Broad-spectrum antivirals against viral fusion

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Abstract | Effective antivirals have been developed against specific viruses, such as HIV, Hepatitis C virus and influenza virus. This 'one bug–one drug' approach to antiviral drug development can be successful, but it may be inadequate for responding to an increasing diversity of viruses that cause significant diseases in humans. The majority of viral pathogens that cause emerging and re-emerging infectious diseases are membrane-enveloped viruses, which require the fusion of viral and cell membranes for virus entry. Therefore, antivirals that target the membrane fusion process represent new paradigms for broad-spectrum antiviral discovery. In this Review, we discuss the mechanisms responsible for the fusion between virus and cell membranes and explore how broad-spectrum antivirals target this process to prevent virus entry.

Zoonoses
Infectious diseases that are transmitted between a reservoir and a non-reservoir host. This usually refers to diseases caused by pathogens that jump from non-human animals to humans.

Broad-spectrum antivirals
For the purposes of this Review: antivirals that act against at least two distinct viruses from two different families via some common mechanism of action.

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Emerging infectious diseases pose a constant threat to global health and the global economy. The vast majority of these diseases are zoonoses that occur when human and environmental factors force the unintended overlap of previously distinct ecological niches. This overlap increases the chances for viruses to jump between host species and/or to generate new crossover species. In most cases, zoonotic viruses, being ill adapted to the new human host, are highly pathogenic, and infections with these viruses lead to rapidly progressing, severe diseases with high fatality rates[1]. Filoviruses (such as Ebola virus (EBOV) and Marburg virus), henipaviruses (such as Hendra virus and Nipah virus (NiV)) and coronaviruses (such as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)) are the most lethal examples of recent zoonoses[2–5]. In addition, viruses in the families *Arenaviridae* (such as Junin virus and Lassa virus), *Bunyaviridae* (including diverse hantaviruses and Rift Valley fever virus (RVFV)) and *Flaviviridae* (such as Dengue virus (DENV) and West Nile virus (WNV)) may represent an even greater threat in the future, as global climate change increases the geographical range of their animal or arthropod hosts[6,7].

HIV is arguably the most obvious example of a zoonosis that has disrupted global health and the economy. Public health interventions combined with concerted efforts to develop specific drugs against HIV have yielded laudable successes. The development of specific drugs that target different aspects of the HIV life cycle has resulted in potent antiretroviral combination drug therapies that have saved innumerable lives. The success of antiretroviral drug development is now being recapitulated by the latest generation of potent disease-eradicating anti-Hepatitis C virus (HCV) drugs that target specific HCV proteins. These successes showcase the power of the traditional pathogen-specific drug development paradigm that is so well practised by the pharmaceutical industry.

Despite the many successes of the 'one bug–one drug' approach to antiviral drug development, this strategy may be inadequate for responding to an increasing diversity of viruses that cause significant diseases in humans. For example, industrial antiviral drug development is understandably driven by economic incentives, which means that, in 2015, the list of clinically active antivirals approved by the US FDA or the European Medicines Agency (EMA) will be dominated by anti-HIV (~35), anti-HCV (~6), anti-herpesvirus (~7) and anti-influenza virus (~3) drugs. Given the increasing number of emerging and re-emerging viral zoonoses, and the close to half a million unknown mammalian viruses that are predicted to be present in wildlife reservoirs and remain to be discovered[8], the traditional virus-specific paradigm of antiviral drug development is unlikely to result in timely and effective therapies against these numerous pathogens that cause rare but lethal infections. These observations underscore the need for broad-spectrum antivirals that act on multiple viruses by targeting some commonality in their life cycle rather than on specific viral proteins. Importantly, ribavirin is currently the only broad-spectrum antiviral that is purportedly effective

Box 1 | **Classical inhibitors of viral fusion**

Blocking cognate virus–receptor interactions is the most obvious antiviral paradigm for entry inhibitors. This strategy is exemplified by maraviroc (Selzentry; Pfizer), a small molecule that inhibits entry of HIV-1 isolates which use CC-chemokine receptor 5 (CCR5) as a co-receptor for virus entry[27]. However, the development of receptor-specific entry inhibitors for the vast majority of viral pathogens is limited by the identification of the actual receptors but also by economic incentives. Furthermore, the broad diversity of viral receptors rules out blocking virus–receptor interactions as a broad-spectrum antiviral strategy.

In addition to specific host cell receptors, an increasing number of ancillary host cell molecules have been highlighted as enabling virus entry. For example, many viruses use glycosaminoglycans, sialic acid, glycosphingolipids, and/or lectins for docking onto and concentration on the surface of target cells in order to facilitate virus entry and invasion. These relatively nonspecific interactions can play crucial parts in virus entry or release, and some appear to be more broadly shared between distinct viruses, thus representing candidate targets for broad-spectrum antivirals.

Sialic acid analogues such as zanamivir and oseltamivir, which act as viral neuraminidase inhibitors, prevent sialic acid cleavage and virion release, and are technically not virus entry inhibitors. Nonetheless, they do reduce lung viral load and have been the mainstay of influenza treatment, despite there being a low barrier to resistance[91]. However, the removal of cell surface sialic acid from lung epithelial cells seems to be a viable broad-spectrum antiviral strategy against parainfluenza viruses and influenza viruses, which depend on sialic acid for entry. For example, DAS181 (Fludase; Ansun Biopharma) is an inhaled recombinant bacterial sialidase that has shown efficacy in Phase II clinical trials against influenza virus[92] and has been used successfully to treat severe parainfluenza virus disease in immunocompromised patients[93]. Importantly, as DAS181 is a host-targeted therapeutic, it should increase the barrier to the emergence of drug-resistant viral strains.

Decoy lectins (for example, cyanovirin-N and griffithsin) or glycomimetics, which compete with viruses for attachment to cell surfaces, have also been recently explored as antivirals and may even prove to be broad-spectrum antivirals[16], although their expected toxicity when administered systematically will probably limit their use to topical microbicides.

Finally, many viruses bind to cell surface receptors but only access the host cell cytosol somewhere along the endosomal trafficking pathway[11,95]. Thus, inhibitors of endosomal trafficking and/or acidification (for example, bafilomycin A1 and NH_4Cl) are commonly used in virology laboratories to study the mechanisms of entry of pH-dependent viruses *in vitro*. Reducing the acidification of endocytic vesicles counteracts the pH-triggered conformational changes in the viral fusion glycoproteins that are required for membrane fusion (see text). These inhibitors have the potential of being broad-spectrum antivirals, even towards non-enveloped viruses[11,95]. However, many chemicals that are fruitfully used in *in vitro* studies seem to be too cytotoxic to translate into effective treatments, although some have been evaluated as antivirals *in vivo* (TABLE 1). New compounds with distinct mechanisms of action may hold more promise. For instance, the recently identified aryl semicarabazone EGA was shown to inhibit trafficking from early to late endosomes rather than block endosomal acidification[96].

Membrane-enveloped viruses
Viruses that are surrounded by a lipid bilayer derived from host cell membranes. This envelope protects the virion content and harbours, among other cell-derived or virally encoded proteins, the viral proteins necessary for attachment, fusion and virus entry.

Membrane fusion
The process of merging two initially distinct lipid bilayers together into a single lipid bilayer.

against various RNA viruses, but there is no consensus on its mechanism of action (MOA)[9], perhaps because ribavirin has multiple modes of action and works in different ways for different viruses.

An ideal broad-spectrum antiviral is one that targets a common but essential viral function or property. Notably, the vast majority of the viral pathogens that are present in the Emerging Infectious Diseases/Pathogens list of the US National Institute of Allergy and Infectious Diseases (NIAID) — such as Smallpox virus, viral haemorrhagic fever viruses (arenaviruses, bunyaviruses, flaviviruses and filoviruses), henipaviruses and coronaviruses, and arboviruses causing encephalitides (such as WNV), to cite only a few — are membrane-enveloped viruses. In order for these viruses to replicate, they need to gain access to the metabolic resources within the host cell. This occurs through a process involving virus and host cell membrane fusion, which requires viral fusion proteins and takes place either directly at the cell surface or in some later endocytic compartment. Despite the diverse array of viral fusion proteins and their varied MOAs, there are underlying biophysical and biochemical features of the membrane fusion process that are common among enveloped viruses. Targeting these conserved features, such as the biophysical properties of the viral lipid membrane or host factors that are required for efficient membrane fusion, is emerging as a new paradigm for the development of broad-spectrum antivirals. Furthermore, these features are not encoded by the viral genome, suggesting that such antiviral strategies will also probably increase the barrier to resistance.

In this Review, we discuss the common features underlying the fusion between virus and cell membranes and present compounds that, owing to their ability to target different steps in this process, have the potential to be broad-spectrum antivirals. We also highlight emerging antivirals with well-established MOAs and with the potential for clinical development.

Virus entry and membrane fusion

The fusion between virus and cell membranes is a molecular choreography requiring cognate interactions between viral envelope proteins and host cell components at the interface between virus and cell membranes (reviewed in REFS 10–14). Here, we describe the core stages of the membrane fusion process and pinpoint the key commonalities that can be targeted by broad-spectrum antivirals which target membrane fusion and virus entry.

Viral attachment and receptor engagement. Viruses dock onto the cell membrane by engaging viral receptors that are located at the cell surface. Achieving such close proximity between enveloped viruses and cell membranes is not trivial, as lipid bilayers naturally repulse each other, mainly owing to electrostatic and hydration repulsion forces[15]. Thus, viruses have co-opted a wide array of auxiliary factors to facilitate cellular adsorption, including soluble factors (such as proteins of the transferrin family and lipoproteins) and cell surface-associated factors (such as lectins and glycosaminoglycans). Competition with these attachment cofactors and/or entry receptors has been the basis of classical inhibitors of virus entry (BOX 1).

Engagement of the proper receptor (or receptors) either results in direct viral fusion at the plasma membrane or induces various endocytic pathways that lead to fusion in some intracellular vesicular compartment most suited to the viral life cycle[10,11]. These intracellular compartments along the endosomal pathway are often processed by endosomal proteases and/or low pH conditions that trigger conformational changes in the viral fusion proteins, and these conformational changes are essential for membrane fusion. By contrast, viruses that fuse directly at the cell surface often rely on host cellular or secreted proteases — secreted from the producing cell

Table 1 | **Examples of potentially broad-spectrum antivirals targeting events in the entry cascade of enveloped viruses**

Target step in entry	Molecular target	Drug examples	Refs
Viral protein maturation	Cellular proteases	Cathepsin B and cathepsin L inhibitors*‡	16
Nonspecific interactions	Glycosaminoglycans	Carrageenan§‖, surfen*, SALPs* and AVPs	16,56,57,94
	Sialic acid	Zanamivir¶, oseltamivir¶ and sialidases	91
	Lectins and the carbohydrate portions of glycoproteins	Cyanovirin-N§, griffithsin§# and AVPs	16,56,57
	Envelope lipids (for example, phosphatidylserine)	Bavituximab§*‡, dominant-negative MFG-E8** and AVPs	56,57,84,97
Endocytosis	Trafficking pathway components‡‡	Chlorpromazine‡, cytochalasin B‡ and EGA	16,96
	Acidification pathway components	Chloroquine diphosphate§¶	16
Conformational changes in the fusion protein	Protein disulfide isomerase	Nitazoxanide¶	50,51
	Fusion protein (triggering)	Arbidol¶§§ and AVP-p‖‖	53,98
	Fusion protein (refolding the trimers of hairpins)	Fusion inhibitor AVPs¶ (for example, T-20 and RVFV-6)	16,32,56
Viral membrane fusion	Cholesterol (depletion)	Statins¶ and PERLs	66
	Membrane components (altering curvature and fluidity)	LJ series and JL series compounds, and RAFIs	72,80
	Membrane components (causing virolysis)	AVPs, C5A, MP7-NH_2 and C31G*‖	56–59

AVPs, antiviral peptides; MFG-E8, milk fat globule-EGF factor 8; PERLs, polyunsaturated endoplasmic reticulum-targeting liposomes; RAFIs, rigid amphipathic fusion inhibitors; RVFV-6, Rift Valley fever virus inhibitory peptide 6; SALPs, synthetic anti-lipopolysaccharide peptides. *In clinics for other uses. ‡Evaluated *in vivo* (human and non-human primates). §Evaluated *in vivo* (non-primates). ‖Evaluated *in vivo* for other diseases (for example, cancer). ¶Mucosal protection. #Aerosolized. **Dominant-negative MFG-E8 has also been shown to bind to phosphatidylserine and block phagocytosis via phosphatidylserine receptors on the cells[97]. ‡‡Cytochalasin B inhibits actin polymerization by blocking actin monomer addition. Chlorpromazine and chloroquine have long been known to lower endosomal pH, but their effects are pleiotropic, and their molecular targets with respect to inhibition of endocytosis remain unknown. EGA is a newly discovered compound that inhibits a host factor involved in early to late endosomal trafficking[96]. The identify of this host factor remains to be identified. §§Arbidol has pleiotropic effects and can also affect several other steps of different viral life cycles[53]. ‖‖Derived from the fusion glycoprotein of a nonpathogenic model arenavirus, AVP-p has demonstrated broad antiviral activity selectively against pseudoviruses bearing Old and New World arenavirus envelope proteins. Reverse of arbidol, AVP-p interestingly appears to prematurely activate viral fusion proteins[98].

or in its environment — for the processing of their envelope proteins. Proteolytic processing of the metastable viral fusion proteins is a common prerequisite that primes the viral fusion proteins to undergo the cascade of conformational changes that forces the merging of virus and host cell membranes. For example, cathepsins have emerged as proteases that are co-opted by several acutely pathogenic viruses, such as EBOV, SARS-CoV and henipaviruses[16]. Therefore, cathepsin inhibitors have been sought as potential broad-spectrum entry inhibitors against cathepsin-dependent viruses (TABLE 1). As cathepsins are ubiquitous proteases that are required for many host activities, whether an inhibitor with an appropriate therapeutic index can be found remains to be determined.

The cascade of conformational changes. Until recently, there were thought to be just three structurally distinct classes of viral fusion proteins involved in the fusion between virus and host cell membranes (FIG. 1). The hallmarks of the α-helical class I fusion proteins include a proteolytically generated amino-terminal fusion peptide that anchors the virus to the host cell membrane targeted for fusion, a trimeric quaternary structure and the triggered formation of complementary α-helical domains that fold into a six-helix bundle structure in the post-fusion conformation. The triggering of the conformational changes of class I fusion proteins can be pH dependent (for example, in influenza virus), pH independent (for example, in HIV) or both (for example, in avian alpharetroviruses; see below). Class II fusion proteins are composed almost entirely of β-sheets in a three-domain architecture (DI, DII and DIII) and have a tightly folded fusion loop in the DII domain that, similarly to the fusion peptide in class I fusion proteins, anchors the virus to the host cell membrane targeted for fusion. Class II fusion proteins, which are found in flaviviruses such as DENV and WNV, are pH dependent and have complex receptor determinants. The structurally mixed class III fusion proteins comprise elements from both class I and class II fusion proteins (α-helical domains, and β-sheet domains with fusion loops, respectively) and are pH dependent; these proteins are found in, for example, Vesicular stomatitis virus (VSV) and herpesviruses.

These three classes of fusion proteins all act as functional trimers when driving the membrane fusion process, and they all fold into stable trimers in the post-fusion conformation (FIG. 1). However, recent structures of the E2 proteins from bovine viral diarrhoea virus (BVDV)[17,18] and HCV[19] indicate that these fusion proteins adopt a novel fold that is distinct from the folds of class I, II and III fusion proteins. Notably, BVDV and HCV are from two divergent genera within the *Flaviviridae* family, which includes the genus Flavivirus, members of which (such as DENV and WNV) possess typical class II fusion proteins. The mechanistic details of this potentially new class of fusion proteins remain

Fusion proteins
Viral envelope proteins that mediate the actual merging of viral and host cell membranes.

Endocytic compartment
A membrane-bound vesicle that shuttles from the plasma membrane to the lysosome and plays a major part in the endomembrane system in eukaryotic cells.

Viral receptors
Host factors that allow specific attachment of the virus to the target cell and initiation of the entry–fusion cascade.

Metastable
Pertaining to the viral fusion protein: in a transient, intermediate and/or energetically unfavourable pre-fusion state. Various triggers and conformational changes enable the metastable pre-fusion protein to fold into the least energetic and most stable post-fusion state.

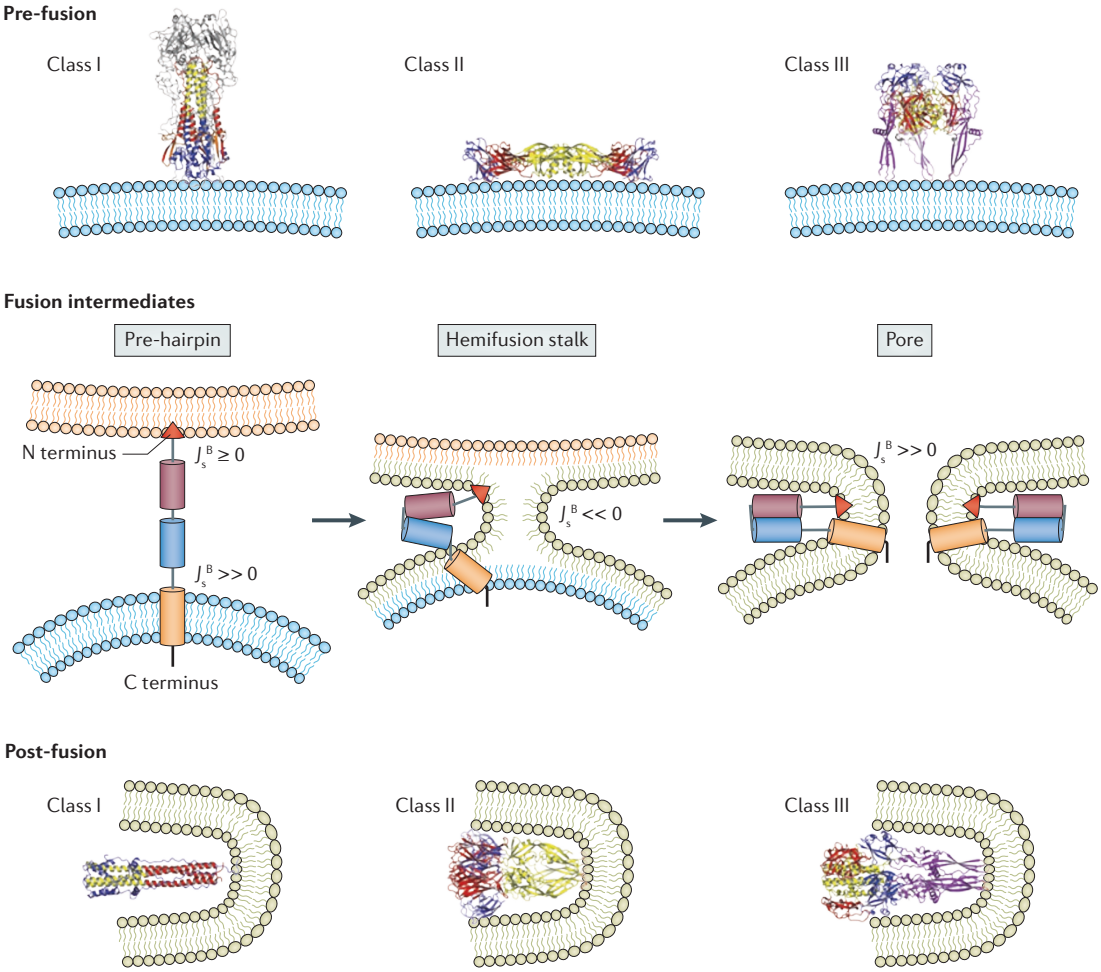


Figure 1 | **The fusion process between viral and cellular membranes.** Viral fusion proteins mediate membrane fusion via divergent structures, as shown by the pre- and post-fusion structures of representative class I fusion proteins (haemagglutinin (HA) from Influenza A virus), class II fusion proteins (E protein from Dengue virus) and class III fusion proteins (G protein from Vesicular stomatitis virus). As the fusion intermediates have not been crystallized, purely schematic models consistent with the body of experimental evidence are presented. Free virions harbour one of the three classes of metastable fusion proteins in their pre-fusion conformations. In this state, the fusion peptides (class I) or loops (classes II and III) are buried inside the proteins. Various triggers, such as receptor binding, protease trimming and low pH, induce conformational rearrangements, resulting in the anchoring of the fusion peptides or loops (red triangle at the amino terminus of the fusion protein) in the juxtaposing cellular membrane. Anchoring leads to concurrent formation of complementary amphipathic domains (purple and cyan cylinders) — α-helices in class I proteins and β-sheets in class II proteins — in the pre-hairpin extended intermediates. For simplicity, only one monomer is represented, but the pre-hairpin intermediates are always trimeric. These newly exposed domains are unstable and refold to form more energetically favourable structures. The enthalpy associated with these conformational changes forces mixing of the outer leaflet of the viral membrane with the outer layer of the cellular membrane, resulting in formation of the hemifusion stalk. The inner leaflets of the lipid bilayers then come into contact and begin mixing, opening a pore between viral and cellular membranes as the trimeric structures refold into a highly stable post-fusion conformation. It is likely that the fusion peptides or loops and the transmembrane domains (orange cylinder) interact to some degree to promote the transition from hemifusion stalk to pore formation. Subsequent pore enlargement allows delivery of the viral contents into the target cell cytosol. The bilayer spontaneous curvature (J_S^B) values of the viral and cellular membranes are indicated to highlight the dramatic changes in membrane curvature that occur during the membrane fusion process. The target cell membrane is almost flat ($J_S^B \geq 0$), or even negatively curved ($J_S^B < 0$) when fusion occurs in endosomal membranes, compared with the highly positively curved virion surface ($J_S^B >> 0$). During membrane fusion, the membrane-bending energetics required to drive the dramatic positive ($J_S^B >> 0$) to negative ($J_S^B << 0$) curvature transitions are substantial (BOX 2). Adapted with permission from REF. 20, Elsevier.

Cathepsins
pH-dependent proteases (for the most part) that are found in the endolysosomal compartments of eukaryotic cells.

Therapeutic index
The ratio between the toxic and effective antiviral concentrations of a compound.

to be determined, as does the structure of the disulfide-linked E1 protein that is thought to be the fusogen in these heterodimeric fusion proteins[20]. Despite the differences between the different classes of fusion proteins, and even though intermediate conformations of the fusion proteins have not been observed directly, all viral fusion proteins seem to mediate membrane fusion through a similar series of conformational changes and

Pre-hairpin intermediate (PHI). An extended and metastable intermediate conformation of the viral fusion protein, just before it folds back into the stable post-fusion conformation.

intermediate structures (FIG. 1). All fusion proteins are metastable in their pre-fusion conformation, and receptor engagement and other triggers (such as low pH) lead to their destabilization. This results in the exposure and concomitant insertion of the fusion peptide (class I) or loop (class II and class III) into the target cell membrane. This extended pre-hairpin intermediate (PHI) also leads to the *de novo* formation of complementary but unstable α-helical or β-sheet domains that refold and/or oligomerize to form more energetically favourable structures (FIG. 1). This cascade of conformational changes powers the merging of the outer leaflet of the virus membrane and the outer leaflet of the host cell membrane (hemifusion), followed by the merging of the inner leaflets of the two membranes, culminating in the formation of the fusion pore[12–14] (FIG. 1). Interestingly, although the triggering of conformational changes in most fusion proteins is either pH dependent or pH independent, the class I fusion proteins of avian alpharetroviruses require both types of trigger. In these cases, binding to the cognate receptor at neutral pH induces the formation of the PHI. However, this PHI is unusually stable and long-lived. A second, low-pH trigger is then required to induce the PHI to refold into the trimer of hairpins that leads to mixing of the lipid bilayers and formation of the fusion pore[21,22].

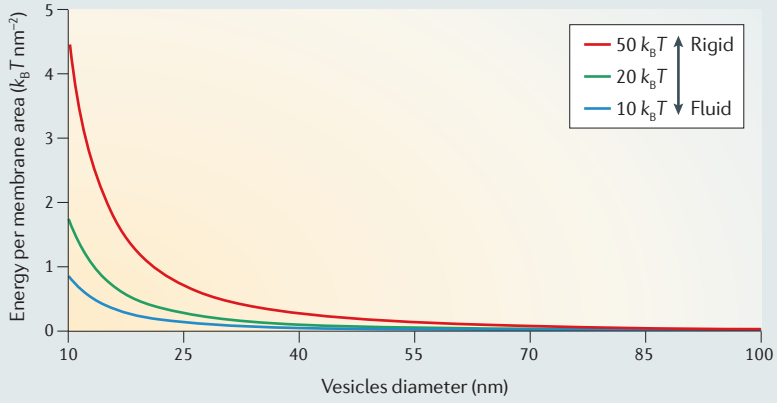
Box 2 | **Energetics of membrane bending**

Most phospholipid components of biological membranes are cyclindrical compounds with a polar head and hydrophobic tail. As such, they self-assemble into planar bilayers. The energy costs associated with bending a piece of flat membrane into a spherical vesicle can be calculated using thermodynamic principles and the known physicochemical properties of lipid bilayers[24,26]. Thus, the energy cost per membrane area of creating a sphere has been estimated as $G_{bending} = 8\pi\kappa/4\pi r^2 = 2\kappa/r^2$, in which κ is the bending rigidity of the membrane and r is the vesicle radius[26]. The bending rigidity can be expressed as k_BT, in which k_B is the Boltzmann constant (which relates energy at the individual particle level with temperature; it is the gas constant (R) divided by the Avogadro constant (N_A)) and T is the absolute temperature.

Sterol components such as cholesterol can result in packing of specific phospholipid species within cholesterol-rich microdomains. This can decrease the intrinsic membrane fluidity that results from free diffusion of phospholipid species in the lipid bilayer. Conversely, phospholipids with unsaturated acyl chains cannot be packed as homogeneously as phospholipids with saturated acyl chains. This results in more dynamic diffusion of the phospholipids in the bilayer. Thus, cholesterol and unsaturated phospholipids can increase and decrease membrane rigidity, respectively. Membrane rigidity ranges from $10\,k_BT$ for highly fluid model membranes comprising only unsaturated phospholipids to $50\,k_BT$ for membranes containing 50% cholesterol[26], which resembles the plasma membrane composition of some mammalian cells, such as erythrocytes.

Several factors influence the energetic cost of bending membranes. For example, for any given membrane rigidity, there is an exponential increase in the amount of absolute energy required ($k_BT\,nm^{-2}$) for bending membranes as the vesicle diameter gets smaller (see the figure). Furthermore, biophysical-modelling data illustrate how even minor increases in membrane rigidity, such as those caused by lipophilic photosensitizers[72,74], when coupled with a large decrease in vesicle radius, as occurs during formation of the fusion stalk, can result in an exponentially insurmountable amount of extra energy required to bend the viral membrane for productive fusion to occur (see the figure). Thus, the energetics of membrane bending provide a rational foundation for broad-spectrum antiviral strategies that exploit the several and considerable energy barriers which must be overcome for productive virus–host cell membrane fusion to occur.

Figure is from *J. Virol.*, 2014, 88, 1849–1853, doi:10.1128/JVI.02907-13 and amended with permission from American Society for Microbiology.



Common elements to target. Regardless of the specific protein–protein interactions or pH conditions that trigger the conformational dynamics which drive membrane fusion, the essential biophysical features that underlie this process are the same[23,24]. These essential features include the dramatic transition of the viral membrane[25] from a positive curvature (bilayer spontaneous curvature (J_S^B) >> 0) to a negative curvature (J_S^B << 0), the appropriate membrane fluidity to maintain and complete the process of lipid bilayer mixing when the fusion process has been triggered, and the energetics required to power these transitions and overcome the electrostatic repulsion between the negatively charged lipidic head groups of the viral and cellular lipid bilayers. The first and second features are dependent on the lipid (and sterol) composition of both virus and host cell membranes, whereas the third feature is provided by various non-exclusive sources, such as temperature, pH and the enthalpy associated with conformational changes in proteins[26] (BOX 2). These essential features of the membrane fusion process provide a framework for understanding the broad-spectrum antivirals that we discuss in this Review, all of which seem to target these common elements either directly or indirectly (TABLE 1). Indeed, modulating these physicochemical properties to raise the energetics of membrane bending, such that virus–cell fusion is energetically disfavoured, would be one general principle for broad-spectrum antiviral strategies that target virus fusion.

Targeting proteins

One strategy to design broad-spectrum antivirals is to target proteins that are involved in the viral fusion process, including the viral fusion proteins themselves.

Fusion inhibitors. A greater understanding of the different steps involved in the cascade of conformational changes that results in membrane fusion and the biochemical characterization of the domains exposed in the PHI of various fusion proteins has led to the rational design of peptide inhibitors that antagonize the transitions and folding of the extended PHIs into their highly stable post-fusion conformations (FIG. 2). The first proof of concept came from the study of a peptide with 36 amino acids derived from the α-helical carboxy-terminal

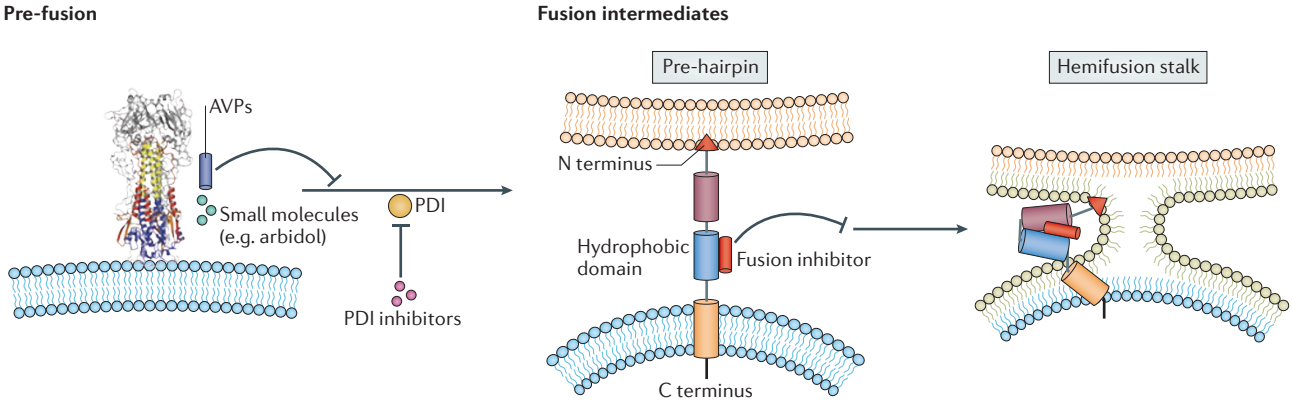


Figure 2 | **Broad-spectrum antivirals targeting fusion proteins.** Small molecules (for example, arbidol) and antiviral peptides (AVPs; most often, α-helical peptides) can interact with the pre-fusion conformations of fusion proteins. These interactions can stabilize or destabilize the fusion proteins, preventing the formation of fusion intermediates. Similarly, inhibitors of enzymes that are specialized in the intramolecular rearrangements of disulfide bonds, such as protein disulfide isomerase (PDI) family of proteins, impair the fine-tuned conformational changes that are required for the subsequent sequence of fusion and thus prevent virus entry. Small fusion inhibitor peptides are AVPs specifically derived from and/or designed to target the hydrophobic domains of fusion proteins. These hydrophobic domains are responsible for the formation of the trimers of hairpins that are necessary to promote progression from the pre-hairpin extended intermediate state to the hemifusion stalk state, and fusion inhibitors therefore impair this progression.

heptad repeat domain (HR2) of gp41, the class I fusion protein of HIV-1. This peptide, called T-20 (also known as enfuvirtide or Fuzeon (Roche))[27], is in clinical use for salvage therapy of patients who are infected with multidrug-resistant HIV. T-20 was designed to target the complementary gp41 HR1 domain in *trans*, fitting in between the interstices of the trimeric HR1 core in the PHI and preventing the refolding of the cognate HR2 domain in *cis*. Thus, T-20 antagonizes the gp41 transition from the PHI to the stable trimer-of-hairpins structure. T-20 is proposed to act as a dominant-negative inhibitor, as it inhibits HIV-1 entry at a much greater potency (that is, with a lower half-maximal inhibitory concentration (IC_{50})) than would be expected from its affinity for the trimeric HR1 core. However, resistance mutations to T-20 can often occur outside the complementary HR1 region, and some viral isolates from treated patients have even been reported to become dependent on T-20 for entry, indicating that we still do not fully understand the T-20 MOA[28,29]. Nevertheless, the paradigm established by T-20 for HIV-1 has not only encouraged the development of analogous HR2-peptide-based fusion inhibitors for other class I viral fusion proteins[30] but has also led to the investigation of DIII-derived peptide inhibitors for class II fusion proteins, on the basis of the functional homology between the HR2 domain of class I fusion proteins and the DIII domain of class II fusion proteins[31].

Although such peptide-based approaches may be broadly applicable across each class of viral fusion proteins, in general the actual peptide therapeutic is likely to be specific to the virus (and closely related variants) that it was developed for. However, some peptides derived from other domains of fusion proteins have unexpectedly displayed activity against unrelated viruses. For example, the recently described RVFV-6 peptide, which is derived from the membrane-proximal stem region of RVFV Gc, a class II fusion protein, inhibited entry not only of RVFV but also of EBOV and VSV, which encode class I and class III fusion proteins, respectively[32]. Structural modelling indicated that the RVFV-6 peptide is compatible with the exposed complementary hydrophobic domains in other classes of fusion proteins, but this proposed MOA remains to be rigorously tested.

Conformational-change inhibitors. Allosteric control of protein function by thiol–disulfide exchange is an emerging theme of broad scientific interest, especially for metastable proteins involved in cell entry[33–35]. For example, thiol isomerases, such as the prototypical cell surface protein disulfide isomerase A1 (PDIA1; encoded by *P4HB*), have been implicated in the rearrangement of intramolecular disulfide bonds that occurs in some viral envelope proteins during the fusion process[36–39] (reviewed in REF. 47) (FIG. 2). There is a large family of PDI proteins that usually reside in the endoplasmic reticulum (ER). Studies on cell surface PDI with regards to viral entry, unless otherwise specified, typically refers to PDIA1. However, the lack of specific inhibitors against PDI family members (with the exception of PDIA1-specific monoclonal antibodies[36,38]), coupled with the lack of a mechanism that accounts for cell surface retention of PDI, has stymied investigations into PDI as a potential therapeutic target. Furthermore, the assumption that PDI is not a druggable target, owing to its ubiquitous function as an ER chaperone — the inhibition of which would lead to unacceptable toxicities — has also hampered the search for specific PDI inhibitors.

Discoveries in recent years may offer new opportunities to investigate PDI as a druggable target for potential broad-spectrum antivirals. For example, the discovery that galectin 9 binds to, retains and increases the activity of PDIA1, and perhaps PDIA3 (also known as ERp57)

Hemifusion
That state of membrane fusion in which only the lipid constituents of the outer leaflets of the two juxtaposing bilayers are mixed; at this point in virus–host cell membrane fusion, the inner leaflets of the two bilayers are still distinct, and the virion content has no access to the host cell cytoplasm.

Enthalpy
The enthalpy (*H*) of a system is a thermodynamic function equivalent to the internal energy of the system (*U*) plus the product of its volume (*V*) and the pressure (*p*) exerted on it by its surroundings; thus, $H = U + pV$. For a small volume such as viral fusion proteins, $H \approx U$.

Heptad repeat domain
A structural motif comprised of seven-residue repeats that follow an HPPHCPC pattern, in which H, P and C represent hydrophobic, polar and charged amino acids, respectively.

and PDIA6 (also known as ERp5), on the surface of T cells[36] provides a mechanistic basis for the long-standing observation of PDI activity on the surface of lymphocytes and macrophages[40]. Galectins effectively function as oligomers, forming glycoprotein lattices on the cell surface by clustering cognate ligands[41] such as PDIA1. Furthermore, galectin 9 enhancement of HIV entry into host $CD4^+$ T cells is inhibited by PDIA1-specific monoclonal antibodies[38,42], suggesting that cell surface PDIA1 does indeed modulate virus entry.

Several studies have identified compounds that form specific thiol covalent adducts to the cysteine residues in the PDIA1 active site and can therefore serve as specific PDIA1 inhibitors[43,44]. PACMA31 (propynoic acid carbamoyl methyl-amide 31) was identified in a screen for novel antitumour agents[44], whereas 16F16, which has activities against PDIA1 and PDIA3, was identified in a screen for cytoprotective compounds in a cellular model of Huntington disease[43]. As PDI secreted by platelets and endothelial cells is thrombogenic, an additional screen analysed potent inhibitors of extracellular PDI as candidates for antithrombotic therapy and identified quercetin-3-*O*-rutinoside (also known as rutin) as a highly specific PDIA1 inhibitor[45]. Quercetin-3-*O*-rutinoside selectively inhibits PDIA1 but not related PDI family members such as PDIA3, PDIA4 (also known as ERp72), PDIA6 or thioredoxin. PACMA31 and quercetin-3-*O*-rutinoside were effective in relevant animal models, with no untoward toxicities at the concentrations used. Thus, PDI inhibitors that have been successfully developed to treat other diseases[43–45] could be leveraged as potential broad-spectrum antivirals[46,47] (FIG. 2). In a reversal of the traditional paradigm that relies on testing the activity of different compounds against a specific virus, these highly specific PDI inhibitors could be used to screen for the set of susceptible viral pathogens, and the pharmacokinetic, safety and toxicity data already obtained with these lead compounds could be applied to relevant animal models of infection. Clearly, the side effects of these drugs have to be balanced against their potential efficacy as antivirals. Nevertheless, the repurposing of FDA-approved drugs to treat rare but lethal emerging infectious diseases is a strategy endorsed by the FDA's Medical Countermeasures Initiative (MCMi), an FDA-wide effort set up to facilitate the availability of drugs and other countermeasures that will be needed to control emerging infectious diseases. More speculatively, nitazoxanide (NTZ), which is a thiazolide antiparasitic agent, was recently proposed as a potential PDI inhibitor[48,49] and has demonstrated broad antiviral activity *in vitro* both as a single drug and in synergy with other antivirals[50–52]. However, it is still unclear whether the antiviral activity of NTZ is directly related to its anti-PDI activity.

Arbidol (ARB; also known as umifenovir) is a well-known indol derivative with broad-spectrum antiviral activity that is a popular treatment for respiratory infections in Russia and China. Unfortunately, the reported MOAs are also broad and pleiotropic[53]. At least in the case of influenza virus, viral resistance to ARB maps to the class I fusion protein haemagglutinin (HA), and ARB-resistant HA variants mediate membrane fusion at a higher pH than the wild-type counterparts[54]. Conversely, ARB-bound HA variants require a lower pH than the wild-type protein to transition to the low-pH form (or PHI). These data indicate that ARB-resistant HAs are more easily destabilized and suggest that ARB inhibits influenza virus entry by stabilizing HA in its pre-fusion conformation (FIG. 2). Whether ARB has a similar MOA against other pH-dependent viruses remains to be seen.

NTZ and ARB exemplify promising broad-spectrum antiviral candidates for immediate use in patients, as they combine good bioavailability with a safe record of use in patients, and oral formulations are available. ARB has been broadly distributed in Russia and China[53] but has yet to be approved by the FDA and the EMA for use in Western countries. NTZ is approved by the FDA for the treatment of infections with *Giardia lamblia* (Alinia; Romark Laboratories, L.C.) and is currently in global Phase III clinical trials against influenza viruses[50,55]. Both NTZ and ARB appear to have safe clinical profiles, and off-label use for the treatment of susceptible viral pathogens may thus be warranted if guided by proper surrogate animal efficacy studies and if no other options are available.

Targeting membranes

In addition to the traditional compounds that target proteins which mediate viral fusion, an emerging paradigm for broad-spectrum antivirals points at the lipids of the virus and host cell membranes as potential targets to block viral fusion and entry.

Virolytic antiviral peptides. Virolytic antiviral peptides (AVPs) are reminiscent of the broad-spectrum and pleiotropic antimicrobial peptides (AMPs) that are part of the innate immune defence mechanisms present in all kingdoms of life. To date, the best-characterized AVPs are generally small (2–5 kDa) cationic, amphiphilic α-helical peptides, the activity of which most probably relies on their interfacial hydrophobicity[56]. Indeed, AVPs engage in electrostatic and/or hydrophobic interactions with the hydrophobic surfaces of fusion proteins that are transiently exposed during the fusion process, but AVPs also interact with membrane lipids[56,57]. The ability of AVPs to interact with fusion proteins is exemplified by the aforementioned fusion inhibitor peptides such as T-20 and RVFV-6 (FIG. 2). By contrast, the ability of AVPs to interact with membrane lipids is exemplified by C5A, a peptide derived from the non-structural protein 5A (NS5A) of HCV, and by the wasp venom secretagogue toxin (mastoparan)-derived peptide MP7-NH_2. Both C5A and MP7-NH_2 have detergent-like properties (FIG. 3) and displayed potent virolytic activity against various enveloped viruses, including members of the *Flaviviridae* and *Paramyxoviridae* families, such as WNV, DENV, HCV and Human respiratory syncytial virus (RSV), but not against others, such as influenza virus[58,59]. No obvious differences could be discerned between sensitive and resistant viruses, and the puzzling selectivity of lytic AVPs towards their targets remains a subject of ongoing investigations[60]. Perhaps, as is the case for

Secretagogue
A substance that triggers the secretion of another substance.

several AMPs[57,61], the selectivity of AVPs resides in the distinctive lipid composition between the viral target membrane and the membrane of the host cell; viral lipidomic profiling will help to address the veracity of this hypothesis. A database of experimentally validated AVPs, AVPdb[62], indicates that AVPs are being increasingly investigated for their therapeutic utility, but the lack of a cohesive MOA is likely to impede their clinical application in the near future.

Membrane curvature and fluidity. Membrane fluidity is an essential parameter that governs the energetics of the membrane curvature transitions which occur during membrane fusion[24,26] (BOX 2). Lipid composition is the main determinant of membrane fluidity, and an imbalance at the virus–cell membrane interface can positively or negatively affect the progression of the fusion process. Indeed, the positive-to-negative membrane curvature transitions that occur during virus–cell fusion (FIG. 1) have been fruitfully interrogated with various lipids that either support or antagonize the geometric constraints during the fusion process. For example, cone-shaped lipids (such as cholesterol and oleic acid) tend to favour membrane fusion by promoting negative curvature and therefore facilitating hemifusion. By contrast, inverted-cone-shaped lipids (also called wedge-like lipids; such as lysolipids, including lysophosphatidylcholine (LPC)) inhibit fusion by increasing the positive curvature of viral membranes (FIG. 3), thus raising the energy barrier that must be overcome for productive fusion mediated by viral fusion proteins[25,26,63,64].

As sterol and lipid composition is essential to membrane curvature and fluidity, removal and/or addition of these species from the viral and/or cellular membranes have been evaluated as antiviral strategies. An interesting example comes from the so-called polyunsaturated ER-targeting liposomes (PERLs), which were originally developed to deliver antiviral drug cargoes to the ER[65]. Serendipitously, PERLs were found to have broad-spectrum antiviral activity against HIV, HCV and Hepatitis B virus (HBV), even when no antiviral drug cargoes were loaded into the PERLs[66]. Mechanistic investigations indicated that PERLs trigger a reduction in the cellular levels of cholesterol, a cone-shaped lipid that tends to favour membrane fusion for some viruses, even more efficiently than the clinically approved cholesterol-lowering drug lovastatin (Mevacor; Merck), which also has an antiviral effect against HCV and HBV[66] (TABLE 1). PERL-triggered cellular cholesterol depletion resulted in the decreased entry of susceptible viruses that are sensitive to reduced levels of cholesterol at the cell surface. In addition, viruses produced from PERL-treated cells had reduced infectivity owing to a lower cholesterol content in the lipid envelopes of budded virions (FIG. 3), a phenotype that could be rescued by the addition of exogenous cholesterol[66]. These results confirmed the importance of cholesterol within both the cellular and viral membranes, at least for the three tested viruses (HIV, HCV and HBV). Thus, PERL-triggered cellular cholesterol reduction seems to impair more than one step in the replication cycle of susceptible viruses. Although this capability is attractive, the exact mechanism (or mechanisms) of PERL antiviral activity against each of the susceptible viruses remains to be characterized, and the off-target effects of the PERL-induced signalling cascades await better definition.

Inverted-cone-shaped compounds. Inverted-cone-shaped species (such as lysolipids) are known to impair fusion by stabilizing viral lipid membranes in a state of positive membrane curvature that raises the energetic cost needed for viral fusion (FIG. 3). However, these compounds may also act at the last step of membrane fusion and stabilize the highly positively curved pore edges (FIG. 1), thereby preventing their expansion[67]. Notably, lysolipids are broadly antiviral but are also cytotoxic and unstable *in vivo*[63]. In addition, their antifusogenic effects are realized only at millimolar concentrations and are readily reversible. Although these properties bode well for the use of lysolipids as experimental tools, they hinder the therapeutic potential of these compounds. Nonetheless, the lysolipid MOA has inspired the rational design of a novel class of broad-spectrum antivirals termed rigid amphipathic fusion inhibitors (RAFIs).

RAFIs are non-lipidic inverted-cone-shaped molecules that are made by conjugation of a large, bulky, hydrophilic head (nucleoside derivatives) to a smaller, rigid and planar hydrophobic tail[68,69]. RAFIs are geometrically reminiscent of lysolipids and exhibit broad-spectrum antiviral activity against several enveloped viruses, such as herpes simplex virus (HSV), influenza virus, VSV and HCV, but not against non-enveloped viruses. Although structure–activity relationship analyses (SAR analyses) showed some consistency with a proposed MOA involving geometric antagonism of negative membrane curvature, only a subset of the RAFIs appeared to be active. Furthermore, RAFIs seemed to act irreversibly, with IC_{50}s in the tens-of-nanomolar range. Collectively, these observations are inconsistent with a purely geometric MOA and suggest that additional mechanisms are responsible for the antiviral effects mediated by RAFIs[70,71]. Therefore, although the greater potency of the inverted-cone-shaped RAFIs relative to lysolipids (IC_{50}s in the nanomolar range for RAFIs versus the millimolar range for lysolipids) supports their potential as broad-spectrum antivirals, additional studies are needed to clarify the MOA of these compounds.

Lipid oxidation. A group of amphiphilic thiazolidine derivatives exemplified by the small molecule LJ001 were found to exhibit broad-spectrum antiviral activity, as they could inhibit all of the 25 viruses from 11 different families of enveloped viruses tested, including various viruses from the *Filoviridae*, *Paramyxoviridae*, *Arenaviridae*, *Flaviviridae* and *Herpesviridae* families, but not non-enveloped viruses[72,73]. These compounds are light-activated membrane-targeting singlet oxygen ($^1O_2$) generators (also known as type II photosensitizers)[72,74]. $^1O_2$ generated by these compounds oxidizes the C=C double bonds present in unsaturated phospholipids, which results in a *cis*-to-*trans* isomerization of the bonds and

Cone-shaped lipids
Lipids that have smaller hydrophilic head groups relative to their hydrophobic acyl chains. They participate in negative membrane curvatures and can thus facilitate hemifusion.

Inverted-cone-shaped lipids
Lipids that have larger hydrophilic head groups relative to their hydrophobic acyl chains. They participate in positive membrane curvatures and can thus impair viral or vesicular fusion.

Lysolipids
Lipid molecules that result from the natural hydrolysis of phospholipids. They have only one acyl chain in their tail region, instead of the two present in phospholipids, giving lysolipids an inverted-cone shape.

Structure–activity relationship analyses
(SAR analyses). Analyses that optimize the pharmacophore and drug-like properties of hit compounds.

Photosensitizers
Molecules that can absorb the radiant energy of light (photons) and transfer it to other molecules.

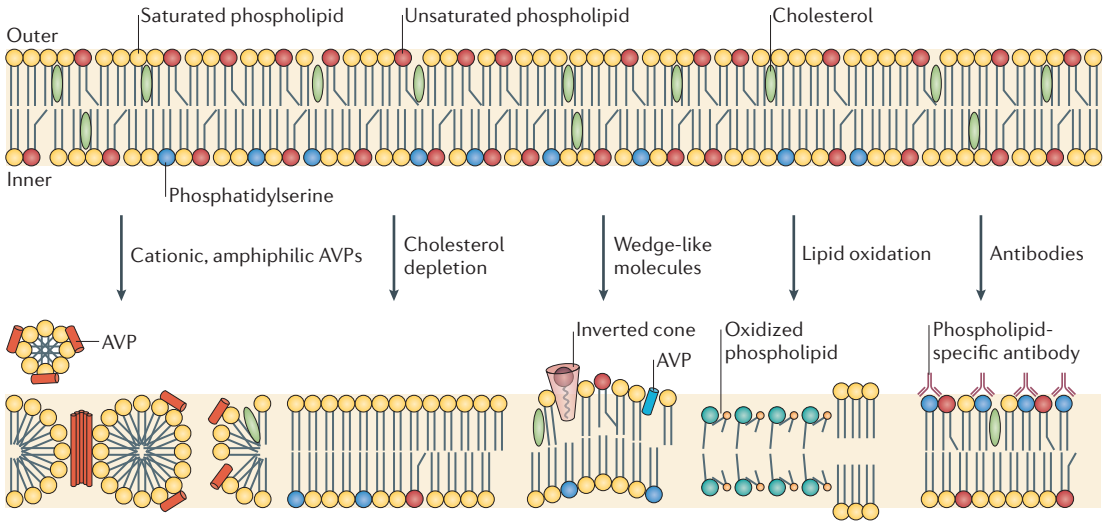


Figure 3 | **Broad-spectrum antivirals targeting viral membranes.** As lipid composition is essential to membrane curvature and fluidity, the removal and addition of lipid species have been evaluated as antiviral strategies. For example, some cationic, amphiphilic antiviral peptides (AVPs) have detergent-like properties at high concentrations and can result in the formation of pores or lead to the micellization of viral membranes. Polyunsaturated endoplasmic reticulum-targeting liposomes (PERLs) have shown potential as broad-spectrum antivirals by depleting cellular and viral membranes of cholesterol; cholesterol depletion reduces the fluidity of the membranes and impairs the negative-curvature transitions that are necessary for the fusion between viral and cellular membranes. Wedge-like or inverted-cone-shaped molecules and some amphiphilic AVPs can increase the spontaneous positive curvature of the viral membrane lipid bilayer, raising the barrier of energy required to power membrane fusion mediated by viral fusion proteins (BOX 2). Similarly, membrane-targeting type II photosensitizers generate singlet oxygen within the plane of the viral membrane, and this singlet oxygen oxidizes unsaturated phospholipids and induces changes in the nanoarchitecture of the viral membrane that are not conducive to membrane fusion. The clustering of oxidized phospholipids results in differential lipid packing, reduced fluidity, increased positive curvature, increased area per lipid molecule and reduced membrane thickness. Phospholipid-specific antibodies can target particular phospholipid species that are enriched in some viral membranes (such as phosphatidylserine) and thus block viral attachment and entry.

introduces polar hydroperoxy (–OOH) groups in the highly hydrophobic acyl chains of the phospholipids[75]. These photochemically induced changes alter critical biophysical properties of membranes, although the exact changes that occur following lipid oxidation are unknown. First, the projected plasma membrane surface area occupied by the oxidized phospholipids is predicted to increase owing to migration of the hydroperoxy group to the polar plane (that is, surface) of the bilayer[76,77]. Second, oxidized phospholipids are predicted to cluster into microdomains in order to reduce hydrophobic repulsion forces against their newly incorporated polar groups[78] (FIG. 3). Indeed, these features were physically observed at the nanoscopic scale using atomic force microscopy, enabling the quantitative assessment of a $^1O_2$-induced decrease in membrane thickness, with an expansion of the area per unsaturated phospholipid[79]. Altogether, these rearrangements of membrane nanoarchitecture result in increased positive curvature and reduced fluidity, which together increase the energetics required for membrane fusion (BOX 2).

Using a panoply of assays involving both model membranes and live viruses treated with LJ001 and its derivatives, the lipid oxidation of viral membranes was shown to be correlated with increased lipid packing and reduced membrane fluidity but also linked to impaired viral fusion[72,74]. Furthermore, these observations extended not only to this group of thiazolidine derivatives and a follow-up class of novel oxazolidine-2,4-dithione derivatives (for example, JL118 and JL122)[72,74] but also to the chemically unrelated RAFI compounds[80]. Indeed, close examination of only the most active RAFIs revealed the consistent presence of a hydrophobic polycyclic perylene group reminiscent of other well-known photosensitizers (for example, hypocrellins and hypericin), suggesting that these RAFIs act as photosensitizers. Follow-up studies confirmed that the antiviral activity of the exemplar RAFI, dUY11, was abolished in the absence of light and is thus strictly dependent on light exposure. Furthermore, the antiviral activity of dUY11 could be reversed by $^1O_2$ scavengers, characterizing dUY11 as a type II membrane-targeting photosensitizer in regard to its antiviral activity[80]. This convergent MOA for chemically distinct classes of broad-spectrum antivirals that target the lipid component of membrane fusion underscores the generalizability of the proposed MOA.

Despite the encouraging *in vivo* activity of JL118 and JL122 (REF. 72), the clinical potential of membrane-targeting photosensitizers as broad-spectrum antivirals *in vivo* seems to be currently limited by photophysical hurdles, which include the depth of tissue penetration by visible light. Nonetheless, advances in photochemistry and nanotechnology may help to overcome some of these hurdles; for example, recent developments include

upconverting fluorescent nanoparticles that are able to convert tissue-penetrating long wavelengths to relatively shorter ones[81,82], which are within the JL118 and JL122 absorption spectra[72].

These studies established a novel paradigm for broad-spectrum antivirals, not only because of the identified MOA of these membrane-intercalating photosensitizers — which involves the photo-oxidation of unsaturated phospholipids, leading to biophysical changes that negatively affect the ability of viral membranes to undergo fusion — but also because these compounds are effective as antivirals at concentrations that do not induce noticeable biophysical changes in metabolically active cell membranes, which also contain the unsaturated phospholipid targets of $^1O_2$ (REFS 72,73). This is due to the fact that biogenic cellular membranes benefit from the cellular reparative capacities, which static viral membranes lack; future classes of broad-spectrum antivirals may exploit this general principle.

Phospholipid-specific antibodies. Finally, bavituximab (PGN401; Peregrine Pharmaceuticals) represents one of the most clinically promising broad-spectrum antiviral paradigms. Bavituximab is an immunoglobulin G3 monoclonal antibody directed against the anionic phospholipid phosphatidylserine. Phosphatidylserine is normally restricted to the inner leaflet of the cellular plasma membrane but is flipped out and exposed at the cell surface during apoptotic events[83]. As viral replication can often trigger a cellular apoptotic state, neo-virions bud out of these producing cells with pieces of membrane that contain phosphatidylserine in their outer leaflet, and phosphatidylserine has indeed been detected in various viral envelopes. By targeting this unique feature of the selective exposure of phosphatidylserine on some viral envelopes but not on the membranes of healthy cells, bavituximab has the potential to be used as a broad-spectrum antiviral (FIG. 3). Indeed, bavituximab has proved to be efficacious against Murine cytomegalovirus (MCMV), the arenavirus Pichinde virus and VSV[84]. Bavituximab is currently being evaluated against various haemorrhagic fever viruses and HIV, and is in Phase I and Phase II clinical trials for the treatment of chronic HCV infection or co-infections with HIV and HCV. The potential to use bavituximab against viral diseases in the future will be greatly enhanced by its current evaluation as an antineoplastic therapeutic. However, the exact range of viruses that display sufficient amounts of phosphatidylserine in the outer leaflet of their membranes to be successfully targeted by bavituximab remains to be determined.

Conclusion and outlook

Despite the diverse array of viral fusion mechanisms, an even wider range of putative broad-spectrum antivirals have been characterized that can target the virus–host cell membrane fusion process. Some of these antivirals are broad spectrum in the sense that they target all viruses within the same family or all proteins within a viral fusion protein class, whereas others are broad spectrum in the sense that they target the requirement for a particular fusion trigger. Furthermore, some antivirals are truly broad spectrum because they target the physicochemical basis that underlies all membrane fusion events, while exploiting the physiological difference between static viral membranes and metabolically active cellular membranes with reparative capacity. For example, AVPs, sterol-binding agents and membrane-intercalating photosensitizers may all damage both virus and cell membranes, and thus be toxic to some extent. However, the key paradigm is to recognize that this physiological difference between inactive viral and active cellular membranes can be exploited for the development of broad-spectrum antivirals. The relevant issue is not whether a drug is toxic or known to be toxic to certain cellular processes at some arbitrary concentration, but whether the drug is toxic at antiviral concentrations (or under treatment conditions). In addition, the risk–benefit calculation of how much toxicity to accept must depend on the acuteness and pathogenicity of the viral infection, and the availability (or lack thereof) of other effective therapeutic options. For example, in chronic infections such as HIV or HCV, for which drugs need to be taken daily for prolonged periods, therapeutic selectivity and long-term safety issues are paramount. In these cases, for which an abundance of highly effective therapies already exists, the barrier to using any broad-spectrum antivirals will be very high. Conversely, as in the current (2014–2015) Ebola epidemic in west Africa, the short-term toxicity of a potential antiviral would have to be weighed against the lack of other effective options, if there are valid reasons to support the efficacy of the potential drug.

The rapid expansion of broad-spectrum antiviral approaches that directly or indirectly target the lipids involved in viral membrane fusion[85] underscores the need for more comprehensive characterization of viral lipidomes. Lipidomic studies have already revealed that cell activity and the lipid composition on primary human cell types differ[86]. Thus, it is more than likely that viruses grown in commonly used laboratory cell lines do not accurately reflect the viral phenotype that causes transmission of infectious diseases. This affects the efficacy of antiviral testing[87] and calls for standardization of the protocols and cell types used for antiviral evaluation. Such standardization will accelerate the translational development of novel broad-spectrum antivirals. For example, the use of more relevant *in vitro* surrogate systems, such as the use of human pluripotent stem cell-derived primary cell types for the determination of antiviral efficacy and therapeutic index (half-maximal cytotoxic concentration (CC_{50})/(IC_{50}), could provide more biologically relevant information, even before antiviral testing in animal models[88]. Similarly, recent advances in three-dimensional bioprinting and microfluidic organs-on-chips have allowed the generation of relevant tissue models for drug discovery and toxicology[89,90]. Thus, SAR studies on broad-spectrum antivirals that target viral fusion can even be honed on these more relevant tissue models before eventual animal testing. Altogether, these technologies will facilitate the translation of our basic understanding of the viral fusion process into broad-spectrum therapies that target this process.

Viral lipidomes
The total lipid compositions of specific viruses.

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Competing interests statement
The authors declare no competing interests.

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