Implications of protein conformations to modifying novel inhibitor Oseltamivir for 2009 H1N1 influenza A virus by simulation and docking studies

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Abstract Recently three FDA approved existing drugs, namely—Oseltamivir, Peramivir and Zanamivir, used against Neuraminidase (NA) for the inhibitory effect on the process of viral progeny release to inhibit infection. All NA subtypes has been divided into two groups (Group 1 and Group 2) based on phylogenetic study. Oseltamivir and Zanamivir drugs are designed for Group 2 NA but are also used against 2009 H1N1 NA that lies in Group 1. There is no specific drug available for H1N1 and, consequently, there is an urgent requirement for the same. The structure-based drug design and fragment-based drug design methods are used for building more effective and economic drug molecules. In this work, the fragment-based drug development followed by fragment evolution on the basis of protein conformations after every 10 ns of 100 ns simulation. There are two analogs of Oseltamivir acid drug discovered in this study. Only analog 1, along with Oseltamivir acid, were then docked with the native protein. The analog 1 (benzoic acid inhibitor 11) exhibited higher binding affinity value of -10.70 kcal/mol in comparison to its predecessor. The concept of conformations and protein–ligand interactions can be useful in designing new drugs for H1N1 with high specific binding.

Keywords Drug design · Neuraminidase · 2009 H1N1 · Computer-aided drug design

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Introduction

The Influenza virus causes highly infectious seasonal viral diseases and, occasionally, pandemic flu [9, 14, 16, 25, 32, 41, 47]. The outbreak of 2009 H1N1 flu has led to serious economic losses and public panic, which indicates the importance of flu prevention and control [46]. Influenza A virus acquires two major glycoproteins, hemagglutinin (HA) and neuraminidase (NA), that protrude from the viral envelope. HA binds to sialic acid-containing host cell receptors to facilitate viral entry, whereas NA cleaves the sialic acid from receptors to facilitate the spread of infection by releasing newly synthesized virions into neighboring cells, thus making it a major target for anti-influenza drugs [33].

Only three FDA approved novel drugs—Zanamivir (a dehydrated sialic acid derivative), Oseltamivir acid (cyclohexane ring) and Peramivir (a cyclopentane)—are currently available for influenza virus. Influenza A and B infections are considerably reduced by these drugs [19, 36]. During the 2009 H1N1 pandemic, Oseltamivir and Zanamivir were used for the treatment of sick individuals. Zanamivir is available for oral inhalation only, primarily due to its high polarity [7, 17]. However, Oseltamivir-resistant variants have also been reported [15]. There is, therefore, a pressing requirement to develop a new analog having high efficacy against resistant variants.

The NA inhibitors design is based on frequently used functional groups such as carboxyl, guanidine and 3-pentyloxy [2, 3, 21, 23, 29, 32, 35, 37–39]. The potency of a ligand depends on the combination of groups [18]. The carboxyl group exists in almost all the NA ligands and the carboxylate anion matches perfectly with the positively charged arginine triad of NA active sites.

The FDA approved drugs mentioned have been developed by the rational structure-based drug design approach and take into consideration the NA structures for group 2 [45]. There is no FDA approved drug specifically meant for group 1 (presence 150-Cavity) and drugs designed for group 2 (deficient 150-cavity) have been administered to counter 2009 H1N1 [1, 24, 44, 46]. This necessitates the development of a more potent drug that is specifically designed for usage in situations involving H1N1 which lies in group 1.

A number of computational approaches are available and used for designing a new potent molecule. One of them is the *Fragment-based Drug Design* that relies on identification of small chemical fragments. These fragments are weakly bound to the target on an individual basis but combining them results in creation of a lead molecule with higher binding affinity [31]. Another approach *Molecular Dynamics (MD) simulation* has been playing an increasingly important role for enhancing our knowledge about 3D structures of biological targets and their potential inhibitors. Molecular dynamics simulations have the capability to show structural flexibility changes around the binding site. The structural flexibility has an occasional large effect on the docking scores and, in addition, helps finding the residues of the target protein that come into contact with a specific fragment of the compound [22]. The combination of potent fragments is important purely from the perspective of a high efficiency lead molecule which binds strongly to a target. These lead molecules act as modified drugs which are better than the original drugs.

This work has its focus on designing new targeted inhibitors by using a computational approach that relies on identifying structural flexibility of a druggable active site at different time intervals through Molecular Dynamics (MD) simulations. The simulation width was 100 ns and the step size used was 10 ns for all conformations. The docking studies are used for understanding the interaction between drugs and different conformations. The knowledge thus acquired is used for designing new analogs / lead compounds.

Materials and Methods

Protein structure preparation

The crystal structure of the complexed neuraminidase with Oseltamivir acid (PDB ID: 3TI6) was retrieved from Protein Data Bank (PDB) [5]. The ligand from crystal structure was removed, protein was prepared, and the resulting structure was used as the input for Molecular Dynamics (MD) simulations.

Ligand preparation

The ligands used in this study were downloaded from Chem Spider Database. Compound structures (Oseltamivir acid, Zanamivir and Peramivir) were downloaded in the 2D MOL format and then converted into the PDB format using PYMOL [34]. The Gasteiger charges and rotatable bonds were allowed to move freely to the PDB ligands using Auto dock tools.

Molecular dynamics setup

MD simulations were done by using GROMACS 5.0 [4, 43]. The topology of protein was generated by using pdb2gmx tool at amino acid protonation state with a standard pH value of 7. The simulation runs under G43a1 force field. The solvation of input protein structure was carried out using the extended single-point charge (SPC) water model in a cubic box with 1.0 nm space around the solute. The system net charge was neutralized by replacing the water molecules with 3Na⁺ ions. The system contained 49,937 atoms and out of them 15,354 atoms belonged to water molecules. Steepest decent method was used for energy minimization. Subsequently, the system was equilibrated for 100 ps under NVT (constant number of particles, volume and temperature) and NPT conditions (constant number of particles, pressure and temperature), using leap-frog integrator. Such all-atom molecular dynamics simulations were carried out for 100 ns each.

The system weakly coupled to an external bath using V-rescale thermostat. The constant temperature was set as 300 K and LINCS algorithm was used for all bond constraints [27]. Long range electrostatic interactions were calculated with Particle Mesh Ewald (PME) and the time step used was 2 fs [12].

The protein stability and flexibility acquired during 100 ns by GROMACS built-in tools was used for the determination of Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and Radius of gyration (Rg) computations.

Prediction of cavity

The surface topology of protein was calculated using CASTp server [11].

Docking studies

The novel drugs (Oseltamivir acid, Peramivir and Zanamivir) were considered for docking studies. The corresponding 2D Structures (MOL format) were obtained from Chem spider database and converted in 2D PDB format by PYMOL. For 3D structure preparation of ligands,

discovery studio 3.1 was employed. 3D protein structures obtained at 10 ns intervals across the simulation span were docked separately with ligands. Blind docking was performed by Auto dock with default grid spacing of 0.375 Å. Genetic algorithm was used as a local search parameter. Docking included flexible ligand and rigid protein structures. Ten docking runs were performed for each ligand and conformation of protein. Atomic contacts between the ligand and the protein were analyzed using Discovery studio 3.1. From the ten docking runs of each of these docking studies, the complex with lowest energy was used for further interpretation.

Results and discussion

Stability of the structure

Molecular dynamics (MD) simulation of neuraminidase was executed for a time period of 100 ns. The protein structure stability was analyzed using RMSD and Rg plots (Fig. 1). The protein stabilized close to 10 ns and remained stable till the end of the cycle at 100 ns. The final RMSD value was observed as 0.329 nm. The average Rg value was determined as 1.95 nm and is an indicator of protein compactness. The results indicate that the simulated structures follow a stable trajectory throughout the 100 ns simulation. The RMSF profile allows the identification of the flexible regions in the protein whereas the RMSF value denotes average displacement of residue (C α atoms) with respect to time [28].

Prediction of cavity

The active site prediction at different time intervals was done by CASTp server. The maximum and minimum cavity volume values were found to be 797.1 Å and 203.2 Å, respectively, as shown in (Table 1 in Supplementary material).

Docking

Docking was performed for different conformations of NA and novel drugs (Oseltamivir acid, peramivir, and Zanamivir). Every docking was repeated 10 times. In docking results, the scoring function is a numeric representation of the binding affinity between all conformations of protein and novel drugs. Out of the 10 dockings at different time intervals for all novel drugs, only that docking is selected that has the minimum binding energy. The minimum binding energy of the starting complex (native structure) is shown to be -7.06 kcal/mol (Oseltamivir), -7.0 kcal/mol (Peramivir) and -7.02 kcal/mol (Zanamivir) respectively.

Selection of common residues for hydrogen bond formation

Molecular simulation of all three drugs for the enzyme H1N1-NA has been carried out to explore the active site. The docking studies at different time intervals, on the other hand, help identify most favorable binding interactions that can be used to modify the existing drugs. Only the high binding affinity docked complexes at different time interval have been selected. In all, 11 residues from all drugs have been identified that participate in hydrogen bond formation. These are: ARG118, GLU119, ASP151, ARG152, SER153, ARG156, TRP178, GLU227, GLU277, ARG371, GLU425 [10, 42] and are shown in (Table 2 in Supplementary material). Out of these 11, those 6 common residues (GLU119, ARG152, GLU227, ARG371, ARG156, GLU277) have been selected that are present for the maximum time duration at different time intervals.

Selection of fragments

To select the fragments, the interaction pattern between all three drugs with selected six residue positions was observed. The results indicated that Oseltamivir covered the maximum residue positions with high binding affinity

Fig. 1 The protein stability representation by **a** Root mean square deviation. **b** Root mean square fluctuation plot based on c-alpha atoms profiles. **c** Radius of gyration shows protein compactness

at different time intervals. For the remaining positions not covered by the fragments of Oseltamivir, other two drug molecule fragments were considered. This leads to new specific analogs, which interact with all selected common residues and play an important role in increasing the binding affinity (Table 3 and 4 in Supplementary material). The fragments selected for modification were analyzed on the basis of uniqueness as well as the interaction frequency (with a minimum value of 4). These drugs showed the four unique interacting functional groups. Out of these, the two functional groups (guanidine amino group and glycerol hydroxyl group) showed significant interaction.

Designing of new analogs

The active site of neuraminidase (N1) is divided into five sub-sites (S1–S5) [40]. The purpose of this study is to design new analogs of Oseltamivir acid by selected fragments that cover the maximum sub-sites. The process of prediction and searching of new analogs is shown in (Fig. 2).

Selection (Guanidine $-CNH(NH_2)_2$) The Oseltamivir single amino group maintains native interaction with only glutamate (GLU119 and GLU227, GLU119 or GLU227) at all-time intervals, but the two amino groups of guanidine maintain native interaction with glutamates (GLU119, GLU227, GLU277) in most time intervals. So these amino groups work as a key substituent to maximize the interactions of negatively charged binding pocket (sub site—S2), in addition to showing interaction with TRP178 and ASP151. Increasing the lipophilicity may strengthen the binding interactions. Addition: the two amino group of guanidine were added in Oseltamivir amino group to determine the energetically favored interactions between

various substituents and within the binding pocket. Similarity: This predicted compound is used to retrieve a similar compound from chemical databases. A similar analog benzoic acid inhibitor 11 (CID: 5275971) with the same chemical scaffolds was picked from PubChem database. This analog increased lipophilicity with the presence of benzene ring. This analog 1 is used for the different type of influenza virus A (H2N2) and B as an inhibitor [2, 8]. This compound may serve as a potent inhibitor for H1N1 influenza virus [20].

Selection (Glycerol group $-CH_2OH-CHOH-CH_2OH$) The selected hydroxyl group of glycerol of Zanamivir maintains the interaction with ARG371, ARG277 and ARG152 at different time intervals. However, the 3-pentanone group of Oseltamivir was not found to participate the maximum number of times for interaction at different time intervals. Replacement: this 3-pentanone group of Oseltamivir is replaced by glycerol hydroxyl groups of Zanamivir. Similarity: a search for the predicted compound 2 in the compound database did not throw information on any similar compound.

Calculation of molecular properties

The molecular properties for these designed compounds were calculated to estimate the drug likeliness (Table 5). The results indicate that the Rule of 5 is followed by only one analog 1 (Benzoic Acid Inhibitor 11) whereas analog 2 does not satisfy the criteria. Consequently, one analog 1 is selected for docking studies.

Fig. 2 Conceptual strategy to achieve new compounds using modification process in selected fragments **a** Guanidine amino group added in place of amino group and search similar compound with same chemical scaffold/groups. **b** Glycerol group in place of amino group

electrostatic and one hydrophobic bond interactions are generated by residues ASP151, GLU277 and ARG224 with bond length 4.07 Å, 3.81 Å and 4.17 Å respectively.

The spread of pathogenic H1N1 has evolved into a significant pandemic threat to the human population over the last few years. Novel NA ligands are being continually synthesized to account for the drug resistance issue. In this work, the fragment-based approach was used to analyze and figure out the specificities of various ligands at the neuraminidase (NA) active sites, combined with docking and molecular dynamics simulations. To understand the relationship between the ligand binding and conformational change of NA better, a fragment-based approach was used to design a new inhibitor employing a minimal 6 residues set of bonding interactions.

The findings of the current work suggest that the functional substituents, rather than the core templates, play a more significant role towards the binding processes of target protein [6, 8, 13]. Two functional groups (glycerol and guanidine) were selected from Peramivir and Zanamivir for modification in Oseltamivir acid. The amide group of Oseltamivir was extended by two amine groups. The Pentan-3-One was replaced by glycerol group.

The current work has predicted two analogs (1 and 2). The analog 1 is found to interact five with out of six selected common residues (GLU119, ARG152, GLU227, ARG371, GLU277), besides interacting with four additional residues (ARG118, ARG292, TYR406, TRP178). This results in formation of 9 hydrogen bonds, with -10.70 kcal/mol being the significantly higher binding affinity in comparison to Oseltamivir acid. It is expected that the novel neuraminidase inhibitor suggested here may serve as a potential focal point for designing future neuraminidase inhibitors against H1N1 virus.

Lead optimization by simulation studies

The Molecular dynamics simulation study was to explore the stability and conformational changes of inhibitor molecule in relation to the binding site that provides insight into the binding stability. Molecular dynamics exposed that this molecule could efficiently activate the biological pathway without changing the conformation in the binding site of NA protein. To evaluate the stabilities of benzoic acid—NA protein complex during the MD simulation, RMSD was calculated with respect to the initial structures along the 3.0 ns (ns) trajectories (Fig. 4). The trajectories indicated the stabilization of the receptor on the binding of benzoic acid inhibitor 11/(DRG) in the active site after 20.0 ns in system with a mean RMSD value of 2.0 nm [30]. In addition, the stability of the system also proved the reliability of the docking results. The average short-range

Fig. 4 The analog 1 stability representation by root mean square deviation during 20 ns simulation

coulombic interaction energy between ligand and protein is -169.613 kJ mol $^{-1}$ during 20 ns simulation [26].

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