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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. DENV-1–4) 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-
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                           | [105]      |
|                                  |                                                                      | Live chimeric vaccine candidates                                          |            |
|                                  |                                                                      | DNA vaccine                                                               |            |
|                                  |                                                                      | Chimeric proteins                                                         |            |
|                                  |                                                                      | Recombinant protein                                                       |            |
|                                  | Next generation dengue vaccines: A review of candidates in preclinical development | Recombinant subunit vaccines                                              | [107]      |
|                                  |                                                                      | DNA vaccines                                                             |            |
|                                  |                                                                      | Virus Like Particle vaccines                                              |            |
|                                  |                                                                      | Virus-vectored vaccines                                                   |            |
|                                  |                                                                      | Live attenuated virus vaccines                                            |            |

(Table 1) contd....
The Development of Peptide-based Antimicrobial Agents against Dengue Virus

| Pathway                                | Title                                                                 | Compounds                      | References |
|----------------------------------------|-----------------------------------------------------------------------|--------------------------------|------------|
| Anti-dengue virus entry                | Targeting host factors to treat West Nile and dengue viral infections | Small molecule, Cytokine, Antibody, Carbohydrate | [23]       |
|                                        | Carbohydrate-related inhibitors of dengue virus entry                 | Carbohydrate                   | [111]      |
|                                        | 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]      |
|                                        | 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, AMHALTGA, EGAMHSALA, GSQEGAMHS, SALAGTEV, SQEGAMHSA, QEGAMHSAL, and GAMHSAAL) within the 19-mer conserved region [79]. They found that a 9-mer AMHALTGA 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: ×103 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-γ 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, inhibited 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 (IC50) 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 (IC50 = 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 the infection of FcRII-expressing human K562 cells (IC50 = 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 IC50 of 2–5 μM without signs of cytotoxicity to mammalian epithelial and mosquito cells at an IC50 of 50 μM [93].

Schmidt et al. proposed that peptides derived from the stem region of the E protein in DENV-2 (DV2419–447) could inhibit viral infectivity by a two-step mechanism [94]. Initially, DV2419–447 bound to a viral membrane was introduced into the cell through endocytosis; leading to subsequent DV2419–447 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 IC50 >500 μM and approximately 35 μM, respectively [99]. Laosuthipong 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    | FERVPGEVTRRALPPVSS | 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    | P1: AKFVAWTLKAAAGGRHLRGLITVNPITG-GEPOQLNWFKGS | The P1 was located in virus-2 E domain III. | [82] |
| CD8+ cell | Capsid: MNVRKQAKNTPLFNLKRERNRVRSTVVQLTRFSLGL-MLQGRGPVLLFLMALVAFRLFILTIPPTAGILKRWGTTIKK-SKAINVLRGFRKEIGRLMLNLRM ORDN M39: ATCGACTCTCAGCGTCTCAGGACGATCGTCGG-GGG | The NLPs-2 consisted of the recombinant capsid protein and ssDNA (ODN M39) (protein:DNA = 3:1). | [76, 78, 83, 86] |
| CD8+ cell | Pep03: LVTFFKATHAHHKQEV-Linker-LVTFFKNAAHAKHQQEV-Linker-LVTFFKPKHAKQKD-VLinker-GGG | The Pep03 was a synthetic peptide that derived from four DENV envelope domain II. | [88] |
| CD8+ cell | NS352–60: VTRGVY (DENV - 1) VTRGAVLMH (DENV - 2) VTRGAVLTY (DENV - 3-1) VTRGAVLTH (DENV - 3-2) VTRGCSV ICH (DENV - 4) NS3358–368: LTVWVFPSIKS (DENV - 1) LTVWVFPSIKA (DENV - 2 - 3 - 4) NS3501–509: TPEGIIPAL (DENV - 1 - 3) TPEGIIPSM (DENV - 2) TPEGIIPTLS (DENV - 4) NS3538–547: MRRGDLPVWL (DENV - 1 - 2 - 3 - 4) NS3575–583: EENMEVEIW (DENV - 2) EENMDVEIW (DENV - 1 - 3 - 4) | The peptides were synthesized based on NS3 protein. | [87] |
| B cell    | pr4: KGKSLFFKTENGVMC | 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% (IC₅₀ = 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].
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 benzyol-NKRR-H was the most suitable candidate for inhibiting NS2B/NS3 (Ki = 5.8 μM) [121, 149]. In 2010, Tambunan and Alamudi used a bioinformatics method to design peptide inhibitors for NS2B/NS3 [71]. Seven cyclotetrapeptides [Disulfide Bridge: 1-5] were designed (CKRRC, CGRRC, CRGRC, CRTRC, CTRRC, CRKRC, and CRRKC) using a molecular-docking method [71]. The optimal cyclotetrapeptide was CRKRC, for which the Ki was estimated at 0.707 μM [71]. In 2011, Schuller et al. modified the benzyol-KRR-H sequence [70, 149] and found that the inhibitory effects of phenylacetyl-KRR-H were more powerful than those of benzyol-KRR-H (IC50 = 6.7 ± 1.1 μM vs 127 ± 2.1 μM). Moreover, they found that this new tripeptide was superior to the previously referenced inhibitor benzyol-NKRR-H with improved IC50 value (IC50 = 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 IC50 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 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 IC50 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% ± 48 h and 38% ± 72 h) or post-treated (30% ± 48 h and 45% ± 72 h) groups [68]. In 2012, Xu et al. designed the cyclic peptide CAGKRKS [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 plec-tasin 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 Pseudoplec-tania 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 (IC50 = 12.68 ± 3.2 μM at 37°C; IC50 = 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 methyl-transferase, which has two critical active sites on its surface:

| Target | Peptide sequence | Origins and Descriptions | References |
|--------|-----------------|--------------------------|------------|
| Stem region of E protein | DN59: MAILGDTA(WDFG)SLGQFSIKALHQVFGAIY | Synthesized | [91-93] |
| The domain II hinge | DNS7opt: RWMVWRHFHRLLPYNPGKNKQNQQWP | Synthesized | [90] |
| Domain I/domain II beta sheet connection | lOA1: FWFTLIKQAKQPYRRFC | Synthesized | [90] |
| E protein | DV2<sup>5</sup>-<sup>44</sup>, AWDFGSGLGQFSIKALHQVFGAIYGA | Synthesized | [94, 95] |
| Domain III of DENV2 E protein | DET2: PWLKGDDLDL, DET4: AQVKGKDLD | Synthesized | [99] |
| Viprolaxikine and host cell | Viprolaxikine: (DDHELQD, DETELQD, DEVMLQD and/or DEVLMQD) common motif: D/D/E-X-X-Q-D | Synthesized | [96] |
| E Protein | EF (Glu-Phe) | Molecular docking | [97] |
| E Protein | BNP(7-32), porcine: DSFGCGRRLDRIGSGLGCNVLRRY | Molecular docking | [98] |
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: RGGRLCYCRRRFCCVGV [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.                   | [69, 122, 150] |
|                | CAGKRKSG [Cyclic bridge:1-8] | This peptide was modified from conotoxins, MrIA, which was produced by cone snail.     | [72]        |
|                | Plectasin: GFGCNWPWDEDMQCHNHSIKGYKGYYCAKGGFVCKCY [Disulfide Bridge:4-30; 15-37; 19-39] | Plectasin is a fungal peptide produced from Pseudoplectania nigrella.                  | [69, 152]   |
|                | Ltc 1: SMWSGMWRKKLKLHNALKKKLKGE | Ltc 1 is a peptide from venom gland of Lachesana tarabaeva.                            | [67]        |
| NS5 methyltransferase | CTWYC for SAM binding site [Disulfide Bridge: 1-5] | These two peptides were designed by used molecular docking approach.                 | [74]        |
|                | CYEFC for RNA-cap site [Disulfide Bridge: 1-5] | These two peptides were screen by molecular dynamics simulation and molecular docking approaches from commercial cyclic peptide | [75]        |
|                | [Tyr123] Prepro Endothelin (110-130), amide, human: CQCASQKDCKWSYCQAGKEI for SAM binding site [Disulfide Bridge: 110-124; 112-120] | Urotensin II, human                                                                 | [75]        |
|                | Urotensin II, human | ETPDCFWKYCV for RNA-cap site [Disulfide Bridge: 5-10]                                     | [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 (ΔG(binding) = -30.72 kcal/mol) and CYEFC for the RNA-capping site (ΔG(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<sub>50</sub>** = 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.

**ACKNOWLEDGEMENTS**

This work was financially supported by a grant from the *Ministry of Science and Technology*, Taiwan.

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