Entry Inhibitors: Efficient Means to Block Viral Infection

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Abstract

The emerging and re-emerging viral infections are constant threats to human health and wellbeing. Several strategies have been explored to develop vaccines against these viral diseases. The main effort in the journey of development of vaccines is to neutralize the fusion protein using antibodies. However, significant efforts have been made in discovering peptides and small molecules that inhibit the fusion between virus and host cell, thereby inhibiting the entry of viruses. This class of inhibitors is called entry inhibitors, and they are extremely efficient in reducing viral infection as the entry of the virus is considered as the first step of infection. Nevertheless, these inhibitors are highly selective for a particular virus as antibody-based vaccines. The recent COVID-19 pandemic lets us ponder to shift our attention towards broad-spectrum antiviral agents from the so-called 'one bug-one drug' approach. This review discusses peptide and small molecule-based entry inhibitors against class I, II, and III viruses and sheds light on broad-spectrum antiviral agents.

Graphic Abstract
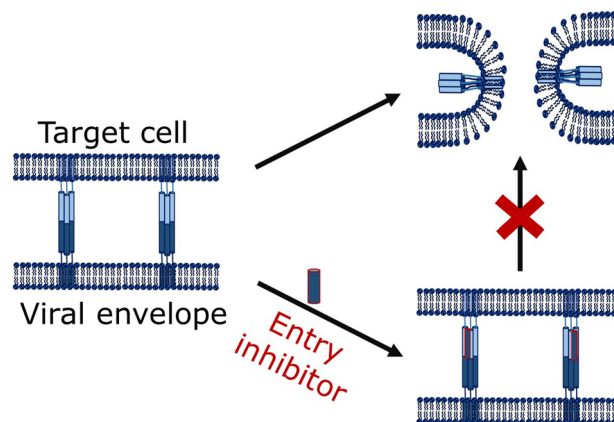
Keywords Entry inhibitor · Viral infection · Membrane fusion

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Viral Infections

In the current scenario, emerging and re-emerging viral infections are the major pandemic threats to humankind. The rate of replication of virion and the rate of transmission play crucial roles for any viral infections. Most of the viruses are zoonotic in nature, which transmit from animals to humans. HIV, filoviruses (such as Ebola and Marburg), henipaviruses (such as Hendra and Nipah), coronaviruses (such as severe acute respiratory syndrome (SARS-CoV)

and middle east respiratory syndrome coronavirus (MERS-CoV), COVID-19), and influenza are some popular examples of zoonoses. In addition, some virus families like *flaviviridae* (such as Dengue virus (DENV), *bunyaviridae* (Hanta virus), and *arenaviridae* (Junin and Lassa virus) are animal or arthropod-borne, and also cause deadliest viral diseases (Vigant et al. 2015). A summary of some viral epidemic is shown in Table 1.

Initiatives have been taken to develop antiviral agents to intervene viral infection, and most of the strategies are based on the 'one bug-one drug' approach. However, this strategy is not adequate for responding to an ever-increasing number of emerging and re-emerging disease-causing viruses. In addition, there are many mammalian viruses present in the wildlife reservoir which remain to be discovered (Vigant et al. 2015). Again, the tendency of drug resistance is a leading parameter to cause failure of the above approach.

This review discusses the efficient ways to block the entry of viruses, thereby providing protection against viral infection. Though the morphology, genome structure, and life cycle of each virus is different, most of the viruses follow a common pathway for their entry into the human cells. Therefore, targeting those conserved features could be a successful strategy to defy the fusion between virus and host cell membranes.

Viral Entry into Host Cells

A majority of viral pathogens that cause emerging and re-emerging infectious diseases are membrane-enveloped viruses. Enveloped viruses are characterized by a lipid bilayer, known as envelope, covering the virus particle i.e. virion. The virion consists of an outer protein coat called capsid which surrounds the inner core that comprises nucleic

acids (either RNA or DNA). One or more glycoproteins are generally decorated on the viral envelope. A general structure of an enveloped virus is shown in Fig. 1.

Viruses are obligate intracellular parasites, which carry out their replication within the host cell, and cannot multiply outside a living cell. Once the virus enters into a host cell, it competes with the immune response of the target and disrupts a variety of cellular proteins and chemical pathways to survive and replicate (Mazzon and Marsh 2019). Therefore, delivery of the viral genome inside the host cell is the earliest stage for any viral infection. At this stage, enveloped viruses first bind to specific surface receptors of the target cell membrane non-covalently, followed by fusion between virus and host cell. This fusion step may occur at the cell surface or after internalization of the virus particle by endocytosis. In either case, fusion is catalysed by one type of viral glycoproteins (in case of influenza viruses, retroviruses) or combinations of multiple viral glycoproteins (in case of herpesviruses, paramyxoviruses). Viral glycoproteins (fusogens), when activated by non-covalent interactions with
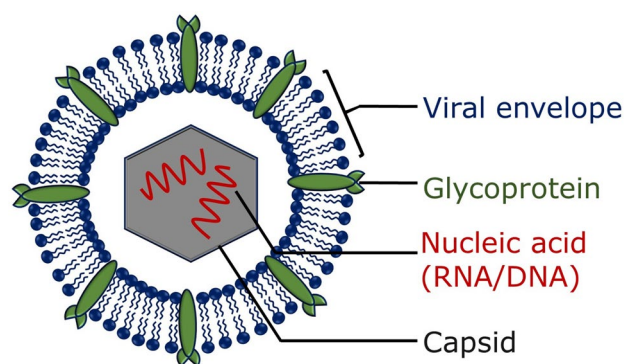

Fig. 1 Schematic representation of an enveloped virus

Table 1 Different viral diseases and their impact on humankind

Viral disease	People infected	People died	Year(s)	References
COVID-19	20 million	> 5,00,000	2019–Present	Johns Hopkins University database
MERS	2494	858	2012–2019	WHO website
Dengue	100 million	38,000	2016	Bloom and Cadarette (2019)
Influenza (Swine flu)	700 million to 1.4 billion	575,000	2009–2010	Saunders-Hastings and Krewski (2016)
SARS	8098	774	2002–2003	Olsen et al. (2003)
HIV/AIDS	75 million	32 million	1960–2008	WHO website
Small Pox	130,000	26,000	1974	Bloom and Cadarette (2019)
Influenza (Hong Kong flu)	> 500 million	500,000–2 million	1968–1970	Saunders-Hastings and Krewski (2016)
Influenza (Asian flu)	> 500 million	1–2 million	1957–1958	Saunders-Hastings and Krewski (2016)
Influenza (Spanish flu)	500 million	50 million	1918–1919	Taubenberger and Morens (2006)

surface receptors and/or by acidic endosomal pH, result in large-scale conformational change which drives the fusion process (Mas and Melero 2013; Melikyan 2008; White et al. 2008). In essence, virus-cell fusion is, therefore, the step at which the virus particle loses its individuality. HIV and herpes simplex virus fuse at the cell membrane, whereas influenza, polio, hepatitis C, and foot-and-mouth disease viruses enter into the cell via endocytosis followed by endosomal fusion.

The structures of pre-fusion and post-fusion conformations of many viral fusogens have been studied using x-ray crystallography and cryo-electron microscopy. It has been believed that the post-fusion conformation is more stable compared to the pre-fusion conformation (Mas and Melero 2013; White et al. 2008). Moreover, there is a high kinetic barrier between the pre- and post-fusion conformation. Upon protonation (at low endosomal pH) or binding to the receptor and/or co-receptor leads to conformational change of the fusogen, and the energy liberated during this transition successfully drives the fusion process (Harrison 2015; Mas and Melero 2013). The free energy profile for membrane fusion process considering the lipid-stalk model is shown in Fig. 2.

The sequence of viral glycoprotein (fusion protein) differs from virus to virus; however, it has been observed that all viral fusion proteins share some common structural characteristics. Usually, upon activation, all viral fusion proteins undergo conformational rearrangement to assume pre-hairpin structure. The lifespan of the pre-hairpin structure varies from seconds to minutes depending upon the virus (Floyd et al. 2008; Munoz-Barroso et al. 1998). Interestingly, the conformational rearrangement of the fusion protein exposes the hydrophobic segment, known as fusion peptide, to the membrane surface (Mas and Melero 2013; Pattnaik et al. 2018). The exposed hydrophobic fusion peptide is ultimately bound to the target membrane, whereas transmembrane domain (TMD) region of the fusion protein remains rooted in viral membranes. Thus, bridging of viral and target membrane occurs by two different portions of fusion protein, followed by the collapsing of the pre-hairpin bridge that results into close apposition of the two lipid bilayers. The close apposition of two lipid bilayers causes merging of the outer leaflets of each bilayer to form the semi-stable stalk intermediate (Pattnaik et al. 2018; Yang and Huang 2003). The stalk follows the formation of a transmembrane contact (TMC), and extended transmembrane contact (ETMC). Finally the ETMC opens to form a fusion pore leaving a highly stable post-fusion hairpin conformation inserted into the target membrane (Lee and Lentz 1997; Pattnaik et al. 2018; Siegel 1999). Figure 3 shows the schematic representation of a fusion process.

There may be one or more viral fusion protein interacting with the target membrane to form the pre-hairpin intermediate as the bending energy depends on the composition of the membrane (Pattnaik et al. 2018; Tristram-Nagle and Nagle 2007). The requirement of number of trimeric proteins to induce fusion differs from virus to virus (Harrison 2015; Kielian and Rey 2006).
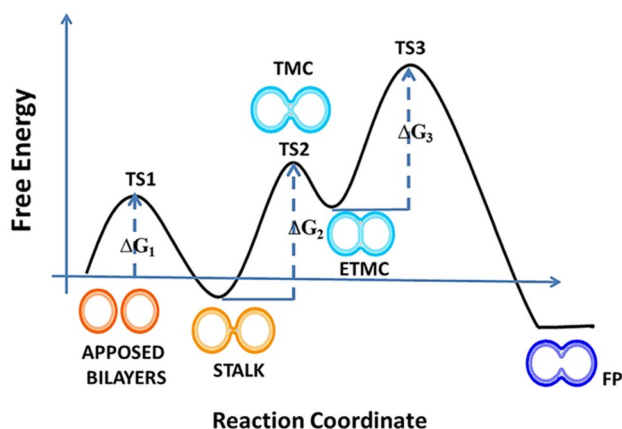

Fig. 2 Schematic representation of the fusion reaction profile considering the lipid-stalk model of membrane fusion. The figure has been adapted from reference Meher et al. (2019b) with permission
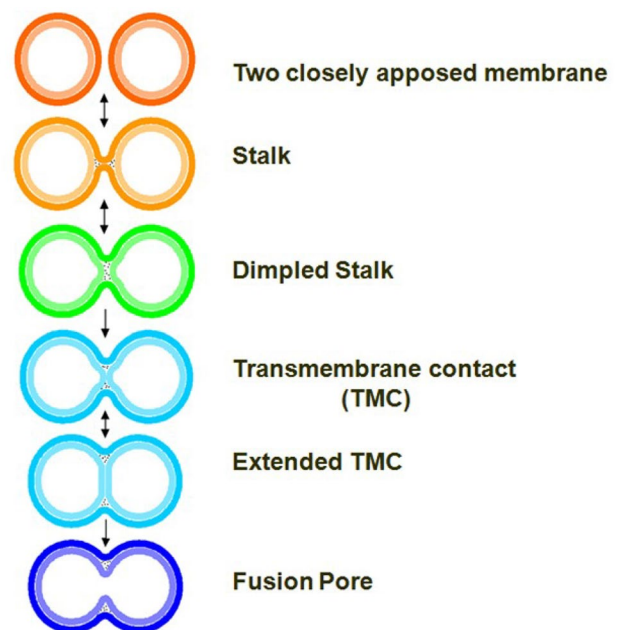

Fig. 3 Schematic representation of stepwise fusion process. The figure has been adapted from reference Pattnaik et al. (2018) with permission
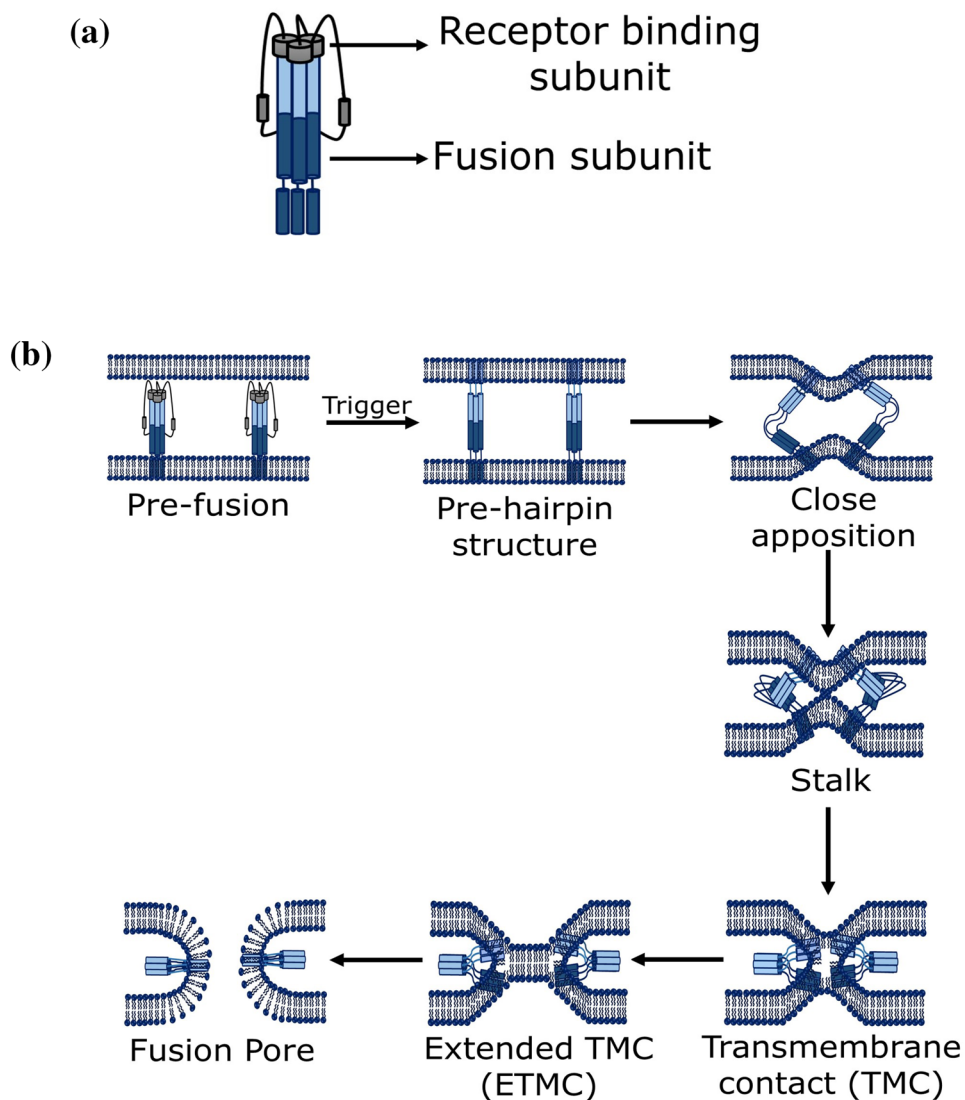
Viral Fusion Proteins

Generally, viral fusion proteins are classified into three different classes, class I, II, and III. Despite little diversity in structural features, they all form a trimeric hairpin which was confirmed by their high-resolution structures (Earp et al. 2005; Harrison 2005; Kielian and Rey 2006; Lamb and Jardetzky 2007; Weissenhorn et al. 2007; White et al. 2008). Structurally all the fusion proteins contain three C-terminal regions that wrap the central N-terminal trimeric core (White et al. 2008).

Class I Fusion Protein

Class I fusion proteins have been shown to maintain homology within a particular family; however, there is no sequence homology across families of class I fusion proteins.

However, all class I fusion proteins are trimers in pre-fusion as well as post-fusion conformations. They are being characterized by a single chain precursor which becomes fusion active by proteolytic cleavage. The cleavage results into two fragments, N-terminal fragment which is generally the receptor-binding domain (e.g. the HA1 fragment of influenza or the gp120 fragment of HIV-1) and the C-terminal fusion facilitating fragment (e.g. HA2 fragment of influenza or the gp41 fragment of HIV-1). The central N-terminal is trimeric α-helical coiled coil adorned by three C-terminal helices easing formation of six-helix bundle (6HB) (White et al. 2008). Formation of this 6HB provides the energy required to overcome coulombic repulsion between two apposing membranes (Mas and Melero 2013). The general pathway of membrane fusion by class 1 fusion protein is shown in Fig. 4. Generally, the fusion peptide locates at the N-terminal of the class 1 fusion protein, However, in influenza HA and paramyxovirus F protein its location differs

Fig. 4 Schematic representation of **a** the fusion protein, **b** membrane fusion steps followed by class 1 fusion protein

(Pattnaik et al. 2018; White et al. 2008). A schematic representation of class I fusion protein from N to C-terminal is shown in Fig. 5. The group of enveloped viruses carrying class I fusion proteins includes *retroviridae* family (Human immunodeficiency virus HIV), *orthomyxoviradae* virus family (Influenza A, B, C, and D), *coronaviridae* family (SARS), *filoviridae* family (Ebola), and *paramyxoviridae* family (Sendai virus) (Rey and Lok 2018). Different class I fusion protein, location of fusion peptide, and their fusion subunits are shown in Table 2 (White et al. 2008).

Entry of HIV

The human immunodeficiency virus (HIV) expresses an envelope glycoprotein gp160 which mediates the entry of HIV. The schematic representation of HIV envelope protein is shown in Fig. 6. Initially, gp160 undergoes receptor-mediated proteolytic cleavage into two subunits viz., gp120 (the surface protein) and gp41 (the transmembrane protein) (Meher et al. 2019b; Skehel and Wiley 2000; White et al. 2008). The heterodimer, gp120 and gp41 organize on the viral membrane as a trimer of three gp41 subunits and three gp120 subunit through non-covalent interaction (Berkhout et al. 2012). First, the surface subunit gp120 binds to the CD4 receptor and undergoes a substantial conformational change leading to higher affinity towards the chemokine co-receptor CCR5 or CXCR4. This induces the activation of a spring-loaded conformational change of gp41. This conformational change leads to the pre-hairpin conformation where the protein gp41 holds both the viral membrane and host cell membrane together through transmembrane domain (TMD) and hydrophobic fusion peptide, respectively (Fig. 4b). In addition to the TMD and fusion peptide, gp41 contains two heptad regions NHR and CHR and their interaction proceeds to the formation of six-helix bundle (6HB) which brings the apposed membrane into closer contact (Fig. 4b) (Chan and Kim 1998). The change in free energy during the formation of 6HB facilitates the formation of fusion pore and hence HIV entry is possible (Berkhout et al. 2012).

Entry of Influenza Virus

The major glycoprotein on the influenza envelope virus is Hemagglutinin (HA) and it is synthesized as a precursor polypeptide, HA0. The schematic representation of influenza virus hemagglutinin is shown in Fig. 7. HA0 is activated upon proteolytic cleavage into two subunits, HA1 (the receptor-binding subunit) and HA2 (the fusion subunit), linked by a disulphide bond (Meher and Chakraborty 2017; Prabhu et al. 2009; Stevens et al. 2006). Sialic acid present on the glycolipids or glycoproteins of the host cell acts as the receptor for HA1 binding, followed by the internalization of
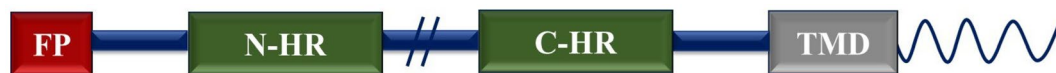

Fig. 5 Schematic representation of a class I fusion protein. Here FP represents fusion peptide, NHR and CHR represent N- and C-heptad regions, respectively, and TMD represents transmembrane domain

Table 2 Different class I fusion protein

Class I virus family	Viruses	Fusion protein	Fusion subunit	Fusion peptide location	Fusion pH
Retroviridae	Human immunodeficiency virus (HIV)	gp160	gp41	N-terminal (mostly)	Neutral/low
Orthomyxoviridae	Influenza virus	HA	HA2	N-terminal	Low
Coronaviradae	Severe acute respiratory syndrome (SARS) virus	S	S2	Internal	Neutral/low
Filoviridae	Ebola virus	GP	GP2	Internal	Low
Paramyxoviridae	Sendai virus	F	F1	N-terminal	Neutral
Arenaviridae	Lassa virus	GP, SSP	GP2	N-terminal	Low

Fig. 6 Schematic representation of HIV envelope protein. Here, FP represents fusion peptide, NHR and CHR represent N- and C-heptad regions, respectively, and TM represents transmembrane domain

Fig. 7 Schematic representation of influenza virus hemagglutinin. Here FP represents fusion peptide, NHR and CHR represent N- and C-heptad regions, respectively, and TM represents transmembrane domain

the virus through endocytosis (Bullough et al. 1994; Kadam et al. 2017). The acidic endosomal environment (pH ~ 5.3) promotes protonation of HA1 subunit that triggers the electrostatic repulsion between HA1 and HA2 resulting in the separation of HA1 from HA2. This detachment of HA1 from HA2 promotes drastic conformational change of HA2 subunit followed by relocation of fusion peptide over 100 Å from its original location (Bullough et al. 1994; Carr and Kim 1993). This major conformational change exposes the fusion peptide (20–25 amino acids in the N-terminal of the HA2 protein), which binds to the target membrane (Fig. 4b) (Gething et al. 1986; Meher and Chakraborty 2017). The further conformational change of HA2 brings the transmembrane domain close to the fusion peptide resulting hairpin structure (Fig. 4b) following the further step of viral entry.

Paramyxovirus Entry

Paramyxoviruses cause critical human diseases, ranging from lower respiratory tract diseases in infants caused by human parainfluenza virus type 1 (HPIV1), type 2 (HPIV2), and type 3 (HPIV3) to highly lethal central nervous system diseases caused by the emerging paramyxoviruses such as Hendra virus and Nipah virus (Collins et al. 1996; Porotto et al. 2009; Williams et al. 2004).

The fusion protein for Paramyxovirus entry is known as 'F' protein. The function of F protein is quite different from other class 1 fusion proteins. There are some other receptor-binding proteins such as HN in HPIV3 and simian virus 5. The fusion protein F is produced as an uncleaved form,

F0, after cleavage F2 (N-terminal) and F1 (C-terminal) are produced which are linked by a disulphide bond previously. During this process, the fusion peptide (N-terminal segment) that was located in the internal region of F1 domain gets exposed (Dutch et al. 2000; Mas and Melero 2013). F1 is equivalent to HA2 chain of influenza virus. In addition to fusion peptide, F1 has two heptad regions (NHR and CHR) in its ectodomain. The location of NHR (or HRA) is adjacent to the fusion peptide, whereas CHR (or HRB) is proximal to the transmembrane region (C-terminus of F1). Upon binding to the receptor, F protein is activated followed by a series of conformational changes leading to separation of CHR coil and refolding of NHR coil to form an elongated trimeric coiled coil (Mas and Melero 2013). The fusion peptide, now at the N-terminal of the F1 segment, is inserted into the target membrane to form the pre-hairpin intermediate (Fig. 4b). This is followed by elongation of fusion peptide and some conformational changes. During this process, the CHR gets inverted and form six-helix bundle with NHR and promotes fusion between viral and host cell (Dutch 2010). The schematic representation of F protein and its cleavage-induced activation is shown in Fig. 8.

SARS-CoV Entry

The coronavirus spike protein (S) plays a crucial role for severe acute respiratory syndrome coronavirus (SARS-CoV) entry into the target cell. The S protein undergoes proteolytic cleavage to produce S1 and S2 subunits associated by non-covalent bond. However, this proteolytic cleavage is not an

Fig. 8 Schematic representation of F protein cleavage. Here FP represents fusion peptide, NHR and CHR represent N- and C-heptad regions, S–S represents the disulphide bond and TM represents transmembrane domain
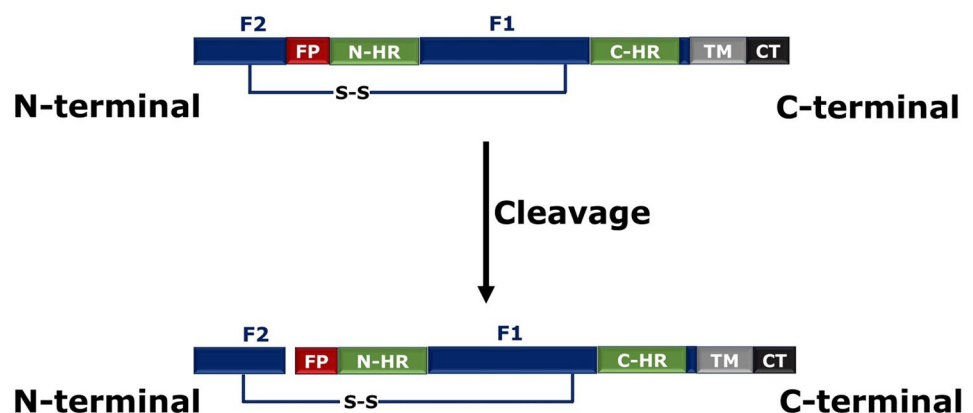
absolute requirement for inducing membrane fusion (Masters 2006). The interaction between S1 spike protein and the cell surface receptor protein, angiotensin-converting enzyme 2 (ACE2) is the primary step for the membrane fusion (Masters 2006; Taguchi and Shimazaki 2000). The receptor binding induces conformational changes in S2 subunit leading to exposure of N-terminal fusion peptide from the interior of S2 subunit, and positions it to interact with the host cell membrane (Cavanagh and Davis 1986; Tripet et al. 2004). The S2 protein contains fusion peptide (FP) at N-terminal region, internal fusion peptide (IFP), and pre-transmembrane peptide (PTM). In addition to these, it comprises highly conserved two heptad regions, NHR (or HR1) and CHR (or HR2), and transmembrane domain at the C-terminus (Bosch et al. 2004). Atomic resolution structure of HR1 and HR2 complex showed the formation of a 6HB, which drives the fusion process (Deng et al. 2006; Hakansson-McReynolds et al. 2006; Meher et al. 2019a; Supekar et al. 2004; Xu et al. 2004). The schematic representation of S protein and its S1/S2 cleavage site is shown in Fig. 9.

Class II Viral Fusion Proteins

Generally, class II fusion proteins do not maintain sequence homology, but their secondary and tertiary structures are conserved. Interestingly, the structures of the class II fusion proteins are different from class I fusion proteins (Kielian 2006; Lescar et al. 2001). Basically, the class II fusion proteins are extended finger like molecules containing three globular domains having mostly β-sheets structures (Fig. 10). The dimeric pre-fusion structures of class II fusion proteins form homotrimers in the post-fusion condition (Kielian 2006). The domain I of class II viral fusion protein is a β-barrel, which contains the N-terminus. The extension of two adjacent β-strands of domain I results in long finger-like structure called domain II. At the tip of domain II, a highly conserved hydrophobic fusion loop (represented as yellow diamond in Fig. 11) is present (Kielian 2006; Mas and Melero 2013). In between domain I and domain II, there exists a hinge region, which is flexible and allows different angles of flexibility between two domains (Kielian 2006; Mukhopadhyay et al. 2005). The domain III participates in the cell surface receptor recognition. Domain

III is terminated in a segment called "stem" and this stem connects domain III with transmembrane anchor (Harrison 2015). Different class II fusion proteins and location of fusion peptide and their fusogenic subunits are shown in Table 3 (Harrison 2015; White et al. 2008).

The class II viral fusion protein (such as E of flaviviruses and E1 for alphaviruses) forms 1:1 dimer with another chaperone protein (prM and pE2 for flaviviruses and alphaviruses, respectively). The initial step of the fusion process is the cleavage of the dimer by the signal proteases and furin in the trans-Golgi network (TGN). The monomeric fusion protein undergoes pH-induced conformational change in endosome. This results into exposure of fusion loop towards the target membrane for bridging the target and viral membranes (Guirakhoo et al. 1991; Harrison 2015; Lobigs and Garoff 1990). The structure of post-fusion homotrimer reveals that the fusion loops insert only marginally into the target membrane (Modis 2013). Few examples of class II viral fusion have been described in the subsequent sections.

Flavivirus Entry

The fusion protein for flavivirus entry is known as E protein, and is synthesized and folds co-translationally with another regulatory chaperone protein, prM (Kielian 2006). The fusion protein E binds the receptor leading to clathrin-mediated endocytosis. The acidic endosomal environment (pH 6) triggers the conformational change facilitating exposure of the fusion loop at the tip of domain II (Mancini et al. 2000; Modis 2013; Zhang et al. 2003). The domains I and II bend 30° with respect to each other resulting swing away of fusion loop from viral surface towards the target membrane. This domain movement releases the constraints executed by the close-packing of E on the viral membrane and hence allowing free diffusion of fusion protein E. Flaviviral E inserts as monomer to the target membrane and this membrane insertion promotes lateral reshuffling of E monomers to form trimer (Modis 2013). This is comparable with the pre-hairpin intermediate of class I fusion protein-mediated fusion process where the two different regions of each E protein are inserted into the two membranes to be fused. There is a flexible hinge region between domain I and domain II and at this stage there is rotation of 33° of Domain III with

Fig. 9 Schematic representation of S protein. Here FP represents fusion peptide, NHR and CHR represent N- and C-heptad regions, SP and TM represent signal peptide and transmembrane domain, respectively

Fig. 10 Schematic representation of stepwise fusion process by class II fusion protein
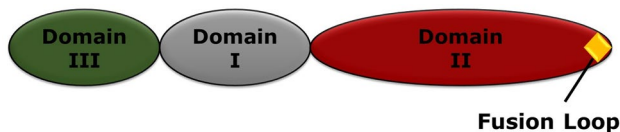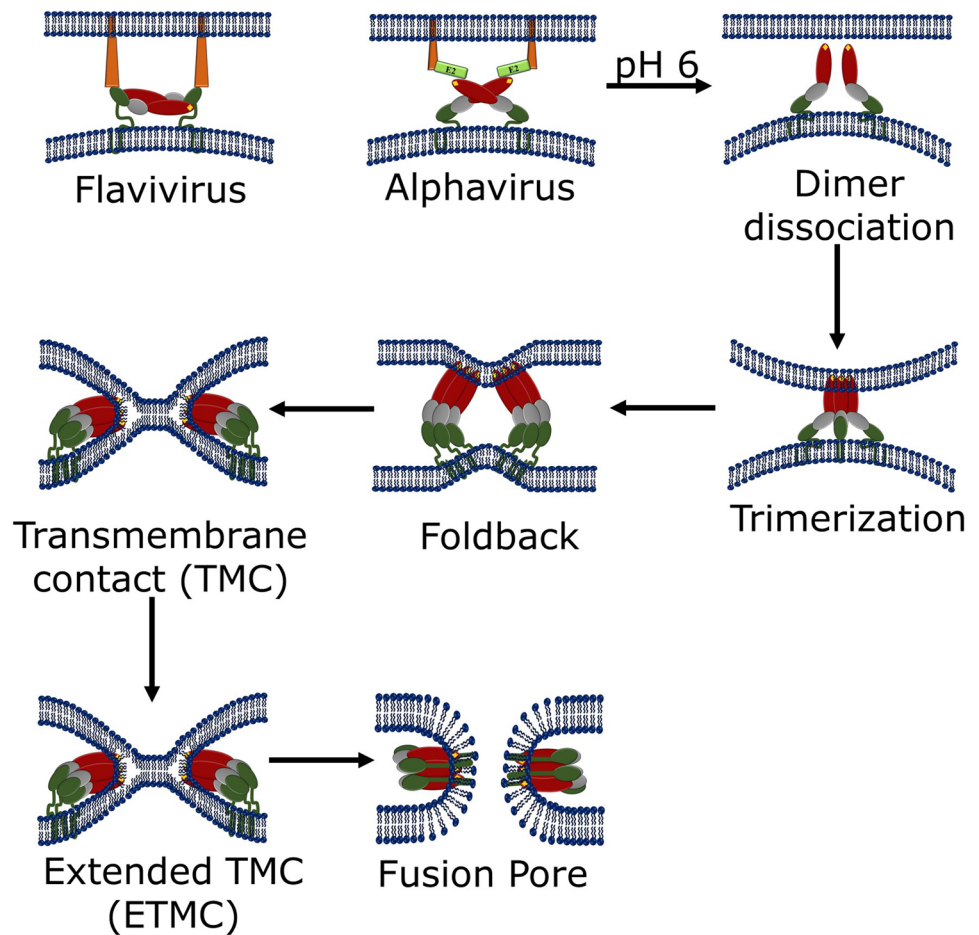

Fig. 11 Schematic representation of class II fusion protein. Here, grey, red, and green colours represent domains I, II, and III respectively. The fusion loop is shown by yellow diamond (Color figure online)

respect to Domain I, bringing the C-terminal stem region and fusion loop peptide (inserted into the target membrane) together. This refolding brings the two membranes together and thus drives fusion (White et al. 2008).

Alphavirus Entry

The fusion protein for alphavirus entry is known as E1 protein. It is being produced and folds co-translationally with another regulatory chaperone protein, p62 (Kielian 2006). The chaperone binds the receptor followed by internalization of the virus to the endosome. Upon acidic endosomal environment, the dissociation of the protector protein E2 occurs resulting in the release of constrains and exposure of the fusion loops. At this stage there is 15-degree hinge movement of domain II relative to domain I resulting swing towards nearest three-fold symmetry axis in virus causing trimerization with adjacent E1. Alphaviral E1 inserts as trimer to the target membrane (Modis 2013). The E1 protein

Table 3 Different class II fusion proteins

Class II virus family	Virus	Fusion protein	Fusion subunit	Fusion peptide location	Fusion pH
Flaviviridae	Dengue virus	E	E	Internal	Low
Alphaviridae	Semliki Forest Virus	E1	E1	Internal	Low
Bunyaviridae	Hantavirus	G_N/G_C (G_N/G_C)	G_C	Internal	Low

undergoes similar conformational rearrangement as flavivirus E protein, leading to fusion of viral and endosomal membranes (Mas and Melero 2013).

Class III Fusion Proteins

Class III fusion proteins have structural features similar to both class I and class II fusion proteins. In pre-fusion state they are trimeric, and mostly contain α-helical coiled-coil structure like class I fusion proteins. In addition, their fusion domains are similar to class II fusion proteins, the fusion loops are found at the tip of extended β-strands. Unlike class I fusion proteins, class III fusion proteins do not require proteolytic processing of either a protein precursor (as in class I) or a regulatory protein (as in class II) to become fusion active, rather they are expressed from individual m-RNA (Mas and Melero 2013; White et al. 2008). Class III fusion proteins are identified in vesicular stomatitis virus (VSV) glycoprotein G, herpes simplex virus glycoprotein B (gB), and baculoviruses. The pre-fusion and post-fusion structures of VSV-G have been studied but only the post-fusion structure of other two viruses are reported so far (Rey and Lok 2018). The schematic representation of VSV-G protein is shown in Fig. 12.

Vesicular Stomatitis Virus Entry

The structures of G protein of VSV in high-pH (pre-fusion) and low-pH (post-fusion) conformations indicate the structural and functional similarity with influenza virus hemagglutinin and/or flavivirus E (Harrison 2008). Upon acidification, a number of conformational changes occur in the fusion protein leading to the exposure of the fusion peptide to the viral membrane. This is followed by the bending of the C-terminus to the N-terminus, forming the six-helix bundle (6HB), and thereby bringing both membranes to the close apposition. The low pH-induced conformational changes are reversible in case of VSV-G. There is thermodynamic equilibrium between pre-fusion (high pH) and post-fusion (low pH) states, and this equilibrium shifts towards the post-fusion state at low pH (White et al. 2008). There is rotation of domain I and domain II with respect to domain IV and a little reshuffling of domain III occurs during transition from pre-fusion to post-fusion conformation. This transition is
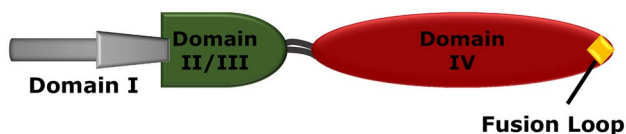
very similar to HA2 refolding as in case of influenza. The rotation of domain I and domain II, and folding of C-terminal can be correlated to the central coiled-coil extension in case of influenza (Harrison 2015).

## Class I Viral Entry Inhibitors

Peptide-Based Inhibitors

Generally, the peptide-based inhibitors prevent the formation of six-helix bundle (6HB), which is a trademark for the class I viral fusion. Therefore, most of the peptide inhibitors have been designed by mimicking the sequence of NHR and CHR, so that the inhibitor molecule interacts with the complementary helix, and blocks the 6HB formation. The discovery of Enfuvirtide (T20) to inhibit entry of HIV gathers the momentum for the development of peptide-based fusion inhibitors. As a result, several peptide-based fusion inhibitors have been developed in last couple of decades with improved stability as well as better pharmacokinetics to block viral entry.

Inhibiting HIV Entry

Peptide-based fusion inhibitors for HIV are classified into two categories viz., C-peptides and N-peptides. C-peptides mimics the C-terminal heptad region (or HR2) sequence to target the NHR (or HR1) of gp41, whereas N-peptides are developed from N-terminal heptad region to target the CHR region of gp41 (Fig. 13) to block the formation of 6HB. Both the C- and N-peptides have potential to inhibit HIV entry, but poor solubility of N-peptides (as they are hydrophobic in nature) makes them less potent as inhibitors (Baldwin et al. 2003; Lu et al. 1995; Root et al. 2001). T20, T1249, Cp32M, Sifuvirtide, and T2635 are some examples of C-peptides and IQN17, IQN23, and NCCG-gp41 are some examples of N-peptides.

T20 (also known as Enfuvirtide or Fuzen) is the first peptide-based fusion inhibitor developed for inhibiting HIV entry. It is a 36-amino acid residue peptide derived from α-helical carboxy terminal heptad domain (CHR) of gp41. It was designed to target the complimentary NHR, thereby, preventing 6HB formation and stops the progress of the subsequent steps of fusion (Wild et al. 1994a, b). The poor bioavailability and lower pharmacokinetic efficacy (plasma half-life of about 4 h) lead to requirement of higher doses of T20 (Berkhout et al. 2012; Kilby et al. 1998, 2002; Lalezari et al. 2003; Stocker et al. 2006; Zhang et al. 2002). T1249 is another peptide, designed based on CHR sequence of gp41 that inhibits HIV entry. T1249 is a 39-amino acid long peptide, which is designed by extending the N-terminal of T20. Interestingly, T1249 has better efficacy than T20 and

Fig. 12 Schematic representation of VSV glycoprotein. The fusion loop is shown by a diamond on Domain IV
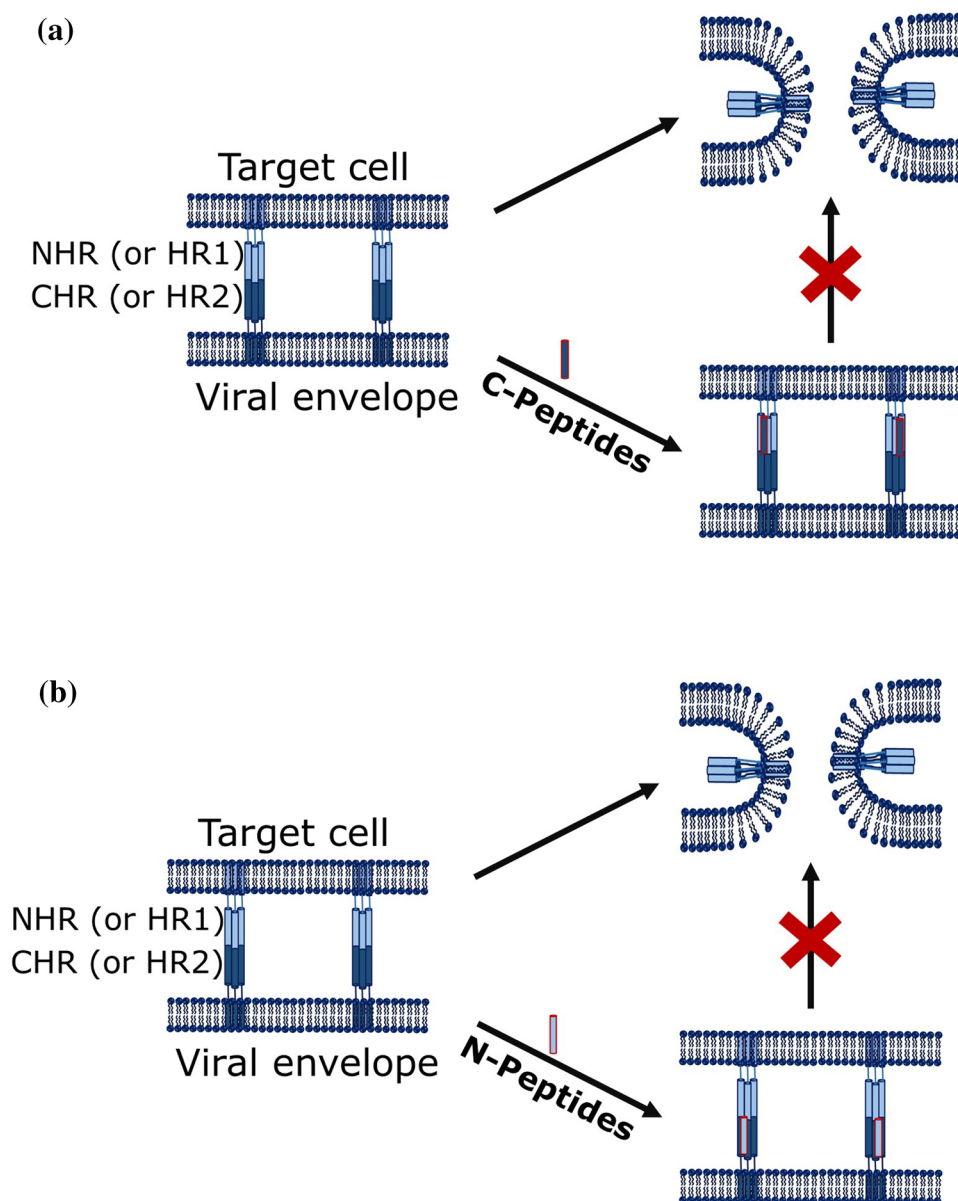
is sensitive against T20-resistant HIV-1 variants (Berkhout et al. 2012; Lalezari et al. 2005).

The low serum half-life of T20 and T1249 necessitates further development of peptide-based inhibitors with better serum half-life. The next-generation inhibitors such as Cp32M, Sifuvirtide, and T2635 are having more helical structure than that of T20 and T1249, and showed better binding with the NHR. In addition, these peptides demonstrated better stability as well as higher serum half-life. Moreover, these peptides are active against virus variants that are resistant to T20 and T1249 (Eggink et al. 2009, 2011; Wang et al. 2009c; Yao et al. 2012).

Work from several groups has shown that CHR of gp41 also acts as a target for HIV entry inhibition. N-peptides (based on NHR) are weak inhibitors (with IC_{50} values in

micromolar range) as compared to C-peptides, which show IC_{50} values in nanomolar range. This could be attributed to the fact that N-peptides are hydrophobic, aggregation prone, and poorly soluble in aqueous medium. Efforts have been made to solve this problem, and to develop inhibitors with better IC_{50} values. The first effort was to develop a chimeric peptide with higher solubility and higher affinity towards CHR. This has been achieved by linking NHR to a GCN4-based trimerization motif. Peptides such as IQN17 (trimeric coiled-coil GCN4-pI$_Q$I fused with N-terminus of a 17-residue NHR (N17)), IQN23 (trimeric coiled-coil GCN4-pI$_Q$I fused with N-terminus of a 23-residue NHR (N23)) were developed to bind CHR with higher affinity (Baldwin et al. 2003; Berkhout et al. 2012; Bianchi et al. 2005; Eckert and Kim 2001; Eckert et al. 1999). Another chimeric N-peptide

N_{CCG}-gp41 was derived from the NHR of gp41 that interacts with the CHR. The trimer of the chimeric peptide forms a stable complex with the CHR via engineered intermolecular disulphide bond, thereby demonstrates IC_{50} values in nano molar range (Baldwin et al. 2003; Berkhout et al. 2012; Eckert and Kim 2001; Louis et al. 2001). A preformed 5-helix peptide containing three NHR helices and two CHR are joined by short peptide linkers to form a 5-HB. This 5-HB interacts with free CHR of gp41 to form the 6HB in the pre-fusion state with nanomolar IC_{50} value, thereby restricting gp41 to form the post-fusion conformation (Baldwin et al. 2003; Berkhout et al. 2012; Root et al. 2001). There are several mutated NHR-based peptides that demonstrate anti-HIV activity. $N36^{Mut(e,g)}$ is one such example that shows nanomolar IC_{50} value. However, this peptide does not affect the 6HB formation directly, rather disrupts the trimeric NHR coiled-coil structure of the pre-hairpin intermediate (Berkhout et al. 2012; Bewley et al. 2002).

Conjugating cholesterol to the inhibitory peptide enhances the membrane partitioning of the peptide to the model membrane as well as in human blood cell, thereby suggesting better inhibitory effect of the cholesterol-conjugated peptide than the parent peptide (Hollmann et al. 2013; Ingallinella et al. 2009). In addition, the cholesterol-conjugated peptides show improved pharmacokinetics (Pessi 2015). Artificial peptides conjugated with cholesterol and pocket-specific small molecules demonstrate improved antiviral activity, even against the strains that are resistant to enfuvirtide (Wang et al. 2014).

Several fusion inhibitors have been designed to inhibit the interaction between gp120 and the host cell receptor, CD4 as the interaction between CD4 and gp120 is crucial for the docking of the virus on the cell surface. Peptide 12p1 (RINNIPWSEAMM) inhibits the interaction between CD4 and gp120 from three different HIV strains (Ferrer and Harrison 1999). This indicates that 12p1 binds a conserved site on gp120, and demonstrates broad-spectrum viral entry against multiple strains. The conserved regions of gp120 have also been utilized to develop peptide and protein-based inhibitors to block the interaction between gp120 and CD4 to avoid the viral entry, which has been extensively reviewed by Pu et al. (2019).

Inhibiting Influenza Entry

Unlike HIV, there are several challenges in developing peptide-based inhibitors for influenza virus. Influenza virus fuses in the presence of acidic endosomal pH. Therefore, the delivery of the inhibitory peptide to the endosome makes the problem more difficult. In addition, stability of the peptide in lower endosomal pH is an added problem (Yang et al. 2013).

Tagging cholesterol with the peptide enhances the partitioning of the peptide to the endosome and reveals anti-influenza properties. Peptides derived from the influenza hemagglutinin (HA) are generally incompetent to inhibit viral entry. However, it has been found that peptides, with a GSGSG linker, and the cholesterol group at C-terminal are potent inhibitors for influenza entry. These peptides trap HA in a transient intermediate state and prevent the subsequent steps to proceed for completion of fusion (Lee et al. 2011). Again, cholesterol-conjugated peptides such as S-KKWK and its derivatives (cholesterol is conjugated to the N-terminus of the short peptide KKWK) exhibited potent and broad anti-influenza A virus (IAV) activity in vitro and in vivo. S-KKWK binds to a conserved hydrophobic pocket on HA2 subunit, thereby preventing the six-helix bundle formation, and required conformational rearrangement of the HA2 subunit, which is an essential step for membrane fusion (Lin et al. 2017).

Generally, peptide inhibitors bind to the highly conserved stem epitope to block the low pH-induced conformational rearrangements required for membrane fusion. A library of cyclic peptides bind to this stem epitope, and inhibit influenza entry by stabilizing the pre-fusion conformation as shown in Fig. 14 (Kadam et al. 2017).

Inhibiting Paramyxovirus Entry

Like HIV and influenza, 6HB is the common feature of the entry of paramyxoviruses. This common mechanism of viral entry is suggestive of a common target to inhibit viral membrane fusion. Indeed, many potent inhibitors are
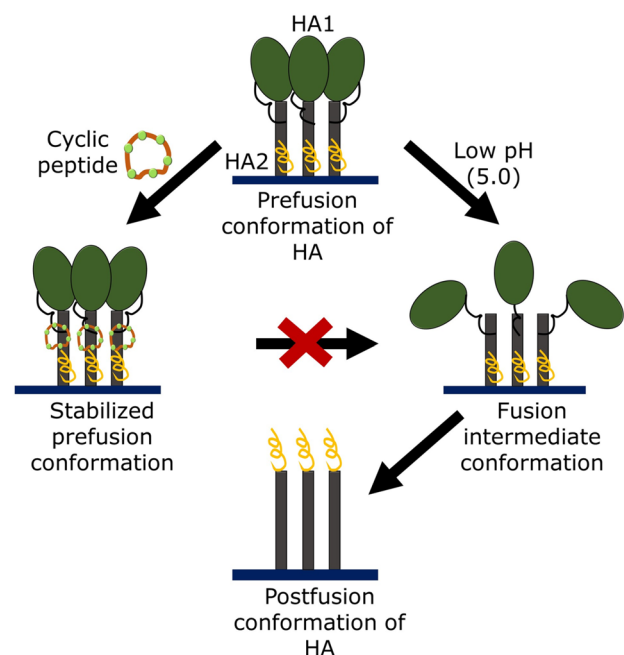

Fig. 14 Schematic representation of mechanism of HA conformational change inhibition by cyclic peptides

designed and developed utilizing the heptad region (HR) of many paramyxoviruses' fusion proteins. Peptides derived from conserved heptad repeat (HR) regions of paramyxovirus fusion (F) proteins inhibit viral fusion by disrupting the formation of 6HB (Fig. 13), which is essential for driving the fusion reaction towards completion (Porotto et al. 2009).

Peptides derived from CHR regions of many paramyxoviruses, including Sendai virus, measles virus, Newcastle disease virus (NDV), respiratory syncytial virus (RSV), simian virus 5 (SV5), Hendra virus (HeV), and Nipah virus (NiV), can inhibit the entry of the homologous viruses (Joshi et al. 1998; Lambert et al. 1996; Porotto et al. 2006, 2010; Rapaport et al. 1995; Wild and Buckland 1997; Yao and Compans 1996; Young et al. 1997, 1999). Peptides such as WT, G12A, Ran, SV-163, and SV-473 were derived from the fusion peptide, and the two HR domains of Sendai virus F protein. It has been reported that SV-473 derived from the CHR inhibits fusion between virus with human red blood cells, though the mechanism is yet to be elucidated (Rapaport et al. 1995). Peptides synthesized from CHR from the F proteins of human parainfluenza virus type 2 (PI2) and type 3 (PI3) showed potential to inhibit virus-induced cell fusion, and control the spread of viral infection. In addition, the longer peptides (34 amino acids for PI2F or 35 amino acids for PI3F), which are extended from CHR to the transmembrane domain showed broad inhibition of cell fusion (Yao and Compans 1996).

Human metapneumovirus (hMPV) was discovered in 2001 (van den Hoogen et al. 2001). Synthetic peptides from NHR and CHR were induced to form a thermostable (melting temperature, 90 °C) helical structure, and showed to form hexameric coiled-coil structure. The peptide synthesized from NHR region demonstrates significant inhibitory effect against hMPV entry with around 50 nM IC_{50} value (Miller et al. 2007). CHR region-derived peptides of the F protein of HPIV3 also display inhibitory effects against HPIV and HeV/NiV entry (Porotto et al. 2006, 2010). Interestingly, the conjugation of cholesterol to the CHR-derived peptide localizes at the site of fusion and deactivates the fusion protein, thereby, enhancing the inhibitory efficiency of the peptide. The cholesterol-tagged peptides create a protective antiviral shield by targeting the fusion protein directly at the site of fusion, therefore elevate the inhibitory potential against paramyxoviruses (Porotto et al. 2010).

Inhibiting SARS-CoV Entry

The inhibitory properties of synthetic peptides derived from NHR and CHR were also tested for SARS-CoV. The CHR peptide was shown to be a potent inhibitor of mouse hepatitis virus (MHV) entry into the cell, confirmed from biological assays. CHR of MHV binds to the NHR region of the spike protein, and inhibits the subsequent interaction with native CHR, consequently, blocks the entry of SARS-CoV (Bosch et al. 2003). Interestingly, the NHR-derived peptides do not show any inhibitory effect. However, CHR peptides of SARS-CoV have reduced efficacy compared to the CHR peptides of the murine coronavirus mouse hepatitis virus (Bosch et al. 2004). Another CHR-based peptide CP-1 having low alpha-helicity also inhibits virus infection in the micromolar range. It has been demonstrated that the equimolar mixture of CP-1 and NP-1 (NHR-based peptide) forms a 6HB, which indicates that the CP-1 is capable to form 6HB with the native NHR, thereby, impeding the formation of post-fusion structure of the spike protein resulting in fusion inhibition (Liu et al. 2004).

A CHR-derived peptide EK1 targets the NHR of human coronavirus (HCoV) S protein, which proved to be effective in inhibiting infection of five different HCoVs, including SARS-CoV and MERS-CoV, and three SARS-related CoVs (SARS-CoVs) (Xia et al. 2019). In addition, the same group have reported that EK1 peptide is effective against SARS-CoV-2 S protein-mediated membrane fusion (Xia et al. 2020b). Again, cholesterol conjugation of EK1 (EK1C4) increases the inhibitory efficacy (Xia et al. 2020a). A higher helical stability of NHR as well as efficient interaction with CHR site to form a stable 6HB structure, which leads to enhanced fusion capacity, was found in the fusion protein of SARS-CoV-2. Modified HR2 sequence-derived peptide IPB01 with a cholesterol group, known as IPB02 has high ability in inhibiting SARS-CoV-2 as well as SARS-CoV (Zhu et al. 2020).

Some synthetic peptides outside the spike protein heptad repeat regions show antiviral activities. Zheng et al. have hypothesized that agents that interfered with, or bound competitively with some specific protein domains would disrupt the function of S protein resulting inhibition of SARS-CoV infections. They synthesized four derivative peptides of S protein fragments that showed antiviral activities in a cell line. In addition, two of these peptides reduced SARS-CoV infectivity over 10,000-fold through pre-incubation. Again, the infections were completely inhibited in the presence of three peptides (Zheng et al. 2005).

The binding of SARS-CoV spike (S) protein to the receptor i.e. cellular angiotensin-converting enzyme 2 (ACE2) is the first step in SARS-CoV infection. Therefore, peptides which block spike protein and ACE2 interaction may inhibit SARS-CoV entry. Small peptides derived from S protein such as SP-4 (residues 192–203), SP-8 (residues 483–494), and SP-10 (residues 668–679) significantly blocked the interaction between S protein and ACE2 with IC_{50} values of 4.30, 6.99, and 1.88 nM, respectively. Using alanine scanning mutagenesis, Han et al. suggested that charged amino acids between residues 22 and 57 were crucial for inhibitory efficiency, and two peptides (residues 22–44 and 22–57) derived from ACE2 showed antiviral activity with IC_{50}

values of 50 and 6 μM, respectively. In addition, a peptide containing two segments of ACE2 i.e. residues 22–44 and 351–357 linked together by glycine revealed a potent antiviral activity with IC_{50} of about 0.1 μM (Han et al. 2006). This has further shown that the serine protease TMPRSS2 plays a pivotal role in priming the S protein, which is important for the entry of SARS-CoV (Glowacka et al. 2011; Matsuyama et al. 2010; Shulla et al. 2011). Therefore, it has been postulated that the inhibition of TMPRSS2 could lead to inhibit the entry of SARS-CoV (Kawase et al. 2012). Camostat mesylate, an inhibitor for TMPRSS2 partially blocks the SARS-CoV-2 entry, however, application of camostat mesylate with E-64d (an inhibitor for CatB/L) completely inhibits the viral entry (Hoffmann et al. 2020). This result indicates that the CatB/L, cysteine protease, also plays an important role in viral entry, and this protein could also be considered as a potential target for development of SARS-CoV-2 entry inhibitors.

Small-Molecule Inhibitors

Even though peptide-based fusion inhibitors have taken the centre stage, however, they have several limitations. Therefore, significant efforts have been made towards developing small-molecule fusion inhibitors.

Inhibiting HIV Entry

The surface protein (gp120), transmembrane protein (gp41), receptor (CD4), and chemokine co-receptor (CCR5 or CXCR4) are actively involved in HIV entry, therefore, these proteins are targets for developing HIV inhibitors. The interaction between the CD4 receptor and surface protein gp120 is the preliminary step of HIV entry. Therefore, quite a few small molecules target gp120 and inhibit HIV entry by blocking the interaction between gp120 and CD4. Small molecules such as BMS-378806 (Chen et al. 2005), BMS-488043 (Ho et al. 2006), NBD-556, NBD-557 (Zhao et al. 2005), and NBD-09027 (Curreli et al. 2012) are coming in this category.

Small-molecule inhibitors such as 5M030, 5M038, and 5M041 (Frey et al. 2006), ADS-J1 (Wang et al. 2009a), a number of N-substituted pyrrole derivatives (NB-2, NB-64, A12, GLS-22, and GLS-23) (Jiang et al. 2004; Liu et al. 2008; Wang et al. 2010), furan-based molecules (NB-293, NB-206) (Katritzky et al. 2009), and indole-based compounds (Balogh et al. 2009; Zhou et al. 2011, 2014) target the deep hydrophobic pocket on the NHR trimer core, and thereby inhibit the formation of 6HB and block membrane fusion (He et al. 2007, 2008; Liu et al. 2007). The binding site of CD4 is exposed at the native state of gp120, while the CHR-binding sites in gp41 are exposed only after the completion of binding of gp120 to the receptor CD4. However,

this exposure is transient and lasts for few seconds to minutes. But, the CHR-binding sites of gp41, particularly the hydrophobic pocket region, are more conserved as compared to CD4 binding site in gp120. Consequently, small-molecule inhibitors targeting conserved gp41 pocket region are expected to have broader antiviral spectrum than those targeting gp120 (Lu et al. 2016).

Chemokine receptors are important cofactor for HIV entry and act as an important target for antiviral small molecule. Significant efforts have been given to develop molecules that block the interaction between gp120 and chemokine receptor, and control the expression of chemokine receptor in the target cells to inhibit HIV entry. Two positively charged peptides, ALX40-4C and T22, and a bicyclam compound called AMD3100 bind directly to CXCR4, thereby, blocking its interaction with gp120 and inhibit entry of HIV (Chan and Kim 1998). Maraviroc is a US FDA and European Medicines Agency (EMA)-approved novel small molecule that blocks gp120 interaction with CCR5 with potent anti-HIV activity (Xu et al. 2014). Ibalizumab (Trogarzo), a monoclonal antibody binds to the CD4 receptor and blocks its interaction with gp120, and inhibits HIV entry which was also approved by FDA (Beccari et al. 2019).

Inhibiting Influenza Entry

Two molecules, MBX2329 and MBX2546, with amino alkyl phenol ether and sulfonamide scaffolds, respectively, inhibit entry of influenza virus with IC_{50} value of 0.3–5.9 μM. They also inhibit a wide spectrum of influenza A viruses, which includes the 2009 pandemic influenza virus A/H1N1/2009, highly pathogenic avian influenza (HPAI) virus A/H5N1, and oseltamivir-resistant A/H1N1 strains. Both MBX2329 and MBX2546 bind to stem region of the HA trimer and inhibit HA-mediated fusion (Basu et al. 2014).

Generally, HA inhibitors are of two types, the first type blocks the interaction of HA1 with the neuraminic acid (Neu) receptors present on the surface of the target host cells, and the other disturbs the HA2-mediated fusion process by inhibiting the low pH-induced conformational change of HA2. Compounds such as BMY-27709 (Luo et al. 1996, 1997; Wu et al. 2017) and stachyflin (Wu et al. 2017; Yagi et al. 1999; Yoshimoto et al. 1999, 2000) come under the first category.

The indole alkaloids such as Neoechinulin B bind to the influenza envelope HA and disrupt its interaction with the sialic acid receptor, and the attachment of viruses to the target host cells (Chen et al. 2015). Thiazolides such as Nitazoxanide blocks the maturation of viral HA and hence active against influenza viruses (Ashton et al. 2010; Belardo et al. 2015; Haffizulla et al. 2014). Three different saponins with 3-O-β-chacotriosyl residue showed significant

inhibitory activity for H5N1 entry into cells with IC_{50} value of 7.22–9.25 mM (Song et al. 2009).

Inhibiting Paramyxovirus Entry

Measles virus (MV) uses both CD46 and CD150 as cellular receptors (de Vries et al. 2010). Plemper et al. suggested that a microdomain in the Measles virus (MV) fusion protein (F protein) is structurally conserved in the paramyxovirus family (Plemper et al. 2003). Therefore, this conserved microdomain is an important target for the development of antiviral molecules. The first compound of this kind was 5-amino-2-benzylbenzoxazole (B17/OX-1), which is having an IC_{50} value of 50 μM against MV. OX-1 also prevents the early step of lipid mixing, or hemifusion formation (Plemper et al. 2004). An acyclic variant of OX-1 (4-nitro-2-phenylaceylaminobenzamide, AS-48) demonstrated approximately 10 to 15-fold increase in antiviral activity (Plemper et al. 2005).

Inhibiting SARS-CoV Entry

Simmons et al. proposed a three-step process for SARS-CoV infection. Receptor binding is the first step followed by conformational change of spike S protein, and cathepsin L proteolysis within the endosome. They targeted the last step for inhibition of SARS-CoV replication by small molecule. Compound MDL28170 inhibited the infection with IC_{50} value of 2.5 nM (Simmons et al. 2005). Using the molecule fingerprint searching method and molecular dynamics simulation Wang et al. suggested that a compound (MOL376) derived from a Chinese medicine herb with the therapeutic efficacy on the human body suitably binds to the binding pocket of cathepsin L and hence it might become a capable inhibitor for SARS-CoV infection (Wang et al. 2007).

Based on the concept of chemical genetics, Kao et al. identified 104 compounds with anti-SARS-CoV activity. Out of these, 2 compounds interact with the SARS-CoV main protease (M^{pro}), 7 interact with helicase (Hel), and 18 target spike (S) protein-ACE2-mediated viral entry. Three selected compounds that showed better efficacies are MP576, HE602, and VE607, which target the SARS-CoV main protease, helicase, and ACE2-mediated viral entry, respectively, with IC_{50} value in micromolar range (Kao et al. 2004).

Class II Viral Entry Inhibition

The control of class II viral infections by vaccination is still not efficient. For example, CYD-TDV (chimeric yellow fever virus-tetravalent dengue vaccine) developed by Sanofi Pasteur was first dengue vaccine, and got the licence in December 2015 for use in individuals between 9 and 45 years of age, living in endemic areas. However, its average efficiency was 50%, 39%, 75%, and 77% for DENV-1,

DENV-2, DENV-3, and DENV-4, respectively (Capeding et al. 2014; Chew et al. 2017; Villar et al. 2015).

Several strategies have been taken to develop inhibitors for class II viruses. One such approach is similar to the antiviral compound against HIV. The peptides derived from stem region of the fusion core may bind to the trimeric core of the post-fusion conformations and disrupt the final folding of the fusion protein which is essential for membrane fusion (Kielian 2006; Modis 2013). Although the exact inhibition mechanism is unknown, it is believed that DN59 (a peptide corresponding to stem domain of DENV) and WN83 (designed from overlapping regions of E protein domain I/II junction) may interfere with intramolecular interaction between stem and other portions of class II viral fusion protein. It has been reported that DN59 is active against both dengue and West Nile virus, but WN83 is only active against West Nile virus (Hrobowski et al. 2005).

The other strategy to inhibit class II viral infection is to target the hinge region of the fusion protein. Hinge region is a flexible region which undergoes conformational change during virus maturation and conversion from neutral to acidic pH to induce trimer formation. In flavivirus, the pH-dependent fusion is also affected by mutation in the hinge region, while in alphaviruses the cholesterol dependency was reduced by mutation in the hinge region. A hydrophobic pocket was identified in the dengue virus E, and its crystal structure at neutral pH suggests that a detergent molecule *n*-octyl-β-glucoside is present in this pocket. Blocking this pocket to reduce the hinge flexibility may inhibit class II fusion protein-induced membrane fusion (Kielian 2006; Modis 2013). High-throughput docking with this hydrophobic pocket led to the identification of compound 6 (Wang et al. 2009b) and compound 1662G07 (Schmidt et al. 2012) to block dengue virus entry. It was reported that compound 6 had an average 50% effective concentration of 119 nM against dengue virus serotype 2 in human cell line (Wang et al. 2009b). Poh et al. identified a small molecule, NITD448, which binds to the hydrophobic pocket and demonstrates antiviral efficiency with IC_{50} of 6.5 μM (Poh et al. 2009).

Class III Viral Entry Inhibition

IFITM3 and Tetherin are located on the plasma membrane. Tetherin restricts infections by retroviruses and filoviruses whereas IFITM3 inhibits influenza virus and flavivirus infection. In addition, Weidner et al. reported that both IFITM3 and Tetherin inhibit VSV infections. IFITM3 blocks the infection after endocytosis during or before primary transcription, while Tetherin inhibits the release of virion particles from the infected cells. Again, the N-terminal 21 amino acids residue and C-terminal transmembrane region of IFITM3 are essential for its antiviral function (Weidner

et al. 2010). The lysosomal inhibitor chloroquine also inhibits VSV infection to 50%, when added after 1.5 h of infection (Miller and Lenard 1980).

Broad-Spectrum Antiviral Agents: Need of the Hour

Viral infections are major pandemic threats in all time to the human civilization. Emerging infectious diseases pose a constant menace to global health and economy. Vaccination is the only way to get away from the cruelty of these emerging and re-emerging viruses, however, the vaccines are highly specific to a particular virus even to a particular strain. The traditional strategy of 'one bug-one drug' in antiviral drug development is inadequate for responding to an increasing diversity of viruses that cause deadly diseases in humans (Vigant et al. 2015). Till date more than 20 million people are suffering from coronavirus disease 2019 (COVID-19) that claimed more than half a million lives, and stalled the livelihood of uncountable. Therefore, the development of broad-spectrum antiviral agent is the need of the hour to combat continuously emerging viral diseases. As viruses use their own fusion machinery, it differs from virus to virus, which makes it challenging to develop broad-spectrum antiviral agent by targeting the fusion machine of the virus. However, the fusion process follows some common pathway in terms of evolution of membrane structure and conformation (Jahn et al. 2003). This prompted us to develop certain class of peptides or small molecules that inhibit the structural changes in the membrane that is essential for fusion process. In search of peptide-based broad-spectrum antiviral agent by targeting the membrane, we recently developed coronin 1-derived tryptophan–aspartic acid (WD)-containing peptides, which show significant inhibition of polyethylene glycol (PEG)-mediated fusion of model membranes (Pattnaik and Chakraborty 2018, 2019).

Pathogenic mycobacteria survive within macrophages for years by evading lysosomal degradation (Jayachandran et al. 2007; Pieters 2008). In addition to different biochemical processes, the role of phagosomal coat protein coronin 1 cannot be undermined. Coronin 1 is recruited to the phagosomal membranes only when live mycobacteria are present in the phagosome (Gruenberg and Stenmark 2004; Pieters et al. 2013). This clearly suggests the putative function of coronin 1 in inhibiting fusion between phagosome and lysosome. Interestingly, in coronin 1, the positions of tryptophan and aspartic acid are conserved across the species (Eckert et al. 2011). Therefore, the inhibitory effect of tryptophan–aspartic acid (WD)-containing peptides derived from coronin 1 were investigated. It has been found that two peptides, TG-23 and GL-22, substantially reduce the PEG-mediated fusion of small unilamellar vesicles by inhibiting

water penetration into the membrane, which is important for membrane fusion (Pattnaik and Chakraborty 2018). Though these results are preliminary, but displays promise for the development of broad-spectrum antiviral agents that can target and modify the membrane physical properties to avoid fusion between virus and the host cell.

Concluding Remark and Future Perspectives

It is not very difficult to identify emerging viral diseases and the genetic fingerprint of viral fusion proteins, however, the discovery of vaccine or drugs against these viruses is extremely time consuming. Moreover, some viral infections such as the ongoing COVID-19 pandemic spread at an extraordinary rate, which overtakes the development of suitable therapies. Thus, the traditional approach of 'one bug-one drug' is not sufficient for combating emerging viral diseases. Therefore, in our opinion, more proactive research on broad-spectrum antiviral agents might be helpful to deal with future pandemic situations.

Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

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