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IN SILICO DOCKING ANALYSIS FOR VIRAL PROTEIN - HEMAGGLUTININ-NEURAMINIDASE AGAINST THE SYNTHETIC DRUGS FOR HUMAN PARAINFLUENZA VIRUS 3

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ABSTRACT

Hemagglutinin-Neuraminidase (HN) protein is a multifunctional protein responsible for attachment to receptors containing Sialic acid, neuraminidase (NA) activity, and the promotion of the fusion protein. The cell membrane fusion of hemagglutinin-neuraminidase protein and F protein interaction regulation is remaining not clear. This work investigates the binding and entry of the cells of parainfluenza virus type 3, focusing on how the receptor-binding molecule triggers the fusion process. We have used computational methods to find the ligand having best interaction with hemagglutinin-neuraminidase protein. HN protein interactions with the drug namely Amantadine, Rimantadine, Oseltamivir and Zanamivir were studied, in which the best drug interaction with the receptor was confirmed by binding energy, number of hydrogen bond formed and Inhibitory constant result given by Autodock 4. Finally, the conformation result shows that Zanamivir has the maximum hydrogen bond formed and lowest binding energy with the receptor.

KEYWORDS

Hemagglutinin - Neuraminidase, Human Parainfluenza virus3, Neuraminidase Activity, Newcastle disease virus.

INTRODUCTION

Influenza disease is highly contagious diseases caused by the Paramyxovirus in the respiratory tract. It can cause cough, headache, sore throat, runny nose and fever. Parainfluenza viruses initiate (paramyxovirus) infection by binding to the surface of the cell receptors through combined action of two viral surface glycoprotein's (HN and Fusion protein). These glycoproteins get fused with the surface of the receptors and initiate viral replication process into host cell's cytoplasm and more number of virion are produced. During this process, binding to the receptor molecule must trigger the viral fusion protein to mediate fusion and entry of the virus into a cell[1].

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The HN protein is present on the cell surface and on the virion as a tetramer composed of disulfide-linked dimmers[2]. The molecule contains a cytoplasmic domain, a membrane-spanning region, a stalk region, and a globular head. Crystal structures of the HN protein of the avian paramyxovirus known as Newcastle disease virus (NDV) [3, 4] and more recently the HPIV3 HN protein[5] demonstrates that the globular head contains the primary Sialic acid–binding site and the neuraminidase active site. The neuraminidase function is critical for the spread of virus to new cells, and if the enzyme activity is inhibited, then virus infection is abrogated[6]. Also, there is evidence that this enzyme plays some role in the introduction of apoptosis to the infected cells[7]. HN protein acts as a receptor by binding to amantadine, rimantadine, oseltamivir and zanamivir.

Antiviral drugs include M2 inhibitors, which are ion channel blockers (Amantadine and Rimantadine), and the Neuraminidase inhibitors (Oseltamivir and Zanamivir) [8]. The neuraminidase inhibitors Oseltamivir and Zanamivir have fewer side effects than the M2 ion channel inhibitors Rimantadine and Amantadine drugs are effective as treatment if started within 24 hours of illness onset, reducing fever and symptoms by 1.2 days[9].

Works related to Neuraminidase inhibitor drugs such as zanamivir and oseltamivir are reported and suggested that preventing of secondary bacterial complications. However, no clinical studies were designed to determine its effects directly[10]. In the drug Zanamivir no interaction studies with other ligands has been reported so far. So, our work mainly focused the interaction of hemagglutinin-neuraminidase protein with the ligands (Amantadine, Rimantadine, Oseltamivir and Zanamivir) try to find the best inhibitor.

MATERIAL AND METHODS

The HN protein was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/). The PDB Id is 1V2I and its crystallographic data reports that only amino acids from 142 to 572 were crystallized.

LIGAND SELECTION

PubChem compound database contains validated chemical depiction information. Structures that are stored in PubChem contain calculated properties and descriptions, which help in searching and filtering of chemical structures. The ligand for HN protein was retrieved from PubChem compound. The ligands are namely Amantadine, Rimantadine, Oseltamivir and Zanamivir. The chemical properties of the selected ligands are given in Table 1. The ligands were downloaded as XML files from PubChem compound. These Xml files were converted into the 3D structures using Open Babel software.

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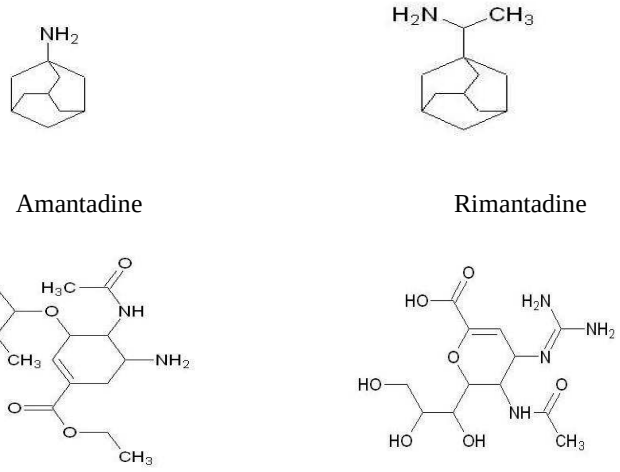

Fig 1 Chemical structure for ligands

Fig (1) Show the PubChem compound chemical structure of ligands namely Amantadine, an antiviral that is used in the prophylactic or symptomatic treatment of influenza A. Rimantadine, an RNA synthesis inhibitor that is used as an antiviral agent in the prophylaxis and treatment of influenza. Oseltamivir, an acetamido cyclohexene that is a structural homolog of Sialic acid inhibit neuraminidase. Zanamivir is a guanido-neuraminic acid that is used to inhibit neuraminidase.

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Table 1

Properties of the ligands used in this study

Properties	Amantadine	Rimantadine	Oseltamivir	Zanamivir
Molecular Weight	151.24868[g/mol]	179.30184[g/mo]	312.40452[g/mol]	332.3098[g/mol]
Molecular Formula	$C_{10}H_{17}N$	$C_{12}H_{21}N$	$C_{16}H_{28}N_2O_4$	$C_{12}H_{20}N_4O_7$
H-Bond Donor	1	1	2	7
H-Bond Acceptor	1	1	5	10
Rotatable Bond Count	0	1	8	6
Tautomer Count	-	-	2	4
Exact Mass	151.1361	179.1674	312.204907	332.133199
Mono Isotopic Mass	151.1361	179.164	312.204907	332.133199
Topological Polar Surface Area	26	26	90.7	201
Heavy Atom Count	11	13	22	23
Complexity	144	180	418	518
Isotope Atom Count	0	0	0	0
Covalently-Bonded Unit Count	1	1	1	1

The various properties of selected ligands are provided in the table

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OPEN BABEL SOFTWARE

Open Babel is freeware software with graphical user interface. This software uses chemical toolbox designed to Chemical data and converts one file into another file format. For example xml file can be converted into pdb file. This software is widely used in areas such as molecular modeling, cheminformatics and bioinformatics. HN protein compound downloaded in XML file format from PubChem Compound was converted into pdb using Open Babel Software.

Q-SITE FINDER

Q-Site Finder is one of the tools for binding site prediction. It uses the interaction energy between the protein and a simple van der Waals probe to locate energetically favourable binding sites. Energetically favourable probe sites are clustered according to their spatial proximity and clusters are then ranked according to the sum of interaction energies for sites within each cluster. These clusters are placed in rank order of the likelihood of being a binding site according to the sum total binding energies for each cluster. Q-Site Finder is freeware software available online (http://www.bioinformatics.leeds.ac.uk/qsitefinder).

PYMOL

PyMol is an open-source tool used to visualize molecules available from (www.pymol.org). It runs on Windows, Linux and MacOs. PyMol producing high quality images from 3D structures of small molecules and biological macromolecules such as proteins. It has well developed functions for manipulating structures and some basic functions to analyze their chemical properties[11]. PyMOL has been written mostly in the Python language (www.python.org), while the time-critical parts of the system have been coded in C. This way, Python programs interact most easily with the PyMOL GUI (Graphical User Interface).

AUTODOCK 4

Autodock 4 is non commercial software for automated docking programs. Autodock actually consists of two main programs Autodock and Auto Grid. Autodock performs the docking of the ligand to a set of grids describing the target protein. Auto Grid pre-calculates these grids. Autodock is used for docking as Flexible docking and Grid docking. The output results are more accurate and reliable. It can optionally model flexibility in the target macromolecule. It enables Auto Dock's use in evaluating protein-protein interactions. Autodock programs run on various platforms using such as Windows, Mac OS X and Linux. Lamarckian Genetic Algorithm-Dock is comprised of a stochastic population generator, a docking routine based on a Lamarckian genetic algorithm, and a local search function based on molecular mechanics (MM) energy minimization[12]. The input files for Auto Grid and Autodock are created, and then the grid map calculation run, followed by the docking calculation in Autodock.

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AUTOGRID

This program used for pre-calculating grid maps of interaction energies for various atom types, such as aliphatic carbons, aromatic carbons, hydrogen bonding oxygen's with a macromolecule such as protein, DNA or RNA. These grid maps are used for Autodock docking calculations to determine the total interaction energy for a ligand with a macromolecule. The Grid points set for X, Y, Z direction and grid spacing 0.375 fixing the binding sites and dock the molecule structures.

RESULTS

The crystal structure of the HN protein was retrieved from PDB. The HN protein for HPIV3 is crystallized and the structure's PDB Id is 1V2I. This protein sequence length of the crystal structure is 142 to 572. The crystal structure had R-Value=0.185(obs), R-Free=0.259 and Resolution [A^o] =2.20. Hence, this structure was used for further analyses in this work.

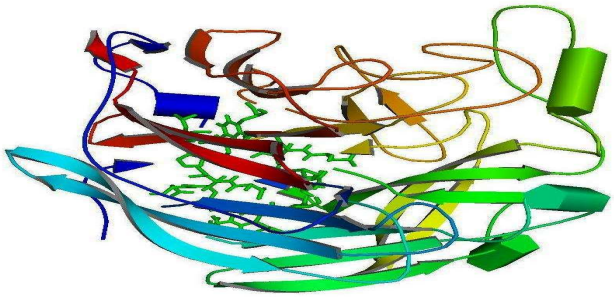

Fig 2 Binding Sites of the Hemagglutinin-Neuraminidase protein

HN protein was visualized using Pymol (Fig 2). This figure represents the number of sheets, helices and loops that are available. The binding site residues were selected which are shown as green sticks in the above fig 2.

Q-SITEFINDER

The Q-Site Finder tool was used for binding site prediction for ligands. The Q-Site takes as input the PDB file and the results show 10 sites predicted as binding sites. Each site is shown in a different color and the top most one which is in green is the best one. The Q-Site Finder predicted sites in 90% of proteins tested is good. HN protein binding site residue as (Fig 3) PRO150, LEU151, ARG173, PRO178, VAL197, ILE198, ASN199, ASP200, THR 230, ASP234, ASP238, LEU239, LEU258, ALA259, LEU260, LEU261, ASN262, PRO324, GLY325, ILE326, TYR327, LEU412, LEU413, LEU414, LEU415, TYR482, SER534, CYS 535, LEU562 and GLU566.

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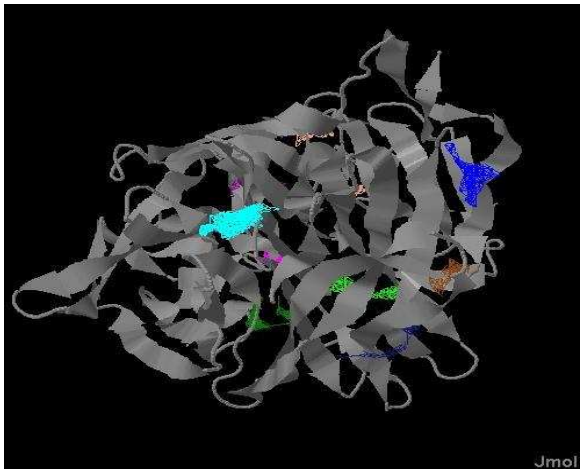
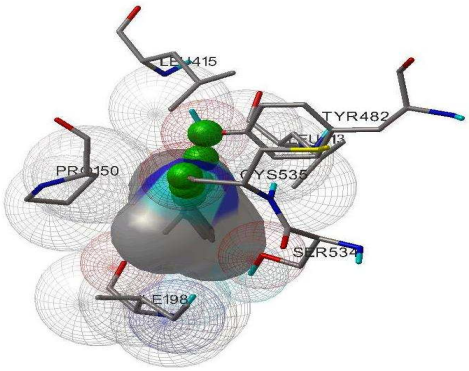


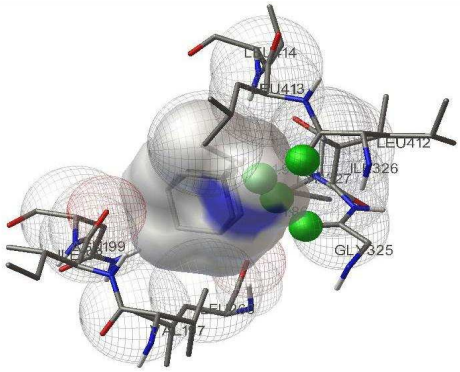
Fig 3 ***Ligand Binding Sites Prediction Using Q-Site Finder***

Fig (3) shows the HN protein and the Binding sites residue in different colors. The best binding site residues are highlighted in green and it was used for docking studies.

AUTODOCK RESULTS



Amantadine



Rimantadine

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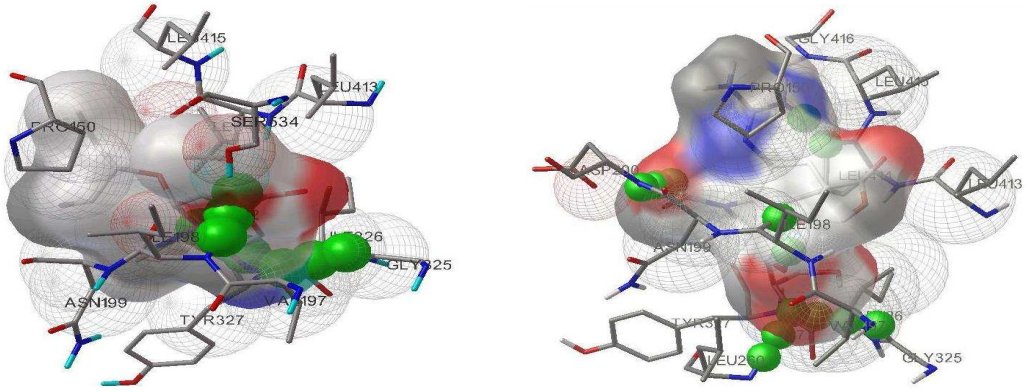

Oseltamivir **Zanamivir**

Fig 4 ***Docking Results for Ligand Using Autodock4***

The HN protein interacts with the four ligands which is shown in the fig (4). The red color region in fig (4) represents the interaction of ligand with the receptor and green color shows hydrogen bonds formed with the receptor.

Amantadine binding energy value is -6.32kcal/mol and two hydrogen bonds were formed. The two residues namely TYR 482 and CYS 535 of the receptor were involved in hydrogen bonding and inhibitory constant of this ligand was found to be 16.62 µM. Rimantadine binding energy value is -6.44 kcal/mol and two hydrogen bonds were formed. The two residues namely GLY 325 and LEU 412of the receptor were involved in hydrogen bonding and inhibitory constant of this ligand was found to be 19.05 µM. Oseltamivir binding energy value is -6.03 kcal/mol and three hydrogen bonds were formed. The three residues namely ILE 198, GLY 325 and TYR 327of the receptor were involved in hydrogen bonding and inhibitory constant of this ligand was found to be 38.13 µM. Zanamivir binding energy value is -6.45 kcal/mol and five hydrogen bonds were formed. The five residues namely ILE 198, ASP 200, LEU 260, LEU 414 and GLY 325 of the receptor were involved in hydrogen bonding and inhibitory constant of this ligand was found to be 18.76 µM. The residues which interact with the different ligands are mentioned in Table 2.

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Table 2. ***Results of the docking analysis for Synthetic compounds***

Ligands	Binding Energy (kcal/mol)	No. of Hydrogen Bond formed	Hydrogen Residue	Inhibit Constant (µm)
Amantadine	-6.32	2	TYR 482, CYS 535	16.62
Rimantadine	-6.44	2	GLY 325, LEU 412	19.05
Oseltamivir	-6.03	3	ILE 198, GLY 325, TYR 327	38.13
Zanamivir	-6.45	5	ILE 198, ASP 200, LEU 260, LEU 414 , GLY 325	18.76

All the Ligand interaction with the receptor is available in the output file. The best conformations results are found out using Binding energy, number of hydrogen bond formed and inhibitory constant value (Table 2). All the ligand compare to the best result for Zanamivir.

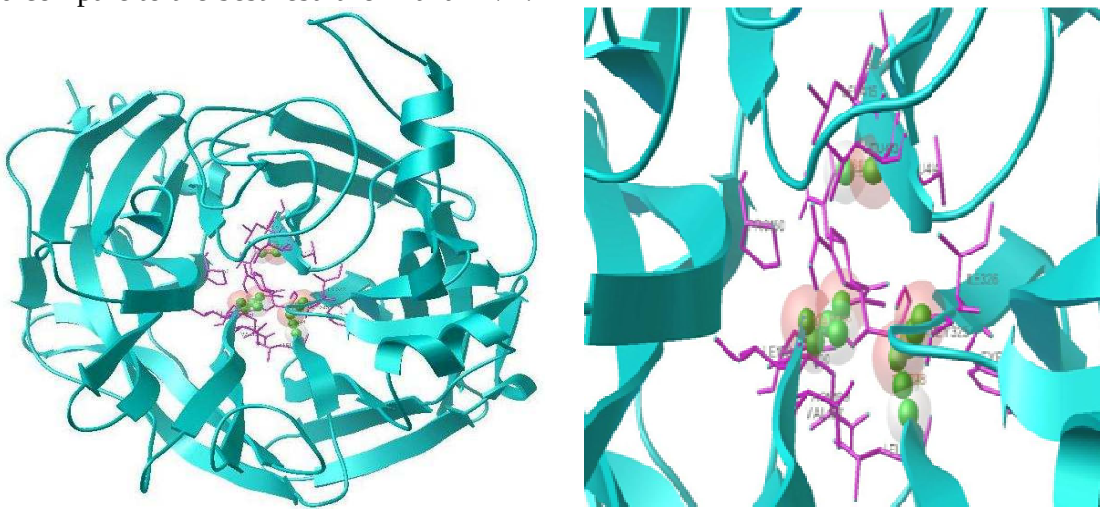

Fig 5 Best docked result for Zanamivir

Fig (5) shows receptor in cyan color, ligand in pink color and receptor interaction with ligand in green color. The lowest binding energy value is -6.45kcal/mol for Zanamivir and mostly numbers of hydrogen bond were formed five. The five hydrogen bonds residues namely ILE 198, ASP 200, LEU 260, LEU 414 and GLY 325 of the receptor were involved in hydrogen bond and inhibitory constant value is 18.76 µm.

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DISCUSSION

The promotion of membrane fusion by Newcastle disease virus requires an interaction between the viral HN and fusion proteins, although the mechanism by which this interaction regulates fusion is not clear[13]. Insight into the mechanism of membrane fusion promoted by paramyxoviruses has been furthered recently by studies of the molecular properties of the two envelope proteins, F and HN[14]. Interaction studies of the four ligands namely Amantadine, Rimantadine, Oseltamivir and Zanamivir with HN protein has been compared in this study. The antiviral drugs currently available for the treatment of influenza A infection (two neuraminidase inhibitors and two M2 ion channel inhibitors), only the neuraminidase inhibitors oseltamivir and zanamivir are also active against influenza B. The neuraminidase inhibitors, oseltamivir and zanamivir, have fewer side effects than the M2 ion channel inhibitors rimantadine and amantadine, and drug resistance seems to develop less frequently[8]. There is no clinical trial study for the drugs till now. Antiviral agents active against paramyxoviruses for this purpose should be studied in humans in both clinical settings. Intervention would be expected to be especially efficacious in children, the elderly population, immunosuppressed persons, and in patients with antibiotic treatment failure. These findings support further drug development and highlight the need for clinical trials with humans to address these issues[15]. Many works have been reported as the Zanamivir dosage give to highly effective person for the best inhibitor[16], but there is no report on comparative interaction studies with other drugs[10]. In our work, we have done the comparative analysis of the interaction between the HN protein and the ligands. Based on our results such as the lowest binding energy -6.45 and five hydrogen bond formed and we can concluded that and also suggested Zanamivir as the better one drugs. Further clinical trials on this work may define the suitable inhibitor without any side effects.

CONCLUSION

The interactions between viral proteins HN against the ligands were studied by using various computational methods. Based on Binding energy, and Hydrogen bond formed and Inhibitory constant value the docking results were analyzed. The results were compared within themselves to find out the best ligand which can inhibit the property of the viral protein. Based on these observations, zanamivir have high values to inhibit the viral protein among the Amantadine, Rimantadine and Oseltamivir ligands. The proximity of the antibody-binding sites suggests that antibodies neutralize virus infectivity by preventing virus-to-cell binding. So, Zanamivir can act as a best inhibitor to prevent the antibody sites. These works can be extended in

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the clinical side to find out the best inhibitor. Further QSAR studies can be done to identify conformational changes.

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