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Short Communication

Homology modeling, docking, and molecular dynamics reveal HR1039 as a potent inhibitor of 2009 A(H1N1) influenza neuraminidase

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ABSTRACT

The neuraminidase of the influenza virus is the target of antiviral drugs oseltamivir and zanamivir. Clinical practices have shown that zanamivir and oseltamivir are effective in treating the 2009 A(H1N1) influenza virus. However, drug resistance strains are also emerging. Herein, we report the findings from homology modeling and molecular simulations of 2009 A(H1N1) neuraminidase complexed with zanamivir, oseltamivir, and several herb extracts with potential activities. Our docked oseltamivir and zanamivir results are consistent with previous studies. Based on the same procedure, the docked results of herb extracts HR1039 and HR1040 suggest that they are potential potent inhibitors of neuraminidase. Also, the binding modes of HR1039/HR1040 are different from those of oseltmivir and zanamivir, and may be effective in treating oseltamivir-resistant influenza virus strains.

1. Introduction

Science Mach 2009, a new strain of influenza A virus (2009 A (H1N1)) has spread rapidly and evolved into global pandemic [1]. The 2009 A(H1N1) influenza virus shares several common characteristics with the 1918 Spanish flu [2]. The pandemic caused by 2009 A(H1N1) influenza may lead to a dramatic burden on global healthcare systems, and may result in high morbidity and mortality rates. Currently two antiviral drugs, oseltamivir and zanamivir, are available for the treatment of influenza, and were reported effective for 2009 A(H1N1) influenza. However, as the influenza virus is evolving fast, some drug resistance strains are emerging. It is thus critical to seek potential alternative treatments, and identify the roots of the drug resistance.

Influenza virus can be classified by the antigenic properties of two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA) [3]. Hemagglutinin binds to the sialic acid receptor on the cell surface and facilitates the entry of the virus [4]. Neuraminidase cleaves the terminal linkage of the sialic acid receptor, which results in the release of the progeny virions from the infected host cells. Neuraminidase may facilitate the early processing of influenza virus infection in lung epithelial cells [5]. Because of its essential role in release of influenza virus particles, neuraminidase has been an attractive target for the anti-influenza drugs [6]. The two antiviral drugs, oseltamivir and zanamivir, both target neuraminidase.

The complex structure of 2009 A(H1N1) neuraminidase and these antiviral drugs is not available yet. Modeling of 2009 A(H1N1) neuraminidase structures thus provides a means to understand the interactions between antiviral drugs and 2009 A(H1N1) neuraminidase. Comparisons among wild type and drug resistance structural models should also provide insights on the molecular mechanism of drug resistance. Finally, compounds with potential therapeutic effects can be screened against the neuraminidase models of wild type and drug resistance strains. These compounds may complement current antiviral drugs when targeting the drug resistance strains.

2. Material and methods

Homology modeling and molecular dynamic (MD) techniques have been utilized to construct the three-dimensional structure of A(H1N1) neuraminidase. The neuraminidase sequence was collected from the NCBI protein database (accession no. ACP44158.1), and a sequence similarity search for the protein against other sequences with available structural information was performed using the NCBI BLAST service [7]. Seventeen structures were identified as homologous from Protein Data Bank (PDB) (PDB ID: 1A4G, 1A4Q, 3CKZ, 3CLO, 2HUO, 2HU4, 3CL2, 3B7E, 3BEQ, 2HT7, 2HT8, 2HTQ, 1L7H, 2QWH, 2QWK, 2CML, and 1V3E). The automated sequence alignment (Fig. 1) and analysis of the template and target was carried out using the Clustal X 2.09 program [8]. The MODELLER package [9] has been used to model the structure of A

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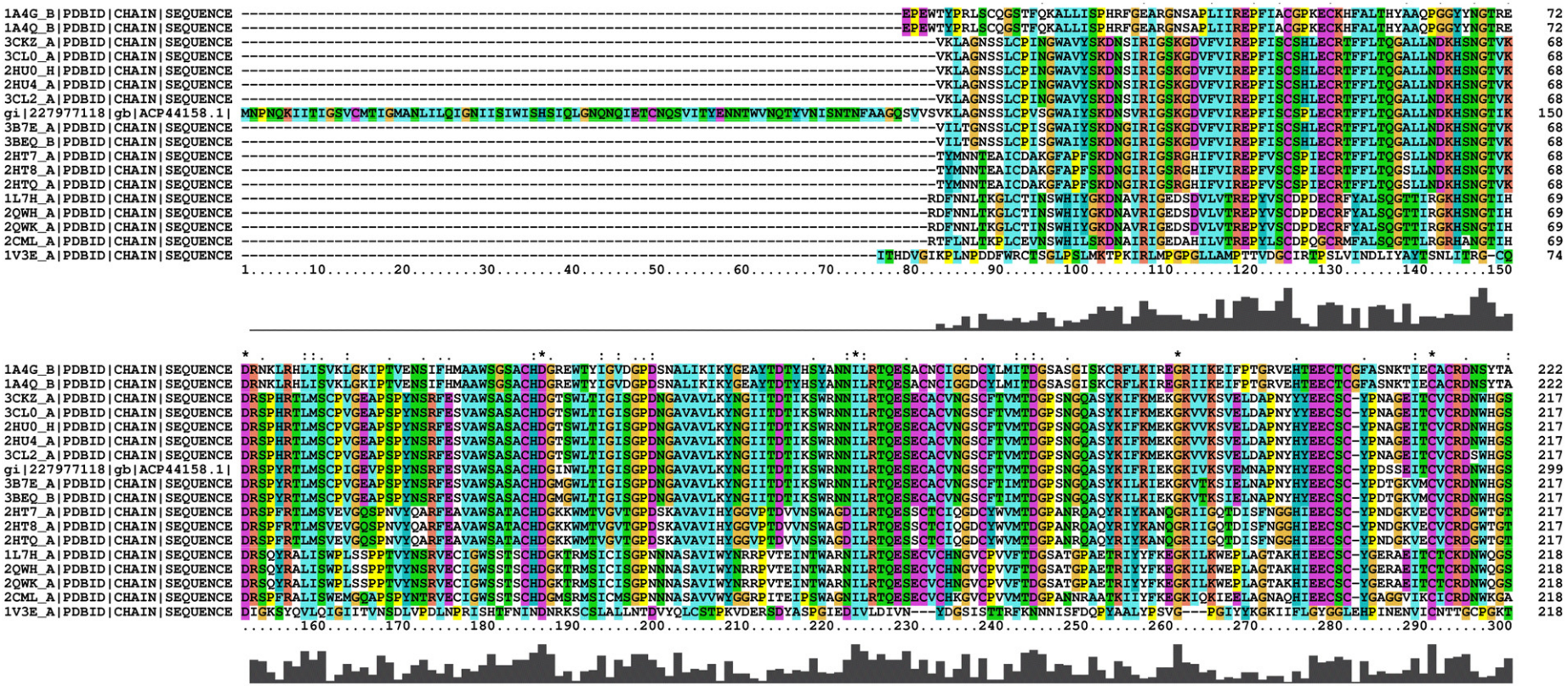

Fig. 1. H1N1 virus neuraminidase protein sequence aligned with seven different template sequences.

(H1N1) neuraminidase. According to the GA341 score functions [10] and a protein sequence similarity equal to 88.831%, the three-dimensional structure of the protein (PDB ID: 3B7E) was chosen for subsequent homology modeling simulations. The MODELLER software implemented comparative protein structure modeling by satisfying spatial restraints [11,12]. The structure of oseltamivir-resistant neuraminidase His275Tyr mutant was also modeled. The three models generated were then subjected to an energy minimization process by using the conjugate gradient method for about 2000 iterations and 2 ns isothermal, constant volume MD simulation, with AMBER FF99 all-hydrogen amino acid parameters and the TIP3P water box in the AMBER [13] program, in order to obtain the relaxed conformation, namely the neuraminidase protein with oseltamivir and zanamivir. To assess the quality of the optimized models, PROCHECK [14] and ERRAT [15] analyses were also undertaken. The Ramachandran plot of our model shows that 92.0% of residues were found in the most favored and additional allowed regions and 8.0% were in the generously allowed region. The quality factor (ERRAT) of our model was equal to 80.657, and thus we consider that the model is acceptable for predicting the binding modes and interactions of oseltamivir and zanamivir with A(H1N1) neuraminidase.

Oseltamivir and zanamivir were constructed and minimized using the SYBYL modeling program [16]. We aligned the structures of the model and the template (PDB ID: 3B7E), and then used the orientation of zanamivir in the template to identify the general binding pocket of the model. Next, the GOLD [17] docking program was used to dock the two drugs into the active site of our model structure, which was defined as all residues within 0.12 nm from alignment with the template structure drug. GOLD is a fast and accurate way to dock small compounds into fixed protein binding sites, utilizing hydrogen binding annealing parameters, GOLDscore and a genetic algorithm. Four thousand conformations were obtained from docking for the two compounds and scored by GOLDscore. The conformations of the best GOLDscores were then selected for subsequent MD simulations.

The software UCSF DOCK 6.2 [18] was then applied in the virtual screening of drug-like compounds and to verify our predicted binding modes. The screening database was derived from a Chinese natural herb product database containing more than 50,000 compounds. After the screening, we found that the DOCK grid scores of ~1000 compounds are better than those of the two drugs (the DOCK grid scores of the two drugs were almost equal to −30.15 kcal/mol). In addition, two compounds (HR1039 and HR1040, Fig. 2) have best DOCK grid scores, which were nearly twice as low as the two drugs. Consequently, these two herb compounds were selected for subsequent MD simulation.

Our models (wild type and His275Tyr drug-resistant mutant) with the two drugs and the two herb compounds (HR1039 and HR1040) were then simulated with the AMBER package using the AMBER FF99 all-hydrogen amino acid and general amber force field (GAFF) parameters. The geometries of the four compounds were fully optimized and their electrostatic potentials were obtained using single-point calculation, both at the B3LYP level with the 6-31G(d,p) basis set using the GAUSSIAN 03 program [19]. Subsequently, their partial charges were obtained with the restrained electrostatic potential (RESP) procedure using Antechamber. From docking simulations, the complex structures were generated and then inserted into the TIP3P water box. All MD simulations were performed in the isobaric, isothermal ensemble (with a simulation temperature equal to 310 K), unless noted, using the leap-frog integrator, an integration time step of 0.002 ps and SHAKE [20] of all covalent bonds involving hydrogen atoms. In electrostatic interactions, atom-based truncation was undertaken using the PME method. In addition, the switch van der Waals function was also used with a 1.8 nm cutoff for atom-pair lists. The complex structures were minimized for 50,000 conjugate gradient steps. The minimized complex structures were then subjected to a 2 ns isothermal, constant volume MD simulation. Over the 2 ns MD trajectories of the neuraminidase with water box and the four compounds, the overall structure of both complexes appeared to be equilibrated after 500 ps. The final structures and trajectories from these simulations were used to investigate the binding modes, pair interaction energies (electrostatic and van der Waals energies) and binding free energies ($\triangle G_{\text{Binding}}$) of the four compounds with the neuraminidase of the A(H1N1) virus. The time evolution of the interaction energies of the four compounds with the neuraminidase of the A(H1N1) virus was performed with NAMD software. [21]. The time evolution of the interaction energies between the neuraminidase and the four compounds was calculated for snapshot structures taken from the MD trajectory of the system. The time evolution of the potential energies and the interaction energies of the four complexed systems is shown in Fig. 3.

The binding free energy calculations were performed with the solvated interaction energy (SIE) method [22]. The SIE method is similar to the linear interaction energy (LIE) methods and other Molecular Mechanics Poisson–Boltzmann Surface Area (MM/PBSA) methods [23–25]. All those are so-called End-Point methods [26], which try to estimate the free energy between the bound and free states by considering only the initial and final states of the system. End-Point methods are less computational expensive than free energy perturbation (FEP) methods. The SIE method differs from LIE method in that SIE considers solvation terms explicitly, in an attempt to reproduce absolute binding free energies [22]. The End-Point methods do not include the translational, rotational and conformational entropic contributions [26], but are suitable for drug lead optimization [22,27]. The binding free energies between the neuraminidase and the four compounds were calculated for snapshot structures taken from the MD trajectory of the system. Fifty snapshots were taken at even intervals from the latter 1500 ps ligand-complexed MD trajectories of the neuraminidase and the four compounds for the binding energy analyses in the study. The $\triangle G_{\text{Binding}}$ of the four compounds are shown in Table 1. The lower $\triangle G_{\text{Binding}}$ of HR1039 suggests that HR1039 may be a more potent inhibitor comparing to oseltamivir and zanamivir. Also, the His275Tyr

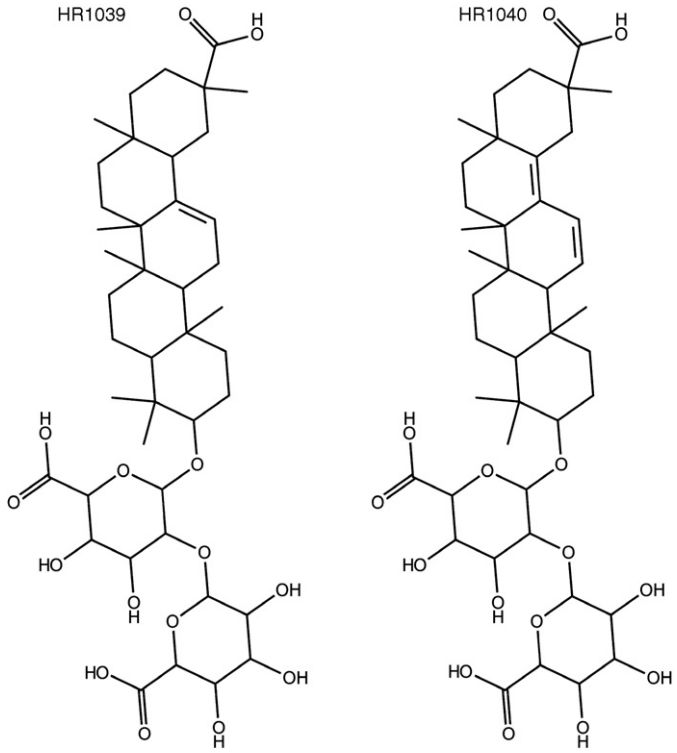

Fig. 2. Molecular structures of herb extract HR1039 (left) and HR1040 (right).

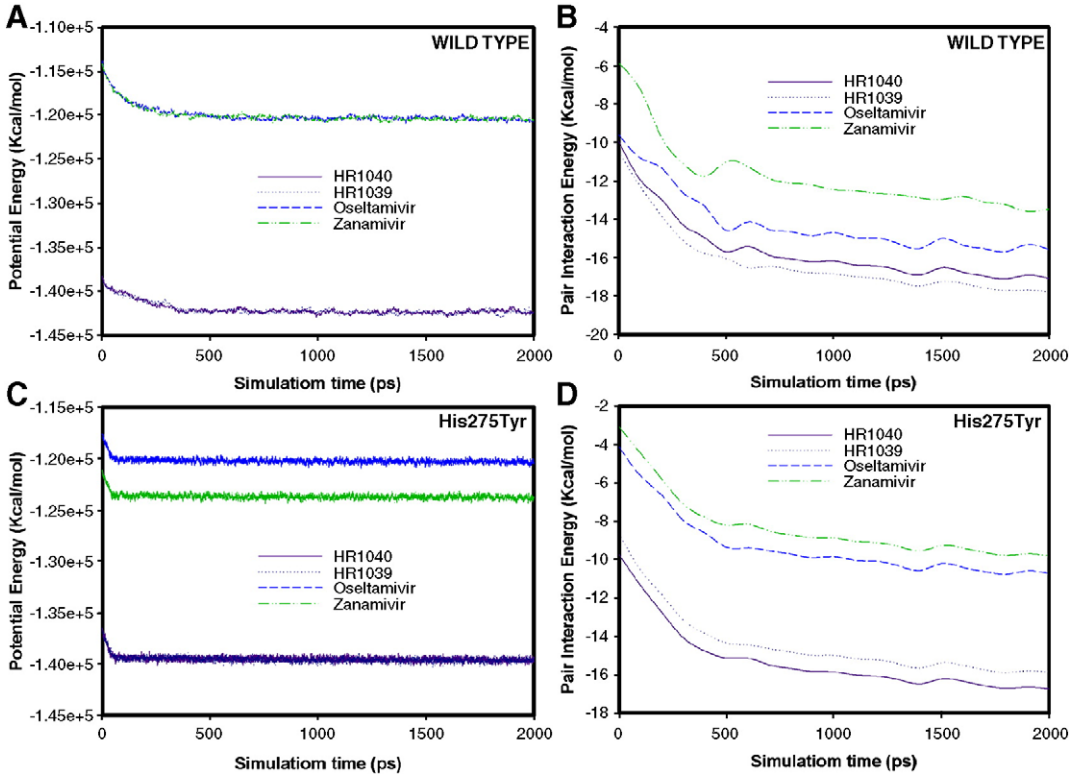

Fig. 3. The time evolution of the potential energies and the interaction energies of the four complexed systems. (A) Potential energies of the four compounds complexed with wild type neuraminidase. (B) Pair interaction energies in wild type nueraminidases. (C) Potential energies in the His275Tyr oseltamivir-resistant mutant. (D) Pair interaction energies in His275Tyr mutant.

mutation has profound effects on binding free energy of oseltamivir and zanamivir, yet HR1039 and HR1040 are not affected (Table 1).

3. Results and discussions

The analysis results of our simulations are consistent with previous studies [28,29]. The simulation results are summarized in Table 2 and Fig. 4. For the zanamivir binding modes, four residues (Arg152, Glu277, Glu278, and Asn295) form five hydrogen bonds with the drug, three residues (Glu119, Asp151, and Glh228) have frequent electrostatic bonding interactions with the drug, and there are no obvious van der Waals interactions between the drug and the neuraminidase protein.

Table 1
Binding free energies of oseltamivir, zanamivir, HR1039, and HR1040 with neuraminidases of the 2009 A(H1N1) virus.

Compound	Zanamivir	Oseltamivir	HR1039	HR1040
$\triangle G_{Binding}$ (kcal/mol) (wild type)	−21.39	−20.54	−26.38	−23.91
$\triangle G_{Binding}$ (kcal/mol) (His275Tyr drug-resistant mutant)	−13.67	−12.94	−25.47	−24.86

Binding free energy calculations were performed with the SIE method.

Table 2
Analysis of the binding modes of oseltamivir and zanamivir with the neuraminidase of the 2009 A(H1N1) virus.

Compound	Zanamivir	Oseltamivir
Elestostatic	Glu119, Asp151,Glh228	Ash151
Van der Waals	Null	Ile149, Arg152, Trp179, Ile223, The438
Hydrogen bonding	Arg152, Glu277, Glu278, Asn295	Gln136, Arg118, Glu278, Tyr402

For oseltamivir binding modes, four residues (Gln136, Arg118, Glu278, and Tyr402) form four hydrogen bonds with the drug, the Ash151 residue has frequent electrostatic bonding interactions with the drug, and five residues (Ile149, Arg152, Trp179, Ile223, and The438) have frequent van der Waals interactions with the drug. The same displacements in His275 and Glu277 (His274 and Glu276 in H5N1 neuraminidase) are found in our modeled structures [29]. These displacements are critical to the oseltamivir-resistance of N1 neuraminidase. Another mutant, Asn295Ser (Asn294 in H3N2 and H5N1), is also involved in oseltamivir-resistance [29]. The interactions between Asn295 and the two compounds are also noted in our modeled results. The consistent results suggest that our approaches may be successfully applied to the screening and identification of novel neuraminidase inhibitors.

The binding modes of the HR1039 and HR1040 are distinctive to those of the two drugs above. The analysis results of our simulations are shown in Table 3 and Fig. 5. The same binding modes were seen in both in HR1039 and HR1040. This is not surprising, since the two only differ in several double bonds not directly involved in the binding of the compound–neuraminidase complex (Fig. 2). For all the binding modes, the eight residues (Arg118, Arg152, Glu278, Arg293, Arg368, Gly401, Tyr402, and Lys432) form five hydrogen bonds with the two herb compounds, the four residues (Arg118, Agr152, Arg368, and Lys432) have frequent electrostatic bonding interactions with the two herb compounds, and the Ile149 residue has frequent van der Waals interactions with the two herb compounds. The bindings of HR1039 and HR1040 to neuraminidase do not involve the two residues critical to oseltamivir-resistance, His275 (mediated by Glu277) and Asn295. This suggests that the oseltamivir-resistant neuraminidases may still be sensitive to the two potential potent inhibitors, HR1039 and HR1040.

The simulation results suggest that the four compounds have different binding modes, thus HR1039 and HR1040 may serve as potential alternative treatment to influenza when the virus shows resistance to oseltamivir or zanamivir. However, one residue

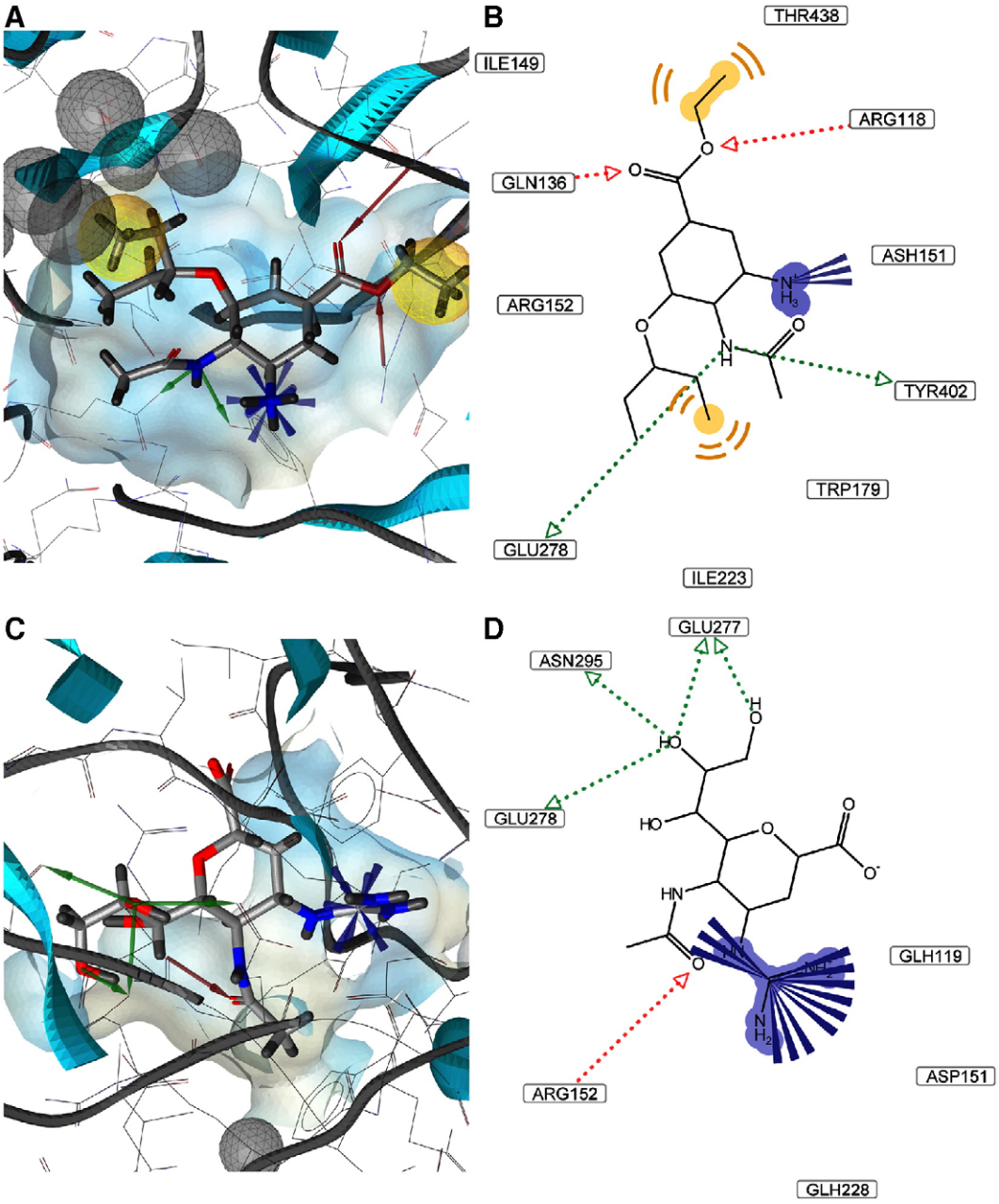

Fig. 4. Modeled structures of the 2009 A(H1N1) neuraminidase: (A) oseltamivir bound to the binding site of neuraminidase. (B) Pharmacophore of oseltamivir. (C) Zanamivir bound to the binding site of neuraminidase. (D) Pharmacophore of zanamivir.

participates in all four compound–neuraminidase complexes. The Glu278 residue forms hydrogen bonds with Arg293 and these compounds. Considering all the residues involved in the binding of these compounds, the contributions of Glu278 are not significant. However, the mutation at this residue may influence the binding affinity of these compounds. Though drug-resistant mutation at this residue has not been reported yet, Arg293Lys mutation has been reported in N2 subtype of neuraminidases [30]. Further examination of mutations at Arg293 and Glu278 may provide hints to possible drug-resistant strains of the N1 subtype in the future.

Table 3
Analysis of the binding modes of HR1039 and HR1040 with the neuraminidase of the 2009 A(H1N1) virus.

Compound	HR1039	HR1040
Elestostatic	Arg118, Agr152, Arg368, Lys432	Arg118, Agr152, Arg368, Lys432
Van der Waals	Ile149	Ile149
Hydrogen bonding	Arg118, Arg152, Glu278, Arg293, Arg368, Gly401, Tyr402, Lys432	Arg118, Arg152, Glu278, Arg293, Arg368, Gly401, Tyr402, Lys432

4. Conclusion

We have carried out molecular modeling, docking, virtual screening and molecular dynamics for neuraminidase from 2009 A(H1N1) influenza virus. The molecular dynamics of oseltamivir– and zanamivir–neuraminidase complexes are consistent with previous studies. Based on the same procedures, we have identified two compounds (HR1039 and HR1040) as potential potent inhibitors of 2009 A(H1N1) neuraminidase. Molecular dynamics suggested that the binding modes of the two compounds are different from those of oseltamivir and zanamivir. With the emergence of oseltamivir-resistant 2009 A(H1N1)

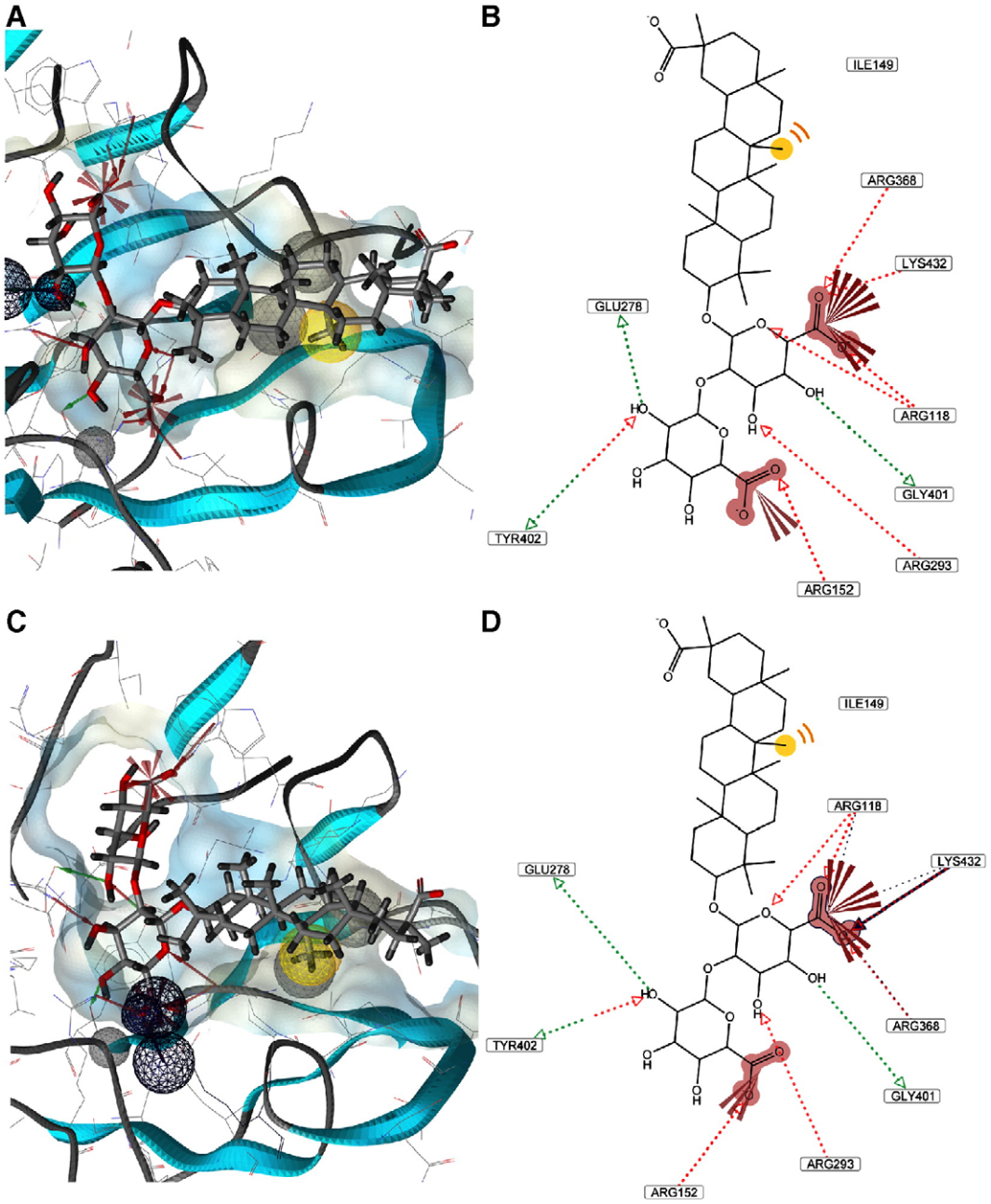

Fig. 5. Modeled structures of the 2009 A(H1N1) neuraminidases: (A) HR1039 bound to the binding site of neuraminidase. (B) Pharmacophore of HR1039. (C) HR1040 bound to the binding site of neuraminidase. (D) Pharmacophore of HR1040.

strains, compounds with alternative binding modes may be of great clinical implications.

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