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Research Article

In Silico prediction of 3D structure of RNA polymerase of H1N1 influenza virus and design its optimal inhibitors

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ABSTRACT:
Influenza is a contagious disease caused by the influenza virus having high fever, runny nose, sore throat, muscle pains, headache, coughing etc. as common symptoms. Influenza virus is associate myxovirus composed of two compound proteins Haemagglutinin and Neuraminidase. RNA polymerase-II (RNA pol-II) of the host is one of the foremost necessary enzymes responsible for transcription of mRNA from a DNA template strand. Viruses obstruct with the host RNA pol-II mechanism either by its ubiquitination and subsequent proteasomal degradation, or inhibit RNA pol-II phosphorylation thereby lowering its performance. Host transcription suppression eventually leads to shutoff of host proteins expression and provides viruses transcripts a combative edge for access to the cellular translation machinery. In this paper, to counteract the antiviral response; a strategy is used by preventing the expression of the host protein along with this the homology modeling and structure based virtual screening were performed to discover new ligand on the basis of 3D structure of target receptor.

Keywords: Influenza virus, RNA polymerase, Homology Modeling, Docking, Virtual Screening, Drug Design.
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[I] INTRODUCTION

Influenza, commonly known as the flu, is an infectious disease caused by influenza virus. This virus was not as equivalent as the 2009 H1N1 outbreak but it was similar insofar as it was associate contagious disease that had similarities to the swine flu virus. H1N1 flu virus inflicting some deaths among a younger population early in the spring of 2009.

It is a myxovirus that is composed of two conjugated protein, Haemagglutinin and Neuraminidase. Various studies have been conducted on Haemagglutinin (HA), Neuraminidase and RNA polymerase protein which is essential for virus to attach and infect cells, especially making RNA polymerase as a key target for developing neutralizing antibodies and vaccines to prevent the infection [1].

Influenza is an old disease caused by influenza virus strains A, B or C. Of these, A is the predominant strain that causes human disease, while B and C do not cause any severe complications in human.

By nature Influenza viruses are unstable and unpredictable, and have the unique capability of antigenic drift by mutation [2].

Influenza A (H1N1) virus is the subtype of influenza A virus that was the most common cause of human influenza (flu) in 2009, and is associated with the 1918 outbreak called as the Spanish Flu.

For this reason, depending on the type of H or N antigen express with metabolic synergy, these molecules are described as H1N1, H1N2 etc. Red blood cells form clump together due to presence of Haemagglutinin, which in return binds the virus to the infected cell. Neuraminidase is a kind of organic compound hydrolase accelerator that helps to move virus particle through the infected cell and assist in budding from the host cell.

RNA polymerase-II (RNA pol-II) of the host is one of the most important enzymes responsible for transcription of mRNA from a DNA template strand. Viruses interfere with host RNA pol-II function, its ubiquitination and subsequent proteasomal degradation or inhibit RNA pol-IIphosphorylation thereby lowering the efficiency. The proposed works suggest that certain antiviral compounds discovered function at least in part by targeting RNA polymerase.

The task of this study is to perform the Homology Modeling and Structure based virtual screening to discover and design the optimal inhibitors of H1N1 Influenza virus.

[II] MATERIALS AND METHODS

In order to predict and identify the RNA polymerase of H1N1 Influenza virus, design of its ligand and optimal inhibitor, the following steps is used that shown in fig. 1.

A) Identification of gene sequence of RNA polymerase of H1N1 Influenza virus

B) Prediction of structure of RNA polymerase of H1N1 Influenza virus using Modeller9.15 by Homology Modeling

C) Blind docking

D) Focused docking for identification of ligand

E) Refinement of docking result

F) proposed optimal inhibitor

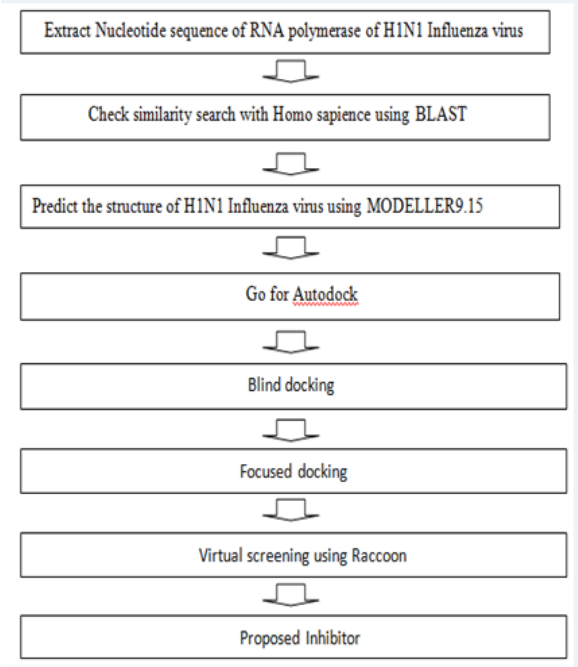


Fig.1: The proposed framework prediction and identification of RNA polymerase of H1N1 influenza virus, their ligand and Design of their optimal inhibitors.

2.1. Identification of gene sequence for RNA polymerase of H1N1 Influenza virus:

One of the most important steps for comparative modeling is the search for a structural template, which is represented by a protein with a previously determined three dimensional structure.

To guide the search for such template, one can rely on similar sequence identification algorithms, such as Basic Local Alignment Search Tool (BLAST [4]).

The sequence of RNA polymerase of H1N1 influenza virus was downloaded from NCBI nucleotide database (accession no. 134048665) and a sequence similarity search for the nucleotide against other such sequences was performed using the NCBI BLAST server [3].

2.2. Prediction of structure of RNA polymerase of H1N1 Influenza virus using Modeller9.15 by Homology modeling:

Homology modeling techniques is powerful approaches that can be utilized to construct the three-dimensional structure of RNA polymerase

of H1N1 Influenza virus. The three dimensional structure of the protein was chosen for subsequent homology modeling simulations.

The Modeller (Fig. 2), a python based software was used to build peptide backbone automatically on a template structure, with subsequent refinement by molecular dynamics and energy minimization.

For finding the best templates and calculating a good alignment, Modeller is necessary before submitting the job to the grid. The work of processing multiple, slightly models is divided among different slave clusters increasing the workflow efficiency [5].

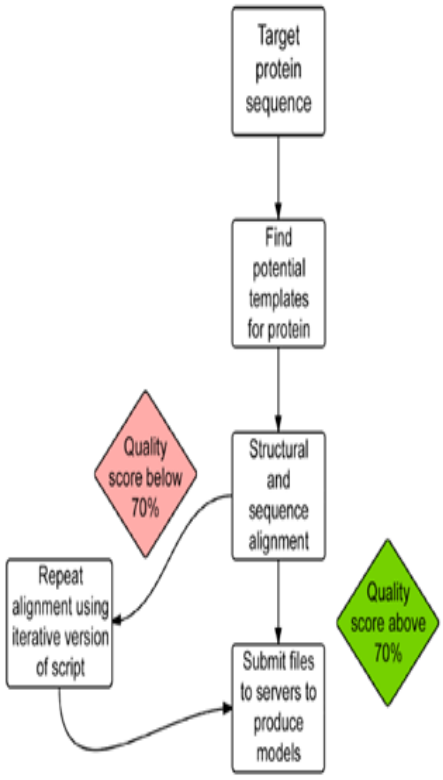


Fig. 2: The workflow to generate the files necessary for MODELLER [5]

2.3. Validation of structure:

After completing the homology modeling, validation of the structure is done by RAMPAGE online tool.

2.4. Blind docking:

To ensure that the ligand orientation and positions obtained from the arrival studies represents valid and affordable potential binding modes of the inhibitors, the docking ways and parameters used were valid [2]. In the present study, the method is tested on a bunch of drug-sized compounds and proteins with up to a thousand amino acid residues. Both proteins form complex structures and ligand- free proteins were used as targets. It is concluded that blind docking can be used for unbiased mapping of the binding patterns of drug candidate.

2.5. Focused docking for ligand identification:

Predicted binding sites (binding sites calculated from the protein structure alone) is used to judge here as a tool to focus the arrival of little molecule ligands into protein structures, simulating cases where the real binding sites were unknown. The focused approach used to identify the correct binding site more frequently than blind docking, produce more accurate docking poses for the ligand with less computational time.

2.6. Refinement of Docking Results:

After a set of candidate structures is generated by Modeller (or a similar algorithm), one must be able to evaluate the accuracy, native-like properties and overall quality of each structure in order to define the best ranked model [4].

2.7. Virtual Screening Using NCI Diversity Set:

NCI database was provided by National Center for High performance Computing. The database included 365602 compounds. Pharmacophore hypothesis is used to map and align the compounds from NCI database by the Catalyst compare/fit algorithm [3]. All the files necessary for virtual screening were prepared by software Raccoon. It is a graphical programme for AutoDock virtual screening (autodock.scripps.edu/resources/raccoon).

Raccoon can split multiple- molecule matter files, convert them into the AutoDock format, and filter them by using common criteria (e.g., Lipinskis rules, fragment-like rule of 3, and drug likeness). A validation check of the input files is performed at every step, which includes checking for the presence of non- customary atom sorts and

making certain that parameters, input filenames, and grid maps have a coherent format [2].

2.8. Proposed optimal inhibitor:

An inhibitor is a substance that delays, slows or prevents a chemical reaction. In case of this work, it can also be said that the optimal inhibitors are the most potent inhibitors that can halt the mechanism of formation of RNA by RNA polymerase enzyme by inhibiting or reducing its binding affinity.

2.8. Evaluation of Physicochemical Properties of Lead Compounds:

The top thirteen screened ligands are used for RNA polymerase of H1N1 Influenza virus are evaluated for important physicochemical properties such as calculated partition coefficient (ClogP), 2D-Polar surface area (2D PSA), molecular weight, hydrogen bond donor and acceptor sites etc. by using Marvin Sketch software.

2.9. Drug Score and Toxicity of Lead Molecules Prediction:

The top thirteen lead molecules were used for finding the toxicity and drug score by TEST (Toxicity estimation software tool) tool. There are different drug relevant properties are calculated by TEST tool like cLogP value, molecular weight, drug likeness, drug score and toxicities like mutagenicity, tumerigenicity, irritant effects and reproductive effect in lead molecules on the basis of functional group present in lead molecules structure.

[III] RESULTS

3.1. Identification of gene sequence for RNA polymerase of H1N1 Influenza virus:

Extract the nucleotide sequence of RNA polymerase of H1N1 Influenza virus (Through http://www.ncbi.nlm.nih.gov/).

3.2. Prediction of structure of RNA polymerase of H1N1 Influenza virus using Modeller9.15 by Homology modelling:

After getting the sequence of RNA polymerase of H1N1 Influenza virus, prediction of its 3D structure was done through Modeller9.15 tool. The predicted molecule was obtained by comparative modeling of target and the template sequence. The 3D structure of predicted protein is shown in Fig. 3.

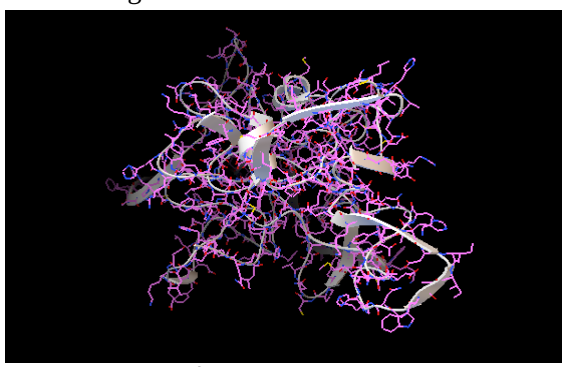


Fig. 3: Structure of predicted protein.

3.3. Validation of structure:

As after modeling of the structure is done, validation of the modeled structure is done by RAMPAGE online tool, which shows the accuracy of this structure is 81.7%.

3.4. Blind docking:

In blind docking whole structure of RNA polymerase is covered under 3D grid box for docking. The coordinates of grid box of RNA polymerase are given in Table 1 and the grid box used for blind docking is shown in Fig. 4.

Receptor	RNA Polymerase
x-D	48
y-D	76
z-D	50
Spacing(Angstrom)	1.000
X center	-7.27
Y center	-18.344
Z center	6.875

Table 1: The coordinates of grid box used in blind docking.

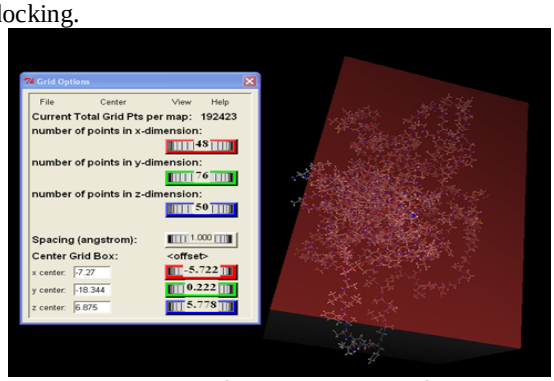


Fig. 4: Grid box used for blind docking of RNA polymerase covering whole molecule involve in binding of ligand.

3.5. Focused docking for ligand identification:

Obtained binding sites from blind docking is again covered by the grid box coordinates given in Table 2 and the grid box used for focus docking is shown in Fig 5.

Receptor	RNA Polymerase
x-D	48
y-D	50
z-D	50
Spacing(Angstrom)	0.286
X center	-3.82
Y center	0.138
Z center	12.155

Table 2: The coordinates of grid box used in focus docking.

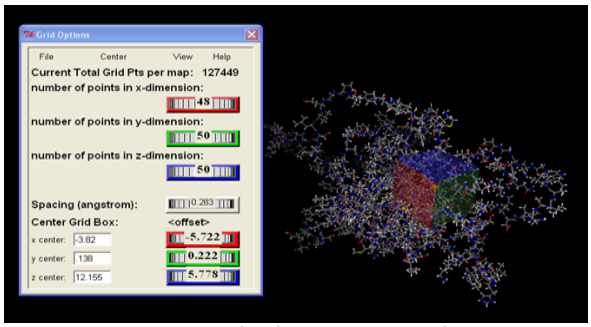
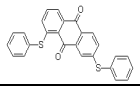
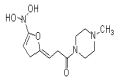
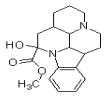
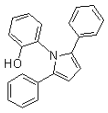
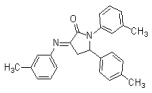
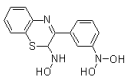
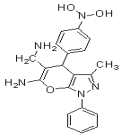
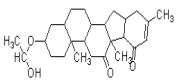


Fig. 5: Grid box used for focus docking of RNA polymerase of H1N1 covering whole molecule involve in binding of ligand.

3.6. The proposed top lead molecules:

After performing the virtual screening, thirteen lead molecules were obtained that will be further used for the prediction of most potent inhibitors shown in Table 3.

S.No.	ZINC ID	Structure	Binding energy
1.	ZINC05124931		-9.01
2.	ZINC01689932		-8.16
3.	ZINC00608128		-8.13
4.	ZINC01674410		-8.01
5.	ZINC13597368		-7.99
6.	ZINC06576501		-7.97
7.	ZINC00728291		-7.80
8.	ZINC12671904		-7.75

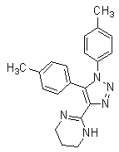
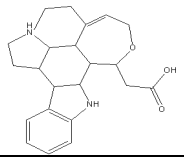
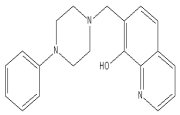
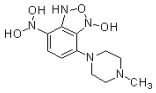
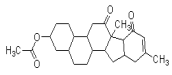
9.	ZINC01629421		-7.71
10.	ZINC01711028		-7.69
11.	ZINC01707109		-7.67
12.	ZINC05180959		-7.65
13.	ZINC12671898		-7.62

Table 3: Thirteen screened lead molecules with their respective binding energies.

After the screening was over, a script summarize results.py from Scripps Research Institute was utilized to sort the binding energies of the docked ligand and select the best hits.

The results of all dockings were evaluated based on hydrophobic and polar interactions between ligand and protein active site residues and the binding energy which must come in the empirical range -5 to -15 kcal/mol. Binding affinity is calculated from the formula: Ki = e[(G/(RT)] where G=change in free energy upon binding, R=gas constant and T=temperature.

3.7. ADME and Toxicity Profiling:

The toxicity and drug scores of all thirteen screened lead molecules predicted by using the OSIRIS online tool for RNA polymerase of H1N1 and select those lead molecules which show no toxicity. Five lead molecules are found to have no toxicity are shown in Table 4.

ID	Mutagenic	Tumorigenic	Irritant	Reproductive effect	Drug score
ZINC05124931	No	No	Low	No	0.08
ZINC01689932	No	No	No	Low	0.57
ZINC00608128	No	No	No	No	0.81
ZINC01674410	No	No	No	No	0.27
ZINC13597368	No	No	No	No	0.49

Table 4: Toxicity and drug score prediction of five lead molecules.

3.8. Physicochemical Properties of Lead Molecules:

In physiochemical properties, the Lipinski's rule of five criteria of five lead molecules has been evaluated in Table 5.

Lead ID	ClogP	PSA	Mol.Wt	HBD	HBA
ZINC05124931	6.68	84.74	424	0	2
ZINC01689932	-0.19	76.48	269	2	6
ZINC00608128	2.1	53.01	328	1	4
ZINC01674410	5.0	25.16	311	1	1
ZINC13597368	4.86	32.67	368	0	2

Table 5: Physiochemical Properties of proposed lead molecules.

[IV] CONCLUSION:

On the basis of predicted values, it can be concluded that these five lead molecules ZINC05124931, ZINC01689932, ZINC00608128, ZINC01674410, ZINC13597368, can act as potential inhibitors against the RNA polymerase of H1N1 Influenza virus as all the five lead molecules are having a good binding energy and having the capability to stop the mechanism of Influenza virus. All the five lead molecules are obtained by molecular docking simulation based virtual screening technique. These five lead molecules can inhibit growth of Influenza virus species by targeting its RNA polymerase enzyme, and this RNA polymerase sequence has not been reported in human beings. Therefore, these designed lead molecules are not likely to interact with mammalian mRNA. These predicted lead molecules can be used for pharmacophore design by Biomedical and pharmaceutical researchers.

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