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CHAPTER

Advances in structure-assisted antiviral discovery for animal viral diseases

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19.1 Introduction

Antiviral molecules interfering with viral replication and multiplication in host come from diverse sources ranging from natural sources like plants, bacteria, and fungi or are produced by chemical synthesis (Kulkarni and Sanghai, 2014). Chemically synthesized antiviral drugs can either be designed or prepared by random synthesis. Latest advancements in drug development are structure-assisted identification with the help of computers, design and the synthesis of target-specific antiviral. The action mechanism of antiviral is diverse ranging from the viral entry and budding steps to the targeting of virus-specific enzymes. The prevalence of animal viruses poses a major potential threat to animal health and also puts the human population at risk as viruses like influenza virus and encephalitis viruses belonging to the genus alphavirus are reemerging (Feldmann et al., 2002). Structural virology of animal viruses has increased our understanding of viruses, viral replication, their evolution, and interaction with the host. Structure−function relation studies are definitely the need of the hour for rational design of drugs and vaccines to effectively treat animal viral diseases. These studies are important for economical, veterinary, and human medical perspectives. In this chapter, briefly structural techniques and advances made in animal virology toward structure-based identification, and development of antiviral against animal viruses is described.

19.1.1 General strategies for identifying viral drug and vaccine targets

The utmost important step in the drug discovery process is the identification of a drug target. In general, particular in vivo binding site of a drug through which the drug exerts its action is known as a drug target. From the therapeutic point of view, understanding the mechanism of drug binding inhibition and regulation of the target protein activity is very important to combat the viral diseases. Once the drug target has been validated, the next step is to identify, characterize, and

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design inhibitory molecules. Various viral targets and the strategies that are used to identify these viral and vaccine targets are summarized below:

1. Viral surface proteins and the strategies used to target these proteins: This includes viral surface glycoprotein and viral receptors. These are potential candidate for antiviral and vaccine development. Strategies used to target these include receptor-based and ligand-based drug designing. In receptor-based drug designing, mapping of ligands is done. Ligand molecules are engineered by assembling little pieces in a stepwise manner within the binding pocket constraint. In ligand-based approach, ligands for a specific receptor are determined using biophysical simulations and construction of chemical libraries. Availability of structural information is expanding the possibilities of identifying vaccine candidate by screening and mapping epitopes using bioinformatics tools such as Epivax, Epimatrix (Tan et al., 2012; De Groot and Moise, 2007).
2. Targeting the viral proteins/enzymes: Here, peptidomimetic drugs are designed against various viral enzymes such as proteases. Various techniques such as X-ray crystallography, nuclear magnetic resonance (NMR), and computational studies have also enabled for the successful designing of antiviral drugs (Tsantrizos, 2008; Wei and Zhou, 2010).
3. Targeting protein−protein interactions: Mutational and cell-based, yeast two hybrids and various biophysical methods such as surface plasmonresonance, isothermal calorimetry, fluorescence energy resonance transfer (FRET)-based, differential scanning calorimetry have helped in studying essential protein−protein interactions that are potential antivirals. Mutational studies at protein interfaces result in the increase or decrease of binding affinity of one viral protein with other protein, hence implying their beneficial role in viral life cycle (Brito and Pinney, 2017).
4. Targeting host factors: Various cellular proteins of the signal transduction pathway are a potential drug target. Antiviral molecules against host factors involved in viral replication are being targeted to prevent the hijack of host system by virus. Strategies like rapid immunoaffinity purification targeting a virus/host protein followed by mass spectrometry to identify associated protein can be implied (Rowles et al., 2013).
5. Targeting RNA-protein interactions: Using riboproteomics approach that profiles RNA-protein interactions and RNA coimmunoprecipitation which helps to identify all the proteins interacting with the viral RNA (Salim et al., 2016; Yeh et al., 2016; Figs. 19.1 and 19.2).

19.1.2 Structure determination techniques

19.1.2.1 X-ray crystallography

X-ray crystallography is the most powerful and reliable method to obtain a macromolecular structure. It is used to determine structures of viruses and viral

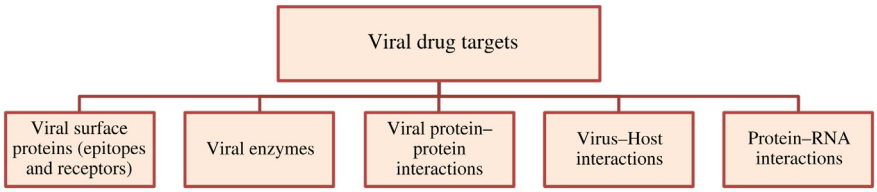

FIGURE 19.1 Antiviral drug targets.

Viral surface proteins, enzymes, various interactions such as protein–protein, protein–RNA, and virus–host are potential antiviral targets.

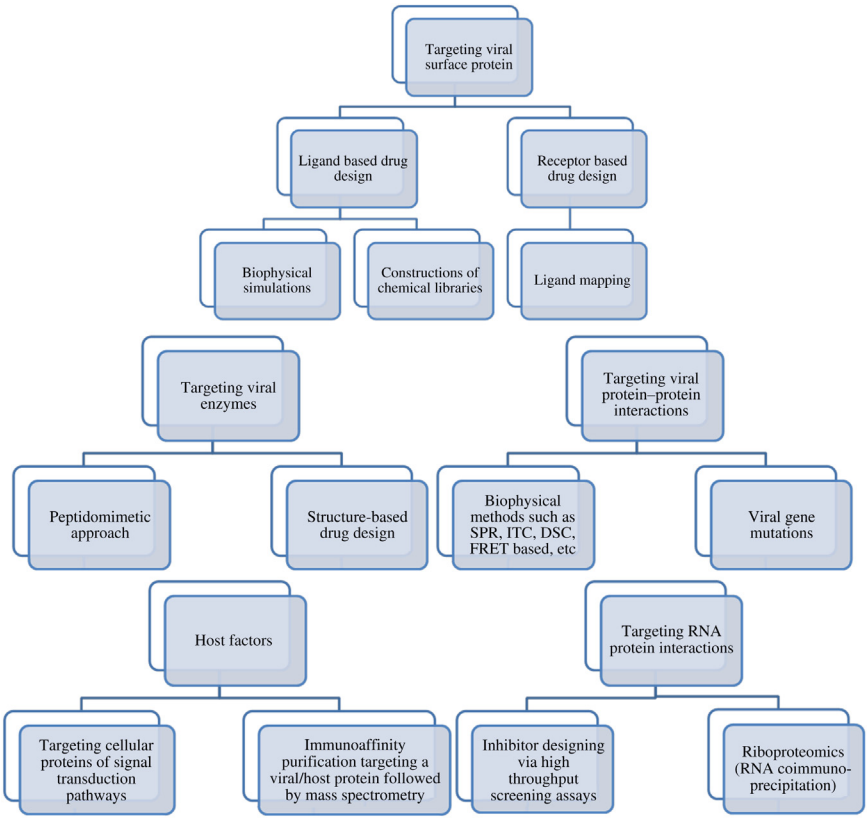

FIGURE 19.2 General strategies used to identify drug targets or vaccine targets.

Various strategies targeting essential virus proteins and interactions are depicted.

proteins by growing crystals (Ilari and Savino, 2008). The three components of X-ray crystallography are protein crystals, source of X-rays, and detector. Crystallographers aim high-powered X-rays at a tiny crystal and molecules arranged in the crystal lattice scatter the X-rays onto an electronic detector.

The intensity of each diffracted ray is detected and fed into a computer, which uses a mathematical equation to calculate the position of every atom in the crystallized molecule. The result is a three-dimensional digital image of the molecule (Rhodes, 2010). X-ray crystallography is helpful in revealing the detailed three-dimensional structures of thousands of proteins. Many advances in drug discovery and medicine are due to the X-ray crystallography by identifying the drug targets in many diseases.

Macromolecular crystallography is a very powerful technique for drug discovery. It has a prominent role in finding drug targets in many diseases that are major challenges today, for example: HIV protease structure was identified by using X-ray crystallography (Klei et al., 2007). Knowledge of the structure led to the identification of various antiviral compounds that interacted with the active site of the enzyme (Munshi et al., 1998). In a similar way, the structure of many proteins of the viruses which can be targeted to stop the viral invasion can be computationally predicted with the help of three-dimensional crystal structures of targeted virus or viral protein. This invites the structures-based drug designing against a particular targeted viral protein. Not only in drug discovery, but X-ray crystallography has also paved a way for making the drugs more effective as molecular details of drug and with targeted site are revealed at the atomic level. Adding to this, atomic structure of the potential viral target in complex drug also elucidates the mechanism of inhibition of the drug, how it interacts at molecular level, what makes it work, and so on (Munshi et al., 1998).

19.1.2.2 Nuclear magnetic resonance

Another powerful biophysical technique is NMR spectroscopy which gives information not only about the structure but also the dynamics of viral proteins and their complexes (Marion, 2013). Nuclei of single atoms absorb different radio frequencies according to the environment of protein. These adsorption signals may be perturbed by adjacent nuclei. This determines the distance between nuclei. These distances, in turn, can be used to determine the overall structure of the protein. NMR is a successful technique used to find out how proteins interact with each other and with small molecules or drugs that can inhibit the interaction (Bakail and Ochsenbein, 2016). It can also be helpful in identifying possible hits for pharmaceutical use. Structures generated from the NMR structure analysis indicate the number of conformations for the protein in solution that are useful in designing small antiviral molecules against the targeted protein active site (Li et al., 2017).

19.1.2.3 Cryo-electron microscopy

In recent years, major advancements have been made in cryo-electron microscopy (cryo-EM) technique and it has emerged as one of the most useful and powerful structural biology techniques that enable the characterization of complex biological systems. cryo-EM has evolved as a powerful tool in structure determination of macromolecular complexes that are not suitable for crystallographic and NMR

studies (Murata and Wolf, 2018). Structure prediction of protein or virus models is done using the electron density of cryo-EM maps. Hence, cryo-EM is a reliable method for structure determination of macromolecules. Macromolecular complexes such as drug bound to viral enzyme active site or neutralizing antibody bound to virus surface epitope can be studied using cryo-EM technique (Ripoll et al., 2016). This availability of structural knowledge leads to rational design and synthesis of more potent drug molecules and vaccines for viral diseases.

19.1.3 Computational structure prediction and drug design

Drug design and drug discovery are of extreme importance in animal and human health care. Computational (computer-based) approaches serve as an important role in structure-based drug design. Structure-based drug design utilizes the three-dimensional structure (3D) of a protein target to design the potential candidate drugs that bind selectively with high affinity to the drug target (Anderson, 2003). Computational approaches utilize various methods for structure-assisted designing of drug molecules. The objective of designing a drug based on the availability of the 3D of protein is to invent or advance a molecule that binds tightly to the drugable site by competing with natural substrates of the protein and further moderating its inhibition function for viral therapy. Such a structure-picked drug molecule found on the basis of the protein structure is more effective and less toxic. Molecular docking methods use the spatial shape of the protein active site to which the drug is expected to bind for selecting a suitable compound that has the potential of being rationally designed as effective antiviral drugs (de Ruyck et al., 2016). For some proteins whose crystallization is difficult to perform, homology modeling can be put to use. It constructs a 3D model of a given protein on the basis of related similar or known structures (homologous structures). However, when the crystal structure of a protein is already known, then the knowledge regarding its active site residues involved in catalysis becomes important followed by computational virtual screening of compound libraries of small molecules. Small molecules are selected by docking them to the target protein. Molecular docking and simulation predicts the binding orientations of potential molecules (drug candidates) to protein targets so as to predict the affinity and activity of such small molecules (Katsila et al., 2016). A number of powerful software programs, for example: AutoDock, HEX, GOLD, FlexX, DOCK, Glide, Surflex, and LigandFit, are being used to predict the docking calculations. Selected in silico potential drug candidates are then tested in vitro for their antiviral effects or so. The use of computers and computational methods forms the core of computational drug design. Availability of protein 3D structures, high-performance computing, etc. is enhancing the modern day drug discovery process. Computational tools offer the advantage in a way that the new drug candidates are more quick and cheap.

Various structure-based approaches in identifying and designing antiviral drugs for some animal viruses are discussed below (Table 19.1).

Table 19.1 Antiviral drug targets with structure-assisted inhibitors and their advantage in antiviral discovery.

S. no.	Targets	Inhibitors	Advantage	References
1	Foot and mouth disease virus (FMDV) RNA-dependent RNA polymerase (RdRp) (3D polymerase)	Nucleotide analogs (ribavirin)	Crystal structure of the FMDV 3D polymerase in both the form-unliganded and bound to a template-primer RNA decanucleotide	Ferrer-Orta et al. (2004, 2010)
2	FMDV 3Dpol-novel binding pocket	Noncompetitive inhibitors (1A8, 3A11, 4H6, 5D9, and 7F8)	They can be used for future structure-based drug design studies and antiviral drugs	Durk et al. (2010)
3	Herpes simplex virus type 1 DNA polymerase	4-Oxo-dihydroquinolines	Identify a series of nonnucleosidic viral polymerase inhibitors	Liu et al. (2006)
4	Severe acute respiratory syndrome coronavirus (SARS-CoV) main protease (M^{pro})	N1	The discovery of a single agent with clinical potential against existing and possible future emerging CoV-related diseases	Yang et al. (2005)
5	Coronavirus $3CL^{pro}$ enzyme	19 ligands	They can be used as a basis for future anti-coronaviral inhibitor discovery experiments	Berry et al. (2015)
6	Alphavirus nsP2 protease	Pep-I and Pep-II	Potent inhibitor against CHIKV and will be used as antiviral drug in future.	Singh et al. (2018)
7	Alphavirus nsP4	Favipiravir	Help in designing other potent broad-spectrum antivirals	Delang et al. (2014)
8	Aura virus capsid protein	Piperazine (small heterocyclic molecule)	Its usefulness for further investigations towards the development of piperazine based antialphaviral drugs	Aggarwal et al. (2017)
9	Alphavirus capsid protease hydrophobic pocket	(*S*)-(+)-Mandelic acid and ethyl 3-aminobenzoate	Serve as the basis for antiviral development against alphaviruses	Sharma et al. (2018)
10	Newcastle disease virus (paramyxovirus) hemagglutinin-neuraminidase (HN)	Neu5Ac2en and the β-anomer of sialic acid	Basis for the structure-based design of inhibitors for a range of paramyxovirus-induced diseases	Crennell et al. (2000)
11	Influenza virus protein NA	Zanamivir, oseltamivir, and peramivir	New strategies in small-molecule drug development to overcome influenza A virus resistance	Shen et al. (2015)
12	Bovine viral diarrhea virus RdRp	VP32947 and 1453	Basis for the structure-based drug design	Choi et al. (2004)
13	Pestivirus envelope glycoprotein E2	PTC12	Crystal structures of the bovine viral diarrhea virus 1 (BVDV1) glycoprotein E2 at neutral and low pH providing structural insight into the pre and postfusion state of the protein.	El Omari et al. (2013), Pascual et al. (2018)

19.2 Animal viruses and viral diseases

19.2.1 Foot and mouth disease virus

Foot and mouth disease virus (FMDV) belongs to the *Picornaviridae* family of viruses. It causes foot and mouth disease (FMD) in cloven-hoofed animals. FMDV can be transmitted by close contact of animals, long-distance aerosol spread, inanimate objects like fodder and motor vehicles. It is highly contagious in cattle, pigs, buffaloes, goats, sheep, etc. It affects every part of the world where livestock are kept and more than 100 countries are still affected by FMDV. It affects wild and domesticated ruminants and therefore is a major concern in trade of livestock and animal products. It can cause acute and prolonged, asymptomatic but persistent infection. FMDV proliferates rapidly in infected species and causes vesicular disease in the feet and mouth. Seven serotypes each including a wide range of variants has been defined for FMDV. FMDV virion consists of nucleic acid and capsid enclosing the genomic positive-strand RNA. The virus genome encodes a single, long open reading frame (ORF) flanked by 5′-untranslated region (5′-UTR) and 3′-untranslated region (3′-UTR). The viral ORF upon translation and processing gives rise to four structural proteins, 10 nonstructural proteins (nsPs), and some cleavage intermediates (Forss et al., 1984). FMDV takes over host control by repressing host translation machinery and innate immune response to infection like many other viruses, by cleaving cellular proteins associated with signaling pathway and blocking protein secretion. A critical role of nsPs and noncoding elements of FMDV regulates these biological processes. Like other viruses, FMDV virus undergoes evolution and mutation, thus one of the hurdles in designing vaccines is between and within the serotypes of FMDV.

19.2.1.1 Clinical signs of foot and mouth disease virus

The incubation period for FMDV usually ranges between 1−12 days. Symptoms include high fever for 2−3 days, blisters inside the mouth leading to foamy saliva, blisters on the feet, swelling in testicles of mature males, and decline in milk production in cows. The disease can also lead to myocarditis (inflammation of the heart muscles) and death in newborn animals. Some asymptomatic-infected domestic animals may also serve as carriers except pig (Jamal and Belsham, 2013).

19.2.1.2 Serotypes of foot and mouth disease virus

FMDV has seven distinct serotypes—O, A, C, Southern African Territories 1, 2, 3 (SAT1, SAT2, SAT3) and Asia-1. Serotypes O and A were discovered by Vallee and Carre. Serotype C was discovered by Waldmann and Trautwein. Later another three serotypes were identified in samples from South Africa. The last serotype was identified from a sample that was collected at Okara, Punjab, and Pakistan from a water buffalo (Longjam et al., 2011).

19.2.1.3 Structure and genome of foot and mouth disease virus

FMDV is a 25−30 nm spherically shaped particle. FMDV virion has a symmetric protein shell called capsid enclosing the nucleic acid. Capsid consists of 60 copies of capsomers and each capsomer is composed of four structural polypeptides namely VP1, VP2, VP3, and VP4. FMDV genome consists of single-stranded positive-sense RNA about 8.3 kb in length. RNA encodes a single but long, ORF about 7 kb. The viral ORF is flanked by a long 5′-UTR and a short 3′-UTR. Viral genome has 3′ poly-A tail. A small protein around 24−25 residues long known as VPg (or 3B), encoded by 3B region of the viral genome, is covalently attached to the 5′ end of the genome. This VPg protein is released into the infected cell and plays no role in translation initiation. Viral ORF is translated into a polyprotein of around 250 kDa which is cleaved by two virus-encoded proteases namely leader (L^{pro}) and $3C^{pro}$ to form structural and nonstructural proteins. Generally, ORF is divided into four areas due to the different functions of mature polypeptides. These regions are—L region, P region, P2 region, and P3 region. L region located at 5′ end to the capsid and encodes for L^{pro}. P region which encodes for precursor capsid polypeptide and generates 4 capsid proteins—VP4, VP2, VP3, and VP1 is cleaved by viral protease. P2 region codes for three viral proteins—2A, 2B and 2C. P3 region codes for four viral proteins—3A, 3B, $3C^{pro}$, and $3D^{pol}$. 3C is a viral protease and 3D acts as RNA-dependent RNA polymerase (RdRp). Viral nonstructural proteins play an important role in virus-mediated host evasion (Longjam et al., 2011).

19.2.1.4 Foot and mouth disease virus nonstructural proteins

The first FMDV nsP that is translated is L^{pro} whose region lies in the polyprotein preceding the capsid precursor protein. This protein has two alternative forms, namely, Lab^{pro} and Lb^{pro} (in vitro and in vivo forms). L^{pro} is a papain-like protease that releases itself from the polyprotein via cleavage between its C-terminus and the N-terminus of VP4. L^{pro} is major virulence factor and not required for viral replication. L^{pro} cleaves host translation initiation factor eIF4G and thus represses host-cell translation. This shuts off the host cap-dependent mRNA translation. L^{pro} also suppresses the host innate immune reaction to viral infection by blocking the interferon activity.

FMDV 2A is 18 amino acid peptides and lacks protease motifs. However, it has characteristic C-terminal motif Glu(x)AsnProGly(2A)/Pro(2B). 2A is cleaved from P1-2A precursor by $3C^{pro}$ or by $3CD^{pro}$ 2A cleavage event occurs only during polypeptide synthesis in such a way that 2A peptide remains connected to P1 structural protein precursor (P1-2A). 2A-2B cleavage is not a proteolytic event but a modification of the translational machinery by 2A peptide. This helps in the release of 2A protein while allowing the synthesis of the downstream proteins to proceed. 2B protein is viroporin that is hydrophobic transmembrane low molecular weight protein. FMDV 2B codes for a 154 aa peptide, slightly longer than other viroporins and contains two predicted putative transmembrane domains

located at positions 83−104 aa and 119−137 aa. The transmembrane hydrophobic domains interact with phospholipid bilayer to increase membrane permeability and facilitate the release of viral particles. 2B is crucial for viral pathogenicity. 2C protein is 318 amino acid long proteins consisting of amphipathic helix in its N-terminal. This nsP is involved in many biological functions linked to membrane targeting.

3A protein, a 153 aa peptide is conserved in most FMDV strains. Most of the coding region in N-terminus (1−75 position) encodes a hydrophilic as well as a hydrophobic domain capable of binding to membranes. That is why FMDV 3A has membrane binding activity based on this hydrophobic motif. 3B protein, known as VPg is covalently bound to 5′-terminus of the genome. The first step in the replication of the picornavirus genome is the uridylylation of the VPg peptide primer. The genome has a 5′ terminal feature of VPgU(pU) covalently linked, which allows the use of VPg as a peptide primer to synthesize viral RNA. $3C^{pro}$ is a chymotrypsin-like cysteine protease that is responsible for most cleavages of viral polyprotein. $3D^{pol}$ is RdRp and synthesizes positive and negative strand viral RNAs. It has a catalytic component of RNA replication and plays an important role in the life cycle of FMDV. $3D^{pol}$ sequences are highly conserved among different serotypes as well as subtypes of FMDV (Gao et al., 2016).

19.2.1.5 Vaccination

Vaccines limited the spread of the virus during epidemics in FMD-free countries as well as in endemic regions thus playing a vital role in FMD control. Recently, vaccines are typically produced by the inactivation of the whole virus. Such vaccines have the quantity and stability of the intact viral capsids in the final preparation. First promising novel FMD vaccine was licensed for manufacture and to be used in the United States (Park, 2013). This adenovirus-vectored FMD vaccine causes in vivo expression of viral capsids in vaccinated animals. Another promising vaccine is composed of stabilized and empty FMDV capsids that are produced in vitro in a baculovirus expression system (Cao et al., 2016). Other areas under research include enhanced adjuvants, vaccine quality control procedures, vaccine protection, and immune correlation.

19.2.1.6 Structure-based drug development against foot and mouth disease virus

Various proteins of FMDV have been targeted to design antivirals against them. Structural-based and computational approaches have been used to find out potent inhibitor molecules against FMDV. In 1994, the first crystal structure of FMDV in reduced form was reported. In 2004, crystal structure of FMDV RdRp (3D polymerase) has been determined in ligand-free and in complex with a template-primer RNA (Ferrer-Orta et al., 2004; Fig. 19.3).

Some conserved amino acid side chains bind to the template-primer in the complex which helps in mediating the initiation of RNA synthesis. This crystal

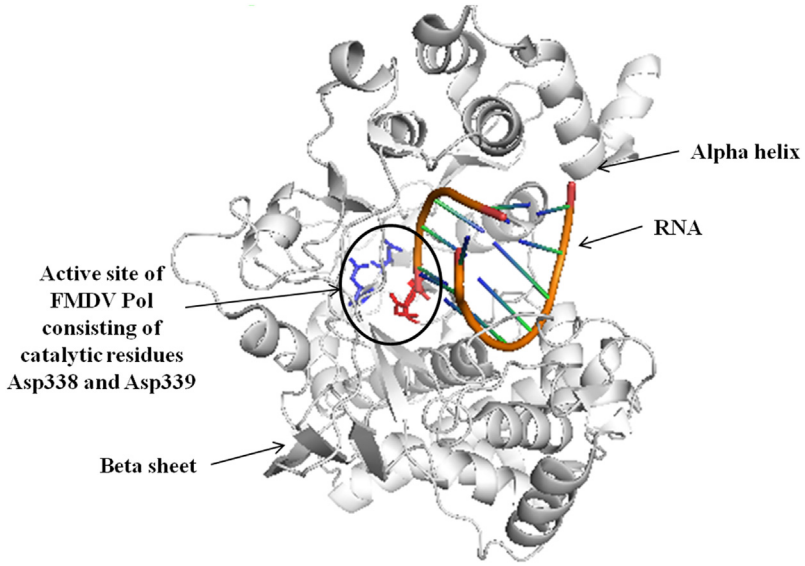

FIGURE 19.3 Complex of foot and mouth disease virus (FMDV) RNA-dependent RNA polymerase (RdRp) with bound RNA at 3 Å.

The bound RNA is represented as stick. Protein is represented as alpha helix and beta sheets. Interaction between catalytic active site residues Asp338 and Asp339 and Asp238 and Asp240 of RNA is also shown (PDB ID: 1WNE) (Ferrer-Orta et al., 2004).

structure sheds light on important information for studies on viral RNA replication and the concept of designing antiviral compounds. In 2006, the crystal structure of FMDV 3C protease was reported. It revealed new insights into the structural-functional aspects of the viral replication enzyme. The crystal structure of FMDV 3Cpro confirmed that it belongs to family of chymotrypsin-like cysteine proteases (Barrett and Rawlings, 2001). The protein comprises of two six-strand barrels and between these barrels, on one face of the protein, lies the peptide binding cleft that has the active site of the enzyme. This FMDV 3C protease crystal structure paved the way for structural-based drug design (Curry et al., 2007; Fig. 19.4).

In 2010, several inhibitor molecules against the crystal structure of FMDV 3Dpol were identified. These targeted a novel binding pocket on 3Dpol which could be used for future structure-based drug design studies (Durk et al., 2010; Fig. 19.5).

Knowledge of the 3D structure of viral capsids allowed for the engineering of the thermostable capsids. However capsid stability and effectiveness of the viral vaccines was a concern. So in 2015, a molecular dynamics (MD)-based strategy for the evaluation of mutations which is designed to increase the stability of capsid through increased noncovalent interactions was developed. Therefore this

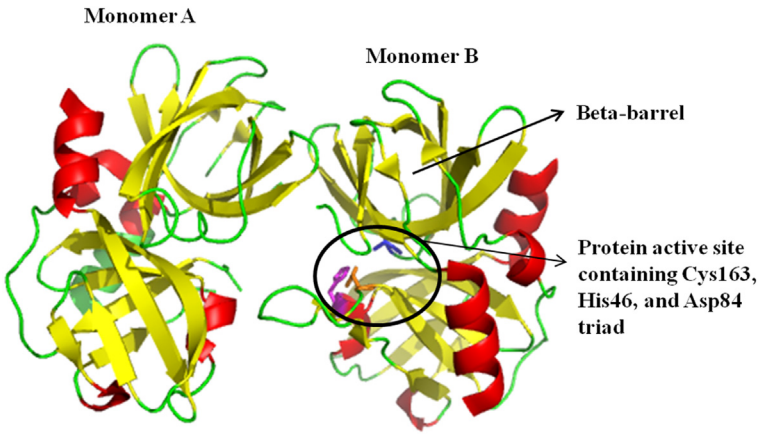

FIGURE 19.4 Structure of foot and mouth disease virus (FMDV) protease at 1.9 Å.

FMDV protease showing two β-barrels. Between these barrels, lies the protein active site. Active site residues include catalytic triad of Cys163, His46, and Asp84. Alpha helices and beta sheets are shown (PDB ID: 2BHG) (Curry et al., 2007).

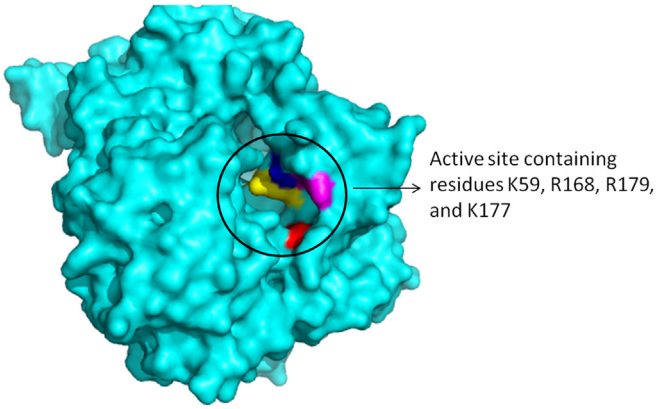

FIGURE 19.5 Structure of foot and mouth disease virus (FMDV) 3D polymerase at 3 Å.

Surface of the protein is shown. Inhibitor binding pocket on the surface of 3D pol of FMDV showing active site catalytic residues K59, R168, R179, and K177 (in circle) (PDB ID: 2E9Z) (Durk et al., 2010).

designed MD protocol allowed derivation of structural-based design of stabilized FMDV viruses and empty capsids which allowed for the development of stable vaccines (Kotecha et al., 2015). In 2018, the crystal structure of mutant viral polymerases with less sensitivity to ribavirin was reported (Ferrer-Orta et al, 2010).

19.2.2 Herpesviruses

Herpesviruses (HSV) are double-stranded (ds) DNA viruses of animals which belong to the *Herpesviridae* family. Their natural host range includes molluscs, fishes, amphibians, birds, reptiles, and mammals including human beings. It causes mucocutaneous lesions inside the oral cavity as well as genital infections in humans. Nine species of herpes virus take humans as their primary host. These cause diseases ranging from mild lesions to serious malignancies. The serologic prevalence percentage is higher in populations of developing countries than in developed countries for many human herpes virus. This widespread of human herpes virus is due to the fact that most virus infections are asymptomatic, and have mild, unnoticed symptoms. Also, the virus is capable of establishing latent, recurrent infections in their hosts. Herpes virus infection is a serious concern and especially dangerous for immunocompromised patients (Jiang et al., 2016). Herpes viruses are grouped into three subfamilies: alpha, beta, and gamma herpesvirus.

19.2.2.1 Structure of herpesvirus

HSV is an enveloped DNA virus that has 150−200 nm diameter and a linear ds DNA around 120 to 230 kbp. The virus has an icosadeltahedral capsid consisting of 162 capsomers. It has a large segment containing viral proteins and an external trilaminar lipid envelope constituting at least 12 glycoproteins. The genome is composed of two regions namely unique long region, UL and unique short region, US linked covalently to each other and flanked by three segments. HSV has three origins of replication (ori)—one copy of oriL and two copies of oriS (Vadlapudi et al., 2013).

19.2.2.2 Herpesviruses lytic and latent cycle

HSV lytic cycle is divided into three steps: viral entry, viral replication, and viral assembly and exit. Viral entry depends on the type of cell. Initially, viral glycoproteins bind to the host-cell receptors and after that viral envelope either fuses with plasma membrane or undergo endocytosis. Only five of the total twelve viral envelope proteins are essential for viral infection such as glycoprotein C (gC), gB, gD, gH, and gL. gB acts as a homooligomer whereas gH and gL form a functional heterooligomer. The binding of gC to heparan sulfate initiates virus contact with the host cell. Postinfection into nucleus, host RNA polymerase II initiates viral gene expression — early and late genes. HSV DNA replicates via a sigma or rolling-circle mechanism. After that, post-DNA replication genes are transcribed forming viral structural components and required for capsid assembly. These are transported into nucleus via nuclear localization sequences. The procapsid is then packaged along with viral DNA to form a mature capsid after being assembled. Inside the cytoplasm, capsids are enveloped by budding into Golgi compartment and are finally secreted out from the infected cells. Herpes viruses have the ability to undergo latency in the hosts for lifetime. During latency, viral transcription is shut off except for 8.3 kb transcript which is associated with

latency and thus called latency associate transcript. This is unstable polyadenylated primary transcript and is further processed into two stable introns with extended half-lives. When reactivated by proper stimuli such as ultraviolet, stress, immune-suppression, these viruses get activated and entered lytic cycle resulting in spreading various diseases (Boehmer and Nimonkar, 2003).

19.2.2.3 Antivirals against herpesviruses

A number of antiviral molecules that target HSV-1 and HSV-2 are present. All are nucleoside analogs except foscarnet and cidofovir, while acyclovir, famciclovir, and valaciclovir are used to treat the majority of cases of HSV-1 and HSV-2 (Bacon et al., 2003). Other medications such as foscarnet, valganciclovir, ganciclovir, and cidofovir have activity against the alpha herpesviruses and are recommended in certain circumstances, such as the treatment of some acyclovir-resistant HSV. Antiviral drugs for the treatment against HSV infections have been developed over the past 40 years. However, most drug-resistant HSV isolates have been reported such as resistance to acyclovir, etc. So this demands the need of highly effective low toxicity drugs in HSV-resistant isolates. Here is the whole antiviral designing process based on the structural findings of HSV (Jiang et al., 2016). Viruses of the family *Herpesviridae* are cause innumerable human diseases. Up to the year 2000, the available treatments were largely ineffective, with the exception of a few drugs for treatment of herpes simplex virus (HSV) infections. However, for some DNA viruses of this family, advancement was made for biochemistry and structural biology of the enzyme viral protease, revealing common features that can be exploited in the development of a new class of antiherpesvirus drugs. So, herpesvirus proteases were identified as a unique class of serine protease with a Ser-His-His catalytic triad. A new, single domain protein fold was determined by X-ray crystallography for the proteases of HSV. It was shown that dimerization is unique for serine proteases and is required for activity of the HSV proteases. With this known fact of dimerization, there was a serious impact on functional analysis and inhibitor discovery. The conserved functional and catalytic properties of the herpesvirus protease enzyme lead to common considerations in the process of inhibitor discovery. Crystal structures of the herpesvirus proteases allowed more direct interpretation of ligand structure−activity relationships (Waxman and Darke, 2000; Fig. 19.6).

In addition, screening of chemical libraries provided some novel structures as starting points for drug development. In 2000, human herpesvirus (HHV) capsids using log-phase cultures of body cavity-based lymphoma 1 cells induced with 12-*O*-tetradecanoylphorbol-13-acetate were obtained for electroncryo microscopy and computer reconstruction. The 3D structure of the HHV-8 capsids revealed that a capsid shell is composed of 12 pentons, 150 hexons, and 320 triplexes arranged on aT516 icosahedral lattice. This structure is similar to those of herpes simplex virus type 1 (HSV-1) and human cytomegalovirus, which are prototypical members of alpha and beta herpesviruses, respectively (Wu et al., 2000). Till 2006, herpesviruses were the second leading cause of human viral diseases,

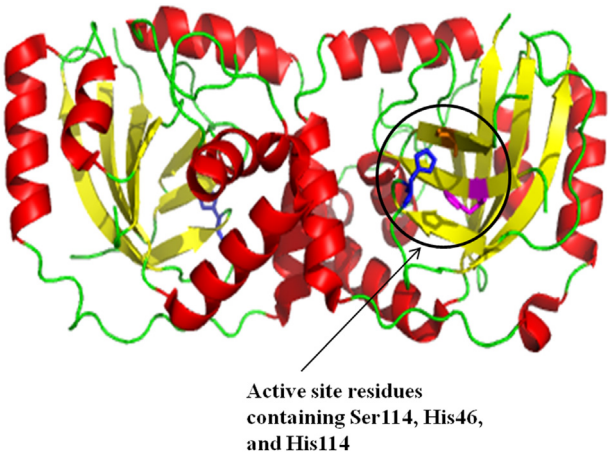

FIGURE 19.6 Structure of herpesvirus protease at 2.1 Å.

Structure of Herpes virus protease solved through X-ray crystallography at 2.1 Å. Catalytic triad of Ser114, His46, and His134 is located on the solvent exposed surface of the protein in the active site. Catalytic site residues are shown in the circle (PDB ID: 1FL1) (Waxman and Darke, 2000).

especially dangerous in immunocompromised individuals. Common therapies for herpes viral infections used nucleoside analogs, such as Acyclovir and target the viral DNA polymerase, essential for viral DNA replication. But the problem is that this class of drugs exhibits a narrow antiviral spectrum, and resistance to these agents was an emerging problem. So the need of the hour was a better understanding of herpes virus replication that could help in the development of safe and effective broad-spectrum antiherpetic. In 2006, the first crystal structure of a herpesvirus polymerase, the herpes simplex virus type 1 DNA polymerase, at 2.7 Å resolution was reported (Liu et al., 2006; Fig. 19.7).

The structural similarity of this polymerase to other polymerases allowed constructing high confidence models of a replication complex (RC) of the polymerase and of Acyclovir as a DNA chain terminator. A novel inhibition mechanism was established in which a representative of a series of nonnucleosidic viral polymerase inhibitors, the 4-oxo-dihydroquinolines, bound at the polymerase active site as well as interacting noncovalently with both the polymerase and the DNA duplex. Most viruses need cell-entry proteins called fusogens in order to get into the host cell. It was known that herpes virus fusogen does not act alone but needs a complex of other viral cell-entry proteins. In 2010, this complex structure was determined. Then it turned out that this protein complex is not a fusogen but it regulates fusogen. It was also established that certain antibodies interfere with this complex so that it cannot regulate fusogen. This gave a clue that certain antiviral can be designed which target this interaction can in turn, prevent viral

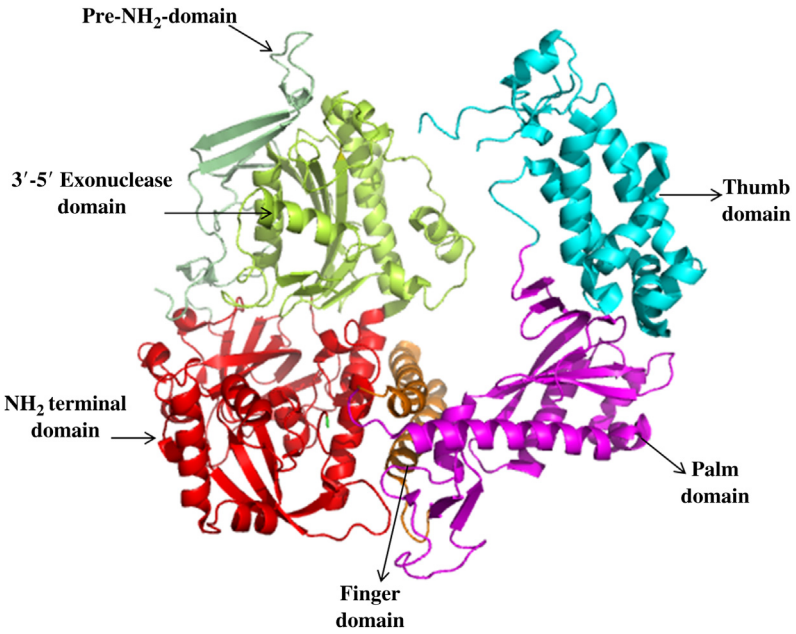

FIGURE 19.7 Herpes simplex virus type 1 DNA polymerase structure at 2.68 Å

DNA Pol is consisting of six domains. Pre-NH_2 −terminal domain is from NH_2 -terminal domain up to residue 140, NH_2 terminal domain is from 141-362 and 594-639 residues, 3'-5' exonuclease domain is from 363-593 residues, palm domain consisting of residues 701-766 and 826-956, finger domain is from residues 767-825 and thumb domain is from 957-1197 (PDB ID: 2GV9) (Liu et al., 2006).

infection (Mesri et al., 2010). The nonnucleoside inhibitors of HSV DNA polymerase target the site that is less important for the binding of a natural nucleoside or nucleoside inhibitor. In 2012, using crystal structure of HSV DNA polymerase, a possibility of new lead molecule based on a-pyrone analogs as nonnucleoside inhibitors came into light using structure-based modeling approach (Karampuri et al., 2012).

In 2012, different in silico approaches were applied to virtually screen for potential inhibitors targeting glycoproteins gB−gH−gL complex formation interface of HSV. Using structure-based virtual screening on gB and gH−gL glycoproteins, many potent inhibitor molecules separately target the active residues involved in their binding activity (Hussain Basha and Naresh Kumar, 2012). In 2016, nature products and new antivirals mechanisms were suggested to target the HSV proteins like DNA helicase/primase complex to fight the drug resistance of HSV. New types of molecules are anti-HSV agents such as flavonoids, sugar-containing compounds, and peptides (Jiang et al., 2016). New antiviral mechanism included the lethal mutagenesis was proposed as a novel chemotherapeutic

strategy for drug resistance. The high frequency of mutations in the viral genome leads to a large danger of genetic mutations causing reduction in viral infective activities. Therefore lethal mutagenesis may be effective in weakening the capacity of the virus for drug resistance. Only one nucleoside analog, ribavirin, exhibits a broad spectrum of antiviral activity against DNA and RNA-based viruses. By 2018, structure of the HSV portal-vertex at subnanometer resolution, solved by cryo-EM and single-particle 3D reconstruction has been reported (McElwee et al., 2018). This led to a number of new discoveries, including the presence of two previously unknown portal-associated structures. Moreover, 3D reconstruction revealed that the viral DNA is packaged within the capsid as a left-handed spool that is arranged in concentric shells. Adding to this, data has also shown a molecular machine that plays a critical role in the replication cycle of an important family of human pathogens. So this can be targeted and can be used in designing antivirals.

19.2.3 Coronavirus (severe acute respiratory syndrome)

Coronaviruses belong to the subfamily *Coronavirinae* under family *Coronavorodae*. Coronaviruses are enveloped viruses containing single-stranded positive-sense RNA as their genetic material. RNA is surrounded by a nucleocapsid of helical symmetry. The genome size of coronaviruses varies between 26−32 kb, being the largest for a RNA virus. Many proteins contribute to the overall structure of all coronaviruses namely spike (S), envelope (E), membrane (M), and nucleocapsid (N). Coronavirus mainly infects mammals and birds (Brian and Baric, 2005). Humans can be infected by six known strains of coronaviruses. In mammals and birds, coronaviruses infect the upper respiratory and gastrointestinal tract. In humans, coronaviruses cause cold, fever, throat congestion, pneumonia, and bronchitis. A human coronavirus called Severe acute respiratory syndrome coronavirus (SARS-CoV) causes severe acute respiratory syndrome (SARS) and can cause unique pathogenesis ranging from lower and upper respiratory tract infections (Cheng et al., 2007). In the case of SARS coronavirus, a defined receptor-binding domain mediates the attachment of virus to its cellular receptor, angiotensin-converting enzyme 2.

19.2.3.1 Replication of coronavirus

Replication of coronavirus occurs in the cytoplasm. Upon viral entry in the host cell, it uncoats and RNA genome is deposited into the cytoplasm. RNA has 5′ methylated cap and a 3′ polyadenylated tail. This allows the RNA to attach to ribosomes for translation. Because of the enzyme replicase encoded by the viral genome, the RNA transcribes into new copies of RNA. A protease, nonstructural protein of coronavirus (CoV) cleaves off each nonstructural protein from the long polyprotein (Fehr and Perlman, 2015). There are following types of human coronaviruses: (1) human coronavirus 229E, (2) human coronavirus OC43, (3) SARS-CoV, (4) human coronavirus NL63 (HCoV-NL63, New Haven coronavirus),

(5) human coronavirus HKU1, (6) Middle East respiratory syndrome coronavirus (MERS-CoV), previously known as novel coronavirus 2012 and HCoV-EMC.

19.2.3.2 Structure-based antivirals against coronavirus

Unfortunately there are no effective antivirals against coronavirus. There is an urgent need to develop new strategies to prevent and to control coronavirus infections, and to understand their biology, replication, and pathogenesis of these viruses. Better understanding of the function of CoV proteins in the virus replication and transcription mechanism may lead to the development of pioneering antivirals. In 2003, structural and functional properties of spike protein of coronavirus were characterized (Bosch et al., 2003). The function the viral spike glycoprotein is to mediate entry of coronavirus. In 2003, the crystal structure of SAR-CoVM^{pro} (main protease) was also solved (Xue et al., 2007). It revealed that the main protease structure forms a homodimer with three domains in each monomer. The antiparallel â-barrel structure of domains I and II is similar to other coronavirus proteases. It forms a chymotrypsin-like fold responsible for catalytic reactions. In 2005, native structure of coronavirus 3CL^{pro} was reported which was solved by X-ray crystallography (Bacha, 2008). SARS-CoV main protease (M^{pro}), is a protein that is required for the maturation of SARS-CoV and is vital for its life cycle, thus, making it an attractive target for structure-based drug design of antiSARS drugs (Lu et al., 2006). Crystal structure of SARS-CoVM^{pro} with inhibitor N1 was also reported (Yang et al., 2005; Fig. 19.8).

In 2006, the structure-based virtual screening on a chemical database containing 58,855 compounds based on the 3D structure of SARS-CoVM^{pro} was performed. Active compounds selected from this virtual screening approach (also confirmed by the bioassay), were taken as the templates to build the core structure for analog search. Finally, the complex structures of potent inhibitors with SARS-CoVM^{pro} were solved by X-ray crystallography. It helped to further study the SARS-CoVM^{pro} inhibition mechanisms of these compounds. In 2012, 2.6-Å crystal structure of the feline coronavirus Nsp7:Nsp8 complex solved by X-ray crystallography was reported (Xiao, 2013). In 2015, many broad spectrum inhibitors against 3CL^{pro} enzyme of coronavirus were identified based on crystal structure of this enzyme and virtual screening methods (Berry et al., 2015). The 3CL^{pro} of coronavirus proved to be an effective drug discovery target. It has even been termed as “the Achilles heel of coronaviruses”. CoV helicase is one of the most evolutionary conserved proteins in nidoviruses, and hence making it an important target for drug development (Hao et al., 2017). In 2018, first full-length crystal structure of the MERS-CoV helicase was reported. CoV helicase has an N-terminal Cys/His-rich domain (CH) with three zincs, a beta-barrel domain, and a C-terminal SF1 helicase core (Durai et al., 2015). These findings are very helpful to provide novel structural information essential for structure-based drug design against CoV.

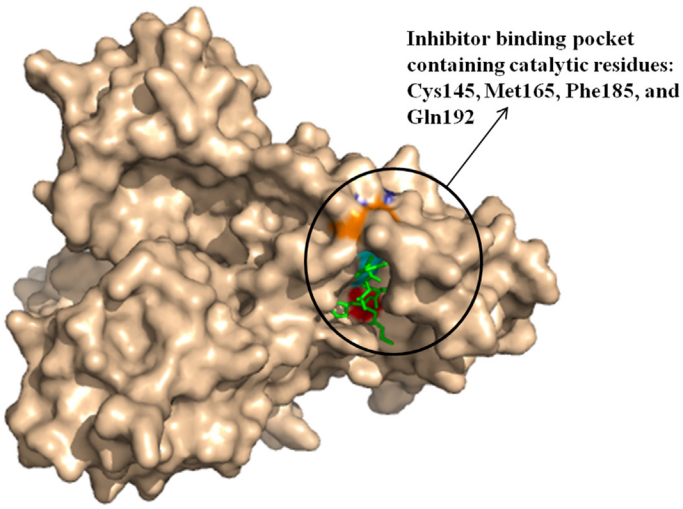

FIGURE 19.8 Structure of severe acute respiratory syndrome coronavirus (SARS-CoV) main protease (M^{pro}) and inhibitor complex at 2 Å.

Inhibitor N1 binding in the active site of SARS-CoV Mpro. Active site residues involved in interaction with N1 constitutes Cys145, Met145, Phe185, and Gln192 are shown in the circle (PDB ID: 1WOF) (Yang et al., 2005).

19.2.4 Alphaviruses

Alphaviruses belong to the *Togaviridae* family of viruses. They are enveloped, positive sense, single-stranded RNA viruses. Alphavirus particles have a 70 nm diameter, tend to be spherical (although slightly pleomorphic), and have a 40 nm isometric nucleocapsid. There are 30 alphaviruses that can infect various vertebrates like humans, rodents, birds, fish, as well as invertebrates. Their transmission between species and individuals occurs mainly through mosquitoes. Hence, they are also called arthropod-born. Alphaviruses are divided into Old World viruses and New World viruses. Chikungunya virus (CHIKV), o'nyong'nyong virus, and sindbis virus (SINV) are the Old World alphaviruses which cause rash, polyarthralgia, and chronic arthritis. New World alphaviruses such as Eastern equine encephalitis virus (EEEV) and Venezuelan equine encephalitis virus (VEEV) are mostly associated with neurological disease. During the Cold War, both the United States biological weapons program and the Soviet biological weapons program researched and weaponized VEEV (Croddy et al., 2008). It causes only moderate morbidity and low mortality in humans but severe morbidity and mortality in animals. EEEV and VEEV caused 70%−90% and 20%−80% mortality in horses, respectively as compared to mortality in humans (Zacks and Paessler, 2010). Salmonid alphavirus (SAV) is a unique group of viruses that causes pancreas disease and severe infection in fish. It is a big problem and

economically important due to high mortality rate from 5% to 60% and poor growth performance in the recovered fish. SAV infection causes the massive loss of biomass in commercial fish farming also (Herath et al., 2016). The alphavirus genome is almost 12 kb in length which exhibits two ORFs—one encoding the nonstructural polyprotein and another encoding the structural polyprotein. nsP1, nsP2, nsP3, and nsP4 are formed as a result of cleavage from the nonstructural polyprotein. These are necessary for the regulatory functions such as transcription and translation of viral mRNA in host. Two nsP precursors (P123 or P1234) are produced by viral RNA using host-cell translational machinery. The carboxyl-terminal protease domain of nsP2 cleaved these precursor polyproteins. At P3/4 junction, cleavage occurs in trans or cis whereas cis at P1/2 junction. Final cleavage at P2/3 junction results in the formation of completely mature nsPs. These nsPs along with host proteins form the positive strand RC. This allows the RNA template to synthesize positive-sense genomic (49S) and subgenomic (26S) RNAs. The structural polyprotein translates into five structural proteins: the capsid (C), E1, E2 (major envelope glycoproteins), E3, and 6K proteins (Shin et al., 2012).

19.2.4.1 Functions of nonstructural proteins

The nsP1 protein, an mRNA capping enzyme, has both guanine-7-methyltransferase (MTase) and guanylyltransferase (GTase) activities. So, nsP1 mediates the methylation and capping functions of viral (Abu Bakar and Ng, 2018). SINV nsP1 protein does not require membrane association for its enzymatic function. The GTase activity of SINV nsP1 is metal-ion dependent, whereas metal is not required for MTase enzymatic activity of nsP1 (Tomar et al., 2011). nsP1 protein, the alphavirus capping enzyme, is a potential drug target because it has a distinct molecular mechanism of capping the viral RNAs than the conventional capping mechanism of host. nsP1 catalyzes the methylation of guanosine triphosphate (GTP) by transferring the methyl group from *S*-adenosylmethionine to a GTP molecule at its N7 position with the help of nsP1 MTase followed by guanylylation reaction which involves the formation of m7GMP−nsP1 covalent complex by nsP1 guanylyltransferase (GTase) (Kaur et al., 2018). The alphavirus nonstructural protein nsP2 possesses various enzymatic activities. The N-terminal region contains a helicase domain that has seven signature motifs of superfamily 1 (SF1) helicases. It functions as RNA triphosphatase that performs the viral RNA capping reactions. It also functions as nucleotide triphosphatase and thus facilitating the RNA helicase activity. The C-terminal region of nsP2 contains a papain-like cysteine protease, which is responsible for processing the viral nonstructural polyprotein (Abu Bakar and Ng, 2018; Narwal et al., 2018; Singh et al., 2018). The crystal structure of the nsP2 cysteine protease of VEEV was reported in the free and E64d-bound states which are the first report of an inhibitor bound alphaviral nsP2 protease structure. The structures and identified active site residues in this study may assist the discovery of potential protease inhibitors against VEEV (Hu et al., 2016). The exact role of alphavirus nsP3 protein is not much

clear in the RC. The nsP3 protein has the N-terminal macro domain (phosphatase activity or nucleic acid binding ability), the alphavirus unique domain, and the C-terminal hypervariable domain. Recently, it has been shown that nsP3 has a role in pathogenicity (Abu Bakar and Ng, 2018). The nsP4 polymerase is the most highly conserved protein in alphaviruses with >50% identity in amino acid. A core RNA-dependent RNA polymerase (RdRp) domain is at the C-terminal end which has RNA synthetic properties of the viral RC. It has TATase activity that suggests a novel function of the alphavirus RdRp in the maintenance and repair of the poly(A) tail, an element required for replication of the viral genome (Tomar et al., 2006).

19.2.4.2 Viral target proteins for drug development

Over the last decade in Asia, Europe, and the Americas, the alphaviruses reemergence has focused on the need of selective inhibitors. At present, no antiviral treatment is available. Viral targets within alphavirus RC include various molecular determinants. Structural and functional analysis of these targets can make the structure-based drug design and development of antivirals against alphaviruses possible.

nsP1−nsP1, nsP1−nsP2, nsP1−nsP3, nsP1−nsP4, nsP2−nsP4, and nsP4−nsP4 interactions are identified in CHIKV. Many of these interactions are shown similarly in SINV and Semliki forest virus (SFV) (Abu Bakar and Ng, 2018). nsP1 recruits other nsPs as discussed in literature and is crucial for SFV replication by membrane association (Lampio et al., 2000). nsP1's interaction with all other nsPs is very important and is an attractive target for drug development. Inhibition of nsP1 anchoring in the spherules occurs when its affinity for the cell membrane is perturbed. This will inhibit the recruitment of the other nsPs and thus preventing the initiation of RC formation. nsP2 protein has viral replication and host evasion strategies which can be targeted for the viral inhibition. nsP2 has RNA helicase, RNA triphosphatase, nucleoside triphosphatase and autoprotease activities. It is an important cofactor for the maturation of viral RC (Kappes, 2014). The nsP2 protease is a good drug target because many viruses are targeted in the same way [human immunodeficiency virus (HIV) and hepatitis C virus]. This has led to the development of many FDA-approved inhibitors (Lenz et al., 2010; Weber and Agniswamy, 2009). nsP4 is virus-specific and is RNA polymerase. It can be a good target to inhibit the viral DNA duplication and its survival. For in silico drug designing, the crystal structure of nsP2 protease is of great interest. nsP2 protease domain crystal structures of the VEEV, CHIKV, and SINV are also present with protein data bank (PDB) entries 2HWK, 5EZQ, 3TRK, and 4GUA respectively. In 2015, crystal structure of nsP2 protease from CHIKV at 2.5 Å was submitted (PDB ID 4ZTB). Using this crystal structure, various peptidomimetic inhibitors were designed against $nsP2^{pro}$ in 2016 (Dhindwal et al., 2017). In 2018, a FRET-based protease assay was used to analyze the proteolytic activity of CHIKV nsP2 protease. This protease assay was used to assess the inhibitory activity of these peptidomimetic compounds identified. It was

concluded that two peptidomimetic compounds, Pep-I (MMsINC database ID MMs03131094) and Pep-II (MMsINC database ID MMs03927237) inhibited CHIKV nsP2 protease activity and can be potential antiviral drugs (Singh et al., 2018). To find out a few potential inhibitors against CHIKV nsp2 protease, homology modeling and computer-aided drug design strategies have been implemented for the first time (Bassetto et al., 2013). Potential inhibitors based on structural studies and molecular simulations have been reported (Nguyen et al., 2015; Singh et al., 2012).

Lopinavir and Nelfinavir, potent HIV protease inhibitors, are FDA-approved inhibitors against CHIKV. A number of highly selective CHIKV and VEEV nsP1 capping enzyme inhibitors are reported recently which interrupt the nsP1-GTase activity (Delang et al., 2016; Gigante et al., 2014, 2017). Nucleoside analogs are also shown to be effective against several alphaviruses, such as ribavirin, which inhibit the CHIKV and SFV genome replication by depleting GTP pools (Briolant et al., 2004). Moreover, the inhibition of nsP4 RdRp by ribavirin through its interaction with Cys483 residue increases the replication fidelity (Coffey et al., 2011). D-N4-hydroxycytidine (NHC), another nucleoside analog is the most potent inhibitor of VEEV. It can serve as a substitute for ribavirin because it cannot develop NHC resistant mutants. Favipiravir is another nsP4 inhibitor and is a potent antiviral against CHIKV infection by inhibiting its interaction with Lys291 residue (Delang et al., 2014). Compound-A could potentially inhibit RdRp's ribonucleotide selection function by targeting Met2295 (Wada et al., 2017). However, it is believed that by chemical modification compound-A may be a starting point for reducing its toxicity. In 2017, 3D structure of capsid protein of aura virus in complex with piperazine was reported at 2.2 Å. Piperazine is a small heterocyclic molecule and docking studies have shown that it binds to hydrophobic pocket of CHIKV capsid protein. It can be effective as an antialphaviral drug (Aggarwal et al., 2017). In 2018, crystal structure of CHIKV capsid protease domain was determined at 2.2 Å. It was found that small molecules such as (S)-(+)-mandelic acid and ethyl 3-aminobenzoate target capsid hydrophobic pocket. These bind to the conserved hydrophobic pocket of CP (Sharma et al., 2018). This may serve as a basis for the development of antivirals against CHIKV infections. In 2018, crystal structure of nsP2pro was determined at 2.59 Å, which revealed that the protein consists of two subdomains: an N-terminal protease subdomain and a C-terminal methyltransferase subdomain. Additionally, structure insights revealed that access to the active site and substrate binding cleft is blocked by a flexible interdomain loop in CHIKV nsP2pro. This may serve beneficial for structure-based drug design and optimization of CHIKV protease inhibitors (Narwal et al., 2018). Recently, a high-throughput ELISA-based assay was developed to screen inhibitors against divalent metal-ion-dependent alphavirus capping enzyme (Kaur et al., 2018). Various inhibitors such as sinefungin, aurintricarboxylic acid, and ribavirin were assessed and their inhibitory effect against nsP1 was reported. In addition, nsP2 protease-based cell-free high-throughput screening assay for evaluation of

inhibitors against emerging CHIKV has been developed (Saha et al., 2018). These successful methods for identifying antiprotease molecules together with a high-throughput screening assay can lead to the development of industrial level large-scale screening platform for identification of protease inhibitors against emerging and reemerging viruses.

19.2.5 Paramyxovirus

The *Paramyxoviridae* family is divided into two subfamilies: *Paramyxovirinar* and *Pneumovirinae*. The *Paramyxovirinae* subfamily has five genera namely *Respirovirus*, *Rubulavirus*, *Avulavirus*, *Morbillivirus*, and *Henipavirus*. This subfamily comprises of various viruses like measles, mumps, Newcastle disease, parainfluenza, Hendra, and Nipah viruses (NiVs). The second subfamily, the *Pneumovirinae*, comprises of two genera: *Pneumovirus* and *Metapneumovirus*. This subfamily also includes new human and animal pathogens, such as the human and bovine respiratory syncytial viruses that specifically affect bovine, caprine, and ovine species, and the human and avian metapneumoviruses. Paramyxoviruses include major disease causing pathogens that cause significant health hazards (Aguilar and Lee, 2011). They have enveloped RNA and infected the host with the help of two surface glycoproteins that fuse their lipid membranes with the host-cell plasma membrane. These viruses have an attachment and a fusion (F) protein. Membrane fusion is probably due to receptor-induced conformational changes within the attachment protein that leads to the activation and folding of fusion protein (Plattet and Plemper, 2013).

19.2.5.1 Antivirals against paramyxovirus

Paramyxoviruses are the main cause of respiratory disease in children. One of two viral surface glycoproteins, the hemagglutinin-neuraminidase (HN) has various functions in addition to being the major surface antigen that induces neutralizing antibodies. In 2000, the crystal structure of multifunctional Newcastle disease virus (Paramyxovirus 1) HN alone and in complex with inhibitor was reported (Fig. 19.9).

The structure provides the basis for the structure-based design of inhibitors for a range of paramyxovirus-induced diseases (Crennell et al., 2000). In 2005, the crystal structure of the secreted, uncleaved ectodomain of the paramyxovirus was presented (Yin et al., 2005). In 2012, combined X-ray crystallography and cryo-electron tomography were done to show the structure of matrix protein of Newcastle disease virus, a paramyxovirus. Structure and sequence conservation imply that other paramyxovirus matrix proteins function similarly (Habchi and Longhi, 2012). In 2012, it was established that favipiravir may serve as antiviral against these viruses. Hendra virus (HeV) and NiV are bat-born paramyxoviruses. In 2013, experimental findings in animals have demonstrated that a human

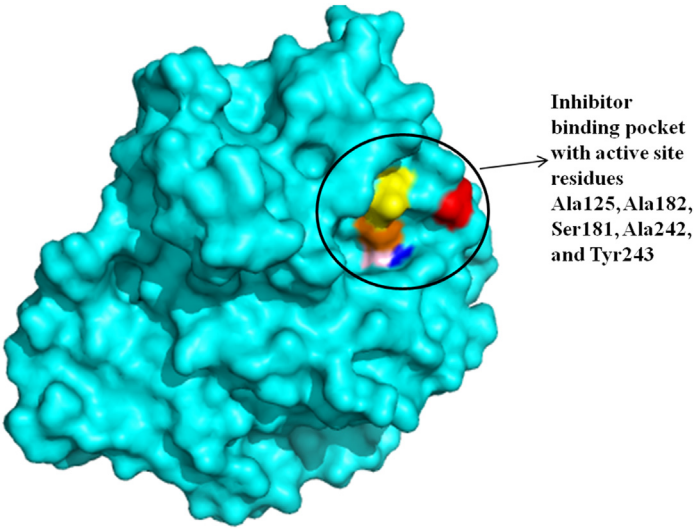

FIGURE 19.9 Crystal structure of multifunctional Newcastle disease virus (Paramyxovirus 1) neuraminidase (NA) at 2.5 Å.

Inhibitor binding pocket on the surface of neuraminidase active site. Active site residues in the protein that are involved in interaction are A125, A182, S181, A242 and T243. These are shown in the circle on the surface of the protein. (PDB ID 1E8T) (Crennell et al., 2000).

monoclonal antibody targeting the viral G glycoprotein is an effective postexposure treatment against Hendra and NiV infection. Also, a subunit vaccine based on the G glycoprotein of HeV affords protection against Hendra and NiV. The vaccine has been developed for use in horses in Australia. It is the first vaccine against a biosafety level-4 (BSL-4) agent to be licensed and commercially deployed. HeV is one of the members of the Henipavirus genus of paramyxoviruses, which are designated BSL-4 organisms because of the high mortality rate of NiV and HeV in humans. Paramyxovirus cell entry is mediated by the fusion protein, F, and this is in response to binding of a host receptor by the attachment protein. During posttranslational processing, the fusion peptide of F is released. Upon receptor-induced triggering, it is inserted into the host-cell membrane. F undergoes a dramatic refolding from its prefusion to postfusion conformation. This brings the host and viral membranes together, allowing entry of the viral RNA. In 2015, the crystal structure of the prefusion form of the HeV F ectodomain was reported. The structure shows great similarity with the structure of prefusion parainfluenzavirus 5 fusion protein (Wong et al., 2016). In 2018, first successful treatment of henipavirus infection in vivo with a small-molecule drug suggests that favipiravir should be evaluated as an antiviral treatment option for henipavirus infections (Dawes et al., 2018).

19.2.6 Avian influenza virus

Avian influenza virus belongs to the family *Orthomyxoviridae*. These are negative sense single-stranded viruses. These are classified into three types: A, B, and C based on the antigenic difference in their nucleoproteins (NPs) and matrix proteins. Influenza A is the major pathogen that causes epidemic influenza. The influenza A genome is composed of eight RNA segments. Five segments code for one protein each and the other three code for two proteins each. The proteins are: hemagglutinin (HA), NA, matrix protein 1 (M1), M2 proton channel, NP, nonstructural protein 1 (NS1), nuclear export protein (NEP; also known as NS2), polymerase acid protein (PA), polymerase basic proteins (PB1 and PB2), and a protein named PB1-F2 which is expressed from a second reading frame (+1) of the PB1 gene. PB1, PB2, and PA form the RNA polymerase. The surface glycoproteins HA and NA provide distinct antigenic properties to the influenza virus. Influenza A viruses are further organized according to HA and NA subtypes (Du et al., 2012). Sixteen HA subtypes (H1−H16) and nine NA subtypes (N1−N9) have been identified. The subtypes of the 1997 bird flu and the 2009 swine flu viruses have been indicated above. In 2001, crystal structure of H5 avian hemagglutinin was reported. In 2008, crystal structure for the NS1 effector domain of avian influenza virus A was reported (Hale et al., 2008). In 2009, crystal structure of an avian influenza polymerase was reported (Fig. 19.10).

This unbolted many possibilities to target the viral protein for designing of new antiinfluenza therapeutics (Yuan et al., 2009). In 2015, three inhibitors against protein NA were approved by FDA namely zanamivir (Relenza, Glaxo Smith Kline), oseltamivir (Tamiflu, Roche), and peramivir (Rapivab, BioCryst). Zanamivir was the first approved inhibitor among these (Shen et al., 2015). In 2017, structure-based drug discovery studies were done by targeting the PA−PB1 interaction. Many potent antiinfluenza drugs were reported with the help of in silico simulation studies (Watanabe et al., 2017).

19.2.7 Pestivirus

The *Flaviviridae* family consists of three genera which include—*Flavivirus* genus (type species, yellow fever virus) as the largest genus, the *Hepacivirus* (type species, hepatitis C virus), and the *Pestivirus* (type species, bovine virus diarrhea). *Pestivirus* belonging to the family *Flaviviridae* is not arthropod-borne and mainly infects mammals. These cause diseases like hemorrhagic syndromes, abortion, and fatal mucosal disease. These are single-stranded viruses with positive-sense RNA. The genome is around 12.5 kb long. There is no poly-A tail at 3′-end of the genome. Hence these viruses have no posttranscriptional modifications, and have simple RNA genomes. The genome contains RNA to encode both structural and

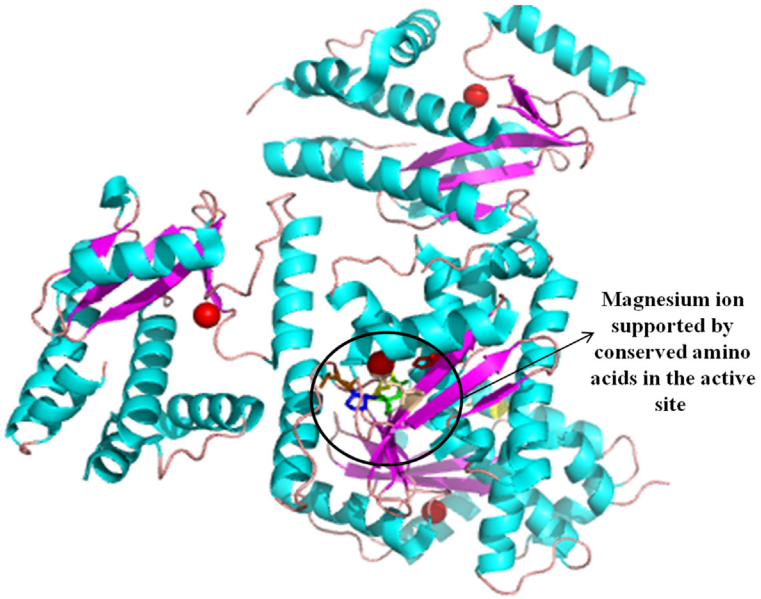

FIGURE 19.10 Crystal structure of avian influenza polymerase at 2.2 Å.

Pol consisting of four polypeptide chains, each containing alpha helices and beta-sheets in presence of magnesium ions (spheres form). Mg ion is coordinated by the acidic residues E80 and D108, HH41, E119, L106, and P107 (in the circle). These amino acids except P107 are conserved in Influenza virus A, B, and C (PDB ID: 3EBJ) (Yuan et al., 2009).

nsPs. These are enveloped viruses. These are icosahedral-like particles with a linear genomic arrangement (Kumar et al., 2015). Their entry into the host-cell is mediated by clathrin-coated endocytosis and is achieved by the attachment of viral envelope protein E to host receptor.

19.2.7.1 Vaccine and structure-based drug design

There are vaccines against pestiviruses and the correct vaccine strain should be given, depending on the herd's location and the endemic strain in that region. The vaccination must be given regularly to maintain immunity. There are various species in the genus pestivirus-border disease virus, bovine viral diarrhea virus (BVDV), classical swine fever virus, etc. (Kumar et al., 2014). In 2004, crystal structure of RdRp from BVDV was reported (PDB ID 1S4F). This structure explained many possibilities where inhibitor molecules could bind. This shed light on various inhibitors binding sited on the protein leading to opportunity of designing many structure-based inhibitor molecule (Choi et al., 2004) (Fig. 19.11).

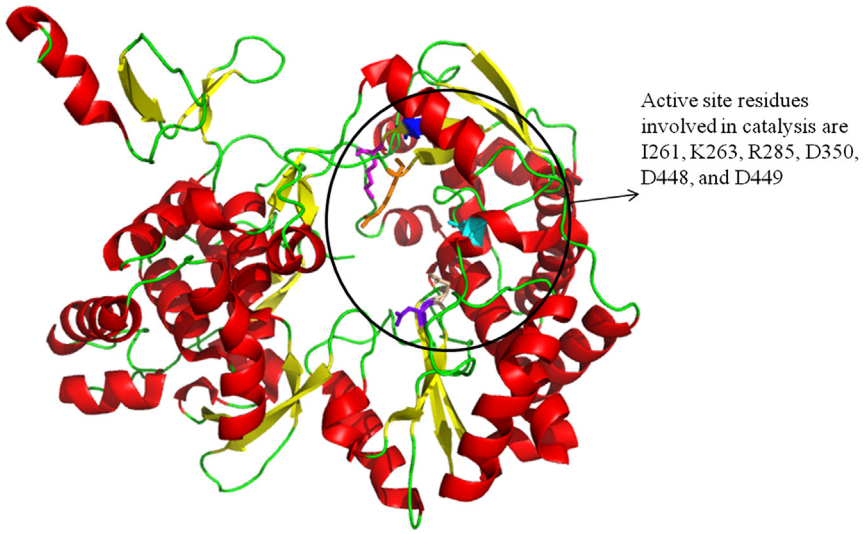

FIGURE 19.11 Crystal structure of bovine viral diarrhea virus (BVDV) RNA-dependent RNA polymerase (RdRp) at 3 Å.

Alpha helices and beta-sheets are shown. Active site residues in protein that are involved in catalysis are I261, K263, R285, D350, D448, and D449 (in the circle) (PDB ID: 1S4F) (Choi et al., 2004).

In 2012, crystal structure of bovine viral diarrhea virus 1 (BVDV1) envelope glycoprotein E2 was reported (PDB ID 2YQ2) (El Omari et al., 2013). On the basis of the crystal structure of BVDV E2 protein, small-molecule high-throughput docking was performed to identify the molecules that likely bind to the envelope protein E2 of BVDV. Several structurally different compounds were purchased as well as synthesized. These were then assayed for antiviral activity against BVDV. Their possible binding determinants were characterized by MD simulations. A common pattern of interactions was observed between active molecules and amino acid residues in the binding site in E2. These findings are believed to offer a better understanding of the interaction of BVDV E2 with these inhibitors, as well as benefit the discovery of novel and more potent BVDV antivirals (El Omari et al., 2013). In 2015, crystal structure of the NS3 helicase of the pestivirus was reported (PDB ID 4CBG) (Tortorici et al., 2015). In 2017, the closed-conformation crystal structure of the full-length pestivirus NS3 with its NS4A protease cofactor segment was established (PDB ID 5WX1) (Zheng et al., 2017) (Fig. 19.12).

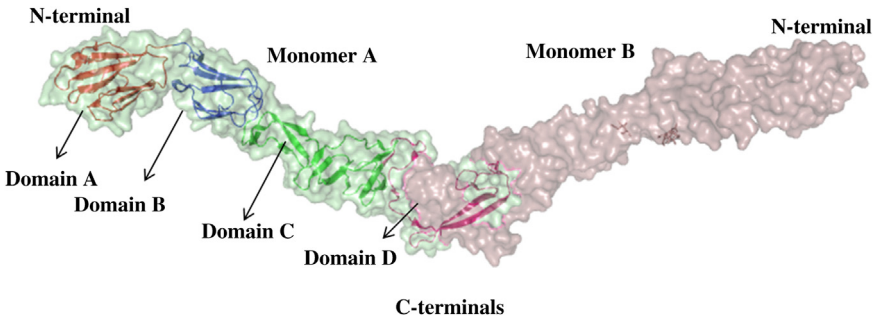

FIGURE 19.12 Crystal structure of bovine viral diarrhea virus 1 (BVDV1) envelope glycoprotein E2, pH 8 at 2.58 Å.

Dimeric structure consisting of two monomeric units: A and B. Domains of monomer A starting from the N terminus are labeled. Domain A is starting from residues 1-87, domain B from 88-164, domain C residues from 165-271 and domain D residues from 272-333 (PDB ID: 2YQ2) (El Omari et al., 2013).

19.3 Conclusion

In this chapter, various viral proteins that are necessary for the survival of virus inside host are discussed such as viral polymerase, viral helicase, protease, etc. The approaches used in predicting the structure of these necessary proteins are elaborated. Various drug molecules or antivirals designed on the basis of structure of these proteins are highlighted. The details of the available inhibitors and the potential antiviral candidates are disclosed. Vaccination status against animal viruses is discussed. Further improvement in these and the need of novel structure-based drugs and their clinical testing is emphasized.

Protein structure-based drug design has been contributing to the drug discovery process since the early 1990s. This structural knowledge of interaction between drugs and the target protein has been applied mainly to predict potency changes of chemically modified lead compounds. With the help of 3D-structural information, additional aspects of the drug discovery process have become predictable. Selectivity of compounds between homologous or orthologous proteins can be predicted. This provides new possibilities to design selective compounds or predict the suitability of animal models for pharmacodynamic studies. Antivirals display a variety of mechanisms of action. Antivirals may enhance the animal immune system or block a specific enzyme or a particular step in the viral replication cycle. As viruses are obligate intracellular parasites that use the host's cellular machinery to survive and multiply, it is essential that antivirals do not harm the host. However, a major concern is that viruses are continually developing new antiviral resistant strains due to their high mutation rate. This demands for mandatory search or development of new antiviral compounds. With the help of structure-based drug designing, there is progress in preclinical drug discovery.

There is a deficit of information between identified hits and the many criteria that must be fulfilled side by side to convert these hits into preclinical candidates that have a real chance to become a drug. This gap can be bridged by investigating and understanding the interactions between the ligands and their receptors. Accurate calculations of the free energy of binding are still elusive. Better knowledge of all these will help in finding good drug candidates to combat the diseases caused by animal viruses.

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