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Virtual screening of eighteen million compounds against dengue virus: Combined molecular docking and molecular dynamics simulations study

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ABSTRACT

Dengue virus is a major issue of tropical and sub-tropical regions. Dengue virus has been the cause behind the major alarming epidemics in the history with mass causalities from the decades. Unavailability of on-shelf drugs for the prevention of further proliferation of virus inside the human body results in immense number of deaths each year. This issue necessitates the design of novel anti-dengue drug. The protease enzyme pathway is the critical target for drug design due to its significance in the replication, survival and other cellular activities of dengue virus. Therefore, approximately eighteen million compounds from the ZINC database have been virtually screened against nonstructural protein 3 (NS3). The incremental construction algorithm of Glide docking program has been used with its features high throughput virtual screening (HTVS), standard precision (SP), extra precision (XP) and in combination of Prime module, induced fit docking (IFD) approach has also been applied. Five top-ranked compounds were then selected from the IFD results with better predicted binding energies with the catalytic triad residues (His51, Asp75, and Ser135) that may act as potential inhibitors for the underlying target protease enzyme. The top-ranked compounds ZINC95518765, ZINC44921800, ZINC71917414, ZINC39500661, ZINC36681949 have shown the predicted binding energies of −7.55, −7.36, −8.04, −8.41, −9.18 kcal/mol, respectively, forming binding interactions with three catalytically important amino acids. Top-docking poses of compounds are then used in molecular dynamics (MD) simulations. In computational studies, our proposed compounds confirm promising results against all the four serotypes of dengue virus, strengthening the opportunity of these compounds to work as potential on-shelf drugs against dengue virus. Further experimentation on the proposed compounds can result in development of strong inhibitors.

1. Introduction

Dengue virus belongs to *Flavivirus* genus which is a member of Flaviviridae family [1,2]. Hosts of dengue virus are humans and monkeys. Severe flu-like symptoms are observed in dengue fever which leads to high body temperature up to 40 °C or over. Symptoms also include muscle and joint pain, severe headache, facial flushing and skin rash. More severe conditions are called Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) [3]. The plasma dengue viral load in patients with severe dengue infection such as DHF and DSS is 1–2 logs higher than those in normal patients [4]. The cause of mortality from dengue infection is mostly due to DHF and DSS. People suffering from DHF have 5% rate of mortality but if turns to DSS then the mortality rate can rise as high as 40% [5]. Dengue virus completes its life cycle within one or two weeks. The symptoms start appearing after five to eight days of the infection [6]. Length of dengue virus genome is 10.7–11 kb. Dengue virus genome is composed of single stranded RNA. The structure of the dengue virus is roughly spherical, with a diameter of approximately 50 nm [7]. The single stranded RNA encodes ten proteins. Three of which are structural proteins (*i.e.* the capsid, envelope and membrane proteins that form the coat of the virus and deliver the RNA to target cells). Seven of them are nonstructural proteins *i.e.*; NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 that cause the production of new viruses once the virus gets inside the cell [8]. Nonstructural protein 3 (NS3) is a bi-functional enzyme. Its serine protease N-terminal domain carries out proteolysis of the polyprotein forming a segment of 40 residues from the NS2B protein acting as a cofactor. The C-terminal domain has ATPase/helicase enzy-

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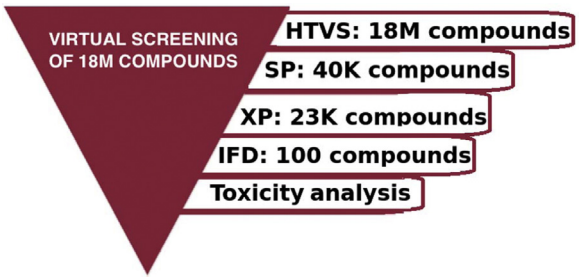

Fig. 1. Hierarchal screening approach using combined molecular modeling approach. HTVS, SP, XP and IFD denote high-throughput virtual screening, standard precision, extra precision and induced-fit docking, respectively.

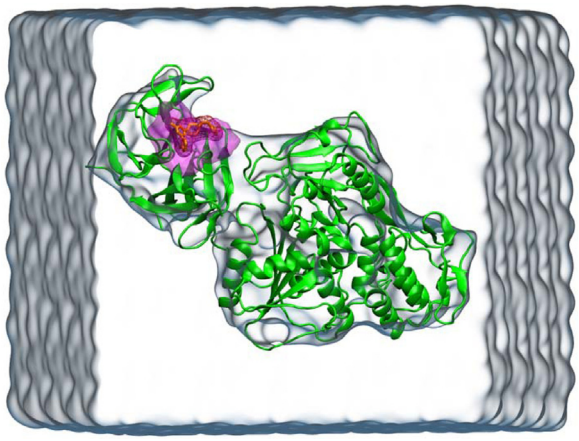

Fig. 2. Initial snapshot of the constructed system which was implemented in the MD simulation. NS3 protease helicase enzyme in complex with ZINC44921800. Protein, ligand and water atoms were rendered by cartoon, licorice and quick surface models of the VMD, respectively.

matic activity. Therefore, the NS3 protease enzyme has a prime importance as an antiviral drugs designing target against dengue virus.

Dengue virus is responsible for infecting 50–100 million people each year. Out of the huge number of hospitalized cases, 500,000 patients develop the more severe condition dengue hemorrhagic fever, leading to approximately 20,000 deaths, mainly in children [9,10].

Dengue virus has four serotypes DEN-1, DEN-2, DEN-3 and DEN-4. Each serotype has different interactions with antibodies in human blood serum [11]. These four serotypes share approximately 65% of their genomes. Although there are variations among these DENV serotypes; the disease and symptoms caused by the infection of these four serotypes are same [12]. NS3 protease enzyme has the conserved triad of catalytically important residues (His51, Asp75, Ser135) among all 4 serotypes. Crystal structures are available for protease enzymes of all four DENV serotypes [13].

Although some inhibitors are proposed for different dengue proteins through various procedures, no "on-shelf" drug or vaccine is available so far [14,15]. The purpose of this study is to discover potential anti-dengue virus drug candidates using virtual screening techniques. Large dataset of small molecules were prepared, virtually screened and molecular dynamics (MD) simulations and post-processing MD analyses were applied for the selected compounds at the current study against Nonstructural Protein 3 (NS3) [16].

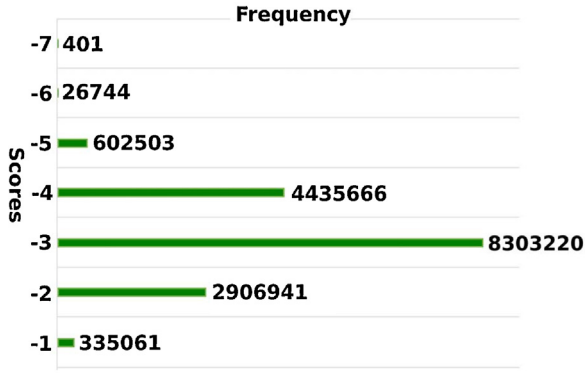

Fig. 3. Frequency distribution graph showing the distribution of ~18 million compounds over range of docking scores. (Scores are in range starting from 0 to −7 kcal/mol). Docking scores are distributed within range of 0 to −1, −1 to −2 kcal/mol and so on.

2. Methods

2.1. Structural preparation of enzyme

NS3 is a bi-functional enzyme. Its serine protease N-terminal domain carries out proteolysis of the polyprotein with a segment of 40 residues from the NS2B protein acting as a cofactor. The C terminal domain has ATPase/helicase enzymatic activity. Full length bi-functional enzyme of Dengue virus IV serotype has crystal structures available in Protein Data Bank (PDB ID: 2VBC) [17]. Crystal structure of the NS3 protease with PDB IDs: 3U1I, 3U1J, 2FOM, and 3L6P of Dengue virus have been taken from PDB, respectively [18–21]. All proteins were prepared for the experimentation using protein preparation module of Schrodinger's Maestro Molecular modeling suit [22]. Hydrogen atoms were added followed by energy minimization and optimization by OPLS2005 force field. Protonation states were determined at physiological pH 7.4 using PROPKA [23,24]. Water molecules around the catalytic triad (His51, Asp75, and Ser135) were kept during protein preparation and rest of the water molecules were removed.

2.2. Ligand preparation and virtual screening

Drug-like chemical structure library of around 17.9 million compounds were downloaded from the ZINC database for molecular docking simulation studies [25]. These structures were prepared by the ligand preparation LigPrep module of the Maestro molecular modeling package. The physiological medium and ionization states were determined for the all ligands and the pH was set to 7.0. Known NS3 reference inhibitors from literature were prepared by the ligand preparation using LigPrep followed by Macromodel minimization and conformational search modules of the Maestro. The lowest energy conformations of NS3 reference inhibitors were selected for the docking studies as control experiments [26,27].

After ligand and protein preparation, and grid generation according to the active site of the protein, the next step was docking simulations. Glide docking program of Maestro molecular modeling package is used at the docking simulations [28]. Ligand screening was based on hierarchal screening approach in docking (Fig. 1). High throughput virtual screening (HTVS), standard precision (SP), extra precision (XP) from Glide module and Induced Fit Docking (IFD) from Maestro has been applied successively on 17.9 million compounds, iteratively.