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Review

NS3 and NS5 Proteins: Important Targets for Anti-Dengue Drug Design

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A dengue é uma doença negligenciada responsável pelo óbito de 22.000 pacientes por ano em áreas onde a doença é endêmica. Embora grandes esforços tenham sido investidos em pesquisas na busca de tratamentos para a dengue, não há ainda nenhuma vacina ou antiviral disponível no mercado para tratamento desta patologia. Entre as estratégias que vêm sendo utilizadas para a busca e desenvolvimento de antivirais contra a dengue, a inibição de enzimas é aquela que tem se mostrado a mais promissora. O presente artigo de revisão descreve os recentes avanços no que tange à utilização de enzimas do vírus da dengue como alvos para o desenho de antivirais.

Dengue is a neglected disease, one that is responsible for 22,000 deaths each year in areas where it is endemic. Despite the tremendous efforts invested in research for dengue treatments, no vaccine or antiviral has reached the market, and disease treatment is limited to supportive care. Among strategies that have been used in the search for and development of an antiviral against dengue, the inhibition of viral enzymes has been proven to be the most successful approach. This review covers the major progresses that have been published concerning compounds that inhibit viral enzymes.

Keywords: dengue, antivirus, NS3 (non-structural 3), NS5 (non-structural 5), viral enzymes

1. Introduction

Dengue fever and dengue hemorrhagic fever are the most rapidly spreading mosquito-borne diseases in the world today, with an observed 30-fold increase of reported cases over the last 50 years.[1] The World Health Organization estimates that, globally, 2.5 billion people are at risk, and annually, 50-100 million people become infected, of which approximately 0.5 million require hospitalization[2] and 22,000 deaths occur each year in areas where it is endemic.[3]

The disease has four viral serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), and its spectrum ranges from asymptomatic infection to dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), and may lead the patient to death.[4] All four serotypes of dengue virus are transmitted to humans by the *Aedes aegypti* and *Aedes albopictus* mosquitoes.[5]

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The cost of dengue in the Western Hemisphere alone is estimated to be $ 2.1 billion *per* year.[6] Despite the tremendous efforts invested in anti-dengue virus (anti-DENV) research, no clinically approved vaccine or antiviral chemotherapeutics are available for humans, and disease treatment is limited to supportive care.[7-9]

One key concern surrounding the utility of antiviral therapy for dengue is the rapid decline in viremia during the natural course of infection.[10-12] The optimal dengue drugs encompass a good safety profile, resolve symptoms rapidly and reduce risks of severity, and achieve comparable inhibition of all four DENV serotypes.[13]

The antiviral agents for dengue can inhibit (*i*) viral entry; (*ii*) the viral protein NS4B; (*iii*) the viral capsid; (*iv*) the viral protease; (*v*) the viral helicase; (*vi*) the viral methyltransferase; (*vii*) the viral polymerase; and (*viii*) the host target. Inhibition of viral enzymes has been proven to be the most successful of antiviral approaches.[8,13,14] Considering this fact, the aim of this review is to summarize major progress towards drugs that inhibit viral enzymes.

2. Viral Structure

The dengue virus particle is about 50 nm in diameter. The 10,723-nucleotide RNA genome encodes an uninterrupted open reading frame (ORF), directing the synthesis of a polyprotein precursor in the order NH_2-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH, where C is the capsid protein, M is the membrane-associated protein, E is the envelope protein, and NS1 through NS5 are nonstructural proteins.[15]

The capsid protein (C) has about 12 and 14 kDa, and has several residues of basic amino acids such as lysine and arginine, which allow these to interact with the viral genome for construction of the nucleocapsid. The prM protein is a glycoprotein presenting 18.1-19.1 kDa which cleavages the N-terminal region, originating the M protein. The cleavage process is associated with the virus maturation, and prM and M are intracellular and extra cellular proteins, respectively. The M protein with 8.2-8.5 kDa is one of the two proteins that form the viral envelope, and it is involved in the penetration of the virus of the host cell.[16]

The glycoprotein E functions on the virus and in the host cells causing cell tropism, a membrane fusion cell, resulting in the neutralization of antibodies and hemaglutination.[17] This glycoprotein has three distinct domains bound by S–S (sulfur-sulfur) connection: I, II, and III domains have 495 amino acids and a molecular weight of 56-57 kDa.[18,19]

The prM protein forms an intracellular heterodimer, that stabilizes the E protein.[16] PrM is cleaved during the exocytose in virion liberation. Such a process originates the M protein,[19] possessing a new conformation, which is necessary for the right disposition of E. This fact suggests a chaperone-like function of the M protein.[16]

The NS1 protein has approximately 45 kDa and exists in multiple forms (monomer, dimer, and hexamer) in different compartments of insect cells and dengue virus-infected mammals. The NS1 protein is found in a membrane-associated or soluble form that is secreted by the infected cells, and therefore can be found in cell culture mediums or serums from infected patients.[20,21] The functions of the NS1 dengue virus infections have not been clearly elucidated.[21]

NS2A is a 22-kDa hydrophobic protein that was previously shown to be important for viral replication and pathogenesis. NS2A participates in viral RNA synthesis,[22] viral assembly,[23] virus-induced membrane formation,[23] contributes to the production of NS1,[24] and inhibits interferon α/β response.[25]

The NS2B is a co-factor for the NS3 protein. NS3 is a multi-functional enzyme, acting as a protease for polyprotein processing, an RNA (ribonucleic acid) triphosphatase for capping nascent viral RNA, and a helicase for unwinding the double-stranded replicative form of RNA.[26] NS3 and NS5 (*vide infra*) are sufficiently conserved within the four serotypes.

The flavivirus proteases, including NS2B-NS3 protease, are essential for viral replication and infectivity. Chambers and collaborators show that yellow fever virus genetically modified to contain only inactive NS2B-NS3 protease is unable to infect target cells.[27] Similarly, Rothan and collaborators show that the treatment of cells with a peptide that inhibits NS2B-NS3 protease decreases dengue virus infection by 80%.[28]

The NS4A protein has been implicated in substantial re-arrangements of internal membranes, permitting facile virus RNA synthesis and assembly; however, the mechanism by which this occurs is unknown.[29,30] NS4B consisting of 248 amino acids has been shown to be part of the viral replication complex and is also implicated in dengue virus pathogenicity.[24,31] NS4B, as one of the dengue virus proteins with RNA interference (RNAi) suppression activity, is independent of the interferon inhibition functions of NS4B.[32]

NS5 is the largest (104 kDa) and the most conserved protein in DENV. It is also a bifunctional enzyme with a methyltransferase domain (MTase; residues 1-296) at its N-terminal end and a RNA-dependent RNA polymerase (RdRp; residues 320-900)[33] at its C-terminal end.[34] Specifically, residues 320-368 are strictly conserved among the flaviviruses. These residues are also implicated to interact with NS3.[35,36]

3. NS2B/NS3 Protease and NS3 Helicase

The bipartite NS3 protease-NS3 helicase is an enzyme that is central to flavivirus replication and polyprotein processing. Dissecting the structural and functional properties of this protein in its full-length state is therefore key to improving our understanding of the flavivirus life cycle and informing the design of effective antiviral drugs. It remains unclear why NS3 harbors several catalytic activities within one polypeptide chain. However, the conservation of this arrangement across the *Flaviviridae* genus suggests some functional relevance.[37]

NS3 protease is a trypsin-like serine protease shown to harbor a classic serine protease catalytic triad comprised of residues histidine 51 (His51), aspartic acid 75 (Asp75), and serine 135 (Ser135).[38] The N-terminal one-third of the dengue virus NS3 protease (NS3pro) is required for protease activity, and the C-terminal two-thirds are associated with the enzymatic functions of a nucleoside triphosphatase and RNA helicase.[39] Falgout and collaborators demonstrated that the activating NS2B cofactor is a prerequisite for

catalytic activity of the NS3 protease.[40] The two-component NS2B-NS3pro protease represents a more structurally relevant target than NS3pro alone for functional studies and drug discovery research. However, the mechanism by which NS2B contributes to high activation of NS3pro remains poorly understood. Elucidation of the prerequisite role of NS2B will pave the way for discovering and designing new drugs against dengue diseases.[41]

The viral protease is responsible for cleavage at a number of sites, including NS2A-NS2B, NS2B-NS3, NS3-NS4A, and NS4B-NS5; it also cleaves just upstream of the signal sequences at the C-prM and NS4A-NS4B junctions, within NS2A, and within NS3 itself.[42]

The fact that NS2B-NS3pro recognizes only sites that contain two cationic residues, whereas trypsin recognizes sites containing a single cationic residue, has necessitated the development of new classes of inhibitors for targeting the active site.[43] The structural boundaries originally annotated within the polyprotein suggested that the NS3 domain encoded the functional protease. Later studies showed that expression of NS3 alone did not lead to production of an active protease; however, including a portion of NS2B with NS3 led to full proteolytic activity.[44]

Viral proteases are proven antiviral targets, as evidenced by the clinical availability of ten human immunodeficiency virus 1 (HIV-1) protease inhibitors[45] and the hepatitis C virus (HCV) protease inhibitors.[44] Thus, it is plausible that a protease inhibitor for dengue virus would be efficacious in the clinic. However, experience with HIV-1 and HCV indicates that there are certain drawbacks associated with protease inhibitors, and these are important considerations in developing dengue virus protease inhibitors.[13]

The helicase domain of NS3 (NS3Hel, residues 180-618) has seven structural motifs reminiscent of superfamily 2 helicases.[46] It has three subdomains with significant sequence identity and structural similarity to other flavivirus helicases.[47,48] Subdomains I and II are also structurally similar to the corresponding domains in the hepatitis C virus, suggesting a common functional mechanism.[49]

The combined activities of a polynucleotide-stimulated helicase and nucleoside triphosphatase (NTPase) in the C-terminal domain are required for melting secondary structures prior to initiation of RNA synthesis and for the unwinding of RNA duplexes, either to separate double stranded RNA (dsRNA) intermediates formed during viral RNA synthesis or as a translocase that can remove proteins bound to viral RNA.[50] Mutational analysis has shown that the adenosine triphosphatase (ATPase), helicase and NTPase activities of DENV NS3 share a common active site.[51] Dengue viruses with impaired helicase activities lose the ability to replicate, demonstrating the importance of the NS3 helicase domain in the viral life cycle[52] and that inhibitors or modulators for these enzymes are potentially of interest as therapeutic agents.[53]

In the HCV NS3 protein, such interdependence of different domains for their respective activities has been extensively characterized, and a strong interdependence in the enzymatic activities of the protease and helicase domains of HCV NS3 protein has been demonstrated. Previous studies have suggested a significant regulatory role of the protease domain on flavivirus NS3 helicase activity,[48-53] but, recently, a study showed that the NS3 protease domain had no significant effect on the ATPase and helicase activities of DENV NS3.[54]

The important role of NS3 for the replication of the dengue virus makes this protein an interesting site for viral inhibiting.

4. NS5 Methyltransferase and NS5 Polymerase

The NS5 is about 900 amino acids long and comprises a methyltransferase domain at its N terminus and an RNA-dependent RNA polymerase domain at its C-terminal end. Both enzymatic activities form attractive targets for antiviral development.[55-58]

The domain RNA-dependent RNA polymerase (RdRp) is responsible for the replication of the positive-strand RNA genome in an asymmetric and semi-conservative process in which the antigenome is only present in a double-stranded RNA replication intermediate.[59] In DENV infections, the NS5 protein is primarily localized within the nucleus. However, not all flavivirus RdRps localize to the nucleus. The rationalization for a viral RdRp localizing to the nucleus when its actual enzymatic functions in the virus life cycle are required in the cytoplasm is currently unknown but is actively being investigated.[60] However, these observations do suggest that, apart from its enzymatic functions, NS5 may also engage in virus-host interactions and actively interact with the host environment.[61]

Viral MTases are involved in the mRNA capping process, transferring a methyl group from the cofactor *S*-adenosyl-L-methionine (AdoMet) to the N7 atom of the cap guanine and onto the 2'OH group of the ribose moiety of the first RNA nucleotide. In the genus *Flavivirus*, both (guanine-N7)-methyltransferase (N7MTase) and (nucleoside-2'-O-)-methyltransferase (2'OMTase) activities have been associated with the N-terminal domain of the viral NS5 protein.[33,62,63]

The linkage between the two NS5 domains does not seem to be relevant to the functions of these two domains, since the RdRp activity is not altered by the presence of

the glucosyltransferases (GTase) and MTase domain, and the GTase and MTase activities are not affected by the RdRp domain.[64,65]

The interdependence of the NS3 and NS5 activities, suggested by the sequence of reactions during genome replication and capping, was shown by experiments in which NS5 stimulates NS3's NTPase and RNA 5'triphosphatase (5'-RTPase) activities,[66-68] and where NS3 stimulates NS5's GTase activity.[64]

The enzymes codified by NS5 show they have important functions in the replication of the virus, playing a key role and suggesting NS5 as a potential antiviral target.

5. Dengue Inhibitors

The best characterized DENV nonstructural proteins are NS3 and NS5, which are multifunctional proteins presenting several enzymatic activities. These proteins are the most conserved ones in all four dengue virus serotypes. Therefore, NS3 and NS5 are considered attractive targets for the search and development of dengue antiviral chemotherapeutics.[36] It is important to mention that inhibition of virus enzymes is one of the most successful approaches for drug antiviral discovery.[13] In this section, several compounds that have been described in the literature as promising dengue antiviral drugs will be presented.

6. Compounds Inhibiting Protease

Several peptides have been screened as inhibitors of DENV NS3 protease. Nitsche and co-authors described the activity of several di and tripeptides.[69] The dipeptides displayed different lysine (Lys) and arginine (Ar) sequences. At 50 μmol L^{-1}, the most active dipeptides corresponded to compounds **1** and **2** (Figure 1) inhibiting, respectively, 63.8 ± 4.3% (inhibitory constant, K_i = 29.0 ± 5.5 μmol L^{-1}) and 68.6 ± 5.6% (K_i = 15.4 ± 1.8 μmol L^{-1}) of enzymatic activity. Based on previous literature reports that the sequence norleucine (Nle), lysine, arginine, arginine (Nle-Lys-Arg-Arg) is a promising lead for the development of a small peptide that inhibits DENV NS3-NS2B protease,[70,71] a series of tripeptides was also prepared and screened. It was found that the enzymatic inhibitory activity of the compounds was in the range of 56.7 to 88.5%, with tripetides **3** (88.5 ± 2.8%; K_i = 4.9 ± 0.3 μmol L^{-1}), **4** (83.4 ± 2.2%; K_i = 6.4 ± 0.4 μmol L^{-1}), **5** (85.6 ± 2.9%; K_i = 6.5 ± 0.6 μmol L^{-1}), **6** (84.6 ± 2.9%; K_i = 10.5 ± 0.8 μmol L^{-1}), and **7** (82.9 ± 6.3%; K_i = 11.9 ± 0.9 μmol L^{-1}) as the most active.

Conotoxins are a mixture of peptide neurotoxins produced by cone snails to paralyze prey and be used for defense. Crude venom extracts from five different *Conus* species were tested for serotype 2 DENV protease inhibitory activity,[72] being that the extract from *Conus marmoreus* was the only effective kind. High performance liquid chromatography (HPLC) fractioning of *C. marmoreus* extract afforded, among others, conotoxin MrIA,[73] which was found to significantly inhibit serotype 2 NS2B-NS3 protease activity (inhibitory constant, K_i = 2.2 μmol L^{-1}). The activity of this conotoxin was attributed to a disulfide bond-mediated loop. Inspired by this structural motif, several cyclic peptides containing a disulfide bond were synthesized. Peptide **8** was the most effective, presenting K_i = 2.2 ± 0.2 μmol L^{-1}.

Studies conducted with tetrapeptides identified compound **9** to possess a boronic acid functionality as the tetrapetide with the highest affinity (K_i = 43 nmol L^{-1}).[74,75]

The various peptides investigated so far exhibit inhibitory activities in biochemical protease assays. However, this is not the case in cell-based viral infection assays. This fact is attributed to their poor permeability and stability. One important fact is that cyclization of the peptides results in improved stability and cell permeability.[72]

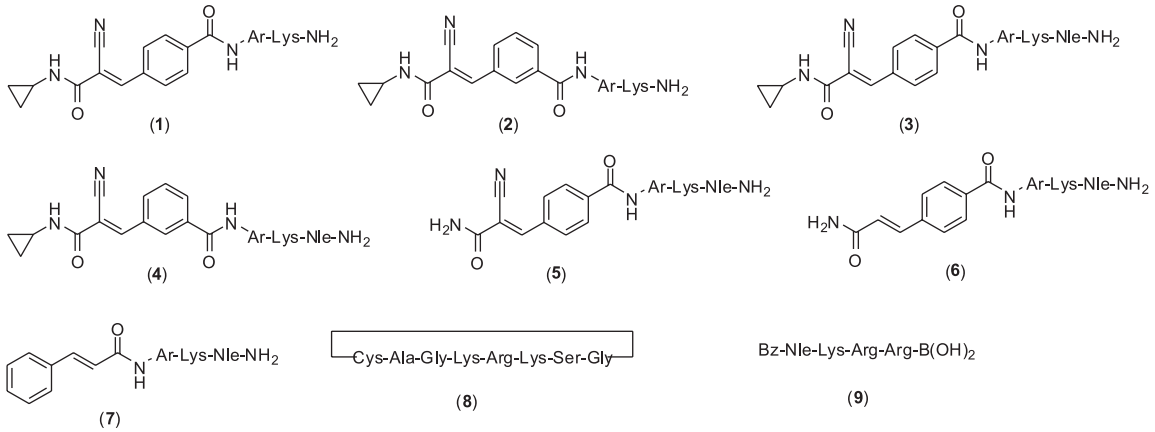

Figure 1. Structures of peptides **1**-**9**.

Screening of small-molecular-weight compounds is another important approach that has been utilized in the search for dengue antiviral drugs. Figure 2 depicts the structure of some lead compounds that have been identified in these screening campaigns.

In screening of DENV protease inhibitors a problem found is the occurrence of false positives. A selective high-throughput bioassay, based on tryptophan fluorescence, was described to overcome this problem.[76] This methodology identified phtalazine as DENV protease inhibitor. In addition, the assays were capable to discriminate between specific and non-specific analogues concerning DENV protease activitiy.[76] It was demonstrated that specific analogues quenched DENV protease fluorescence and showed variation in IC_0 (inhibitory concentration) values. In contrast, nonspecifically binding compounds did not quench its fluorescence and showed similar IC_{50} values with steep dose-response curves.

A screening of approximately 12,000 small-molecular-weight compounds resulted in the selection of guanidine derivatives, such as **11** (Figure 2), as competitive inhibitors of pan-dengue and West Nile virus NS3 protease inhibitors with IC_{50} values in the 1-70 μmol L^{-1} range. The compounds displayed low cytotoxicity. Although from *in vitro* assays the derivatives showed protease activity, antiviral activity against DENV-2 on Vero cells was not detectable.[77]

A docking investigation screening of a small set of compounds that dock into the P1 pocket and the catalytic site of the DENV-2 NS3 protease domain apo-structure led to the discovery of the compounds ADRP0006 (**12**) and ARDP0009 (**13**).[78] The 50% effective concentration (EC_{50}), 50% cytotoxic concentration (CC_{50}), and selective index (SI) were determined for both compounds (compound **12**: EC_{50} = 4.2 ± 1.9 μmol L^{-1}, CC_{50} = 69 ± 4 μmol L^{-1}, SI = 16.6; compound **13**: EC_{50} = 35.8 ± 8 μmol L^{-1}, CC_{50} > 300 μmol L^{-1}, SI > 8.1). In addition to inhibiting protease activity *in vitro*, both compounds were also capable of inhibiting viral replication in cell culture experiments.[78]

Twenty three analogues of compounds **12** were purchased for testing against DENV-2 NS2B-NS3 protease. Due to solubility problems, only ten analogues were selected for a preliminary protease screening. Among these compounds, four displayed protease inhibitory activities comparable to ADRP 0006 (**12**), while other four analogues (structures **14**-**17**, Figure 2) presented superior activity (~2 to ~60-fold higher).[79]

Steuer and co-workers investigated the biological effects of α-ketoamides and α-ketoamides derivatives on DENV virus protease.[80] All the compounds evaluated presented moderate activities in the *in vitro* DENV protease assays. However, compound **18,** which presented the highest inhibitory activity (39.1%), displayed a remarkably inhibited DENV replication in a cell-culture assay in a dose-dependent manner, achieving a more than 1000-fold reduction of virus titers at non-cytotoxic concentrations.[80]

Tomlinson and Watowich conducted a high-throughput screening of a library of 2,000 compounds aiming to find new leads for dengue antiviral development.[81] In this study, they described ivermectin (**19**), selamactin (**20**), methylbenzethonium chloride (**21**), tyrothricin (**22**) and alexidine hydrochloride (**23**) (Figure 3) as a lead for DENV-2 NS3-NS2B protease inhibitors. The lowest

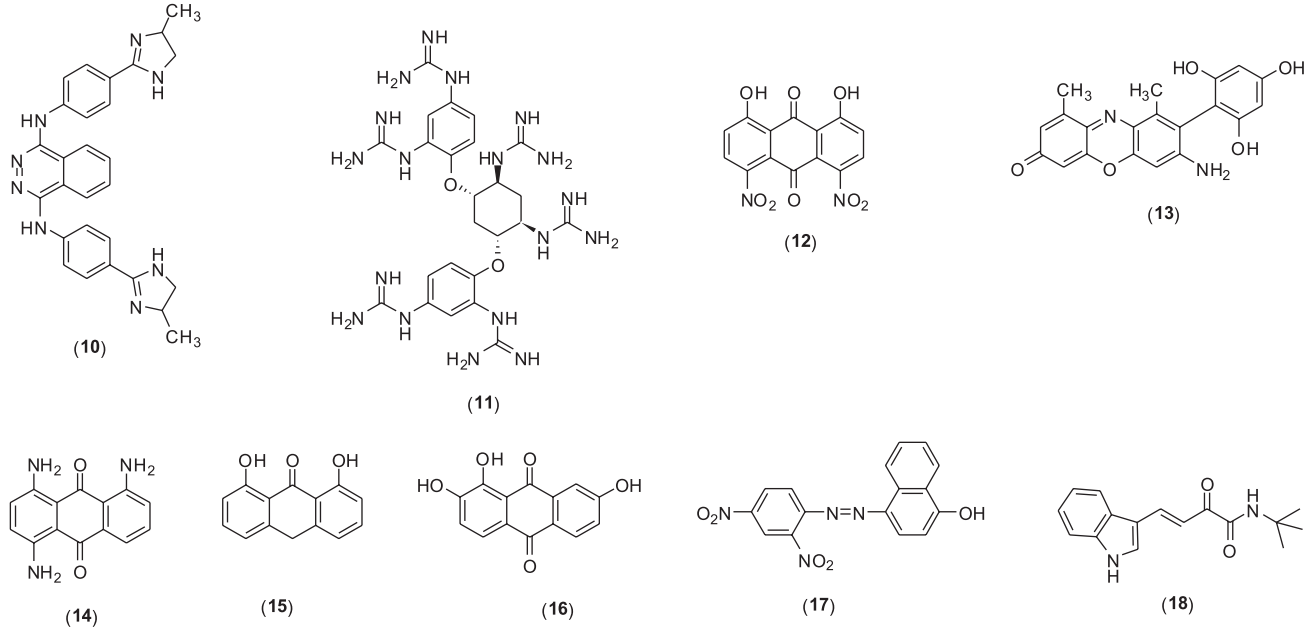

Figure 2. Structures of DENV protease inhibitors **10**-**18**.

determined K_i value was 12 ± 1.2 μmol L^{-1}, associated with compound **23**. It is also important to mention that compounds **18**-**23** were also effective on the related NS3 protease of West Nile virus.[81]

The exploitation of aminobenzamide, quinoline, and benzotiazole scaffolds has led to the discovery of compounds that can be explored in dengue antiviral discovery (Figure 4).

Compound **24,** an amidobenzamide,[82] is active against dengue and West Nile virus proteases, presenting K_i values, respectively, equal to 8.77 and 5.55 μmol L^{-1}.

The quinoline derivative **25** described by Deng and co-workers is effective in inhibiting DENV NS2B-NS3 protease activity (IC_{50} = 9.45 ± 0.78 μmol L^{-1}) as well as virus replication (IC_{50} = 24.7 μmol L^{-1}).[83] The selective index (SI) determined for this compound was superior to 4.1.

Investigations by Lai and colleagues disclosed benzoathiazole derivatives **26** and **27** as DENV protease inhibitors.[84,85] Chemical **26** presents an IC_{50} value equal to 0.91 ± 0.05 μmol L^{-1}, and a K_i value of 2.36 ± 0.13 μmol L^{-1}. Dengue and West Nile virus NS2B/NS3 proteases are inhibited by compound **27** (IC_{50} values were found to be 3.75 ± 0.06 and 4.22 ± 0.07 μmol L^{-1}, respectively). Kinetics studies support a competitive mode of inhibition by this compound.

As one last example of DENV, NS3 protease inhibitor corresponds to the ammonium species BP2109 (**28**) (Figure 4).[86] It is capable of inhibiting DENV protease activity, presenting an IC_{50} of 15.43 ± 2.12 μmol L^{-1}. BP12109 (**28**) has moderate cytotoxicity and reduces the reporter expression of the DENV-2 replicon with an EC_{50} of 0.17 ± 0.01 μmol L^{-1}.

7. Compounds Inhibiting Helicase

The *in silico* study was conducted with a selected region of the ssRNA (single stranded RNA) site in the crystal structure of the NS3 helicase domain of the Kunjin virus, an Australian variant of the West Nile Virus, and identified ivermectin (**19**) (Figure 3), as a DENV helicase inhibitor (IC 50 of 500±70 nmol L^{-1}). Kinetic studies proved that the mechanism of inhibition is uncompetitive. Moreover, it was demonstrated that ivermectin (**19**) is capable of inhibiting *in vitro* dengue, West Nile Virus, and yellow fewer virus replication.[87]

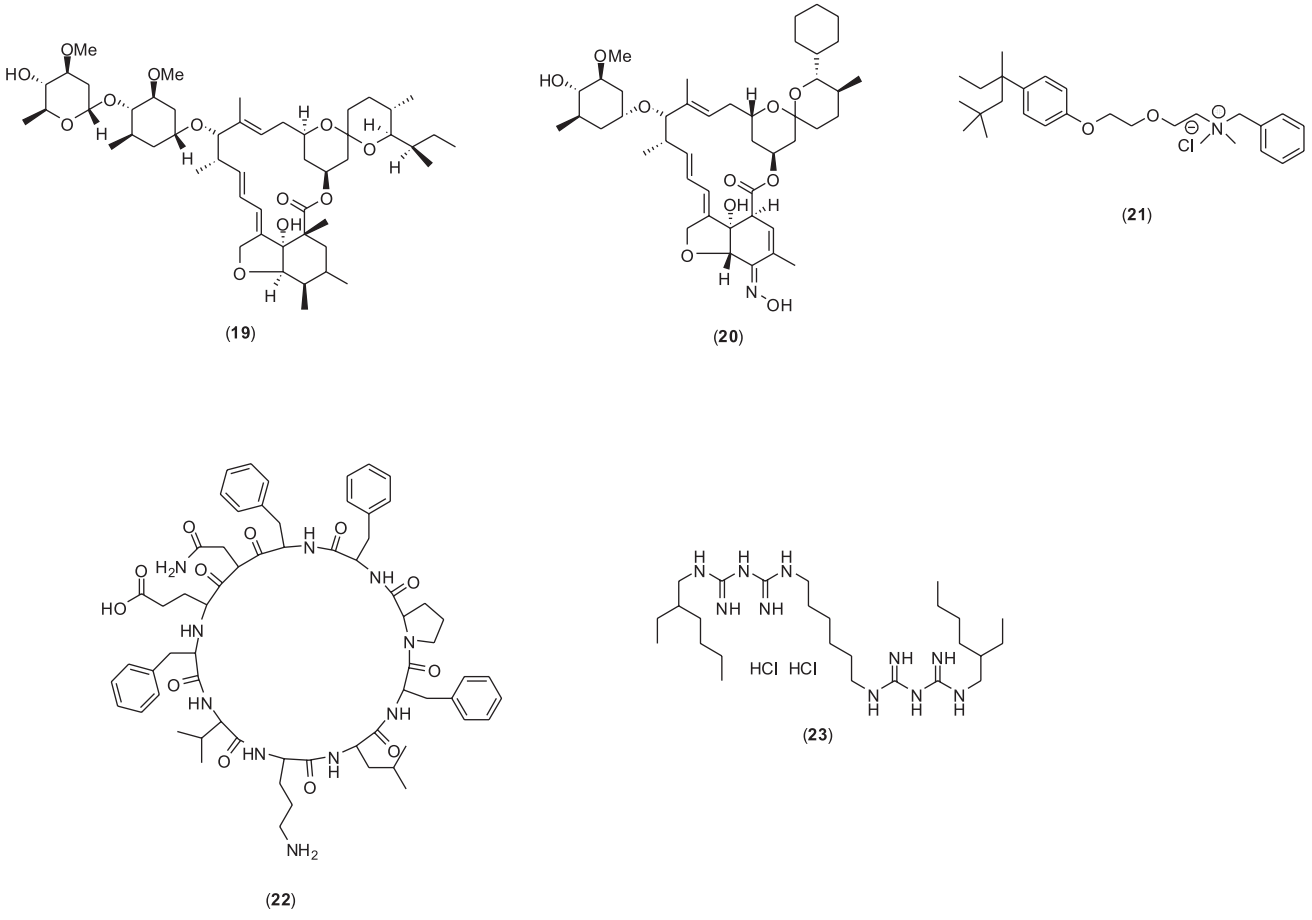

Figure 3. Structures of ivermectin (**19**), selamectin (**20**), benzothonium chloride (**21**), tyrothricin (**22**), and alexidine hydrochloride (**23**).

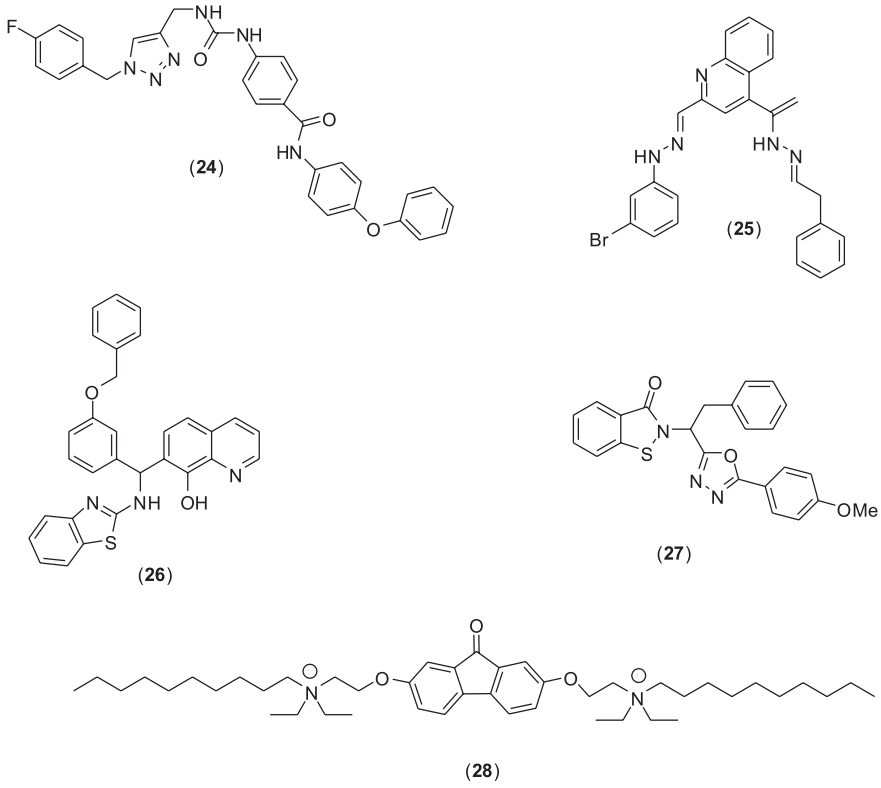

Figure 4. Structures of DENV protease inhibitors **24**-**28**.

Recently, a high-throughput screening of a 200,000 compounds library identified the benzoxazole ST-610 (**29**) (Figure 5) as a dengue helicase inhibitor.[87] The compound inhibited all four dengue serotypes in cell culture. In addition, ST-610 is non-cytotoxic and non-mutagenic. Finally, this benzoxazole showed marginal effects in the DENV AG-129 mouse model, with < 10-fold viremia reduction.[88]

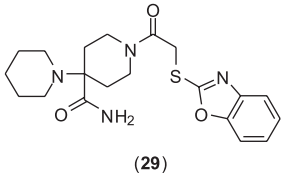

Figure 5. Structure of ST-610.

8. NS5 Methyltransferase and NS5 Polymerase Inhibitors

A series of small-molecular weight compounds possessing the general structure **30** (Figure 6) are compounds that could block dengue virus methyltransferase.[89,90] The investigators tested twelve compounds for its inhibitory activity against DENV-3 methyl transferase (for both N7 and 2'-O methyl transferase activities). Among the evaluated compounds, the chlorinated derivative **31** was the most active, presenting $K_i = 0.82 \pm 0.06$ for N7-methylation and $K_i = 0.17 \pm 0.02$ for 2'-O methylation.

Figure 6 depicts the structures of DENV RdRp inhibitors. They can be divided into two major categories, namely nucleoside and non-nucleoside inhibitors.

Compounds **32** and **33** are nucleoside inhibitors. NITD-008 (**32**) corresponds to an adenosine analogue and presents high potency against DENV replication. *In vivo* experiments with DENV-infected mice showed that peak viremia was suppressed and completely prevented death in a lethal mouse model. Moreover, no adverse effects were noticed when rats were orally treated with this nucleoside. However, severe side-effects were observed in both rats and dogs after 2 weeks of application. As a consequence, further development of compound **32** was ended.[91,92]

Balapiravir (**33**) is another example of nucleoside originally developed by Hoffmann-La Roche for treatment of hepatitis C virus. However, this clinical development was finished because of side effects on patients.[93] This nucleoside was reconsidered for a phase II trial for DENV. The nucleoside presented low efficiency at different doses applied to two groups of adult dengue patients. The reasons for this failure remain unclear.[12]

The non-nucleoside **34** (Figure 6) was identified after a high-throughput screening of about 1.8 million compounds. This N-sulphonylanthranilic acid derivative

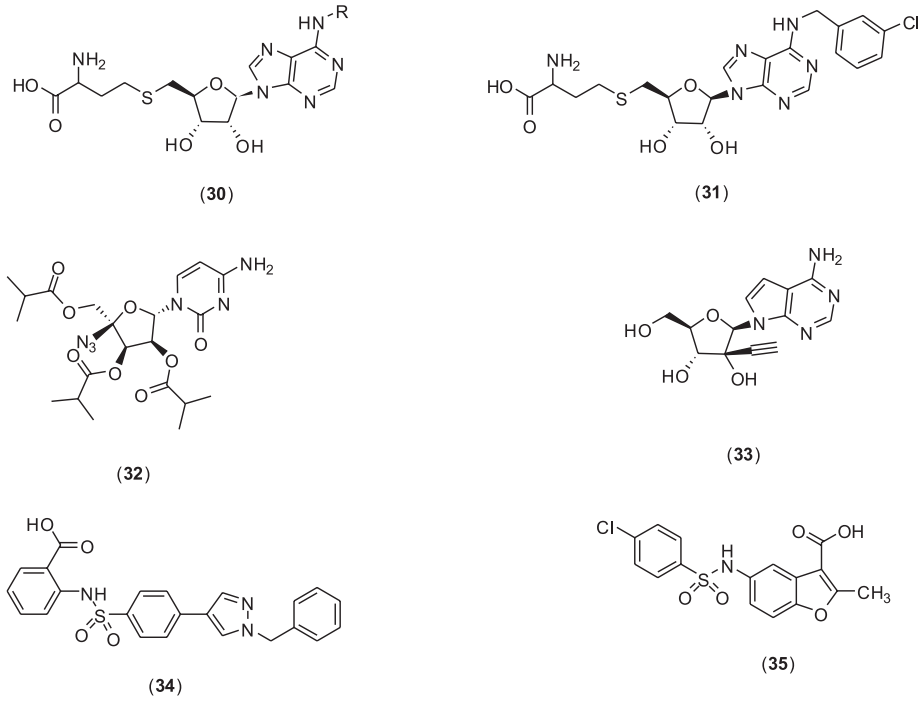

Figure 6. Nucleoside and non-nucleoside.

inhibits DENV-4 RdRp activity in a dose-responsive manner, with IC_{50} of 113 μmol L^{-1}. Using a replicon assay, it was noticed that compound **34** also inhibits DENV-2 replication (EC_{50} of 100 μmol L^{-1}). It was also demonstrated that this compound binds selectively, though weakly, to DENV RdRp. The related compound **35** also inhibited DENV-2 RdRp activity more efficiently with an IC_{50} of 7.2 μmol L^{-1}.[94,95]

9. Conclusions

Two general strategies can be pursued for any antiviral therapy. The first is to inhibit viral targets. The second is to inhibit host targets. Inhibition of targets in the host continues to be risky since it is not yet known what all the mechanisms of infection and disease development in the host are, particularly in cases of dengue hemorrhagic fever. Another point is that the action of the drug should be well known to minimize side effects.

In this case, inhibition of viral targets is a good way. The best characterized DENV proteins are nonstructural proteins NS3 and NS5, which are multifunctional proteins presenting several enzymatic activities. These proteins are the most conserved ones in all four dengue virus serotypes. It is a good point to create an efficient drug that acts in all four serotypes.

Even with forthcoming advances, a drug for clinical trials has not yet been obtained. A greater understanding of the viral replicative cycle in the host and the variations that occur in different serotypes will be necessary for the choice and improvement of a suitable drug.

Although challenging, recent research has generated optimistic results and has encouraged researchers to develop an effective therapy against the dengue virus in the near future.

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