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In silico molecular modeling of neuraminidase enzyme H1N1 avian influenza virus and docking with zanamivir ligands

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ABSTRACT

Objective: Neuraminidase is an enzyme aspartic protease that is essential for the life cycle of H1N1. **Methods:** Constructed a model of Neuraminidase enzyme the 3D structure as template using with Modeller software. The Neuraminidase enzyme model was predicted and validated by Procheck, What check, Errat, Verify-3D and AutoDock web server for reliability. **Results:** The Modeller homology-modeling algorithm was demonstrated excellent accuracy in blind predictions. The Neuraminidase enzyme model built with little, 35% identity could be accurate enough to be successfully used in receptor based rational drug design. The closest homologue with the highest sequence identity 100% was selected. Zanamivir drug and analogues were retrieved from PubChem database, as well as subjected to docking interaction with Neuraminidase enzyme used AutoDock programme. Based on the root mean square deviation and lowest binding energy values the best docking orientation was selected. The better lowest binding energy value -6.91 was selected of CID_25209232. **Conclusions:** This study will be used in broad screening of inhibitors of the protein. However, further implemented experimental and clinical verification is needed to establishment these analogues as drug.

1. Introduction

Influenza A viruses belong to the family Orthomyxoviridae and possess 8 negative-sense RNA segments encoding 11 known proteins. Of these, the 2 viral surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA) form the basis of multiple serologically distinct virus subtypes. Currently, 16 HA and 9 NA subtypes have been identified in wild water birds, the natural host for all influenza A viruses and the reservoir from which viruses emerge to infect domestic poultry and occasionally mammals[1]. The subtypes of influenza virus are H1N1, H1N2, H3N1, H3N2, H2N3 and H5N1, *etc*[2-4]. The current H1N1 virus strain is a mixture of human, pig and bird genes and has proved to be very contagious, but no more deadly than common seasonal flu viruses. However, it could theoretically become more dangerous if it adds virulence by combining with H5N1, which is far more deadly but harder to pass along among humans[5,6].

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.

The current anti-influenza drugs, zanamivir and oseltamivir are successful examples of structure-based drug design, and both exert its antiviral effects through the inhibition of neuraminidase activities of the influenza A and B viruses[7,8]. The influenza A neuraminidases are divided phylogenetically into two distinct subtypes, group-1 and group-2[9]. Both oseltamivir and zanamivir were developed on the basis of the crystal structures of group-2 neuraminidase proteins (N2 and N9). Because those drugs have shown similar activity against the N1 neuraminidase, it was thought that the active sites of those

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proteins are similar. 3D structure of Neuraminidase enzyme is not available in protein databank until date. The quality of the homology model depends on the quality of sequence alignments, template structure and extent of identity between the template and target sequences. The present study, we have used the homology modeling to construct an atomic resolution model of the target protein from its amino acid sequence based on an experimental 3-D structure of a related homologous protein and then Docking program has been used to show the interaction between Neuraminidase enzyme models with zanamivir drug compounds.

2. Materials and methods

2.1. Template selection and sequence alignment

The target protein sequence Accession No.ACD88519 was retrieved from NCBI in fasta format. Target protein sequence involved in homology search in PSI BLAST for sequence similarity and identify template sequence with existing 3D structure file of template protein sequence from RCSB protein databank (PDB:3B7E). The zanamivir drug was collected from drug bank database (http://www.drugbank.ca) Accession No.DB00558. Zanamivir analogue structures were retrieved from NCBI Pubchem database.

2.2. Target sequence

>gi|189312997|gb|ACD88519.1| neuraminidase [Influenza A virus /turkey/Kansas/ 4880/1980 (H1N1))]

MNTNQRIITIGTICLIVGIVSLLLQIGNIVSLWISHSIQTGEKNH
PEVCNQSVITYENNTWVNQTYVNISNTNIAAGQGVTPIILAGN
SSLCPISGWAIYSKDNSIRIGSKGDIFVMREPFISCSHLECRTFF
LTQGALLNDRHSNGTVRDRSPYRTLMSCPIGEAPSPYNSRFES
VAWSASACHDGMGWLTIGISGPDNGAVAVLKYNGIITDTIKS
WRNKILRTQESECVCINGSCFTIMTDGPSNGQASYKIFKMEKG
KIIKSIELDAPNYHYEECSCYPDTGKVVCVCRDNWHASNRPW
VSFDQNLDYQIGYICSGVFGDNPRSNDGKGNCGPVLSNGANG
VKGFSFRYGNGVWIGRTKSISSRSGFEMIWDPNGWTETDSSFS
MKQDIIALTDWSGYSGSFVQHPELTGMNCIRPCFWVELIRGQ
PKESTIWTSGSSISFCGVNSETASWSWPDGADLPFTIDK

2.3. Template sequence

>3B7E:A|PDBID|CHAIN|SEQUENCE

VILTGNSSLCPISGWAIYSKDNGIRIGSKGDVFVIREPFISCSH
LECRTFFLTQGALLNDKHSNGTVKDRSPYRTLMSCPVGEAPS
PYNSRFESVAWSASACHDGMGWLTIGISGPDNGAVAVLKYN
GIITDTIKSWRNNILRTQESECACVNGSCFTIMTDGPSNGQASY
KILKIEKGKVTKSIELNAPNYHYEECSCYPDTGKVMCVCRDN
WHGSNRPWVSFDQNLDYQIGYICSGVFGDNPRPNDGTGSCG
PVSSNGANGIKGFSFRYDNGVWIGRTKSTSSRSGFEMIWDPN
GWTETDSSFSVRQDIVAITDWSGYSGSFVQHPELTGLDCMRP
CFWVELIGQPKENTIWTSGSSISFCGVNSDTVGWSWPDGAEL
PFSI

2.4. Homology model building

Homology modeling was done in Modeller9v7, which requires three input files: 1) Query or Target sequence in FASTA format; 2) Template sequence in FASTA format; 3) Atomic coordinate's files (PDB file). Pair wise sequence alignment of Template and Target sequences were performed as well as alignment of the protein sequences were constructed by the software Modeller9v7. Structure refinement and energy minimization was performed with Modeller9v7 itself by using the Regularization macro. Regularization is a procedure for fitting a protein model with the ideal covalent geometry of residues to the atomic positions of the structure[10].

2.5. Evaluation and validation

Modeled protein was predicted and evaluated using Procheck, What check, Errat[11] and Verify_3D[12]. Procheck was used to perform full geometric analysis as well as stereo chemical quality of protein structure by analyzing residue-by-residue geometry and overall structure geometry. Ramachandran plot statistics was analyzed to evaluate the stability of the model and confirmation of the residues. Errat server predicted an overall quality factor of modeled protein[13].

2.6. Molecular docking

The 3D structure of modeled protein Neuraminidase Enzyme was used to molecular docking with 3D structure of zanamivir analogues using AutoDock program[14]. Zanamivir and its analogues were taken from Pubchem database, SDF file format of all ligands were converted to 3D structure using Chemdraw program. The primary task of docking is to find the exact binding position and orientation of ligand molecule in the active site of protein. Autodock is a suit of program being used for flexible docking of ligand to protein. It included ligand flexibility allowing the ligand to change conformation during the docking simulation. During docking, all torsions were allowed to rotate and water molecules were excluded, because the position of water molecules cannot be conserved in docking process. Modeled protein was used as input for autogrid program. Moreover, grid map was generated; these maps were chosen to be sufficiently large to include significant portions of the ligand. Docking study was carried out using the empirical free energy function and the Lamarckian genetic algorithm. Autodock generates different conformers for each docking simulation. The result of docking simulation provided the orientation and specific position of best binding of the ligand in the active site, which were used to determine nearest neighbors, hydrogen bonding and van der waals interactions.

3. Results

Homology sequence searching was done against query sequence using Basic Local Alignment Search Tool (BLAST), which was performed to identify the template sequence and 3D structure for target sequence. The best template structure was identified PDB ID: 3B7E with 1.46 Å resolutions with maximum score (963), and lowest E-Value (0.0) was better obtained with alignment with 100% sequence identity. The homology model was generated using Modeller9v7 program. The secondary structure of protein model was predicted 100% coil structure, helix - sheets no percentages, ribbon style of poly peptide chains were found Arg (8) residues, Ala (28) residues. The orientation of Alpha 20.918, beta 45.286, and gamma 3.692 degree was predicted. The atom properties of carbon(C) 20.00, Bfactor with 100% residue, ASP (A) residues (20.0) as well as totally 1542 atoms of residues were analyzed.

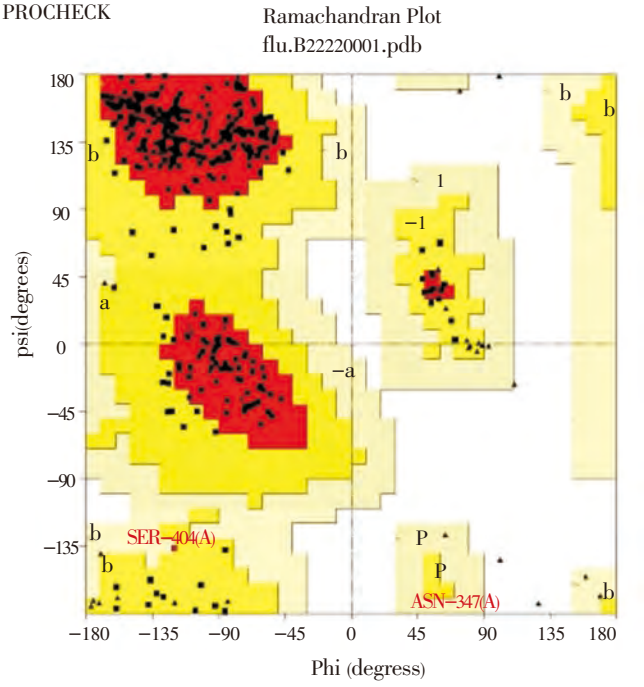

Figure 1. Ramachandran plot prediction of the Neuraminidase enzyme.

The modeled protein flu.B22220001.pdb was validated by Ramachandran plot[14] for geometric as well as stereochemical quality of protein. Most favoured regions of residues 85.2% allowed regions of additional residues 14.2%, generously allowed regions 6.0%, non-proline residues, non-glycine residues regions 100.0% and most disallowed regions 0.0% respectively. Therefore best model model was validated by Procheck, Ramachandran plot (Figure 1). Whatcheck result had predicted better Ramachandran Z-score for bond angle Z-score and bond length Z-score for modeled protein. The profile score above zero in the Verify3D graph corresponded to acceptable environment of the model. Moreover, the high score value 0.57 indicates that environment profile of the model was good. The Errat program was predicted for overall quality factor of validated model 96.899 that was better-predicted model of protein (Figure 2).

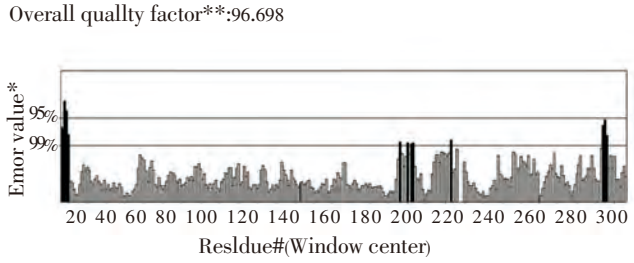

Figure 2. Illustration of ERRAT plot analysis f or overall quality factor of Neuraminidase enzyme model.

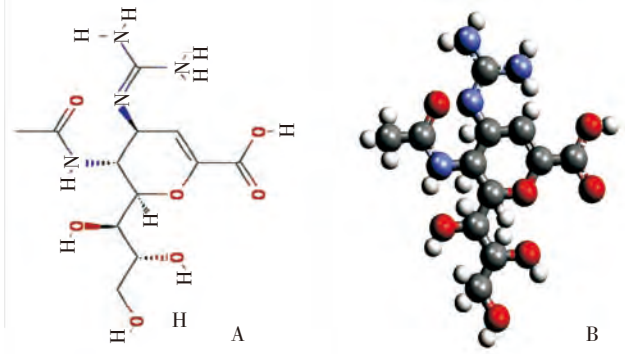

Figure 3. Zanamivir Ligand.
A: Chemical structure of zanamivir. B: 3D Structure of zanamivir

Table 1
Properties of energy minimized zanamivir and analogues of lead compound were determined by MOPAC algorithm of Chem3D Ultra software.

Ligand ID number	CID_53324370	CID_ 25241228	CID_ 25209232	CID_ 10735239	CID_118043618	CID_60855
Final heat of formation	-320.97332	-279.43836	-272.56606	-310.92947	-256.36254	-321.72866
Kcal (in KJ)	-1342.95237	-169.17009	-1140.41638	-1300.92889	-1072.62088	-1346.11270
Cosmo area (in Square Angstroms)	279.21	346.05	338.37	335.89	309.87	343.47
Cosmo volume (in Cubic Angstroms)	205.35	263.23	248.97	254.83	234.16	257.25
Total energy (in EV)	-4885.75772	-5811.28539	-5451.18636	-5771.53275	-5360.37547	-5772.00145
Electronic energy (in EV)	-5202.26402	-6179.17514	-5818.51285	-6247.41670	-5759.46702	-6082.18012
Core-core repulsion (in EV)	316.50630	367.88974	367.32650	475.88395	399.09155	310.17867
Dielectric energy (in EV)	-3.69303	-4.43453	-3.69970	-4.21040	-4.02801	-3.97237
Gradient norm	141.68760	78.91538	46.72238	31.71385	37.54909	37.32507
Ionization potential	9.56065	9.99213	10.02318	10.11373	9.88142	10.00173
No. Of filled levels	65	78	75	78	72	78
Molecular weight g/mol	334.302	336.334	335.328	331.310	371.349	332.309

Note: MOPAC- Molecular Orbital Package.

Table 2

Docking Results of Neuraminidase enzyme model with zanamivir ligands

Ligand ID number	CID_ 53324370	CID_25241228	CID_ 25209232	CID_ 10735239	CID_118043618	CID_60855
Binding Energy	−6.12	−6.07	−6.91	−6.58	−5.73	−7.42
kI (in uM)	32.74	35.78	8.64	15.15	63.11	3.65
Intermolecular Energy	−7.34	−7.36	−8.39	−6.74	−7.13	−7.29
Internal Energy	−2.42	−3.46	−3.05	−3.31	−3.55	−5.32
Torsion Energy	2.74	3.57	3.29	3.29	3.29	3.84
Unbounded Extended Energy	−0.89	−1.19	−1.24	−0.18	−1.66	−1.35
Cluster RMS	0.0	0.0	0.0	0.0	0.0	0.0
Reference RMS	35.96	34.92	33.92	33.98	34.5	34.76

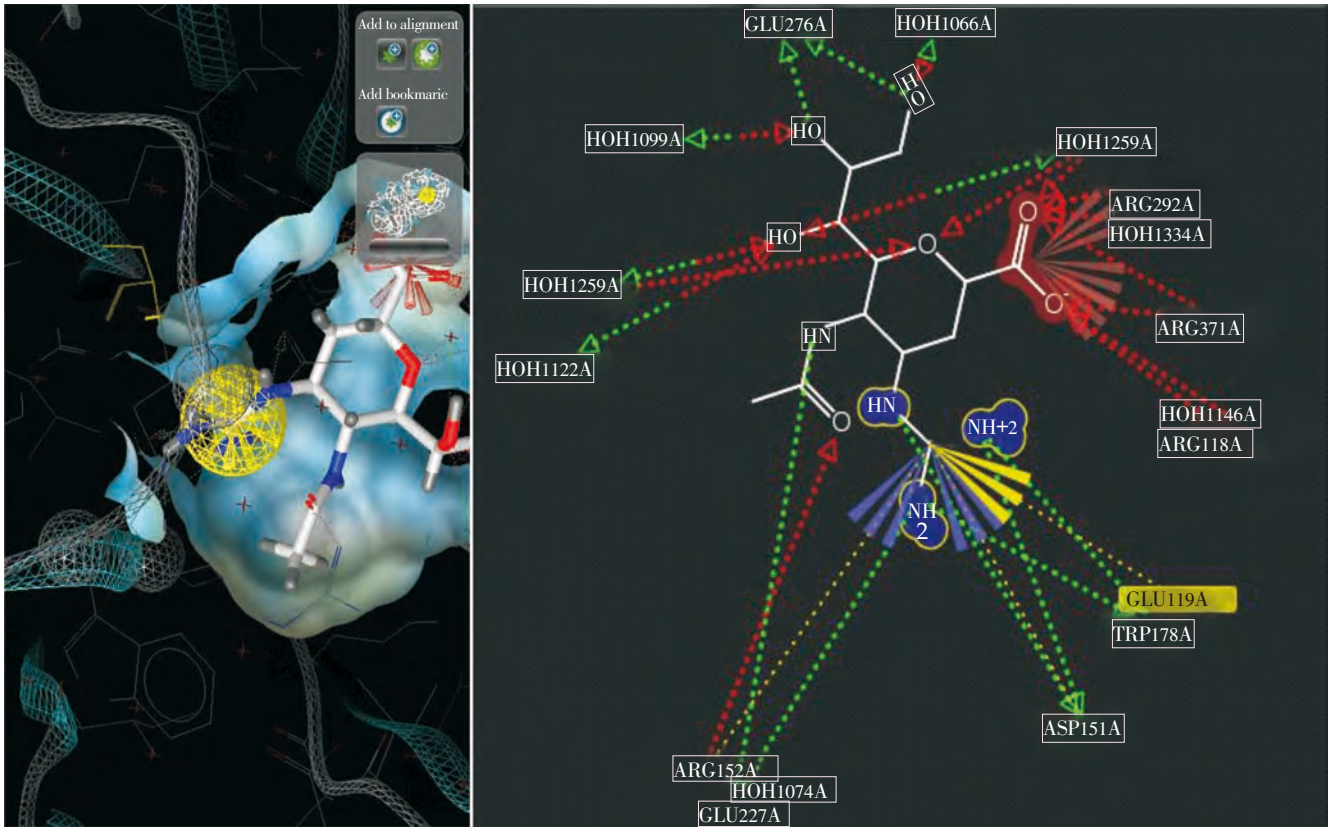

Figure 4. Pharmacophore site identification of zanamivir ligand in.

The zanamivir (ZMR) drug properties were found such as average weight (332.3098), chemical formula (C12H20N4O7), IUPAC name(2R,3R,4S)-4-[(diaminomethylidene)amino]-3-acetamido-2-[(1R,2R)-1,2,3-trihydroxypropyl]-3,4-dihydro-2H-pyran-6-carboxylic acid, water solubility (7.31e+00 g/l), logP (−2.30), logS (−1.66), pKa (12.79), hydrogen acceptor count (10), hydrogen donor count (7), polar surface area (200.72), rotatable bond count (6), refractivity (76.19) and polarizability (31.24) from the drugbank database web server. Moreover, predicted and validated protein model of Neuraminidase Enzyme was involved to docking study with zanamivir drugs (Figure 3). The interaction results between proteins and ligand analogues complexes were illustrated each other (Figure 4).

The Zanamivir drug analogues were energy minimized separately by the MOPAC (Molecular Orbital PACkage algorithms) of Chem 3D Ultra software (Table 1). Totally three bindig sites residues GLU (119), GLU (277), and ASP (151) were identified. Yellow color was indicated for pharmacophore active site of Zanamivir ligand used Ligandscout programme. In this docking simulation, both Zanamivir drug and analogues were successfully docked with Neuraminidase enzyme model (Figure 5). The ligands were subjected to docking using AutoDock program. Based on the lowest energy values the best docking orientation was selected. while Zanamivir analogues were docked with the predicted 3D model of the enzyme flu.B22220001.pdb, result showed that (Table 2) CID_25209232 ligand ranked one with the lowest binding energy (−6.91) in comparison to existing other analogues except original Zanamivir drugs CID_60855 having the binding energy (−7.42) which, commercially available in market. Other binding parameters of CID_25209232 ligand

such as intermolecular energy (−8.39), Kinetic energy (8.64), Internal energy (−3.05), Torsion energy (3.29), Unbounded extended energy (−1.24), Cluster RMS (0.0) and Reference RMS (33.92) were determined and suggested that the greater binding affinity was obtained than others analogues. The different docking simulations were illustrated strong binding affinities of zanamivir analogues with Nuraminidase enzyme (Figure 6).

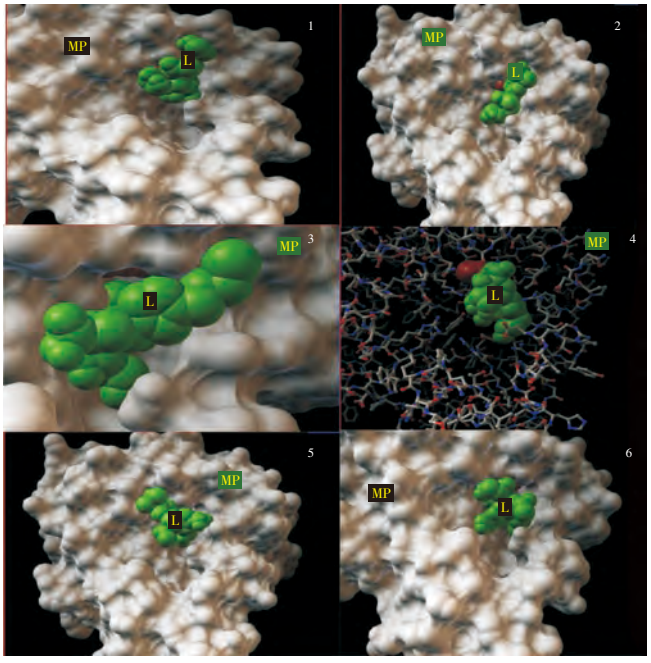

Figure 6. Various docking poses of zanamivir drug analogues with neuraminidase enzyme model. MP- Modeled Protein, L-Ligand.

4. Discussion

In the present study, the reliable protein structure of neuraminidase enzyme was generated and validated by various bioinformatics softwares and tools. Predicted protein homology model had 85.2% residues in favored and allowed region of Ramachandran plot along with overall quality factor of 96.899. Further, modeled x-ray crystallography structure of Neuraminidase enzyme with zanamivir ligand and its analogues using a docking of Autodock programme. Docking results showed that zanamivir drug CID_25209232 was found lowest binding energy −6.91 among the other zanamivir analogues. This study may be subject of experimental validation and clinical trial to establish these said analogues as more potent drug for the treatment of different viral diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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