes can result in the emergence of drug-resistant strains. Shin and Seong., reviewed the underlying mechanisms of FDA-approved anti-influenza drugs and patterns of drug resistance. The authors also explored potential novel targets for the development of broad-spectrum antiviral drugs and reviews recent advancements in drug design strategies to combat drug resistance in influenza infections [3]. Antimicrobial peptides (AMPs) and their mimics can potentially be used to combat the spread of viruses and as therapeutic agents. AMPs, and more specifically antiviral peptides (AVPs), act either directly on the virus or via the host. AVPs have been shown to target steps in the viral life cycle, especially attachment, entry, and fusion. Researchers have been investigating the synthesis of new AVPs and studying their antiviral activities, as well as unraveling the underlying mechanisms by which these peptides and modified versions (peptidomimetics) exert their antiviral effects against the dengue virus [4]. Peptides are suitable antiviral drugs as they are small, highly active, specific, effective in the nanomolar range, easy to synthesize, and can easily be degraded by body peptidases thus reducing the chance of accumulation and long-term side effects. Due to the significant benefits associated with peptides, researchers have directed their attention towards identifying peptides targeting viral proteins. Furthermore, studies reported advanced techniques for validating the binding affinity of

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peptides to their respective targets [5]. Other reviews have assessed the structural characteristics of peptides with antiviral and antibacterial properties. This was achieved by examining the diverse primary and secondary structures associated with host defence peptides [6]. These previous reviews focused on the sources of peptides, the various methods that have aided in the quest for potential antiviral peptides, and their benefits. This review highlights associations between physical features of antiviral peptides such as charge, hydrophobicity and amphipathicity, with their mode of action, including interactions with viral envelopes, cellular membranes, and host immune components. By consolidating the current knowledge on cationic antiviral peptides with information on their sequence, activity, and therapeutic index, and physical features with their mode of action, this review provides valuable insights for researchers, clinicians, and drug developers designing and testing new antiviral therapies based upon AVPs.

2. Method

This is a narrative review focusing on the antiviral activities of AMPs. A summary of the literature search criteria is presented as a flow chart in Fig. 1.

2.1. Literature and Antimicrobial peptide database search

Two electronic databases, PubMed and Scopus, were used for the initial literature search and two manually curated databases, the database of Antimicrobial Activity and Structure of Peptides (DBAASP) (https://dbaasp.org/home) and the Antimicrobial Peptide Database (APD) (https://aps.unmc.edu/), were used to generate a final list of papers. In terms of literature search, Boolean search terms "cationic antimicrobial peptide" AND "virus" AND "mechanism of action" were used whereas for databases search "antiviral peptide" search term was used. Further article selection followed the PRISMA guidelines as shown in Fig. 1 [7]. All in vitro, *in vivo* or *in silico* studies that reported the antiviral activity of any peptide were included. Studies were excluded if they reported on the activity of AMPs against plant or animal (non-human) viruses, bacteria, fungus, or concerned anionic peptides. The final inclusion criteria were that the articles must be published in English and available in full-text format. Non-English articles, abstracts, letters, commentaries, reports, short communications, and synopses were excluded from this study.

3. Results

3.1. Peptide selection

The literature and database searches initially generated a total of 1769 journal articles, of which 166 were duplicates and removed. Subsequent screening of titles and abstracts of the remaining 1603 records lead to the removal of 1302 papers. A total of 246 articles were extracted excluding 55 articles that presented peptides active against animal or plant viruses (but had no information about activity against human viruses). After that, to ensure only cationic antimicrobial peptides were evaluated, each peptide identified from the search was imported into the Expasy ProtParam tool [8] from where their net charge, aliphatic index (which measures the hydrophobicity of the peptide) and GRAVY score (which measures the overall hydropathy of the peptide, with negative values indicating non-polar peptides and positive values indicating polar peptides) were extracted and imported to online tools (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_seccons.html) to determine the secondary structure of the peptides. As antimicrobial peptides are typically short (12–50 amino acids), with a

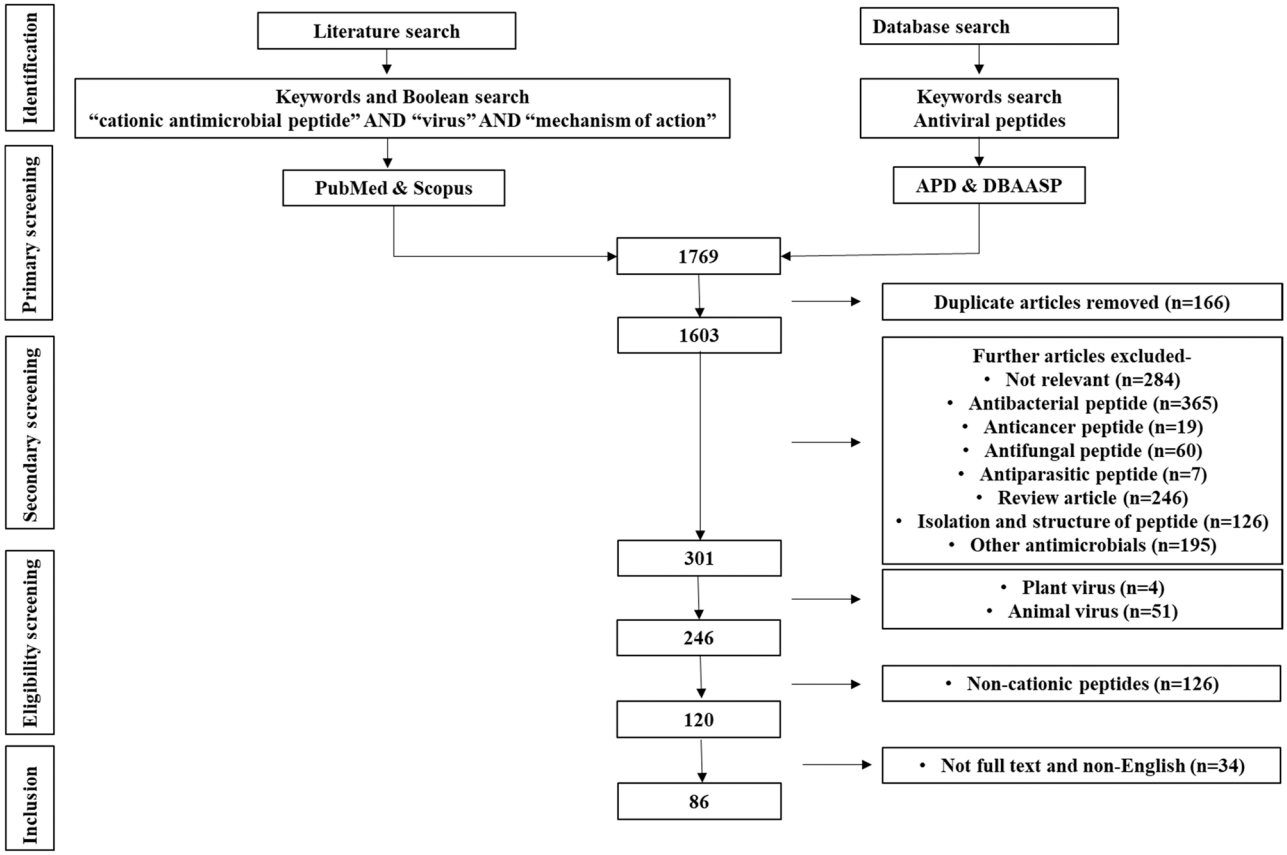

Fig. 1. A flow chart representing the collection procedures of antiviral peptides from scientific journals and databases. APD = Antimicrobial Peptide Database; DBAASP = Database of Antimicrobial Activity and Structure of Peptides.

+ 2 to + 9 charge, and amphiphilic [6], only peptides with a charge of ≥ + 2 were included in the study, and this excluded a further 126 articles. Finally, 86 articles were extracted and used for further analysis. Information about the peptides mode of action, concentration that was cytotoxic to 50% mammalian cells (CC_{50}) and the concentration that inhibited viral replication (IC_{50}), or their half maximal effective concentration (EC_{50}) was extracted from these articles so that a therapeutic index (TI) CC_{50}/IC_{50} or EC_{50}) could be calculated.

3.2. Antiviral peptides and their mechanism of action

Natural AVPs can be derived from animals, plants, marine life, amphibians, or other sources. Synthetic peptides can be produced either by synthesizing peptides, often based on information from molecular docking studies, or by using peptidomimetics (adding chemical groups or non-natural amino acids) to enhance antiviral activity. Using bioinformatics tools and protein engineering strategies, synthetic AVPs can be designed to bypass most of the limitations that occur in natural AVPs [1].

A total of 156 antimicrobial peptides with cationic properties were investigated for their antiviral activity and mode of action against multiple viruses. Twenty-one different viruses have been reported to be affected by AVPs (as defined in the current study; Fig. 2). Most studies have examined the effect of AVPs on human immunodeficiency virus (HIV) [9–38], 73 peptides demonstrated antiviral activity against HIV, 47 peptides were active against herpes viruses [39–53], 27 peptides active against hepatitis C virus (HCV) [54–59], 11 were active against human coronaviruses [39, 52, 53, 60–65], and 9 showing antiviral activity against the influenza virus [39, 61–63, 66–69]. The other viruses that have been studied were Dengue virus (DEV) [53, 70–75] (8 active peptides); Zika virus (ZIKV) [75–80] (8 active peptides); Vaccinia virus [81–83] (8 active peptides), Human papilloma virus (HPV) [51,84] (6 active peptides); Ebola virus [85] (5 active peptides); Measles virus [39, 52, 61] (3 active peptides); Junin virus [47] (2 active peptides); Enterovirus 71 (EV71) [86] (2 active peptides); Hepatitis B virus [87,88] (HBV) (2 active peptides); Human rhinovirus (HRV) [63] (2 active peptides); Japanese encephalitis virus (JEV) [79,89] (2 active peptides); BK virus [90] (1 active peptide); Adenovirus [91] (1 active peptide); Aichi virus [92] (1 active peptide); Human cytomegalovirus [93] (HCMV) (1 active peptide); and Venezuelan equine encephalitis virus (VEEV) [94] (1 active peptide).

Most (15) of these viruses are enveloped viruses, which have a lipid bilayer membrane on the outer surface of virus (HIV, Herpes virus, HCV, coronavirus, influenza virus, DEV, HBV, Ebola virus, ZKV, JEV, Vaccinia virus, Measles virus, HCMV, VEEV, Junin virus) and the remainder are non-enveloped (Aichi virus, BK virus, human adenovirus, human enterovirus, HPV, and human rhinovirus), which lack a membrane.

A summary of where some of the AVPs act to prevent replication in host cells is shown in Fig. 3. AVPs can directly target the structural components of the virus outside of the host cells or target various stages of viral replication cycle such as blocking host cell receptors, inhibiting virus binding, attachment or entry, replication, transcription, translation, other post entry processes, modulating the immune response to be protective against viruses, or preventing virus release (Fig. 3). Some AVPs can act on multiple stages. For example (Fig. 3), LL-37 has been shown to be able to directly inactive viruses (step-1), prevent viral attachment, fusion, and entry (step-3), affect post binding entry processes (step- 4 and 6) and prevent viral replication (step- 8), although they may act at each stage only for specific viral types.

3.3. Enveloped viruses

The envelope of a virus is a lipid bilayer that surrounds the viral capsid that contains its genetic material and is essential for the virus's entry into host cells, replication, and transmission. As a result, the envelope is a crucial target for antiviral agents and is a prime location for AVPs to bind to and neutralize the virus. By targeting the envelope, AVPs can prevent the virus from infecting host cells and replicating, ultimately reducing the spread of the virus. This makes the envelope an important focus for antiviral research and the development of new antiviral agents. Enveloped viruses that had been investigated for susceptibility to AVPs are discussed below.

3.3.1. Human immunodeficiency virus (HIV)

HIV is the causative agent of acquired immunodeficiency disease syndrome (AIDS). Seventy-three AVPs have been reported to be active against HIV. The AVPs listed in Table 1 most likely target the envelope of the HIV virus. Dermaseptin-S4 (isolated from frog skin) and its synthetic analogs (S4a, K4-S4, S4-(1−16)a, K4-S4-(1−16)a, S4-(1−12)a, S4-(1−9)a, and S4-(6−28)) [32], indolicidin [14] (isolated from neutrophil blood cells of cows) and a scorpion venom peptide Kn2–7 [37] directly bind with the HIV envelope when preincubated with the virus. Most of these AVPs are alpha-helical, with only a few exceptions (indolicidin and K4-S4). The AVPs K4-S4 and Kn2–7 were likely to be most appropriate to progress to further testing as they had high TI values of 12 and 13.9, respectively.

The entry steps of HIV were the second most targeted site for AVPs. The synthetic peptide T22 (derived from polyphemusin II) interfered

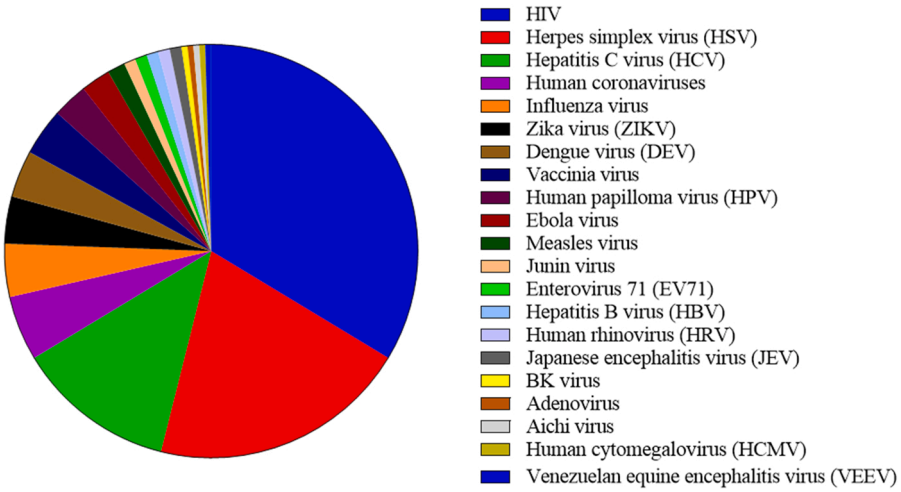

Fig. 2. The number of antiviral peptides active against various viruses. As some peptides have a broad spectrum of antiviral activity, they are counted as active against more than one virus.

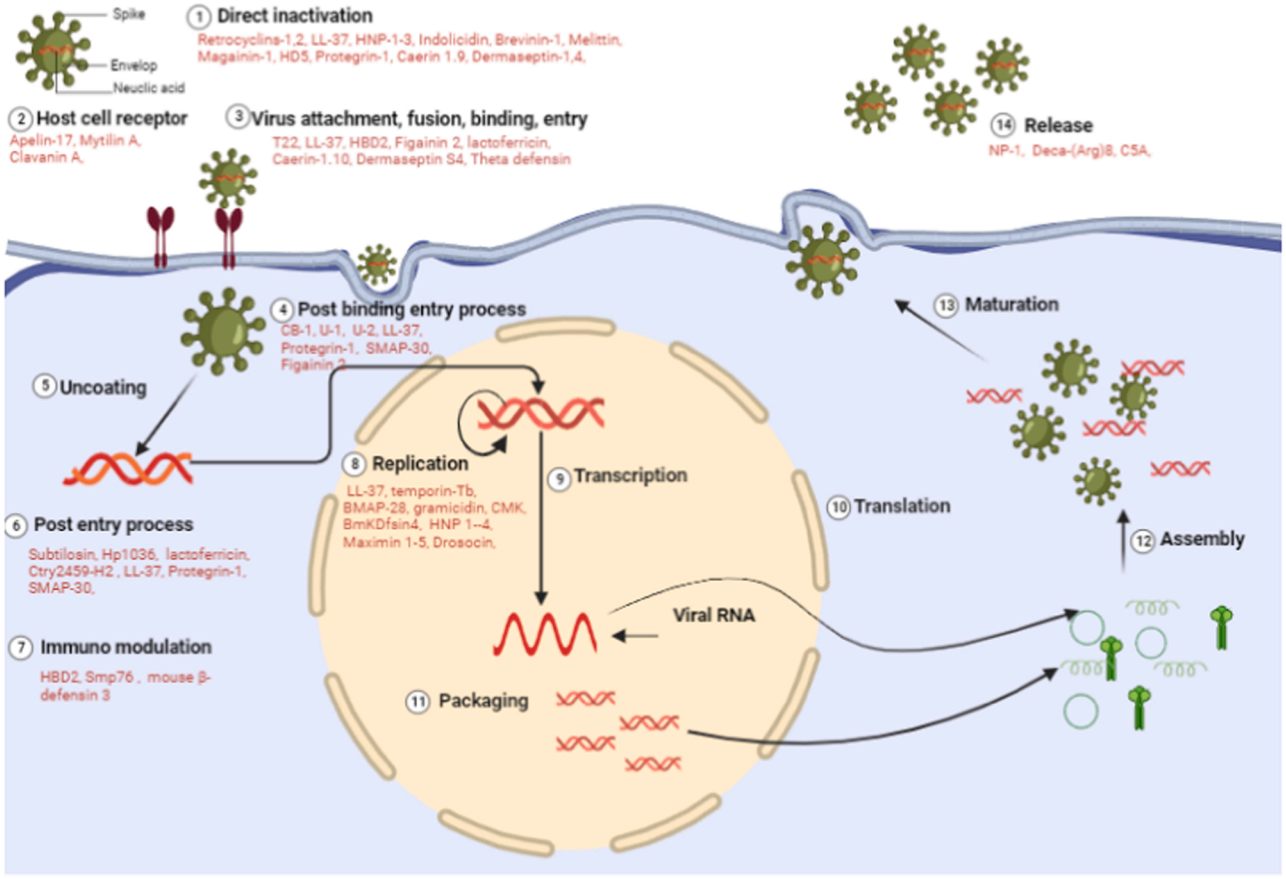

Fig. 3. Key steps in a viral replication cycle targeted by antiviral peptides; 1. Direct inactivation of virus; 2. Blocking host cell receptors; 3. Viral attachment, fusion, binding and entry processes; 4. Post binding entry processes; 5. Uncoating; 6. Post entry processes; 7. Immune modulation; 8. Viral replication; 9. Viral gene transcription; 10. Viral gene translation; 11. Packaging of viral genome; 12. Viral assembly; 13. Maturation; and 14. Virus release.

Table 1
Characteristics of AVPs that directly targeted the envelop of HIV.

Peptide name (References)	Amino acid Sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%))	CC_{50} (μM)	IC_{50} or EC_{50} (μM)	Therapeutic index (TI)
Dermaseptin-S4 [32]	ALWMTLLKKVLKAAAKAALNAVLVGANA	4	146.79	1.032	85.71/14.29	4.5	2.25	2
K4-S4 [32]	ALKMTLLKKVLKAAAKAALNA	5	140	0.667	0	16.8	1.4	12
S4a [32]	ALWMTLLKKVLKAAAKAALNA	4	140	0.81	95.24/4.76	5.6	2	2.8
S4-(1–16)a [32]	ALWMTLLKKVLKA	3	157.69	0.892	84.62/ 15.38	19.2	> 19.2	< 1
K4-S4-(1–16)a [32]	ALKMTLLKKVLKA	4	157.69	0.662	76.92/15.38	> 100	28	> 3.6
S4-(1–12)a [32]	ALWMTLLKKV	2	156	0.99	70/20	> 100	> 100	1
S4-(1–9)a [32]	ALWMTLLKK	2	141.11	0.633	55.56/ 22.22	> 100	> 100	1
S4-(6–28) [32]	ALWMTLLKKVLKAAAKAALNAVLV	4	162.92	1.217	91.67/8.33	> 100	> 100	1
Kn2–7 [37]	FIKRIARLLRKIF	5	157.69	0.554	84.62/ 15.38	23	1.65	13.9
Indolicidin [14]	ILPWKWPWWPWRR	3	60	-1.069	0/100.00	ND	35.13–52.43	ND

*GRAVY score = Grand average hydrophobicity; CC_{50} = cytotoxic concentration by 50%; EC_{50} = effective concentration reducing viral replication by 50%; IC_{50} = inhibitory concentration by 50%; TI= therapeutic index (EC_{50} or IC_{50}/CC_{50}), ND = not determined.

with gp120 and CD4 mediated virus fusion and entry, blocked the CXCR4 coreceptor and inhibited HIV infection [10–13]. The Rev-derived synthetic peptides Rev(34−50)-A(4)C and Rex(1−21) also inhibited HIV entry by blocking the CXCR4 coreceptor [35]. Human alpha-defensins (HNP 1–4), rhesus theta defensins (RTD1–3), retrocyclin-1–3 and their analogues RC-101 & RC-112 interfered in gp120 and CD4 binding [23, 24, 28–31]. Although both the peptides retrocyclin-1 and RC-112 share an identical sequence, RC-112 is composed solely of D-amino acids, while retrocyclin-1 contains only L-amino acids [24]. Retrocyclin-1 was active in a late stage of the virus fusion process most likely by inhibiting 6-helix bundle formation [25]. RC-101 also inhibited HIV entry by forming patch-like aggregates on the surface of CD4 + cells [21]. The apelin peptides 36, 17, 13 and 12 inhibited HIV entry by targeting the orphan G protein-coupled receptor APJ which serves as a coreceptor for HIV infection [20]. One synthetic peptide (Caps-MT) from the membrane-proximal helical motif of the extracellular domain of gp41 (which was an AVP as defined in the current article), blocked gp41 mediated cell fusion and entry [19].

Table 2 summarizes the results of the studies that evaluated different antiviral peptides for their potential to target the fusion and entry stage of the HIV virus. The results indicate that having an alpha-helix was rare among these AVPs, with only two peptides (Caps-MT and Rev (34−50)-A (4)C) being alpha-helical, and they had the lowest IC_{50} values (1 and 0.42 μM, respectively). The random coil structure was found to be the most prevalent secondary structure in the case of HIV entry inhibition.

HIV is transmitted via the exchange of body fluids. Perhaps due to this, most of the AVPs tested for anti-HIV activity were examined for their ability to inhibit viral replication rather than attacking the virus outside of the host cell (e.g., directly acting on its envelope). The human defensin beta-defensin (HBD)− 2, HBD-3, alpha-defensin, human neutrophil peptide (HNP)− 1, and two synthetic arginine-rich peptides (Rev(34−50)-A(4)C and Rex(1−21)) derived from the Rev protein of HIV probably worked by inhibiting replication as their action occurred after the viruses had infected cell lines [27, 28, 35], although the exact mechanisms have yet to be established. Protegrin-1(isolated from porcine neutrophil) interferes at the early stages of replication of HIV [33]. The synthetic peptides I33, EBR28 and IN-1 can inhibit HIV's integrase enzyme that the virus uses to integrate its genome into the host's during its replication cycle [22,38]. Cecropin A (derived from Tobacco budworm moth) and melittin (isolated from honeybee venom) were able to inhibit HIV-1 gene transcription, at concentrations that did not affect the host cells or the virus directly, by suppressing the activity of the long terminal repeat genes in HIV [16]. Synthetic derivatives of LL-37 and other human and bovine cathelicidins can inhibit HIV replication [34], and one mechanism was to inhibit HIV-1 reverse transcriptase [36]. Table 3 demonstrates that the characteristics of AVPs that affected HIV replication machinery varied considerably. The net charge of AVPs ranged from + 2 to + 10, the aliphatic index from 0 to 162.5 and the GRAVY score from − 2.552 to + 0.87 which represents the hydrophobicity value of peptides. Whilst most of the peptides were predicted to have an α-helical secondary structure, HBD-2, HBD-3, HNP-1 Protegrin-1, IN-1 and Rex (1−21) were predicted to have no helix. For an antiviral to be effective in human, it should have a high therapeutic index (TI; CC_{50}/ (IC_{50} or EC_{50}), i.e. the ratio of the dose that provides a therapeutic effect against a virus is greater than the dose that causes harmful or toxic effects in the host). In general, a high therapeutic index for an antiviral peptide is desirable, as it indicates a wide margin of safety between the antiviral effect and toxic effects. Determining the therapeutic index for an antiviral peptide is an important aspect of its development and clinical testing. The AVPs BMAP-18, GI-20, GI-20Q16, BMAP-18X6X10 and LL-37 showed the highest TI values of 24, 15, 15 and 12, respectively (Table 3), indicating that further evaluation of their safety and efficacy may be useful.

Antiviral activity can be measured by determining reductions in the cytopathic effect of HIV in the presence and absence of AVPs. Tachyplesin and polyphemusin derived from horseshoe crab species, and peptides synthesized from them, and Circulin C (derived from a tropical tree) had potent anti-HIV cytopathic effects [9, 15, 18]. All these peptides, except T131, had a random coil structure (Table 4), and most of them showed good therapeutic index (TI) values. T22, T131, T121, T130, T123, TW70, and T11 were the best candidates for further evaluation, with the highest TI values of 6771, 2125, 1419, 526, 480, 320, and 112, respectively. Additionally, a retrovirus protease inhibitor that was designed from the HIV Vif domain was an AVP as it had a net charge of + 3. Its characteristics are given in Table 4, however there is no information on its CC_{50}, although it does have a relatively low IC_{50} value of 3.31 μM [17].

3.3.2. *Herpes Simplex Virus 1 & 2*

Herpes simplex viruses cause mouth or genital ulcers. Several AVPs target herpes simplex viruses HSV-1 and HSV-2. Some of these AVPs directly interact with the virion structure, disrupting or disturbing membrane integrity or aggregating virion particles, including temporin L (derived from European frog), TL1 (a synthetic peptide) [39], MCP-1,2 (isolated from rabbit leukocytes) [40], MOD-1 (synthetic) [41], hectate (synthetic peptide analogue of melittin) [42], indolicidin [43,47], CAM-brevinin-1 (brevinin-1 synthetic analog) [43], dermaseptin S4 [44] and it's synthetic derivatives [51], bomodin (a recombinant AMP based upon BMAP-27) [53], and gB122 (a synthetic peptide) [50]. The net charge of these peptides ranged from + 2 to + 10, aliphatic index from 19.33 to 146.79, and the GRAVY score from − 1.121 to + 1.288. Most of the AVPs were alphahelical. Bomidin had the highest TI value of

Table 2
Characteristics of AVPs that inhibit HIV entry and fusion.

Peptide name (References)	Amino acid Sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%))	IC_{50} (μM)
Apelin-12 [20]	RPRLSHKGPMPF	3	32.5	-1.133	0/100	44.28
Apelin-13 [20]	QRPRLSHKGPMPF	3	30	-1.315	0/100	16.77
Apelin-17 [20]	KFRRQRPRLSHKGPMPF	6	22.94	-1.6	0/100	2.24
Apelin-36 [20]	LVQPRGPRSGPGPWQGGRRKFRRQRPRLSHKGPMPF	10	29.72	-1.508	0/100	0.072
HNP-1 [30]	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	3	65.33	0.3	0/73.33	ND
HNP-2 [30]	CYCRIPACIAGERRYGTCIYQGRLWAFCC	3	64.14	0.248	0/72.41	ND
HNP-3 [30]	DCYCRIPACIAGERRYGTCIYQGRLWAFCC	2	62	0.123	0/73.33	ND
HNP-4 [30,31]	VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTR	4	85	0.55	0/40.62	ND
Retrocyclin-1 [23–25]	GICRCICGRGICRCICGR	4	86.67	0.744	0	0.67
Retrocyclin-2 [30]	CICGRGICRCICGRRICR	5	86.67	0.517	0	1.13 ± 1.41
Retrocyclin-3 [30]	RICRCICGRRICRCICGR	6	86.67	0.289	0	ND
RC 101 [21,29]	GICRCICGKGICRCICGR	4	86.67	0.778	0	1.20
RC 112 [24]	GICRCICGRGICRCICGR	4	86.67	0.744	0	0.88
RTD-1 [30]	GFCRCLCRRGVCRCICTR	5	59.44	0.35	0/100	1.72 ± 0.85
RTD-3 [30]	GFCRCICTRGFCRCICTR	4	43.33	0.522	0/94.44	1.55 ± 0.58
RTD-2 [30]	GVCRCLCRRGVCRCICRR	6	75.56	0.217	0/94.44	2.46 ± 1.58
Rev (34–50)-A (4)C [35]	TRQARRNRRRRWRERQRAAAAC	9	22.73	-2.232	77.27/22.73	0.42
Rex (1–21) [35]	MPKTRRRPRRSQRKRPPTPWP	9	0	-2.552	0/100	> 10
Caps-MT [19]	APKEWMAWAREIAAYAKLIAALIKQGI	2	116.3	0.374	81.48/ 18.52	1
T22, Polyphemusin II [Tyr-5,12, Lys-7] [10–13]	RRWCYRKCYKGYCYRKCR	8	0	-1.706	0	ND

*GRAVY score = Grand average hydrophobicity; IC_{50} = inhibitory concentration by 50%.

Table 3
Characteristics of AVPs that affect the replication machinery of HIV-1.

Peptide name (References)	Amino acid Sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%))	CC_{50} (μM)	IC_{50} or EC_{50} (μM)	Therapeutic index (TI)
Protegrin-1 [33]	RGGRLCYCRRRFCVCVGR	6	53.89	-0.25	0/38.89	ND	7.77	ND
LL-37 [33,34]	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	6	89.46	-0.724	78.38/21.62	18.4	1.6	11.5
BMAP-18 [34]	GRFKRFRKKFKKLFKKIS	10	43.33	-1.25	72.22/27.78	8.45	0.35	24.1
BMAP-18X6X10 [34]	GRFKRXRKKXKKLFKKIS	10	43.33	-1.561	72.22/27.78	10.2	0.68	15
GI-20Q16 [34]	GIKQFKRIVQRIKDFLRNLV	5	126.5	-0.225	80/20	13.7	0.91	15
GF-17 [34]	GFKRIVQRIKDFLRNLV	4	125.88	-0.094	70.59/29.41	8.9	0.98	9
GI-20 [34]	GIKEFKRIVQRIKDFLRNLV	4	126.5	-0.225	80/20	22.7	1.08	21
GI-20EF [34]	GIKEFKREFQRIKDFLRNLV	3	92.5	-0.695	80/20	9.9	1.6	6.2
BMAP-18P9 [34]	GRFKRFRKPFKKLFKKIS	9	43.33	-1.122	55.56/44.44	18.9	3.2	5.9
FK-13 [34]	FKRIVQRIKDFLR	4	112.31	-0.438	61.54/23.08	10.4	3.4	3
GI-20W17 [34]	GIKEWKRIVQRIKDFLRNLV	4	126.5	-0.41	80/20	23.6	7.4	3.2
SK-21 [34]	SKEKIGKEFKRIVQRIKDFLR	5	88.1	-1.005	71.43/28.57	22.5	10.8	2
LL-23 [34]	LLGDLLRKSKEKIGKEFKRIVQR	5	114.35	-0.757	86.96/13.04	> 35.4	> 35.4	1
KR-20 [34]	KRIVQRIKDFLRNLVPRTES	4	107	-0.755	60/40	> 40.5	> 40.5	1
Retro-FK-13 [34]	RLFDKIRQVIRKF	4	112.31	-0.438	69.23/ 23.08	33.7	> 58.1	< 0.6
KR-12 [34]	KRIVQRIKDFLR	4	121.67	-0.708	58.33/25	> 63.5	> 63.5	1
HBD-2 [26–28]	GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP	6	64.15	-0.102	0/90.24	ND	ND	ND
HBD-3 [26]	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	11	67.11	-0.7	0/82.22	ND	ND	ND
HNP-1 [28]	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	3	65.33	0.3	0/73.33	ND	ND	ND
Inhibition of virus replication by suppressing gene expression								
Cecropin A [16]	RWKVFKKIEKVGRNIRDGVIKAAPAIEVLGQAKAL	6	114.29	-0.111	77.14/22.86	ND	ND	ND
Melittin [16]	GIGAVLKVLTTGLPALISWIKRKRQQ	5	135	0.273	69.23/30.77	ND	ND	ND
Inhibition of integrase								
EBR28 [22]	YQLLIRMIYKNI	2	162.5	0.417	50/25	ND	5	ND
I33 [22]	QLLIRMIYKNILFYLVPGPGHGAEPERRNIKYL	3	118.18	-0.127	51.52/ 36.36	ND	9	ND
IN-1 [38]	WQCLTLTHRGFVLLTITVLR	2	146	0.87	0/35	ND	12	ND
Inhibition of reverse transcriptase								
LL-37 [33]	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	6	89.46	-0.724	78.38/21.62	ND	15	ND
LL13–37 [36]	IGKEFKRIVQRIKDFLRNLVPRTES	4	101.2	-0.624	64/36	ND	7	ND
LL17–32 [36]	FKRIVQRIKDFLRNLV	4	133.75	-0.075	75/18.75	ND	70	ND

* , GRAVY score = Grand average hydrophobicity; CC_{50} = cytotoxic concentration by 50%; EC_{50} = effective concentration reducing viral replication by 50%; IC_{50} = inhibitory concentration by 50%; TI= therapeutic index (EC_{50} or IC_{50}/CC_{50}), ND = not determined.

16 (Table 5). Six other peptides were found to inhibit virus replication, namely hecate [42], BMAP-28 [45], melittin, magainin-1,2 [47] and AR-23 [52], however their exact mechanism of action was not specified. These replication inhibitors had charges ranging from + 3 to + 9, aliphatic indexes from 72.17 to 148.7, and GRAVY scores from + 0.083 to + 0.73, and were a alpha helical. AR-23 and melittin showed the highest TI values at 32 and 6 respectively (Table 6).

Human alpha, theta and rhesus theta defensins were reported to interfere with virus entry into host cells [48]. Retrocyclin 2 bound with HSV-2 glycoprotein to inhibit attachment whereas human alpha-defensins (HNP 1–3) showed antiviral activity when added during the post-binding period [48]. Human apolipoprotein E (apoEdp) and 11 synthetic peptides from it showed anti-HSV activity by inhibiting virus entry when cells were co incubated with both peptides and virus [49]. Rabbit alpha defensin, NP-1 showed no affinity towards virus glycoproteins or host cell receptors but still stopped virus entry and cell-to-cell transmission [46]. This peptide prevented translocation of VP16 to the cell nucleus thereby interfering with early post binding steps (fusion of the viral envelope and cell membrane). Another peptide, AR-23, was reported to inhibit HSV entry and attachment [52] and the synthetic peptide gB122 from HSV-1 glycoprotein B gave cellular protection from infection by inhibiting HSV-1 entry [50]. The overall characteristics of these peptides were presented in Table 6.

3.3.3. *Hepatitis C virus*

Worldwide hepatitis C virus remains the major cause of chronic hepatitis and liver cirrhosis [59]. Table 7 represents the overall features and mode of action of the anti-HCV peptides. Hp 1090 (isolated from scorpion venom) and six peptides designed from Hecate targeted the lipid envelope of HCV. Hp 1090 is a hydrophilic alpha-helical peptide with an IC_{50} value of 5 μM [56,58] (Table 7). Hecate and its derivatives had net charges from + 8 to + 10, aliphatic indeces of 137.2–144.78, and GRAVY scores + 0.05 to + 0.26. These peptides had IC_{50} values ranging from 2.6 to 34.24 μM (Table 7). Scorpion defensin peptide BmKDfsin3 inhibited HCV attachment and subsequent replication by suppressing p38 activation during infection thereby regulating P38 mitogen-activated protein kinase (MAPK) signaling pathway [59]. Ten cationic hydrophobic peptides [54,55] targeted a specific region of the HCV polyprotein and prevented NS3 protease activation. All these synthetic peptides showed a net positive charge between + 4 to + 5, an aliphatic index between 104.62 and 156.92, and a GRAVY score between + 0.069 and + 0.815. Most of the peptides were predicted to have an alpha-helix structure (Table 7). Peptides P49, P50 and 33 had the lowest IC_{50} values of 3–3.4 μM (Table 7). A study evaluating the anti-HCV effect of human-alpha, β, and synthetic linear avian alpha-defensins found that human defensins were more active than avian ones, providing cellular protection, neutralization of viruses, and allowing recovery of any HCV-infected cells [57]. Human-alpha and

Table 4
Characteristics of AVPs which reduced HIV induced cytopathic effect or protease inhibition.

Peptide name (References)	Mode of action/amino acid Sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%))	CC_{50} (μM)	IC_{50} or EC_{50} (μM)	Therapeutic index (TI)
Reduction of HIV induced cytopathic effect								
T22 [9]	RRWCYRKCYK GYCYRKCR	8	0	-1.706	0/100.00	21.67	0.0032	6771.8
Tachyplesin I [9]	KWCFRVCYRGICYRRCR	6	40	-0.518	0/ 58.82	12.78	3.70	3.5
Tachyplesin II [9]	RWCFRVCYRGICYRKCR	6	40	-0.518	0/41.18	15.87	2.91	5.5
Polyphemusin I [9]	RRWCFRVCYRGFCYRKCR	7	16.11	-0.833	0/44.44	13.83	2.40	5.8
Polyphemusin II [9]	RRWCFRVCYKGFCYRKCR	7	16.11	-0.8	0/55.56	13.57	0.78	17.4
T5 [9]	WCFRVCYRGICYRRCR	5	42.5	-0.306	0/37.50	17.28	4.11	4
T8 [9]	KWCFRVCFRGICFRRCR	6	40	-0.035	0/47.06	12.07	2.41	5
T9 [9]	KWCFRVAYRGIAYRRCR	6	51.76	-0.6	0/70.59	14.06	2.63	5.34
T11 [9]	RWCYRKCYRGICYRKCR	7	22.94	-1.235	0/64.71	16.86	0.15	112.4
T12 [9]	WCYRKCYRGICYRKCR	6	24.38	-1.031	0/ 93.75	95.47	3.19	29.9
T13 [9]	AWCYRKCYRGICYRKCR	6	28.82	-0.865	0/100	134.60	3.09	43.6
TW70 [15]	RRWCYRKKPYRKCR	8	0	-2.45	0/100.00	8	0.025	320
T121 [15]	RRWCYRKKGYRKCR	8	0	-2.364	0/100.01	45.42	0.032	1419.4
T123 [15]	RRWCYRKKGYRECR	6	0	-2.336	0/85.71	81.62	0.17	480
T130 [15]	RRWCYRKKGERKCE	5	0	-2.45	0/71.43	57.95	0.11	526.8
T131 [15]	RRYCYRKKPYRKCR	8	0	-2.479	0	42.50	0.02	2125
circulin c [18]	NGIPCGESCVWIPCITSVAGCSCKSKVCYR	2	71.33	0.437	0/86.67	ND	0.048	ND
Inhibition of protease								
Vif 88–98 [17]	EWRKKRYSTQV	3	26.36	-2.118	0/100	ND	3.31	ND

* , GRAVY score = Grand average hydrophobicity; CC_{50} = cytotoxic concentration by 50%; EC_{50} = effective concentration reducing viral replication by 50%; IC_{50} = inhibitory concentration by 50%; TI= therapeutic index (EC_{50} or IC_{50}/CC_{50}), ND = not determined.

Table 5
Characteristics of AVPs that directly target the envelop of herpes simplex virus.

Peptide name (References)	Amino acid Sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%))	CC_{50} (μM)	IC_{50} or EC_{50} (μM)	Therapeutic index (TI)
DRS-S1 [51]	ALWKTMLKKLGTMALHAGKAALGAAADTISQGTQ	3	92.35	0.185	88.24/11.76	25	3	8.3
[Thr4Lys12]-DRS-S1(1–29)-NH2 [51]	ALWTTMLKKLGKMALHAGKAALGAAADTI-NH_2	3	108.28	0.524	89.66/10.35	20	1.3	15.4
DRS-S1(1–29)-NH2 [51]	ALWKTMLKKLGTMALHAGKAALGAAADTI-NH_2	3	108.28	0.524	89.66/10.34	20	1.7	11.8
[1Nal3]-DRS-S1 (1–15)-NH2 [51]	AL-1Nal-KTMLKKLGTMAL-NH_2	3	125.71	0.65	100/0	50	2.7	18.5
[His3]-DRS-S1 (1–15)-NH2 [51]	ALHKTMLKKLGTMAL-NH_2	3	117.33	0.393	93.33/ 6.67	> 100	5	> 20
[Lys01Nal3]-DRS-S1(1–15)-NH2 [51]	KAL-1Nal-KTMLKKLGTMAL-NH_2	4	117.33	0.394	93.33/ 6.67	50	5	10
DRS-S1(1–19)-NH2 [51]	ALWKTMLKKLGTMALHAGK-NH_2	4	97.89	0.132	84.21/15.79	80	9	8.9
DRS-S1(1–13)-NH2 [51]	ALWKTMLKKLGTM-NH_2	3	97.69	0.2	69.23/15.38	100	10	10
DRS-S1(1–14)-NH2 [51]	ALWKTMLKKLGTMA-NH_2	3	97.86	0.314	92.86/7.14	85	10.5	8
DRS-S1(1–12)-NH2 [51]	ALWKTMLKKLGT-NH_2	3	105.83	0.058	66.67/25	25	ND	ND
MOD-1 [41]	KLWKKWAKWLKLWLAW	5	110	-0.325	0	18	4.6	3.9
Temporin L[39]	FVQWFSKFLGRIL	2	112.31	0.823	53.85/15.38	19.61	8.55	2.3
TL-1 [39]	FVPWFSKFLGRIL	2	112.31	0.969	46.15/23.08	42.18	9.99	4.2
Bomidin [53]	MGRFKRFRKKFKKLFKKLS	10	41.05	-1.121	78.95/21.05	169.6	10	17
gB122 [50]	GHRRYFTFGGGYVYF	2	19.33	-0.387	0/33.33	ND	138	ND
Indolicidin [43,47]	ILPWKWPWWPWRR	3	60	-1.069	0/100.00	ND	29,360.08	ND
CAM brevinin-1 [43]	FLPVLAGIAAKVVPALFCKITKKC	4	134.17	1.288	79.17/20.83	ND	39,322.60	ND
Dermaseptin-S4 [44]	ALWMTLLKKVLKAAAKAALNAVLVGANA	4	146.79	1.032	85.71/14.29	ND	ND	ND
MCP-1 [40]	VVCACRRALCLPRERRAGFCRIRGRIHPLCCRR	9	85.76	-0.112	6.06/81.82	ND	ND	ND
MCP-2 [40]	VVCACRRALCLPLERRAGFCRIRGRIHPLCCRR	8	97.58	0.139	9.09/ 78.79	ND	ND	ND
Hecate [42]	FALALKALKKALKKLKKALKKAL	9	144.78	0.222	95.65/4.35	ND	ND	ND

* , GRAVY score = Grand average hydrophobicity; CC_{50} = cytotoxic concentration by 50%; EC_{50} = effective concentration reducing viral replication by 50%; IC_{50} = inhibitory concentration by 50%; TI= therapeutic index (EC_{50} or IC_{50}/CC_{50}), ND = not determined.

Table 6
Characteristics of AVPs targeting HSV following different mode of actions.

Peptide name (References)	Mode of action/ amino acid sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%))	CC_{50} (μM)	IC_{50} or EC_{50} (μM)	Therapeutic index (TI)
Inhibition of replication								
AR-23 [52]	AIGSILGALAKGLPTLISWIKNR	3	148.7	0.73	69.57/30.43	25	0.78	32
Melittin [47]	GIGAVLKVLTTGLPALISWIKRKRQQ	5	135	0.273	69.23/30.77	8.51	1.35	6.3
Magainin 2 [47]	GIGKFLHSAKKFGKAFVGEIMNS	3	72.17	0.083	78.26/ 21.74	> 100	22.16	> 4.5
Magainin 1 [47]	GIGKFLHSAGKFGKAFVGEIMKS	3	72.17	0.217	60.87/39.13	> 100	36.59	> 2.7
BMAP-28 [45]	GGLRSLGRKILRAWKKYGPIIVPIIRI	7	144.44	0.256	40.74/40.74	ND	ND	ND
Hecate [42]	FALALKALKKALKKLKKALKKAL	9	144.78	0.222	95.65/4.35	ND	ND	ND
Inhibition of virus adhesion and entry								
ApoEdpL-W [49]	WRKWRKRWWWRKWRKRWW	10	0	-2.767	0	ND	3.1	ND
MU92 (Hepta 1 dp) [49]	RWWRWWRRWWRWWR	6	0	-2.443	0	ND	3.3	ND
RLLR5 [49]	RLLRRLLRRLLRRLLRRLLR	10	195	-0.35	0	ND	<3.3	ND
MU94 (Deca 1 dp) [49]	YRWWRWARRWYRWWRWARRW	8	10	-2.11	0	ND	5	ND
N-[RLLR]3 [49]	APKAMRLLRRLLRRLLR	7	149.41	-0.247	82.35/17.65	ND	7.3	ND
MU89 (Octa 1 dp) [49]	RRWYRWWRRRWYRWWR	8	0	-2.75	0	ND	8.3	ND
MU104 [49]	RRRRRRRRRRRRRRWWW	15	0	-3.9	0/ 77.78	ND	9.25	ND
Deca 1 [49]	YRWWRWARRW	4	10	-2.11	50/40	ND	> 20	ND
Hepta 1 [49]	RWWRWWR	3	0	-2.443	0/42.86	ND	> 20	ND
MU103 [49]	RRRRRRRWWWRRRRRRRR	15	0	-3.9	0	ND	> 20	ND
Octa 1 [49]	RRWYRWWR	4	0	-2.75	0/50	ND	> 20	ND
HNP-1 [48]	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	3	65.33	0.3	0/73.33	ND	ND	ND
HNP-2 [48]	CYCRIPACIAGERRYGTCIYQGRLWAFCC	3	64.14	0.248	0/72.41	ND	ND	ND
HNP-3 [48]	DCYCRIPACIAGERRYGTCIYQGRLWAFCC	2	62	0.123	0/73.33	ND	ND	ND
NP-1 [46]	VVCACRRALCLPRERRAGFCRIRGRIHPLCCRR	9	85.76	-0.112	6.06/ 81.82	ND	ND	ND
Retrocyclin-2 [48]	CICGRGICRCICGRRICR	5	86.67	0.517	0	ND	ND	ND
Retrocyclin 1 [48]	GICRCICGRGICRCICGR	4	86.67	0.744	0	ND	ND	ND
RTD-3 [48]	GFCRCICTRGFCRCICTR	4	43.33	0.522	0/94.44	ND	ND	ND
gB122 [50]	GHRRYFTFGGGYVYF	2	19.33	-0.387	0/33.33	ND	18	ND
AR-23 [52]	AIGSILGALAKGLPTLISWIKNR	3	148.7	0.73	69.57/30.43	25	3.125	8
Inhibition of virus attachment								
AR-23 [52]	AIGSILGALAKGLPTLISWIKNR	3	148.7	0.73	69.57/30.43	25	6.25	4
Cell line pretreatment to inhibit infection								
gB122 [50]	GHRRYFTFGGGYVYF	2	19.33	-0.387	0/33.33	ND	72	ND
Inhibition of cell-to-cell transmission								
NP-1 [46]	VVCACRRALCLPRERRAGFCRIRGRIHPLCCRR	9	85.76	-0.112	6.06/ 81.82	ND	ND	ND

* , GRAVY score = Grand average hydrophobicity; CC_{50} = cytotoxic concentration by 50%; EC_{50} = effective concentration reducing viral replication by 50%; IC_{50} = inhibitory concentration by 50%; TI= therapeutic index (EC_{50} or IC_{50}/CC_{50}), ND = not determined.

recombinant beta defensins had net positive charges ranging from + 2 to + 11, aliphatic indexes between 46.11 and 85, and GRAVY scores between − 1.008 and + 0.55. All alpha defensins were hydrophobic, whereas all synthetic beta defensins were hydrophilic. Additionally, the structure of all these peptides were random coils (Table 7).

3.3.4. *Human coronaviruses*

The human coronaviruses SARS-CoV, SARS-CoV-2 and MERS-CoV infect host cells through the fusion of the virus spike protein with a host cell receptor, angiotensin-converting enzyme 2 (ACE-2 for SARS-CoV and SARS-CoV-2) [65] and dipeptidyl-peptidase 4 (DPP4 for MERS-CoV) [95] and have caused epidemics and pandemics of severe respiratory disease. AVPs active against human coronaviruses showed four modes of action: direct inactivation, inhibition of virus entry, inhibition of replication, and immunomodulation. Mucroporin-M1 (synthetic analog of mucroporin) directly interacts with the SARS-CoV envelope [61] and TL-1 (a temporin L analog) directly inactivates SARS-CoV-2 [39]. Peptides AR-23 (isolated from skin secretion of frog) [52] and bomidin [53] also directly interact with the SARS-CoV-2 envelope to inhibit infection. Human cathelicidin (LL-37) and alpha and theta defensins interfere with the SARS-CoV-2 entry process [64,65]. LL-37 inhibits virus entry by binding with both the receptor binding domain of the spike protein and the ACE2 receptor on the host cell [65].

Synthetic AVPs P9 and P9R bind to SARS-CoV, SARS-CoV-2 and MERS-CoV glycoproteins to prevent endosomal acidification, blocking membrane fusion and RNA release [62,63]. SARS-CoV infected mice treated with rhesus theta defensin-1 were protected and had reduced a mortality rate as the result of immunomodulation [60]. The cytokine responses observed in lung tissue homogenates at 2 and 4 days after infection demonstrated elevated levels of interleukin-6 (IL-6), keratinocyte chemoattractant, and granulocyte colony-stimulating factor in mice infected with SARS-CoV and treated with RTD-1, as compared to mice infected with SARS-CoV alone. Although RTD-1 did not show any significant direct inhibitory effect on the virus titer in vivo or in vitro, the altered cytokine responses appear to have impacted the outcome of the disease [60].

Table 8 presents the characteristics of peptides that directly target the coronavirus envelope. These peptides have a net charge ranging from + 2 to + 10, aliphatic index ranging from 41.05 to 160.59, and GRAVY scores ranging from − 1.121 to + 0.969. Most of the antiviral peptides (AVPs) have an alpha helix structure, except for P9 and P9R, which have the highest TI values of 158 and > 333, respectively. On the other hand, human defensins and cathelicidin, which act as virus entry inhibitors, have a positive charges ranging from + 3 to + 6, aliphatic indeces ranging from 64.06 to 89.46, and GRAVY scores ranging from − 0.724 to + 0.778. Among these, human alpha defensins have a random coil structure, theta defensins have neither an alpha helix nor a random coil structure, and LL-37 has an alpha helix structure. Additionally, the immunomodulating peptide RTD-1 has a net charge of + 5, aliphatic index of 59.44, and GRAVY score of + 0.35. It has a random coil secondary structure.

Table 7
Characteristics and mode of action of Anti-HCV peptides.

Peptide name (References)	Mode of action/ amino acid sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%)	CC_{50} (μM)	IC_{50} or EC_{50} (μM)	Therapeutic index (TI)
Inhibition of NS3 protease and NS2/3 Processing								
P49 [55]	KKKKAVAATYVLV	4	120	0.323	0/38.46	ND	3	ND
P50 [55]	KKKKVAAATYVLV	4	120	0.323	0/30.77	ND	3	ND
P90 [55]	KKKKVTAATYVLV	4	112.31	0.131	0/30.77	ND	4.3	ND
P83 [55]	KKKKVVKATYVLV	5	126.92	0.069	0/30.77	ND	6.5	ND
P163 [55]	KKKKVVLPFLFFF	4	104.62	0.769	0	ND	7	ND
P63 [55]	KKKKFVAATYVLV	4	112.31	0.4	0/30.77	ND	7	ND
P58 [55]	KKKKVVLATYVLV	4	156.92	0.662	0/30.77	ND	7.5	ND
P165 [55]	KKKKLLAAFLFFF	4	105.38	0.815	0	ND	7.6	ND
P179 [55]	KKKKLVLPFLFVF	4	134.62	0.846	0	0	8.3	ND
33 [54]	KGSVVIVGRIILSGRK	4	151.87	0.644	0/25	ND	5.7	ND
37 [54]	RGGSVVIVGRIILSGRK	4	142.94	0.547	0/41.18	ND	3.4	ND
Cell line pretreatment to reduce infection, neutralization, & treatment to infected cell								
HNP-1 [57]	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	3	65.33	0.3	0/73.33	ND	ND	ND
HNP-2 [57]	CYCRIPACIAGERRYGTCIYQGRLWAFCC	3	64.14	0.248	0/72.41	ND	ND	ND
HNP-3 [57]	DCYCRIPACIAGERRYGTCIYQGRLWAFCC	2	62	0.123	0/73.33	ND	ND	ND
HNP-4 [57]	VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTR	4	85	0.55	0/40.62	ND	ND	ND
RHBD-1 [57]	DHYNCVSSGG QCLYSACPIF TKIQGTCYRG KAKCCK	4	46.11	-0.272	0/91.67	ND	ND	ND
RHBD-2 [57]	GIGDPVTCLK SGAICHPVFC PRRYKQIGTC GLPGTKCCKK P	6	64.15	-0.102	0/90.24	ND	ND	ND
RHBD-3 [57]	GIINTLQKYY CRVRGGRCAV LSCLPKEEQI GKCSTRGRKC CRRKK	11	67.11	-0.7	0/82.22	ND	ND	ND
RHBD-4 [57]	EFELDRICGY GTARCRKKCR SQEYRIGRCP NTYACCLRKW DESLLNRTKP	6	50.8	-1.008	42/58	ND	ND	ND
Directly targeted envelop/membrane								
Hecate [58]	FALALKALKKALKKLKKALKKAL	9	144.78	0.222	95.65/4.35	12.17	8.57	1.4
Lys-Hecate [58]	K-FALALKALKKALKKLKKALKKAL	10	138.75	0.05	91.67/ 8.33	7.97	2.6	3
Glu-Hecate [58]	E-FALALKALKKALKKLKKALKKAL	8	138.75	0.067	95.83/4.17	9.12	3.30	2.7
Acetyl-Hecate [58]	Acetyl-FALALKALKKALKKLKKALKKAL	9	144.78	0.222	95.65/4.35	9.43	3.79	2.5
GA-Hecate [58]	GA-FALALKALKKALKKLKKALKKAL	9	137.2	0.26	92/8	34.95	11.18	3.1
GA2-Hecate [58]	GA2-FALALKALKKALKKLKKALKKAL	9	137.2	0.26	92/8	> 100	34.24	> 2.9
Hp1090 [56]	IFKAIWSGIKSLF	2	127.69	1.077	61.54/15.38	ND	5	ND
Inhibition of virus replication								
BmKDfsin3 [59]	GFGCPFNQGKCHRHCRSIRRRGGYCDGFLKQRCVCYRK	9	28.16	-0.924	0/ 92.11	61.99	4.36	14.2

* , GRAVY score = Grand average hydrophobicity; CC_{50} = cytotoxic concentration by 50%; EC_{50} = effective concentration reducing viral replication by 50%; IC_{50} = inhibitory concentration by 50%; TI= therapeutic index (EC_{50} or IC_{50}/CC_{50}), ND = not determined.

Table 8
Characteristics and mode of actions of AVPs that are active against human coronaviruses.

Peptide name (References)	Mode of action/amino acid Sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%))	CC_{50} (μM)	IC_{50} or EC_{50} (μM)	Therapeutic index (TI)
Directly targeted envelop/membrane								
P9R [63]	NGAICWGPCPTAFRQIGNCGRFRVRCCRIR	6	55.33	-0.15	0/70	> 87.92	0.26	> 338
P9 [62,63]	NGAICWGPCPTAFRQIGNCGHFKVRCCKIR	5	55.33	-0.067	0/ 73.33	113.88	0.72	158
TL-1 [39]	FVPWFSKFLGRIL	2	112.31	0.969	46.15/23.08	42.18	4.62	9.1
mucroporin-M1 [61]	LFRLIKSLIKRLVSAFK	5	160.59	0.794	88.24/11.76	34.70	7.12	4.9
AR-23 [52]	AIGSILGALAKGLPTLISWIKNR	3	148.7	0.73	69.57/30.43	25	12.5	2
Bomidin [53]	MGRFKRFRKKFKKLFKKLS	10	41.05	-1.121	78.95/21.05	169.6	ND	ND
Inhibition of virus entry								
RC-101 [65]	GICRCICGKGICRCICGR	4	86.67	0.778	0	ND	2.48	ND
HNP-1 [65]	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	3	65.33	0.3	0/73.33	ND	ND	ND
HD5 [65]	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR	4	64.06	-0.113	0/65.62	ND	ND	ND
LL-37 [64]	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	6	89.46	-0.724	78.38/21.62	ND	ND	ND
Immunomodulation to reduce infection in vivo								
RTD-1 [60]	GFCRCLCRRGVCRCICTR	5	59.44	0.35	0/100	ND	ND	ND

* , GRAVY score = Grand average hydrophobicity; CC_{50} = cytotoxic concentration by 50%; EC_{50} = effective concentration reducing viral replication by 50%; IC_{50} = inhibitory concentration by 50%; TI= therapeutic index (EC_{50} or IC_{50}/CC_{50}), ND = not determined.

3.3.5. *Influenza virus*

Antigenic shifts and drifts in the influenza genome make it challenging to manage. There are several anti-influenza drugs and vaccines available, but strains can become resistant over time. The AVPs TL-1 [39], P9 [62,63], P9R [63], Urumin (isolated from the skin of south Indian frog) [67] and mucroporin-M1 [61] disrupt the virus envelop and

interfere with endosomal acidification. The AVPs BF-30 (isolated from snake venom), retrocyclin-2 and HBD-3 inhibit virus entry by blocking hemagglutinin-mediated fusion [66,69]. Short synthetic lipopeptides such as S-KKWK can inhibit virus entry by interfering with the conformational rearrangements of the hemagglutinin-2 subunit [68].

Peptides that directly target the influenza virus envelope have a net charge ranging from + 2 to + 6, aliphatic indeces ranging from 55.33 to 160.59, and GRAVY scores ranging from − 0.15 to + 0.794 (Table 9). Most of these antiviral peptides have a random coil structure, except for mucroporin-M1 and TL-1. All the peptides showed good antiviral activity, with IC_{50} values ranging from 0.26 to 6.74. BF-30, HBD-3, and retrocyclin-2 act as fusion inhibitors and have a positive charge ranging from + 5 to + 11, aliphatic index ranging from 67.11 to 86.67, and GRAVY scores ranging from − 0.7 to + 0.517. Among these, HBD-3 has a random coil structure, theta defensins have neither an alpha helix nor a random coil structure, and BF-30 has an alpha helix structure. In addition, one lipopeptide that acts as an entry inhibitor has a net positive charge of 3, an aliphatic index of 0, and a GRAVY score of − 2.68. It has a random coil secondary structure with a lower IC_{50} value of 2.52 (Table 9).

3.3.6. Zika virus

Eight AVPs have been identified with the ability to inhibit Zika virus infection. The AVP brevinine-2GHk (isolated from skin of southeast Asian frog) disrupts the envelope integrity by binding with the ZIKV E protein [80]. Hc-CATH, a cathelicidin from snake skin, targets the envelope and interferes with the downregulation of the AXL kinase receptor. AXL plays an important role in ZIKV infection either by acting as a cofactor for the entry of ZIKV to host cells or by regulating the innate immune response of host cells by negatively regulating interferon (IFN) signaling and the expression of inflammatory factors [76]. Both mouse cathelicidin CRAMP and human cathelicidin LL-37 directly bind with the virions and cause increased leakage of genomic material (RNA). These cathelicidins also inhibit Zika virus replication if they are applied to cells after entry of the virus, but the specific target site is unknown [77]. Two other synthetic cathelicidins GF-17 and BMAP-18 show anti-Zika activity when preincubated with the virus (presumably by affecting its envelop, but this has yet to be verified) and stimulating the interferon pathway to provide protection [78]. The AVP RC-101 and An1a (identified from spider venom) inhibit NS2B-NS3 serine protease. The NS2B-NS3 protease is a complex of two viral proteins, NS2B and NS3, that work together to cleave the viral polyprotein at specific sites. The viral precursor polyprotein is a long chain of amino acids that is translated from the viral RNA and needs to be cleaved into smaller functional proteins in order for the virus to replicate and produce new virus particles [75,79]. RC-101 also binds with the DE loop of the E protein, preventing virus entry [79].

Antiviral peptides that directly target Zika virus have a positive net charge ranging from + 4 to + 12, an aliphatic index between 43.33 and 125.88, and a GRAVY score from − 1.25 to + 0.033. All these AVPs have an alpha helix secondary structure. Two cathelicidins, CRAMP and LL-37, that inhibit virus replication have a positive net charge of + 4 and + 6, an aliphatic index of 91.76 and 89.46, and a GRAVY score of − 0.682 and − 0.724, respectively. Both peptides also have an alpha helix secondary structure. The AVP immunomodulators during Zika virus infection contain two members, BMAP-18, and GF-17, which have a positive net charge of + 10 and + 4, an aliphatic index of 43.33 and 125.88, and a GRAVY score of − 1.25 and − 0.094, respectively with an alpha helix secondary structure. Two peptides, An1a and RC-101, act specifically on the NS2B-NS3 protease, with RC-101 also acting as an entry inhibitor. An1a has mostly random coil sheet secondary structure, while RC-101 does not have an alpha helix or random coil structure. An1a has a positive net charge of + 3, an aliphatic index of 29.72, and a GRAVY score of − 0.481, while RC-101 has a positive net charge of + 4, an aliphatic index of 86.67, and a GRAVY score of + 0.778. (Supplementary Table 1).

3.3.7. Dengue virus

Eight antiviral peptides were found to have anti-dengue virus activity. Bomidin directly targeted the dengue virus by interacting with its envelope and disrupting the integrity of the virion [53]. Two synthetic peptides DN57opt and 1OAN1 inhibited virus binding and fusion with the host cell and caused structural modifications of the virus [70]. During dengue infection, there is an increased expression of the β3 integrin receptor on the surface of the host cell, which the virus uses to gain entry. The β3 integrin receptor interacts directly with the binding domain of the viral E protein located specifically on domain III of the envelope protein (EDIII). LL-37 inhibited virus entry by binding to the E protein, while P7 (a synthetic peptide) interfered with the interaction between β3 integrin and EDIII [73,74]. The AVPs protegrin-1, latarcin-1 (isolated from spider venom) and An1a inhibited dengue virus replication by targeting NS2B-NS3 serine protease. The NS2B/NS3 protease is a serine protease that belongs to the chymotrypsin family, and it is responsible for cleaving the viral precursor polyprotein at 8 out of 13 specific cleavage sites. This proteolytic processing is critical for the maturation of the viral particle and the production of functional viral

Table 9
Characteristics and mode of actions of anti-influenza virus peptides.

Peptide name (References)	Mode of action/amino acid Sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%))	CC_{50} (μM)	IC_{50} or EC_{50} (μM)	Therapeutic index (TI)
Directly targeted envelop/membrane								
P9R [63]	NGAICWGPCPTAFRQIGNCGRFRVRCCRIR	6	55.33	-0.15	0/70	> 87.92	0.26	> 338
P9 [62,63]	NGAICWGPCPTAFRQIGNCGHFKVRCCKIR	5	55.33	-0.067	0/ 73.33	113.87	0.36	316.3
mucroporin-M1 [61]	LFRLIKSLIKRLVSAFK	5	160.59	0.794	88.24/11.76	34.70	1.03	33.7
Urumin [67]	IPLRGAFINGRWDSQCHRFSNGAIACA	2	72.59	-0.033	0/81.48	2450	3.8	644.7
TL-1 [39]	FVPWFSKFLGRIL	2	112.31	0.969	46.15/23.08	42.18	6.74	6.3
Inhibition of fusion								
BF-30 [69]	KFFRKLKKSVKKRAKEFFKKPRVIGVSIPF	11	71.33	-0.537	56.67/23.33	67.7	5.2	13
HBD-3 [66]	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	11	67.11	-0.7	0/82.22	ND	ND	ND
Retrocyclin-2 [66]	CICGRGICRCICGRRICR	5	86.67	0.517	0	ND	ND	ND
Inhibition of virus entry								
Lipopeptide [68]	S-KKWK	3	0	-2.68	0/80	43.59	2.52	17.3

* , GRAVY score = Grand average hydrophobicity; CC_{50} = cytotoxic concentration by 50%; EC_{50} = effective concentration reducing viral replication by 50%; IC_{50} = inhibitory concentration by 50%; TI= therapeutic index (EC_{50} or IC_{50}/CC_{50}), ND = not determined.

proteins necessary for viral replication [71, 72, 75].

The charge of these peptides ranged from + 2 to + 10, with an aliphatic index of 29.72–76.43 and GRAVY score of − 0.25 to − 1.248. All peptides were hydrophilic, with equal prominence of alpha-helix and random coil structures (Supplementary Table 2).

3.3.8. Other enveloped viruses

Data for the AVPs that act against other enveloped viruses can be found in Supplementary Table 3. For the hepatitis B virus, two AVPs, mucroporin-M1 and BmKDfsin4 (a scorpion defensin), had antiviral activity. Mucroporin-M1 inhibited virus replication by inducing the mitogen-activated protein kinase (MAPK) pathway and downregulating HNF4α expression, while BmKDfsin4 inhibited virus DNA and protein production [87,88]. Mucroporin-M1 is a hydrophobic peptide with an alpha helical structure and a TI value of 7.9, while BmKDfsin4 is hydrophilic with a random coil structure with a much higher TI value of 103.6 (Supplementary Table 3).

LL-37 and its derivatives have activity against Ebola virus. These AVPs impair cathepsin B-mediated processing of the Ebola virus glycoprotein, which is necessary for the virus to enter and infect host cells [85]. The peptides are all alpha helixes and share properties such as a net charge ranging from + 4 to + 6, an aliphatic index ranging from 89.46 to 126.5, and a GRAVY score ranging from − 0.724 to − 0.094. Among these peptides, the GI-20d* and 17-BI derivatives had higher TI values than other peptides, indicating that they have greater potential as antiviral agents against Ebola (Supplementary Table 3).

The AVPs mucroporin-M1, TL-1 and AR-23 have activity against measles virus. Mucroporin-M1 disrupts the virus envelope [61]. The AVPs TL-1 and AR-23 directly inactivated the virus [39,52] but the mechanism has not been investigated in detail. These AVPs have a positive net charge of + 2 to + 5, an aliphatic index within the range of 112.31–160.59, and a GRAVY score from + 0.73 to + 0.969. This means that they are relatively hydrophobic and contain more positively charged amino acids than negatively charged ones. All three peptides have an alpha helical structure. The results show that Mucroporin-M1 and AR-23 were found to be more potential AVPs, with TI values of 9.8 and 8, respectively compared to TL-1 which showed a lower TI value of less than 0.8 [39, 52, 61] (Supplementary Table 3).

Synthetic peptide HD5(1−9) has been found to inhibit the entry and attachment of human cytomegalovirus to host cells. It has a net positive charge of + 2, an aliphatic index of 11.11, and a GRAVY score of − 0.589. Its structure is a random coil, which means that it lacks a fixed and ordered conformation and is more flexible. The reported IC_{50} value of HD5(1−9) is 40 μM, and its CC_{50} is 150 μM, indicating a therapeutic index (TI) of 3.75. These characteristics are significant because they show the potential of HD5(1−9) as an antiviral agent, specifically against HCMV [94] (Supplementary Table 3).

LL-37 has been found to be active against Venezuelan equine encephalitis virus (VEEV) by inhibiting its replication and entry into host cells. However, the authors did not report the exact IC_{50} value. Nonetheless, the finding that LL-37 is active against VEEV is significant, as it suggests that this AVP may have potential as an antiviral agent against this virus [94]. Two AVPs, P2 (synthetic peptide) and RC-101 inhibited Japanese encephalitis virus (JEV) infection, with P2 being directly active against the virus with an IC_{50} of 0.0941 μM [89]. RC-101 inhibited this virus entry and replication into the cell with an IC_{50} values of 10.67 μM [79] (Supplementary Table 3).

Eight peptides were active against vaccinia virus. The AVPs LL-37, magainin-2B (synthetic analog of magainin), uperin-3.1 (isolated from dorsal glands of the Australian floodplain frog), CaLL (a synthetic form of cecropin-LL37 hybrid), and three synthetic peptides PD3, PD4, and RW3 targeted the envelope of the virion and inhibited infection [82,83]. These peptides have a positive net charge ranging from + 2 to + 8, an aliphatic index ranging from 0 to 143.33, and a GRAVY score ranging from − 2.7 to + 0.9. Except for RW3, all the peptides have an alpha helix structure, which is a common structural motif in proteins. On contrary two cathelicidin, LL-37 and CRAMP inhibited virus replication both in vitro and in vivo [81]. CRAMP has a positive net charge of + 4, an aliphatic index of 91.76, and a GRAVY score of − 0.682, while LL-37 has a positive net charge of + 6, an aliphatic index of 89.46, and a GRAVY score of − 0.724 (Supplementary Table 3).

Both cecropin A and melittin have antiviral activity against Junin virus, with the ability to inhibit virus replication within host cells. Melittin has a positive net charge of + 5, an aliphatic index of 135, and a GRAVY score of + 0.273, while cecropin A has a positive net charge of + 7, an aliphatic index of 74.71, and a GRAVY score of − 0.582. However, when comparing the two peptides, cecropin A had a higher potential for further research, with a higher TI value of 29.4, compared to melittin's TI value of 9.9 [47].

3.4. Non enveloped virus

As their name suggests, non-enveloped viruses lack a lipid layer surrounding their viral capsid. Examples of non-enveloped viruses include adenoviruses, papillomaviruses, BK virus, and human rhinovirus. Due to the lack of a lipid enveloped, the mechanisms of antiviral action and the types of antiviral compounds that are effective against non-enveloped viruses can differ from those that target enveloped ones. Also, perhaps due to this difference, there are fewer studies on the action of AVPs against non-enveloped viruses.

The AVP LL-37 produced 96% inhibition of Aichi virus infection, with an IC_{50} values of 73 μM (TI=3.4) [92]. It has a positive net charge of + 6, an aliphatic index of 89.46, and a GRAVY score of − 0.724 (Table 10). Human alpha defensin HD5 has activity against BK virus. HD5 aggregates the virion, thereby inhibiting its infectivity. HD5 is a cationic peptide with a net positive charge of + 4, an aliphatic index of 64.06, a GRAVY score of − 0.113, and a random coil secondary structure [90] (Table 10).

The peptides HNP-1, dermaseptin-S1 synthetic derivatives, and RTD-1 are active against human adenovirus type 5 and human papillomavirus. HNP-1 reduces the production of human adenovirus type 5, with an IC_{50} of 4.35 μM [91]. Dermaseptin-S1 derivatives inhibited human papillomavirus entry into host cells, but only when added before infection. On the other hand, RTD-1 clustered the papilloma capsid and prevented the virus from binding with cell surface receptors [51,84]. The AVPs active against the papilloma virus peptides have similar characteristics in terms of their net charge range, which ranged between + 3 and + 5, and their aliphatic index, which ranged from 59.44 to 108.28. However, there is some variation in their GRAVY score, which ranges from + 0.058 to + 0.524. All the peptides are alpha helical, except for RTD-1, which has a random coil structure (Table 10).

The AVPs LL-37 and CRAMP inhibited human enterovirus 71 production by reducing binding to the cells, and subsequently its cytopathic effects, intracellular viral RNA copy number and levels of viral VP1 protein [86]. Both AVPs also increased expression of interferon beta (IFN-β), interferon regulatory transcription factor 3, and significantly decreased generation of interleukin-6 (IL-6) and activation of p38 MAPK [86]. CRAMP has a net charge of + 4, an aliphatic index of 91.76, and a GRAVY score of − 0.682, while LL-37 has a net charge of + 6, an aliphatic index of 89.46, and a GRAVY score of − 0.724.

Two synthetic AVPs, P9 and P9R, inhibited human rhinovirus infection either by hindering virus-host endosome acidification or by inhibiting virus replication [63]. Both AVPs are cationic, hydrophilic and have random coil structure. In terms of activity P9R showed higher potency with a lower IC_{50} value of 1.55 μM (TI = >56.6) than P9 with higher IC_{50} value of 10.19 μM (TI = >8.8) [63] (Table 10).

4. Discussion

Many diseases caused by viruses are difficult to treat due to the emergence of new strains, making it important to find new and effective treatments. The possibility of viral pandemics that pose a significant

Table 10
Characteristics and mode of actions of peptides against non-enveloped viruses.

Peptide name (References)	Mode of action/amino acid Sequence	Charge	Aliphatic index	GRAVY Score	Structure prediction (α-helix/ random coil (%))	CC_{50} (μM)	IC_{50} or EC_{50} (μM)	Therapeutic index (TI)
Aichi virus								
Mechanism of action not specified								
LL-37 [92]	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	6	89.46	-0.724	78.38/21.62	250	73	3.4
BK Virus								
Virion aggregation								
HD5 [90]	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR	4	64.06	-0.113	0/65.62	ND	ND	ND
Human adenovirus								
Intracellular treatment after adenovirus infection								
HNP-1 [91]	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	3	65.33	0.3	0/73.33	ND	4.35	
Human enterovirus 71 (EV71)								
Inhibition of virus replication, binding and caused immunomodulation								
CRAMP [86]	GLVRKGGEKFGEKLRKIGQKIKEFFQKLALEIEQ	4	91.76	-0.682	73.53/26.47	ND	ND	ND
LL-37 [86]	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	6	89.46	-0.724	78.38/21.62	ND	ND	
Human papilloma virus								
Inhibition of virus entry by interfering binding between virion and cell surface complexes								
DRS-S1(1–29)-NH2 [51]	ALWKTMLKKLGTMALHAGKAALGAAADTI-NH2	3	108.28	0.524	89.66/10.34	40	ND	ND
[Thr4Lys12]-DRS-S1 (1–29)-NH2 [51]	ALWTTMLKKLGKMALHAGKAALGAAADTI-NH2	3	108.28	0.524	89.66/10.35	40	ND	ND
DRS-S1(1–14)-NH2 [51]	ALWKTMLKKLGTMA-NH2	3	97.86	0.314	92.86/7.14	> 100	ND	ND
DRS-S1(1–13)-NH2 [51]	ALWKTMLKKLGTM-NH2	3	97.69	0.2	69.23/15.38	> 100	ND	ND
DRS-S1(1–12)-NH2 [51]	ALWKTMLKKLGT-NH2	3	105.83	0.058	66.67/25	> 100	ND	ND
RTD-1 [84]	GFCRCLCRRGVCRCICTR	5	59.44	0.35	0/100	ND	14	ND
Human rhinovirus								
Inhibition of virus–host endosome acidification/ replication								
P9R [63]	NGAICWGPCPTAFRQIGNCGRFRVRCCRIR	6	55.33	-0.15	0/70	> 87.92	1.55	> 56.7
P9 [63]	NGAICWGPCPTAFRQIGNCGHFKVRCCKIR	5	55.33	-0.067	0/ 73.33	> 89.90	10.19	> 8.8

* , GRAVY score = Grand average hydrophobicity; CC_{50} = cytotoxic concentration by 50%; EC_{50} = effective concentration reducing viral replication by 50%; IC_{50} = inhibitory concentration by 50%; TI= therapeutic index (EC_{50} or IC_{50}/CC_{50}), ND = not determined.

danger to the global population is a real concern. There are many ongoing research efforts aimed at better understanding of viruses and developing new treatments. Cationic peptides are a class of antiviral compounds that contain positive charges. These charges allow the peptides to interact with the negatively charged surfaces of viruses, leading to a range of antiviral effects such as disrupting the viral envelop, blocking virus attachment to host cells, and inhibiting virus replication. Cationic antiviral peptides are a promising area of research for the development of new antiviral drugs, as they have broad-spectrum activity against a range of viruses, low toxicity to host cells, and the potential for low resistance development by the virus.

This review highlighted the fact that most studies of AVPs' activity had been conducted in vitro studies, with very few experiments using animal models, thus it is difficult to conclusively state that AVPs can prevent or reduce viral infections. However, AVPs were active against a wide range of viruses, including both enveloped and non-enveloped viruses. Most studied AVPs were found to be active against RNA viruses rather than DNA viruses, but this might simply be due to the RNA viruses being studied more. AVPs had either been selected from natural sources or synthesized using bioinformatics tools.

The main objective of this review was to emphasize the correlations between the physical characteristics of antiviral peptides, such as charge, hydrophobicity, and amphipathicity, with their mechanisms of action. To achieve this, the peptides were submitted to two online tools to determine their physicochemical properties. After analysing the physicochemical properties and mode of action of the peptides, this study identified three prominent target sites (virus particles, entry processes and post entry replication stages) for antiviral peptides, and that the secondary structure and hydrophobicity of the peptides varied depending on the target site. The peptides that target the virus directly, commonly the viral envelope, tend to have an alpha helix structure and be hydrophobic. The alpha helix structure can provide stability to the peptide, which may enhance its ability to interact with the viral target. The hydrophobicity of these peptides may also play a role in their interactions with the viral envelope. On the other hand, the peptides that target virus entry steps were mostly hydrophilic and random coils or with an unstable secondary structure. This may be because the peptides need to be able to interact with multiple targets and have more flexibility in their structure to do so. Finally, peptides that target the virus replication machinery tended to have an alpha helix structure but are hydrophilic. This may be because the peptides need to interact in a cell with the viral replication machinery and disrupt its function. Overall, these findings suggest that the secondary structure and hydrophobicity of antiviral peptides can play an important role in their efficacy and specificity for different target sites along with positive charges.

Several peptides had demonstrated broad spectrum antiviral activity against multiple viruses through various mechanisms. These peptides mainly targeted viruses directly or by disrupting the viral envelope, interfering or inhibiting replication, and targeting virus attachment, fusion or entry. These group of peptides included TL-1 (directly targeted HSV-1 & 2, human coronaviruses, influenza, and measles virus) [39], indolicidin (directly targeted HSV-1 & 2 and HIV) [14,43], dermaseptin S4 (targeting the envelope of HIV and HSV-1) [32,44], synthetic derivatives of dermaseptin (effective against HSV-1 via directly targeting, and interfered within the replication process of HPV) [51], AR-23 (inhibiting replication of HSV-1 and directly targeting human coronaviruses, and measles virus) [52], bomidin (effective against HSV-2 by inhibiting replication and directly targeting human coronaviruses and dengue virus) [53], BMAP-18 (inhibited HIV replication and effective against Zika virus by directly targeting it and causing immunomodulation) [34,96], P9 and P9R (effective against influenza, and SARS-CoV-2 by directly targeting them, and inhibited human rhinovirus replication)

[63], mucroporin-M1 (directly targeted SARS-CoV, influenza, measles virus, and interfered within hepatitis B virus replication) [61,87], LL-37 (effective against HIV, Zika virus, vaccinia virus, enterovirus 71 via directly targeting them and coronavirus, dengue, Ebola, and VEEV by inhibiting their entry) [34, 64, 73, 77, 82, 85, 86, 94], melittin (inhibited HIV and HSV-1 & 2 replication) [16,47], CRAMP (directly inhibited Zika virus, Vaccinia virus, and Enterovirus 71) [77, 81, 86], protegrin-1 (active against HIV and dengue virus by inhibiting replication) [33, 71], An1a (inhibited NS2B-NS3 protease of Zika and dengue virus) [75], HNP-2,3,4 (active against HIV, HSV-1&2 by inhibiting fusion and entry and provided cell line protection to reduce HCV infection) [30, 48, 57], retrocyclin 1 and RTD-3 (inhibited fusion and entry of HIV and HSV) [25, 30, 48], HD5 and its derivatives (inhibited entry of HCMV and SARS-CoV-2) [65,93], GI-20 and GF-17 (targeted entry steps of HIV and Ebola virus and caused changes in the immune response of cells infected with the Zika virus while also directly targeting the virus) [34, 78, 85], and RC-101 (inhibited entry of HIV and SARS-CoV-2) [29,65].

Some peptides were able to target multiple viruses directly, despite the differences in the composition of their envelopes. Usually, enveloped viruses are known to obtain their envelopes from various parts of the host cell. For example influenza virus acquires its envelope from the plasma membrane [97], HIV from the plasma membrane [98], hepatitis B virus from the plasma membrane of hepatocytes [99], dengue virus from the membrane of rough endoplasmic reticulum (ER) [100], human coronaviruses from the ER-Golgi intermediate compartment - a mobile complex responsible for delivering cargo from the ER to the Golgi [101], and HSV-1 from the inner nuclear membrane coated with nuclear egress complex (NEC) protein [102]. The composition of the envelope can vary greatly among different viruses due to their diverse origin. However, some peptides were effective against a wide range of viruses by targeting their envelopes, despite these differences. Additionally, some peptides targeted multiple steps of the virus entry and replication process. Further research is required to identify the precise targets and modes of action of many of these peptides against diverse viruses. This is necessary to draw definitive conclusions regarding their ability to combat multiple viruses through the same mechanism.

Future investigations should concentrate not only on in vitro experiments of single peptides but also on animal models and the possible synergistic impacts of using a combination of several peptides with distinct mechanisms of action to combat viruses and diminish the likelihood of developing resistance.

5. Conclusions

The objective of this review was to shed light on the potential of cationic AVPs as antiviral therapies. In view of the ongoing threat of viral pandemics, there is a pressing need for novel treatment options. Cationic AVPs have shown promise as antiviral agents, both on their own and in combination with other peptides or conventional drugs. The three primary targeting sites for AVPs were the virus itself, its entry steps, and replication. From the information obtained through this review, the physiochemical properties of peptides targeting these three groups varied: peptides directly targeting the virus tended to be alpha helical and hydrophobic, peptides targeting virus entry steps tended to be random coils and hydrophilic, while peptides targeting replication tended to be alpha helical and hydrophilic. The information provided in this review may serve as a guide for the development of new improved cationic AVPs. For example, these linear peptides can be allowed to tune for making shorter versions of amino-acid sequences, provide conformational constraint by synthesizing cyclic peptides and to develop hyper-branched peptides such as dendrimers to overcome limitations and shortage of antiviral therapeutics.

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Conflict of interest

None of the authors declared competing or conflict of interest.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.peptides.2023.171024.

References

[1] L.C.P. Vilas Boas, M.L. Campos, R.L.A. Berlanda, N. de Carvalho Neves, O. L. Franco, Antiviral peptides as promising therapeutic drugs, Cell Mol. Life Sci. 76 (18) (2019) 3525–3542, https://doi.org/10.1007/s00018-019-03138-w.
[2] M.S. Mousavi Maleki, S. Sardari, A. Ghandehari Alavijeh, H. Madanchi, Recent patents and FDA-approved drugs based on antiviral peptides and other peptide-related antivirals, Int J. Pept. Res Ther. 29 (1) (2023) 5, https://doi.org/10.1007/s10989-022-10477-z.
[3] W.J. Shin, B.L. Seong, Novel antiviral drug discovery strategies to tackle drug-resistant mutants of influenza virus strains, Expert Opin. Drug Discov. 14 (2) (2019) 153–168, https://doi.org/10.1080/17460441.2019.1560261.
[4] M.F. Chew, K.S. Poh, C.L. Poh, Peptides as therapeutic agents for dengue virus, Int J. Med Sci. 14 (13) (2017) 1342–1359, https://doi.org/10.7150/ijms.21875.
[5] G. Agarwal, R. Gabrani, Antiviral peptides: identification and validation, Int J. Pept. Res Ther. (2020) 1–20, https://doi.org/10.1007/s10989-020-10072-0.
[6] H. Jenssen, P. Hamill, R.E. Hancock, Peptide antimicrobial agents, Clin. Microbiol Rev. 19 (3) (2006) 491–511, https://doi.org/10.1128/cmr.00056-05.
[7] R. Liberati, D.G. Altman, J. Tetzlaff, C. Mulrow, P.C. Gøtzsche, J.P. Ioannidis, M. Clarke, P. Devereaux, J. Kleijnen, D. Moher, 1Guidelines and Guidance The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration, 2009.
[8] Expasy ProtParam tool, 2022. ⟨https://web.expasy.org/protparam/⟩. (Accessed 12 May 2022).
[9] M. Masuda, H. Nakashima, T. Ueda, H. Naba, R. Ikoma, A. Otaka, Y. Terakawa, H. Tamamura, T. Ibuka, T. Murakami, et al., A novel anti-HIV synthetic peptide, T-22 ([Tyr5,12,Lys7]-polyphemusin II, Biochem Biophys. Res Commun. 189 (2) (1992) 845–850, https://doi.org/10.1016/0006-291x(92)92280-b.
[10] H. Nakashima, M. Masuda, T. Murakami, Y. Koyanagi, A. Matsumoto, N. Fujii, N. Yamamoto, Anti-human immunodeficiency virus activity of a novel synthetic peptide, T22 ([Tyr-5,12, Lys-7]polyphemusin II): a possible inhibitor of virus-cell fusion, Antimicrob. Agents Chemother. 36 (6) (1992) 1249–1255, https://doi.org/10.1128/aac.36.6.1249.
[11] B.S. Weeks, M. Nomizu, A. Otaka, C.A. Weston, A. Okusu, H. Tamamura, N. Yamamoto, N. Fujii, The synthetic [Tyr5,12,Lys7]-polyphemusin II peptide (T22) binds to the CD4 cell surface molecule, Biochem Biophys. Res Commun. 215 (2) (1995) 626–631, https://doi.org/10.1006/bbrc.1995.2510.
[12] H. Tamamura, A. Otaka, T. Murakami, T. Ishihara, T. Ibuka, M. Waki, A. Matsumoto, N. Yamamoto, N. Fujii, Interaction of an anti-HIV peptide, T22, with gp120 and CD4, Biochem Biophys. Res Commun. 219 (2) (1996) 555–559, https://doi.org/10.1006/bbrc.1996.0272.
[13] T. Murakami, T. Nakajima, Y. Koyanagi, K. Tachibana, N. Fujii, H. Tamamura, N. Yoshida, M. Waki, A. Matsumoto, O. Yoshie, T. Kishimoto, N. Yamamoto, T. Nagasawa, A small molecule CXCR4 inhibitor that blocks T cell line-tropic HIV-1 infection, J. Exp. Med 186 (8) (1997) 1389–1393, https://doi.org/10.1084/jem.186.8.1389.
[14] W. Edward Robinson Jr, B. McDougall, D. Tran, M.E. Selsted, Anti-HIV-1 activity of indolicidin, an antimicrobial peptide from neutrophils, J. Leukoc. Biol. 63 (1) (1998) 94–100, https://doi.org/10.1002/jlb.63.1.94.
[15] H. Tamamura, R. Arakaki, H. Funakoshi, M. Imai, A. Otaka, T. Ibuka, H. Nakashima, T. Murakami, M. Waki, A. Matsumoto, N. Yamamoto, N. Fujii, Effective lowly cytotoxic analogs of an HIV-cell fusion inhibitor, T22 ([Tyr5,12, Lys7]-polyphemusin II), Bioorg. Med Chem. 6 (2) (1998) 231–238, https://doi.org/10.1016/s0968-0896(97)10037-2.
[16] M. Wachinger, A. Kleinschmidt, D. Winder, N. von Pechmann, A. Ludvigsen, M. Neumann, R. Holle, B. Salmons, V. Erfle, R. Brack-Werner, Antimicrobial peptides melittin and cecropin inhibit replication of human immunodeficiency virus 1 by suppressing viral gene expression, J. Gen. Virol. 79 (Pt 4) (1998) 731–740, https://doi.org/10.1099/0022-1317-79-4-731.
[17] A. Friedler, I. Blumenzweig, L. Baraz, M. Steinitz, M. Kotler, C. Gilon, Peptides derived from HIV-1 Vif: a non-substrate based novel type of HIV-1 protease inhibitors, J. Mol. Biol. 287 (1) (1999) 93–101, https://doi.org/10.1006/jmbi.1998.2585.
[18] K.R. Gustafson, L.K. Walton, R.C. Sowder Jr., D.G. Johnson, L.K. Pannell, J. H. Cardellina, Jr, M.R. Boyd, New circulin macrocyclic polypeptides from

Chassalia parvifolia, J. Nat. Prod. 63 (2) (2000) 176–178, https://doi.org/10.1021/np990432r.
[19] B.S. Jin, J.R. Ryu, K. Ahn, Y.G. Yu, Design of a peptide inhibitor that blocks the cell fusion mediated by glycoprotein 41 of human immunodeficiency virus type 1, AIDS Res Hum. Retrovir. 16 (17) (2000) 1797–1804, https://doi.org/10.1089/08892220050195757.
[20] M.X. Zou, H.Y. Liu, Y. Haraguchi, Y. Soda, K. Tatemoto, H. Hoshino, Apelin peptides block the entry of human immunodeficiency virus (HIV, FEBS Lett. 473 (1) (2000) 15–18, https://doi.org/10.1016/s0014-5793(00)01487-3.
[21] A.M. Cole, T. Hong, L.M. Boo, T. Nguyen, C. Zhao, G. Bristol, J.A. Zack, A. J. Waring, O.O. Yang, R.I. Lehrer, Retrocyclin: a primate peptide that protects cells from infection by T- and M-tropic strains of HIV-1, Proc. Natl. Acad. Sci. USA 99 (4) (2002) 1813–1818, https://doi.org/10.1073/pnas.052706399.
[22] V.R. de Soultrait, A. Caumont, V. Parissi, N. Morellet, M. Ventura, C. Lenoir, S. Litvak, M. Fournier, B. Roques, A novel short peptide is a specific inhibitor of the human immunodeficiency virus type 1 integrase, J. Mol. Biol. 318 (1) (2002) 45–58, https://doi.org/10.1016/s0022-2836(02)00033-5.
[23] C. Münk, G. Wei, O.O. Yang, A.J. Waring, W. Wang, T. Hong, R.I. Lehrer, N. R. Landau, A.M. Cole, The theta-defensin, retrocyclin, inhibits HIV-1 entry, AIDS Res Hum. Retrovir. 19 (10) (2003) 875–881, https://doi.org/10.1089/088922203322493049.
[24] S.M. Owen, D. Rudolph, W. Wang, A.M. Cole, M.A. Sherman, A.J. Waring, R. I. Lehrer, R.B. Lal, A theta-defensin composed exclusively of D-amino acids is active against HIV-1, J. Pept. Res 63 (6) (2004) 469–476, https://doi.org/10.1111/j.1399-3011.2004.00155.x.
[25] S.A. Gallo, W. Wang, S.S. Rawat, G. Jung, A.J. Waring, A.M. Cole, H. Lu, X. Yan, N.L. Daly, D.J. Craik, S. Jiang, R.I. Lehrer, R. Blumenthal, Theta-defensins prevent HIV-1 Env-mediated fusion by binding gp41 and blocking 6-helix bundle formation, J. Biol. Chem. 281 (27) (2006) 18787–18792, https://doi.org/10.1074/jbc.M602422200.
[26] M.E. Quiñones-Mateu, M.M. Lederman, Z. Feng, B. Chakraborty, J. Weber, H. R. Rangel, M.L. Marotta, M. Mirza, B. Jiang, P. Kiser, K. Medvik, S.F. Sieg, A. Weinberg, Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication, Aids 17 (16) (2003) F39–F48, https://doi.org/10.1097/00002030-200311070-00001.
[27] L. Sun, C.M. Finnegan, T. Kish-Catalone, R. Blumenthal, P. Garzino-Demo, G. M. La Terra Maggiore, S. Berrone, C. Kleinman, Z. Wu, S. Abdelwahab, W. Lu, A. Garzino-Demo, Human beta-defensins suppress human immunodeficiency virus infection: potential role in mucosal protection, J. Virol. 79 (22) (2005) 14318–14329, https://doi.org/10.1128/jvi.79.22.14318-14329.2005.
[28] A. Seidel, Y. Ye, L.R. de Armas, M. Soto, W. Yarosh, R.A. Marcsisin, D. Tran, M. E. Selsted, D. Camerini, Cyclic and acyclic defensins inhibit human immunodeficiency virus type-1 replication by different mechanisms, PLoS One 5 (3) (2010), e9737, https://doi.org/10.1371/journal.pone.0009737.
[29] S.M. Owen, D.L. Rudolph, W. Wang, A.M. Cole, A.J. Waring, R.B. Lal, R.I. Lehrer, RC-101, a retrocyclin-1 analogue with enhanced activity against primary HIV type 1 isolates, AIDS Res Hum. Retrovir. 20 (11) (2004) 1157–1165, https://doi.org/10.1089/aid.2004.20.1157.
[30] W. Wang, S.M. Owen, D.L. Rudolph, A.M. Cole, T. Hong, A.J. Waring, R.B. Lal, R. I. Lehrer, Activity of α-and θ-defensins against primary isolates of HIV-1, J. Immunol. 173 (1) (2004) 515–520.
[31] Z. Wu, F. Cocchi, D. Gentles, B. Ericksen, J. Lubkowski, A. Devico, R.I. Lehrer, W. Lu, Human neutrophil alpha-defensin 4 inhibits HIV-1 infection in vitro, FEBS Lett. 579 (1) (2005) 162–166, https://doi.org/10.1016/j.febslet.2004.11.062.
[32] C. Lorin, H. Saidi, A. Belaid, A. Zairi, F. Baleux, H. Hocini, L. Bélec, K. Hani, F. Tangy, The antimicrobial peptide dermaseptin S4 inhibits HIV-1 infectivity in vitro, Virology 334 (2) (2005) 264–275, https://doi.org/10.1016/j.virol.2005.02.002.
[33] L. Steinstraesser, B. Tippler, J. Mertens, E. Lamme, H.H. Homann, M. Lehnhardt, O. Wildner, H.U. Steinau, K. Uberla, Inhibition of early steps in the lentiviral replication cycle by cathelicidin host defense peptides, Retrovirology 2 (2005) 2, https://doi.org/10.1186/1742-4690-2-2.
[34] G. Wang, K.M. Watson, R.W. Buckheit Jr, Anti-human immunodeficiency virus type 1 activities of antimicrobial peptides derived from human and bovine cathelicidins, Antimicrob. Agents Chemother. 52 (9) (2008) 3438–3440.
[35] K. Shimane, E.N. Kodama, I. Nakase, S. Futaki, Y. Sakurai, Y. Sakagami, X. Li, T. Hattori, S.G. Sarafianos, M. Matsuoka, Rev-derived peptides inhibit HIV-1 replication by antagonism of Rev and a co-receptor, CXCR4, Int J. Biochem Cell Biol. 42 (9) (2010) 1482–1488, https://doi.org/10.1016/j.biocel.2010.05.005.
[36] J.H. Wong, A. Legowska, K. Rolka, T.B. Ng, M. Hui, C.H. Cho, W.W. Lam, S.W. Au, O.W. Gu, D.C. Wan, Effects of cathelicidin and its fragments on three key enzymes of HIV-1, Peptides 32 (6) (2011) 1117–1122, https://doi.org/10.1016/j.peptides.2011.04.017.
[37] Y. Chen, L. Cao, M. Zhong, Y. Zhang, C. Han, Q. Li, J. Yang, D. Zhou, W. Shi, B. He, F. Liu, J. Yu, Y. Sun, Y. Cao, Y. Li, W. Li, D. Guo, Z. Cao, H. Yan, Anti-HIV-1 activity of a new scorpion venom peptide derivative Kn2-7, PLoS One 7 (4) (2012), e34947, https://doi.org/10.1371/journal.pone.0034947.
[38] A. Armon-Omer, A. Levin, Z. Hayouka, K. Butz, F. Hoppe-Seyler, S. Loya, A. Hizi, A. Friedler, A. Loyter, Correlation between shiftide activity and HIV-1 integrase inhibition by a peptide selected from a combinatorial library, J. Mol. Biol. 376 (4) (2008) 971–982, https://doi.org/10.1016/j.jmb.2007.11.095.
[39] C. Zannella, A. Chianese, L. Palomba, M.E. Marcocci, R. Bellavita, F. Merlino, P. Grieco, V. Folliero, A. De Filippis, M. Mangoni, L. Nencioni, G. Franci, M. Galdiero, Broad-Spectrum Antiviral Activity of the Amphibian Antimicrobial Peptide Temporin L and Its Analogs, Int J. Mol. Sci. 23 (4) (2022), https://doi.org/10.3390/ijms23042060.
[40] R.I. Lehrer, K. Daher, T. Ganz, M.E. Selsted, Direct inactivation of viruses by MCP-1 and MCP-2, natural peptide antibiotics from rabbit leukocytes, J. Virol. 54 (2) (1985) 467–472, https://doi.org/10.1128/jvi.54.2.467-472.1985.
[41] Y. Aboudy, E. Mendelson, I. Shalit, R. Bessalle, M. Fridkin, Activity of two synthetic amphiphilic peptides and magainin-2 against herpes simplex virus types 1 and 2, Int J. Pept. Protein Res 43 (6) (1994) 573–582, https://doi.org/10.1111/j.1399-3011.1994.tb00559.x.
[42] A. Baghian, J. Jaynes, F. Enright, K.G. Kousoulas, An amphipathic alpha-helical synthetic peptide analogue of melittin inhibits herpes simplex virus-1 (HSV-1)-induced cell fusion and virus spread, Peptides 18 (2) (1997) 177–183, https://doi.org/10.1016/s0196-9781(96)00290-2.
[43] B. Yasin, M. Pang, J.S. Turner, Y. Cho, N.N. Dinh, A.J. Waring, R.I. Lehrer, E. A. Wagar, Evaluation of the inactivation of infectious Herpes simplex virus by host-defense peptides, Eur. J. Clin. Microbiol Infect. Dis. 19 (3) (2000) 187–194, https://doi.org/10.1007/s100960050457.
[44] A. Belaid, M. Aouni, R. Khelifa, A. Trabelsi, M. Jemmali, K. Hani, In vitro antiviral activity of dermaseptins against herpes simplex virus type 1, J. Med. Virol. 66 (2) (2002) 229–234, https://doi.org/10.1002/jmv.2134.
[45] M. Benincasa, B. Skerlavaj, R. Gennaro, A. Pellegrini, M. Zanetti, In vitro and in vivo antimicrobial activity of two alpha-helical cathelicidin peptides and of their synthetic analogs, Peptides 24 (11) (2003) 1723–1731, doi:10.1016/j.peptides.2003.07.025.
[46] S. Sinha, N. Cheshenko, R.I. Lehrer, B.C. Herold, NP-1, a rabbit alpha-defensin, prevents the entry and intercellular spread of herpes simplex virus type 2, Antimicrob. Agents Chemother. 47 (2) (2003) 494–500, https://doi.org/10.1128/aac.47.2.494-500.2003.
[47] V.C. Albiol Matanic, V. Castilla, Antiviral activity of antimicrobial cationic peptides against Junin virus and herpes simplex virus, Int J. Antimicrob. Agents 23 (4) (2004) 382–389, https://doi.org/10.1016/j.ijantimicag.2003.07.022.
[48] B. Yasin, W. Wang, M. Pang, N. Cheshenko, T. Hong, A.J. Waring, B.C. Herold, E. A. Wagar, R.I. Lehrer, Theta defensins protect cells from infection by herpes simplex virus by inhibiting viral adhesion and entry, J. Virol. 78 (10) (2004) 5147–5156, https://doi.org/10.1128/jvi.78.10.5147-5156.2004.
[49] B.A. Kelly, S.J. Neil, A. McKnight, J.M. Santos, P. Sinnis, E.R. Jack, D. A. Middleton, C.B. Dobson, Apolipoprotein E-derived antimicrobial peptide analogues with altered membrane affinity and increased potency and breadth of activity, Febs J. 274 (17) (2007) 4511–4525, https://doi.org/10.1111/j.1742-4658.2007.05981.x.
[50] R. Akkarawongsa, N.E. Pocaro, G. Case, A.W. Kolb, C.R. Brandt, Multiple peptides homologous to herpes simplex virus type 1 glycoprotein B inhibit viral infection, Antimicrob. Agents Chemother. 53 (3) (2009) 987–996, https://doi.org/10.1128/aac.00793-08.
[51] D. Savoia, M. Donalisio, A. Civra, S. Salvadori, R. Guerrini, In vitro activity of dermaseptin S1 derivatives against genital pathogens, Apmis 118 (9) (2010) 674–680, https://doi.org/10.1111/j.1600-0463.2010.02637.x.
[52] A. Chianese, C. Zannella, A. Monti, A. De Filippis, N. Doti, G. Franci, M. Galdiero, The broad-spectrum antiviral potential of the amphibian peptide AR-23, Int J. Mol. Sci. 23 (2) (2022), https://doi.org/10.3390/ijms23020883.
[53] R. Liu, Z. Liu, H. Peng, Y. Lv, Y. Feng, J. Kang, N. Lu, R. Ma, S. Hou, W. Sun, Q. Ying, F. Wang, Q. Gao, P. Zhao, C. Zhu, Y. Wang, X. Wu, Bomidin: an optimized antimicrobial peptide with broad antiviral activity against enveloped viruses, Front Immunol. 13 (2022), 851642, https://doi.org/10.3389/fimmu.2022.851642.
[54] P.L. Darke, A.R. Jacobs, L. Waxman, L.C. Kuo, Inhibition of hepatitis C virus NS2/3 processing by NS4A peptides, Implic. Control Viral Process., J. Biol. Chem. 274 (49) (1999) 34511–34514, https://doi.org/10.1074/jbc.274.49.34511.
[55] S. Portal-Núñez, C.J. González-Navarro, M. García-Delgado, J.L. Vizmanos, J. J. Lasarte, F. Borrás-Cuesta, Peptide inhibitors of hepatitis C virus NS3 protease, Antivir. Chem. Chemother. 14 (5) (2003) 225–233, https://doi.org/10.1177/095632020301400501.
[56] R. Yan, Z. Zhao, Y. He, L. Wu, D. Cai, W. Hong, Y. Wu, Z. Cao, C. Zheng, W. Li, A new natural α-helical peptide from the venom of the scorpion Heterometrus petersii kills HCV, Peptides 32 (1) (2011) 11–19, https://doi.org/10.1016/j.peptides.2010.10.008.
[57] E.H. Mattar, H.A. Almehdar, V.N. Uversky, E.M. Redwan, Virucidal activity of human α- and β-defensins against hepatitis C virus genotype 4, Mol. Biosyst. 12 (9) (2016) 2785–2797, https://doi.org/10.1039/c6mb00283h.
[58] M.N. Batista, P. Sanches, B.M. Carneiro, A.C.S. Braga, G.R.F. Campos, E.M. Cilli, P. Rahal, GA-Hecate antiviral properties on HCV whole cycle represent a new antiviral class and open the door for the development of broad spectrum antivirals, Sci. Rep. 8 (1) (2018) 14329, https://doi.org/10.1038/s41598-018-32176-w.
[59] Y. Cheng, F. Sun, S. Li, M. Gao, L. Wang, M. Sarhan, M.A. Abdel-Rahman, W. Li, H.F. Kwok, Y. Wu, Inhibitory activity of a scorpion defensin BmKDfsin3 against Hepatitis C virus, Antibiotics 9 (1) (2020) 33.
[60] C.L. Wohlford-Lenane, D.K. Meyerholz, S. Perlman, H. Zhou, D. Tran, M. E. Selsted, P.B. McCray Jr., Rhesus theta-defensin prevents death in a mouse model of severe acute respiratory syndrome coronavirus pulmonary disease, J. Virol. 83 (21) (2009) 11385–11390, https://doi.org/10.1128/jvi.01363-09.
[61] Q. Li, Z. Zhao, D. Zhou, Y. Chen, W. Hong, L. Cao, J. Yang, Y. Zhang, W. Shi, Z. Cao, Y. Wu, H. Yan, W. Li, Virucidal activity of a scorpion venom peptide variant mucroporin-M1 against measles, SARS-CoV and influenza H5N1 viruses, Peptides 32 (7) (2011) 1518–1525, https://doi.org/10.1016/j.peptides.2011.05.015.
[62] H. Zhao, J. Zhou, K. Zhang, H. Chu, D. Liu, V.K. Poon, C.C. Chan, H.C. Leung, N. Fai, Y.P. Lin, A.J. Zhang, D.Y. Jin, K.Y. Yuen, B.J. Zheng, A novel peptide with

potent and broad-spectrum antiviral activities against multiple respiratory viruses, Sci. Rep. 6 (2016) 22008, https://doi.org/10.1038/srep22008.
[63] H. Zhao, K.K.W. To, K.H. Sze, T.T. Yung, M. Bian, H. Lam, M.L. Yeung, C. Li, H. Chu, K.Y. Yuen, A broad-spectrum virus- and host-targeting peptide against respiratory viruses including influenza virus and SARS-CoV-2, Nat. Commun. 11 (1) (2020) 4252, https://doi.org/10.1038/s41467-020-17986-9.
[64] C. Wang, S. Wang, D. Li, P. Chen, S. Han, G. Zhao, Y. Chen, J. Zhao, J. Xiong, J. Qiu, D.Q. Wei, J. Zhao, J. Wang, Human cathelicidin inhibits SARS-CoV-2 infection: killing two birds with one stone, ACS Infect. Dis. 7 (6) (2021) 1545–1554, https://doi.org/10.1021/acsinfecdis.1c00096.
[65] C. Xu, A. Wang, M. Marin, W. Honnen, S. Ramasamy, E. Porter, S. Subbian, A. Pinter, G.B. Melikyan, W. Lu, T.L. Chang, Human defensins inhibit SARS-COV-2 infection by blocking viral entry, Viruses 13 (7) (2021), https://doi.org/10.3390/v13071246.
[66] E. Leikina, H. Delanoe-Ayari, K. Melikov, M.S. Cho, A. Chen, A.J. Waring, W. Wang, Y. Xie, J.A. Loo, R.I. Lehrer, L.V. Chernomordik, Carbohydrate-binding molecules inhibit viral fusion and entry by crosslinking membrane glycoproteins, Nat. Immunol. 6 (10) (2005) 995–1001, https://doi.org/10.1038/ni1248.
[67] D.J. Holthausen, S.H. Lee, V.T. Kumar, N.M. Bouvier, F. Krammer, A.H. Ellebedy, J. Wrammert, A.C. Lowen, S. George, M.R. Pillai, J. Jacob, An amphibian host defense peptide is virucidal for human H1 hemagglutinin-bearing influenza viruses, Immunity 46 (4) (2017) 587–595, https://doi.org/10.1016/j.immuni.2017.03.018.
[68] D. Lin, Y. Luo, G. Yang, F. Li, X. Xie, D. Chen, L. He, J. Wang, C. Ye, S. Lu, L. Lv, S. Liu, J. He, Potent influenza A virus entry inhibitors targeting a conserved region of hemagglutinin, Biochem Pharm. 144 (2017) 35–51, https://doi.org/10.1016/j.bcp.2017.07.023.
[69] J. Xu, S. Chen, J. Jin, L. Ma, M. Guo, C. Zhou, J. Dou, Inhibition of peptide BF-30 on influenza A virus infection in vitro/vivo by causing virion membrane fusion, Peptides 112 (2019) 14–22, https://doi.org/10.1016/j.peptides.2018.10.004.
[70] J.M. Costin, E. Jenwitheesuk, S.M. Lok, E. Hunsperger, K.A. Conrads, K. A. Fontaine, C.R. Rees, M.G. Rossmann, S. Isern, R. Samudrala, S.F. Michael, Structural optimization and de novo design of dengue virus entry inhibitory peptides, PLoS Negl. Trop. Dis. 4 (6) (2010), e721, https://doi.org/10.1371/journal.pntd.0000721.
[71] H.A. Rothan, A.Y. Abdulrahman, P.G. Sasikumer, S. Othman, N.A. Rahman, R. Yusof, Protegrin-1 inhibits dengue NS2B-NS3 serine protease and viral replication in MK2 cells, J. Biomed. Biotechnol. 2012 (2012), 251482, https://doi.org/10.1155/2012/251482.
[72] H.A. Rothan, H. Bahrani, N.A. Rahman, R. Yusof, Identification of natural antimicrobial agents to treat dengue infection: In vitro analysis of latarcin peptide activity against dengue virus, BMC Microbiol 14 (2014) 140, https://doi.org/10.1186/1471-2180-14-140.
[73] K. Alagarasu, P.S. Patil, P. Shil, M. Seervi, M.B. Kakade, H. Tillu, A. Salunke, In-vitro effect of human cathelicidin antimicrobial peptide LL-37 on dengue virus type 2, Peptides 92 (2017) 23–30, https://doi.org/10.1016/j.peptides.2017.04.002.
[74] X. Cui, Y. Wu, D. Fan, N. Gao, Y. Ming, P. Wang, J. An, Peptides P4 and P7 derived from E protein inhibit entry of dengue virus serotype 2 via interacting with β3 integrin, Antivir. Res 155 (2018) 20–27, https://doi.org/10.1016/j.antiviral.2018.04.018.
[75] M. Ji, T. Zhu, M. Xing, N. Luan, J. Mwangi, X. Yan, G. Mo, M. Rong, B. Li, R. Lai, L. Jin, An antiviral peptide from alopecosa nagpag spider targets NS2B-NS3 protease of flaviviruses, Toxins 11 (10) (2019), https://doi.org/10.3390/toxins11100584.
[76] J. Wang, B. Jiang, K. Wang, J. Dai, C. Dong, Y. Wang, P. Zhang, M. Li, W. Xu, L. Wei, A cathelicidin antimicrobial peptide from Hydrophis cyanocinctus inhibits Zika virus infection by downregulating expression of a viral entry factor, J. Biol. Chem. 298 (10) (2022), 102471, https://doi.org/10.1016/j.jbc.2022.102471.
[77] Z. Liu, J. Wu, Z. Qin, C. Dong, H. Yang, J. Sun, W. Xu, L. Wei, Endogenous cathelicidin is required for protection against ZIKV-caused testis damage via inactivating virons, Antivir. Res 198 (2022), 105248, https://doi.org/10.1016/j.antiviral.2022.105248.
[78] M. He, H. Zhang, Y. Li, G. Wang, B. Tang, J. Zhao, Y. Huang, J. Zheng, Cathelicidin-derived antimicrobial peptides inhibit zika virus through direct inactivation and interferon pathway, Front Immunol. 9 (2018) 722, https://doi.org/10.3389/fimmu.2018.00722.
[79] X. Jia, J. Guo, W. Yuan, L. Sun, Y. Liu, M. Zhou, G. Xiao, W. Lu, A. Garzino-Demo, W. Wang, Mechanism through which retrocyclin targets flavivirus multiplication, J. Virol. 95 (15) (2021), e0056021, https://doi.org/10.1128/jvi.00560-21.
[80] W. Xiong, J. Li, Y. Feng, J. Chai, J. Wu, Y. Hu, M. Tian, W. Lu, X. Xu, M. Zou, Brevinin-2GHk, a peptide derived from the skin of fejervarya limnocharis, inhibits zika virus infection by disrupting viral integrity, Viruses 13 (12) (2021), https://doi.org/10.3390/v13122382.
[81] M.D. Howell, J.F. Jones, K.O. Kisich, J.E. Streib, R.L. Gallo, D.Y. Leung, Selective killing of vaccinia virus by LL-37: implications for eczema vaccinatum, J. Immunol. 172 (3) (2004) 1763–1767, https://doi.org/10.4049/jimmunol.172.3.1763.
[82] R.E. Dean, L.M. O'Brien, J.E. Thwaite, M.A. Fox, H. Atkins, D.O. Ulaeto, A carpet-based mechanism for direct antimicrobial peptide activity against vaccinia virus membranes, Peptides 31 (11) (2010) 1966–1972, https://doi.org/10.1016/j.peptides.2010.07.028.
[83] K.V. Mohan, S.S. Rao, C.D. Atreya, Antiviral activity of selected antimicrobial peptides against vaccinia virus, Antivir. Res 86 (3) (2010) 306–311, https://doi.org/10.1016/j.antiviral.2010.03.012.
[84] J.G. Skeate, W.H. Segerink, M.D. Garcia, D.J. Fernandez, R. Prins, K.P. Lühen, F. O. Voss, D.M. Da Silva, W.M. Kast, Theta-defensins inhibit high-risk human papillomavirus infection through charge-driven capsid clustering, Front Immunol. 11 (2020), 561843, https://doi.org/10.3389/fimmu.2020.561843.
[85] Y. Yu, C.L. Cooper, G. Wang, M.J. Morwitzer, K. Kota, J.P. Tran, S.B. Bradfute, Y. Liu, J. Shao, A.K. Zhang, L.G. Luo, S.P. Reid, S.H. Hinrichs, K. Su, Engineered human cathelicidin antimicrobial peptides inhibit ebola virus infection, iScience 23 (4) (2020), 100999, https://doi.org/10.1016/j.isci.2020.100999.
[86] J. Yu, Y. Dai, Y. Fu, K. Wang, Y. Yang, M. Li, W. Xu, L. Wei, Cathelicidin antimicrobial peptides suppress EV71 infection via regulating antiviral response and inhibiting viral binding, Antivir. Res 187 (2021), 105021, https://doi.org/10.1016/j.antiviral.2021.105021.
[87] Z. Zhao, W. Hong, Z. Zeng, Y. Wu, K. Hu, X. Tian, W. Li, Z. Cao, Mucroporin-M1 inhibits hepatitis B virus replication by activating the mitogen-activated protein kinase (MAPK) pathway and down-regulating HNF4α in vitro and in vivo, J. Biol. Chem. 287 (36) (2012) 30181–30190, https://doi.org/10.1074/jbc.M112.370312.
[88] Z. Zeng, Q. Zhang, W. Hong, Y. Xie, Y. Liu, W. Li, Y. Wu, Z. Cao, A scorpion defensin BmKDfsin4 inhibits hepatitis B virus replication in vitro, Toxins 8 (5) (2016), https://doi.org/10.3390/toxins8050124.
[89] L. Chen, Y. Liu, S. Wang, J. Sun, P. Wang, Q. Xin, L. Zhang, G. Xiao, W. Wang, Antiviral activity of peptide inhibitors derived from the protein E stem against Japanese encephalitis and Zika viruses, Antivir. Res 141 (2017) 140–149, https://doi.org/10.1016/j.antiviral.2017.02.009.
[90] A.S. Dugan, M.S. Maginnis, J.A. Jordan, M.L. Gasparovic, K. Manley, R. Page, G. Williams, E. Porter, B.A. O'Hara, W.J. Atwood, Human alpha-defensins inhibit BK virus infection by aggregating virions and blocking binding to host cells, J. Biol. Chem. 283 (45) (2008) 31125–31132, https://doi.org/10.1074/jbc.M805902200.
[91] A. Bastian, H. Schäfer, Human alpha-defensin 1 (HNP-1) inhibits adenoviral infection in vitro, Regul. Pept. 101(1-3) (2001) 157–161, https://doi.org/10.1016/s0167-0115(01)00282-8.
[92] L.C. Vilas Boas, L.M. de Lima, L. Migliolo, G.D. Mendes, M.G. de Jesus, O. L. Franco, P.A. Silva, Linear antimicrobial peptides with activity against herpes simplex virus 1 and Aichi virus, Biopolymers 108 (2) (2017), https://doi.org/10.1002/bip.22871.
[93] R. Böffert, R. Businger, H. Preiß, D. Ehmann, V. Truffault, C. Simon, N. Ruetalo, K. Hamprecht, P. Müller, J. Wehkamp, M. Schindler, The human α-defensin-derived peptide HD5(1-9) inhibits cellular attachment and entry of human cytomegalovirus, Antivir. Res 177 (2020), 104779, https://doi.org/10.1016/j.antiviral.2020.104779.
[94] A. Ahmed, G. Siman-Tov, F. Keck, S. Kortchak, A. Bakovic, K. Risner, T.K. Lu, N. Bhalla, C. de la Fuente-Nunez, A. Narayanan, Human cathelicidin peptide LL-37 as a therapeutic antiviral targeting Venezuelan equine encephalitis virus infections, Antivir. Res 164 (2019) 61–69, https://doi.org/10.1016/j.antiviral.2019.02.002.
[95] Y. Li, Z. Zhang, L. Yang, X. Lian, Y. Xie, S. Li, S. Xin, P. Cao, J. Lu, The MERS-CoV Receptor DPP4 as a candidate binding target of the SARS-CoV-2 Spike, iScience 23 (8) (2020), 101400, https://doi.org/10.1016/j.isci.2020.101400.
[96] M. He, H. Zhang, Y. Li, G. Wang, B. Tang, J. Zhao, Cathelicidin-derived antimicrobial peptides inhibit zika virus through direct inactivation and interferon pathway, Front Immunol. 9 (2018) 722.
[97] I.A. Schaap, F. Eghiaian, A. des Georges, C. Veigel, Effect of envelope proteins on the mechanical properties of influenza virus, J. Biol. Chem. 287 (49) (2012) 41078–41088.
[98] M.A. Checkley, B.G. Luttge, E.O. Freed, HIV-1 envelope glycoprotein biosynthesis, trafficking, and incorporation, J. Mol. Biol. 410 (4) (2011) 582–608, https://doi.org/10.1016/j.jmb.2011.04.042.
[99] J. Inoue, K. Sato, M. Ninomiya, A. Masamune, Envelope proteins of hepatitis b virus: molecular biology and involvement in carcinogenesis, Viruses 13 (6) (2021), https://doi.org/10.3390/v13061124.
[100] S. Mukhopadhyay, R.J. Kuhn, M.G. Rossmann, A structural perspective of the flavivirus life cycle, Nat. Rev. Microbiol 3 (1) (2005) 13–22, https://doi.org/10.1038/nrmicro1067.
[101] N.A. Wong, M.H. Saier Jr., The SARS-coronavirus infection cycle: a survey of viral membrane proteins, their functional interactions and pathogenesis, Int J. Mol. Sci. 22 (3) (2021), https://doi.org/10.3390/ijms22031308.
[102] W.W. Newcomb, J. Fontana, D.C. Winkler, N. Cheng, J.B. Heymann, A.C. Steven, The primary enveloped virion of herpes simplex virus 1: its role in nuclear egress, mBio 8 (3) (2017), https://doi.org/10.1128/mBio.00825-17.