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DRUG REPURPOSING: IN SILICO BASED STUDY ON REPURPOSING PHYTOCHEMICALS ON INFLUENZA

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Abstract

By and by, the world is in a battle with the novel Influenza and with no prompt medicines accessible the scourge brought about by the disease is expanding step by step. A ton of researchers are continuing for the potential medication up-and-comer that could help the medical care framework in this battle. In recent years, studies of phytoconstituents have gradually increased worldwide because the natural sources and variety of such plants allow them to complement modern pharmacological approaches. As computer technology has developed, in silico approaches such as virtual screening and network analysis have been widely utilized in efforts to elucidate the pharmacological basis of the functions of phytoconstituents. We present a docking-based screening using a quantum mechanical scoring of a library built from approved drugs and compounds that Curcumin, Gallic Acid, Phenethyl Isothiocyanate, Piperine, with Proteins Neuraminidase, Hemagglutinin, M1 and M2 Matrix proteins having PDB IDs 3BEQ, 4WE8, 5V6G, 6BKL respectively, could display antiviral activity against Influenza. Clearly, these compounds should be further evaluated in experimental assays and clinical trials to confirm their actual activity against the disease. We hope that these findings may contribute to the rational drug design against Influenza.

Keywords: PDB IDs 3BEQ, 4WE8, 5V6G, 6BKL

Introduction

Influenza malady has become a significant public issue over the globe since the beginning. The World Health Organization (WHO) estimates that worldwide the annual influenza epidemics result in about 3-5 million cases of severe illness and about 250,000 to 500,000 deaths.As of twelfth of November 2020, more than 10.79 million cases have been accounted for in 210 nations and domains (World-o-meter, 2020). Due to this high global mortality of influenza infections it represents a major and recurrent public health threat with high economic burden. It influences individuals worldwide and there is no immunization yet for these infections as the virus has mutated in the recent years and also shows drug resistance against some antiviral medications which is viewed as a major threat to worldwide general wellbeing. There is a pressing need to create an intense enemy of this disease, specialists for the avoidance of the flare-up and stop viral contaminations. Repurposing of realized phytoconstituents is by all accounts an exceptionally productive path as it is a cost and time-efficient approach for new indications, so as to create strong medications to battle diseases in this brief timeframe. As of late, various endeavors have been made to plan novel inhibitors or utilize drug repurposing ways to deal with recognition hostile to medications.

Targeted proteins for Influenza :

PDB ID -3BEQ

Neuraminidase of A/Brevig Mission/1/1918 H1N1 strain

3BEQ is a 2 chain structure with a sequence from influenza virus A and a resolution of 1.64A. Influenza virus neuraminidase (NA) plays a crucial role in facilitating the spread of newly synthesized virus in the host and is an important target for controlling disease progression. Neuraminidase catalyzes the removal of terminal sialic acid residues from viral and cellular glycoconjugates to facilitate virus release. Additionally, it helps the virus to spread through the circulation by further removing sialic acids from the cell surface. These cleavages prevent self-aggregation and ensure the efficient spread of the progeny virus from cell to cell. An additional cavity adjacent to the substrate-binding site is observed in N1. This cavity arises from an open conformation of the 150 loop (Gly147 to Asp151) and appears to be conserved among group 1 NAs (N1, N4, N5, and N8).

PDB ID - 4WE8

The crystal structure of hemagglutinin of influenza virus A/Victoria/361/2011

4WE8 is a single chain structure with a sequence from influenza virus A with a resolution of 2.10A. Since the major antigenic sites of the HA overlap into the receptor binding site (RBS) of the molecule, the virus constantly struggles to effectively adapt to host immune responses, without compromising its functionality. It has been structurally assessed the evolution of the A(H3N2) virus HA RBS, using an established recombinant expression system. The results uncovered that while its receptor-binding site has evolved from one that can bind a broad range of human receptor analogs to one with a more restricted binding profile for longer glycans, the virus continues to circulate and transmit efficiently among humans.

PDB ID -5V6G

Crystal structure of Influenza A virus Matrix Protein M1(NLS-88R)

5V6G is a 4 chain structure with a sequence from influenza virus A with a resolution of 2.00A.The M gene segment of influenza A virus has been shown to be a contributing factor to the high growth phenotype. However, it remains largely unknown why matrix protein 1 (M1), the major structural protein encoded by M gene, exhibits pH-dependent conformational changes during virus replication. Understanding the mechanisms underlying efficient virus replication can help to develop strategies not only to combat influenza infections but also to improve vaccine supplies.The results suggested that maintaining M1 pH-dependent conformational flexibility is critical for efficient virus replication, and position 88 is a key residue controlling M1 pH-dependent conformational changes. The findings provided insights into developing M1-based antiviral agents.

PDB ID -6BKL

Influenza A M2 transmembrane domain bound to rimantadine

6BKL is a 8 chain structure with a sequence from influenza virus A with a resolution of 2.00A.The M2 proton channel of influenza A is the target of the antiviral drugs amantadine and rimantadine. Structural studies of drug binding to the channel using X-ray crystallography have been limited because of the challenging nature of the target, with the one previously solved crystal structure limited to 3.5 Å resolution.These X-ray crystal structures of the M2 proton channel with bound inhibitors reveal that ammonium groups bind to water-lined sites that are hypothesized to stabilize transient hydronium ions formed in the proton-conduction mechanism.

Procedure:

1. Ligand Screening

For the initial Ligand screening purposes, a web-based tool named SwissADME (https ://www.swiss adme.ch/) was used to eliminate a few compounds according to Lipinski's rule of five parameters. For a compound to qualify as ligand it should Have < 500 Da molecular weight, a high lipophilicity i.e. value of Log P being less than 5, hydrogen bond acceptors being less than 10 and H-bond donors less than 5. Any compound with more than 2 violations was ruled out for further study (Lipinski2004).

2. Protein Preparation and Active site Determination.

Required protein in pdb format was downloaded from the website **rcsb.org**, commonly known as the **Protein Data Bank**. 3D conformers of the ligand were downloaded from PubChem.

Using **PyMOL (Version 2.4.1)** software water molecules as well as native ligands from the protein were removed, defined as cleaning/purification of the protein for further application. **Using a web server called Deep Site** Active Pockets of the proteins were calculated. The results calculated by the web server were in the form of different ids, centers and scores.

Scoring in deep site was using neural networking based on following instructions using DCNN architecture. https://academic.oup.com/bioinformatics/article/33/19/3036/3859178 Center values for the grid were selected keeping score greater than 0.98.

UCSF Chimera (Version 1.14) was used to prepare the receptor using DockPrep function. **Dock Prep** prepared structures for Docking using these functions:

- deleting water molecules
- repairing truncated sidechains
- adding hydrogens
- assigning partial charges
- writing files in Mol2 format

1. In silico Docking Using Auto dock Vina

Auto dock Vina (Version 1.1.2) along with UCSF Chimera (Version 1.14) was used for molecular **Docking Studies**. Center values and size of the grid of different scores were used from **DEEPSITE** calculations done above.

Following Parameters were set in auto dock vina.

Receptor options –

- **Add hydrogens in Chimera** (**true**/false) – whether to add hydrogens in Chimera before calling the script. The receptor prep script will check for hydrogens and add them if they are missing. AutoDock Vina needs the polar

(potentially H-bonding) hydrogens to identify atom types for scoring purposes.

- **Merge charges and remove non-polar hydrogens** (**true**/false) – note AutoDock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the processed receptor
- **Merge charges and remove lone pairs** (**true**/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the processed receptor (and there may not have been any lone pairs to start with)
- **Ignore waters** (**true**/false)
- **Ignore chains of non-standard residues** (**true**/false) – ignore chains composed entirely of residues other than the 20 standard amino acids.
- **Ignore all non-standard residues** (true/**false**) – ignore all residues other than the 20 standard amino acids.

For Ligands

- **Merge charges and remove non-polar hydrogens** (**true**/false) – note Auto Dock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the ligand output files
- **Merge charges and remove lone pairs** (**true**/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the ligand output files (and there may not have been any lone pairs to start with)

Docking parameters

- **Number of binding modes** (1-10, **10**) – maximum number of binding modes to generate
- **Exhaustiveness of search** (1-8, **8**) – thoroughness of search, roughly proportional to time
- **Maximum energy difference (kcal/mol)** (1-3,**3**) – maximum score range; binding modes with scores not within this range of the best score will be discarded.

The docking results were calculated by Auto dock vina using it's Scoring function and results were displayed in the form of Scores and RMSD values. Docking results with the highest value score accompanied by negative sign and least RMSD values were chosen for further studies.

4. Residue Analysis

PyMOL was used for visualization of interactions of the docked structure at the ligand sites. **Discovery Studio 2020** was used to study the ligand interactions and total number of residues. It was also used to plot the 2D structure of the interactions and residues.

5. Statistical Analysis: Descriptive, estimation and Hypothesis testing with confidence interval of 95% was applied to data using formula 1 given below.

$$CI = \bar{x} \pm z\frac{s}{\sqrt{n}}$$

CI = confidence interval
$\bar{x}$ = sample mean
z = confidence level value
s = sample standard deviation
n = sample size

Formula 1 used for calculation of confidence interval

Results and Discussion:

Molecular Docking:

The docking result was obtained from Auto dock vina in the form of Dock score for all the four proteins docked with above mentioned ligands.

Influenza Protein Docking Results:

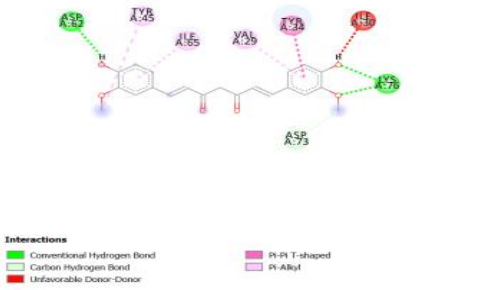
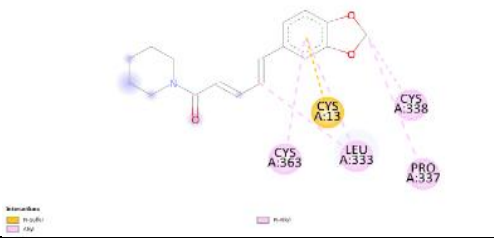
PDB-ID 3BEQ

For 3BEQ, four active sites were selected out of which the 2nd active site was selected with a Deep site score of 0.988. The selection was made on the basis of the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 1 and Table 2 shows the post statistical docking scores with Ligand Protein Interactions.

Table 1:

Ligands	Dock score
Curcumin	-7.3
Gallic Acid	-5.8
Phenethyl Isothiocyanate	-4.6
Piperine	-6.9

Table 2:

Ligands	Dock score	Interactions
Curcumin	-7.3	
Piperine	-6.9	

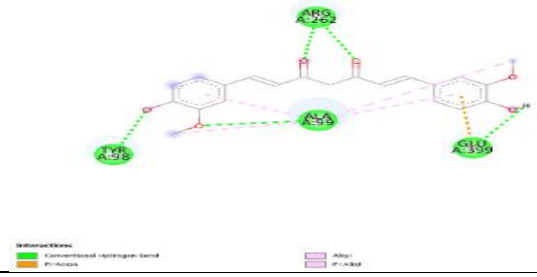
PDB-ID 4WE8

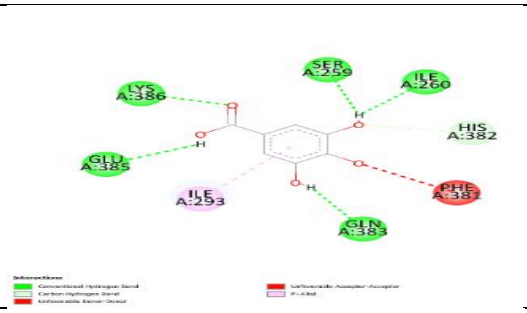
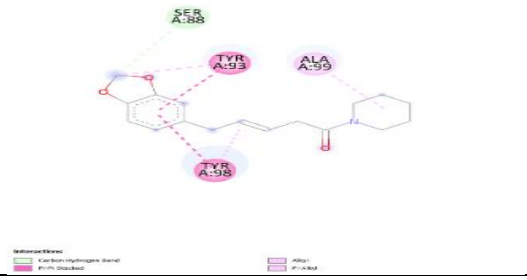
For 4WE8 , out of the three active sites the 1st active site was selected with a Deep site score of 0.98 (rounded up to 2nd decimal place). The selection was made on the basis of the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 3 and Table 4 shows the post statistical docking scores with Ligand Protein Interactions.

Table 3:

Ligands	Dock score
Curcumin	-7.4
Gallic Acid	-6.5
Phenethyl Isothiocyanate	-4.8
Piperine	-7

Table 4:

Ligands	Dock score	Interactions
Curcumin	-7.4	

Gallic Acid	-6.5	
Piperine	-7.0	

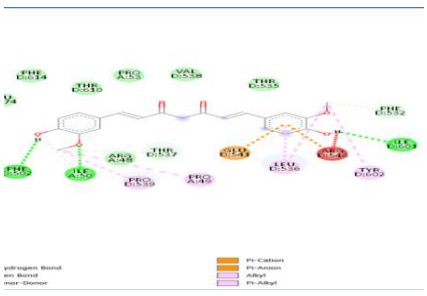
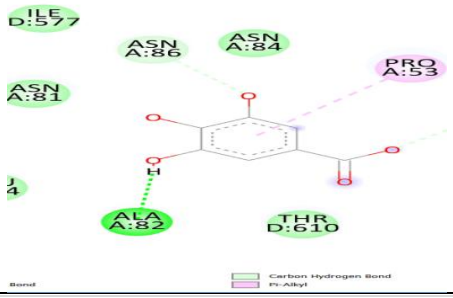
PDB-ID 5V6G

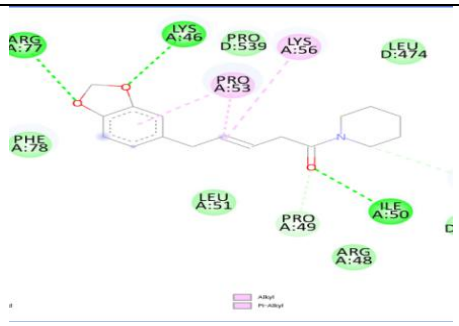
For 5V6G, five active sites were selected out of which 1st active site was selected with a Deep site score of 0.995, the selection was made on the basis of highest binding energy of ligand-receptor. The docking results before statistics are shown in Table 5 and Table 6 shows the post statistical docking scores with Ligand Protein Interactions.

Table 5:

Ligands	Dock score
Curcumin	-8.1
Gallic Acid	-5.9
Phenethyl Isothiocyanate	-5.3
Piperine	-7.8

Table 6:

Ligands	Dock score	Interactions
Curcumin	-8.1	
Gallic acid	-5.9	

Piperine	-7.8	

PDB-ID 6BKL

For 6BKL, three active sites were selected out which 1st active site was selected with Deep site score of 0.999, the selection was made on the basis of highest binding energy of ligand-receptor, docking results before statistics are shown in Table 6 and Table 7 shows the post statistical docking scores with Ligand Protein Interactions.

Table 7:

Ligands	Dock score
Curcumin	-8.1
Gallic Acid	-6.4
Phenethyl Isothiocyanate	-5.7
Piperine	-6.1

Table 8:

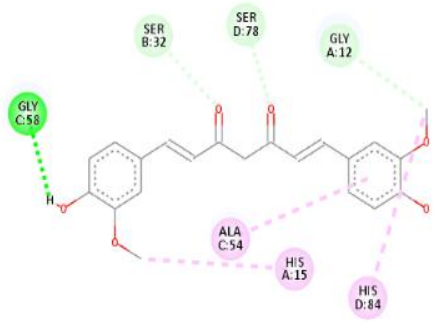
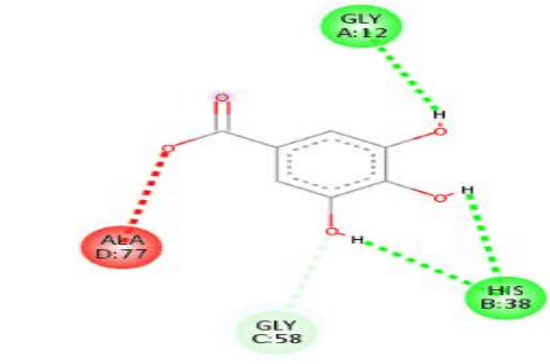
Ligands	Dock score	Interactions
Curcumin	-8.1	
Gallic acid	-6.4	

Table 9: summarizes the results showing ligands and their interacted proteins that were considered in the study for the targeted diseases.

Ligand	Proteins Interacted	Target Disease(s)
-	-	-
Curcumin	3BEQ, 4WE8, 5V6G, 6BKL	Influenza
Gallic acid	4WE8, 5V6G, 6BKL	Influenza
Phenyl isothiocyanate	None	Influenza
Piperine	3BEQ, 4WE8, 5V6G	Influenza

Conclusion:

All four ligands were studied using bioavailability radar. Our results proposed Curcumin, Gallic acid and Piperine showed best docking results however Phenethyl Isothiocyanate didn't show standardized results with any of the proteins included in the study. Influenza protein with PDB ID 3BEQ showed standardized results for Curcumin and Piperine. Other influenza proteins included in study with PDB ID 4WE8 showed best docking results with Curcumin, Gallic acid, and Piperine. Another Influenza protein 5V6G produces standardized results in this study for Curcumin, Gallic acid and Piperine, with Curcumin showing a significantly considerable Dock score. Lastly protein 6BKL showed the best dock score for Curcumin and Gallic acid with Curcumin having a considerably good Dock score. To find the effectiveness and to propose the exact mechanism in-vitro studies can be encouraged on Curcumin, Gallic acid and Piperine for targeting various proteins in the Influenza that are discussed above to understand the mechanism and a potential cure for Influenza.

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