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Topical Perspectives

Allosteric pocket of the dengue virus (serotype 2) NS2B/NS3 protease: *In silico* ligand screening and molecular dynamics studies of inhibition

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ABSTRACT

The allosteric pocket of the Dengue virus (DENV2) NS2B/NS3 protease, which is proximal to its catalytic triad, represents a promising drug target (Othman et al., 2008). We have explored this binding site through large-scale virtual screening and molecular dynamics simulations followed by calculations of binding free energy. We propose two mechanisms for enzyme inhibition. A ligand may either destabilize electronic density or create steric effects relating to the catalytic triad residues NS3-HIS51, NS3-ASP75, and NS3-SER135. A ligand may also disrupt movement of the C-terminal of NS2B required for inter-conversion between the "open" and "closed" conformations. We found that chalcone and adenosine derivatives had the top potential for drug discovery hits, acting through both inhibitory mechanisms. Studying the molecular mechanisms of these compounds might be helpful in further investigations of the allosteric pocket and its potential for drug discovery.

1. Introduction

Dengue virus (genus *Flavivirus)* is a human pathogen transmitted by the mosquitoes *Aedes aegypti* and *A. albopictus*. It poses a public health threat to 2.5 billion people worldwide, leading to 50–100 million human infections annually [1,2]. Approximately 500,000 dengue fever (DF) cases progress to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), resulting in ~25,000 deaths, mainly in children.

Dengue virus type 2 (DENV2) is the most prevalent of the four dengue serotypes [2]. The 10,723 nt genome of DENV2 encodes 3391 amino acid residues of a single polyprotein precursor C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5 [3] (Fig. 1A).

DENV2 NS2B/NS3 is a serine protease of the trypsin superfamily with a catalytic triad (NS3-HIS51, NS3-ASP75 and NS3-SER135) [4]. DENV2 NS2B/NS3 protease is a complex of two separate proteins – the NS2B protein and the protease domain of the NS3 protein [5]. Forty amino acid residues of the hydrophilic segment of NS2B are vital for structural stability and enzymatic activity [5,6]. The protease adopts two critical conformational states – a "closed" conformation in which the C-terminal of NS2B is bound to the allosteric pocket proximal to the catalytic triad, and an "open" conformation with the NS2B chain coiled with its C-terminal away from the allosteric pocket (Fig. 1B). It has been proposed that the "open" structural conformation is favored in the DENV2 NS2B/NS3 protease [7,8]. Based on the published data, it has been postulated that there are several stages in native enzyme activity: "open" conformation > substrate binding > reaction > motion of the C-terminal of NS2B into the "closed" conformation > release of reaction products > return to the "open" conformation [5–10]. Our analysis of the homology model of the "closed" conformation of the protease [11] reveals an essential role for NS3-ASN152 in keeping the C-terminal of the NS2B chain fixed in the allosteric pocket of the NS3 protease domain.

NS2B-GLY_4-SER-GLY_4-NS3pro, which is commonly used in experimental studies, shows decreased enzymatic activity compared to the native DENV2 NS2B/NS3 protease [12]. This is likely due to it being fixed in the "open" conformation.

Few allosteric inhibitors of DENV2 NS2B/NS3 protease have been experimentally identified, and their binding modes were elucidated through *in silico* docking [13,14]. We conclude that NS3-LYS74, NS3-LEU149, and NS3-ASN152 are essential for interactions with inhibitors.

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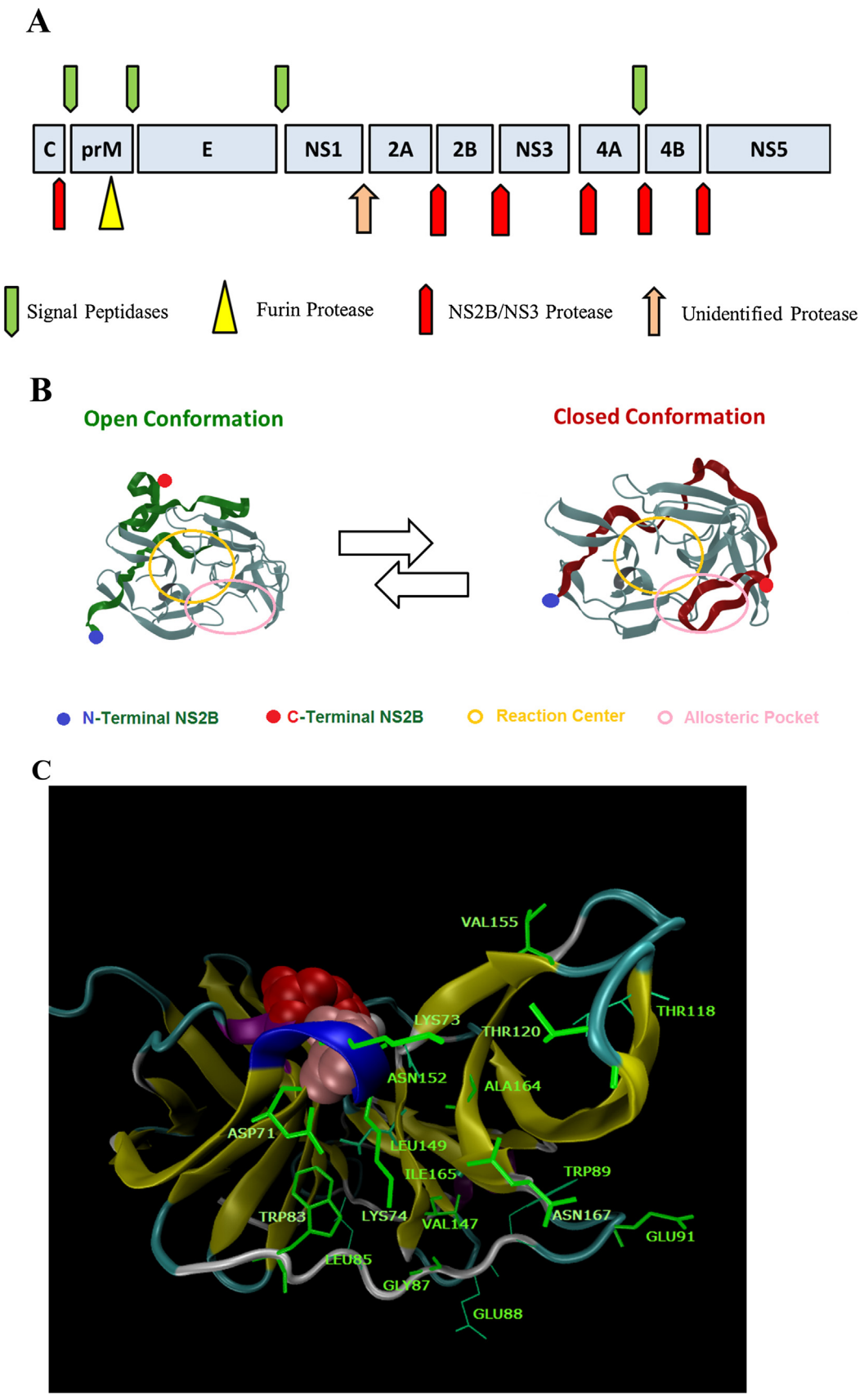

Fig. 1. Function and structure of DENV2 NS2B/NS3 protease. (A) Proteins comprising DENV polyprotein sequence, and enzymes in charge of its cleavage; (B) feasible role of "open"–"closed" conformational move in protease function in native condition. (C) Molecular structure of DENV2 NS2B/NS3 protease: reaction center residues are shown in VDW representation – NS3-ASP75 (pink), NS3-HIS51 (red), and NS3-SER135 (gray, behind); residues of the allosteric pocket in NS3 domain are shown with green lines – NS3-ASP71, NS3-LYS73, NS3-LYS74, NS3-TRP83, NS3-LEU85, NS3-GLY87, NS3-GLU88, NS3-TRP89, NS3-GLU91, NS3-THR118, NS3-THR120, NS3-VAL147, NS3-LEU149, NS3-ASN152, NS3-VAL155, NS3-ALA164, NS3-ILE165, and NS3-ASN167. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)