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In Silico Phytochemical Screening for Hantavirus

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Abstract

Several rodent species are carriers of hantavirus which are present throughout the world. Hantavirus was a group of unknown class of viruses which was first seen in the United States of America in 1993 as an acute respiratory disease now termed hantavirus pulmonary syndrome (HPS). Earlier HPS hantavirus were known as the agents of hemorrhagic fever with renal syndrome which was seen in eastern hemisphere of the world .A lot of research is going around in and around hantavirus Their ubiquity and potential for causing severe human illness make these viruses an important public health concern.We present a docking based screening using a quantum mechanical scoring of a library built from approved drugs and compound that limonin ,curcumin, luteolin, rutin, Baicalin, Quercetin, Resveratrol, Kaempferol, Naringenin, Atropine, 4 Hydroxy Coumarin, Colchicine, Thymoquinone, Glabridin ,Hypericin, Lycorine with proteins having PDB id's 5E04 and 5E05 could display that the phytochemicals can act against Hantavirus. We hope that our findings may contribute to rational drug design against Hantavirus.

Introduction

Hantavirus has become a serious issue all over the world for some time. As of till now about 2 million people have been affected around the world. Till today no immunizations have been found for these infections, which remain a threat to the world. There is an immediate need to find a solution that is both economic and ecological. As of late, various endeavors were made to plan to novel inhibitors or utilize drug repurposing.

In the past century, two major outbreaks of diseases led to the discovery of Hantavirus in the old and the new worlds. The first outbreak occurred during the Korean war (1950-1953) and the second outbreak occurred in the four corner regions of the United States in1993 and was initially referred to as Four Corners disease which is now called Hantavirus Pulmonary Syndrome (HPS). In South America fatality rates of HPS can reach up to 35%–50%. The transmission of pathogenic Hantaviruses to humans occurs mainly via inhalation of aerosolized excreta from infected rodents. Since there are no vaccines currently available nor specific therapeutic treatments, prevention of Hantavirus infection. There is a pressing need to create an intense enemy of these diseases, to avoid the viral contamination. So here certain phytochemical Drugs were used against Hantavirus by means of Bioinformatics Applications.

Procedure

1. Ligand screening

For the Initial Ligand Screening purposes, a web based tool named SwissADME (http://www.swissadme.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 <500Da 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.

2. Protein Preparation and Active site Determination.

Required protein in pdb format was downloaded from the website **rcsb.org** commonly known as **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 proteins 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 (Version1.14) was used to prepare the receptor using the **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 the auto dock vina.

Receptor option-

- **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 atoms 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 the 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 for 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 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 AutoDockVina 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 its 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 95% was applied to data using formula1 given below:

Formula 1 used for calculation of confidence interval

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

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

Results and Discussion:

Molecular Docking

The docking result was obtained from AutoDock Vina in the form of Dock Score for all the three proteins docked with above mentioned ligands.

Hantavirus Protein Docking Result

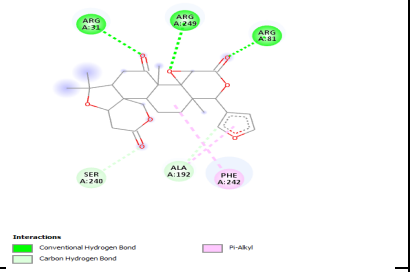
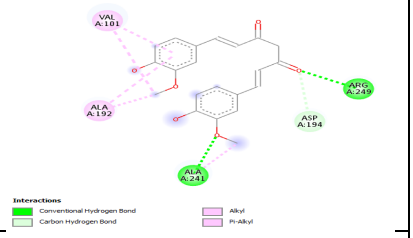
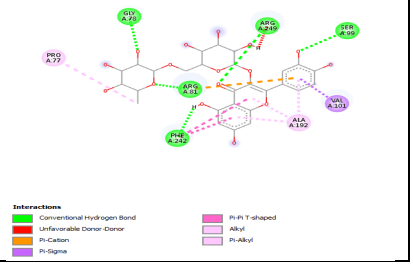
PDB-ID 5E04 for 5E04, Three active sites were selected out of which 0th active site was selected with a Deep site score of 0.986. Table 1 the selection was made on the basis of the highest binding energy of the ligand- receptor. The docking results before statistics are shown in Table1 and Table 2 shows the post-statistical docking scores with Ligand Protein Interactions.

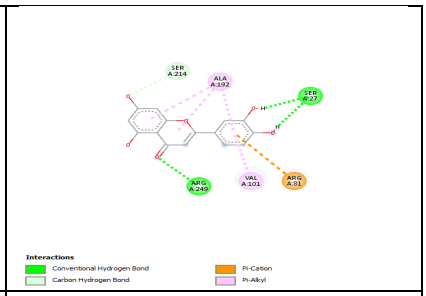
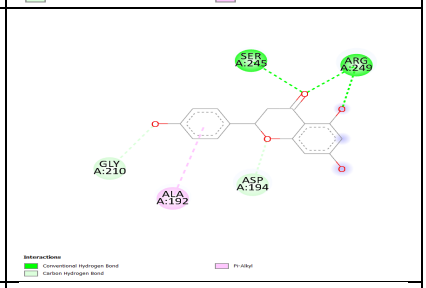
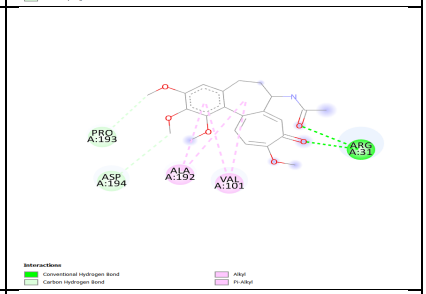
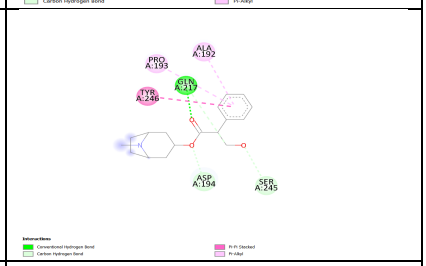
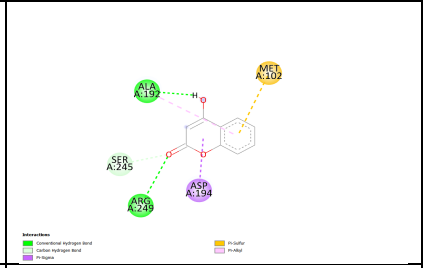
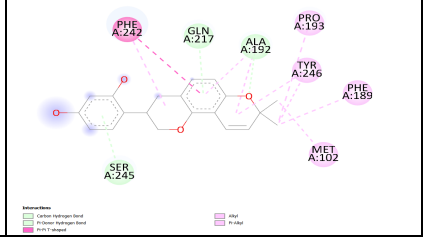
Sites	score	Selected/Not Selected
0	0.986	
1	0.765	
2	0.753	
3	0.578	

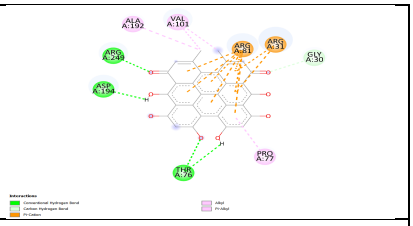
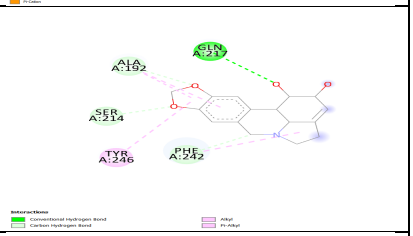
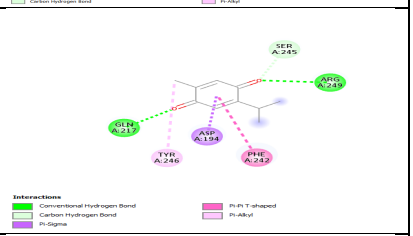
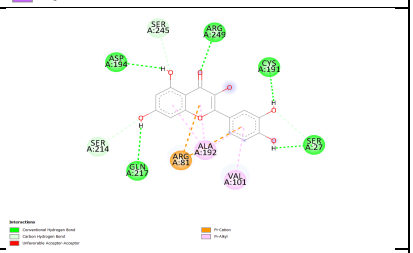
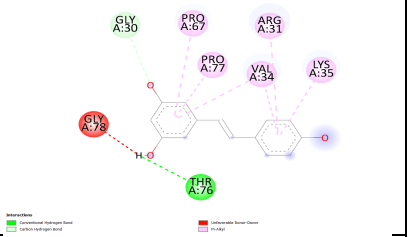
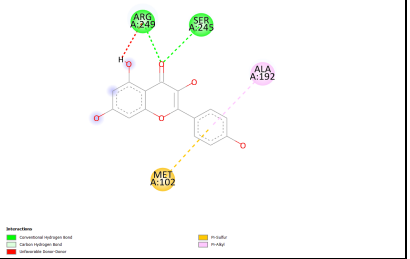
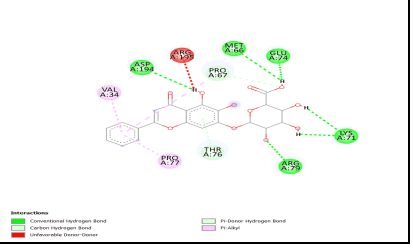
Ligands	Dockscore
Limonin	-9

Curcumin	-7.3
Rutin	-9.6
Luteolin	-8.3
Naringenin	-7.9
Atropine	-6.4
4 Hydroxycoumarins	-6.9
Colchicine	-6.6
Glabridin	-8.5
Hypericin	-8.7
Lycorin	-8.3
Thymoquinone	-6.5
Quercetin	-8.2
Reservatol	-5.9
Kaempferol	-8.1
Baicalin	-8.1

5E04

Ligands	**Dockscore**	**Interactions**
Limonin	-9	
Curcumin	-7.3	
Rutin	-9.6	

Luteolin	-8.3	
Naringenin	-7.9	
Atropine	-6.4	
4 Hydroxycoumarins	-6.9	
Colchicine	-6.6	
Glabridin	-8.5	

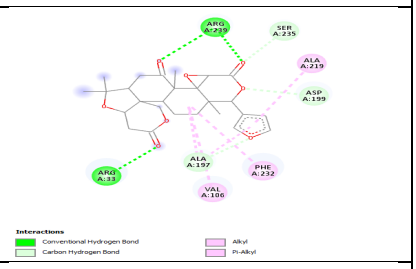
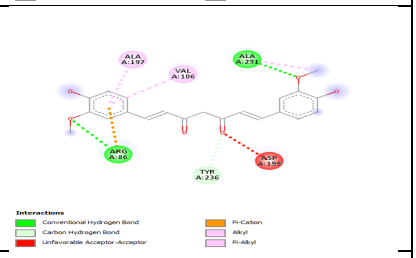
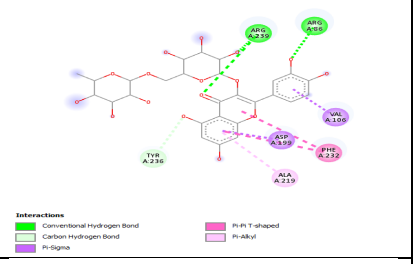
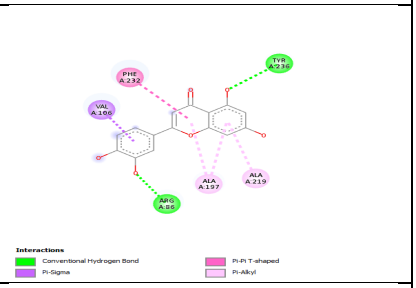
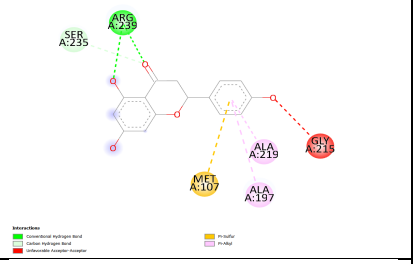
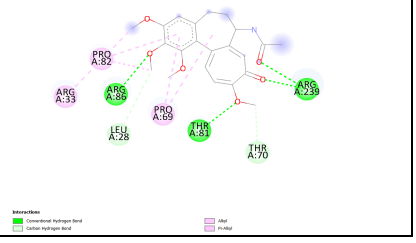
Hypericin	-8.7	
Lycorin	-8.3	
Thymoquinone	-6.5	
Quercetin	-8.2	
Reservatol	-5.9	
Kaempferol	-8.1	
Baicalin	-8.1	

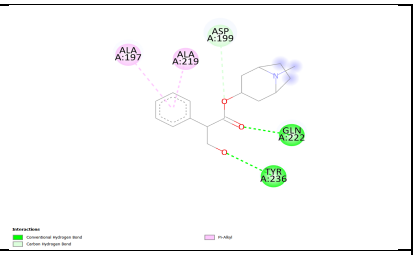
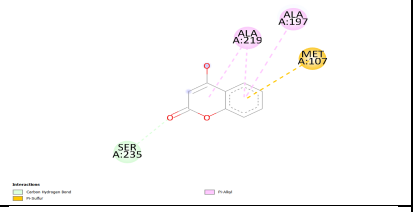
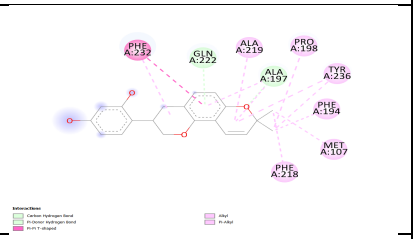
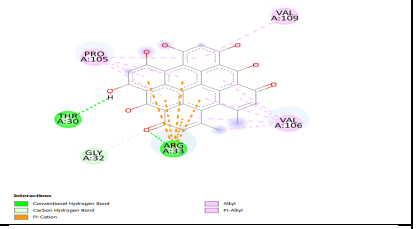
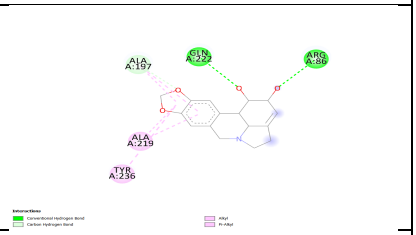
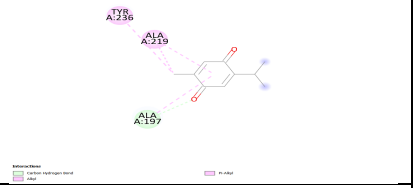
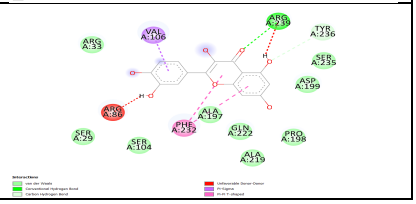
PDB-ID 5E05 for 5E05, Three active sites were selected out of which 0th active site was selected with a Deep site score of 0.988. Table 1 the selection was made on the basis of the highest binding energy of the ligand- receptor. The docking results before statistics are shown in Table1 and Table 2 shows the post-statistical docking scores with Ligand Protein Interactions.

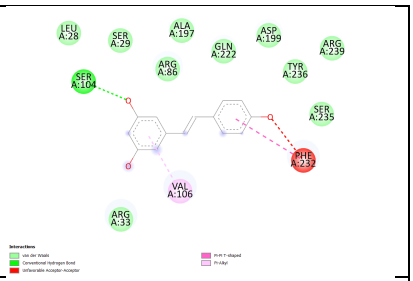
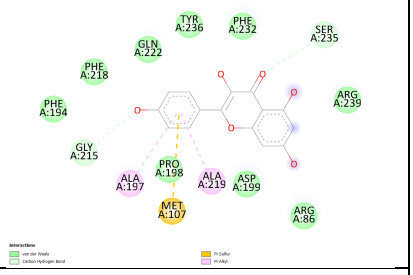
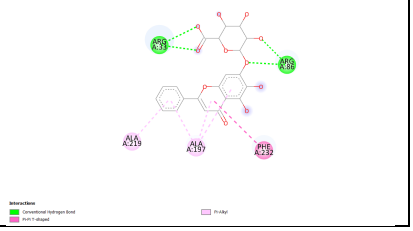
Sites	**Score**	**Selected/Not selected**
0	0.988	
1	0.842	
2	0.816	
3	0.464	

Ligands	**Dockscore**
Limonin	-10
Curcumin	-6.8
Rutin	-7.9
Luteolin	-7.8
Naringenin	-7.8
Atropine	-7
4 Hydroxycoumarins	-6.8
Colchicine	-6.2
Glabridin	-8.2
Hypericin	-9
Lycorin	-8.2
Thymoquinone	-5.8
Quercetin	-7.4
Reservatol	-6.7
Kaempferol	-7.7
Baicalin	-9.2

5E05

Ligands	Dockscore	Interactions
Limonin	-10	
Curcumin	-6.8	
Rutin	-7.9	
Luteolin	-7.8	
Naringenin	-7.8	
Atropine	-7	

4 Hydroxycoumarins	-6.8	
Colchicine	-6.2	
Glabridin	-8.2	
Hypericin	-9	
Lycorin	-8.2	
Thymoquinone	-5.8	
Quercetin	-7.4	

Reservatol	-6.7	
Kaempferol	-7.7	
Baicalin	-9.2	

Ligands	**Acceptance**	
	PDB ID- 5E04	PDB ID-5E05
Limonin	Accepted	Accepted
Curcumin	Accepted	Strongly Accepted
Rutin	Accepted	Accepted
Luteolin	Accepted	Accepted
Naringenin	Accepted	Accepted
Atropine	Strongly Accepted	Strongly Accepted
4 Hydroxycoumarins	Strongly Accepted	Strongly Accepted
Colchicine	Strongly Accepted	Strongly Accepted
Glabridin	Accepted	Accepted
Hypericin	Accepted	Accepted
Lycorin	Accepted	Accepted
Thymoquinone	Strongly Accepted	Strongly Accepted
Quercetin	Accepted	Accepted
Reservatol	Strongly Accepted	Strongly Accepted
Kaempferol	Accepted	Accepted
Baicalin	Accepted	Accepted

Conclusion:

All the Sixteen Ligands were studied using Bioavailability Radar. Our Result Proposed Atropine, 4 Hydroxycoumarins, Colchicine, Thymoquinone and Reservatol showed the best docking result for Hantavirus Proteins with PDB Ids 5E04. While, Curcumin, 4Hydroxycoumarin, Colchicine and Thymoquinone showed the best docking result for Hantavirus proteins with PDB IDs 5E05.To find the effectiveness and to propose the exact mechanism In-Vitro Studies can be encouraged on Curcumin, Thymoquinone, 4 Hydroxycoumarin, Colchicine, Atropine and Rervatol targeting respective diseases that are discussed above to understand the mechanism and potential cure for HantaVirus Disease.

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