International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:5, No:11, 2011

Computer Aided Drug Design and Studies of Antiviral Drug against H3N2 Influenza Virus

Aditi Shukla [1]*, Ambarish S. Vidyarthi [2] and Subir Samanta [3]

Abstract**—**The worldwide prevalence of H3N2 influenza virus and its increasing resistance to the existing drugs necessitates for the development of an improved/better targeting anti-influenza drug. H3N2 influenza neuraminidase is one of the two membrane-bound proteins belonging to group-2 neuraminidases. It acts as key player involved in viral pathogenicity and hence, is an important target of anti-influenza drugs. Oseltamivir is one of the potent drugs targeting this neuraminidase. In the present work, we have taken subtype N2 neuraminidase as the receptor and probable analogs of oseltamivir as drug molecules to study the protein-drug interaction in anticipation of finding efficient modified candidate compound. Oseltamivir analogs were made by modifying the functional groups using Marvin Sketch software and were docked using Schrodinger's Glide. Oseltamivir analog 10 was detected to have significant energy value (16% less compared to Oseltamivir) and could be the probable lead molecule. It infers that some of the modified compounds can interact in a novel manner with increased hydrogen bonding at the active site of neuraminidase and it might be better than the original drug. Further work can be carried out such as enzymatic inhibition studies; synthesis and crystallizing the drug-target complex to analyze the interactions biologically.

Keywords**—**H3N2 Influenza, Neuraminidase, Oseltamivir analogs, structure based drug designing

I. Introduction

INFLUENZA rapidly spreads around the world in seasonal epidemics and imposes a considerable economic burden to the affected population. Although difficult to assess, these annual epidemics are thought to result in between 3-5 million cases of severe illness and 250,000 to 500,000/year (According to WHO's report) [1].The annual flu (also called "seasonal flu" or "human flu") kills an estimated 36,000 people in the United
States each year [2]. In the past ten years, H3N2 has tended to remain prevalent.

The enzyme neuraminidase is one of two glycoproteins found on the surface membrane of influenza virus. It plays an important role in facilitating the spread of viral infection. It cleaves the terminal sialic acid moieties from the receptors and hence, results, into the release of progeny virus from infected cells. The active site of neuraminidase is highly conserved and so targeting this glycohydrolase provides efficient means of controlling influenza infections [3, 4, 5, 6]. Adamantanes were earlier used to treat influenza, but now the strain has become almost 90% resistant and no longer is a recommended drug to influenza [2],[7].The two neuraminidase inhibitors which are already commercially available are Zanamivir and Oseltamivir. These are used to treat influenza and also appear to be quite effective in Prophylaxis [8]. However, they are completely different in terms of mode of delivery, pharmacological action and side effects. Zanamivir has poor oral availability and is therefore administered by inhalation only and has limited usage when treating the elderly person because it may induce bronchospasm. Also there are reports of Zanamivir resistance [9]. Oseltamivir is an orally active influenza neuraminidase inhibitor approved for treating and preventing influenza virus infection [6, 8. 9]. Modification of oseltamivir to improvise its effectiveness is the primary aim of this study.

In order to develop improved and effective drug against viral targets, various computational and bioinformatics approaches has been used to study and simulate protein receptor-drug interaction at molecular level. Computer Aided Drug Design (CADD), a specialized discipline that uses computational methods to simulate drug-receptor interactions, could be utilized for such development program [10, 11, 12]. Computational docking operation further enhances the scope to study molecular binding interactions. In present study we have taken various analogs of Oseltamivir as possible drug candidiate and neuraminidase as target. Glide was used as the docking software to study the drug-protein interaction and to design an efficient anti-influenza drug molecule.

II. Materials And Methods

In the present study, bioinformatics online databases like PubMed, PubChem and PDB were used to collect biological, chemical and protein structural information on neuraminidase subtype 2 (N2) and Oseltamivir. Marvin Sketch software was used to create structure of possible drug analog.Glide was used for protein-drug docking studies. Docking is the process of fitting together of two molecules in 3-Dimensional space.

Neuraminidase-2 Protein Sequence

>Neuraminidase

MNPNQKIITIGSICMTIGIISLILQIGNIISIWVSHSIQTGSQNH
TGICNQRIITYENSTWVNQTYVNINNTNVVAGKDTTSVTLAG
*NSSLCPIRGWAIYSKDNSIRIGSKGDVFVI**R**EPFISCSHLECR*
*TFFLTQGALLNDKHSNGTVK**D**RSPYRALMSCPIGEAPSPYN*
SRFESVAWSASACHDGMGWLTIGISGPDDGAVAVLKYNGII
TETIKSWRKRILRTQESECVCVNGSCFTIMTDGPSNGPASY
*RIFKIEKGKITKSIELDAPNSHY**E**ECSCYPDTGTVMCVC**R**D*
NWHGSNRPWVSFNQNLDYQIGYICSGVFGDNPRPKDGKG
*SCDPVTVDGADG**V**KGFSYRYGNGVWIGRTKSNSS**R**KGFE*
*MIWDPNGWTDTDSNFLVKQDVVAMTDWSG**Y**SGSFVQHP*
*ELTGLDCMRPCFWV**E**LIRGRPREKTTIWTSGSSISFCGVNS*
DTANWSWPDGAELPFTIDK

The structure of neuraminidase was retrieved from a neuraminidase-inhibitor complex from PDB (PDB id: 1ING) [11] Neuraminidase was extracted from the complex and was

1 *Corresponding Author: Aditi Shukla is with Department of Biochemistry, University of Luebeck, Ratzeburger Allee 160 - 23558, Luebeck, Schleswig-Holstein, Germany. (Mobile-(+49) 1602361495, email – aditi@biochem.uni-luebeck.de)

2 Ambarish S. Vidyarthi is with Department of Biotechnolgy, Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, India (phone:+91-651-2276223, email- asvidyarthi@bitmesra.ac.in)

3 Subir Samanta is with Department of Pharmaceutical Science, Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, India (phone:+919431596870email:samanta_bitmesra@yahoo.co.in)

analyzed by using RasMol [Raster Display of Molecules]. There are 8 residues in the active site of N2 which are conserved in almost all the neuraminidase subtypes. These 8 residues are Asp 151, Arg 118, Glu 277, Arg 292, Val (or Ile) 349, Arg 371, Tyr 406, Glu 425 [3, 4, 5,13].

The structures of conventional drug Oseltamivir was retrieved from PubChem. Structural analogs of these drug molecules were created by using Marvin Sketch, a Java based chemical drawing tool which allows creating and editing of molecules in various file formats.

Docking analysis of Oseltamivir structural analogs with N2 was carried out by Glide Docking software. Oseltamivir analogs and N2 receptor complexes was identified via docking and their relative stabilities were evaluated using number of hydrogen bonds formed in the active sites as well as the docking score. The overall approach for selection of potential Oseltamivir analog is shown in Fig 1.

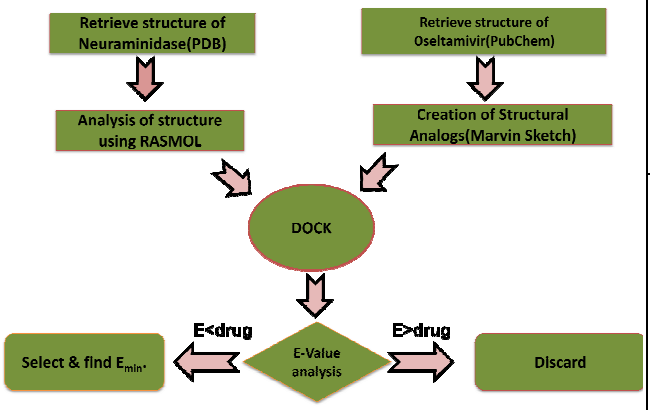
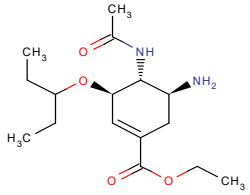
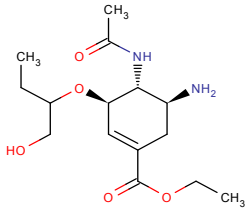
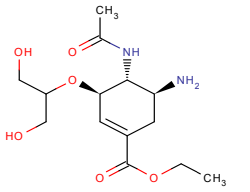
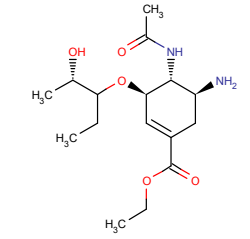
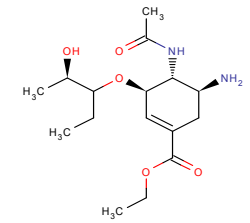


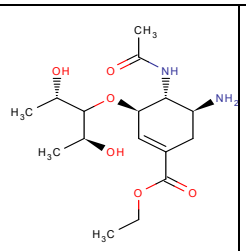
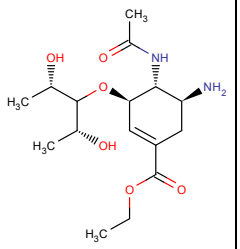
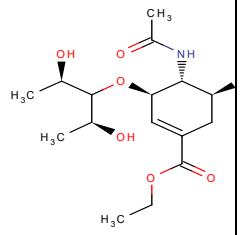
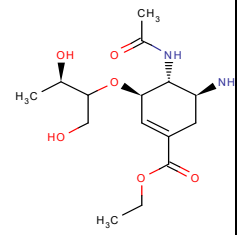
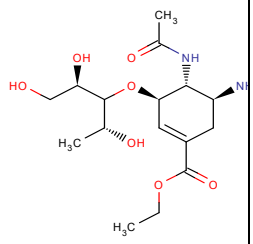
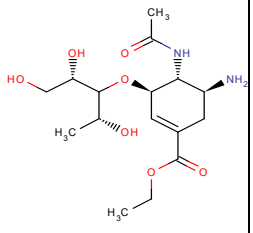
Fig. 1 Flowchart showing the Steps used in Drug Designing

III. Results

The interaction of neuraminidase of H3N2 influenza virus and Oseltamivir has been studied using the docking software Glide. Glide was chosen for the docking purpose because of its rapidness, accuracy and reproducibility. Ten analogs of Oseltamivir namely adiasvssn1 to adiasvssn 10 were created using Marvin Sketch and docked with Glide to study the drug-protein interaction based on Least Energy Value Complex. Docking results of N2 and the Conventional drug Oseltamivir as well as its analogues are shown in Table I.

Table I
Showing the docking result after performing the docking on Glide. The scores in bold are the best score obtained in Least Energy Value Complex

Name	Structure	Glide Score	Energy Model Score
Oseltamivir		-7.53104	-49.0089
Adiasvssn1		-7.48395	-51.3685
Adiasvssn2		-7.62781	-50.7944
Adiasvssn3		-8.16035	-51.7149
Adiasvssn 4		-7.84823	-49.4418

Adiasvssn 5		-8.22504	-51.7149
Adiasvssn 6		-7.97201	-54.9563
Adiasvssn 7		-8.64708	-53.2847
Adiasvssn 8		-8.24278	-52.9433
Adiasvssn 9		-8.46651	-53.1232
Adiasvssn 10		**-9.58916**	**-56.7963**

The hydrogen bonds interaction of adiasvssn 10 with N2 is shown in Figure 2.

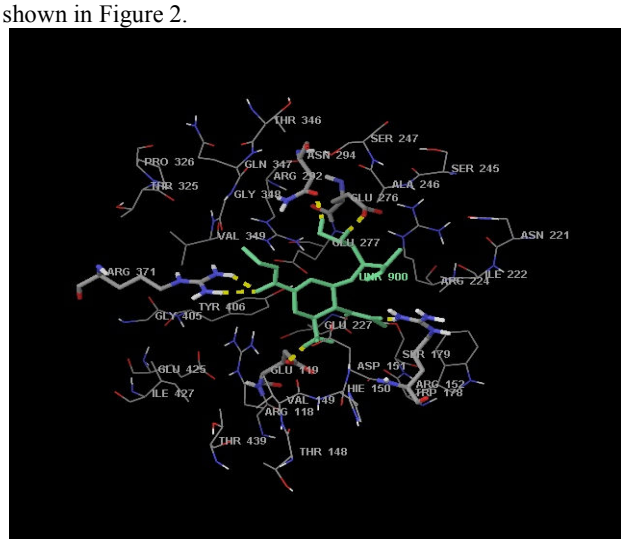


Fig. 2 Snapshot showing the docked position of Oseltamivir analog adiasvssn 10 with N2. The yellow dashed lines are the Hydrogen bonds between the protein and the drug

IV. Discussions

Based on the literatures it has been clearly shown that Oseltamivir have been used as a standard drug which acts against influenza virus neuraminidase surface protein. Oseltamivir on docking with N2 produced an energy value of -49.0089. Good binding affinity of the carboxylic acid of oseltamivir with the triad guanidine groups of the three conserved arginine residues Arg118, Arg371, and Arg292 in neuraminidase was observed. Also, the methyl of N-acetyl group of the oseltamivir is perfectly fitted in the hydrophobic pocket formed by the side chains of Trp178 and part of Arg224 in neuraminidase. So, no modification was needed for the carboxylic acid and the methyl of N-acetyl group in the original drug. Furthermore, the C8 chirality was not taken into account due to its flexibility in C6–O–C8 [5].

Applying these restrictions, 10 analogs of oseltamivir were designed and drawn using Marvin Sketch i.e. - adiasvssn 1-adiasvssn 10. The C8-pentyl group of the inhibitor plays a role in binding to the neuraminidase. Hydroxyl groups were attached which favor hydrogen bonding at different orientations. The π value of substitution of –OH group is -0.67 for aromatic substitution that means the substance becomes hydrophilic and thus would help in H- bond formation with the amino acid residues. After designing, the analogs were energy minimized. The decrease in the energy value, during the protein-drug interaction, was due to modifications in the Oseltamivir structure. This way the pharmacophoric part of the drug was partially identified.

Adiasvssn 10 had Energy score of -56.7963 inferring that it had better binding with the active sites of N2 than the standard drug. The binding sites of the analog at the active site of N2 were almost similar to that of the standard drug. It means that functional groups involved were the same and by preparing the analog only the steric compatibility was increased with the increase in additional two hydrogen bonds. This resulted in the

increase in interaction of the protein with the modified drug and hence an improved glide score was obtained.

Glide score is obtained by adding the score of all XP descriptors such as hydrophobic enclosure rewards, rewards for hydrophobically packed hydrogen bonds, electrostatic rewards, low molecular weight rewards, polar atom burial penalty, exposed hydrophobic ligand group penalty etc.

Energy Model Score is the energy state of the docked conformer and takes into account the structure of the receptor and ligand complex.

The order of binding interactions according to the Glide Score is - adiasvssn 10 > adiasvssn 7 > adiasvssn 9 > adiasvssn 8 > adiasvssn 5. However, the order of energetically favored structure is - adiasvssn 10 > adiasvssn 6 > adiasvssn 7 > adiasvssn 9 > adiasvssn 8. Taking both the score into consideration we have selected adiasvssn 10, adiasvssn 7, and adiasvssn 9 as the best three analogs which have lesser energy than the standard drug (oseltamivir).

V. Conclusion

The Protein-Ligand interaction plays a significant role in the structure based drug designing. In the present work we have taken H3N2 influenza virus neuraminidase as the drug target and oseltamivir as the standard drug that is used against influenza. On the neuraminidase receptor, oseltamivir was docked and the energy value was obtained. When the modified analogs of the drugs were docked against the same receptor the energy value obtained was lesser than the standard drug. Hence, more stable interaction complexes were formed. From this work we can conclude that some of the modified inhibitor candidates make selective interaction with the conserved residues with increased hydrogen bonding at the active site. Hence, they might be better acting than the reference drugs. Of these molecules adiasvssn 10, adiasvssn 7, adiasvssn 9 are probable lead molecules and can be tested against influenza owing to their high-energy value as well as glide score. We can infer that the lead molecule is one which should have maximum interaction including H-bonding and have good docking score. The concept of protein-ligand interaction may help to design and establish a new series of compounds active against H3N2 influenza virus.

Acknowledgment

BTISNet Sub DIC facility at Department of Biotechnology and Facility created under UGC SAP at Department of Pharmaceutical Science of BIT, Mesra are gratefully acknowledged.

References

[1] G. WHO Fact sheet No. 211 Accessed 16 January 2009. URL: http://www.who.int/mediacentre/factsheets/fs211/en/
[2] Infection Control Today, Aug 2010, Alert on seasonal Influenza (H3N2) Virus Infections. URL:http://www.infectioncontroltoday.com/news/2010/08/cdc-issues-alert-on-seasonal-influenza-a-h3n2-virus-infections.aspx
[3] JN Varghese, Development of neuraminidase inhibitors as anti-influenza drugs. Drug Dev. Res. 46 (1999)
[4] JN Varghese, VC Epa, PM Colman, Three-dimensional structure of the complex of 4-guanidino-NeuSAc2en and influenza virus neuraminidase Prot. Sci. 4 (1995) 1081-1087.
[5] QS Du, SQ Wang, KC Chou, Analogue inhibitors by modifying oseltamivir based on the crystal neuraminidase structure for treating drug-resistant H5N1 virus, Biochem. Biophys. Res. Commun. 362 (2007) 525-531
[6] KC Chou, QS Du, DQ Wei, H Sun, Insights from modeling the 3D structure of H5N1 influenza virus neuraminidase and its binding interactions with ligands, Biochem. Biophys. Res. Commun. 344 (2006) 1048-1055
[7] Influenza Strain, H3N2, Resistant To Antivirals Amantadine and Rimantadine Main Category: Flu / Cold / SARS Article Date: 16 Jan 2006 - 0:00 PDT (Medical News Today)
[8] I Stephenson, TW Clark, M Pareek, Antiviral treatment and prevention of seasonal influenza: A comparative review of recommendations in the European Union, J. Clin. Vir. 42 (2008) 244-248.
[9] K Bauer, M Richter, P Wutzler, M Schmidtke, Different neuraminidase inhibitor susceptibilities of human H1N1, H1N2, and H3N2 influenza A viruses isolated in Germany from 2001 to 2005/06, Antivir. Res. 82 (2009) 34-41.
[10] DBM Virupakshaiah, C Kelmani, R Patil and P Hegade, Computer Aided Docking Studies on Antiviral Drugs against SARS, PWASET 24 (2007) 297-299.
[11] S. Singh, MJ Jedrzejas, GM Air, M Luo, WG Laver, WJ Brouillette, Structure - based inhibitors of Influenza Virus sialidase. A benzoic acid lead with novel interaction. J. Med. Chem. 38 (1995) 3217-3225.
[12] M Luo, GM Air, WJ Brouillette, Design of Aromatic Inhibitors of Influenza Virus Neuraminidase, J. Infect. Dis. 176 (1997) S62-S65.
[13] PM Colman, Structural basis of antigenic variation: Studies of influenza virus neuraminidase. Immunol. Cell Biol. 70 (1992) 209-214.