5

Flaviviral Infections and Potential Targets for Antiviral Therapy

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1. Introduction

The family *Flaviviridae* includes human and animal pathogenic viruses of global importance, e.g. the human flaviviruses West-Nile virus (WNV), dengue virus (DENV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV) and yellow fever virus (JEV) as well as hepacivirus hepatitis C virus (HCV). This virus family was named after the jaundice occurring in course of YFV infection, the first identified virus of the *Flaviviridae* (Monath, 1987; Halstead, 1992). In humans infections with *Flaviviridae* may lead to fulminant, hemorrhagic diseaes [YFV, DENV and omsk hemorrhagic fever virus (OHFV)], viral encephalitis [JEV, TBEV, WNV, St. Louis encephalitis virus (SLEV)] or chronic hepatitis C, formerly referred to as non-A, non-B hepatitis (HCV) (Monath & Heinz, 1996; Rice, 1996). Viruses belonging to the third genus, pestivirus, infect only animals, leading to severe disease of the host, usually followed by death [bovine viral diarrhea virus (BVDV), classical swine fever virus (CSFV) and border disease virus (BDV)] (Nettelton & Entrican, 1995).

The genus flavivirus consists of more than 70 species that are, on the basis of phylogenetic analyses, divided into 14 classes which in turn are grouped into three clusters: the mosquito-borne cluster, the tick-borne cluster and the non-vector cluster. All flaviviruses of human importance are mosquito- or tick-borne viruses. They enter through the skin by the bite of an infected arthropod, proliferating locally and spreading through the blood circulation and cross the blood-brain barrier and finally entering the central nervous system. This fact is important for further pathogenesis and unfavorable clinical outcome of the infection (King et al., 2007). Most pathogenic flaviviruses are associated with neurological diseases. The mosquito-borne encephalitic flaviviruses are grouped phylogenetically in the Japanese encephalitis serocomplex. Most tick-borne flaviviruses cause encephalitis and are mainly spread through Europe and Asia. Approximately up to 200 million new cases of infections caused by viruses of the *Flaviviridae* family are registered annually. Up to date, there is no effective antiviral therapy directed against *Flaviviridae* viruses.

Members of the family of *Flaviviridae* are small (40 to 50 nm), spheric, enveloped RNA viruses of similar structure. The genome of the viruses consists of one single-stranded, positive-sense RNA with a length of 9100 to 11000 bases [e.g. 10862 for YFV (strain 17D), 10477 for Russian spring-summer encephalitis virus (RSSEV) and approx. 9100 for HCV].

The RNA possesses a single open reading frame (ORF) flanked by 5′- and 3′- terminally located untranslated regions (5′UTR and 3′UTR respectively). In course of infection with flaviviruses the polyprotein is cleaved co- and post-translationally by proteases (Pryor et al., 1998). The amino terminus of the polyprotein is processed into 3 structural proteins. The spheric nucleocapsid is composed of one viral capsid protein (C-protein). Furthermore, it is surrounded by a lipid bilayer that is acquired for the budding of the virus particle into the lumen of intracellular vesicles. The lipid membrane contains two species of glycoproteins. Protein M, processed from a larger precursor protein (Pre-M), and the envelope protein (E-protein) form homodimers that determine the form of the virus particle. Beside the structural proteins the polyprotein is additionally processed into seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) (Westaway, 1987; Leyssen et al., 2000; Monath & Heinz, 1996; Rice, 1996). Figure 1 presents, schematically, the structure of the flavivirus polyprotein.

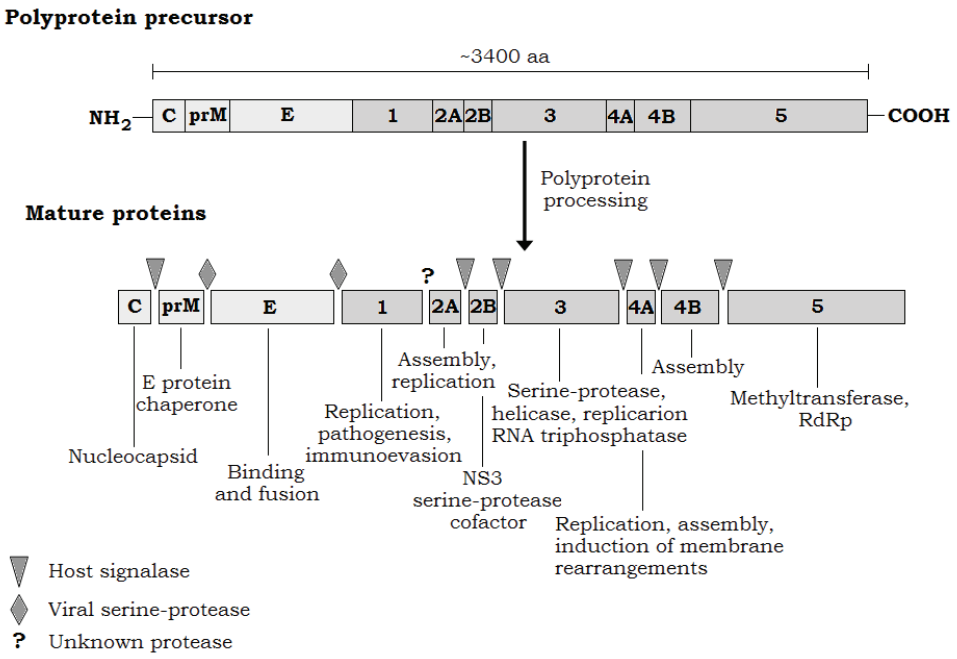

Fig. 1. Genome organization and functions of viral proteins. The polyprotein is co- and posttranslationally cleaved by host and viral proteases (as shown in the figure) into structural (light) and nonstructural (dark) protein. Putative functions of the viral proteins during viral replication are indicated

2. Viral life cycle

Genomic replication proceeds very similarly within the *Flaviviridae* family. After binding to the cell by specific receptors and entry of the virus *via* receptor-mediated endocytosis the viral RNA is uncoated by acid-catalyzed membrane fusion and translation is initiated by

cap-mediated processes. The viral RNA is translated into one viral polyprotein which is co- and posttranslationally cleaved by viral and host proteases. RNA synthesis occurs in cytoplasmic replication complexes localized at the perinuclear membranes. All NS proteins appear to be involved in RNA replication. The blockade of their activities leads to inhibition of the virus propagation. The genomic RNA is encapsidated into the core shell consisting of capsid proteins, enveloped by viral surface protein embedded cellular derived lipid membranes.

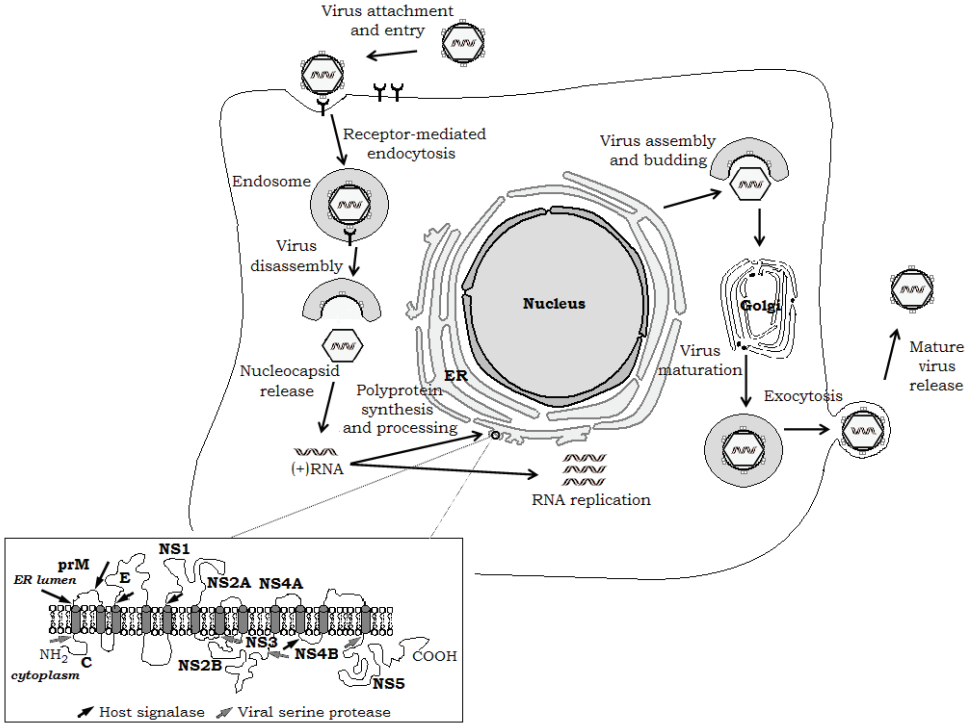

Fig. 2. Flaviviral replication cycle. After binding of the virion to the specific host receptor the virus enters the cell *via* receptor-mediated endocytosis. The low pH in the endosomes mediates fusion of viral membranes with endosomal membranes and lead to release of viral RNA. The viral RNA is translated into one polyprotein and proteolytically processed. Virus assembly consisting of coating and formation of immature virion occurs at the ER. Maturation of the immature virion particle to the mature virion occurs in the trans Golgi network. Mature virions are finally released by exocytosis

The virus particle is budded through intracellular membranes into cytoplasmic vesicles. By the cellular secretory pathway the virus particles are transported to the plasma membrane and undergo a maturation procedure. Virus release into the extracellular compartment is connected with fusion of the vesicles with the plasma membrane. Figure 2 presents the flaviviral life cycle.

3. Promising targets for antiviral therapy

The presumed replication cycle of flaviviruses consists of: I) Adsorption and receptor-mediated endocytosis; II) low pH-dependent fusion in lysosomes and uncoating; III) cap-mediated initiation of translation of the viral RNA into viral precursor polyprotein; IV) co- and post-translational proteolytic processing of the viral polyprotein by cellular and viral proteases; V) membrane-associated synthesis of template minus-strand RNA and progeny plus-strand RNA; VI) assembly of the nucleocapsid, budding of virions in the endoplasmic reticulum (ER), transport and maturation of virions in the ER and the Golgi complex, vesicle fusion and release of mature virions. Some of these steps could be potential targets for antiviral compounds.

3.1 Adsorption and receptor-mediated endocytosis

To date a broad range of receptors for members of the *Flaviviridae* have been identified. Predominantly there are oligo- and polysaccharides, particularly heparin, heparan sulfate and glycosoaminoglycans (GAGs) (Lee & Lobigs, 2000). Nevertheless, in some cases membrane proteins, especially receptor proteins, serve as binding site(s) for viruses of the *Flaviviridae* family.

The entry of flaviviruses into their target cells is mediated by the interaction of the E glycoprotein with cellular surface receptor molecules. Receptor usage is both cell type and virus-specific and contributes to host range, tissue tropism and viral pathogenicity. Highly sulfated, negatively charged glycosaminoglycans, such as heparan sulfate, can be utilized by several flaviviruses as low-affinity attachment factors that concentrate the virus on the cell surface (Agnello et al., 1999; Germi et al., 2002; Kroschewski et al., 2003; Lee & Lobings, 2008; Chen et al., 2010; Kozlovskaya et al., 2010). Similar to observations made for other *Flaviviridae*, low-density lipoprotein receptor may play a role as an attachment receptor of nonheparan sulfate adapted JEV strains in mammalian cells, and might be responsible for the neurovirulence of the virus (Chien et al., 2008). For WNV and JEV, $\alpha_v\beta_3$ integrin has been documented as a functional receptor in permissive mammalian cells. Recent studies have reported that WNV entry into embryonic mouse fibroblasts and hamster melanoma cells is independent from $\alpha_v\beta_3$ integrin, suggesting alternative receptor molecules for different cell types or strain differences (Medigeshi et al., 2008).

C-type lectin receptors (CLR) are host pathogen-recognition receptors (PRR) that are specialized in sensing invading pathogens. Several members of this family are highly expressed on myeloid cells, including monocytes macrophages and dendritic cells (DCs), and thus play a central role in activating host immune defenses (Robinson et al., 2006). Dendritic-cell specific ICAM-3-grabbing nonintegrin (DC-SIGN, CD209), one of the CLRs, possesses a critical function between viral replication in insect vectors and infection of the vertebrate host (Lozach et al., 2005; Navarro-Sanchez et al., 2003; Tassaneetrithep et al., 2003). DC-SIGN is a tetrameric C-type lectin specialized in pathogen capture and antigen presentation and is constitutively expressed on DCs, including those residing in the skin, the anatomical site of initial infection (van Kooyk & Geijtenbeek, 2003). DC-SIGN recognizes carbohydrate structures present on flavivirus glycoproteins. The remarkable capacity of DC-SIGN to distinguish between high-mannose glycans typical for insect-derived glycoproteins and the complex glycosylation of host-derived proteins (Lozach et al., 2005; van Kooyk & Geijtenbeek, 2003) implies that flaviviruses have evolved an elegant strategy to initiate infection of human cells by taking advantage of the ligand specificity of this PRR.

3.2 Low pH-dependent fusion in lysosomes and uncoating

After the attachment of an enveloped virus to the cell surface receptors, the fusion of the viral envelope with the host membrane follows. This process is mediated by virus-specific fusion proteins that merge the viral and cellular membranes. The fusion proteins contain a striking motif, so-called fusion peptide, that becomes exposed in course of conformational changes, and is inserted into the target membrane. To date two different classes of fusion proteins have been described. The proteins (class I and II) differ dramatically in their structure and molecular architecture (Weissenhorn et al., 1999; Colman & Lawrence, 2003). The fusion protein of class I is represented by hemagglutinin of influenza virus and of other related viruses (Wilson et al., 1981; Weissenhorn et al., 1999). Class II fusion protein is represented by the E protein of flaviviruses (Modis et al., 2003). The fusion of the viral and host membranes may occur at neutral or alternatively at lowered pH in endocytic vesicles. *Flaviviridae* require acidic pH for successful fusion with the cell membrane, followed by uncoating of the virus (Bressanelli et al., 2004). As demonstrated for DENV-2 and TBEV at neutral pH, the envelope glycoproteins (in the form of homodimers or homotrimers, respectively) are so closely packed that the viral membrane is practically inaccessible and fusion does not occur. The lowered pH leads to changes of the conformation of envelope glycoproteins. In course of these conformational alterations, fragments of the surface area of the viral membrane will be exposed, making penetration of the virus particle into the cell possible (Bressanelli et al., 2004). A major role in this process is given to several conserved histidine residues of protein E. Mutagenesis confirmed the function and importance of initial pH-sensing and fusion-protein refolding (Fritz et al., 2008; Harrison, 2008).

3.3 Cap-mediated initiation of translation of the viral RNA into viral precursor polyprotein

Signals required for replication of plus-strand RNA viruses are usually located in the 5'-terminal regions of the template strands. They act as promoter elements for initiation of minus- and plus-strand RNA synthesis. In flaviviruses, the translation is initiated by a process called capping. The cap is an unique structure found at the 5´-terminus of viral and cellular eukaryotic mRNA, which is important for mRNA stability and binding to the ribosome during translation. The viral mRNA capping is a cotranscriptional modification resulting from three reactions mediated by viral enzymes: (1) 5´-triphosphate of the mRNA is converted to diphosphate by an RNA triphosphatase (encoded in the NS3 protein). (2) Transfer of guanosine monophosphate (GMP) from GTP to the 5´-diphosphate RNA to form a 5′-5′ triphosphate linkage, typical for cap structures. This reaction is mediated by a guanylyltransferase. (3) The transferred guanosine moiety is methylated at the N7 position by guanine-N7 RNA MTase and forms the cap0 structure m^7GpppN (Egloff et al., 2002; Ray et al., 2006). A second methylation at the first nucleotide yields a cap1 m^7GpppN^m and additionally at the second to cap2 $m^7GpppN^mN^m$. Sequence analysis revealed the presence of RNA GTase, N7 MTase and 2′-O MTase within the NH_2-terminal domain of the NS5 protein of flaviviruses.

3.4 Co- and post-translational proteolytic processing of the viral polyprotein by cellular and viral proteases

The flaviviral polyprotein is processed by host and viral proteases. The viral trypsin-like serine protease is localized at the NH_2-terminal domain of NS3. The viral enzyme catalyzes the cleavages at C-prM, NS2A-NS2B, NS2B-NS3, NS3-NS4A, NS4A-NS4B and NS4B-NS5. It

has a preference for basic amino acids, like Arg-Arg, Arg-Lys, Lys-Arg or Gln-Arg) at position P1 and P2 followed by Gly, Ala or Ser at P1' (Chambers et al., 1990). The host signal peptidase is responsible for cleavage at prM-E and E-NS1 (Speight et al., 1988; Nowak et al., 1989; Falgout & Markoff, 1995). The NS3 protease activity is dependent from its cofactor, a 40-amino acid region of NS2B. This protein fulfils the function of a chaperone, like stabilization of the latter protein, and is responsible for membrane association of the NS2B/NS3 complex. It was shown that a non-cleavable, soluble form obtained by substitution of the linker between NS2B and NS3 revealed a very active protease (Leung et al., 2001). From this results that the cleavage of NS2B-NS3 is not necessary for protease activity. Comparison of NS2B/NS3 full-length protein and NS2B/NS3 protease domain demonstrated the marginal influence of the NS3 nucleoside triphosphatase (NTPase)/helicase domain towards the protease activity. On the opposite site, the unwinding activity of the NS3 protein is significantly decreased by fusion with NS2B using DNA substrate (Chernov et al., 2008). Since this protein is essentially involved in the formation of the replication complex, it is an attractive target for potential antivirals.

3.5 Metabolism of RNA - membrane-associated synthesis of templated minus-strand RNA and progeny plus-strand RNA

The negative-stranded RNA of viruses is synthesized with the use of the parental positive-strand RNA as template. The resulting negative-strand RNA is then used as template for the synthesis of the positive-strand progeny RNA, that is then assembled into viral particles. Since the negative and positive oriented RNA strands are complementary, the NS3-associated helicase activity appears to be necessary for strand separation. NTPase/helicases are in general nucleoside triphosphate-dependent ubiquitous proteins, capable of enzymatic unwinding of double-stranded DNA or RNA structures by disrupting the hydrogen bonds that keep the two strands together. Approximately 80% of all known plus-strand RNA viruses possess at least one NTPase/helicase. The protein consists of three equally-sized structural domains separated by deep clefts. Domains 1 and 3 share with each other a more extensive interface than either of them shares with domain 2. In consequence, the clefts between domains 1 and 2 and domains 2 and 3 are the largest. The domain 2 is flexibly linked to the other two and could undergo a rigid movement relative to domains 1 and 3.

Domains 1 and 2 host seven conserved amino acid sequences, characteristic for the majority of known NTPase/helicases within superfamily II (Kadare & Haenni, 1997).

Some of the motifs are attributed to defined function of the enzyme. The motifs I and II, so called Walker motifs A and B, have been described as a key part of the NTP binding pocket. Walker motif A binds to the terminal phosphate group of the NTP and the Walker motif B builds a chelate complex with the Mg^{2+} ion of the Mg^{2+} – NTP complex (Walker et al., 1982). In the absence of substrate, the residues of the Walker motifs bind one to the other, and to the residues of the conserved T-A-T sequence of motif III. This motif is part of a flexible ''switch sequence'' connecting domains 1 and 2, which transduces the energy resulting from NTP hydrolysis and participates in the conformational changes induced by NTP binding (Matson & Kaiser-Rogers, 1990).

Another main target for potential antivirals within the replication complex is the RNA-dependent RNA polymerase. The enzyme facilitates the synthesis of both the negative-strand RNA intermediate, complementary to the viral genome, and the the positive-strand RNA complementary to the negative-strand intermediate. The investigated fragment of NS5 is

folded in the characteristic fingers, palm, and thumb subdomains. The finger subdomain contains a region, the "fingertips", that displays the same fold with reverse transcriptases (RTs). Comparison with the known structures of the RTs showed that residues from the palm and fingertips are structurally equivalent (Bressanelli et al., 1999). Conserved clusters between *Flaviviridae*, *Picornaviridae* families and retroviruses in defined regions of the molecular surface are: (1) the RNA and NTP binding groove, (2) the back of the thumb, (3) the NTP tunnel, and (4) acidic path at the top-front of the fingers. The back surface of the thumb could be conceivably a site of interaction with other components of the replication complex mentioned above or cellular proteins (Bressanelli et al., 1999). Due to the fact that human cells lack RNA-dependent RNA or DNA polymerases, this protein is the most promising and beside the protease activity best examined enzyme within the flaviviral nonstructural proteins.

3.6 Assembly of the nucleocapsid, budding of virions in ER, transport and maturation of virions in the ER and the Golgi complex, vesicle fusion and release of mature virions

Progeny virions are assembled by encapsidating the genomic RNA into the core shell of capsid proteins, enveloped by two viral surface proteins (prM and E) embedded into host-derived lipid membranes. Although the intracellular assembly of flaviviruses is not completely understood, the viral morphogenesis takes place by budding through intracellular membranes into cytoplasmic vesicles (Heinz & Allison, 2003). Virus assembly intermediates or nucleocapsid particles during the replication process of the flaviviruses have rarely been visualized by electron microscopy, and viral particles first become visible at the intracellular membrane compartment in infected cells. A study using cryo-immunoelectron microscopy has described the nucleocapsid particles of Kunjin virus. Intracellular viral particles appear to be present within the lumen of the rough endoplasmic reticulum, which are then transported to the plasma membrane *via* the cellular secretory pathway (Mackenzie & Westaway, 2001). During the transport to the plasma membrane, the intracellular viral particles undergo the maturation process. Glycans on prM and E proteins are modified by trimming and terminal addition of sugar residues (Mason, 1989; Heinz et al., 1994). The N-terminal fragment of prM is cleaved by furin or a related protease in the trans-Golgi apparatus, and this cleavage is prevented by elevation of the pH in acidic intracellular compartments (Heinz et al., 1994; Stadler et al., 1997). This prM cleavage is generally considered to distinguish extracellular viral particles from intracellular viral particles and is required for converting immature virions into mature virions characterized by the ability being highly infectious and to induce cell fusion (Allison et al., 1995; Elshuber et al., 2003). The uncleaved prM interacts with E protein, forms a fusion-inactive prM/E heterodimer, and keeps E protein from undergoing an acid-induced conformational change during the transport to the plasma membrane (Guirakhoo et al., 1991; 1992). Fusion of the vesicles with the plasma membrane releases the large amount of progeny virions into the extracellular compartment (Mason, 1989).

4. Potential inhibitors

4.1 Viral attachment and entry

The lowered pH leads to conformational changes of envelope glycoprotein. In course of these conformational alterations, fragments of the surface area of the viral membrane will be

exposed, making penetration of the virus particle into the cell possible (Bressanelli et al., 2004). In this context, it is likely that compounds or short peptides competing with the regions of E protein which mediate the low pH-induced rearrangements of the structure of the virus surface would be potential antivirals. Another mechanism of action of potential flavivirus inhibitors of fusion and uncoating could be a reduction of the pH-gradient between acidified and pH-neutral cell compartments. The macrolide antibiotic bafilomycin A1 (Baf-A1, Fig. 3), a specific inhibitor of vacuolar-type H(+)-ATPase, is commonly used to demonstrate the requirement of low endosomal pH for viral uncoating (Bayer, et al., 1998; Nawa, 1998; Natale & McCullough, 1998). Treatment of the cell with the compound induced complete disappearance of acidified cell compartments. The effect of Baf-A1 is concentration-dependent. As demonstrated for JEV growth in Vero cells, the rate of infection decreases proportionally to the degree of depletion of the pH-lowered compartments (Andoh, 1998). Nevertheless, there are indications that Baf-A1 acts additionally on further intracellular processes, like blockade of transport from early to late endosomes. Since the early endosomes are suspected to lack components essential for uncoating, this activity of Baf-A1 could result in a further antiviral mechanism of action of the compound (Bayer et al., 1998).

Stem peptides and their interaction sites along the core trimer are potential targets for inhibition. The presence of a DENV derived peptide blocks infection by the flaviviruses DENV and WNV (Hrobowski et al., 2005). The hydrophobic pocket in the hinge region between domains I and II is thought to be a highly conserved structure amongst the flaviviruses. By virtual screening and followed biological tests Kampmann et al. identified two peptides (Fig. 3) which are able to block WNV and/or YFV infection with IC_{50} values in low micromolar range (Kampmann et al., 2009).

4.2 Enzyme activities associated with NS3 and NS5 proteins

The flavivirus proteases recognize cleavage sites containing dibasic amino acid residues (at positions P1 and P2) and a small amino acid side chain (position P1'). Therefore protease inhibitors like benzamidine and PMSF are inactive against flaviviral NS3 proteases. Several compounds capable of inhibiting the protease activity of NS3 have been identified. Some are short peptides that mimic the protease cleavage site, and thus competitively inhibit NS3 protease activity by binding to its catalytic site. Recently, experiments using palmatine (Fig. 3) as protease inhibitor showed inhibitory effects towards WNV (Jia et al., 2010).

Using a high-throuput system and molecular modelling a substrate competitive inhibitor (Fig. 3) of the WNV protease activity was detected by Mueller et al. (Mueller et al., 2008). An extensive study of pyrazole derivatives (Fig. 3) showed effectiveness in low micromolar range (Sidique et al., 2009). These tested analogues possess higher stability in aqueous buffer then former tested compounds (Johnston et al., 2007).

The NS3-associated NTPase is inhibited by a broad range of nucleotide analogues (Fig. 3) either with a modified nucleobase, e.g. ribavirin-5′-triphosphate, paclitaxel and some ring expanded nucleosides (RENs) triphosphates (Borowski et al., 2000; Borowski et al., 2002; Zhang et al., 2003a; Zhang et al., 2003b), or by nucleotide derivatives that possess a nonhydrolysable bound between the beta- and gamma-phosphates (unpublished data). Nevertheless, inhibition was mostly obtained under selective reaction conditions and the antiviral effect of the compounds as well as the exact *in vivo* mechanism of action is unclear.

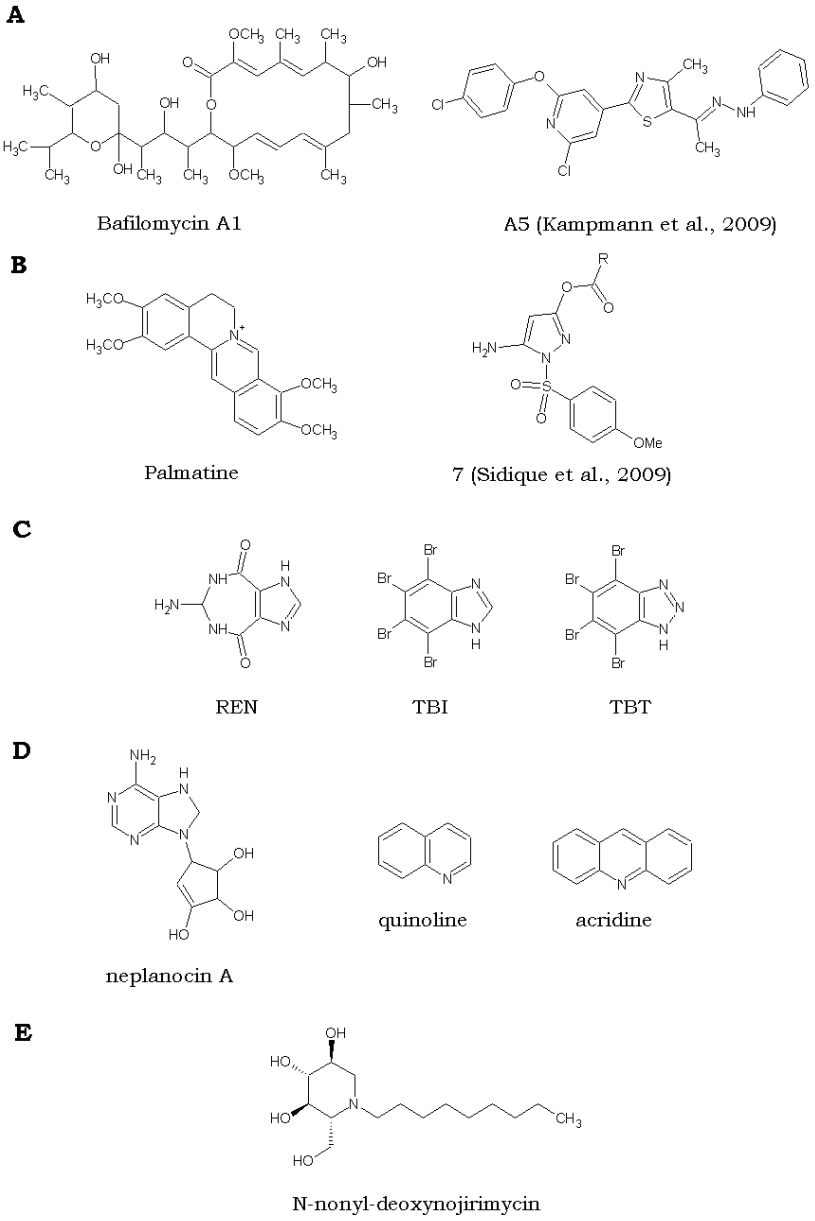

Fig. 3. Structures of antiflaviviral substances. Each panel shows inhibitors from different steps during viral replication: (A) entry, (B) polyprotein processing, (C) replication (NS3 NTPase/helicase activity), (D) replication (NS5 MTase/RdRp activities), and (E) viral maturation and release

The second promising target for potential antivirals is the viral NS5 protein-associated methyltransferase. The majority of the viral and cellular methyltransferases could be inhibited by derivatives of S-adenosylmethionine (SAM), the S-adenosylhomocysteine (SAH) (De Clercq, 1993). In this context, specific inhibitors of a cellular SAH hydrolase might inhibit the replication of *Flaviviridae* RNA, as demonstrated for rhabdoviruses or reoviruses (De Clercq, 1993). Such substances have already been found. For example, neplanocin A (Fig. 3), a naturally occurring carbocyclic nucleoside, and related abacavir and carbovir, in which the absence of a true glycosidic bond makes the compounds chemically more stable, as they are not susceptible to enzymatic cleavage by SAH hydrolase (Song et al., 2001). A further possibility to inhibit the interactions between cap-structure and its target protein, eucaryotic initiation factor 4E (eIF4E), is the reduction of the affinity of the last to the ligand. There are findings suggesting strongly that this affinity is regulated by intracellular protein phosphorylation taking place in the cap-structure-binding pocket of eIF4E (Scheper & Proud, 2002).

The majority of currently known HCV polymerase inhibitors fall into two main categories, according to their chemical structure and their mechanism of action. There are nucleoside analogue inhibitors and non-nucleoside inhibitors. Recently a third class of compounds, mimicking the pyrophosphate group and displaying an ability to inhibit RdRp, was separated.

All nucleoside analogues appear to inhibit the polymerase activity in a similar manner. After penetration into the cell, the compounds undergo intracellular phosphorylation to the corresponding triphosphate. Subsequently the nucleotide analogues are incorporated by the viral polymerase into the growing nucleic acid chain. This leads, in turn, to an increased error frequency of the polymerase and, in consequence, to early termination of the elongation reaction. The second category of RdRp inhibitors comprises structurally and chemically heterogenous compounds, not related to the non-nucleosides or nucleotides. The substances are not incorporated into growing DNA or RNA strand. The compounds inhibit the polymerase indirectly by binding to the enzyme in a reversible and non-competitive manner. A third category of polymerase inhibitors consists of the chemically and structurally homogenous pyrophosphate mimics possessing a diketo acid moiety. The mechanism by which the compounds exert their inhibitory effect is the blockade of the active site of the enzyme. Thus, the binding of the phosphoryl groups of the nucleotide substrate is blocked and formation of complexes Mg^{2+}-NTP or Mn^{2+}-NTP is abolished.

Recently, several small anti-WNV compounds were identified belonging to four different structural classes including pyrazolines, xanthanes, acridines, and quinolines (Goodell et al., 2006, Fig. 3).

4.3 Maturation and release

Endoplasmic reticulum alpha-glucosidase inhibitors block the trimming stem in the course of N-linked glycosylation, and eliminate the production of several ER-budding viruses. The iminosugar derivative N-nonyl-deoxynojirimycin (Fig. 3) was found to inhibit the replication of JEV and DENV significantly (Wu et al., 2002). This effect was probably mediated by inhibition of secretion of the viral glycoproteins E and NS1. The latter protein is known to be essential for flavivirus replication (Lindenbach & Rice, 1997).

5. Conclusion

Due to the fact that flaviviruses are arthropod-borne viruses their spreading around the world is difficult to control. Several strategies in case of YFV or DENV were successful for a short period but there is no way for complete eradication of all those arthropods. Especially the health infrastructure in most epidemic countries in South-East Asia, Africa or Latin America makes the fight against the arthropods and viruses difficult.

Our aims for the future should be to force all strength to develop vaccines against the most severe infection which are available for people living in endemic areas of the world, not only for industrial countries.

Until today no antiviral therapies are effective against flaviviruses. There is an urgent need for new molecules that block replication and/or modulate immune response. Much effort was done during last decades to resolve the virus structures and to examine their life cycles. Further step is the identification of potential antiviral targets. Screening of huge libraries of compounds for their inhibitory effect have been proven as the most promising strategy in the search of antiflaviviral therapy. Unfortunately, at the moment no candidates are detected possessing high activity on the one hand and being less toxic towards human cells. Furthermore, another problem which is already known from HIV therapy is the resistance development by viruses through mutation at the target site.

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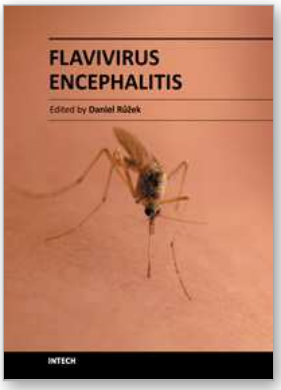

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Encephalitis is an inflammation of the brain tissue associated with clinical evidence of brain dysfunction. The disease is of high public health importance worldwide due to its high morbidity and mortality. Flaviviruses, such as tick-borne encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, or St. Louis encephalitis virus, represent important causative agents of encephalitis in humans in various parts of the world. The book Flavivirus Encephalitis provides the most recent information about selected aspects associated with encephalitic flaviviruses. The book contains chapters that cover a wide spectrum of subjects including flavivirus biology, virus-host interactions, role of vectors in disease epidemiology, neurological dengue, and West Nile encephalitis. Special attention is paid to tick-borne encephalitis and Japanese encephalitis viruses. The book uniquely combines up-to-date reviews with cutting-edge original research data, and provides a condensed source of information for clinicians, virologists, pathologists, immunologists, as well as for students of medicine or life sciences.

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