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REVIEW ARTICLE

The Development of Peptide-based Antimicrobial Agents against Dengue Virus

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Abstract: Dengue fever has become an imminent threat to international public health because of global warming and climate change. The World Health Organization proclaimed that more than 50% of the world's population is at risk of dengue virus (DENV) infection. Therefore, developing a clinically approved vaccine and effective therapeutic remedy for treating dengue fever is imperative. Peptide drug development has become a novel pharmaceutical research field. This article reviews various peptides-based antimicrobial agents targeting three pathways involved in the DENV lifecycle. Specifically, they are peptide vaccines from immunomodulation, peptide drugs that inhibit virus entry, and peptide drugs that interfere with viral replication. Many antiviral peptide studies against DENV have been conducted in animal model trials, and progression to clinical trials for these promising peptide drugs is anticipated.

Keywords: Dengue fever, dengue virus, peptide drug, peptide vaccine, antimicrobial agents, clinical trials.

1. INTRODUCTION

1.1. Dengue Virus and Dengue Fever

Dengue virus (DENV), an arthropod-borne human pathogen that can infect people through a mosquito vector (*Aedes aegypti* or *A. albopictus*), has four serotypes (*i.e.* DENV1–4) and is of the genus *Flavivirus*, a member of the family *Flaviviridae* [1]. The global distribution and incidence of DENV infection have increased considerably over recent decades. The World Health Organization (WHO) estimated that 390 million dengue infections occur each year (95% credible interval 284–528 million), of which 96 million (67–136 million) cases manifest clinically at any level of severity [2, 3]. A previous study used a systemic analysis to estimate the global prevalence and economic burden of DENV, revealing that approximately 5840 million symptomatic DENV infections in 141 countries resulted in annual global cost US$8.9 billion [4]. Moreover, according to the previous report containing a systemic review and meta-analysis, the odds ratio of dengue fever incidence increased rapidly from 22 °C to 29 °C, indicating that the risk of in DENV infection is significantly associated with temperature change [5]. In other words, approximately 50% of the world's population is at risk, even with practical preventive strategies such as vector control programs and public health policies [6-9]. Moreover, the present situation has worsened since the newly genetic variant serotype (DENV-5) was discovered in Southeast Asia and identified in October 2013. This situation has made the development of therapeutics and vaccine for DENV into priority, while complicating vector control and dengue surveillance measures [10-12]. The world's first approved vaccine trial, Dengvaxia®, developed by Sanofi Pasteur, has recently been shown to lead to severe disease following vaccination and subsequent DENV infection. [https://www.nytimes.com/2017/12/17/health/sanofi-dengue-vaccine-philippines.html]. Therefore, the Philippines suspended its large-scale dengue vaccination effort, and Sanofi releasing an updated recommends that people who have never been infected with any strain of dengue not to be vaccinated.

Dengue fever has an incubation period of 3–7 days, and is clinically characterized by fever, chills, muscle pain, frontal headache, retro-orbital pain, arthralgia, nausea, and vomiting [13]. A skin rash often presents on the third or fourth day of fever, and its typical cutaneous feature may begin on the extremities or the trunk and spread to other areas including the face. Severe DENV infection can progress to dengue hemorrhagic fever or dengue shock syndrome, which is characterized by hemorrhage and plasma leakage. This life-threatening complication can lead to shock or death in patients who have had a dengue infection episode and are sub-

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sequently infected by a different serotype [14, 15]; this phenomenon is thought to be caused by antibody-dependent enhancement [16-19]. Several reports on the various clinical manifestations of dengue infection in several endemic countries have indicated that ocular complication, oral lesions, cardiovascular impairment, and hepatic injury may be involved in the progression of dengue fever [20-22]. The presence of the DENV-5 serotype could have critical implications for human health and complicate the development of effective therapeutics and vaccines.

1.2. Search Strategy and Selection Criteria

References of this review were selected through searches of PubMed for articles published from May 1, 1991 to Oct 1, 2016, with the terms "dengue fever", "dengue virus", "peptide drug", "peptide vaccine", "antimicrobial peptides", "antiviral peptides", "antiviral drug", "immunomodulation", "antidengue strategy". We also search Antimicrobial Peptide Database (APD, http://aps.unmc.edu/AP/main.php) and animal model trials documented for relevant antiviral research. Publications from searches of websites released patented drugs are included. Selected review articles are cited to provide readers with more details and references than this review can accommodate.

1.3. Structure and Lifecycle of Dengue Virus

To design a vaccine or antiviral drug, the virological structure and lifecycle of DENV must first be understood [23, 24]. Similar to other flaviviruses, DENV has an approximately 11-kb positive single-stranded RNA genome encoding a single polyprotein that is processed into three structural proteins, namely capsid (C), premembrane (prM), and envelope (E) glycoproteins, and seven nonstructural proteins (*i.e.* NS1, NS2A/B, NS3, NS4A/B, and NS5) that are crucial for viral propagation [25, 26]. Specifically, the structural proteins are responsible for viral particle assembly and budding, and the nonstructural proteins participate in replication of viral genomic RNA [27-30]. Antidengue strategies are mainly aimed at targeting replication proteins with enzymatic functions, such as NS2B/NS3 (protease), NS3 (helicase), and NS5 (RNA-dependent RNA polymerase, RdRp) [31, 32].

Under the present understanding of the molecular structure and lifecycle of DENV, several antidengue strategies have targeted specific steps of key components of the viral life cycle. The main anti-DENV infection strategies are i) activating relevant immune cells of host defense system through vaccines designed using various methods; ii) interfering with the interaction between DENV and host cell receptors or interrupting membranous fusion, which is necessary for infection to occur through the E protein; and iii) interfering with viral replication or recruitment of the viral components necessary for propagation through NS2B/NS3 and NS5 proteins (Fig. **1**) [31, 33-35].

1.4. Antimicrobial and Antiviral Peptides

Antimicrobial peptides (AMPs) are a heterogeneous group of primeval molecules comprising highly conserved components found in various living organisms ranging from prokaryotes to humans [36-38]. Generally, AMPs can be defined as relatively small (6–100 amino acids) and positively charged amphipathic molecules with varying amino acid sequences and lengths according to their biochemical characteristics [37, 39]. AMPs play multifunctional roles in the innate immune defense system of mammals, including their capability to directly kill microbes, suppress microbial growth, and stimulate various immune cells [39-46]. AMPs are promising treatment options because of their broad antimicrobial spectrum against microorganisms such as bacteria, fungi, protozoa, and viruses; furthermore, they have been classified into four major groups according to their secondary structure (*i.e.* α-helical, β-sheet, loop and extended peptides) [47-52]. More than 2500 AMPs have been documented or predicted in various organisms, and are available through the Antimicrobial Peptide Database (APD, http://aps.unmc.edu/AP/main.php). In this database, groups of promising peptides are classified as antiviral peptides (AVPs). There are more than 150 AVPs, including over 90 peptides with anti-HIV features. Because of the importance of antiviral drug discovery, an AVP web server was established to predict highly effective AVPs to assist in developing AVP drugs [53]. The extraordinary properties of AVPs and AMPs demonstrate the following advantages: i) resistance is less likely compared to conventional antibiotics because of the highly diverse and abundant AVPs and AMPs produced by multicellular organisms; ii) the membrane-disturbing activity of AVPs/AMPs is difficult for microbes to evade while maintaining functional cellular membrane and structural integrity; and iii) degradation toward AVPs and AMPs is difficult because microbes would require a designer protease to destroy AVPs and AMPs without devastating host proteins that are necessary for attachment or pathogenic proteins that are essential in the virus lifecycle [36, 54-57].

Regarding AVPs, human defensins demonstrate viral antagonist activities by blocking virus entry to neutralize pathogens such as herpes simplex virus (HSV) and influenza A virus [58, 59]. The α- and θ- defensins exhibit extraordinary anti-HIV potential by interacting with receptors on a host cell to oppose viral attachment or entry [60, 61]. Because the skin is the first line of immune defense, several well-characterized AVPs are produced by skin-residing cells, such as cathelicidin and β-defensin, for defeating most viral infections and microbial invasions through mucosa or cutaneous tissue [62].

Many studies have focused on discovering novel drugs and vaccine candidates for treating DENV [23, 28, 33, 35, 63-104]. We listed the most widely adopted approaches already mentioned in other reviews, such as immunomodulation, anti-DENV entry, and anti-DENV replication (Table **1**) [23, 105-116]. Among these articles, one recommended publication “Peptides as Therapeutic Agents for Dengue Virus” released by Chew *et al.* in 2017 documents and cites all therapeutic peptides from current literature [117]. Before Dengvaxia®, the first dengue vaccine to be licensed, despite the considerable effort toward mitigating the growing threat of a DENV epidemic in the tropical zone, no effective therapeutic agent or licensed vaccine had been developed to prevent DENV infection or treat dengue fever [2, 10, 23, 28, 33, 35, 65, 67, 70-73, 76-99, 102, 105-116, 118-132]. Bioactive peptides isolated from human tissue or body fluid have been excavated from peptide libraries in order to design inhibitors

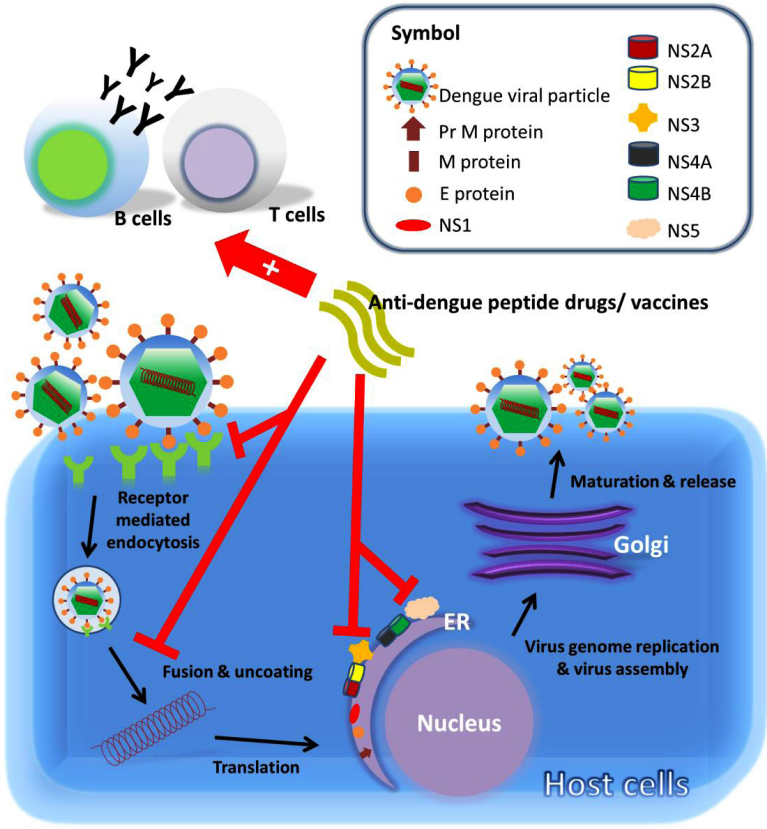

Fig. (1). The lifecycle of DENV and major strategies for anti-DENV peptide drug development. The lifecycle of DENV involves complex interactions between viral proteins and host factors. Interaction begins from the E protein contacting the target host cell through receptor-mediated and clathrin-dependent endocytosis [135-138]. After internalization, viral genomic RNA is released from the endosome because the conformational change in the E protein, which is necessary for membrane fusion to occur, is triggered by pH alteration [138-140]. A single polyprotein is subsequently translated from the genomic RNA and autocatalytically cleaved into structural and nonstructural proteins through the recruitment of viral NS2B/NS3 and host proteases [25, 141]. All processed viral protein subunits are translocated to the endoplasmic reticulum (ER) membrane. Nonstructural proteins are transported to ER-derived vesicular parcels to form a replication complex, while the structural proteins prM and E are embedded into the ER membrane to enclose the nucleocapsid derived from the association of newly synthesized viral RNA with C proteins [24, 139, 142]. Subsequently, an immature viral particle is generated from the assembly of C, prM, E, and genomic RNA, and then buds into the ER-lumen to enable transport through the secretory pathway. Ultimately, the mature DENV virion is released into the cytoplasm under a low pH through furin-mediated cleavage of prM to M in the trans-Golgi network and can infect the next host cell [33, 141, 143-145]. According to the DENV lifecycle, the three major strategies for developing anti-DENV peptide drugs are immunomodulation, anti-DENV entry, and anti-DENV replication [63-99].

Table 1. Recent review articles of anti-DENV drugs and vaccines.

Pathway	Title	Compounds	References
Immunomodulation	Dengue and soluble mediators of the innate immune system	Cytokine and antibody	[106]
	Dengue vaccine- priorities and progress	Live attenuated, whole-virus vaccine candidates Live chimeric vaccine candidates DNA vaccine Chimeric proteins Recombinant protein	[105]
	Next generation dengue vaccines: A review of candidates in preclinical development	Recombinant subunit vaccines DNA vaccines Virus Like Particle vaccines Virus-vectored vaccines Live attenuated virus vaccines Heterologous prime-boost approaches	[107]

(Table 1) contd….

Pathway	Title	Compounds	References
	Targeting host factors to treat West Nile and dengue viral infections	Small molecule Cytokine Antibody Carbohydrate	[23]
Anti-dengue virus entry	Carbohydrate-related inhibitors of dengue virus entry	Carbohydrate	[111]
	Dengue fever: Natural managemen	Herbal/natural drugs	[114]
	Natural compounds against flaviviral infections	Herbal/natural drugs	[109]
	Potential antidengue medicinal plants, a review	Herbal/natural drugs	[108]
	Targeting host factors to treat West Nile and dengue viral infections	Small molecule Cytokine Antibody Carbohydrate	[23]
	Ten years of dengue drug discovery- progress and prospects	Small molecule Peptide	[112]
	Therapeutic antibodies as a treatment option for dengue fever	Antibody	[110]
	Dengue virus entry as target for antiviral therapy	Peptide Small molecule	[115]
	Progress in the identification of dengue virus Entry/Fusion inhibitors	Peptide Small molecule	[116]
	Peptides as therapeutic agents for dengue virus	Peptide	[117]
Anti-dengue virus replication	A perspective on targeting non-structural proteins to combat neglected tropical diseases- Dengue, West Nile and Chikungunya viruses	Small molecule and peptide	[113]
	Dengue and soluble mediators of the innate immune system	Cytokine and antibody	[106]
	Dengue fever: Natural management	Herbal/natural drugs	[114]
	Natural compounds against flaviviral infections	Herbal/natural drugs	[109]
	Potential antidengue medicinal plants, a review	Herbal/natural drugs	[108]
	Targeting host factors to treat West Nile and dengue viral infections	Small molecule Cytokine Antibody Carbohydrate	[23]
	Ten years of dengue drug discovery- progress and prospects	Small molecule Peptide	[112]
	Peptides as therapeutic agents for dengue virus	Peptide	[117]

against HIV-1 infection [133]. Human cathelicidin LL-37 derived from host defense peptides was demonstrated to show protective effect against pulmonary respiratory syncytial virus (RSV) infection in murine model [134]. The potential of peptide libraries offers scientists insight into developing immunomodulatory therapeutic agents and additional antiviral or antimicrobial candidates. Despite the number of high-quality reviews that have been published, only a few of them have specifically highlighted the group of peptide drugs that are among the most promising antidengue strategies to date. In the present review, we focus on discussion as follow: i) categorize one feasible antidengue strategy based on DENV lifecycle; ii) peptides with certain bioactive epitopes having potential served as vaccination candidates; iii) the most effective and promising therapeutic peptides against DENV targeting specific antigenic domain; and iv) the drug discovery potential employing Antimicrobial Peptide Database (APD) to overcome emerging antimicrobial drug resistance.

2. STRATEGIES TO DEVELOP ANTIDENGUE PEPTIDE VACCINES AND DRUGS

2.1. Development of Peptide Vaccine for Dengue Virus in Immunomodulation

The four types of dengue vaccine are live attenuated viruses, inactivated viruses, recombinant subunit antigens, and

DNA vaccines [82, 124-129]. Compared to vaccines that entail using whole proteins or live attenuated viruses, the advantages of using synthetic peptides vaccines include: i) they contain no infectious agent; ii) lower the biological risk (*e.g.* recombination, reassortment, or genome integration); iii) have less potential for adverse effects (*e.g.* allergenicity or oncogenicity); iv) ease of administration; v) exhibit a high degree of specificity; and vi) are flexible to synthesize [130, 131]. Thus, various studies investigating anti-DENV peptides for developing a peptide vaccine are listed in Table **2**. In 2009, Amin *et al.* screened a phage-display random peptide library to identify dengue-specific B-cell epitopes [77]. They identified two peptides with sequences similar to specific regions of NS3 and NS4B proteins; the two peptides could be used for developing diagnostic tools or potential vaccines for DENV infection [77]. In 2010, Chakraborty *et al.* adopted a computational approach to locating 19 amino acids in a conserved region of the envelope protein in all four serotypes of DENV, and identified eight overlapping putative cytotoxic T-cell (CTL) epitopes (*i.e.* LGSQEGAMH, AMHTALTGA, EGAMHSALA, GSQEGAMHS, SALAGATEV, SQEGAMHSA, QEGAMHSAL, and GAMHSALAG) within the 19-mer conserved region [79]. They found that a 9-mer AMHTALTGA epitope exhibited the highest affinity with human leukocyte antigen molecules among the eight epitopes [79]. In 2011, Li *et al.* employed bioinformatics approaches and *in vitro* assays to design and synthesize a multi-epitope peptide (P1) containing the DENV-2 envelope domain III as a vaccine. The P1 induced lympho-proliferation *in vitro*, generated $CD4^+$ cell immune responses, and inhibited viral replication [82]. In addition, P1 reduced DENV-2 RNA in the blood of mice compared with a control (Day 1: 5.56 ± 3.302 *v.s.* 8.44 ± 6.89, Day 3: 0 *v.s.* 75 ± 24.59, Day 5: 0 *v.s.* 2.86 ± 0.948; unit: $\times 10^3$ copied/mL) [82]. In 2014, Gil *et al.* used mixtures (protein:DNA = 3:1) of the recombinant capsid protein (C) and ssDNA oligonucleotides from DENV-2 to produce recombinant nucleocapsid-like particles (NLPs-2) as a C-based vaccine that induced a protective $CD4^+$ and $CD8^+$ cell-mediated immunity in mice without assistance from neutralizing antibodies [76, 78, 83, 86]. In one of three monkeys, NLPs-2 induced interferon-gamma secretion and cytotoxic capacity and reduce the viral load [86]. In 2014, Rocha *et al.* showed that the peptide Pep03, which was derived from the DENV1–4 envelope domain II, induced a humoral response and cytotoxic activity against all DENV serotypes [88]. The viral single ORF polyprotein was post-translationally processed into 10 nonstructural (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) and structural proteins (capsid, premembrane, and envelope), all containing $CD8^+$ CTL epitopes [81]. NS3 contains more epitopes (31% of the total) having the most immunogenic effect as compared with the other nine DENV proteins, such as NS5 (22% of the total) [80, 81, 84, 85]. Piazza *et al.* found five NS3 peptide epitopes (three novel and two known) that were recognized by CTL in DENV-infected dendritic cells [87]. In 2015, Luo *et al.* used peptide scanning and a comprehensive bioinformatics analysis to reveal the premembrane protein pr4, which induced high titer antibodies in Balb/c mice [89].

2.2. Development of Peptides Inhibiting DENV Entry

Viral entry, the first stage of the DENV lifecycle, is crucial for the virus to reproduce and thereby establish infection. The E protein is considered a major mediator of viral entry and membrane fusion; thus, considerable efforts have been devoted to developing antiviral agents targeting the E protein [115, 116, 146]. This section reviews several peptide drug candidates to elucidate research on targeting the E protein (Table **3**).

Michael *et al.* investigated viral entry inhibition agents and their application [91]. For example, in 2005, they identified five regions on the E protein with high Wimley–White interfacial hydrophobicity scale scores as potential targets for antiviral peptides. The DN59 peptide, which is related to the stem domain of DENV, inhabited DENV and West Nile virus in a sequence-specific manner. In a study on the LLC-MK2 cell line, the DN59 peptide interfered with viral entry with a 50% inhibitory concentration (IC_{50}) in the 10-μM range. DN59 demonstrated >99% inhibition of plaque formation at concentrations below 25 μM, and no peptide cytotoxicity was observed in concentrations up to 100 mg/mL [91]. In 2010, they applied a computational method to design peptide inhibitors of the E protein. The two peptides with highly inhibitory activity, DN57opt and 1OAN1 (IC_{50} = 8 μm and 7 μm, respectively, in a focus-forming unit assay), hindered virus–host cell binding, interacted with the E protein, and altered the viral surface morphology [90]. In 2011, they evaluated the activity of two anti-DENV entry peptides, DN59 and 1OAN1, as an inhibitor of antibody-dependent enhancement, which can cause severe DENV disease symptoms. An *in vitro* study revealed that the two peptides inhibited infection of FcRII-expressing human K562 cells (IC_{50} = 3 and 6 μm for DN59 and 1OAN1, respectively), and neither peptide was cytotoxic [92]. In 2012, they elaborated on the inhibitory mechanism of peptide DN59; showing that the peptide induces pores in the viral membrane, causing viral RNA leakage. The results showed that DN59 peptide could inhibit all four DENV serotypes at an IC_{50} of 2–5 μM without signs of cytotoxicity to mammalian epithelial and mosquito cells at an IC_{50} of 50 μM [93].

Schmidt *et al.* proposed that peptides derived from the stem region of the E protein in DENV-2 ($DV2^{419–447}$) could inhibit viral infectivity by a two-step mechanism [94]. Initially, $DV2^{419–447}$ bound to a viral membrane was introduced into the cell through endocytosis; leading to subsequent $DV2^{419–447}$ binding to the E protein, and interruption of the conformational rearrangement in the acidic internal environment of the endosome, and consequently blocking the infection [94]. In another study, Schmidt *et al.* further confirmed that $Residues^{441–447}$ mainly interact with the viral membrane and $residues^{419–440}$ bound to the E protein [95]. They also suggested that the potency of stem-derived peptides could be markedly enhanced through appropriate modifications of $residues^{441–447}$ [95].

Alhoot *et al.* employed a bioinformatics algorithm (BioMoDroid) to design peptides targeting domain III of the E protein [99]. The sum of the hydrophobic and charge compatibility indices was calculated, and four peptide candidates were synthesized and investigated. An *in vitro* experiment showed that two peptides, DET2 and DET4, exhibit inhibitory activity against DENV-2 with an IC_{50} >500 μM and approximately 35 μM, respectively [99]. Laosutthipong *et al.* reported that viprolaxikine, antiviral peptide filtrates from

Table 2. Target and sequence of various peptide vaccines for dengue virus.

Target	Peptide sequence	Origins and Descriptions	References
B cell	FERVPGEVT RRALPPVSS	Two peptides were located in virus nonstructure protein (NS4b and NS3).	[77]
$CD8^+$ cell	AMHTALTGA	The synthetic peptide was selected from the 19-mer peptide sequence which is a conserved region in four DENV by computational approach.	[79]
B cell $CD4^+$ cell	P1: AKFVAAWTLKAAAGGRHVLGRLITVNPIVTG-GEPGQLNWFKKGSS	The P1 was located in virus-2 E domain III.	[82]
$CD4^+$ cell $CD8^+$ cell	Capsid: MNNQRKKAKNTPFNMLKRERNRVSTVQQLTKRFSLG-MLQGRGPLKLFMALVAFLRFLTIPPTAGILKRWGTIKK-SKAINVLRGFRKEIGRMLNILNRRRR ODN M39: ATCGACTCTCGAGCGTTCTCGGGGGACGATCGTCGG-GGG.	The NLPs-2 consisted of the recombinant capsid protein and ssDNA (ODN M39) (protein:DNA = 3:1).	[76, 78, 83, 86]
B cell $CD8^+$ cell	Pep03: LVTFKTAHAKKQEV-Linker-LVTFKNAAHAKKQEV-Linker-LVTFKNPHAKKQDV-Linker-LVTFKVPHAKRQDV Linker: GGGG	The Pep03 was a synthetic peptide that derived from four DENV envelope domain II.	[88]
$CD8^+$ cell	$NS3_{52–60}$: VTRGVY (DENV - 1) VTRGAVLMH (DENV - 2) VTRGAVLTY (DENV - 3·1) VTRGAVLTH (DENV - 3·2) VTRGSV ICH (DENV - 4) $NS3_{358–368}$: KTVWFVPSIKS (DENV - 1) KTVWFVPSIKA (DENV - 2 - 3 - 4) $NS3_{501–509}$: TPEGIIPAL (DENV - 1 - 3) TPEGIIPSM (DENV - 2) TPEGIIPTL (DENV - 4) $NS3_{538–547}$: MRRGDLPVWL (DENV - 1 - 2 - 3 - 4) $NS3_{575–583}$: EENMEVEIW (DENV - 2) EENMDVEIW (DENV - 1 - 3 - 4)	The peptides were synthesized based on NS3 protein.	[87]
B cell	pr4: KGKSLLFKTENGVNMC	The pr4 is a short sequence of pre-membrane protein.	[89]

DENV2 infected mosquito cell cultures, protected both insect and mammalian cells against DENV [96]. Further investigation into viprolaxikine indicated that the antiviral activity derived from three to four strongly anionic heptapeptides (*i.e.* DDHELQD, DETELQD, DEVMLQD and/or DEVLMQD) with the common sequence motif D-D/E-X-X-X-Q-D. The inhibitory activity was attributed to the interaction between viprolaxikine and the host cells, but not to directly acting on DENV2 [96]. Panya *et al.* adopted a molecular-docking method to identify small peptides that specifically bind to the hinge region of the E protein. The inhibitory activity of the seven peptide candidates was tested in Vero cells, and the peptide EF (Glu-Phe) reduced the foci formation by nearly 90% (IC_{50} = 96.5 μM). The results showed that EF inhibited all four DENV serotypes but was most effective against DENV-2 [97]. In 2013, Parikesit *et al.* used the molecular-docking method to screen commercial cyclic peptides in which the possible ligands targeted the E protein. They suggested that porcine BNP(7–32) might be an optimal ligand interacting with the E protein at temperature 310 and 312 K [98].

Table 3. Target and sequence of various peptides for anti-DENV entry.

Target	Peptide sequence	Origins and Descriptions	References
Stem region of E protein	DN59: MAILGDTAWDFGSLGGVFTSIGKALHQVFGAIY	Synthesized	[91-93]
The domain II hinge	DN57opt: RWMVWRHWFHRLRLPYNPGKNKQNQQWP	Synthesized	[90]
Domain I/domain II beta sheet connection	1OAN1: FWFTLIKTQAKQPARYRRFC	Synthesized	[90]
E protein	$DV2^{419-447}$: AWDFGSLGGVFTSIGKALHQVFGAIYGAA	Synthesized	[94, 95]
Domain III of DENV2 E protein	DET2: PWLKPGDLDL DET4: AGVKDGKLDF	Synthesized	[99]
Viprolaxikine and host cell	Viprolaxikine: (DDHELQD, DETELQD, DEVMLQD and/or DEVLMQD) common motif: D-D/E-X-X-X-Q-D	Filtrate from persistently infected cells	[96]
E Protein	EF (Glu-Phe)	Molecular docking	[97]
E Protein	BNP(7-32), porcine: DSGCFGRRLDRIGSLSGLGCNVLRRY	Molecular docking	[98]

2.3. Development of Peptides for Anti-DENV Replication

Table **4** lists some of the peptides tested for inhibition of DENV replication. NS2B/NS3 is an essential protease for viral replication in host cells [63-73]. NS2B is a highly conserved nonstructural protein in flaviviruses, acting as a cofactor for NS3 protease activity [118, 120, 147, 148]. In 2005, Li *et al.* identified a suitable substrate for NS2B/NS3 protease by using a tetrapeptide library [73]. Following that study, 2006 Yin *et al.* designed a tetrapeptide inhibitor for reducing the enzyme activity of NS2B/NS3 [121, 149]. They tested tetrapeptides with various warheads and found that benzoyl-NKRR-H was the most suitable candidate for inhibiting NS2B/NS3 (K_i = 5.8 μM) [121, 149]. In 2010, Tambunan and Alamudi used a bioinformatics method to design peptide inhibitors for NS2B/NS3 [71]. Seven cyclopentapeptides [Disulfide Bridge: 1-5] were designed (CKRRC, CGRRC, CRGRC, CRTRC, CTRRC, CKRKC, and CRRKC) using a molecular-docking method [71]. The optimal cyclopentapeptide was CKRKC, for which the K_i was estimated at 0.707 μM [71]. In 2011, Schuller *et al.* modified the benzoyl-KRR-H sequence [70, 149] and found that the inhibitory effects of phenylacetyl-KRR-H were more powerful than those of benzoyl-KRR-H (IC_{50} = 6.7 ± 1.1 μM *vs* 127 ± 2.1 μM). Moreover, they found that this new tripeptide was superior to the previously referenced inhibitor benzoyl-NKRR-H with improved IC_{50} value (IC_{50} = 6.7 ± 1.1 μM *vs* 9.5 ± 0.21 μM) [70]. In 2012, they reported the anti-DENV effect of protegin (PG-1), a cationic cyclic peptide [Disulfide Bridge: 6-15; 8-13] isolated from porcine leukocytes [66], finding that the IC_{50} of PG-1 to NS2B/NS3 was 11.7 ± 2.23 μM and the inhibition reached 95.7% at 40 μM [66]. In addition, they demonstrated that PG-1 reduced DENV-2 infection in MK-2 cell lines in a dose-dependent manner and the inhibition reached nearly 100% at 12.5 μM [66]. In 2012, Rothan *et al.* found that retrocyclin-1 (RC-1, Table **4**) can act as an anti-DENV peptide containing 18 amino acids [Disulfide Bridge: 3-16; 5-14; 7-12] [68, 122]. The RC-1 was θ-defensin with broad-spectrum antimicrobial activity [150]. An *Escherichia coli* expression system and refolding environment for recombinant RC-1 were established, and the recombinant RC-1 was employed to test the inhibitory effect of NS2B/NS3 and DENV-2 infection in Vero cells [68]. The results showed that the IC_{50} of recombinant RC-1 was relative to the temperature (46.1 ± 1.7 μM at 28 °C; 21.4 ± 1.6 μM at 37 °C ; 14.1 ± 1.2 μM at 40 °C), and that the most remarkable reduction in infection rate was achieved when Vero cells were treated simultaneously with recombinant RC-1 (150 μM) and DENV-2 (70% ± 6.3 at 48 h and 85% ± 7.1 at 72 h) compared with pre-treated (40% at 48 h and 38% at 72 h) or post-treated (30% at 48 h and 45% at 72 h) groups [68]. In 2012, Xu *et al.* designed the cyclic peptide CAGKRKSG [Cyclic bridge:1-8] inhibitor against DENV based on MrIA, a conotoxin that can inhibit NS2B/NS3 [72, 123]. In 2013, Rothan *et al.* reported that recombinant plectasin peptide could act as an anti-DENV peptide [69]. Plectasin is a 40-amino acid fungal cationic cyclic peptide [Disulfide Bridge:4-30; 15-37; 19-39] produced by *Pseudoplectania nigrella* [69, 151, 152]. The results showed that the recombinant plectasin inhibited NS2B/NS3 activity (Ki = 5.031 ± 0.98 μM) and enhanced inhibition in a dose-dependent manner (approximately 40% at 5.0 μM; approximately 60% at 10 μM; approximately 80% at 20 μM) [69]. In 2014, Rothan *et al.* reported that latarcin (Ltc 1), the natural antimicrobial agent isolated from spider venom, could inhibit dengue infection *in vitro* [67]. Ltc 1 inhibits NS2B/NS3 activity related to the temperature effect (IC_{50} = 12.68 ± 3.2 μM at 37°C; IC_{50} = 6.58 ± 4.1 μM at 40°C). In addition, peptide against DENV replication assay showed that Ltc 1 treated simultaneously or post-infected HepG2 cells having better inhibitory effects than pre-infected group [67].

Another target for peptide drug design is the NS5 methyltransferase, which has two critical active sites on its surface:

Table 4. Target and sequence of various peptides for anti-DENV replication.

Target	Peptide sequence	Origins and Descriptions	References
NS2B/NS3	Benzoyl-NKRR-H	This peptide was modified from known tetrapeptide substrate benzoyl-NKRR-AMC (7-amido-4-methylcoumarin).	[64, 73, 121, 149]
	CKRKC [Disulfide Bridge: 1-5]	This peptide was designed by used molecular docking approach.	[71]
	Phenylacetyl-KRR-H	This peptide was modified from known tri-peptide substrate Bz-KRR-H.	[70, 149]
	PG1: RGGRLCYCRRRFCVCVGR [Disulfide Bridge: 6-15; 8-13]	PG-1 was isolated form porcine leukocytes.	[66]
	RC-1: GICRCICGRGICRCICGR [Disulfide Bridge: 3-16; 5-14; 7-12]	RC-1 is encoded in the human genome by a theta-defensins pseudogene.	[68, 122, 150]
	CAGKRKSG [Cyclic bridge:1-8]	This peptide was modified from conotoxins, MrIA, which was produced by cone snail.	[72]
	Plectasin: GFGCNGPWDEDDMQCHNHCKSIKGYKGGY-CAKGGFVCKCY [Disulfide Bridge:4-30; 15-37; 19-39]	Plectasin is a fungal peptide produced from *Pseudoplectania nigrella.*	[69, 152]
	Ltc 1: SMWSGMWRRKLKKLRNALKKKLKGE	Ltc 1 is a peptide from venom gland of *Lachesana tarabaeve*.	[67]
NS5 methyltransferase	CTWYC for SAM binding site [Disulfide Bridge: 1-5] CYEFC for RNA-cap site [Disulfide Bridge: 1-5]	These two peptides were designed by used molecular docking approach.	[74]
	[Tyr123] Prepro Endothelin (110-130), amide, human: CQCASQKDKKWSYCQAGKEI for SAM binding site [Disulfide Bridge: 110-124; 112-120] Urotensin II, human ETPDCFWKYCV for RNA-cap site [Disulfide Bridge: 5–10]	These two peptides were screen by molecular dynamics simulation and molecular docking approaches from commercial cyclic peptide	[75]

a SAM binding site and an RNA-capping site [74, 75]. In 2012, Idrus *et al.* employed a molecular-docking methodology to design a peptide inhibitor for NS5 methyltransferase and proposed two cyclic peptides [Disulfide Bridge: 1-5]: CTWYC for the SAM-binding site ($\Delta G_{binding}$ = -30.72 kcal/mol) and CYEFC for the RNA-capping site ($\Delta G_{binding}$ = -22.89 kcal/mol) [74]. In 2014, Tambunan *et al.* screened commercial cyclic peptide to inhibit NS5 methyltransferase by molecular dynamics simulation and molecular docking [75]. They suggested that the commercial cyclic peptides [Tyr123] Prepro Endothelin (110-130), amide, human [Disulfide Bridge: 110-124; 112-120]" would bind stably to SAM sites (ΔG = -24.73kcal/mol) and "Urotensin II [Disulfide Bridge: 5–10]" would bind stably to RNA-capping sites (ΔG = -19.04 kcal/mol).

CONCLUSION

DENV infection has reemerged as a major public health concern with significant socioeconomic impact resulting in a worldwide changes in prevalence and increase epidemics [153, 154]. AVPs and AMPs, a group of evolutionarily conserved small peptides are promising therapeutic candidates for DENV infections because of their known antiviral effects. Three major pathways involved in the DENV lifecycle have been explored as potential targets of peptide-based drugs; immunomodulation, inhibition of viral entry, and interference with specific steps in the viral replication cycle. This review provides a summary of the efforts to develop AVPs for their antiviral and immunogenic capabilities for dealing with DENV infection. Two patented peptides that interfere with DENV entry are currently in development, suggesting that peptide drugs could play a critical role in combating this globally prevalent tropical disease and illustrate the design principles for developing novel drugs as an alternative strategy to overcome microbial drug resistance. Current challenges with AVPs and AMPs reveal that only a few have entered into clinical trials or have been approved by the US Food and Drug Administration (FDA). AVPs and AMPs can be digested by proteolytic enzyme in the gastrointestinal tract following oral administration; short half lives *in vivo*, protease degradation and rapid kidney clearance are

limitations for systemic administration [155]. Feasible strategies to circumvent these drawbacks include chemical modification and the use of delivery vehicles [156, 157]; therefore, future investigation is warranted to improve their efficacy as well as clinical utility.

LIST OF ABBREVIATIONS

AMPs = Antimicrobial peptides

APD = Antimicrobial Peptide Database

AVPs = Antiviral peptides

C = Capsid glycoprotein

CTL = Cytotoxic T-cell

DENV = Dengue virus

E = Envelope glycoprotein

ER = Endoplasmic reticulum

HSV = Herpes simplex virus

IC_{50} = Half maximal inhibitory concentration

Ltc 1 = Latarcin

NS = Nonstructural proteins

PG-1 = Protegin

prM = Premembrane glycoprotein

RC-1 = Retrocyclin-1

WHO = World Health Organization

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Messina, J.P.; Brady, O.J.; Scott, T.W.; Zou, C.; Pigott, D.M.; Duda, K.A.; Bhatt, S.; Katzelnick, L.; Howes, R.E.; Battle, K.E.; Simmons, C.P.; Hay, S.I. Global spread of dengue virus types: Mapping the 70 year history. *Trends Microbiol.,* **2014**, *22*(3), 138-146.

[2] Bhatt, S.; Gething, P.W.; Brady, O.J.; Messina, J.P.; Farlow, A.W.; Moyes, C.L.; Drake, J.M.; Brownstein, J.S.; Hoen, A.G.; Sankoh, O.; Myers, M.F.; George, D.B.; Jaenisch, T.; Wint, G.R.; Simmons, C.P.; Scott, T.W.; Farrar, J.J.; Hay, S.I. The global distribution and burden of dengue. *Nature,* **2013**, *496*(7446), 504-507.

[3] Molyneux, D.H.; Savioli, L.; Engels, D. Neglected tropical diseases: Progress towards addressing the chronic pandemic. *Lancet,* **2017**, *389*(10066), 312-325.

[4] Shepard, D.S.; Undurraga, E.A.; Halasa, Y.A.; Stanaway, J.D. The global economic burden of dengue: A systematic analysis. *Lancet. Infect. Dis.,* **2016**, *16*(8), 935-941.

[5] Hales, S.; de Wet, N.; Maindonald, J.; Woodward, A. Potential effect of population and climate changes on global distribution of dengue fever: An empirical model. *Lancet,* **2002**, *360*(9336), 830-834.

[6] Schaffner, F.; Mathis, A. Dengue and dengue vectors in the WHO European region: Past, present, and scenarios for the future. *Lancet. Infect. Dis.,* **2014**, *14*(12), 1271-1280.

[7] Messina, J.P.; Brady, O.J.; Pigott, D.M.; Golding, N.; Kraemer, M.U.; Scott, T.W.; Wint, G.R.; Smith, D.L.; Hay, S.I. The many projected futures of dengue. *Nat. Rev. Microbiol.,* **2015**, *13*(4), 230-239.

[8] Guzman, M.G.; Harris, E. Dengue. *Lancet,* **2015**, *385*(9966), 453-465.

[9] Luz, P.M.; Vanni, T.; Medlock, J.; Paltiel, A.D.; Galvani, A.P. Dengue vector control strategies in an urban setting: An economic modelling assessment. *Lancet,* **2011**, *377*(9778), 1673-1680.

[10] Mustafa, M.S.; Rasotgi, V.; Jain, S.; Gupta, V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. *Med. J. Armed Forces India,* **2015**, *71*(1), 67-70.

[11] Normile, D. Tropical medicine. Surprising new dengue virus throws a spanner in disease control efforts. *Science,* **2013**, *342*(6157), 415.

[12] Vasilakis, N.; Cardosa, J.; Hanley, K.A.; Holmes, E.C.; Weaver, S.C. Fever from the forest: Prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nat. Rev . Microbiol.,* **2011**, *9*(7), 532-541.

[13] Simmons, C.P.; Farrar, J.J.; Nguyen v, V.; Wills, B. Dengue. *N. Engl. J. Med.,* **2012,** *366*(15), 1423-1432.

[14] Rothman, A.L. Immunity to dengue virus: A tale of original antigenic sin and tropical cytokine storms. *Nat. Rev. Immunol.,* **2011**, *11*(8), 532-543.

[15] St John, A.L.; Abraham, S.N.; Gubler, D.J. Barriers to preclinical investigations of anti-dengue immunity and dengue pathogenesis. *Nat. Rev. Microbiol.,* **2013**, *11*(6), 420-426.

[16] Pulendran, B. Learning immunology from the yellow fever vaccine: Innate immunity to systems vaccinology. *Nat. Rev. Immunol.,* **2009**, *9*(10), 741-747.

[17] Screaton, G.; Mongkolsapaya, J.; Yacoub, S.; Roberts, C. New insights into the immunopathology and control of dengue virus infection. *Nat. Rev. Immunol.,* **2015**, *15*(12), 745-759.

[18] Murphy, B.R.; Whitehead, S.S. Immune response to dengue virus and prospects for a vaccine. *Annu. Rev. Immunol.,* **2011**, *29*, 587-619.

[19] Dejnirattisai, W.; Jumnainsong, A.; Onsirisakul, N.; Fitton, P.; Vasanawathana, S.; Limpitikul, W.; Puttikhunt, C.; Edwards, C.; Duangchinda, T.; Supasa, S.; Chawansuntati, K.; Malasit, P.; Mongkolsapaya, J.; Screaton, G. Cross-reacting antibodies enhance dengue virus infection in humans. *Science,* **2010,** *328*(5979), 745-748.

[20] Carod-Artal, F.J.; Wichmann, O.; Farrar, J.; Gascon, J. Neurological complications of dengue virus infection. *Lancet Neurol.,* **2013**, *12*(9), 906-919.

[21] Yacoub, S.; Wertheim, H.; Simmons, C.P.; Screaton, G.; Wills, B. Cardiovascular manifestations of the emerging dengue pandemic. *Nat. Rev. Cardiol.,* **2014**, *11*(6), 335-345.

[22] Bich, T.D.; Pham, O.K.; Hai, D.H.; Nguyen, N.M.; Van, H.N.; The, T.D.; Wills, B.; Yacoub, S. A pregnant woman with acute cardiorespiratory failure: Dengue myocarditis. *Lancet,* **2015**, *385*(9974), 1260.

[23] Krishnan, M.N.; Garcia-Blanco, M.A. Targeting host factors to treat West Nile and dengue viral infections. *Viruses,* **2014**, *6*(2), 683-708.

[24] Kuhn, R.J.; Zhang, W.; Rossmann, M.G.; Pletnev, S.V.; Corver, J.; Lenches, E.; Jones, C.T.; Mukhopadhyay, S.; Chipman, P.R.; Strauss, E.G.; Baker, T.S.; Strauss, J.H. Structure of dengue virus: Implications for flavivirus organization, maturation, and fusion. *Cell,* **2002**, *108*(5), 717-725.

[25] Stevens, A.J.; Gahan, M.E.; Mahalingam, S.; Keller, P.A. The medicinal chemistry of dengue fever. *J. Med. Chem.,* **2009**, *52*(24), 7911-7926.

[26] Akey, D.L.; Brown, W.C.; Dutta, S.; Konwerski, J.; Jose, J.; Jurkiw, T.J.; DelProposto, J.; Ogata, C.M.; Skiniotis, G.; Kuhn, R.J.; Smith, J.L. Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system. *Science,* **2014**, *343*(6173), 881-885.

[27] Smit, J.M.; Moesker, B.; Rodenhuis-Zybert, I.; Wilschut, J. Flavivirus cell entry and membrane fusion. *Viruses,* **2011**, *3*(2), 160-171.

[28] Dong, H.; Zhang, B.; Shi, P.Y. Flavivirus methyltransferase: A novel antiviral target. *Antiviral Res.,* **2008**, *80*(1), 1-10.

[29] Iglesias, N.G.; Filomatori, C.V.; Gamarnik, A.V. The F1 motif of dengue virus polymerase NS5 is involved in promoter-dependent RNA synthesis. *J. Virol.,* **2011**, *85*(12), 5745-5756.

[30] Issur, M.; Geiss, B.J.; Bougie, I.; Picard-Jean, F.; Despins, S.; Mayette, J.; Hobdey, S.E.; Bisaillon, M. The flavivirus NS5 protein is a true RNA guanylyltransferase that catalyzes a two-step reaction to form the RNA cap structure. *RNA,* **2009**, *15*(12), 2340-2350.

[31] Nitsche, C.; Holloway, S.; Schirmeister, T.; Klein, C.D. Biochemistry and medicinal chemistry of the dengue virus protease. *Chem. Rev.,* **2014**, *114*(22), 11348-11381.

[32] Schleich, K.; Nurnberger, C.; Sobanski, A.; Efferth, T. Vaccination and antiviral treatment of neglected diseases caused by flaviviral infections. *Curr. Med. Chem.,* **2011**, *18*(4), 604-614.

[33] Perera, R.; Khaliq, M.; Kuhn, R.J. Closing the door on flaviviruses: Entry as a target for antiviral drug design. *Antiviral Res.,* **2008**, *80*(1), 11-22.

[34] Rawlinson, S.M.; Pryor, M.J.; Wright, P.J.; Jans, D.A. Dengue virus RNA polymerase NS5: A potential therapeutic target? *Curr. Drug Targets,* **2006**, *7*(12), 1623-1638.

[35] Thomas, S.J. Developing a dengue vaccine: Progress and future challenges. *Ann. N. Y. Acad. Sci.,* **2014**, *1323*, 140-159.

[36] Zasloff, M. Antimicrobial peptides of multicellular organisms. *Nature,* **2002**, *415*(6870), 389-395.

[37] Giuliani, A.; Pirri, G.; Nicoletto, S.F. Antimicrobial peptides: An overview of a promising class of therapeutics. *Cent. Eur. J. Biol.,* **2007**, *2*(1), 1-33.

[38] Sitaram, N.; Nagaraj, R. The therapeutic potential of host-defense antimicrobial peptides. *Curr. Drug Targets,* **2002**, *3*(3), 259-267.

[39] Cruz, J.; Ortiz, C.; Guzman, F.; Fernandez-Lafuente, R.; Torres, R. Antimicrobial peptides: Promising compounds against pathogenic microorganisms. *Curr. Med. Chem.,* **2014**, *21*(20), 2299-2321.

[40] Diamond, G.; Beckloff, N.; Weinberg, A.; Kisich, K.O. The roles of antimicrobial peptides in innate host defense. *Curr. Pharm. Des.,* **2009**, *15*(21), 2377-2392.

[41] Mookherjee, N.; Hancock, R.E. Cationic host defence peptides: Innate immune regulatory peptides as a novel approach for treating infections. *Cell Mol. Life Sci.,* **2007**, *64*(7-8), 922-933.

[42] Mollica, A.; Stefanucci, A.; Costante, R. Strategies for developing tuberculosis vaccines: Emerging approaches. *Curr. Drug Targets,* **2013**, *14*(9), 938-951.

[43] Schuerholz, T.; Domming, S.; Hornef, M.; Dupont, A.; Kowalski, I.; Kaconis, Y.; Heinbockel, L.; Andra, J.; Garidel, P.; Gutsmann, T.; David, S.; Sanchez-Gomez, S.; Martinez de Tejada, G.; Brandenburg, K. Bacterial cell wall compounds as promising targets of antimicrobial agents II. Immunological and clinical aspects. *Curr. Drug Targets,* **2012**, *13*(9), 1131-1137.

[44] Galdiero, S.; Falanga, A.; Berisio, R.; Grieco, P.; Morelli, G.; Galdiero, M. Antimicrobial peptides as an opportunity against bacterial diseases. *Curr. Med. Chem.,* **2015**, *22*(14), 1665-1677.

[45] Ong, P.Y.; Ohtake, T.; Brandt, C.; Strickland, I.; Boguniewicz, M.; Ganz, T.; Gallo, R. L.; Leung, D.Y. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N. Engl. J. Med.,* **2002**, *347*(15), 1151-1160.

[46] Yang, D.; Biragyn, A.; Hoover, D.M.; Lubkowski, J.; Oppenheim, J.J. Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. *Annu. Rev. Immunol.,* **2004**, *22*, 181-215.

[47] Hancock, R.E.; Lehrer, R. Cationic peptides: A new source of antibiotics. *Trends Biotechnol.,* **1998**, *16*(2), 82-88.

[48] Peters, B.M.; Shirtliff, M.E.; Jabra-Rizk, M.A. Antimicrobial peptides: Primeval molecules or future drugs? *PLoS Pathog.,* **2010**, *6*(10), e1001067.

[49] Oyinloye, B.; Adenowo, F.; Gxaba, N.; Kappo, A. The promise of antimicrobial peptides for treatment of human schistosomiasis. *Curr. Drug Targets,* **2014**, *15*(9), 852-859.

[50] Torrent, M.; Pulido, D.; Rivas, L.; Andreu, D. Antimicrobial peptide action on parasites. *Curr. Drug Targets,* **2012**, *13*(9), 1138-1147.

[51] Gomara, M.J.; Haro, I. Updating the use of synthetic peptides as inhibitors of HIV-1 entry. *Curr. Med. Chem.,* **2014**, *21*(10), 1188-1200.

[52] Nizet, V.; Ohtake, T.; Lauth, X.; Trowbridge, J.; Rudisill, J.; Dorschner, R.A.; Pestonjamasp, V.; Piraino, J.; Huttner, K.; Gallo, R.L. Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature,* **2001**, *414*(6862), 454-457.

[53] Thakur, N.; Qureshi, A.; Kumar, M. AVPpred: Collection and prediction of highly effective antiviral peptides. *Nucleic Acids Res.,* **2012**, *40*(Web Server issue), W199-W204.

[54] Menard, S.; Forster, V.; Lotz, M.; Gutle, D.; Duerr, C.U.; Gallo, R.L.; Henriques-Normark, B.; Putsep, K.; Andersson, M.; Glocker, E.O.; Hornef, M.W. Developmental switch of intestinal antimicrobial peptide expression. *J. Exp. Med.,* **2008**, *205*(1), 183-193.

[55] Peschel, A.; Sahl, H.G. The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nat. Rev. Microbiol.,* **2006**, *4*(7), 529-536.

[56] Martinez de Tejada, G.; Sanchez-Gomez, S.; Razquin-Olazaran, I.; Kowalski, I.; Kaconis, Y.; Heinbockel, L.; Andra, J.; Schurholz, T.; Hornef, M.; Dupont, A.; Garidel, P.; Lohner, K.; Gutsmann, T.; David, S.A.; Brandenburg, K. Bacterial cell wall compounds as promising targets of antimicrobial agents I. Antimicrobial peptides and lipopolyamines. *Curr. Drug Targets,* **2012**, *13*(9), 1121-1130.

[57] Cudic, M.; Otvos, L., Jr. Intracellular targets of antibacterial peptides. *Curr. Drug Targets,* **2002**, *3*(2), 101-106.

[58] Hazrati, E.; Galen, B.; Lu, W.; Wang, W.; Ouyang, Y.; Keller, M.J.; Lehrer, R.I.; Herold, B.C. Human alpha- and beta-defensins block multiple steps in herpes simplex virus infection. *J. Immunol.,* **2006**, *177*(12), 8658-8666.

[59] Doss, M.; White, M.R.; Tecle, T.; Gantz, D.; Crouch, E.C.; Jung, G.; Ruchala, P.; Waring, A.J.; Lehrer, R.I.; Hartshorn, K.L. Interactions of alpha-, beta-, and theta-defensins with influenza A virus and surfactant protein D. *J. Immunol.,* **2009**, *182*(12), 7878-7887.

[60] Cole, A.M.; Hong, T.; Boo, L.M.; Nguyen, T.; Zhao, C.; Bristol, G.; Zack, J.A.; Waring, A.J.; Yang, O.O.; Lehrer, R.I. Retrocyclin: A primate peptide that protects cells from infection by T- and M-tropic strains of HIV-1. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*(4), 1813-1818.

[61] Furci, L.; Sironi, F.; Tolazzi, M.; Vassena, L.; Lusso, P. Alpha-defensins block the early steps of HIV-1 infection: Interference with the binding of gp120 to CD4. *Blood,* **2007**, *109*(7), 2928-2935.

[62] Afshar, M.; Gallo, R.L. Innate immune defense system of the skin. *Vet. Dermatol.,* **2013**, *24*(1), 32-8 e8-9.

[63] Idrees, S.; Ashfaq, U.A. Discovery and design of cyclic peptides as dengue virus inhibitors through structure-based molecular docking. *Asian Pac. J. Trop. Med.,* **2014**, *7*(7), 513-516.

[64] Lohr, K.; Knox, J.E.; Phong, W.Y.; Ma, N.L.; Yin, Z.; Sampath, A.; Patel, S.J.; Wang, W.L.; Chan, W.L.; Rao, K.R.; Wang, G.; Vasudevan, S.G.; Keller, T.H.; Lim, S.P. Yellow fever virus NS3 protease: Peptide-inhibition studies. *J. Gen. Virol.,* **2007**, *88*(Pt 8), 2223-2227.

[65] Prusis, P.; Junaid, M.; Petrovska, R.; Yahorava, S.; Yahorau, A.; Katzenmeier, G.; Lapins, M.; Wikberg, J.E. Design and evaluation of substrate-based octapeptide and non substrate-based tetrapeptide inhibitors of dengue virus NS2B-NS3 proteases. *Biochem. Biophys. Res. Commun.,* **2013**, *434*(4), 767-772.

[66] Rothan, H.A.; Abdulrahman, A.Y.; Sasikumer, P.G.; Othman, S.; Rahman, N.A.; Yusof, R. Protegrin-1 inhibits dengue NS2B-NS3 serine protease and viral replication in MK2 cells. *J. Biomed. Biotechnol.,* **2012**, *2012*, 251482.

[67] Rothan, H.A.; Bahrani, H.; Rahman, N.A.; Yusof, R. Identification of natural antimicrobial agents to treat dengue infection: *In vitro* analysis of latarcin peptide activity against dengue virus. *BMC Microbiol.,* **2014**, *14*, 140.

[68] Rothan, H.A.; Han, H.C.; Ramasamy, T.S.; Othman, S.; Rahman, N.A.; Yusof, R. Inhibition of dengue NS2B-NS3 protease and viral replication in Vero cells by recombinant retrocyclin-1. *BMC Infect. Dis.,* **2012**, *12*, 314.

[69] Rothan, H.A.; Mohamed, Z.; Suhaeb, A.M.; Rahman, N.A.; Yusof, R. Antiviral cationic peptides as a strategy for innovation in global health therapeutics for dengue virus: High yield production of the biologically active recombinant plectasin peptide. *OMICS,* **2013**, *17*(11), 560-567.

[70] Schuller, A.; Yin, Z.; Brian Chia, C.S.; Doan, D.N.; Kim, H.K.; Shang, L.; Loh, T.P.; Hill, J.; Vasudevan, S.G. Tripeptide inhibitors of dengue and West Nile virus NS2B-NS3 protease. *Antiviral Res.,* **2011**, *92*(1), 96-101.

[71] Tambunan, U.S.; Alamudi, S. Designing cyclic peptide inhibitor of dengue virus NS3-NS2B protease by using molecular docking approach. *Bioinformation,* **2010**, *5*(6), 250-254.

[72] Xu, S.; Li, H.; Shao, X.; Fan, C.; Ericksen, B.; Liu, J.; Chi, C.; Wang, C. Critical effect of peptide cyclization on the potency of peptide inhibitors against Dengue virus NS2B-NS3 protease. *J. Med. Chem.,* **2012**, *55*(15), 6881-6887.

[73] Li, J.; Lim, S.P.; Beer, D.; Patel, V.; Wen, D.; Tumanut, C.; Tully, D.C.; Williams, J.A.; Jiricek, J.; Priestle, J.P.; Harris, J.L.; Vasudevan, S.G. Functional profiling of recombinant NS3 proteases from all four serotypes of dengue virus using tetrapeptide and octapeptide substrate libraries. *J. Biol. Chem.,* **2005**, *280*(31), 28766-28774.

[74] Idrus, S.; Tambunan, U.S.; Zubaidi, A.A. Designing cyclopentapeptide inhibitor as potential antiviral drug for dengue virus ns5 methyltransferase. *Bioinformation,* **2012**, *8*(8), 348-352.

[75] Tambunan, U.S.; Zahroh, H.; Utomo, B.B.; Parikesit, A.A. Screening of commercial cyclic peptide as inhibitor NS5 methyltransferase of dengue virus through molecular docking and molecular dynamics simulation. *Bioinformation,* **2014**, *10*(1), 23-27.

[76] Lazo, L.; Hermida, L.; Zulueta, A.; Sanchez, J.; Lopez, C.; Silva, R.; Guillen, G.; Guzman, M.G. A recombinant capsid protein from Dengue-2 induces protection in mice against homologous virus. *Vaccine,* **2007**, *25*(6), 1064-1070.

[77] Amin, N.; Aguilar, A.; Chamacho, F.; Vazquez, Y.; Pupo, M.; Ramirez, J.C.; Izquierdo, L.; Dafhnis, F.; Stott, D.I.; Perez, E.M.; Acosta, A. Identification of Dengue-specific B-cell epitopes by phage-display random peptide library. *Malays. J. Med. Sci.,* **2009**, *16*(4), 4-14.

[78] Lopez, C.; Gil, L.; Lazo, L.; Menendez, I.; Marcos, E.; Sanchez, J.; Valdes, I.; Falcon, V.; de la Rosa, M.C.; Marquez, G.; Guillen, G.; Hermida, L. *In vitro* assembly of nucleocapsid-like particles from purified recombinant capsid protein of dengue-2 virus. *Arch. Virol.,* **2009**, *154*(4), 695-698.

[79] Chakraborty, S.; Chakravorty, R.; Ahmed, M.; Rahman, A.; Waise, T.M.; Hassan, F.; Rahman, M.; Shamsuzzaman, S. A computational approach for identification of epitopes in dengue virus envelope protein: A step towards designing a universal dengue vaccine targeting endemic regions. *In Silico Biol.,* **2010**, *10*(5-6), 235-246.

[80] Duangchinda, T.; Dejnirattisai, W.; Vasanawathana, S.; Limpitikul, W.; Tangthawornchaikul, N.; Malasit, P.; Mongkolsapaya, J.; Screaton, G. Immunodominant T-cell responses to dengue virus NS3 are associated with DHF. *Proc Natl. Acad. Sci. USA,* **2010**, *107*(39), 16922-16927.

[81] Vaughan, K.; Greenbaum, J.; Blythe, M.; Peters, B.; Sette, A. Meta-analysis of all immune epitope data in the Flavivirus genus: Inventory of current immune epitope data status in the context of virus immunity and immunopathology. *Viral Immunol.,* **2010**, *23*(3), 259-284.

[82] Li, S.; Peng, L.; Zhao, W.; Zhong, H.; Zhang, F.; Yan, Z.; Cao, H. Synthetic peptides containing B- and T-cell epitope of dengue virus-2 E domain III provoked B- and T-cell responses. *Vaccine,* **2011**, *29*(20), 3695-3702.

[83] Gil, L.; Bernardo, L.; Pavon, A.; Izquierdo, A.; Valdes, I.; Lazo, L.; Marcos, E.; Romero, Y.; Guzman, M.G.; Guillen, G.; Hermida, L. Recombinant nucleocapsid-like particles from dengue-2 induce functional serotype-specific cell-mediated immunity in mice. *J. Gen. Virol.,* **2012**, *93*(Pt 6), 1204-1214.

[84] Rivino, L.; Kumaran, E.A.; Jovanovic, V.; Nadua, K.; Teo, E.W.; Pang, S.W.; Teo, G.H.; Gan, V.C.; Lye, D.C.; Leo, Y.S.; Hanson, B.J.; Smith, K.G.; Bertoletti, A.; Kemeny, D.M.; MacAry, P.A. Differential targeting of viral components by CD4+ versus CD8+ T lymphocytes in dengue virus infection. *J. Virol.,* **2013**, *87*(5), 2693-2706.

[85] Weiskopf, D.; Angelo, M.A.; de Azeredo, E.L.; Sidney, J.; Greenbaum, J.A.; Fernando, A.N.; Broadwater, A.; Kolla, R.V.; De Silva, A.D.; de Silva, A.M.; Mattia, K.A.; Doranz, B.J.; Grey, H.M.; Shresta, S.; Peters, B.; Sette, A. Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ T cells. *Proc. Natl. Acad. Sci. USA,* **2013**, *110*(22), E2046-E2053.

[86] Gil, L.; Izquierdo, A.; Lazo, L.; Valdes, I.; Ambala, P.; Ochola, L.; Marcos, E.; Suzarte, E.; Kariuki, T.; Guzman, G.; Guillen, G.; Hermida, L. Capsid protein: Evidences about the partial protective role of neutralizing antibody-independent immunity against dengue in monkeys. *Virology,* **2014**, *456-457*, 70-76.

[87] Piazza, P.; Campbell, D.; Marques, E.; Hildebrand, W.H.; Buchli, R.; Mailliard, R.; Rinaldo, C.R. Dengue virus-infected human dendritic cells reveal hierarchies of naturally expressed novel NS3 CD8 T cell epitopes. *Clin. Exp. Immunol.,* **2014**, *177*(3), 696-702.

[88] Rocha, R.P.; Livonesi, M.C.; Fumagalli, M.J.; Rodrigues, N.F.; da Costa, L.C.; Dos Santos, M.C.; de Oliveira Rocha, E.S.; Kroon, E.G.; Malaquias, L.C.; Coelho, L.F. Evaluation of tetravalent and conserved synthetic peptides vaccines derived from Dengue virus Envelope domain I and II. *Virus Res.,* **2014**, *188*, 122-127.

[89] Luo, Y.; Guo, X.; Yan, H.; Fang, D.; Zeng, G.; Zhou, J.; Jiang, L. Comprehensive mapping infection-enhancing epitopes of dengue pr protein using polyclonal antibody against prM. *Appl. Microbiol. Biotechnol.,* **2015**, *99*(14), 5917-5927.

[90] Costin, J.M.; Jenwitheesuk, E.; Lok, S.M.; Hunsperger, E.; Conrads, K.A.; Fontaine, K.A.; Rees, C.R.; Rossmann, M.G.; Isern, S.; Samudrala, R.; Michael, S.F. Structural optimization and de novo design of dengue virus entry inhibitory peptides. *PLoS Negl. Trop. Dis.,* **2010**, *4*(6), e721.

[91] Hrobowski, Y.M.; Garry, R.F.; Michael, S.F. Peptide inhibitors of dengue virus and West Nile virus infectivity. *Virol. J.,* **2005**, *2*, 49.

[92] Nicholson, C.O.; Costin, J.M.; Rowe, D.K.; Lin, L.; Jenwitheesuk, E.; Samudrala, R.; Isern, S.; Michael, S.F. Viral entry inhibitors block dengue antibody-dependent enhancement *in vitro*. *Antiviral Res.,* **2011**, *89*(1), 71-74.

[93] Lok, S.M.; Costin, J.M.; Hrobowski, Y.M.; Hoffmann, A.R.; Rowe, D.K.; Kukkaro, P.; Holdaway, H.; Chipman, P.; Fontaine, K.A.; Holbrook, M.R.; Garry, R.F.; Kostyuchenko, V.; Wimley, W.C.; Isern, S.; Rossmann, M.G.; Michael, S.F. Release of dengue virus genome induced by a peptide inhibitor. *PLoS One,* **2012**, *7*(11), e50995.

[94] Schmidt, A.G.; Yang, P.L.; Harrison, S.C. Peptide inhibitors of dengue-virus entry target a late-stage fusion intermediate. *PLoS Pathog.,* **2010**, *6*(4), e1000851.

[95] Schmidt, A.G.; Yang, P.L.; Harrison, S.C. Peptide inhibitors of flavivirus entry derived from the E protein stem. *J. Virol.,* **2010**, *84*(24), 12549-12554.

[96] Laosutthipong, C.; Kanthong, N.; Flegel, T.W. Novel, anionic, antiviral septapeptides from mosquito cells also protect monkey cells against dengue virus. *Antiviral Res.,* **2013**, *98*(3), 449-456.

[97] Panya, A.; Bangphoomi, K.; Choowongkomon, K.; Yenchitsomanus, P.T. Peptide inhibitors against dengue virus infection. *Chem. Biol. Drug Des.,* **2014**, *84*(2), 148-157.

[98] Parikesit, A.A.; Kinanty; Tambunan, U.S. Screening of commercial cyclic peptides as inhibitor envelope protein dengue virus (DENV) through molecular docking and molecular dynamics. *Pak. J. Biol. Sci.,* **2013**, *16*(24), 1836-1848.

[99] Alhoot, M.A.; Rathinam, A.K.; Wang, S.M.; Manikam, R.; Sekaran, S.D. Inhibition of dengue virus entry into target cells using synthetic antiviral peptides. *Int. J. Med. Sci.,* **2013**, *10*(6), 719-729.

[100] Villar, L.; Dayan, G.H.; Arredondo-Garcia, J.L.; Rivera, D.M.; Cunha, R.; Deseda, C.; Reynales, H.; Costa, M.S.; Morales-Ramirez, J.O.; Carrasquilla, G.; Rey, L. C.; Dietze, R.; Luz, K.; Rivas, E.; Miranda Montoya, M.C.; Cortes Supelano, M.; Zambrano, B.; Langevin, E.; Boaz, M.; Tornieporth, N.; Saville, M.; Noriega, F. Efficacy of a tetravalent dengue vaccine in children in Latin America. *N. Engl. J. Med.,* **2015**, *372*(2), 113-123.

[101] Dejnirattisai, W.; Wongwiwat, W.; Supasa, S.; Zhang, X.; Dai, X.; Rouvinsky, A.; Jumnainsong, A.; Edwards, C.; Quyen, N.T.; Duangchinda, T.; Grimes, J.M.; Tsai, W. Y.; Lai, C.Y.; Wang, W.K.; Malasit, P.; Farrar, J.; Simmons, C.P.; Zhou, Z.H.; Rey, F.A.; Mongkolsapaya, J.; Screaton, G.R. A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virus. *Nat. Immunol.,* **2015**, *16*(2), 170-177.

[102] Fibriansah, G.; Tan, J.L.; Smith, S.A.; de Alwis, R.; Ng, T.S.; Kostyuchenko, V. A.; Jadi, R.S.; Kukkaro, P.; de Silva, A.M.; Crowe, J.E.; Lok, S.M. A highly potent human antibody neutralizes dengue virus serotype 3 by binding across three surface proteins. *Nat. Commun.,* **2015**, *6*, 6341.

[103] Capeding, M.R.; Tran, N.H.; Hadinegoro, S.R.; Ismail, H.I.; Chotpitayasunondh, T.; Chua, M.N.; Luong, C.Q.; Rusmil, K.; Wirawan, D.N.; Nallusamy, R.; Pitisuttithum, P.; Thisyakorn, U.; Yoon, I.K.; van der Vliet, D.; Langevin, E.; Laot, T.; Hutagalung, Y.; Frago, C.; Boaz, M.; Wartel, T.A.; Tornieporth, N.G.; Saville, M.; Bouckenooghe, A. Clinical efficacy and safety of a novel

tetravalent dengue vaccine in healthy children in Asia: A phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet,* **2014**, *384*(9951), 1358-1365.

[104] Sabchareon, A.; Wallace, D.; Sirivichayakul, C.; Limkittikul, K.; Chanthavanich, P.; Suvannadabba, S.; Jiwariyavej, V.; Dulyachai, W.; Pengsaa, K.; Wartel, T.A.; Moureau, A.; Saville, M.; Bouckenooghe, A.; Viviani, S.; Tornieporth, N. G.; Lang, J. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: A randomised, controlled phase 2b trial. *Lancet,* **2012**, *380*(9853), 1559-1567.

[105] Guzman, M.G.; Mune, M.; Kouri, G. Dengue vaccine: Priorities and progress. *Expert. Rev. Anti Infect. Ther.,* **2004**, *2*(6), 895-911.

[106] Espada-Murao, L.A.; Morita, K. Dengue and soluble mediators of the innate immune system. *Trop. Med. Health,* **2011**, *39*(4 Suppl), 53-62.

[107] Schmitz, J.; Roehrig, J.; Barrett, A.; Hombach, J. Next generation dengue vaccines: A review of candidates in preclinical development. *Vaccine,* **2011**, *29*(42), 7276-7284.

[108] Abd Kadir, S.L.; Yaakob, H.; Mohamed Zulkifli, R. Potential anti-dengue medicinal plants: A review. *J. Nat. Med.,* **2013**, *67*(4), 677-689.

[109] Abubakr, M.; Mandal, S.C.; Banerjee, S. Natural compounds against flaviviral infections. *Nat. Prod. Commun.,* **2013**, *8*(10), 1487-1492.

[110] Chan, K.R.; Ong, E.Z.; Ooi, E.E. Therapeutic antibodies as a treatment option for dengue fever. *Expert Rev. Anti Infect. Ther.,* **2013**, *11*(11), 1147-1157.

[111] Hidari, K.I.; Abe, T.; Suzuki, T. Carbohydrate-related inhibitors of dengue virus entry. *Viruses,* **2013**, *5*(2), 605-618.

[112] Lim, S.P.; Wang, Q.Y.; Noble, C.G.; Chen, Y.L.; Dong, H.; Zou, B.; Yokokawa, F.; Nilar, S.; Smith, P.; Beer, D.; Lescar, J.; Shi, P.Y. Ten years of dengue drug discovery: Progress and prospects. *Antiviral Res.,* **2013**, *100*(2), 500-519.

[113] Bhakat, S.; Karubiu, W.; Jayaprakash, V.; Soliman, M.E. A perspective on targeting non-structural proteins to combat neglected tropical diseases: Dengue, West Nile and Chikungunya viruses. *Eur. J. Med. Chem.,* **2014**, *87*, 677-702.

[114] Qadir, M.I.; Abbas, K.; Tahir, M.; Irfan, M.; Raza Bukhari, S.F.; Ahmed, B.; Hanif, M.; Rasul, A.; Ali, M. Dengue fever: Natural management. *Pak. J. Pharm. Sci.,* **2015**, *28*(2), 647-655.

[115] Alen, M.M.; Schols, D. Dengue virus entry as target for antiviral therapy. *J. Trop. Med.,* **2012**, *2012*, 628475.

[116] De La Guardia, C.; Lleonart, R. Progress in the identification of dengue virus entry/fusion inhibitors. *Biomed. Res. Int.,* **2014**, *2014*, 825039.

[117] Chew, M.F.; Poh, K.S.; Poh, C.L. Peptides as therapeutic agents for dengue virus. *Int. J. Med. Sci.,* **2017**, *14*(13), 1342-1359.

[118] Perera, R.; Kuhn, R.J. Structural proteomics of dengue virus. *Curr. Opin. Microbiol.,* **2008**, *11*(4), 369-377.

[119] Botting, C.; Kuhn, R.J. Novel approaches to flavivirus drug discovery. *Expert Opin. Drug Discov.,* **2012**, *7*(5), 417-428.

[120] Wu, C.F.; Wang, S.H.; Sun, C.M.; Hu, S.T.; Syu, W.J. Activation of dengue protease autocleavage at the NS2B-NS3 junction by recombinant NS3 and GST-NS2B fusion proteins. *J. Virol. Methods,* **2003**, *114*(1), 45-54.

[121] Yin, Z.; Patel, S.J.; Wang, W.L.; Wang, G.; Chan, W.L.; Rao, K.R.; Alam, J.; Jeyaraj, D.A.; Ngew, X.; Patel, V.; Beer, D.; Lim, S.P.; Vasudevan, S.G.; Keller, T.H. Peptide inhibitors of Dengue virus NS3 protease. Part 1: Warhead. *Bioorg. Med. Chem. Lett.,* **2006**, *16*(1), 36-39.

[122] Munk, C.; Wei, G.; Yang, O.O.; Waring, A.J.; Wang, W.; Hong, T.; Lehrer, R.I.; Landau, N.R.; Cole, A.M. The theta-defensin, retrocyclin, inhibits HIV-1 entry. *AIDS Res. Hum. Retroviruses,* **2003**, *19*(10), 875-881.

[123] Sharpe, I.A.; Gehrmann, J.; Loughnan, M.L.; Thomas, L.; Adams, D.A.; Atkins, A.; Palant, E.; Craik, D.J.; Adams, D.J.; Alewood, P.F.; Lewis, R.J. Two new classes of conopeptides inhibit the alpha1-adrenoceptor and noradrenaline transporter. *Nat. Neurosci.,* **2001**, *4*(9), 902-907.

[124] Sariol, C.A.; White, L.J. Utility, limitations, and future of non-human primates for dengue research and vaccine development. *Front. Immunol.,* **2014**, *5*, 452.

[125] Clyde, K.; Kyle, J.L.; Harris, E. Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis. *J. Virol.,* **2006**, *80*(23), 11418-11431.

[126] Welsh, R.M.; Fujinami, R.S. Pathogenic epitopes, heterologous immunity and vaccine design. *Nat. Rev. Microbiol.,* **2007**, *5*(7), 555-563.

[127] Guy, B.; Almond, J.W. Towards a dengue vaccine: Progress to date and remaining challenges. *Comp. Immunol. Microbiol. Infect. Dis.,* **2008**, *31*(2-3), 239-252.

[128] Thomas, S.J.; Hombach, J.; Barrett, A. Scientific consultation on cell mediated immunity (CMI) in dengue and dengue vaccine development. *Vaccine,* **2009**, *27*(3), 355-368.

[129] Webster, D.P.; Farrar, J.; Rowland-Jones, S. Progress towards a dengue vaccine. *Lancet Infect. Dis.,* **2009**, *9*(11), 678-687.

[130] Olsen, L.R.; Zhang, G.L.; Keskin, D.B.; Reinherz, E.L.; Brusic, V. Conservation analysis of dengue virus T-cell epitope-based vaccine candidates using Peptide block entropy. *Front. Immunol.,* **2011**, *2*, 69.

[131] Purcell, A.W.; McCluskey, J.; Rossjohn, J. More than one reason to rethink the use of peptides in vaccine design. *Nat. Rev. Drug Discov.,* **2007**, *6*(5), 404-414.

[132] Comber, J.D.; Karabudak, A.; Huang, X.; Piazza, P.A.; Marques, E.T.; Philip, R. Dengue virus specific dual HLA binding T cell epitopes induce CD8+ T cell responses in seropositive individuals. *Hum. Vaccin. Immunother.,* **2014**, *10*(12), 3531-3543.

[133] Munch, J.; Standker, L.; Forssmann, W.G.; Kirchhoff, F. Discovery of modulators of HIV-1 infection from the human peptidome. *Nat. Rev. Microbiol.,* **2014**, *12*(10), 715-722.

[134] Currie, S.M.; Gwyer Findlay, E.; McFarlane, A.J.; Fitch, P.M.; Bottcher, B.; Colegrave, N.; Paras, A.; Jozwik, A.; Chiu, C.; Schwarze, J.; Davidson, D.J. Cathelicidins have direct antiviral activity against respiratory syncytial virus *in vitro* and protective function *in vivo* in mice and humans. *J. Immunol.,* **2016**, *196*(6), 2699-2710.

[135] van der Schaar, H.M.; Rust, M.J.; Waarts, B.L.; van der Ende-Metselaar, H.; Kuhn, R.J.; Wilschut, J.; Zhuang, X.; Smit, J.M. Characterization of the early events in dengue virus cell entry by biochemical assays and single-virus tracking. *J. Virol.,* **2007**, *81*(21), 12019-12028.

[136] Bressanelli, S.; Stiasny, K.; Allison, S.L.; Stura, E.A.; Duquerroy, S.; Lescar, J.; Heinz, F.X.; Rey, F.A. Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. *EMBO J.,* **2004**, *23*(4), 728-738.

[137] Modis, Y.; Ogata, S.; Clements, D.; Harrison, S.C. A ligand-binding pocket in the dengue virus envelope glycoprotein. *Proc. Natl. Acad. Sci. USA,* **2003**, *100*(12), 6986-6991.

[138] Nemesio, H.; Palomares-Jerez, F.; Villalain, J. The membrane-active regions of the dengue virus proteins C and E. *Biochim. Biophys. Acta,* **2011**, *1808*(10), 2390-2402.

[139] Welsch, S.; Miller, S.; Romero-Brey, I.; Merz, A.; Bleck, C.K.; Walther, P.; Fuller, S.D.; Antony, C.; Krijnse-Locker, J.; Bartenschlager, R. Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell Host Microbe,* **2009**, *5*(4), 365-375.

[140] Modis, Y.; Ogata, S.; Clements, D.; Harrison, S.C. Structure of the dengue virus envelope protein after membrane fusion. *Nature,* **2004**, *427*(6972), 313-319.

[141] Mukhopadhyay, S.; Kuhn, R.J.; Rossmann, M.G. A structural perspective of the flavivirus life cycle. *Nat. Rev. Microbiol.,* **2005**, *3*(1), 13-22.

[142] Elshuber, S.; Allison, S.L.; Heinz, F.X.; Mandl, C.W. Cleavage of protein prM is necessary for infection of BHK-21 cells by tick-borne encephalitis virus. *J. Gen. Virol.,* **2003**, *84*(Pt 1), 183-191.

[143] Miller, S.; Krijnse-Locker, J. Modification of intracellular membrane structures for virus replication. *Nat. Rev. Microbiol.,* **2008**, *6*(5), 363-374.

[144] Yu, I.M.; Zhang, W.; Holdaway, H.A.; Li, L.; Kostyuchenko, V.A.; Chipman, P. R.; Kuhn, R.J.; Rossmann, M.G.; Chen, J. Structure of the immature dengue virus at low pH primes proteolytic maturation. *Science,* **2008**, *319*(5871), 1834-1837.

[145] Li, L.; Lok, S.M.; Yu, I.M.; Zhang, Y.; Kuhn, R.J.; Chen, J.; Rossmann, M.G. The flavivirus precursor membrane-envelope protein complex: Structure and maturation. *Science,* **2008**, *319*(5871), 1830-1834.

[146] Perera-Lecoin, M.; Meertens, L.; Carnec, X.; Amara, A. Flavivirus entry receptors: An update. *Viruses,* **2014**, *6*(1), 69-88.

[147] Chambers, T.J.; Nestorowicz, A.; Amberg, S.M.; Rice, C.M. Mutagenesis of the yellow fever virus NS2B protein: Effects on

proteolytic processing, NS2B-NS3 complex formation, and viral replication. *J. Virol.,* **1993**, *67*(11), 6797-6807.

[148] Falgout, B.; Pethel, M.; Zhang, Y.M.; Lai, C.J. Both nonstructural proteins NS2B and NS3 are required for the proteolytic processing of dengue virus nonstructural proteins. *J. Virol.,* **1991**, *65*(5), 2467-2475.

[149] Yin, Z.; Patel, S.J.; Wang, W.L.; Chan, W.L.; Ranga Rao, K.R.; Wang, G.; Ngew, X.; Patel, V.; Beer, D.; Knox, J.E.; Ma, N.L.; Ehrhardt, C.; Lim, S.P.; Vasudevan, S.G.; Keller, T.H. Peptide inhibitors of dengue virus NS3 protease. Part 2: SAR study of tetrapeptide aldehyde inhibitors. *Bioorg. Med. Chem. Lett.,* **2006**, *16*(1), 40-43.

[150] Lehrer, R.I. Primate defensins. *Nat. Rev. Microbiol.,* **2004**, *2*(9), 727-738.

[151] Schmitz, J.; Holzgrabe, U. Plectasin - a new peptide antibiotic with high therapeutic potential. *Pharm. Unserer. Zeit.,* **2010**, *39*(5), 336-338.

[152] Mygind, P.H.; Fischer, R.L.; Schnorr, K.M.; Hansen, M.T.; Sonksen, C.P.; Ludvigsen, S.; Raventos, D.; Buskov, S.; Christensen, B.; De Maria, L.; Taboureau, O.; Yaver, D.; Elvig-Jorgensen, S.G.; Sorensen, M.V.; Christensen, B.E.; Kjaerulff, S.; Frimodt-Moller, N.; Lehrer, R.I.; Zasloff, M.; Kristensen, H.H. Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. *Nature,* **2005**, *437*(7061), 975-980.

[153] The China Post reports. Outbreak of dengue fever is escalating in Kaohsiung. 28 October 2014. Available at: https://chinapost.nownews.com/ 20141028-69309.

[154] BBC NEWS. Taiwan: Dengue fever mosquito competition offers prizes. *News From Elsewhere* 31 October 2014, http://www.bbc.com/news/blogs-news-from-elsewhere-29850539.

[155] Jenssen, H.; Hamill, P.; Hancock, R.E. Peptide antimicrobial agents. *Clin. Microbiol. Rev.,* **2006**, *19*(3), 491-511.

[156] Gentilucci, L.; De Marco, R.; Cerisoli, L. Chemical modifications designed to improve peptide stability: Incorporation of non-natural amino acids, pseudo-peptide bonds, and cyclization. *Curr. Pharm. Design.,* **2010**, *16*(28), 3185-3203.

[157] Nordstrom, R.; Malmsten, M. Delivery systems for antimicrobial peptides. *Adv. Colloid Interface Sci.,* **2017**, *242*, 17-34.