Antiviral Peptides: Identification and Validation

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Abstract
Despite rapid advances in the human healthcare, the infection caused by certain viruses results in high morbidity and mortality accentuate the importance for development of new antivirals. The existing antiviral drugs are limited, due to their inadequate response, increased rate of resistance and several adverse side effects. Therefore, one of the newly emerging field “peptide-based therapeutics” against viruses is being explored and seems promising. Over the last few years, a lot of scientific effort has been made for the identification of novel and potential peptide-based therapeutics using various advanced technologies. Consequently, there are more than 60 approved peptide drugs available for sale in the market of United States, Europe, Japan, and some Asian countries. Moreover, the number of peptide drugs undergoing the clinical trials is rising gradually year by year. The peptide-based antiviral therapeutics have been approved for the Human immunodeficiency virus (HIV), Influenza virus and Hepatitis virus (B and C). This review enlightens the various peptide sources and the different approaches that have contributed to the search of potential antiviral peptides. These include computational approaches, natural and biological sources (library based high throughput screening) for the identification of lead peptide molecules against their target. Further the applications of few advanced techniques based on combinatorial chemistry and molecular biology have been illustrated to measure the binding parameters such as affinity and kinetics of the screened interacting partners. The employment of these advanced techniques can contribute to investigate antiviral peptide therapeutics for emerging infections.

Keywords Binding evaluation techniques · High throughput screening methods · In silico approaches · Peptide based therapeutics

Introduction
The infections caused by viral pathogens including clinical viruses or naturally emerging viruses pose a serious threat worldwide. Unfortunately, only few therapeutics are available for limited viruses like Human immunodeficiency virus (HIV), Hepatitis virus, Herpes simplex virus (HSV) and Influenza virus (Rider et al. 2011). Researchers are currently working to extend the range of specific and novel antivirals to other families of pathogens. Since, viruses depend on host cell organism for replication, the selection of target for the designing of effective and safe antiviral drugs without harming the host cell, is an extremely difficult process. Besides this, owing to the evolution, mutations occur in the viral genome, which contribute to the development of resistance to drugs and thus rendered many drugs ineffective (Lee et al. 2019). The peptides can block infection by targeting either virus or its host. The virus specific antiviral peptides are known as virucidal, as they directly target the viral proteins. Most of the antivirals have been reported to inhibit the development of viruses by targeting its specific regions or components. Various steps of viral life cycle have been targeted for the discovery of novel antiviral drugs, such as viral entry, viral synthesis, or assembly. Due to the extracellular site of action and blockage of viral infection, the viral entry inhibition is marked as an attractive strategy (Chew et al. 2017). Protein–protein interactions (PPIs) are the foundation of important cellular processes and are considered as primary targets for the drug discovery over the last decade (Lee et al. 2019; Teissier et al. 2011). The knowledge of crucial interactors involved in PPIs and their mechanism is necessary to pave way for the selection of suitable target for drug discovery. New approaches in therapeutics include the use of small cyclic molecules, proteins/peptides, nucleic
acids such as small interfering RNA (siRNA) and small hairpin RNA (shRNA) molecules (Teissier et al. 2011). Among these advanced approaches, peptides as therapeutics is a promising field in the drug discovery (Lau and Dunn 2018). Peptides are the biologically active molecules composed of amino acids residues that disrupt the PPIs. They are small (less than 100 amino acids), and they can be easily synthesized. They are also highly specific and effective even in nanomolar range. The main benefit of using peptide as therapeutics is its hydrolysis by peptidases present in the body, which prevents its accumulation in specific organs and minimizes the toxic side effects (Ali et al. 2013). Previously, the pharmaceutical industries have shown poor interest for the expansion of peptide-based therapeutics because of their extremely poor ADME (absorption, distribution, metabolism and elimination) properties. However, the advanced research enables modifications of the peptides such as synthesis of amino acid enantiomers, addition of chemical compounds and their nanoparticle formulation to overcome the pharmacodynamic flaws of peptides (Gentilucci et al. 2010; Zeng et al. 2018). The advantages offered by the modified peptides have sparked the interest amongst the researchers and companies. Now-a-days peptide as therapeutics has come to forte with nearly 20 new peptides added in clinical trials annually. In fact, the global market of peptides as drugs has reached to billion dollars with currently more than 60 peptides approved by US Food and Drug Administration (FDA) and over 400 peptides being under clinical phase trials (Lau and Dunn 2018). Peptide as therapeutics are approved or are being considered for the treatment of diseases such as cancer, diabetes, cardiovascular diseases and even infection caused by few viruses such as HIV, Herpes, Hepatitis and Influenza virus. Thus, over the years peptide-based therapeutics has added a new dimension as the potential antiviral candidate. This review focuses on the types of peptides approaches that can be used for the identification of the lead peptides against the target protein and the selected advanced techniques reported for the validation of the peptide binding affinity to their targets.

**Antiviral Peptides as Therapeutics**

The peptides possessing potential to inhibit the virus are considered as antiviral peptides (AVPs). Usually, the AVPs exhibit antiviral effects by inhibiting the virus directly, but their inhibition sites and the mechanism of action vary within the viral replication cycle (Rider et al. 2011). The AVPs can be obtained through different approaches: (1) Computational approach (2) Natural sources and (3) Biological source such as High-throughput screening (Fig. 1). There are many online databases available which contain information regarding experimentally tested antiviral peptides such as Antiviral peptide database (AVPdp) (Qureshi et al. 2013) where 2683 entries of peptides including 624 modified AVPs are compiled till December 2019, while many others are unreported. Since the field of peptides as antiviral is not entirely explored, therefore, many research studies are being undertaken to elucidate the role of peptides in blocking viral infections. The first peptide drug approved for clinical indication, Enfuvirtide (Enf), a 36-amino acid residue peptide, against HIV corresponds to the heptad-repeat (HR2) domain of gp41 (HIV envelope protein). Enf prevents the fusion of HR1 domain to HR2 during HIV formation and blocks HIV infection (Teissier et al. 2011). Similarly, Boceprevir and Telaprevir, both synthetic peptides against Hepatitis C virus (HCV), got approval by FDA in 2011. These peptides act on NS3/4, a protease inhibitor, and interfere with viral replication (Divyashree et al. 2020). Other peptide candidates such as Myrcludex for Hepatitis B and D viruses (HBV and HDV) (Bogomolov et al. 2016), Flufirvitide for Influenza virus (Skalickova et al. 2015), Sifuvirtide for HIV-1 (Yu et al. 2018), IM862 and SCV-07 for HCV and Thymosin α-1 for HBV as well as HCV (Jenssen 2009), are under various phase trials of pre-clinical and clinical studies. We have discussed the different approaches used for the identification of AVPs. Moreover, the few selected methodologies used for the validation of identified peptides as potential AVPs have also been described here. The techniques used in identification and validation of peptides are compared in Table 1.

**Antiviral Peptides Designed from Computational Approach**

Computer assisted drug designing process is based on the understanding of structural and functional aspects of the viral machinery. The rational knowledge of the viral proteins and the interactors/cellular partners assists in the selection of target protein. This approach has expedited the process of
| S. No | Approach     | Technique        | Characteristics                                                                 | Advantages                                                                 | Disadvantages                                                                 | References                        |
|-------|--------------|------------------|--------------------------------------------------------------------------------|----------------------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------------------|
| 1     | Computational| Docking         | Virtual screening of target based hits                                         | Expedite the process of drug discovery                                        | Requires multiple runs to obtain reliable results                           | Nevola and Giralt (2015)          |
|       |              |                  | Size, shape, charge distribution, polarity, hydrogen bonding, and hydrophobic interactions of ligand-receptor complexes can be identified | Identification of target sites of the ligand and the receptor molecule        | Limits the flexibility of receptor                                           |
|       |              |                  |                                                                              |                                                                             | Less accurate                                                                 |                                   |
|       |              |                  |                                                                              |                                                                             | Docking calculations are complex                                             |                                   |
| 2     | Biological   | Phage display    | Utilizes phages to display foreign protein/peptide                           | Rapid identification of target specific phages                               | Library size is limited by phage transformation efficiency (~10^6)            | Matsubara (2012) and Fukunaga and Taki (2012) |
|       |              |                  | Most adopted system                                                          | System suitable for delivering small peptides (<20 aa)                      | Allows only natural amino acids                                             |                                   |
|       |              |                  |                                                                              | High throughput screening approach                                           | Complicated affinity maturation process because of large diversity of proteins/peptides displayed on surface |                                   |
|       |              |                  |                                                                              | Selection of disease specific antigen mimics                                | Limits the rapidity of library generation                                    |                                   |
|       |              |                  |                                                                              | Selection of organ specific peptides                                         | Restricts the size of expressed proteins                                    |                                   |
|       |              |                  |                                                                              | Used in B-cell and T-cell epitope mapping                                    | Limits the intractability of some targets                                   |                                   |
|       |              |                  |                                                                              |                                                                              | Solid surface based biopanning often results in non specific binding         | Wang and Liu (2011)             |
|       |              |                  |                                                                              |                                                                              | Likely to interfere with other molecules due to single stranded form of m-RNA |                                   |
|       |              |                  |                                                                              |                                                                              | Display larger proteins (> 300 aa) with lower efficiency                    |                                   |
|       |              |                  |                                                                              |                                                                              | Not suitable for displaying membrane bound proteins                         |                                   |
|       |              |                  |                                                                              |                                                                              | Ribonuclease free environment is required                                     |                                   |
|       | Peptidomimetics | Mimics of the natural peptides Prevent the protein–protein/ protein-peptide interaction by competitive binding | Design the mimic of peptide with enhanced bioavailability                      | Offers restricted conformational structures                                   | Sen et al. (2019)                |
|       |              |                  | Overcome the proteolytic instability of natural peptides                      |                                                                              | Termini are exchanged with the inversion of their sequence                   |                                   |
|       |              |                  | Improved receptor selectivity                                                |                                                                              | Requires entire understanding of target interaction                          |                                   |
|       | m-RNA display |                  | Uses the transcription and translation machinery extracted from prokaryotic/ eukaryotic cells Utilizes covalent mRNA-polypeptide complexes linked through puromycin | Library size as large as (~ 10^{12–15})                                     |                                                                              |                                   |
|       |              |                  |                                                                              | Protein expression free from cellular constraints                             |                                                                              |                                   |
|       |              |                  |                                                                              | Allows the incorporation of unnatural amino acids                             |                                                                              |                                   |
|       |              |                  |                                                                              | Greater diversity as transformation is not required                           |                                                                              |                                   |
|       |              |                  |                                                                              | Increased probability of higher affinity hits                                 |                                                                              |                                   |
| S. No | Approach          | Technique                                | Characteristics                                                  | Advantages                                                                                                                                  | Disadvantages                                                                                      | References                      |
|-------|------------------|------------------------------------------|-------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|---------------------------------|
|       | Ribosome display | Living cell free technology             | Utilizes non-covalent ribosome-mRNA-polypeptide complexes for display system | Library size as large as (~ $10^{12-15}$)  
Greater diversity due to not being dependent on transformation efficiency  
Increased probability of higher affinity hits  
Suitable for generating toxic, proteolytically sensitive and unstable proteins  
Random mutations can be introduced  
Allows the incorporation of unnatural amino acids | Selection stringency is limited  
System is very sensitive to RNase activity  
Ribonuclease free environment is required | Dreier and Pluckthun 2011 |
|       | Yeast display    | Proteins/peptides are usually fused to Aga2 protein subunit of yeast | Can be displayed as N- or C-terminal fusion | Displays fully monomeric as well as oligomeric proteins on their surface due to eukaryotic machinery  
More diverse library as compared to phage system | Library size is smaller (~ $10^{6-7}$)  
than other display systems  
Allows only natural amino acids  
Allows the expression of extracellular proteins  
Complicated affinity maturation process because of large diversity of proteins/peptides displayed on surface | Linciano et al. (2019) |
| 3     | Advanced Techniques | Surface Plasmon resonance (SPR) | Label-free binding technique  
Quantitatively analyzes the real time binding kinetics of two bimolecular interactions | Label-free detection  
Real-time data monitoring  
Sensitive and accurate  
Small sample quantity  
Ability to Handle Complex Samples  
Ability to Replicate Measurements | Expensive instrument and sensors  
Expensive maintenance  
Low throughput | Patching (2014) |
|       |                  | Biolayer interferometry | Optical analytical technique  
Quantify real time binding kinetics of bimolecular interactions | Label-free detection  
Real-time data monitoring  
No reference channel required  
Crude sample compatibility  
System requires less maintenance  
High throughput  
Low vibrational/mechanical noise  
Simple, fast and more accurate results | Requires immobilization of ligand to surface of tip  
No temperature control  
Low sensitivity as compared to SPR  
Poor reproducibility  
Relatively high sample consumption  
Results should be cross-validated with SPR | Shah and Duncan (2014) |
drug designing. Peptides can be identified computationally; via in silico screening using molecular docking. A docking program predicts the target site which is usually known as pocket or protrusion with hydrogen bond donors and acceptors, hydrophobic characteristics and different molecular shapes. Subsequently, library of peptides docked with these pockets results in the highest binding peptide (Nevola and Giralt 2015). For example, peptides have been designed computationally using virtual docking against the surface protein of Zika virus (ZIKV) for its detection. Tetra, penta, hexa and heptapeptide libraries were docked using Open Eye Scientific Software against envelope protein of ZIKV; subsequently, eight peptides were selected. They were further tested by Direct ELISA and out of them, three were delineated with best performance for Zika detection (Mascini et al. 2019). Another method is based on peptidomimetics which mimics the designed targets, prevents the interaction of proteins by competitive binding. Four putative peptide inhibitors were designed against Nipah virus (NiV) proteins using the approach of peptidomimetics and the stability of peptide-protein complexes were analysed using MD simulation (Sen et al. 2019). The in silico methods (Docking and peptidomimetics) can predict the peptide sequence but further validation using in vitro/in vivo approaches is required to establish its biological activity.

Antiviral Peptides Derived from Natural Source

The peptides that exhibit immunomodulatory and inhibitory properties against infection caused by bacteria, fungi, viruses or protozoa, are expressed as naturally occurring antimicrobial peptides (AMPs). The AMPs have been extensively used as substitutes of antibiotics for bacterial infections, but recently their use has been expanded to antiviral therapeutics also (Ahmed et al. 2019a). The naturally occurring AVPs are amphipathic and cationic in nature with net positive charge (Bulet et al. 2004). Moreover, it has been proven that the hydrophobicity of the peptides is an essential property for targeting enveloped viruses (Badani et al. 2014; Wang et al. 2017). These AVPs can be derived from different sources such as plants, bacteria, arthropods, amphibians, marine organisms and mammals with their varied mechanism of action (Zhang and Gallo 2016). A peptide family called cyclotides derived from different plant sources has been proven successfully for their antiviral activity against HIV, Influenza virus and Dengue virus (Ireland et al. 2008; Sencanski et al. 2015; Gao et al. 2010). The small size, cationic and amphipathic nature of the cyclotides facilitates its effective binding to the target and rupture the membrane. This allows the leakage of cell components which further leads to the cell death (Weidmann and Craik 2016). In a study, kalata B1, a cyclotide isolated from the leaves of Oldenlandia affins plant, showed destruction of viral particle
at entry step along with inhibition of viral-host membrane fusion, thus exhibiting anti-HIV activity (Henriques et al. 2011). Similarly, a peptide derived from arthropod, Hyalophora cecropia, known as cecropin A showed the inhibitory activity against HIV, Junin virus (JUNV) and HSV by suppression of their gene expression (Wachinger et al. 1998; Albiol Matanic and Castilla 2004). Moreover, in recent studies bovine lactoferrin (bLF) has showed the antiviral activity against three Aedes mosquito transmitted viruses: Dengue (DENV), Chikungunya (CHIKV) and Zika virus apart from anti-HBV activity established in a previous study (Li et al. 2009; Carvalho et al. 2017; Chen et al. 2017). The bLF blocks the viral binding to its target site and thus prevents its spread to host cells. Many other AVPs originated from natural source are summarized in Table 2 with their varied mode of action. However, despite promising efficacy, the utility of these peptides is constrained due to weak binding, low stability, other side effects and virus resistance. The shortcomings of AVPs from natural resource need to be addressed so that they can be considered as mainstay antiviral therapeutics.

### Antiviral Peptides Identified Through Biological Approach

Methodologies based on in vitro display approach, usually offer genetically encoded peptides with superior quality and high affinity to their targets. Among these methodologies, phage display, mRNA display, ribosome display, yeast display and bacteria display are the most common technologies to generate peptides. The phage display technology is widely used and considered as the most appropriate for the screening of high efficiency peptides. While these technologies have already been thoroughly illustrated in other reviews (Nevolà and Giralt 2015), we have focused on the use of selected methodologies for the identification of antiviral peptides and compared them in Table 1.

#### Phage Display

Phage display technology is an efficient in vitro screening method for the selection of high affinity and target specific peptide binder from a randomly displayed peptide library. The technology involves the fusion of exogenous peptide sequence into the genome of phage, its expression on the surface as fusion product to phage surface protein. The phage displayed libraries thus constructed have $10^{8-10}$ variants at a time. In this method, biopanning is performed in which the target molecule is immobilized on surface and incubated with phage library. The unbound or excess of phage particles are removed by washing and potentially bound phages are eluted by acidic/basic buffer or with appropriate ligand. These recovered phages are amplified by infecting bacterial cells *Escherichia coli* and are used for subsequent rounds of biopanning to obtain target specific phages using affinity selection. The sequencing of DNA isolated from binding phage, validated by ELISA, helps to identify peptide sequence (Fukunaga and Taki 2012; Matsubara 2012). A peptide named P3 against Japanese encephalitis virus (JEV) host fusion has been identified as the potential AVP using phage display library. The screened peptide has shown the highest affinity to domain III of JEV envelope glycoprotein assessed by Biolayer interferometry and IC$_{50}$ of ~ 100 µM and IC$_{50}$ of ~ 1 µM in JEV infected BHK-21 cells (Wei et al. 2019). Similarly, an analogous study conducted by de la Guardia et al. (2017) identified three peptides against the domain III of DENV envelope protein to block the DENV infection. Further these peptides were non-toxic to the target cells. Moreover, the same approach has also been used to identify peptides targeting non-structural viral protein: RNA-dependent RNA polymerase (NS5B) of HCV, by screening a library composed of disulfide-constrained heptapeptides (Amin et al. 2003). In another study, a novel heptapeptide was identified using random peptide phage library which inhibited the integration of HIV genome into the host (Desjobert et al. 2004). The most important advantage of this technology over others is its high rate of mutability with affinity selection, which widely employs the screening of phage displayed peptides for identification of potential AVPs. There are many other AVPs derived from the utilization of phage display technology which are summarised in Table 3.

#### mRNA Display

mRNA display technology utilizes the covalently bonded mRNA-polypeptide complexes formed during in vitro translation, which are linked through puromycin (an analogue of the 3′-tyrosyl-tRNA along with mimics of adenosine and tyrosine) via A-site of ribosome. The complexes with desired functions are allowed to bind to the immobilized target protein, reverse transcribed to cDNA and amplified via Polymerase chain reaction (PCR). This enables the reinforcement of DNA template library for next round of screening (Cotten et al. 2012; Newton et al. 2019). The most successful use of mRNA displayed peptide library was described by Litovchick and Szostal (2008), in which they have screened potential AVPs using cyclic peptide-mRNA fusion library targeting Internal ribosomal entry site (IRES) of HCV for inhibition of virions. Another use of this technology was reported for the reverse engineering of peptide vaccines for HCV. High affinity peptides to neutralizing monoclonal antibodies (mAbs) of HCV were selected in this study and used for peptide-based vaccine development (Guo et al. 2015).
| S. No | Peptide | Source | Characteristics | Targeted virus | Mode of action/activity | Reference |
|-------|---------|--------|-----------------|----------------|--------------------------|-----------|
| **I** | **Plant** | | | | | |
| 1 | 2 kDa peptide | Seeds of *Sorghum bicolor* | 2 kDa, cationic and amphipathic peptide | HSV-1 | Inhibition of cell entry by masking essential viral envelope proteins | Camargo et al. (2008) |
| 2 | Cyclotide: vhl-1 | Leaves of *Viola hederaceae* | 31 amino acid cyclic cystine knot like peptide | HIV | Inhibition of viral fusion by disrupting the lipid envelope | Wang et al. (2008) |
| 3 | Cycloviolacin VY1 | *Viola yedoensis* | 31 amino acid peptide, three conserved disulphide bonds, bridge like 3-D structure | Influenza H1N1 | Inhibition of virus at cell entry | Liu et al. (2014) |
| 4 | Kalata B1 | Leaves of *Oldenlandia affinis* | Cyclic backbone, knot-like rigid structure, three conserved disulphide bonds | HIV | Inhibition of viral-host membrane fusion | Henriques et al. (2011) |
| 5 | Kalata B1-inspired peptide | Derivative of Kalata B1 peptide | Amino acid modifications in kalata B1 peptide | DENV | Inhibition of viral replication | Gao et al. (2010) |
| 6 | Peptides 2 and 4 | *Acacia catechu* | 15 amino acids, cationic and amphipathic peptide | DENV | Inhibition at early steps of viral entry | Panya et al. (2019) |
| 7 | Sesquin | Seeds of *Vigna sesquipedalis* | 7 kDa, cationic, defensin like peptide | HIV | Inhibition of viral replication by hindering reverse transcriptase activity | Jack and Tzi (2005) |
| **II** | **Bacteria** | | | | | |
| 8 | Locillomycin | *Bacillus subtilis* | Cyclic lipononapeptide | PEDV | Unknown | Luo et al. (2015) |
| 9 | Surfactin | *Bacillus subtilis* | Cyclic lipopeptide, amphiphilic | HIV, HSV-1, HSV-2, VSV, SIV, NDV, PEDV | Disintegration of lipid envelope and, capsid through ion channel formations | Yuan et al. (2019) |
| **III** | **Arthropod** | | | | | |
| 10 | Alloferon 1 and 2 | Hemolymph of blowfly: *Calliphora vicina* | Linear, nonglycosylated oligopeptide of 13 and 12 amino acid residues, respectively | Influenza virus | Inhibition by activation of natural killer cells and release of interferon | Chernysh et al. (2002) |
| 11 | Alloferon 1-derived peptide | Hemolymph of blowfly: *Calliphora vicina* | Modifications in their N-terminal portions of Alloferon 1 peptide | HSV-1 | Inhibition of viral replication | Kuczer et al. (2010) |
| 12 | Bmkn2-7 | Venom of scorpion: *Mesobuthus martensi* | 13-amino acid residues, basic, alpha-helical peptide | HIV-1 | Inhibition by direct interaction with viral particle | Chen et al. (2012) |
| 13 | Cecropin A | Moth *Hyalophora cecropia* | 37-amino acid linear, cationic peptide | HIV; HSV-1 and 2; JUNV | Suppression of viral gene expression | Wachinger et al. (1998) and Hultmark et al. (2005) |
| S. No | Peptide          | Source            | Characteristics                                              | Targeted virus | Mode of action/ activity                                                                 | Reference                        |
|-------|------------------|-------------------|--------------------------------------------------------------|----------------|-----------------------------------------------------------------------------------------|----------------------------------|
|       | Ctry2459         | Chaerilus tryznai | 13-amino-acid residue, helical and amphipathic peptide       | HCV            | Inhibition by inactivating the viral particles, virucidal activities and suppressed the established infection at cellular level | Hong et al. (2013)               |
| 15    | Ctry2459-H2      |                   | Histidine-rich Ctry2459 peptide                              |                |                                                                                         |                                  |
| 16    | Ctry2469-H3      |                   | Histidine-rich Ctry2459 peptide                              |                |                                                                                         |                                  |
| 17    | Eva1418          | Venom of scorpion: Euscorpiops validus | Helical and amphipathic peptide                           | HSV-1          | Inhibition by disruption of initial steps of infection                                    | Zeng et al. (2018)               |
| 18    | Eva1418-FH5      |                   | Histidine rich derivative of Eva1418 peptide                |                | Enhanced inhibition activity with lowest cytotoxicity                                    |                                  |
| 19    | Hp1090           | Venom of Scorpion: Heterometrus petersii | Amphipathic α-helical peptide                             | HCV            | Inhibition of viral replication                                                         | Yan et al. (2011)                |
| 20    | Hp1239           |                   | Amphipathic α-helical peptide                               | HSV-1          | Inhibition of cell entry by blocking viral-host membrane fusion                          | Hong et al. (2014)               |
| 21    | Hp1036           |                   | Amphipathic α-helical peptide                               | HSV-1          | Inhibition of cell entry by blocking viral-host membrane fusion                          | Hong et al. (2014)               |
| 22    | Lactarcin 1      | Venom of spider: Lachesana tarabaeva | Amphipathic α-helical peptide                             | DENV           | Inhibition of viral replication by binding to viral protease                             | Rothan et al. (2014)             |
| 23    | Mastoparan       | Venom of wasp: Vespula lewisi | 14 amino acid residues, cationic, amphipathic α-helical peptide | VSV, HSV-1, flaviviruses | Inhibition of cell entry by disruption of envelope                                       | Moreno and Giralt (2015)         |
| 24    | Mastoparan 7     | Venom of wasp: Vespula lewisi | Derivative of Mastoparan peptide                            | VSV            | Inhibition of cell entry by disruption of envelope                                       | Sample et al. (2013)             |
| 25    | Melittin         | Venom of bee: Apis melifera | 26 amino acid linear cytolytic peptide with no disulfide bridge | HIV-1; HSV-1 and 2; JUNV | Inhibition of cell entry by disruption of envelope                                       |                                   |
| 26    | Mucroporin-M1    | Scorpion: Lychas mucronatus | Cationic host defense peptide                              | MeV, Influenza-H5N1; SARS-CoV; HIV-1 HBV | Virucidal activity                                                                        | Li et al. (2011)                 |
|       | Amphibians       |                   |                                                             |                | Inhibition of viral replication by decreasing expression of important HBV replication factors |                                   |
| 27    | Dermaseptins S3, S4 | Frogs of Phylomedusa genus | Cationic, amphipathic and α-helical peptide                 | HSV-1 and 2, HIV | Inhibition at cell entry step by targeting viral envelopes                               | Lorin et al. (2005)             |
| 28    | Dermaseptin derived peptide | Frogs of Phylomedusa genus | Cationic, amphipathic and α-helical peptide                 | Rabies virus   | Inhibition at cell entry step                                                            | Bergaoui et al. (2013)           |
| 29    | HS-1             | Skin of Anuran: Hypsiboas semilineatus | Cationic, amphipathic and α-helical peptide                  | DENV 2 and 3   | Inhibition at cell entry step by targeting viral envelopes                               | Monteiro et al. (2018)           |
| S. No | Peptide                  | Source                  | Characteristics                                                                 | Targeted virus          | Mode of action/ activity                                                                                   | Reference                      |
|-------|--------------------------|-------------------------|---------------------------------------------------------------------------------|-------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------|
| 30    | Magainin I and II        | Frog: *Xenopus laevis*  | 23 amino acid residues, lysine-rich, cationic, amphipathic and α-helical peptide | HSV-1 and 2             | Inhibition at cell entry step by disrupting the structure of viral envelope proteins; virucidal activity | AlbiolMatanic and Castilla (2004) |
| 31    | Magainin-II derived peptide | Frog: *Xenopus laevis* | Alanine-substituted magainin-2 amide along with three other peptides            | VV                      | Virucidal activity by attacking its envelope                                                             |                                 |
| 32    | Temporin B               | Frog: *Rana temporaria* | 10–50 amino acids, cationic, amphipathic and α-helical peptide                 | HSV-1                   | Inhibition at entry step by interfering in cell-to-cell spread of the virus                             |                                 |
| 33    | Urumin                   | Indian frog: *Hydropylax bahuvistara* | 27-residues, cationic, amphipathic and α-helical peptide | Influenza-H1N1 and H1N2 | Inhibition at cell entry step by targeting cell receptors                                               | Holthausen et al. (2017)        |
| 34    | Callipeltin A            | *Callipeltin sp.*       | Cyclic depsidecapeptide                                                         | HIV-1                   | Inhibition of virion entry                                                                               | Zampella et al. (1996)          |
| 35    | Celebeside A-C           | *Siliquariaspongia mirabilis* | Cyclic depsipeptides                                                        | HIV-1                   | Inhibition of virion entry                                                                               | Plaza et al. (2009)             |
| 36    | Clavanin A               | Tunicate: *Styelaclava* | 23 residues alpha-helical peptide with amidated C-terminal                     | Rotavirus, Denovirus    | Inhibition by interfering with viral membranes                                                           | Carriel-Gomes et al. (2007)     |
| 37    | Homophymin A             | *Homophymina sp.*       | Cyclic depsideptide                                                            | HIV-1                   | Inhibition of virion entry                                                                               | Plaza et al. (2008)             |
| 38    | Koshikamides F           | *Theonella swinhoei*    | 17-residue cyclic depsipeptides                                                 | HIV-1                   | Inhibition by blocking HIV entry into T cells                                                           | Plaza et al. (2010)             |
| 39    | Koshikamides H           | *Theonella cupola*      | 17-residue cyclic depsipeptides                                                 | HIV-1                   | Inhibition by blocking HIV entry into T cells                                                           |                                 |
| 40    | LvHcL48                  | Hemocyanin of shrimp: *Litopenaeus vannamei* | 79 amino acid fragment                                                        | WSSV                    | Inhibition of the transcription and proliferation possibly by binding to the viral envelope protein     | Zhan et al. (2018)              |
| 41    | Microspinosamide         | *Sidonops microspinosa* | Cyclic depsideptide incorporating 13 amino acid residues                      | HIV                     | Inhibition of cytopathic effects of the infection                                                       | Rashid et al. (2001)            |
| 42    | Mirabamide A, C and D    | *Siliquaria spongia mirabilis* | Cyclic depsipeptides                                                        | HIV-1                   | Inhibition at the early stages of virus entry                                                           | Plaza et al. (2007)             |
| 43    | Mirabamides E, F, G, and H | *Sponge: Stelletta clavosa* | Cyclic depsipeptides                                                         | HIV-1                   | Inhibition at entry step by disruption of viral membrane fusion                                        | Lu et al. (2011)                |
| 44    | Mollamides B             | Tunicate: *Didemmummolle* | Cyclic hexapeptide                                                              | HIV                     | Unknown                                                                                                 | Donia et al. (2008)             |
| 45    | Mutrendamide A           | *Theonella swinhoei*    | Sulfated cyclic depsipeptide                                                   | HIV-1                   | Inhibition by blocking HIV entry into T cells                                                           | Plaza et al. (2010)             |
| 46    | Neamphamide A            | *Neamphius huxleyi*     | Cyclic depsipeptide                                                            | HIV-1                   | Inhibition of virion entry                                                                               | Oku et al. (2004)               |
| S. No | Peptide       | Source                           | Characteristics                                                                 | Targeted virus | Mode of action/activity                                                                 | Reference                  |
|-------|---------------|----------------------------------|-------------------------------------------------------------------------------|----------------|----------------------------------------------------------------------------------------|----------------------------|
| 47    | Nkl71-100     | Turbot: *Scophthalmus maximus*    | five-helix bundled structure stabilized by three intra chain disulphide bonds | SVC            | Inhibition by not binding of viral particles to host cells and fusion of virus and cell membranes | Falco et al. (2019)        |
| 48    | Papuamide A   | Tunicate: *Didemnum molle*       | Cyclic depsipeptides                                                          | HIV            | Virucidal mechanism                                                                     | Andjelic et al. (2008)     |
| 49    | Piscidin 1    | Mast cells of hybrid Striped bass (fish) | 22 amino acid, α-helical and amphipathic peptide | PRV            | Inhibition by direct interaction with virus                                               | Hu et al. (2019)           |
| 50    | Pa-MAP 1      | Polar fish: *Pleuroproctus americana* | an alanine-rich α-helix peptide composed of eleven amino acid residues with three imperfect motif repetitions | HSV-1 and 2    | Virucidal mechanism of action, Inhibition at entry step by interacting viral surface glycoprotein | Migliolo et al. (2012)     |
| 51    | P34           | Intestinal contents of *Leporinus sp.* (fish) | Anionic, thermostable, hydrophobic, lipidic peptide | EAV, FHV-1     | Virucidal activity                                                                      | Castro et al. (2014)       |
| 52    | Stelletapin A and B | *Stelletta clavosa*             | Cyclic and nonribosomal depsipeptides                                          | HIV-1          | Inhibition of cytopathic effects of the infection                                         | Shin et al. (2015)         |
| 53    | Theopapuamide A | *Theonella swinhoei*            | Cyclic depsipeptides                                                          | HIV-1          | Inhibition of virion entry                                                               | Andjelic et al. (2008)     |
| 54    | Theopapuamide B-D | *Siliquariaspongia mirabilis*   | Undecapeptides with an N-terminal fatty acid moiety                          | HIV-1          | Inhibition by disruption of viral membrane                                               | Plaza et al. (2009)        |
| VI    | Mammals       |                                   |                                                                               |                |                                                                                        |                            |
| 55    | α-Defensin HNPs 1, 2 and 4 | Human neutrophil              | 18 to 45 amino acid residues cationic charge, amphipathic properties and predominance of β sheets stabilized by three disulfide bonds | HIV-1          | Inhibition at cell entry step                                                           | Wu et al. (2005)           |
| 56    | α-Defensin HNPs 1 | Human neutrophil              |                                                                               | Influenza A    | Inhibition of viral replication                                                          | Salvatore et al. (2007)    |
| 57    | β-defensins hDB-2 | Epithelial cells              | Cysteine-rich, cationic peptides                                              | HPV; VZV; HIV  | Inhibition at cell entry as well as viral replication by late reverse transcripts and nuclear import | Meyer-Hoffert et al. (2008) and Crack et al. (2012) |
| 58    | β-defensins hBD-3 | Epithelial cells              |                                                                               | HPV; VV; VZV; HIV | Inhibition of viral replication                                                        | Quinones-Mateu et al. (2003), Howell et al. (2007), Gwyer Findlay et al. (2015) |
Table 2 (continued)

| S. No | Peptide       | Source                   | Characteristics                                      | Targeted virus | Mode of action/activity                                      | Reference                                                                 |
|-------|---------------|--------------------------|------------------------------------------------------|----------------|-------------------------------------------------------------|---------------------------------------------------------------------------|
| 59    | Cathelicidin LL-37 | Human neutrophil granules | 12 to 88 amino acid residues, cationic, α-helical and amphipathic peptide | VZV; VV; HSV-1; HIV; RSV; Influenza A; HCV; DENV; ZIKV; VEEV | Inhibition of cell entry by disruption of envelope | Sørensen et al. (2001), Barlow et al. (2011), Tripathi et al. (2015), Matsumura et al. (2016), Alagarasu et al. (2017) |
|       |                |                          |                                                      |                | Adenovirus; Aichi virus; Rhinovirus | Inhibition of cell entry | Gordon et al. (2005), Sousa et al. (2017), Ahmed et al. (2019b) |
| 60    | CYVIP         | Human hemofiltrate       | 71-amino-acid, cationic peptide                     | HCMV           | Inhibition of cell entry by interacting the host cell receptors | Borst et al. (2013)                                                       |
| 61    | Indolicidin   | Bovine neutrophils       | Tridecapeptide amide                                | HIV            | Inhibition by membrane-disruption                          | Robinson et al. (1998)                                                   |
| 62    | Lactoferrin   | Mammals’ milk            | Hydrophobic, cationic, and helical peptide         | CMV; HSV-1 and 2; Adenovirus; Rotavirus; Poliovirus; RSV; HIV; Influenza; HCV; HBV DENV; CHIKV | Inhibition at cell entry as well as viral replication | Van der Strate et al. (2001), Li et al. (2009), Carvalho et al. (2017), Chen et al. (2017) |
| 63    | Lactoferricin | Derivative of lactoferrin | Amphipathic, cationic peptide corresponds to lactoferrin fragment 17–41 | CMV, HIV-1, HPV | Inhibition at cell entry step | Andersen et al. (2001), Misra et al. (2007), Li et al. (2009), Wang et al. (2016) |
|       |                |                          |                                                      | HSV-1 and 2    | Inhibition of viral replication by interfering the host cell microtubules | Marr et al. (2009)                                                        |
| 64    | Protegrin-1   | White blood cells of swine | 18 amino acid residues, cyclical, β-sheets and cationic | DENV           | Inhibition of viral replication by binding to viral protease | Rothan et al. (2012)                                                       |

Human immunodeficiency virus: HIV; Dengue virus: DENV; Herpes simplex virus 1 and 2: HSV-1 and HSV-2; Porcine epidemic diarrhea virus: PEDV; Vesicular stomatitis virus: VSV; Simian immunodeficiency virus: SIV; Newcastle disease virus: NDV; Coxsackie virus B2: CBV-2; Junin virus: JUNV; Hepatitis C virus: HCV; Measles morbillivirus: MeV; Severe acute respiratory syndrome coronavirus: SARS-CoV; Hepatitis B virus: HBV; Vaccinia virus: VV; White spot syndrome virus: WSSV; Carp siphivirus: SVC; Pseudorabies virus: PRV; Equine arteritis virus: EAV; Feline herpes virus type-1: FHV-1; Bovine herpesvirus1: BoHV-1; Human papillomavirus: HPV; Varicella zoster virus: VZV; Respiratory syncytial virus: RSV; Zika virus: ZIKV; Venezuelan equine encephalitis virus: VEEV; Human cytomegalovirus: HCMV; Cytomegalovirus: CMV; Chikungunya virus: CHIKV
Ribosome Display

Ribosome display is an entirely in vitro and cell-free system which makes it efficient in comparison to other display systems (Nevola and Giralt 2015). In this system, the coupling of genotype and phenotype is essential for the selection of high affinity peptides from their pool. During in vitro translation, the association between the mRNA, ribosome and the nascent polypeptide leads to a stabilized protein-ribosome-mRNA complex. This ternary complex is feasible due to the presence of spacer sequence, without stop codon, inserted into the DNA library coding for proteins/peptides. The spacer ensures that the peptide folds properly and stays attached to the mRNA and ribosomes. These specific ribosomal complexes that display folded peptides are then allowed to bind to the immobilized target and the non-specific ones are washed off. The mRNA complexes having bound polypeptide chains are recovered and their sequences are obtained (Zahnd et al. 2007). A large library that contains $10^{13} - 10^{14}$ clones can be screened as it is not dependent on the living cell system and is free of any bias. This technology has numerous advantages over others as the diversity of library depends on the number of available ribosomes and mRNA in the system rather restricted by the bacterial transformation efficiency (Dreier and Plückthun 2011). Moreover, such system allows insertion of random mutations at any round of selection since library has not been transformed after any diversification step. The ribosomal display technology has opened a new insight for using peptide inhibitors for early diagnostic as well as therapeutic agent. For instance, the peptide inhibitor against envelope protein E2 of HCV was identified using ribosomal display library. After extensive selection of 13 rounds, 12-mer peptides were generated. This peptide named PE2D has not only being verified to bind E2 protein but also blocks the virus entry inside hepatocyte cells (Chen et al. 2010).

Yeast Display

The main advantage of the yeast display system over the others is the complete exposure of the peptide/protein for fusion and its compatibility with the fluorescence-activated cell sorting (FACS), which enables the high-throughput screening and characterization of protein/peptide combinatorial libraries (Linciano et al. 2019). Moreover, it also allows the expression of proteins with post translational modifications which has encountered the problem of misfolding in the field of antibody engineering (Mei et al. 2017). Saccharomyces cerevisiae strain based on the Aga1–Aga2 proteins is the most widely used display system. In this system, the protein/peptide is displayed either as N- or C-terminal fusion to the Aga2 protein of yeast cell, which is linked to the Aga1 via disulphide bonds. Every yeast cell exhibits $10^4 - 10^5$ copies of the Aga2 fusion protein/peptide on its surface though the expression of individuals may vary. The construct of yeast cell also contains two epitope tags at the N and C terminus of Aga2 fusion protein, which facilitates the real time quantification of their expression using flow cytometry. Moreover, the tags also enable to estimate and quantify the binding of the target via different labelling approaches (Linciano et al. 2019). Though yeast display system is a valuable platform for screening purpose, however, the library size is restricted due to limited transformation efficiency of yeast. Another major limitation of yeast display is complicated affinity maturation process in comparison to other systems. Besides, these drawbacks, this technology has provided a wide application of high throughput screening in peptide engineering and a platform to study protein–protein/peptide interactions in vivo. In a recent study, this technology was used to screen the hits from a grafted C-peptide library of HIV gp41 against N-peptide trimer of HIVgp41. As a result, four hits suppressed the HIV entry better than others (Tennyson et al. 2018).

Application of Advanced Techniques to Validate Identified Peptides

Another challenging task in the intervention of antiviral peptides is the corroboration of the binding of selected peptides to the target protein, and their antiviral efficacy. Various techniques have been developed to evaluate the PPIs in vitro/in vivo such as Surface Plasmon Resonance (SPR), Optical based Biolayer interferometry, Fluorescence Resonance Energy Transfer (FRET), Nuclear Magnetic Resonance (NMR), Isothermal Calorimetry (ITC), yeast two hybrid display, microscopic visualization and many more. Some of them can be used for the binding evaluation of the peptides to their target proteins. Since various reviews and reports are available on PPIs detection methods in detail (Nevola and Giralt 2015), this review focuses only on the recent techniques used to determine/ validate the peptide binding efficiency (Table 1).

Surface Plasmon Resonance (SPR)

SPR is an optical based detection and label-free technique which utilizes the protein in small amount for the real time quantification and evaluation of the binding affinity as well as kinetics between peptide and target protein (Patching 2014). The binding affinity between interacting partners is measured via small variation in the refractive index at sensor surface. This response change is calculated as the change in the angle of resonance of refracted light when flowing analyte binds to the immobilized ligand. The change in the angle of resonance is measured in the form of resonance unit.
| S. No | Peptide sequence  | Library used                                   | Targeted virus                      | Targeted protein                          | References               |
|-------|-------------------|-----------------------------------------------|-------------------------------------|-------------------------------------------|--------------------------|
| 1     | GSHHRHVHSPFV      | 12-mer peptide library: New England Biolabs (NEB) | Avian infectious bronchitis virus    | Purified whole virus                       | Peng et al. (2006)       |
| 2     | HAWDPIARDPF       | 12-mer peptide library (NEB)                  | Avian influenza A virus-subtype H5N1 | H5N1 viruses                              | Wu et al. (2011)         |
| 3     | AAWHIVALAPN       | 12-mer peptide library (NEB)                  | Avian infectious bronchitis virus    | Purified whole virus                       | Peng et al. (2006)       |
| 4     | ATSHLHVRPSK       | 7-mer disulfide constrained peptide library (NEB) | Avian influenza virus H9N2       | AIV sub-type H9N2 virus particles        | Rajik et al. (2009)      |
| 5     | NDFRSKT           | 7-mer disulfide constrained peptide library (NEB) | Avian influenza virus H9N2       | AIV sub-type H9N2 virus particles        | Rajik et al. (2009)      |
| 6     | HSIRYDF           | 7-mer Peptide Library                          | Bovine ephemeral fever virus       | Neutralization site 1 of glycoprotein: G1 | Hou et al. (2018)        |
| 7     | YSLRSDY           | Octapeptides peptide library                  | Classical swine fever virus        | Envelope protein: E2                       | Yin et al. (2014)        |
| 8     | DRATSSNA          | 7-mer peptide Library                          | Dengue virus                       | Recombinant dengue envelope protein and its domain III | de la Guardia et al. (2017) |
| 9     | SYQSHYY           | 7-mer peptide Library                          | Dengue virus                       | Recombinant dengue envelope protein and its domain III | de la Guardia et al. (2017) |
| 10    | STSFWIT           | 7-mer peptide Library                          | Dengue virus                       | Recombinant dengue envelope protein and its domain III | de la Guardia et al. (2017) |
| 11    | ELLASPSW          | 7-mer disulfide constrained peptide library (NEB) | Hepatitis B virus                  | Full-length HbcAg                          | Ho et al. (2003)         |
| 12    | CWSFFSNIC         | 7-mer disulfide constrained peptide library (NEB) | Hepatitis B virus                  | Full-length HbcAg                          | Ho et al. (2003)         |
| 13    | KHMHWHPALNT       | 12-mer peptide library (NEB)                  | Hepatitis B virus                  | PreS1 region of L-protein                 | Wang et al. (2011)       |
| 14    | WTDMMFAWSPTP      | M13-based 12-mer peptide library              | Hepatitis B virus                  | Thio-PreS                                 | Deng et al. (2007)       |
| 15    | FPWGNNTW          | 7-mer disulfide constrained peptide library (NEB) | Hepatitis C virus                  | NS5B (del 21-His) protein                 | Amin et al. (2003)       |
| 16    | ATWVCGPCT         | Phage-displayed nonapeptide library (PVIII9aa) | Hepatitis C virus                  | mAb JS-81 against CD81                    | Cao et al. (2007)        |
| 17    | WPWHNHR           | heptapeptide M13 phage-display library        | Hepatitis C virus                  | Truncated envelope protein E2             | Lu et al. (2014)         |
| 18    | RINNIPWSEAMM      | libraries of random 12-mers, 7-mers, and cyclic 9-mers | Human immunodeficiency virus     | Envelope glycoprotein gp120               | Ferrer and Harrison (1999)|
| 19    | VSWPELYKWITWS     | 7-mer disulfide constrained peptide library; 12-mer peptide library (NEB) | Human immunodeficiency virus     | mAb VRC01                                 | Chikaev et al. (2015)   |
| 20    | FHNHGKQ           | 7-mer peptide library (NEB)                   | Human immunodeficiency virus       | HIV-1 Integrase                           | Desjoubert et al. (2004) |
| 21    | GWWYKGRAPVS-AVA   | Pentadecapeptides peptide library             | Influenza virus A                  | Monolayer of the ganglioside:GM3          | Matsubara et al. (2009)  |
| 22    | RAVWRHSATPSHSV    | libraries of random 12-mers, 7-mers, and cyclic 9-mers | Human immunodeficiency virus     | mAb VRC01                                 | Chikaev et al. (2015)   |
| 23    | SENRKVPFYSHS      | 12-mer peptide library (NEB)                  | Japanese encephalitis virus        | Domain III of the virus envelope glycoprotein | Zu et al. (2014)         |
| 24    | TPDCCTRWCPLT      | 12-mer peptide library (NEB)                  | Japanese encephalitis virus        | Domain III of the virus envelope glycoprotein | Zu et al. (2014)         |
| 25    | RLRNRAIIIRADA      | 12-mer peptide library (NEB)                  | Mink enteritis virus               | Purified whole virus                       | Zhang et al. (2012)      |
| 26    | LAHKSRLYERHM      | 7-mer disulfide constrained peptide library (NEB) | Newcastle disease virus            | Inactivated whole virus                    | Ramanujam et al. (2002) |
| 27    | CTLLTKLYC         | 7-mer disulfide constrained peptide library (NEB) | Newcastle disease virus            | Inactivated whole virus                    | Ramanujam et al. (2002) |
| 28    | EVSHPKVG          | Heptapeptide library-pSKAN8-HyA library       | Newcastle disease virus            | Inactivated whole virus                    | Ozawa et al. (2005)      |
| 29    | SGGSNRP          | Heptapeptide library-pSKAN8-HyA library       | Newcastle disease virus            | Inactivated whole virus                    | Ozawa et al. (2005)      |
| 30    | WVTTSNQW         | Heptapeptide library-pSKAN8-HyA library       | Newcastle disease virus            | Inactivated whole virus                    | Ozawa et al. (2005)      |
(RU), where 1RU is equivalent to the $10^{-4}$ deg/10$^{-12}$ gmm$^{-2}$ angle shift. It has become the gold standard in research, typically characterizes the interaction between two molecules in which one is in mobile state and the other is fixed on a gold film. This technique can be used to screen the library of molecules for their binding affinity against a single soluble protein which is immobilized on the sensor surface (Tang et al. 2010). Thus, SPR has emerged as a powerful technique in therapeutic intervention. It can also be adapted to study the interactions involving complicated proteins in situ, such as, membrane-bound proteins, ion channels and other growth, immune and cellular receptors, which are considered as potential targets for drug discovery (Patching 2014). Bai et al. (2007) have investigated the affinity interaction of screened peptides to the Envelope protein of West Nile virus (WNV) using SPR, in which they found peptide P9 to have the highest affinity to the target. Besides this, in another study, the binding of Helix-A peptide to the neuronal microtubules (MTs): β-tubulin was determined by SPR. Helix-A peptide prevents the binding of the gp120 protein of HIV to the β-tubulin, a neuronal MT and possesses neuroprotective activity (Avdoshina et al. 2019). Moreover, the SPR has been used as a ligand screening strategy for Influenza virus and HSV-1, in which the technique enables the continuous screening of inhibitors that inhibit the viral entry. The major advantage of this technique is the use of minimal amount of immobilized viral surface proteins or receptors as compared to other techniques (Kumar 2017).

**Biolayer Interferometry**

Another optical based and label-free technique is the Biolayer interferometry (BLI) which validates the interaction between two molecules by quantifying the change in an interference pattern. The target molecule is immobilized on the tip of fiber optic biosensor that moves toward the wells containing the binding partner present in solution. The association and dissociation of the binding partner with the immobilized molecule is monitored by BLI, leading to the generation of optical thickness at the tip of biosensor that produces an optical interference pattern. This pattern can be quantified and used to determine real time kinetic rates of binding and dissociation (Shah and Duncan 2014). Thus, it has become a valuable tool for monitoring interactions between small molecules in the field of drug discovery. This technique is advantageous over others as nonspecific and non ideal interactions can be differentiated in initial steps by examining their binding response and moreover, it has low false positive rate. Besides, the varied flow rate, available unbound molecules and the refractive index of adjacent medium do not affect the obtained interference pattern, which is the unique property of this technique (Wartchow et al. 2011). In this context Zu et al. (2014) have analysed the real time binding affinity of chemically synthesized screened peptides to the Domain III of JEV envelope protein and reported peptide P3 possessed the highest affinity.

**Fluorescence Resonance Energy Transfer (FRET)**

FRET is a sensitive method to investigate the interaction of proteins with large diverse set of peptides/proteins libraries for high throughput screening efficiently. This technique is reliable on the distance-dependent transfer of energy between dye-labelled molecules, where the excited donor fluorophore transfers its energy to an acceptor chromophore (Rogers et al. 2012). This energy transfer determines the ratio metric signal generated by the reduction in fluorescence of donor molecule and the increment in fluorescence of acceptor molecule. The technique of FRET can be used as both screening as well as validation method. In view of this, various FRET based studies have been reported for the identification of potent inhibitors against several viral proteases such as SARS coronavirus 3CLpro protease, DENV NS2B-NS3 protease, WNV Serine Protease, HCV NS3/4A protease.
and HIV protease. Similarly, a FRET based proteolytic assay was used to screen the compounds against CHIKV capsid protein (Aggarwal et al. 2015).

Challenges to the Peptide as Therapeutic Use

Several limitations that obstruct the way of peptide to be a successful therapeutic drug, are its instability, short half-life, lower potency, inability to cross membrane barriers and poor bioavailability due to protease degradation (Ali et al. 2013). The main challenge is to overcome these limitations and to achieve the desired efficacy for the required time span. Various modifications have been employed to enhance the stability and physicochemical properties of the peptides (Gentilucci et al. 2010). For instance, the conjugation of peptide to polymers such as polyethylene glycol (PEG) has enhanced the stability of peptides by increasing their molecular weight (Chew et al. 2017). Likewise, the bioavailability was improved by balancing the aqueous solubility via replacement of redundant hydrophobic amino acids to charged/polar residues (Mant et al. 2009; Wu et al. 2010). Moreover, there are two computational softwares based on support-vector machine (SVM) to predict the solubility of the peptides, thus assisting in the designing and optimising the peptide bioavailability (Lee et al. 2019). In addition to the strategies involved in the improvement of peptide properties, the delivery of peptides has also been improved by linking of peptides to the cell penetrating peptides (CPPs) to enhance their cell permeability (Chew et al. 2017). CPPs are general peptides (<30 amino acids) derived from natural/unnatural sources or chimeric sequences, considered as promising carrier for successful delivery of therapeutic molecules varying from small chemical molecules, liposomes, proteins, peptides and nucleic acids for in vitro as well as in vivo applications (Heitz et al. 2009). Alternatively, peptides can be encapsulated in nanoparticles for efficient delivery, or administered through primary parental or transdermal routes with variations such as prefilled syringes, auto injectors and biodegradable micro needles (Lee et al. 2019). These modifications help to address the challenges of poor ADME properties of non-modified peptides (Lau and Dunn 2018).

Conclusion

In summary, the discovery of the peptide-based therapies have made a significant impact in the research. Many peptide therapies are available in the clinical and pre-clinical trials which are expected to yield positive results. The various approaches including computational, natural and biological sources provide a wide repository for identification of peptides involved in viral therapeutics. These promising therapeutics/inhibitors are advantageous because of their high specificity, selectivity against target and can be easily developed without the prior structural knowledge of target (except docking). Novel technologies like SPR, BLI and FRET validate the binding of identified peptides to their target which further aid in the selection of high affinity potential AVPs. However, there is a dearth of knowledge in the field of identification and characterization of antiviral therapeutics; further advancement and commercialization of peptide-based therapeutics is warranted.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

Ethical Approval This is a review article which does not contain any type of studies related to human or animal participants.

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