SCREENING OF H1N1 NEURAMINIDASE INHIBITORS EMPLOYING PHYTOCHEMICALS OBTAINED FROM MANGIFERA INDICA: A MOLECULAR DOCKING STUDY

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ABSTRACT

Swine Flu is a seasonal viral infectious disease caused due to H1N1. The aim of the present study is to identify natural compounds found in dietary resources that can inhibit neuraminidase, a protein that plays a crucial role in the H1N1 infection and invasion into the human host tissues. The X-Ray Crystallographic structure of the protein Neuraminidase was retrieved from RCSB-PDB (PDB ID: 3B7E) and 3D structures of the selected ligands were prepare by chemsketch and then minimize the free energy and save as PDB file. Docking studies are performed using Auto Dock Vina to study the interactions of the protein with the ligands. Control of phenolic derivatives inhibitors release through the inhibition of neuraminidase has been identified as a potential target for the treatment of H1N1 influenza disease. We have employed molecular dynamics simulation techniques to optimize the H1N1 influenza neuraminidase X-ray crystal structure. Molecular docking of the compounds revealed the possible binding mode. The compounds having affinity towards the protein’s active site region were identified. Docking results indicate that all the compounds interact with neuraminidase with varied binding energies. Our further studies suggest that the activity of neuraminidase can be inhibited by Mangiferin since they have a better binding energy and interact with active site residues.

KEYWORDS: Auto Dock, Docking, H1N1, Influenza, Neuraminidase, Swine flu.
INTRODUCTION
Swine Flu is a seasonal infectious disease caused due to H1N1 virus. Since March 2009, a new strain of the influenza “A” virus (H1N1) has rapidly spread to, many countries from the initial outbreak in South America. In July 2009, the WHO (World Health Organization) declared that the spread of H1N1 influenza virus had become a serious global pandemic.

Swine influenza is known to be caused by influenza “A” subtypes H1N1, H1N2, H2N3, H3N1 and H3N2.[5,6] All Influenza A viruses has Similar Physical Structure. The virions or virus particles are enveloped and can be either spherical or filamentous in form. The total genome size of influenza “A” virus is 13,588 bases and is contained eight RNA strands that code for eleven proteins.[7] Influenza virus, an enveloped virus, has an outer lipid layer membrane which is taken from the host cell within which the virus multiplies. Embedded into this lipid layer are a group of glycoproteins which not only determine the type and subtype of the influenza virus but also facilitate the attachment and release of the viral particle from the host cell. Two main types of glycoprotein exist on the capsid of these viruses, they are, hemagglutinin (HA) and neuraminidase (NA). Hemagglutinin helps the virus bind to the sialic acid residues present on the epithelial cells of the lungs and throat, results in the infection of the upper respiratory tract. Neuraminidase is an enzyme sialidase with the active site in a pocket, hydrolysing the glycosidic linkage between the hemagglutinin and sialic acid residue. It consists of a single polypeptide chain made up of six conserved polar amino acids, followed by hydrophilic and variable amino acids. The orientation of this polypeptide is generally opposite to that of the hemagglutinin antigen. This is indispensable for the virus to infect neighbouring host cells. HA and NA are essential for the proper identification and binding of the virus to the host cell surface, which is the initial stage of viral infection.

Because of the relative deep active site in which low molecular weight inhibitors can make multiple favourable interactions and approachable methods of designing transition state analogues in the hydrolysis of sialosides, the sialidase (NA) becomes more attractive anti-influenza drug target than the hemagglutinin (HA).[8] Two of the most commonly used drugs as neuraminidase inhibitors include ‘Zanamivir (Relenza) and Oseltamivir (Tamiflu)’. These inhibitors occupy the active sites of the neuraminidase protein thus preventing the cleavage of the bond between sialic residues and hemagglutinin. This prevents the release of new viral particles from the infected cells. Recently, the H1N1 influenza associated reports state that
several mutation strains of H1N1 influenza “A” viruses are resistant to Oseltamivir and Zanamivir.\textsuperscript{[18-20]}

Natural products, either as pure compounds or as standardized \textit{MANGIFERA} plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity\textsuperscript{[11]} It is shown that naturally available compounds can be used as anti-viral agents to treat many viral infections.\textsuperscript{[12, 13]} Flavonoids derivatives are low molecular weight compounds that are widespread in the plant kingdom. Those compounds have been shown to possess several biological effects in mammals.\textsuperscript{[21–23]} In particular \textit{in vitro} studies, scientists report that phenolic derivative may inhibit several enzymes related to the cardiovascular system\textsuperscript{[24–28]}, especially inhibiting the Matrix Metalloproteinase (MMPs).\textsuperscript{[29]} Therefore, using flavonoids derivatives as antivirals should be carefully considered in addition to these other proposed activities. In general, phenolic derivatives are interesting molecules combining an aromatic nature with several hydrophilic groups. These aromatic interactions play a key role in protein-protein and protein-ligand interactions.\textsuperscript{[30–32]} The hydrophilic nature (hydroxyl (OH) functional group of flavonoids/water molecules) of the flavonoids shows that water displacement is key for determining ligand affinity.\textsuperscript{[33–37]}

\textbf{MANGIFERA INDICA}

\textbf{Taxonomical classification}

\begin{center}
\textbf{Fig 1. Picture of Mangifera indica plant}
\end{center}

Kingdom: Plantae  
Class: Mangoliopsida  
Phylum: Mangoliophyta  
Order: Spindles
Family: Anacardiaceae
Genus: Mangifera
Species: indica

Mangifera indica (MI), also known as mango, it has been an important herb in Ayurvedic and indigenous medical system for over 4000 years. Mangoes belong to genus mangifera which consists of about 30 species of tropical fruiting tree in the flowering plant family anacardiaceae. According to Ayurveda, varied medicinal properties are attributed to different parts of mango tree.

Various parts of plant are used as a dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge (an anthelmintic medicine), tonic, laxative, diuretic and treat diarrhoea, dysentery, anaemia, asthma, bronchitis, cough, piles, etc.

Objective: Our main objective is to study the function of H1N1 in causing SWINE FLU disease and to perform docking studies using phytochemicals so as to obtain best docking results. Our protein target is H1N1 neuraminidase (PDB ID: 3B7E). The phytochemicals compounds from the plant were extracted by GC-MS chromatography and their respective ligands were downloaded from PUBCHEM database.

MATERIAL AND METHODS
In the present study, docking studies were performed with the enzyme neuraminidase from H1N1 virus with the natural ligands to identify a compound that can be considered as a potential neuraminidase inhibitor. Docking studies are carried out using Auto Dock Vina.

Protein
The three dimensional crystallographic structure of the protein Neuraminidase in complex with Zanamivir was retrieved from RCSB-PDB (3B7E).

Ligands
Mango is one of the most popular of all tropical fruits. Chemical constituent of MI are always of an interest. The different chemical constituents of the plant, especially the polyphenolic, flavonoids, triterpenoids.

Herbal compounds that were known to have antiviral properties were identified from the available literature.\textsuperscript{[38-42]} The CID files of these ligands were obtained from NCBI PubChem.
The protein and ligands were subjected to energy minimization so as to refine them and prepare for docking. The compounds that were selected for analysis are
Table 1. Molecular properties of phytochemicals and auto dock vina score against neuraminidase (pdb id: 3B7E)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ligand</th>
<th>Pub chem id no:</th>
<th>Structures</th>
<th>M.W.</th>
<th>Auto dock vina score(kcal/mole)</th>
<th>H bond acceptor</th>
<th>H bond donar</th>
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<tr>
<td>1</td>
<td>Mangostin</td>
<td>5281650</td>
<td><img src="image" alt="Mangostin Structure" /></td>
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<td>14034474</td>
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<td>8</td>
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<td>424.10</td>
<td>-9.0</td>
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<td>8</td>
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<td>9</td>
<td>Protocatechuic acid</td>
<td>72</td>
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<td>10</td>
<td>Glycine</td>
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<td>Value</td>
<td>Charge</td>
<td>MW</td>
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<td>375.99</td>
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<td>1</td>
<td>0</td>
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</table>

**Active Site Analysis**

After getting the PDB (3B7E) structure from RCSB (http://www.rcsb.org/pdb/home/home.do).

Active site analysis of the Protein Neuraminidase with Zanamivir was performed using Swiss PDB Viewer. The residues found in the binding site of Neuraminidase were identified as ARG-118, ASP-151, ARG-152, TRP-178, GLU-227, GLU-276, ARG-292, and ARG-371.
Docking Studies

The Software’s used for the docking studies are AUTODOCK VINA. Auto Dock Vina is a new open source program for drug discovery, molecular docking and virtual screening, offering multi-core capability, high performance and enhanced accuracy and ease of use. Auto Dock Vina significantly improves the average accuracy of the binding mode predictions compared to Auto Dock 4.2.

Analyzing the Docking Results

The search for the best ways is to fit ligand molecules, into H1N1 neuraminidase structure. Using AUTO DOCK VINA resulted in docking files that contained detailed records of docking. The obtained log files were read in ADT (Auto Dock Tool) to analyze the results of docking. The similarity of docked structures was measured by computing the root mean square deviation (RMSD) between the coordinates of the atoms and creating clustering of the conformations based on the RMSD values. The lowest binding energy conformation in all cluster were considered as the most favourable docking pose. Binding energies that are reported represent the sum of the total intermolecular energy, total internal energy and torsional free energy minus the energy of the unbound system. The top five ligands were selected based auto dock vina score after virtual screening. The protein which is used, known as NEURAMINIDASE retrieved from RCSB-PDB (PDB ID: 3B7E).

RESULTS

Docking results (Table 2, Fig 2, Fig 3, Fig 4, Fig 5, Fig 6, Fig 7 and Fig 8) indicate that all the compounds interact with neuraminidase with best binding energies.

Table 2. Lead molecule with amino acids interaction.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Ligand</th>
<th>Amino acid residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mangostin</td>
<td>Tyr 406, arg 371, arg 224</td>
</tr>
<tr>
<td>2.</td>
<td>Mangiferonic acid</td>
<td>Ser 246, asn 294</td>
</tr>
<tr>
<td>3.</td>
<td>Mangiferin</td>
<td>Tyr 406, glu 227, trp 178, arg 292, asn 294, asp 151</td>
</tr>
<tr>
<td>4.</td>
<td>Isomangiferin</td>
<td>Asp 151, arg 118, trp 178, arg 292, arg 371, arg 152</td>
</tr>
<tr>
<td>5.</td>
<td>Freidilin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zanamivir (known anti H1N1)</td>
<td>Arg 156, tyr 406, arg 118, arg 371</td>
</tr>
</tbody>
</table>
Fig 2. Graphical Representation of Docking Score.

Fig 3. Zanamivir and amino acid interaction (by ligplot)

Fig 4. Mangiferin and amino acid interaction, (by ligplot)
Fig 5. Mangostin and amino acid interaction, (by ligplot)

Fig 6. Isomangiferin and amino acid interaction, (by ligplot)

Fig 7. Mangiferonic acid and amino acid interaction, (by ligplot)
DISCUSSION

Neuraminidase enzymes are glycoside hydrolase enzymes that cleave the glycosidic linkages of neuraminic acids. Neuraminidase enzymes are a large family, found in a range of organisms. The best known neuraminidase is the viral neuraminidase. At least four mammalian sialidase homologs have been described in the human genome (NEU1, NEU2, NEU3 and NEU4).

Neuraminidase, also called sialidases, catalyze the hydrolysis of terminal sialic acid residues from the newly formed virions and from the host cell receptors. Sialidase activities include assistance in the mobility of virus particles through the respiratory tract mucus and the elution of virion progeny from the infected cell.

The enzymatic mechanism of influenza virus sialidase has been studied by Taylor et al, shown in below figure. The enzyme catalysis process has four steps. The first step involves the distortion of the alpha-sialosides from a $^2c5$ chair conformation (the lowest energy form in solution) to a pseudo boat conformation when the sialosides binds to the sialidase. The second step leads to an oxocaroccation intermediate, the sialosyl cation. The third step is the formation of Neu5Ac initially as the alpha-anomer and then mutarotation and release as the more thermodynamically stable betNeu5Ac.

Recent emergence of ZANAMIVIR resistant human influenza A(H1N1) H274Y has emphasized the need for suitable expression systems to obtain large quantities of highly pure & stable, recombinant neuraminidase through two separate artificial tetramerization domains.
that facilitate the formation of catalytically active neuraminidase homo tetramers from yeast & *Staphylothermus marinus*, which allow for secretion of FLAG-tagged proteins and further purification.

It has recently been demonstrated that highly pathogenic H5N1 avian influenza can easily adapt to become airborne-transmissible in mammals, reminding the world about the pandemic potential of avian influenza viruses. Moreover, ZANAVIMIR and that function as influenza neuraminidase (NA) inhibitors are currently recommended for use worldwide.

In this study, we first confirmed the catalytic role of the conserved active-site tyrosine (Tyr406) of influenza NA in order to explore the possibility of developing covalent influenza inhibitors.[43]

Auto dock vina binding energy for mangiferin was -8.4(kcal/mol). Mangiferin is forming hydrogen bonds with Arg 292, Asn 294, asp 151, Tyr 406, glu 227, trp 178 which are active site residues. Isomangiferin has a binding energy of -9.0(kcal/mole) with auto dock Vina binding energies. The compound was found to interact with many residues of which Arg 371, asp 151, arg118, 152, 292, 371, trp178 are known active site residues. Freidilin has a binding energy of -7.8(kcal/mole) in Auto dock Vina. The binding energy obtained for Mangiferonic acid with Auto dock Vina was -8.4(kcal/mole). The binding energy obtained for mangostin with Auto dock Vina was -8.2(kcal/mole).

**CONCLUSION**

The 3-dimensional structure of the Protein neuraminidase in complex with Zanamivir was used in the present study. The binding energy of the protein neuraminidase with various natural compounds obtained from *Mangifera indica* such as mangiferin, mangiferonic acid, freidlin, isomangiferin, mangostin, kainic acid, Gallic acid etc. was obtained using docking software’s namely Auto dock Vina. The interactions were also visualized using Ligplot and auto dock tools. It can be concluded from our observations mangiferin, isomangiferin, mangiferonic acid, freidlin, d mangostin are found to interact with few active site residues like ARG 371, GLU 227,TYR 406, ASP 151, etc. Of all these compounds mangiferin, isomangiferin can be considered as potent inhibitors since they have a better binding energy and also interact with active site residues. This has to be further investigated by wet lab studies.
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