



## COMPARATIVE DOCKING ANALYSIS OF NEURAMINIDASE WITH VARIOUS INHIBITORS

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### ABSTRACT

The H1N1 influenza A virus lifted the global and marketed the drugs of zanamivir and oseltamivir. The study of the above mentioned drugs is along with the specific inhibitor neuraminidase. The expected outbreak of the observation has identified the sisomycin and other derivatives have a better resistance of comparing to the other chemical molecules. Thus, the structures with various complexes produced some reports against influenza, open conformation also analyzed in the same regions of the 150-loop (Gly147-Asp151). We investigated the binding position and pharmacophoric properties of sisomycin resistance of influenza A (H1N1) virus. This is aimed for comparing the related structures of sisomycin with similarity and docked with various algorithms; it is useful for designing neuraminidase inhibitors

**Keywords:** H1N1, Zanamivir, Influenza Virus, pandemic disease, neuraminidase inhibitors.

### INTRODUCTION

Influenza virus is highly contagious and can cause severe respiratory illness and death. Of the three types of influenza virus, type A infects a wide range of avian and mammalian species and can be further classified into subtypes according to the serological reactivity of its surface glycoprotein antigens, hemagglutinin (HA) and neuraminidase (NA). Sixteen serotypes of HA (H1 to H16) and 9 of NA (N1 to N9) circulate in avian and mammalian hosts. Of nine avian NA subtypes, only N1 and N2 have been seen in human viruses responsible for pandemics and recurrent annual epidemics. Type A viruses account for all of the human pandemics of the last century: the 1918 H1N1 "Spanish," the 1957 H2N2 "Asian," and the 1968 H3N2 "Hong Kong" influenza viruses.

Neuraminidase inhibitors are a class of antiviral drugs targeted at the influenza virus, which work by blocking the function of the viral neuraminidase protein, thus preventing the virus from reproducing by budding from the host cell. Inhibition of NA function appears critical in limiting the progression of influenza virus infection in the host, crystallographic analyses of NAs have provided a platform for structure-based drug design. These structures contributed to the successful development of two potent and selective inhibitors, zanamivir (Relenza) and oseltamivir (Tamiflu). Both drugs were designed to mimic transition state analogues based on crystal structures of N2 and N9 NAs. Notwithstanding, both compounds proved to be highly potent inhibitors of NAs from other strains, including N1, as well as for influenza B viruses. This anticipated success was based on the premise that the active-site residues are highly conserved across all subtypes of type A as well as type B influenza virus. However, recent studies have now shown that drug-resistant mutants can be subtype specific, which suggests some variation in the active site and its geometry and in subsequent inhibitor binding modes among the different subtypes.

### Influenza A

Swine influenza is known to be caused by influenza A subtypes H1N1, H1N2, H3N1, H3N2, and H2N3. In pigs, three influenza A virus subtypes (H1N1, H3N2, and H1N2) are the most common strains worldwide<sup>5</sup>. In the United States, the H1N1 subtype was exclusively prevalent among swine populations before 1998; however, since late August 1998, H3N2 subtypes have been isolated from pigs. As of 2004, H3N2 virus isolates in US swine and turkey stocks were triple reassortants, containing genes from human (HA, NA, and PB1), swine (NS, NP, and M), and avian (PB2 and PA) lineages.

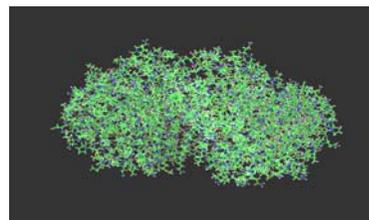
### Birth of viruses

In early June, Oxford University's Department of Zoology, reported test results that show that this strain has been circulating among pigs, possibly among multiple continents, for many years prior to its transmission to humans." The research team that worked on this report also believed that it was "derived from several viruses circulating in swine," and that the initial transmission to humans occurred several months before recognition of the outbreak. The team concluded that "despite widespread influenza surveillance in humans, the lack of systematic swine surveillance allowed for the undetected persistence and evolution of this potentially pandemic strain for many years." According to the researchers, movement of live pigs between Eurasia and North America "seems to have facilitated the mixing of diverse swine influenza viruses, leading to the multiple reassortment events associated with the genesis of the (new H1N1) strain<sup>8</sup>. Transmission of swine influenza virus from pigs to humans is not common and does not always cause human influenza, often only resulting in the production of antibodies in the blood. The meat of the animal poses no risk of transmitting the virus when properly cooked. If transmission does cause human influenza, it is called zoonotic swine flu. People who work with pigs, especially people with intense exposures, are at increased risk of catching swine flu. In the mid-20th century, identification of influenza subtypes became possible, which allows accurate diagnosis of transmission to humans. Since then, fifty

### MATERIALS AND METHODS

#### The information about macromolecule

The PDB is a key resource in areas of structural biology, is a key repository for 3D structure data of large molecules. The molecule which taken is Neuraminidase of A/Brevig Mission/1/1918 H1N1 strain in complex with sisomycin, zanamivir for our consideration. The pdb Id is 3B7E and a resolution factor is 1.45Å and the method of incorporation is X-ray diffraction method.



### The Information about drug molecules

The small molecules were retrieved from pubchem substances and checked for similarity between the small molecules and analyzed here for better understanding with a Neuraminidase enzyme, here categorized into six types. Out of the list of molecules, the sisomycin makes a better binding than other small molecules. It produces -4.15 Kcal/mol by using Argus Lab 4.0 docking software using genetic algorithm. The similarity between the sisomycin and other molecules were displayed and also produces a better pharmacophoric properties while comparing to other molecules

### Determination of active site

The active site was defined from the coordinates of the ligand in the original PDB files 3B7E for the receptor protein. Residues which lie within 3 Angstrom unit area of ligand that interact with it through their side chain were identified and were considered as Active site residues. Out of these The residues Gly 147, Thr 148, Val 149, Lys 150 and Asp 151, (150 loop binding) were determined as to be

active site in the model. The sisomycin makes better towards the neuraminidase inhibitor.



Fig. 1: Structure of Sisomycin

Table 1: Chemical molecules used in the study were taken in pubchem

Small molecules	Similarity
Zaminivir	0.87
Netilmicin	0.42
Mitomycin	0.32
Paclitaxel	0.33
Sisomycin	0.40
Hyaluronic Acid	0.34

Table 2: Properties of small Molecule (sisomycin)

Compound ID	36119
Molecular Weight	447.52638 [g/mol]
Molecular Formula	C <sub>19</sub> H <sub>37</sub> N <sub>5</sub> O <sub>7</sub>
XLogP3-AA	-5.1
H-Bond Donor	8
H-Bond Acceptor	12

## RESULTS AND DISCUSSION

### Docking

The docking between receptor and ligand was performed using the "Dock a ligand" option. Five residues that lie within 4 Angstrom unit area of ligand that interacts with it through their side chain were considered as Active site residues. Docking simulations were performed by selecting "ArgusDock" as the docking engine. An exhaustive Search was performed by enabling "High precision" option in Docking precision menu, "Dock" was chosen as the

calculation type, "flexible" for the ligand and the AScore was used as the scoring function. At maximum 150 poses were allowed to be analyzed, binding site box size was set to 10 x 15 x 15 angstroms so as to encompass the entire active site. The AScore function, with the parameters read from the AScore.prm file was used to calculate the binding energies of the resulting docked structures. This file contains the coefficients for each term in the scoring function. Each docking run was repeated five times to get best results. The docking results are described in Table. The resulting docking molecules were saved as pdb files and best scored results were displayed.

Table 3: Docked results comparison with Sisomycin

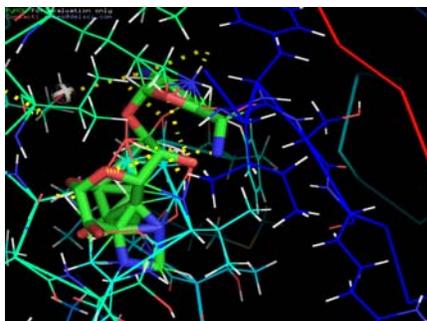
Chemical Molecule	Energy Kcal/mol
Zanamivir	-4.00 k/cal
Sisomycin	-4.15 k/cal
Mitomycin	-4.82 k/cal



### Analyzing pharmacophoric properties

The Pharmacophoric properties of the Neuraminidase inhibitor with the sisomycin shows the better conformations of Hydrogen bonds analysis done in HBAT version 1.1, it displays hydrogen bonds like

N-H...O, O-H...O, N-H..H properties are more. This results that binding between the inhibitor and small molecule shows the strong interactions. The Threonine, Lysine and aspartate show the strong interacting with the ligand. So, it has a better pharmacophoric activity of binding towards the ligand.



**Table 4: Distance calculated the sites of ligand with proteins.**

S.no	Residues involved	H-bond distance (Å)
1	Asp151	2.98
2	Gly147	2.51
3	Lys150	2.51
4	Val 149	3.44
3	Thr148	3.39

The field of molecular docking has emerged during the last three decades and now is becoming an integral aspect in drug discovery and development area. Molecular docking is utilized for the prediction of protein-ligand complexes which is composed of two components: a search algorithm, an algorithm that creates possible protein-ligand complex geometries, and thus performs the process of "pose generation" and a scoring function that predicts the binding affinity of the ligand to the protein based on the complex geometry. ArgusLab provides the stochastic search, analogous to the genetic algorithm normally used as a standard in Molecular docking studies, along with this an exhaustive search method based on identification of complementary shapes of the ligand and the receptor, referred to as ArgusDock is also utilized. The docking results are described in Table. It is evident that the binding energy of mutated complex is more than that of unmutated complex. Binding energies are most widely used mode of measuring binding affinity of a ligand. Here, the sisomycin is the potential target compared with other targets. Thus the drug binding energy makes least energy and which increases the resistant towards NA, making virus resistant to NA inhibitors.

### CONCLUSION

Novel H1N1 (Referred to as "Swine flu" early on) is a new influenza virus causing illness in people. This new virus was first detected in people in the United States in April 2009. A June 10, 2009 update by the U.N.'s World Health Organization (WHO) states that 74 countries have officially reported 27,737 cases of influenza A (H1N1) infection, including 141 deaths. The anti-influenza drugs currently being used to treat infected patients are zanamivir (Relenza) and compared with other targets, out of which zanamivir produces better results on binding with the target Neuraminidase inhibitor, our reports says that the sisomycin the potential target compared with zanamivir and mitomycin which produces low energy and having better pharmacophoric activity. Reports of the emergence of drug resistance make the development of new anti-influenza molecules a priority. This is aimed for comparing the related structures of sisomycin with similarity and docked with various algorithms; it is useful for designing neuraminidase inhibitors which will help to combat H1N1 Pandemic.

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