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Virtual Screening of Potential Inhibitors of Anti-Influenza A H1N1

Praveen Kumar Guttula[a]*, Soumyakantapanda[b]
[a]Department of Biotechnology and Medical Engineering,
National Institute of Technology Rourkela, Odisha, India.
[b]Utkal university, Bhubaneshwar,Odisha,India.

ABSTRACT

Influenza, a viral infection of the upper respiratory tract in humans, has plagued mankind since the dawn of history. The disease in modern times continues to affect a significant proportion of the population irrespective of age or previous infection history. At present in the market, the available drugs which are acting against H1N1 influenza A are Oseltamivir (Tamiflu™) and Zanamivir (Relenza™). Both the anti-influenza A drugs target the viral neuraminidase enzyme (NA), which is responsible for cleavage of terminal sialic acids on the cell surface of the host cell during virus budding and release. Reports of emergence of resistance towards oseltamivir make the development of new anti-influenza H1N1 viral drugs a priority. In the present study, multiple sequence analysis and phylogenetic analysis of NA protein sequences revealed that Influenza A [A/Niigata/F95/2007(H1N1)] has the most distant evolutionary similarity amongst the other NA proteins of H1N1. A model was therefore built for the Influenza A Niigata NA protein sequence using MODELLER9.v6. Analysis of Ramachandran Plot showed 77.0% accuracy of the built model. Active sites of NA protein was identified and were docked with known anti-influenza drugs (oseltamivir and Zanamivir) and analogues of Peramivir designed in silico using Chemsketch. The docking results showed that Protoverine, an antibiotic, has the highest global energy (binding energy) with NA. Three new analogues of Peramivir were also identified out of which one (P3NAH1NI) had the better binding energy in comparison to the native drug (-40.55 vs. -68.77). The ADMET assay for pharmacokinetic and toxicity confirmed their utility as potential anti-influenza A drug. Future study would synthesize these potential inhibitors and evaluate their activity in vivo.

***Key words:* H1N1; neuraminidase inhibitor; virtual screening; peramivir analogue; protein modelling; docking.**

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INTRODUCTION:

Influenza virus is highly contagious and can cause severe respiratory illness and death. There are threetypes of influenza virus classified on their serological cross-reactivity with viral matrix proteinsand soluble nucleoprotein (A, B, and C)[1]. 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 in the last century[2].

Influenza is caused by RNA viruses of the family Orthomyxoviridae [3]. Two of the segments code or the surfaces glycoproteins hemagglutinin HA (which binds to terminal sialic acid) and neuraminidase NA (which cleaves terminal sialic acid) which appear as spikes protruding out of the viral envelope. The viral target in humans is the upper respiratory tract epithelial cells.

Science Mach 2009, a new strain of influenza A virus (2009 A (H1N1)) has spread rapidly and evolved into global pandemic [4]. The 2009 (H1N1) influenza virus shares several common characteristics with the 1918 Spanish flu [5]. 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 [6]. Influenza virus can be classified by the antigenic properties of two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA) [7]. Hemagglutinin binds to the sialic acid receptor on the cell surface and facilitates the entry of the virus [8]. 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 [9]. Because of its essential role in release of influenza virus particles, neuraminidase has been an attractive target for the anti-influenza drugs [10]. The two antiviral drugs, oseltamivir and zanamivir, both target neuraminidase. Thus, creating a model structure of the identified mutant H1N1 Neuraminidase strain will provide us with data required for further research. Selection of a potent inhibitor from a list of in-use antibiotics and anti-influenza A drugs, is done using docking tools.

MATERIALS AND METHODS

Neuraminidase is one of the two enzymes primarily present in the envelope of the Influenza virus. In the present work, we focused on neuraminidase enzyme as it facilitates the exit of the newly synthesized viral particles, from the effected host cell. Because of mutations observed in the active site of neuraminidase enzyme, commercially available swine flu drugs like Oseltamivir(Tamiflu) and Zanamivir(Relenza) are unable to bind optimally. This creates a necessity to develop novel drugs for the existing strains of Influenza A virus that show inhibitory effects on the enzyme.

Collection of Influenza AH1N1 Neuraminidase protein sequences:

Neuraminidase protein sequences of Influenza A H1N1 virus were downloaded from NCBI influenza virus sequence database. In present work, we collected 200 sequences to carrying comparative gene analysis.

Identification of Essential Genes:

The collected protein sequences of Neuraminidase from NCBI were analyzed using DEG [12] (Database for Essential Genes). From DEG, we retrieved the essential genes of H1N1 Neuraminidase that encode similarity with the prokaryotic organisms in the Database.

Phylogenetic Tree analysis and Multiple Sequence Alignment:

The collected Neuraminidase sequences from NCBI are analyzed using the Phylogenetic analysis tools like PHYLIP (http://www.phylip.com/). The symmetry analysis of PHYLIP was carried out using default parameters. The output from PHYLIP is to identify the evolutionary relationship between the all influenza neuraminidase sequences of H1N1 and its ancestral sequences. The NA sequences of H1N1 strains that are deviated from the branching pointi.e the strains which show the lack of symmetry or reliabilitywould be identified by computing the parameters like tree length and Boot strap value.The sequences which have deviated fromother strains of neuraminidase were analyzed by tool CLUSTALX (http://www.clustal.org/) with the default parameters. The aim is to search and identify the amino acid sequences of mutated strains which have least conserved.

Homology Modelling of Influenza A(A/Niigata/F95/2007(H1N1)):

A suitable 3-Dimensional structure of our selected neuraminidase protein sequence ismade by using a modeling tool MODELLER9v6, of Andrej Salilab Org [13] [14].The following MODELLER script aligns the**neura** sequence in file "**neura.ali**" with the template **3B7EA.**

Model evaluation OR Validation

The Model which selected based on Highest Dope score from Mod 9v6 is neuraB99990004.pdb could be evaluated by RAMACHANDRAN PLOT 2.0 software(http://dicsoft1.physics.iisc.ernet.in/rp/select.html).

Identification of active site:

There are a variety of tools available to detect the active site in the model that we have built. Here we found the active site in our model neuraB99990004.pdb, using POCKET-FINDER. **Preparation of ligand:**

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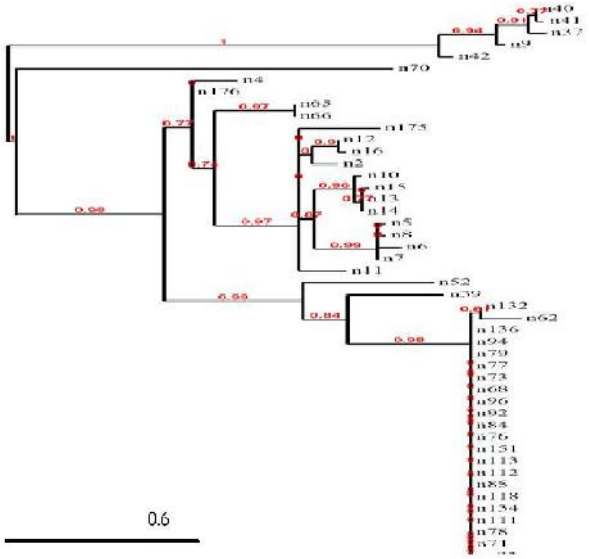
Antibiotics like Protoverine, Gentamicin, Kanamycin, Desmosine and Netlimicin; along with anti-influenza A H1N1 viral drugs like Oseltamivir, Zanamivir and Peramivir were obtained from DrugBank and PubChem in mol format. They were viewed using CHEMSKETCH. We modified the Peramivir structures to prepare its analogues. All of them were converted to pdb formatfor further docking procedure.

Docking:

Protein–ligand docking is a molecular modelling technique. The goal of protein-ligand docking is to predict the position and orientation of a ligand (a small molecule) when it is bound to a protein receptor or enzyme. Pharmaceutical research employs docking techniques for a variety of purposes, most notably in the virtual screening of large databases of available chemicals in order to select likely drug candidates.It is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. There are four steps involved: (a) Preparation of receptor; (b) Preparation of ligand; (c) Sphere preparation & Gridding (d) Docking. We used FIREDOCK tool for the same [15].

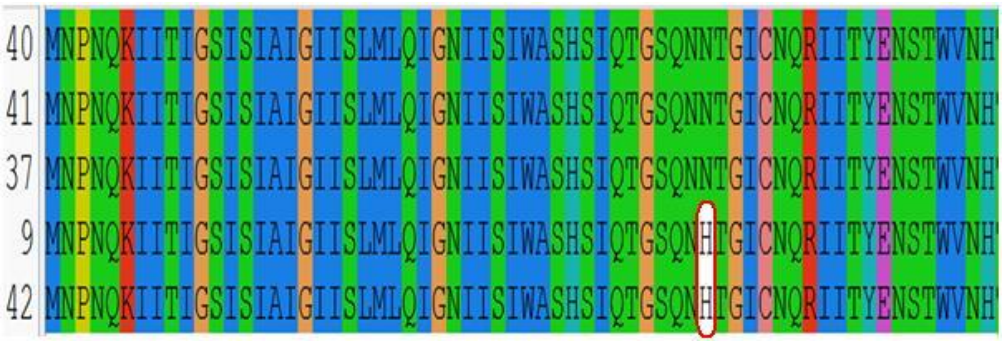
RESULTS & DISCUSSION

Phylogenetic analysis of Influenza A H1N1 neuraminidase proteinsequences:



Basing on the above tree guide, the strains of influenza A H1N1 have deviated and show the lack of symmetry from the others by a noticeable distance are: n40 [Influenza A/Kentucky/UR07-0061/2008], n37 [Influenza A/Boston/47/2009], n41 [Influenza A/Florida/Ur07-0022/2008].

Multiple Sequence Alignment of selected sequences using CLUSTALX



The above output depicts that, sequence 9 i.e. Influenza A virus (A/Niigata/F95/2007(H1N1)) and 42 i.e. Influenza A virus (A/Kentucky/2e/2006(N1)), show non-conserved regions.

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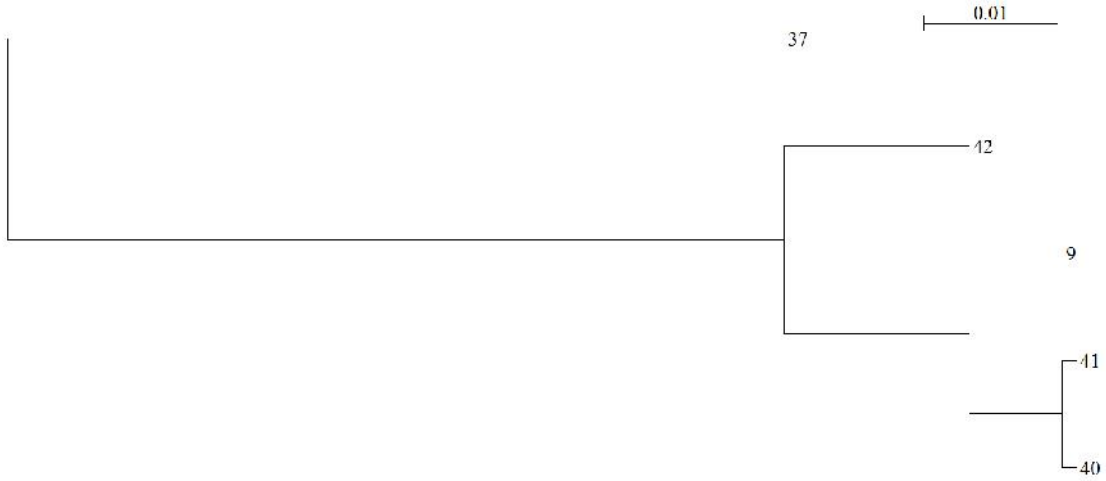
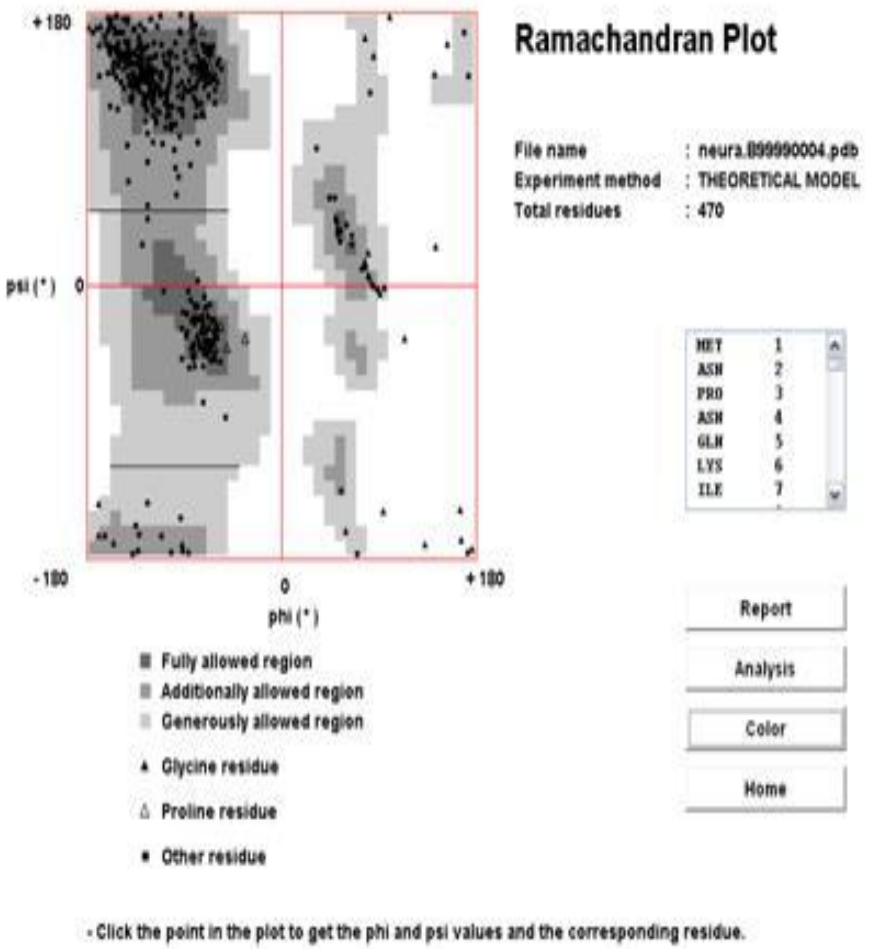


The tree is a representation of the clustalx output of our selected sequence

Validation of model neuraB99990004.pdb by using RAMACHANDRAN-PLOT 2.0



The model neuraB99990004 as viewed on CHIMERA1.5.3:

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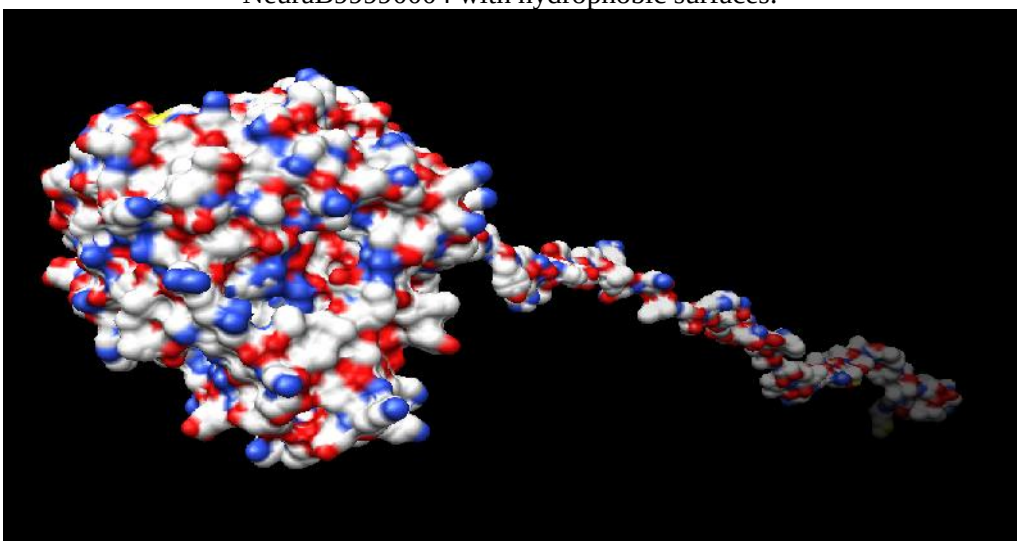
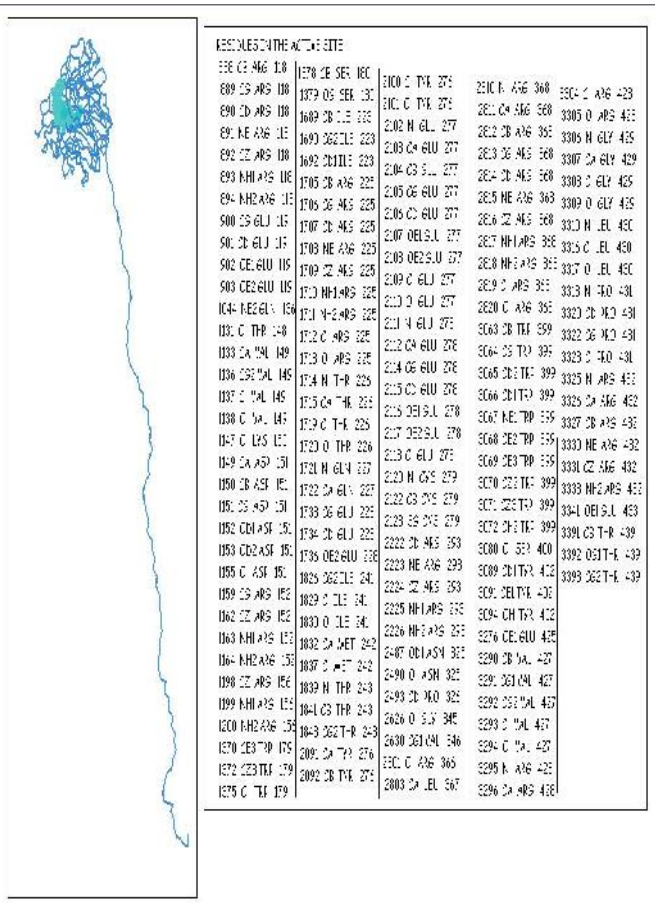
NeuraB99990004 with hydrophobic surfaces.



NeuraB99990004 with all atoms preset.

Identification of Active site using POCKET-FINDER



Active site of neuraB99990004 as seen using SWISS-PDB VIEWER

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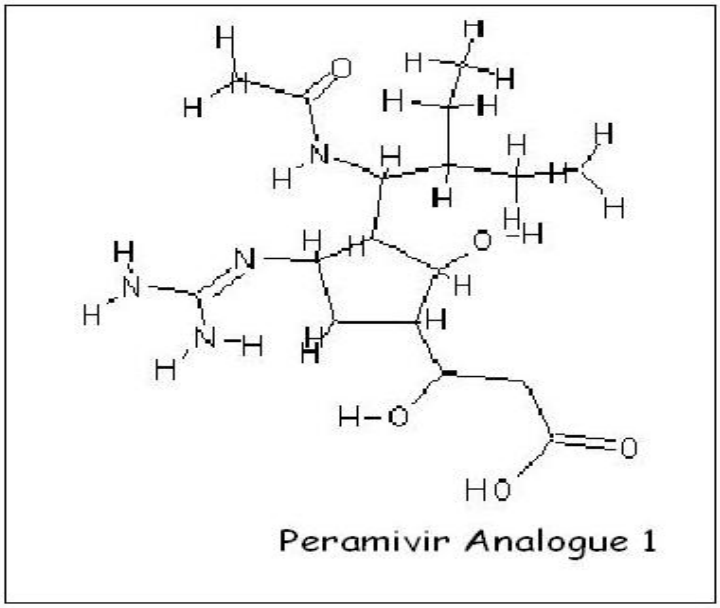
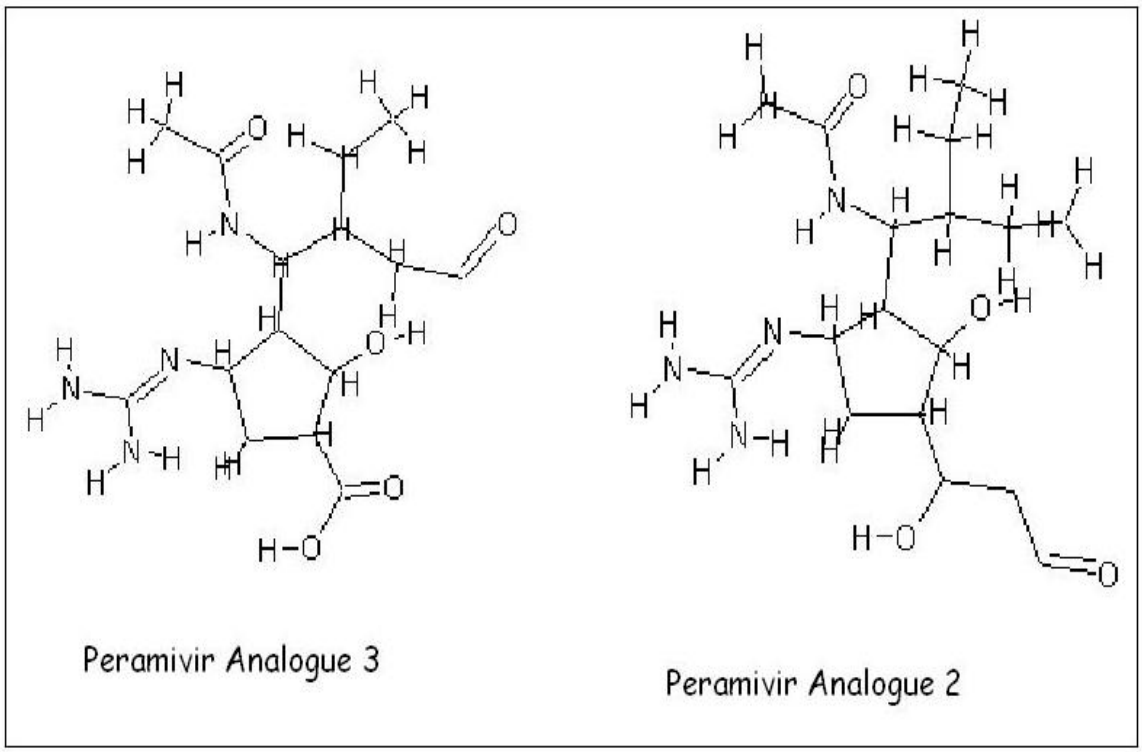
Preparation H1n1 Anti influenza inhibitors:

Modified analogue ofPeramivir structures as drawn using ChemSketch:





Virtual screening of Analogues withModeled NA by Docking:

Oseltamivir

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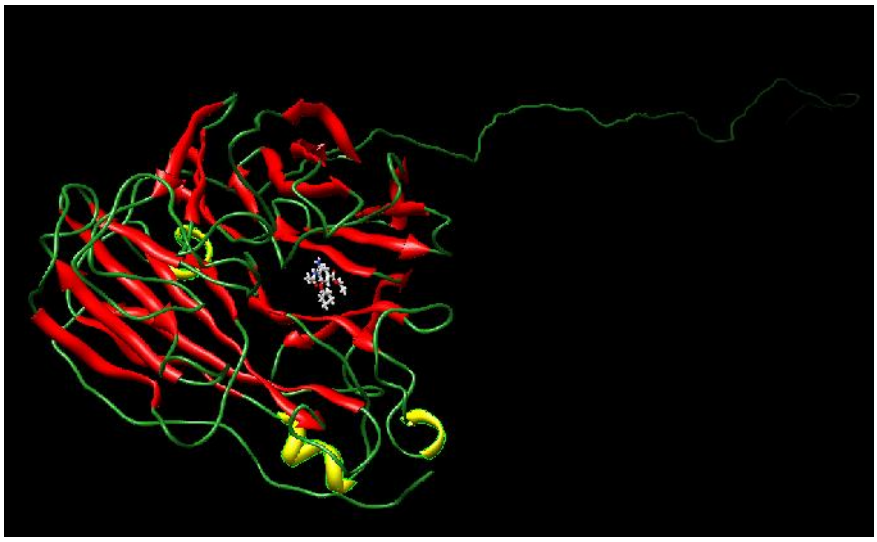

Prediction of potent inhibitors fromDockingoutput:

RANK	DRUG	GLOBAL ENERGY	ATTRACTIVE VdW	REPULSIVE VdW	ATOMIC CONTACT ENERGY
1	**Protoverine**	-68.77	-37.29	24.44	-21.63
2	**Desmosine**	-63.38	-28.89	6.99	-19.26
3	**Zanamivir**	-52.07	-24.83	8.19	-14.88
4	**Netlimicin**	-43.73	-29.74	4.80	-16.57
5	**Gentamicin**	-41.55	-33.1	5.24	-9.90
6	**Kanamycin**	-41.55	-33.1	5.24	-9.90
7	**Peramivir Analogue 3**	-40.55	-19.70	6.54	-12.78
8	**Peramivir**	-37.59	-21.56	5.45	-15.51
9	**Oseltamivir**	-31.56	-20.97	10.08	-14.15
10	**Peramivir Analogue 2**	-24.85	-22.36	12.69	-13.75
11	**Peramivir Analogue 1**	-17.15	-15.70	7.43	-7.86

Resistance to oseltamivir is suspected to be caused due to mutation H247Y in the enzyme neuraminidase of H1N1 strain of influenza Avirus. This work involves building a model for the mutated strain of NA and designing of various analogues of a new drug called Peramivir and finding an effective inhibitor against Oseltamivir resistant neuraminidase by using drug designing tool through the virtual screening method.

A DEG analysis shows the similar sequences present between H1N1 viral sequence and the other prokaryotic organisms. Most of the sequences show similarity with *Mycoplasma genitalium*. These similar sequences mainly encode for lipopolysaccharides and a few proteins present in the cell wall of prokaryotes. Based on the results, we identified two neuraminidase sequences viz. Influenza A (A/Niigata/F95/2007(H1N1)) and Influenza A virus (A/Kentucky/2e/2006(N1)) showing the least conserved

regions. These two were shortlisted for further work. Influenza A(A/Niigata/F95/2007(H1N1)) is taken as the input sequence to build a model neuraB99990004.pdb and it is evaluated by means of RAMACHANDRAN PLOT 2.0.

Molecular docking is utilized for the protein ligand complex 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 a protein based on the complex geometry [16]. Ranking was done based on the least Global Energy (Binding Energy) values. On inferring the above table, we can say that Protoverine binds most effectively with neuraB99990004.

CONCLUSION

In the present work, we identified the mutated Influenza A H1N1 Neuraminidase strain amongst the downloaded H1N1 NA protein sequences from NCBI. We built a neuraminidase model by using MODELLER9v6 software, taking Influenza A virus (A/Niigata/F95/2007(H1N1)) as the input sequence. The model protein structure obtained is neuraB99990004, which has high reliability as per the Ramachandran plot. The developed anti-influenza drugs with prepared PDB, were analyzed by using FIREDOCK. After Docking, the reports suggest that among the antibiotics and Peramivir analogues,Protoverine and Peramivir analogue 3 respectively, werepotent inhibitors with highest global binding energy in comparison to Oseltamivir, Zanamivir and Peramivir (native).Finally we conclude that, Protoverine and Peramivir analogue 3 can be considered as potent H1N1 NA inhibitors and further research is to be carried out, to make them effective in treating swine flu.

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