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MINI REVIEW ARTICLE

Antiviral Drug Targets of Single-Stranded RNA Viruses Causing Chronic Human Diseases

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Abstract: Ribonucleic acid (RNA) viruses associated with chronic diseases in humans are major threats to public health causing high mortality globally. The high mutation rate of RNA viruses helps them to escape the immune response and also is responsible for the development of drug resistance. Chronic infections caused by human immunodeficiency virus (HIV) and hepatitis viruses (HBV and HCV) lead to acquired immunodeficiency syndrome (AIDS) and hepatocellular carcinoma respectively, which are one of the major causes of human deaths. Effective preventative measures to limit chronic and re-emerging viral infections are absolutely necessary. Each class of antiviral agents targets a specific stage in the viral life cycle and inhibits them from its development and proliferation. Most often, antiviral drugs target a specific viral protein, therefore only a few broad-spectrum drugs are available. This review will be focused on the selected viral target proteins of pathogenic viruses containing single-stranded (ss) RNA genome that causes chronic infections in humans (*e.g*. HIV, HCV, Flaviviruses). In the recent past, an exponential increase in the number of available three-dimensional protein structures (>150000 in Protein Data Bank), allowed us to better understand the molecular mechanism of action of protein targets and antivirals. Advancements in the *in silico* approaches paved the way to design and develop several novels, highly specific small-molecule inhibitors targeting the viral proteins.

Keywords: RNA viruses, chronic diseases, drug targets, inhibitors, antivirals, HIV, hepatitis, flaviviruses, protein structures.

1. INTRODUCTION

Human chronic viral infections are responsible for millions of deaths worldwide, motivating both academia and industry towards developing highly effective, novel antiviral drugs [1]. Ribonucleic acid (RNA) viruses are the causative agents of several notable chronic diseases in humans. These viruses contain RNA as their genetic material and can be further classified based on the type of RNA molecule, namely single-stranded (ss) RNA, double-stranded (ds) RNA and circular RNA (circRNA). ssRNA viruses can be classified further based on the sense of nucleic acid as plus (+) and minus (-) RNA viruses or retroviruses if a DNA stage is present in their life cycle. Some RNA viruses can subvert the innate and adaptive immune system and remain persistent, mostly in several immune privileged (*e.g*. ocular tissue, liver, central nervous system, *etc*.) and in non-privileged (*e.g*. lungs, myocardium, *etc*.) tissues causing chronic infections [2]. The molecular mechanisms behind persistent viral infections and the factors driving selected RNA viruses to be capable of causing chronic infections are not fully understood [3]. Human immunodeficiency virus-1, hepatitis, and re-emerging viruses like Zika virus (ZIKV) are well-known examples of RNA viruses causing chronic infection in humans [2]. The direct-acting antivirals (DAA) are the most effective treatment targeting chronic diseases such as HIV-1 and HCV [1, 4]. The non-structural (NS) proteins mostly act as essential components of the viral replication machinery and also contain crucial enzymes required for the viral life cycle. The viral enzyme inhibition approach seems to be extremely successful in the case of chronic HIV-1 and HCV infections [4].

In addition, new developments such as single-cell sequencing aided by multiparameter flow cytometry, which can assist in understanding the amount of heterogeneity in immune cell compartments during acute and chronic viral infections, as well as other high-throughput approaches will provide a better understanding of the adaptations of immune cells to chronic conditions [5]. RNA viruses mutation rate is approximately a million times higher than their host because during evolution the viruses select for faster-replicating polymerases which can eventually error-prone but under the fatal threshold [6]. Therefore, antiviral therapies can induce drug resistance due to the extremely high mutation rates of RNA viruses and also circumvent vaccine-induced immu-

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nity, which implicates re-design of novel therapeutics in order to combat RNA viral infections [7]. Recent advancements in available traditional instruments and new cutting-edge technologies in the fields of structural biology include, tools and techniques such as X-ray crystallography, nuclear magnetic resonance (NMR) and cryo-electron microscopy (cryo-EM) and Cheminformatics/computational drug discovery (CDD) by molecular docking as well as structure-based drug design (SBDD) approach with additional pharmacodynamics data and pharmacokinetic analyses have enabled us to develop novel and next-generation antivirals that are more effective against the already exploited viral protein targets [8, 9].

This review article will mainly highlight chronic viral infections in humans caused by selected single-stranded RNA viruses and describe how the available atomic structures of viral target proteins are being scrutinized to combat chronic viral infections to develop novel antiviral drugs. Although several target proteins are available for each selected RNA virus, for clarity, we will focus on only one specific viral protein for each (Table **1**).

2. RNA VIRUSES CAUSING CHRONIC INFECTIONS IN HUMANS

2.1. (+) ssRNA VIRUSES

2.1.1. Human Immunodeficiency Virus (HIV-1)

HIV belongs to the *Retroviridae* family and the genus *Lentivirus*. It was discovered in 1983 and causes a chronic and life-threatening condition called Acquired Immunodeficiency Syndrome (AIDS). Chronic disease conditions can last for 1-10 years or more and result in several other reported clinical complications, including cancer, osteoporosis, psychiatric disorders, *etc* [1, 10]. HIV can be classified into two types, HIV type 1 (HIV-1) and type 2 (HIV-2) based on the genetic and viral antigen differences. Globally, HIV-1 along with its less widespread cousin HIV-2 has infected more than 70 million individuals, since the beginning of the epidemic. About ~36.9 million individuals were reported to be HIV positive at the end of 2017 and caused millions of deaths every year, according to the World Health Organization (WHO). The viral particle is spherical and ~100-130 nm in diameter. HIV contains two copies of a < 10 kb (+) ssRNA genome which encodes sixteen mature proteins including envelope glycoproteins, the capsid protein, and the replication enzymes called protease – PR, reverse transcriptase – RT, and integrase – IN [11-13]. Apart from the structural polyprotein Gag that is later processed into a MA – matrix, CA – capsid, NC – nucleocapsid and p6, non-structural proteins, and envelope proteins (surface subunit – SU and transmembrane region – TM). HIV genome also code for regulatory proteins namely, tat (transactivator protein) and rev (RNA splicing regulator) that are crucial for viral replication and others are nef (negative regulating factor), vif (viral infectivity factor), vpr (virus protein r) and vpu (virus protein unique) which play a role during viral replication, budding or pathogenesis [14] (Fig. **1A**).

Commercially available anti-HIV drugs target different stages of the viral life cycle: (i) entry, (ii) reverse transcription (reverse transcriptase), (iii) integration (integrase), and

Table 1. Consolidated table of the RNA viruses causing chronic infections in humans and selected target protein discussed in this current review article with/without antiviral compounds.

Viruses Causing Chronic Infections	Target Protein	PDB ID	Antivirals / Small Molecule Inhibitors	Refs.
Human Immunodeficiency virus (HIV-1)	Reverse Transcriptase (RT)	3V81, 6HAK	Nevirapine	[26, 27]
Human T-lymphotropic virus (HTLV-1)	Protease (PR)	3WSJ	Indinavir	[37]
Hepatitis C Virus (HCV)	NS3/4 protease (PR)	3SV6	Telaprevir	[52]
Flaviviruses (ZIKV)	MTase (N-terminal of NS5)	5MRK	Sinefungin	[67]
Coxsackievirus (CV)	RdRp (3D polymerase)	4K4Y	NA	[77]
Rubella virus (RV/RUB)	Capsid protein (CP)	4HAR	NA	[88]
Measles virus (MeV)	Prefusion protein (F)	5YZC, 5YZD	AS-48, FIP	[93]
Ebola virus (EBOV)	Glycoprotein (VP30)	5VAP, 5VAO	NA	[108]
Respiratory Syncytial Virus (RSV)	Nucleoprotein (N)	2WJ8	NA	[115]

(iv) maturation (protease). All of these proteins may mutate rapidly leading to increased drug resistance [15]. Therefore, combinatorial antiretroviral therapies (cART) targeting multiple stages are more effective [16]. The highly active antiretroviral therapy (HAART) consists of 30 antiviral drug molecules as a cocktail that targets four crucial steps in the HIV-1 replication cycle [9]. Several approved RT inhibitor drugs are commercially available including Abacavir, Emtricitabine, Didanosine, and Lamivudine [17]. Persistence and latency of HIV infection is multifactorial and eradication requires combinatorial approaches [16]. Nucleoside RT inhibitors (NRTI) were the first ART used to treat HIV patients but were not highly effective [15]. Similarly there are also several effective anti-HIV drugs that have been developed to combat HIV infection. Therefore, administration of additional inhibitors targeting the other targets either increases life expectancy or reduces the disease progression to AIDS or death. In addition, HIV-1 nuclear import mechanism is another important event for an antiviral target as it is quite crucial for its replication but still not completely understood as it involves multiple viral and host factors [18].

2.1.1.1. HIV-1 Protein Drug Target - Reverse Transcriptase (RT)

RT is a key enzyme which converts the viral RNA (vRNA) into linear dsDNA (RNA-directed DNA polymerase) and has additional roles in the HIV life cycle. The critical step of reverse transcription of the HIV-1 genome is primed by $tRNA^{Lys3}$, whose 3' end is complementary to the viral primer-binding site (PBS) and the RNA structural rearranges upon binding [19, 20]. The RNA structure plays an essential role in altering RT conformation during reverse transcription [21, 22]. Several RT structures shed light on the function of this enzyme, *e.g.* RT bound to commercially available drug nevirapine and DNA complex structure (Fig. **1C**) [23, 25, 26]. The RT antiviral drugs include two classes, the nucleoside and non-nucleoside RT inhibitors (NRTIs and NNRTIs, respectively). Initiation of HIV-1 RT is the first step in the replication cycle. The initiation of reverse transcription involves recruitment of host t-RNA^{Lys3} to the PBS of vRNA to initiate DNA synthesis, which was also recently elucidated from the structure of HIV-1 RT co-crystallized with dsRNA which mimics Reverse Transcriptase initiation complex (RTIC) before incorporation of nucleotides and this confirmation is also quite similar to the RT bound to NNRTI and dsDNA. Therefore, RTIC initiation complex revealed structural features prior to nucleotide incorporation and the dsRNA interactions in the primer binding site, and should facilitate the design of new anti-HIV drugs [21, 27] (Fig. **1B**).

Another recent structure of a drug-resistant HIV-1 RT mutant co-crystallized with NNRTI thiophene [3,2-d] pyrimidine should aid in developing effective NNRTIs even against drug-resistant HIV-1 variants [28]. Recent studies have shown that individuals infected by HIV-2 can also develop AIDS without proper antiretroviral therapy [29]. Therefore, computational methods such as homology model-

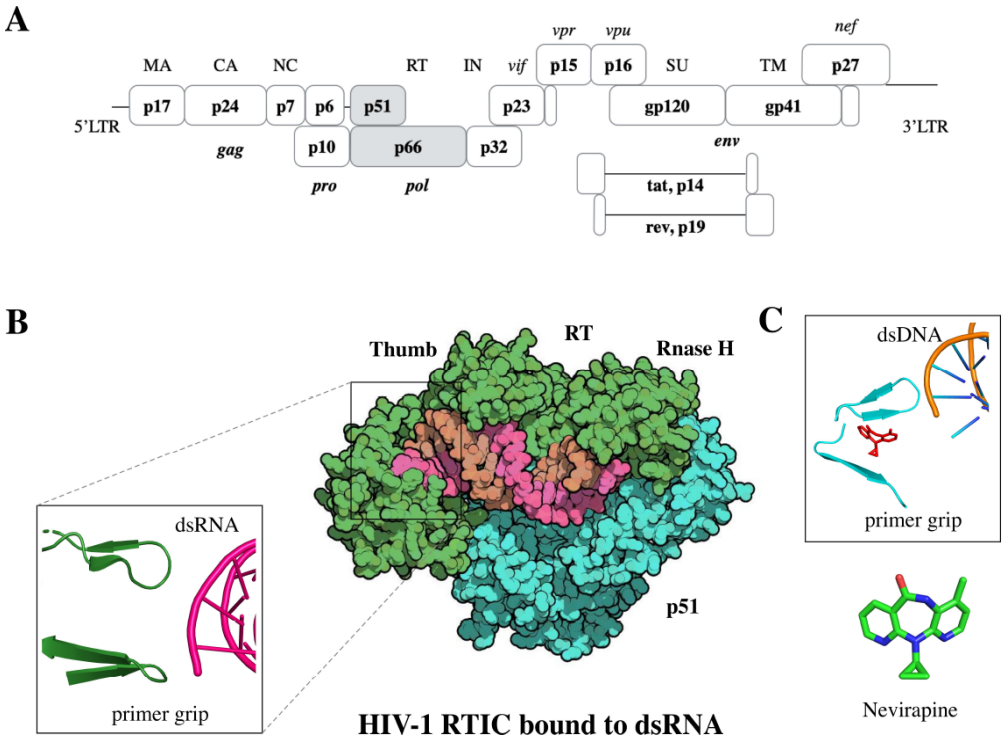

Fig. (1). HIV-1 genome architecture and protein drug target - Reverse Transcriptase (RT). A. HIV-1 genome architecture with labelled structural, non-structural and regulatory genes (selected target shaded in grey), **B**. Overall surface representation of the structure of HIV-1 RT (green) complexed with dsRNA displaying the RNA (red and orange) binding cleft in the initiation complex (PDB ID: 6HAK) and the lower molecule p51 (blue), a cleaved RT with inactive fold, inset 1 shows the zoomed region from the RTIC bound to dsRNA, a potential region for designing novel antiviral compound, and **C**. the inset 2 shows similar zoomed in region from a RT structure bound to antiviral compound Nevirapine and dsDNA very close to the primer grip region [26] (PDB ID: 3V81). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

ling, *in silico* mutation, virtual screening, and quantitative structure-activity relationship (QSAR), docking studies, also play essential roles in anti-HIV drug and vaccine development against both types of HIV infections [30].

2.1.2. Human T-cell Lymphotropic virus Type 1 (HTLV-1)

HTLV-1 belongs to the *Retroviridae* family and the genus delta type *Retrovirus*. It was discovered in 1980 and was the first oncogenic virus identified. It infects ~10-20 million people globally with $CD4^{+}$T cells as the primary target and causes adult T-cell lymphoma (ATL), tropical spastic paraparesis, HTLV-1 associated myelopathy and other inflammatory diseases [31-34]. The virion ranges from 80-100 nm in diameter and contains two covalently bonded RNA molecules and an 8.5 kb genome that encodes trans-regulatory proteins (tax/rex), accessory proteins (p12, p13, p30 and HTLV-1 bZIP factor - HBZ) and three structural proteins (gag, pol and env). HTLV-1 contains an ssRNA which is reverse transcribed into dsDNA. Delta retroviruses use a frameshift mechanism and encode the precursor proteins Gag-Pro and Gag-Pro-Pol. The Gag-Pro precursor proteins encode both structural proteins (matrix – MA, capsid – CA and nucleocapsid – NC). During maturation, non-structural proteins are also produced (transframe protein – TF, protease – PR and two small proteins, p1 and p2). The Gag-Pro-Pol precursor proteins produce MA, CA, TF, PR, p1, RT, RNaseH – RH and integrase – IN. The HTLV-1 envelope proteins include surface subunit – SU and transmembrane region – TM. The pX region in the genome contains a sequence for both regulatory and accessory proteins which are responsible for HTLV-1 infection and pathogenesis [31]. The vRNA integrates into the host genome and encodes the trans-acting factor, tax (a 40 kD phosphoprotein in the pX region), which is believed to trigger a number of transformation events in the early stages of infection (Fig. **2A**) [34, 35].

As in the case of other retroviruses, the most attractive drug targets are the enzymes PR, RT, and IN [31]. There is no approved antiviral drug targeting HTLV-1 viral entry. Chondroitin sulfate type E interacts with the C-terminal of SU (env), blocks viral binding and exhibit antiviral activity [36].

2.1.2.1. HTLV-1 Protein Drug Target - Protease (PR)

HTLV-1 protease is an aspartic acid PR that plays an important role in processing Gag and Gag-(Pol)-Pol polyproteins during maturation and replication. The PR functions as a homodimer and the substrate-binding site comprise of three regions: the C-terminal region, the flap region, and the active site [31]. HTLV-1 protease is closely related to the HIV protease, but still, the amino-acid residues in the flap and C-terminal regions are different. The two aspartic acid residues in the active site are responsible for the hydrolysis of polyprotein by the acid-base mechanism. HIV PR inhibitor Indinavir has shown an inhibitory effect also against HTLV-1 PR [37] (Fig. **2B**).

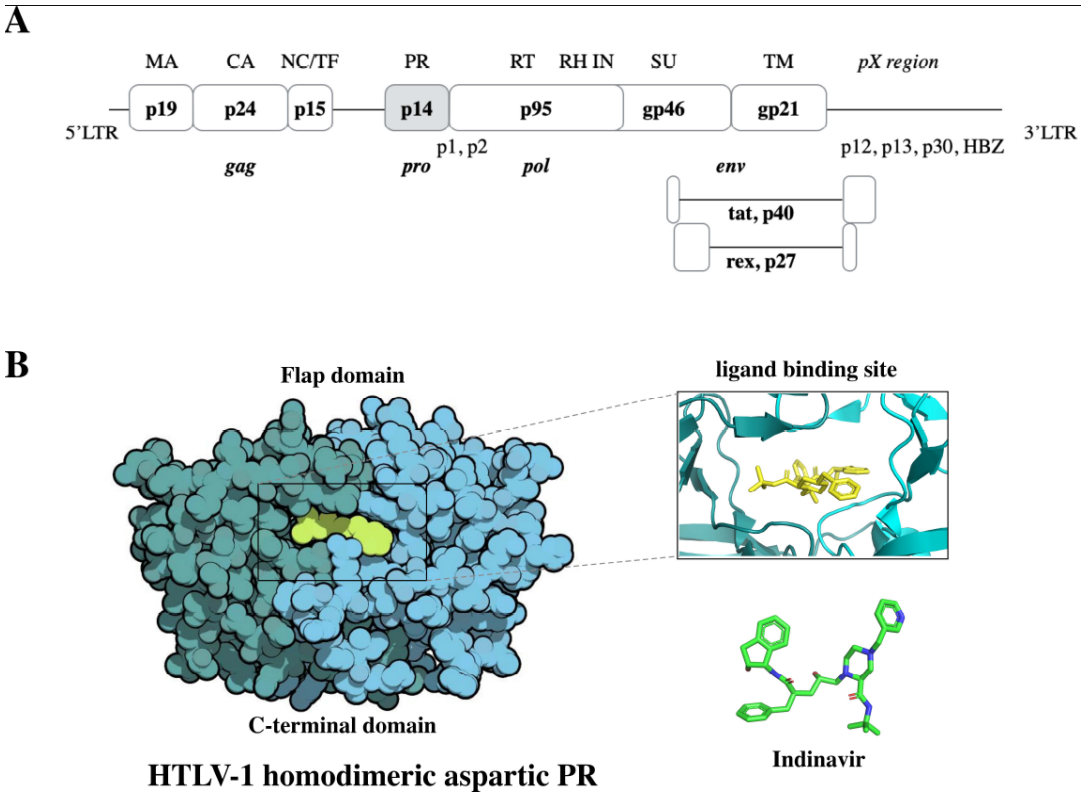

Fig. (2). HTLV-1 genome architecture and protein drug target - Protease (PR). A. HTLV-1 genome architecture with labelled structural, non-structural and regulatory genes (selected target shaded in grey), and **B**. Overall structure of homodimeric HTLV-1 aspartic PR structure (teal and blue) displaying the ligand binding active site boxed, flap and C-terminal region (PDB ID: 3WSJ), inset shows the zoomed in active site region with the non-peptide antiviral PI Indinavir (yellow) and its chemical structure below. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The major target proteins of HTLV-1 are the enzymes involved in viral replication, which seems to be very effective in treating HIV-1 infection. Anti-HIV drugs against RT, PR, and IN have also been repurposed and used to treat HTLV-1 infection. For example, Indinavir is the first non-peptide inhibitor, which can be used in developing more effective inhibitors. In addition, accessory proteins tax and HBZ can also be targeted to develop anti-HTLV-1 drugs.

2.1.3. Hepatitis C Virus (HCV)

HCV belongs to the family *Flaviviridae* and the genus *Hepacivirus*. It was discovered in 1989 and has an extremely high mutation rate [38]. The viral particle is spherical, ~68 nm in diameter, and contains (+) ssRNA, the genome is ~10 kb and it encodes 10 viral proteins. The HCV genome encodes three structural proteins called Core (C) and Envelope (E1 and E2), and seven non-structural proteins called NS1, NS2, NS3, NS4A, NS4B, NS5A and NS5B [39] (Fig. **3A**). Hepatitis C infection leads to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma, causing ~500,000 deaths per year, according to the WHO [1, 40, 41]. Chronic disease or relapses of acute diseases are common for persistent HCV infections, which is difficult to diagnose [42]. HCV can also produce carcinogenic effects *via* chronic inflammation, even though the virus lacks a mechanism of integrating vRNA into the host genome [35]. The most important HCV protein targets are NS5 and NS3/4A. NS5B mediates genome replication by using its RNA dependent RNA polymerase activity (RdRp). For this reason, NS5B is considered an important drug target for anti-HCV inhibitors. An NS3 protein with its cofactor, the serine protease NS4A, cleaves the viral polyprotein to generate proteins essential for replication of the RNA genome, making it a potent drug target for antivirals [43, 44] (Fig. **3B**).

In addition, other hepatitis viruses (A, B, D and E) are known to cause chronic infections in humans, among which satellite Hepatitis D virus (HDV) can result in severe chronic conditions of all viral hepatitides. HDV is a small (36 nm in diameter) spherical virusoid/defective RNA virus that contains (-) ssRNA with 70% self-complementarity forming circRNA (1.7 kb) that are partially double-stranded, rod-like RNA genome. HDV being a satellite virus, can only co-infect along with HBV causing delta hepatitis. Molecular and structural understanding of these satellite ssRNA viruses can help in identifying novel therapeutic targets for combating such severe forms of hepatitis co-infection [45].

2.1.3.1. HCV Protein Drug Target: NS3/4A Serine Protease

The HCV protease NS3/4A is essential for protein processing and replication of HCV [43]. Protease inhibitors are small molecules that inhibit the activity of proteases such as NS3. Several protease inhibitors (Boceprevir, Paritaprevir and Telaprevir) have been approved in the USA to use with approved antivirals such as pegylated interferon and Ribavirin. Several co-crystal structures of NS3/4A PR wildtype and mutants with Telaprevir display the Telaprevir binding site, which includes the catalytic triad residues that explained the better potency of Telaprevir to a mutant variant (Fig. **3B**). In direct-acting antiviral treatments, NS3/4A protease inhibitors such as Glecaprevir and Voxilaprevir have exhibited high antiviral activity when combined with an NS5A inhibitor *e.g.* Velpatasvir [43-45]. Sofosbuvir is a direct-acting antiviral inhibitor against NS5B. Currently available

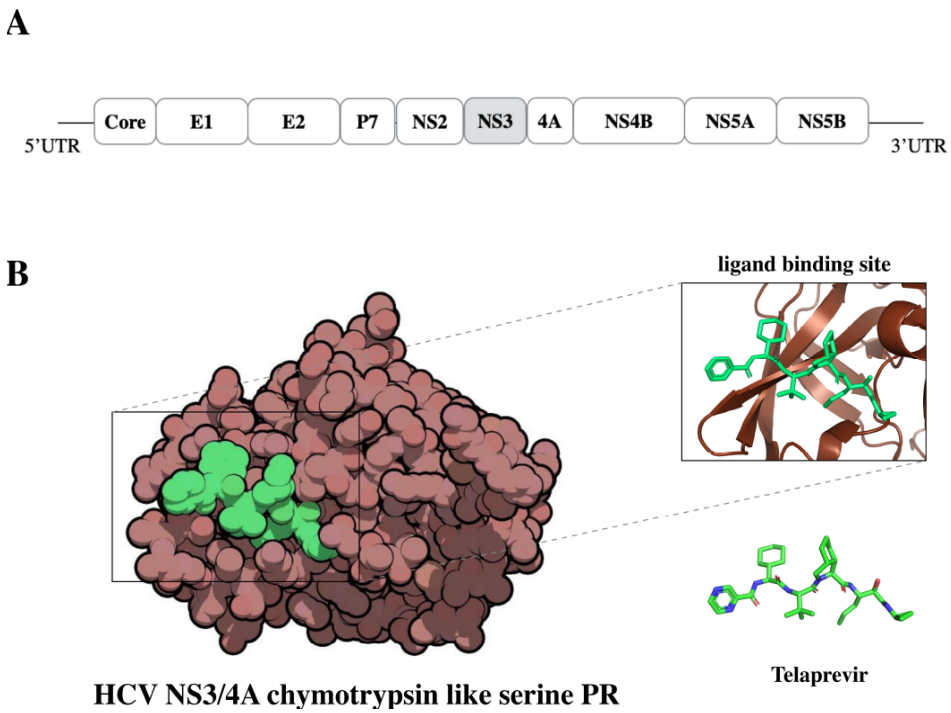

Fig. (3). HCV genome architecture and protein drug target - NS3/4A protease (PR). A. HCV genome architecture with labelled structural and non-structural proteins (selected target shaded in grey), and **B**. Overall structure of HCV NS3/4A protease (brown) structure displaying the ligand binding site (PDB ID: 3SV6), inset shows the zoomed in region of the antiviral Telaprevir (green) binding site and its chemical structure below. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

antiviral combination therapies can cure all genotypes of chronic Hepatitis C virus Sofosbuvir and Velpatasvir (Epclusa) or triple combination in addition with Voxilaprevir (Vosevi) [46-49].

Computational and structural biology approaches provide novel strategies to design antivirals less susceptible to drug resistance and side-effects, that can be used to fight evolving viruses and to treat chronic HCV infections more effectively, and the best *e.g.* FDA approved Vosevi (Sofosbuvir, Velpatasvir and Voxilaprevir) [49, 50].

2.1.4. Flaviviruses

The genus *Flavivirus* of the family *Flaviviridae* includes several arthropod-borne viruses such as Zika virus (ZIKV), dengue virus (DENV), West Nile virus (WNV), yellow fever virus (YFV), and many more. *Flaviviruses* cause several diseases which can result in chronic infections in humans. These viral infections are a a threat to public health and re-emerging viruses are a global risk [53-55]. The virion is spherical with a diameter of ~50 nm and contains non-segmented (+) ssRNA. The genome is ~10-11 kb, and encodes three structural proteins (capsid – C, membrane protein precursor – prM and envelope – E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Fig. **4A**) [56, 57]. Clinical manifestation of flavivirus infection is diverse. For example, Dengue infection ranges from asymptomatic, undifferentiated or mild dengue fever to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [58, 59]. WNV causes acute febrile illness and its progression results in meningitis, encephalitis, and death. Chronic gastrointestinal dysmotility syndrome is commonly observed after most flaviviral infections [60].

Promising targets for drugs that inhibit viral replication include the NS2B-NS3 endoprotease and the NS5 enzymes (Table **2**) [61-63]. Flaviviral enzymes exhibit high structural and functional similarities which should facilitate the design of pan-flaviviral inhibitors [64, 65]. For example, Sinefugin and S-adenosyl homocysteine and few more inhibitors have shown to bind MTase efficiently but had problems with cell permeability and toxicity [56, 62]. By using the available MTase structures in combination with computational docking, fragment-based and SBDD methods, it should be possible to develop specific inhibitors that bind the lipophilic cavity of viral MTase [66, 67].

2.1.4.1. Flavivirus Protein Drug Target - MTase Domain of NS5

NS5 is crucial for viral RNA synthesis and modification, performing two different enzymatic functions. Its methyltransferase (MTase) domain is responsible for the methylation of the nascent RNA that is synthesized by the RNA dependent RNA polymerase (RdRp) domain [57, 62]. The N-terminal MTase domain has both N7-methyltransferase and guanylyltransferase activities and produces cap structures in mature RNAs [68]. The crystal structures of ZIKV, DENV, WNV MTases reveal a cavity next to the S-adenosyl-L-methionine (SAM) binding site, which might be helpful in developing effective inhibitors against MTase and better understanding this central player in replication [54, 68, 69]. For example, ZIKV MTase bound to Sinefugin is shown in Fig. **4B**. The RNA capping structure includes a guanosine residue linked to the 5' end of mRNA through a 5'-5' triphosphate bond (RNA cap – mGpppNm). Methylation of the N-7 position of guanosine is crucial for stability and stimulates translation, while 2'-O methylation of the ribose

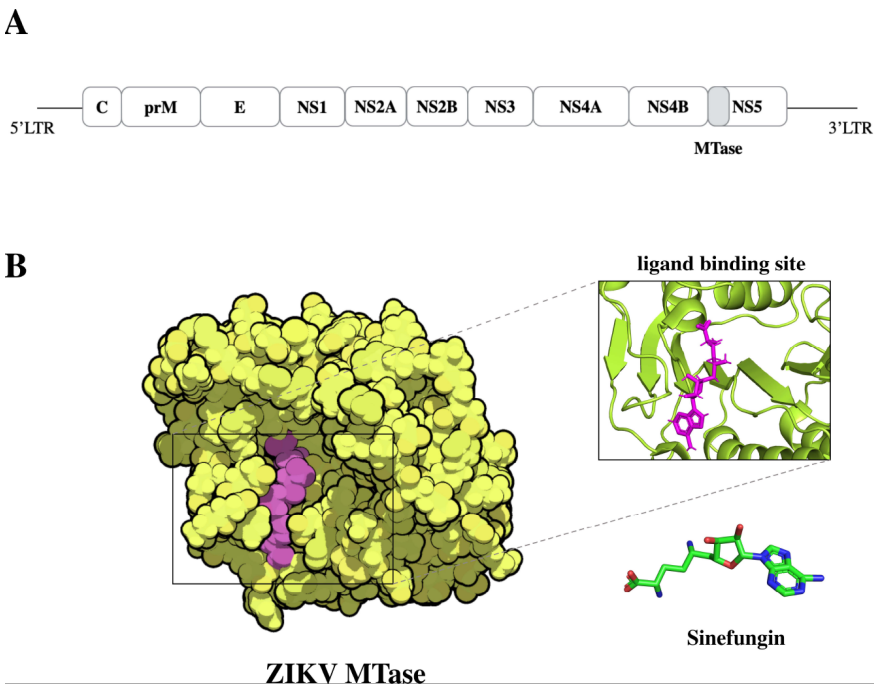

Fig. (4). Flavivirus genome architecture and protein drug target - ZIKV MTase. A. General *Flaviviruses* genome architecture with labelled structural and non-structural proteins (selected target shaded in grey), and **B**. Overall structure of ZIKV MTase structure (yellow) displaying the ligand binding site (PDB ID: 5MRK), inset shows the zoomed in region of the inhibitor Sinefungin (magenta) in the binding site and its chemical structure below. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

protects the vRNA from the host-cell nucleases [70]. Therefore, targeting enzymes responsible for viral RNA capping is an attractive and important target to develop antivirals.

To date, no effective clinical treatment has been developed for any flaviviral infection. Therefore, developing novel therapeutic agents will be a public health priority and a major challenge in the 21st century [56]. Developing vaccines against these flaviviruses is a challenge due to antibody-dependent enhancement (ADE), and only vaccines for selected flaviviruses are currently available [70, 71]. Example of advances in ZIKV vaccine development was recently reviewed in detail [57, 72].

2.1.5. Coxsackievirus (CV)

CV belongs to the family *Picornaviridae* and the genus *Enteroviruses*, CV is a common pediatric virus mainly responsible for aseptic meningitis causing severe morbidity and mortality in infants. The viral particle is ~30 nm in diameter, contains a ssRNA genome of ~7.5kb, which encodes 11 proteins comprising the four structural proteins VP1, VP2, VP3 and VP4 and seven non-structural proteins 2A, 2B, 2C, 3A, 3B, 3C and 3D (Fig. **5A**) [73]. CV infections cause common cold (flu-like) symptoms and mild gastroenteritis. Coxsackievirus type B (CVB) is also responsible for chronic conditions like type-1 diabetes [74]. CVB is widely known to establish cytolytic infection, and persistent CVB infections lead to chronic inflammation of infected organs and are also associated with autoimmune-related disorders such as chronic myocarditis, diabetes and chronic inflammatory myopathy [75]. CVB RNA persistence in heart tissue is associated with chronic disease conditions [76]. Two important non-structural drug targets of CVB are the 3C and 3D proteins. 3C is a cysteine protease that catalyzes co-translational cleavage of the polypeptide into mature viral proteins. It also cleaves an array of cellular transcription and translation factors, thereby facilitating the viral life cycle and making it a potent antiviral target. 3D is an RNA-dependent RNA polymerase (RdRp). CVB RdRp is highly similar to poliovirus and rhinovirus $3D^{pol}$ [77-79].

Although several attractive and highly conserved protein drug targets involved in different points of the life cycle of picornavirus exist, there is still no effective broad-spectrum antiviral molecules available [82] except for compounds targeting the host factor PI4KB [83, 84]. Pleconaril was found to be an effective antiviral compound against resistant CVB strains and Ribavirin is an effective broad-spectrum antiviral compound (reviewed in [75]). Fluoxetine is found to be effective in treating persistent pancreas infection by CVB3 [74]. Other examples, antivirals found to target CVB 2C protein which include Pirlindole, Dibucaine, Zuclopenthixol, *etc*. (Table **2**) [81].

2.1.5.1. CVB Protein Drug Target - RdRp/$3D^{pol}$

$3D^{pol}$ is an RNA-dependent RNA polymerase (RdRp). It plays a key role in transcription and also in catalyzing viral genome replication. Crystal structures of CVB $3D^{pol}$ and CVB $3D^{pol}$ complexed with the protein primer 3B (also known as VPg – viral protein-genome linked) are available [74-76]. They illustrate the characteristic fold of a picornavirus polymerase, which comprises a cupped normal

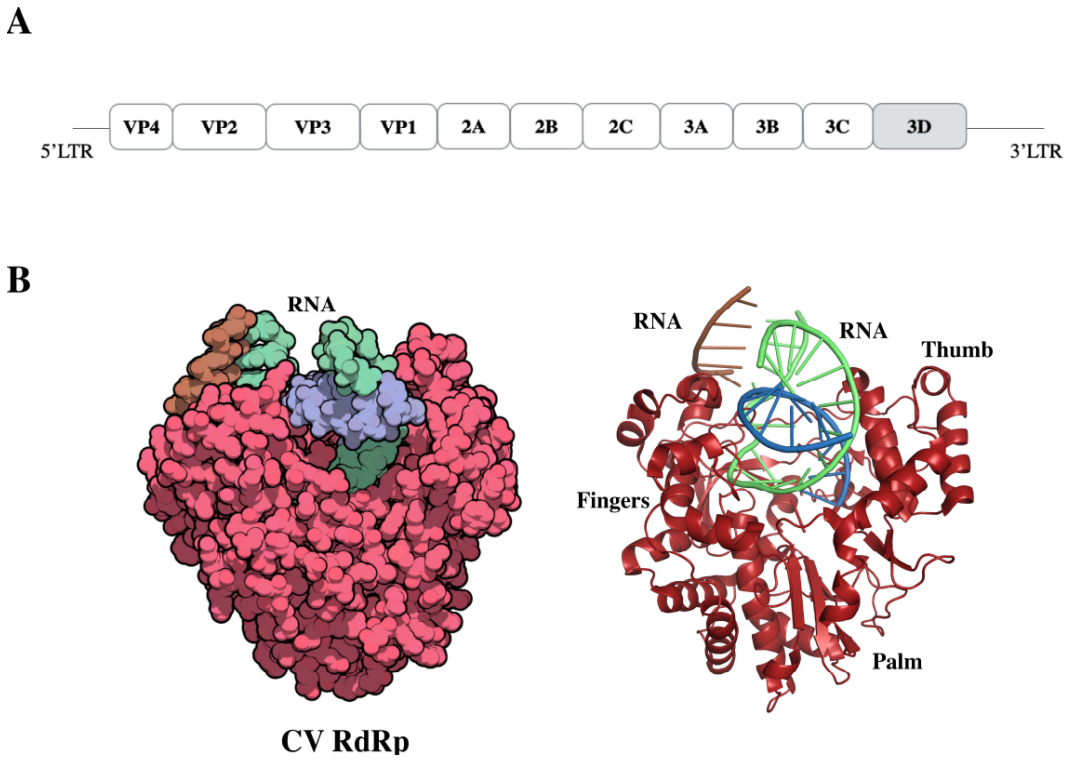

Fig. (5). Coxsackievirus (CV) genome architecture and protein drug target - RdRp/$3D^{pol}$. **A**. CV genome architecture with labelled structural and non-structural proteins (selected target shaded in grey), and **B**. Overall surface and cartoon representation of CVB RdRp (red) elongation complex (EC) displaying the RNA-RNA junction (PDB ID: 4K4Y). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 2. Selected RNA viruses with available viral target proteins and their representative examples of antiviral compounds.

Virus Name	Target Protein	Antivirals / Small Molecule Inhibitor	Refs.
Human Immunodeficiency Virus (HIV-1)	MA	2-(4-{[3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl]methyl})-1-piperazinyl)-N-(4-methylphenyl)acetamide / compound 7	[136]
	CA	Ebselen, PF74, CAP-1, BD-1, BM-1, I-XW-053, compound 34, PF 74, BI-1 and BI-2; CAF, NYAD-1, NYAD36/66/67, CAC-1, NYAD-201	[137-139]
	NC	DiselenoBisBenzamides - DISeBAs, Azodicarbonamide	[140, 141]
	PR	Amprenavir, Tipranavir - TPV, Darunavir, - DRV/Cobicistat, Indinavir - IDV, Atazanavir - ATV, Saquinavir - SQV, Nelfinavir - NFV, Ritonavir - RTV, Lopinavir/ritonavir - LPV/r , Fosamprenavir - FPV	[142-145]
	RT	NRTIs: Abacavir - ABC, Emtricitabine - FTC, Didanosine -DDI, Lamivudine - 3TC, Stavudine - d4T, Tenofovir Disproxil Fumerate - TDF, Zalcitabine - ddC, Azidothymidine/Zidovudine - AZT/ ZDU, and NNRTIs: Nevirapine - NVP, Delavirdine - DLV, Efavirenz - EFV, Etravirine - TMC125, Rilpivirine - TMC278, Dapivirine - DPV, (+)-Calanolide A	[26, 27], [145, 146]
	IN	Doultegravir - DTG, Elvitegravir - EVG, Bictegravir - BIC, Raltegrivir - RAL	[145]
	E (gp120 & gp41)	BMS-378806, BMS-626529, BMS-488043,NBD-556, JRC-II-191,compound 6, 18A, Enfuvirtide - EFV, ADS-J1, NB-2, NB-64, O2N-[Ala]-Nap-OH, 14g, SM038, D9, SB-C09, SB-C01	[147-150]
Hepatitis C virus (HCV)	C	Biotinylated SL209	[151]
	E1	Iminodipyridinopyrimidine - IDPP	[152]
	E2	281816	[153]
	p7	BIT225	[154]
	NS2	Epoxide derivative	[155]
	NS3/4A	Telaprevir - TVR, Boceprevir - BOC, Simeprevir - SMV, Vaniprevir - MK-7009, Asunaprevir - ASV, Asunvepra, Paritaprevir - PTV, Grazoprevir - GZR, Glecaprevir/ABT493 - GLE, Voxilaprevir/GS-9857 - VOX, Danoprevir/ITMN-191 - INN	[49, 52, 156]
	NS4B	Clemizole	[157-159]
	NS5A	Ledipasvir, Ombitasvir, Daclatasvir, Elbasir, Velpatasvir, Pibrentasvir, Odalasvir/ACH-3102, Ravidasvir/PPI-668, MK-8408	[160]
	NS5B	Nucleos(t)ide analogues: Valopicitabine/NM-283, Balapiravir/R-1626, PSI-6130/R-7128, 7-Deaza-2′-c-methyl-adenosine, Sofosbuvir, Uprifosbuvir/MK-3682 - UPR, ALS-2200/VX-135, ACH 3422, Adafosbuvir/AL 335. Non-nucleos(t)ide analogues: Nesbuvir/HCV-796, XTL-2125, A-837093, AG-021541, Dasabuvir - DSV, Beclabuvir/BMS-791325, ABT-072, GS-9669, *Ritonavir*/TMC647055, MBX-700	[160, 161]

(Table 2) contd….

Virus Name	Target Protein	Antivirals / Small Molecule Inhibitor	Refs.
Flaviviruses	C	ST-148	[162]
	E	Nanchangmycin, 2,4-diamino and 4,6-disubstituted pyrimidines, NITD-448, Celgosivir	[163-165]
	NS2B–NS3	Temoporfin, NSC157058, BP13944, Aprotinin	[165-167]
	NS3	Suramin, Ivermectin, ST-610, Pyrrolone	[165, 168]
	NS4	Spiropyrazolopyridone, Aminothiazole NITD-618, SDM25N, CCG-3394, CCG-4088	[169, 170]
	NS5	RdRp: Nucleoside analogues: 7-deaza-2'-*C*-methyladenosine (7DMA), 2-C methyladenosine (2-CMA), 2-C-methylcytidine (2-CMC), 2-C-methylguanosine (2-CMG), 2-C-methyluridine(2-CMU), Favipiravir, Sofosbuvir - SOF, BCX4430, DMB213, 2'-C-ethylnyl-UDP, and MTase: Sinefungin, S-adenosyl homocysteine, Ivermectin, BG-323, 4-HPR	[165, 67], [171-173]
Ebola Virus (EBOV)	GP	Bafilomycin A1, Chlorpromazine Toremifene, Benztropine, Bepridil, Paroxetine, Sertraline, MBX2254, MBX2270	[174-176]
	VP24	Ouabain, Nilotinib, Miglustat, Clomiphene	[177]
	VP35	Favipiravir - T-705, GS-5734, BCX4430, AS1369, ERDRP-0519, GS-441524, GS-5734	[178]
Respiratory syncytial virus (RSV)	N	RSV604, ALN-RSV01	[179, 180]
	G	MBX-300, Microbiotix/NMSO3	[181, 182]
	F	MDT-637, JNJ-53718678,GS-5806, AK0529, TMC353121, BMS-433771, BTA-C585, P13 and C15, RFI-641, VP-14637	[181, 183-185]
	L-protein	Nucleoside inhibitor: ALS-8176 Non nucleoside inhibitors: BI-compound D, YM-53403, AZ-27	[181, 186-188]

"right hand" structure with palm, fingers and thumb domains forming channels for template entry, NTP entry and product exit (Fig. **5B**) [76, 79, 80]. Picornaviral and flaviviral $3D^{pol}$ active site closure occurs by an NTP-induced conformational change in the palm domain [80].

Available RdRp structures of the CVB and other currently available picornaviral polymerase structures will help in developing novel effective antivirals. In addition, there is a therapeutic window between acute and chronic infection, which allows us to treat an acute illness using small molecule inhibitors [79].

2.1.6. Rubella Virus (RV/RUB)

Rubella virus belongs to the family *Togaviridae* and the genus *Rubivirus*. RV is the causative agent of "German Measles". Due to its teratogenic nature, RV/RUB remains the major cause of developmental anomalies globally. In 1941, Australian ophthalmologist Norman Gregg discovered the association between rubella infection during maternity and Congenital Rubella Syndrome (CRS) [82]. The viral particle ranges from ~50-85 nm in diameter and contains a (+) ssRNA genome of ~9.7 kb which encodes five proteins [85]. The RV genome encodes three structural proteins: a nucleocapsid (CP) and the envelope glycoproteins E2 and E1. It also encodes the non-structural proteins p150 containing methyltransferase (M), Y, proline hinge (PH), X, protease (PR) domains, p90 harboring helicase domain and RdRp. The NS proteins are generated by the viral protease cleavage of the single precursor polyprotein p200 (Fig. **6A**) [82, 86]. Disease symptoms include low-grade fever, swollen lymph nodes, and a morbilliform rash. However, RV/RUB infection during pregnancy (first trimester) eventually leads to miscar-

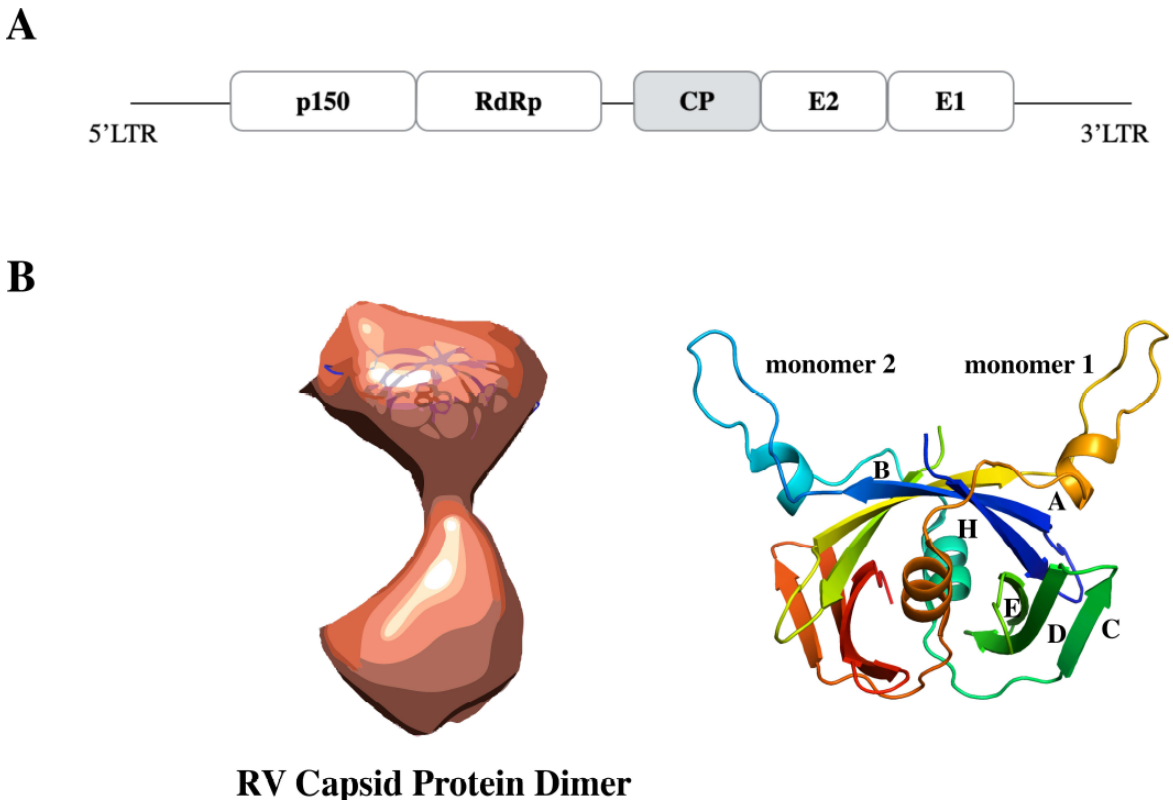

Fig. (6). RV/RUB genome architecture and protein drug target - Capsid protein (CP). A. RV genome architecture with labelled structural and non-structural proteins (selected target shaded in grey), and **B**. RV cryo-EM structure (EMD-8250) of single capsid unit density with C-terminal domain of the capsid protein fitted inside and (right) cartoon representation of capsid protein dimer, right monomer beta strands are labelled A-E and H for two-turn alpha helix (PDB ID: 4HAR). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

riage or CRS, resulting in blindness, deafness, congenital heart defects, mental retardation, and diabetes [82]. RV/RUB virus infects the upper respiratory tract through receptor-mediated endocytosis and low pH-dependent membrane fusion [87].

2.1.6.1. RV Protein Drug Target - Capsid Protein (CP)

The CPs are not only responsible for packing the RNA genome, but they are also involved in viral transcription and replication processes by interacting with NS proteins. RV virions are of irregular shape and size. The three-dimensional structure of CP and cryo-EM structure of virion will hopefully facilitate the development of novel antiviral therapies [88, 89]. The monomeric capsid protein contains five anti-parallel beta-strands and a two-turn alpha helix. The two monomers interact strongly by introducing beta strands A and B into the adjacent monomer's BH loop and further interaction with neighbouring monomers are mediated through hydrogen bonding and hydrophobic interactions (Fig. **6B**). Due to the importance of RV capsid proteins in viral assembly, these can be an important target protein for developing antivirals.

Rubella vaccines exist as live attenuated single-virus vaccines or in combination with other viral vaccines. RV vaccines are administered with measles, mumps and varicella vaccines as MMRV vaccine [90]. Although vaccination strategies effectively reduced the risk of RV infection, RV is endemic in many countries [88, 91]. A few additional complications linked with attenuated vaccines include post-infection encephalopathy, Guillain–Barré syndrome, and haematological complications such as transient thrombocytopenia, purpuric rash, and haemolytic anemia [92]. Rubella vaccine has been available since the 1960s, but still its RV infections are not been completely eradicated. Moreover, no effective antiviral drugs are available for the treatment of RV. Therefore, computation approaches can greatly help in developing novel antivirals against RV infection.

2.2. (-) ssRNA VIRUSES

2.2.1. Measles Virus (MeV)

Measles virus belongs to the family *Paramyxoviridae* and the genus *Morbillivirus*. It was first isolated in 1954. MeV is the most contagious human virus and is associated with the highest child mortality or deaths [93, 94]. The virions are spherical, 100-300 nm in diameter and contain a non-segmented (-) ssRNA genome. The genome is ~15.9 kb and it encodes eight mature viral proteins [95]. The two non-structural proteins V and C are produced by alternatively translating the vRNA and structural phosphoprotein (P) from edited RNA. The nucleocapsid include phosphoprotein (P), a large protein (L) and nucleoprotein (N). The viral envelope include the haemagglutinin protein (H), fusion protein (F) and matrix protein (M) (Fig. **7A**). Symptoms of MeV infection include fever, cough, coryza and conjunctivitis [94]. MV infection leads to immune suppression which enhances

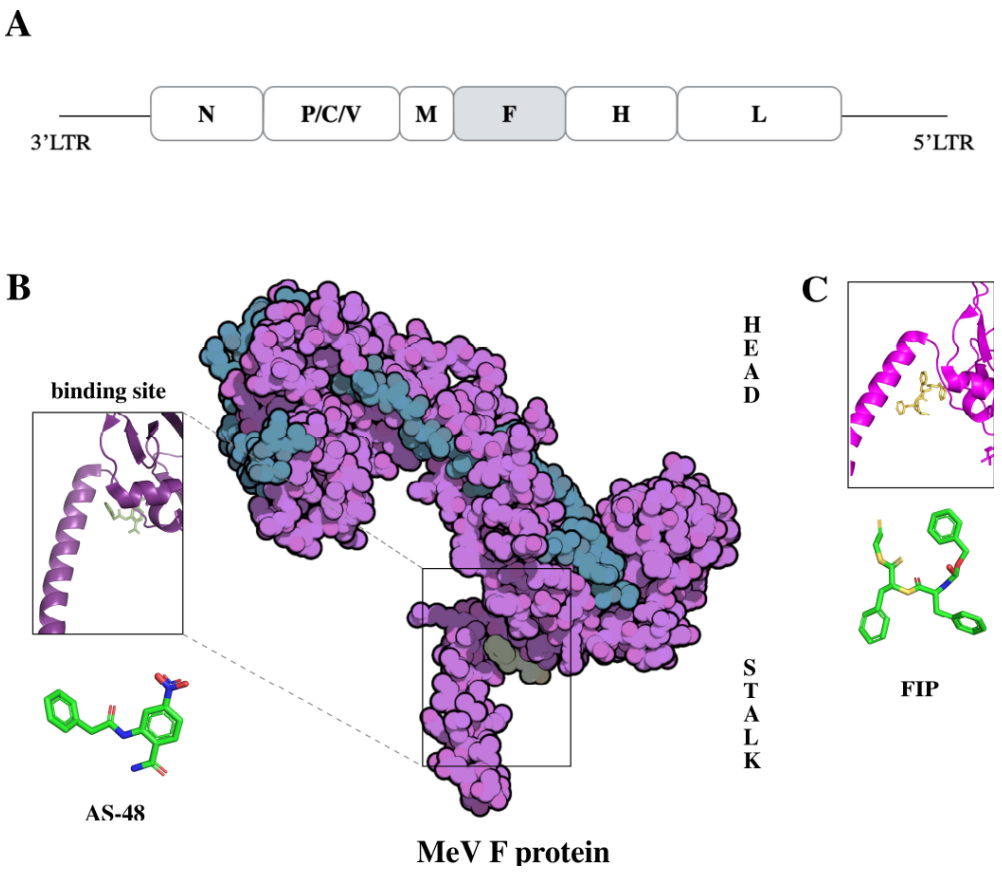

Fig. (7). MeV virus genome architecture and protein drug target - prefusion protein (F). A. MeV virus genome architecture with labelled viral proteins (selected target shaded in grey), **B**. MeV prefusion F protein overall structure (magenta), surface representation (PDB ID: 5YZC) and inlet displays the inhibitor binding site, and **C**. Inlet shown from another independently solved MeV-F protein bound to FIP peptide molecule (PDB ID: 5YZD), and corresponding inhibitor chemical structure below. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

susceptibility to secondary infections from pathogens, which is the major cause of death in infected individuals [95]. The persistent infection affects the central nervous system, resulting in fatal neurodegenerative diseases such as subacute sclerosing panencephalitis and measles inclusion body encephalitis [93].

2.2.1.1. MeV Protein Target - Fusion Protein (F)

The trimeric fusion protein (F) is the crucial component of the fusion machinery and consist of hetero-oligomeric complexes together with the tetrameric attachment (H) protein. The virus binds to the cellular receptor, and entry into the host cell is the pivotal step to establish infection. The MeV deploys viral membrane fusion machinery to penetrate the host cell for successful infection similar to other *Morbillivirus*es. The recent co-crystal structures of F protein with its small molecule inhibitor (AS-48) and a fusion inhibitor peptide, both binds to the same region in between the head and stack, providing a better molecular understanding of the membrane fusion and making possible the design of novel inhibitors for treating MeV-induced neurodegenerative diseases [93] (Fig. **7B**).

No effective antiviral drugs exist for the treatment of MeV infection. A broad-spectrum antiviral drug Ribavirin displayed limited success with side effects. Most of the promising experimental drugs target to the L protein (RdRp) subunit, a lead drug AS-136a and ERDRP-0519 is highly effective, however with several limitations such as potency, aqueous solubility and low bioavailability [96]. Although effective vaccines have been available for more than 40 years, since 2012 MeV infections have still caused 110,000 to 120,000 deaths per year [97]. In immunocompromised individuals, attenuated measles vaccines could even cause serious lung or brain infections [94, 98].

2.2.2. Ebola Virus (EBOV)

EBOV belongs to the family *Filoviridae* and the genus *Ebolavirus*, and causes severe and acute systemic infection in humans with high mortality. The WHO has defined EBOV as a Biosafety level 4 pathogen and the Center for Disease Control and Prevention (CDC) has classified it as a category A bioweapon pathogen [101]. The first reported Ebola outbreak took place in 1976 in the Democratic Republic of Congo and caused Ebola hemorrhagic fever (EHF) [102]. The virion is filamentous, 80 nm in diameter and ~1400 nm in length. EBOV contains a non-segmented (-) ssRNA genome of ~19 kb and it encodes eight viral proteins, called nucleoprotein (NP) involved in transcription and replication, glycoprotein (GP) responsible for virus entry into host cell; pathogenicity, polymerase (L), viral protein (VP) VP24 mediates nucleocapsid formation and is responsible

for viral assembly, VP30 – transcription activator, VP35 – polymerase cofactor involved in RNA synthesis, type 1 interferon antagonist, virulence factor, and VP40 – matrix protein involved in viral assembly and budding (Fig. **8A**) [101, 103]. The most common symptoms are high fever, malaise, fatigue, body aches andgastrointestinal symptoms. EBOV eventually damages the host immune system leading to malfunctioning of multiple organs such as the liver and kidneys. Severe EBOV infection eventually leads to critical gastrointestinal disorder resulting in substantial fluid and electrolyte losses [103, 104].

Available EBOLA antivirals targeting the viral polymerase VP35, include GS-5734, BCX4430 and Favipiravir, were shown to be effective against EBOV infection [105, 106]. Other available inhibitors include Ouabain, Nilotinib, Miglustat, and Clomiphene against VP24 protein and Bafilomycin A1, Chlorpromazine, *etc* against GP proteins [107] (Table **2**).

2.2.2.1. EBOLA Protein Target - VP30 Protein

The eVP30 protein plays a key role in transcription initiation, and its interaction with the Ebola virus nucleoprotein eNP is crucial. The structure of eVP30-eNP reveals details of this interaction and can be a novel target for antiviral drugs [108]. The primary sequence of the eNP is quite unique for Ebola and Marburg virus and not commonly seen in any eukaryotes. In addition, the eNP binding groove is quite narrow and involves several hydrophobic and electrostatic interactions. The interaction between the eVP30-eNP is shown to be important for transcription initiation in the process of RNA synthesis [108], which makes it an attractive target to design and develop antiviral therapy (Fig. **8B**).

So far, no FDA approved antiviral treatments or effective vaccination scheme exists to minimize the mortality of EBOV infections [109]. However, recently, there were cases in which individuals were cured by using Remdesivir (GS-5734) made by Gilead Science of Foster City, California, U.S.A.

2.2.3. Respiratory Syncytial Virus (RSV)

RSV belongs to the family *Paramyxoviridae* and the genus *ortho-Pneumovirus*. RSV causes severe acute respiratory tract infections in infants with significant morbidity and mortality. The RSV was first isolated in chimpanzees in 1956 and infants with severe lower respiratory diseases [110, 111]. The virion is spherical/filamentous, ~150 nm in diameter and contains a non-segmented (-) ssRNA genome. RSV genome is 15.2 kb and it encodes eleven mature proteins called nonstructural proteins (NS-1 and NS-2) responsible for precluding host immune response. Nucleoprotein – N (RNA binding), phosphoprotein – P (polymerase cofactor), large protein/polymerase – L and a transcription factor M2-1 and M2-2 (modulates RNA replication) together comprise the nucleocapsid complex. Inner envelope forming matrix protein–M and small hydrophobic protein – SH forms transmembrane ion channel. The glycoprotein – G and fusion protein – F are required to form the surface epitopes. These surface epitopes can block the RSV fusion with host cells, thereby targeting the viral entry mechanism and making it an important target (Fig. **9A**) [112, 113]. Symptoms of RSV infec-

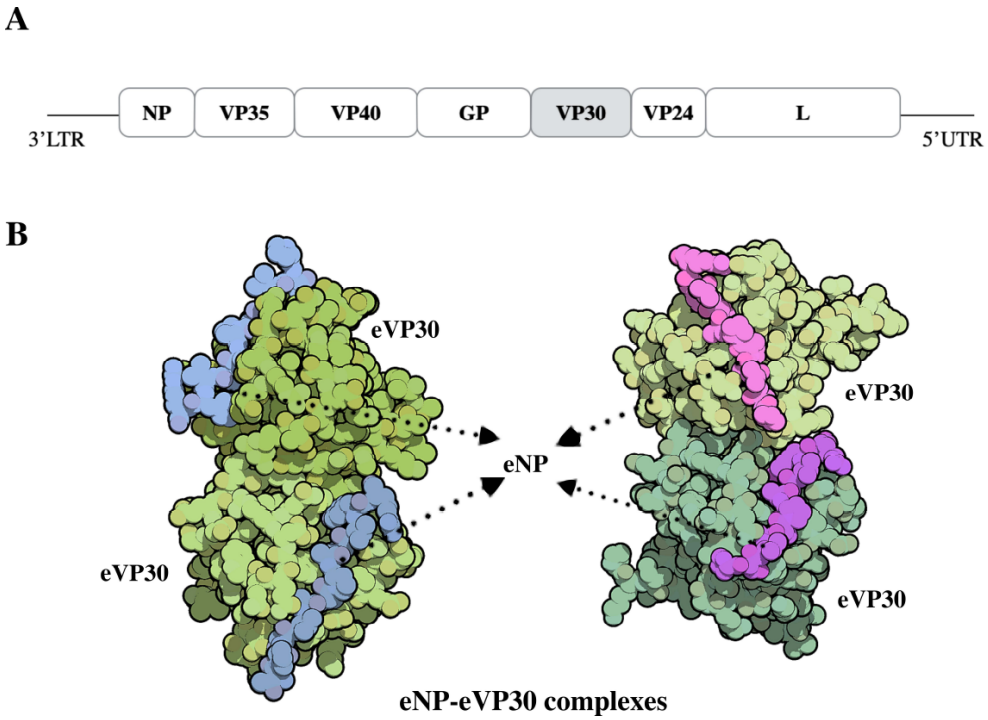

Fig. (8). EBOV genome architecture and protein drug target - eVP30-eNP. A. EBOV virus genome architecture with labelled viral proteins (selected target shaded in grey), and **B**. EBOV glycoprotein (green shades) in complex with its nucleoprotein peptide (blue shades, magenta and pink), overall eVP30-eNP complex structures are shown in surface representation from two asymmetric units but similar interface (PDB ID: 5VAP and 5VAO) and the eNPs are labelled with pointed arrows. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

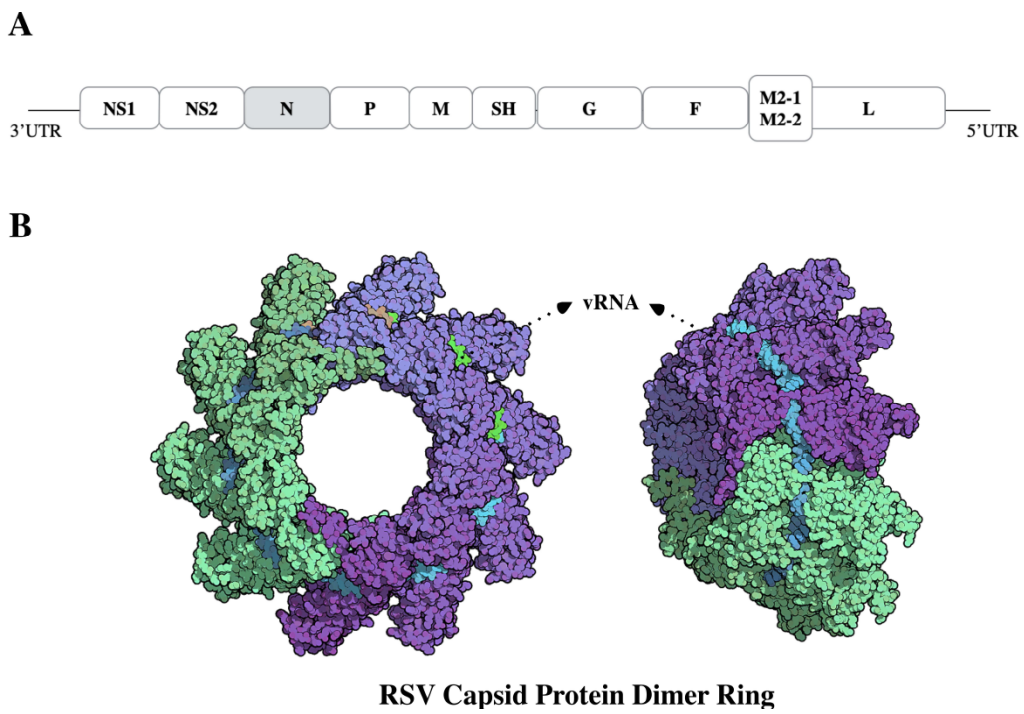

Fig. (9). RSV virus genome architecture and protein drug target - Nucleoprotein (N). A. RSV genome architecture with labelled viral protein (selected target shaded in grey), and **B**. RSV nucleoprotein (N) ring assembly overall structure (green and purple) surface representation (PDB ID: 2WJ8) and side view showing the nucleic acid wound around forming nucleocapsid like structure. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

tions are highly variable from mild upper respiratory tract infections to severe bronchiolitis and pneumonia. Infants with severe RSV infection mostly linked to chronic lung diseases, congenital heart diseases, immunodeficiency, prematurity and birth defects [114]. In addition, RSV was known to cause serious respiratory illness to aged people over 65 years. Nucleoprotein inhibitor RSV604 blocks the viral RNA synthesis and perturbs viral infectivity.

2.2.3.1. RSV Protein Target - Nucleoprotein (N)

Nuclease-resistant ribonucleoprotein (RNP) helical complex, containing both vRNA and N protein is called the nucleocapsid. This nucleocapsid is used as a template during viral RNA synthesis and these helical complexes are nuclease resistant [115]. The recombinant N-RNA complex forms ring with 10-11 protomers that mimic the virion's helical nucleocapsid. The RNA molecule is bound around the protein ring (RNA belt) because each subunit contains a basic surface groove that interacts with seven nucleotides (Fig. **9B**). Cryo-EM structure of RSV nucleocapsid revealed left-handed helical filaments with 45 degree protruding spikes, resembling the head of wheat [115-117]. Nucleocapsid 3'end together with 3'terminal RNA sequence are responsible for vRNA synthesis initiation. Hopefully, these available nucleocapsid structures will pave the way in designing novel antiviral drugs.

A broad-spectrum antiviral, a nucleoside analogs Ribavirin that appears to block RSV replication could improve the brain weight recovery in persistent infection in the CNS. Other than Ribavirin, there are no licensed commercially available antiviral drugs or vaccines available for RSV. Palivizumab (monoclonal antibody – mAb) is administered prophylactically to infants suffering from chronic lung disease of prematurity, congenital heart disease or premature birth [118]. At present, no licensed commercially available vaccines or antiviral drugs exist for RSV infection.

CONCLUSION

Structural virology attained a new era because of the rapidly evolving advancements in the molecular, structural and computational biology tools and techniques. These developments have paved a significant role in elucidating novel structures of viral and host protein targets which were previously very difficult to determine. The currently available structural information supports the modern drug development pipeline and assist in identifying potential direct-acting antivirals [9].

Many different RNA and DNA viruses are responsible for causing chronic diseases in humans and it is estimated that approximately 20% of cancers are caused by viruses. In this current review, the paper focuses on the (+) and (-) ssRNA viruses [119]. The persistent viral infections are increasingly common and eventually result in severe/chronic conditions like cancer or immune disorders depending on several factors including, lifestyle habits, dietary intake, genetics, *etc* [120]. The impact of currently available therapeutic alternatives and herbal medicines is debatable. Moreover, consuming additional supplements (*e.g*. vitamins or minerals) might interact with antivirals and reduce its effectiveness. Selected RNA viruses' other available protein targets and their representative examples of existing direct-acting antivirals are summarized in Table **2**.

Drug repurposing with available antivirals is a promising method to cure viral infections [56, 121]. For example, recent *in silico* studies of the ZIKV RdRp:RNA complex, using structural information, computer simulations and docking studies can guide the medicinal chemists to develop novel nucleotide analogs as a DAA inhibitor for ZIKV RdRp. Together with biochemical and site-directed mutational analyses provide a better understanding of the flavivirus RdRp:RNA complex [122].

Recent reports have shown that HIV-1 has developed resistance against the NNRTI drugs Efavirenz and Nevirapine, which are considered to be the backbone of anti-HIV therapy. Therefore, the WHO has suggested to use Dolutegravir, an integrase inhibitor that can be a potential alternate. HAART is also a major cause of emerging HIV drug-resistant mutations [123, 124]. Apart from the viral target protein, inhibitors targeting the viral-interacting host-factors are being constantly developed to combat viral infections in humans. As viruses interact only with specific host-factors, therefore these identified interacting proteins can also act as a target to developing host-directed drugs [83, 125, 126]. For example, in the case of HIV-1, the trans-acting activator of transcription (Tat) peptide is one of the most extensively studied structural peptides which specifically binds to the trans-activation response element, against which the antiviral peptide mimics are being developed [127, 128].

Novel RNA based drugs through si/miRNA approaches against viral infection appear to be effective in experimental studies, however, due to several limitations they are not commercially available [129-131]. The importance of viral UTRs in regulating viral infection is very well documented yet antivirals targeting UTRs are yet to come [132, 133].

Latest advances in structural biology, translational bioinformatics and virtual ligand design can be used to combat neglected, highly mutating and re-emerging viruses and to design novel antivirals and vaccines [134, 135].

LIST OF ABBREVIATIONS

AIDS	=	Acquired Immunodeficiency Syndrome
ATL	=	Adult T-cell Lymphoma
cART	=	combinatorial Anti-Retroviral Therapy
CDD	=	Computational Drug Discovery
circRNA	=	circular RNA
CP	=	Capsid Protein
CRS	=	Congenital Rubella Syndrome
cryo-EM	=	Cryogenic Electron Microscopy
CVB	=	Coxsackievirus B
DAA	=	Direct-Acting Antivirals
DENV	=	Dengue Virus
ds	=	double-stranded
EBOV	=	Ebola virus
F	=	Fusion protein
FDA	=	Food and Drug Administration
HAART	=	Highly Active Antiretroviral Therapy
HBV	=	Hepatitis B Virus
HCV	=	Hepatitis C Virus
HDV	=	Hepatitis D Virus
HIV	=	Human Immunodeficiency Virus
HTLV-1	=	Human T-cell Lymphotropic Virus type-1
mAb	=	monoclonal Antibody
MeV	=	Measles Virus
MTase	=	Methyltransferase
NP	=	Nucleoprotein
NNRTI	=	Non-Nucleoside RT Inhibitor
NRTI	=	Nucleoside RT Inhibitors
NS	=	Non-structural
PBS	=	Primer-Binding Site
PDB	=	Protein Data Bank
PR	=	Protease
QSAR	=	Quantitative Structure-Activity Relationship
RdRp	=	RNA dependent RNA polymerase
RNA	=	Ribonucleic acid
RSV	=	Respiratory Syncytial Virus
RT	=	Reverse Transcriptase
RTIC	=	Reverse Transcriptase Initiation Complex
RV/RUB	=	Rubella Virus
SAM	=	S-Adenosyl-L-Methionine
ss	=	single-stranded
VP	=	Viral Protein
vRNA	=	viral RNA
WHO	=	World Health Organization
WNV	=	West Nile virus
YFV	=	Yellow fever virus
ZIKV	=	Zika virus

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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