



Targeting Human Bocavirus VP-2 Protein by Combining Pharmacophore, Molecular Docking and ADME-Tox Approaches to Identify Best-Optimized Novel Drug Candidate

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Abstract

Human bocavirus (HBoV) belongs to a parvoviridae family. It has a single-stranded DNA etiologic agent and is known to cause lower respiratory tract infections in small and young children all over the world. Currently, there is no treatment or vaccination. The aim of the study was to describe the Human bocavirus VP 2 protein structure and discover the possible drug candidates that can be further used as possible drugs for dealing with infections caused by Human bocavirus. Molecular docking was performed using AutoDock. The molecule presenting the finest binding energies (Ribavirin -7.72, Amantadine -6.64, and Anabasine -6.61) were finally selected as good drug candidates. These possible drug candidates were further used to produce a pharmacophore model for antiviral ligands using LigandScout. Later the pharmacophore based virtual screening was done against the pre-selected ligand library from zinc database, as a result the finest hits or leads for pharmacophore molecules were obtained. These hits or leads are used as ligands and subjected to molecular docking. The finest molecule was found to be ZINC41029220 -8.42 (Binding Energy), after obtaining the best hits ADME-Tox analysis performed. These molecules were used as inhibitors for Human bocavirus VP 2 protein.

Keyword: *Human Bocavirus VP2 protein, molecular docking, pharmacophore, LigandScout, SiteHound-web, antivirals, binding energies, ADME-Tox analysis*

Introduction:

Human bocavirus 1 is from the family parvoviridae and genus bocavirus. This virus is related to severe acute respiratory infection. Noticed largely in respiratory secretions of the offspring with lower respiratory tract infections. It is spotted through the PCR amplification of sample form the upper respiratory tract. Human bocavirus affects the human respiratory tract and it replicates in the epithelial cells [1]. The pathophysiology of human bocavirus remains unidentified owing to doubt. It was found that virus protein 2 (VP2) displayed chief immunoreactivity. Consequently have taken into thought to learn the organization of VP2 protein.

Protein interaction depends on the very complex intermolecular interaction with different types of ligands, proteins or other surfaces[2]. In receptor, there are certain favorable sites (known as pocket) having high binding affinity with the ligand known as pocket. [3]. The binding energies of the molecules utilized to recognize interactions amongst the target and the ligand molecule. To find the interaction between receptor and ligand, AutoDock tool [4] was used. It is widely non-commercial software that is mostly used for molecular docking as well as virtual screening [5]. The binding energies were found using pharmacophore [6]. Pharmacophore is a significant tool in drug discovery [7]. It is defined by ehrlich in year 1909 as a molecular conformation that carries (phoros) the vital aspect liable for drug (pharmacon) biological activity.

Antiviral drugs are used in handling of viral infections [8]. There are about 15 antivirals used in this studies some of the antivirals used were

Acyclovir: Acyclovir sodium is crystal-like white powder having molecular formula $C_8H_{10}N_5NaO_3$ and M.W-247.19. It is used in dealing of infections formed by herpes simplex (HSV-1 as well as HSV-2) virus, herpes zoster (shingles) and varicella (chickenpox) [9]. Acyclovir used to study effect on canine parvovirus (CPV2) infection in the puppies.

It was effective in stopping of CPV2 replication in puppies.

Rimantadine: Rimantadine is from amantadine class of drugs, which have antiviral properties against influenza A viruses. It prevents viral replication and inhibits ion channel membrane protein. They are not effective against influenza B viruses [10]. Rimantadine used in the treatment and prophylaxis of influenza A strains other than H5N1

Ribavirin: For hepatitis C virus ribavirin is a nucleoside purine analog that is used in treatment but incompletely understood. Ribavirin is very important for hepatitis C therapy. It is used in presence of interferon ribavirin reduces relapse rates and improves sustained virological responses [11].

Amantadine: Amantadine is antiviral, inhibits influenza virus replication and is important treatment of human Parkinson's disease. The recent studies have been made on amantadine to study its clinical applications, in numerous dissimilar virus infections e.g. herpes, herpes zoster neuralgia and form neuroleptic extrapyramidal movement disease to depression and cocaine dependence [12].

Anabasine: Anabasine hydrochloride is also a strong as well as important nicotinic acetylcholine receptor agonist. The chemical name of Anabasine is 3-Piperidin-2-ylpyridine hydrochloride with M.W-198.73. Its molecular formula is $C_{10}H_{14}N_2.HCl$. It starts cholinergic nicotinic and muscarinic receptors and stimulates Ca-dependent catecholamine release from rat adrenomedullary cells. Anabasine has trans-3-hydroxynicotinine, 5-hydroxycotinine, and cotinine-N-oxide. Norcotinine, anabasine, and nicotine are compounds with structural resemblance and may yield mutual product ions Nicotine as well as anabasine have common ion change while trans-3-hydroxycotinine and cotinine-N-oxide have a mutual ion transition [13].

Materials and Methods:

Target Selection: Human bocavirus belongs to parvoviridae family. The size of animal *viruses* is approximately 20 nm, non-enveloped virus through single stranded negative sense DNA genome. The FASTA sequence of Human bocavirus 1 capsid protein 2 (VP2) was acquired from NCBI database [14].

Protein structure prediction:

Protein structure prediction of Human Bocavirus VP2 protein was performed with the help of Modeller tool. It is an important tool for protein structure prediction. It helps us to find the 3D

structure of protein [15]. Thus, it helps us to study the properties of the protein structure. After using the Max Planck Modeller (<https://toolkit.tuebingen.mpg.de/modeller>) [15] we got the protein structure given into fig. 1.

Identification of Active Sites:

SiteHound-web tool (scbx.mssm.edu/sitehound/) [16] is used to identify binding sites of Human Bocavirus VP2 protein. It runs on the SITEHOUND algorithm [16]. This algorithm works by finding the potential ligand binding sites by identifying regions characterized by favorable non-bonded interactions with specific chemical probe [17].

Perform molecular docking:

Molecular docking of antiviral ligands with Human bocavirus 1 VP2 protein was performed using

AutoDock. Auto Dock is a well-known non-commercial docking program that is generally used at research scale. Auto-Dock has the power to deliver novel drug candidates more quickly at a small cost [5]. After studying, the docking results top three ligands showing minimum binding energies were selected which were processed for Pharmacophore modelling.

Pharmacophore Modelling:

The ligands showing best 3 binding energies was used for—creating a pharmacophore using LigandScout software [18]. LigandScout is a very user friendly and integrated platform for molecular design and accurate virtual screening [18]. It offers hassle-free work, contains high performance alignment consisting both ligand and structure based pharmacophore modelling [19]. These algorithms have excellent prediction quality with a great screening speed.

Once the pharmacophore has been generated the same model is used to perform virtual screening against ligand library. A total of 3963 ligands were obtained from virtual screening. These were further subjected to docking with Human bocavirus VP2 protein. From final result the best ligands selected on the basis of best binding energies.

ADME-Tox analysis: ADME-tox analysis was performed to selected top ligands. PreADMET online tool- (<https://preadmet.bmdrc.kr/>) was used for ADMET analysis [20].

Results and Discussion

Max Planck modeller is an online tool used for designing Human bocavirus 1 VP2 protein. This structure is later used to perform the docking studies with antiviral ligands

Fig 1- 3D structure of predict of human bocavirus VP2 protein by Max Planck Modeller



For docking of ligands, active sites were identified using SiteHound-web tool.

The active sites are as follows **Active sites:** GLN 101, SER 103, GLU 174, ASP 175, VAL 176, MET 177, PRO 178, ASP179, LEU 180, LYS 153, THR

184, TRP 185, LYS 186, GLN 361, THR 362, PRO 363, GLU 507, TYR 522, GLN 524

All the active sites shown in the figure are the pockets of the structure. The docking was performed on to these sites and the results obtained were tabulated the Table 1:

Table 1- Human bocavirus VP2 protein molecular docking result

Sr.no	Antiviral ligands	Binding energies
1	Ribavirin	-7.72
2	Amantadine	-6.64
3	Anabasine	-6.61
4	Nicotine	-5.91
5	Acyclovir	-5.7
6	Myosmine	-5.26
7	Troxacitabine	-4.24
8	Valganciclovir	-4.14
9	Ganciclovir	-4.09
10	Oseltamivir	-3.30
11	Vidarabine	-3.23

The best 3 ligand molecules were selected having the most stable binding energies. These molecule were used to create a pharmacophore model with the help of LigandScout.

Fig. 2- (A) Basic structure of antivirals Ribavirin, Amantadine and Anabasine
(B) Pharmacophore model of best 3 antiviral Ribavirin, Amantadine and Anabasine

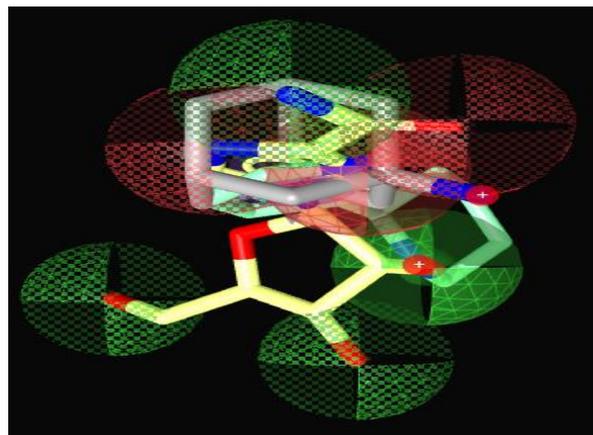
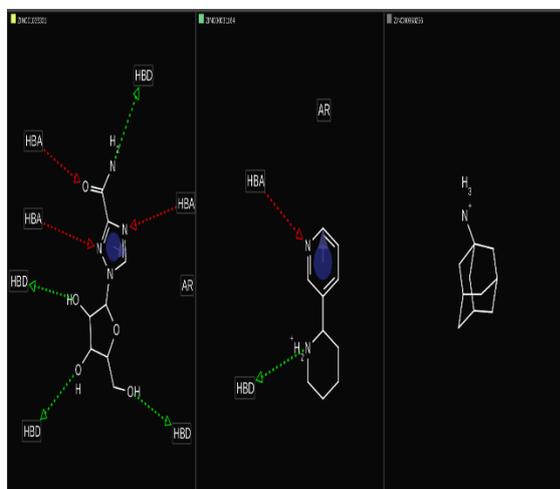


Fig -3 Pharmacophore screening of antiviral molecule by LigandScout software.

Figure 4 is a screenshot of the LigandScout software interface. The main window displays a table of screened ligands with columns for Rank, Name, Matching Features, Pharmacophore Fit Score, Stereo Database, Active Class, #Cofcs, Mol. Weights, and Score. The top 10 results are shown, with Ribavirin (Rank 1) having the highest score of -7.72. The interface also shows various toolbars and a sidebar with 'Pharmacophores' and 'Screening Databases'.

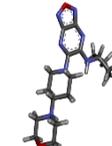
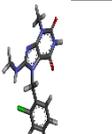
After performing the virtual screening of pharmacophore models created from 3963 ligand library a molecular docking was performed for all 3963 ligands. From the results obtained top 10 molecules were selected on the basis of higher binding energies as shown in Table 2. These ligands were subjected to ADME-Tox analysis. The comparative analysis of the antiviral ligands and the ligands obtained from the screening of pharmacophore model, was performed which shows that binding energies of ligands obtained by (Table-2) pharmacophore model was more stable than antiviral ligands (Table-1).

Table 2 – Best 10 docking result of molecules got from pharmacophore modelling

Sr.no	Ligands we got after pharmacophore screening	Binding energies
1	ZINC41029220	-8.46
2	ZINC12385960	-7.90
3	ZINC00041375	-7.87
4	ZINC41029219	-7.38
5	ZINC35337493	-7.20
6	ZINC91460618	-7.17
7	ZINC91412459	-7.12
8	ZINC20738311	-7.10
9	ZINC65433719	-7.02
10	ZINC71781030	-6.88

The ligands were selected from the pharmacophore screening showing the best binding energies, thus used for the further studies.

Table 3 – Best three ligands were selected for ADME-Tox Analysis

Sr. no	ZINC ID	Chemical structure	Binding Energy
1	ZINC 41029220		-8.46
2	ZINC 12385960		-7.90
3	ZINC 00041375		-7.87

The above selected structures were further used for ADME-Tox Analysis using the online tool tool Pre-Admet (<https://preadmet.bmdrc.kr/>) [20] and following results were obtained.

Table 4 – ADMET analysis of molecule. (A) ADME properties. (B) Drug likeliness (C) Toxicity

Ligands From Pharmacophore Result				
Sr. No.	Zinc ID's	ADME properties		
		BBB	HIA	CACO-2
1.	zinc41029220	0.0704	93.661	0.827549
2.	zinc123850960	0.1902	77.27	1.01002
3.	zinc123850960	0.1575	88.387	4.53762

Ligands From Pharmacophore Result				
Sr. No.	Zinc ID's	Drug likeliness		
		Rule of 5	MDDR	WDI
1.	zinc41029220	Suitable	Mid-Structure	Failed
2.	zinc123850960	Suitable	Mid-Structure	Failed
3.	zinc123850960	Suitable	Mid-Structure	Failed

Ligands From Pharmacophore Result				
Sr. No.	Zinc ID's	Toxicity		
		AMES	Rat	Mouse
1.	zinc41029220	Mutagen	Positive	Positive
2.	zinc123850960	Mutagen	Positive	Positive
3.	zinc123850960	Mutagen	Positive	Positive

In the following result analyzed about ADME properties, druglikeliness and toxicity, the ligand molecule ZINC41029220 showed minimum absorption in the central nervous system and high human intestinal absorption of 93.661 and low permeability seen in CaCO-2 test. In druglikeliness all the molecules followed Lipinski rule of 5 in mid-structure range. In toxicity test all the ligand molecules failed in the WDI (World Drug Index) and mutagenic. The ligand molecule ZINC41029220 displayed non-carcinogenicity in Rat and Mouse respectively.

Conclusion

Till date there is no potential drug available that can cure the infections caused due to bocavirus 1. In the current work researchers have targeted the VP2 protein by combining pharmacophore, molecular docking and ADME-Tox approaches. The antiviral molecule is docked with the receptor. Top three molecules (Ribavirin- -7.72, Amantadine- -6.64, and Anabasin- 6.61) were used for pharmacophore modelling. A Total of 9 lacs molecules were screened through pharmacophore models which were obtained from top 3 antiviral molecules. Once running through pharmacophore model 3963 molecules were obtained. These molecules were also docked with main receptor vp2 protein and top 3 results were selected and those molecules were used. 1. ZINC41029220 - -8.46, 2. ZINC12385960 - -7.90, 3. ZINC00041375- -7.87. The modelled pharmacophores were proved to be more promising than the antiviral compounds themselves. The combination of these can used in clinical trials for developing a novel drug for Human bocavirus.

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