Analysis of binding properties of VP2 protein of Human *parvovirus B19* through *in silico* molecular docking

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Key messages

- Human *parvovirus B19*, a member of the *Erythrovirus* genus *Parvoviridae* (Ballou *et al.*, 2003) infects predominantly erythroid precursor cells in human bone marrow and fetal liver tissues and induces cell death to the infected cell directly (Brown *et al.*, 1994).
- The B19 genome has two large open reading frames (ORFs), with the single nonstructural (NS1) protein encoded by genes on the left side of the genome and the two capsid proteins (VP1 and VP2) on the right side (Ozawa *et al.*, 1987). Sequence analysis, functional family annotation and proteomic analysis for VP2 protein reveals its importance in viral capsid formation, pathogenicity and its usefulness as a drug target against the viral infection.
- Currently there is no vaccine and no specific treatment for *parvovirus B19* infection. The initial symptoms can be treated using over counter medications, such as acetaminophen or ibuprofen.
- Unavailability of proficient vaccine or drug to combat *B19* virus, the availability of whole genome sequence of human *parvovirus B19* on open source to discover conserved protective antigens encouraged to follow a Bioinformatics approach to identify a drug target through structure based drug designing.

Material and methods

RETRIEVAL OF VP2 PROTEIN OF HUMAN *PARVOVIRUS B19* FROM UNIPROT PROTEIN DATABASE

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FUNCTIONAL FAMILY ANNOTATION OF THE VP2 PROTEIN USING SVMPROT

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PROTEOMIC ANALYSIS OF VP2 PROTEIN

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PREDICTION OF ANTIGENIC PEPTIDES USING JEMBOSS

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PREDICTION OF HUMAN *PARVOVIRUS B19* VP2 PROTEIN AS AN EFFECTIVE DRUG TARGET

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RETRIEVAL OF VP2 PROTEIN CRYSTAL STRUCTURE FROM PROTEIN DATA BANK

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PROTEIN PREPARATION AND BINDING SITE PREDICTION USING BINDING TOOL OF DISCOVERY STUDIO 2.0

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PREPARATION FROM HARVARD'S CHEMBANK LIGAND DATASET USING PREPARE LIGAND PROTOCOL

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STRUCTURE BASED VIRTUAL SCREENING IN DISCOVERY STUDIO LIGANDFIT

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RANKING THE DOCKED LIGANDS BASED ON DOCKING SCORE

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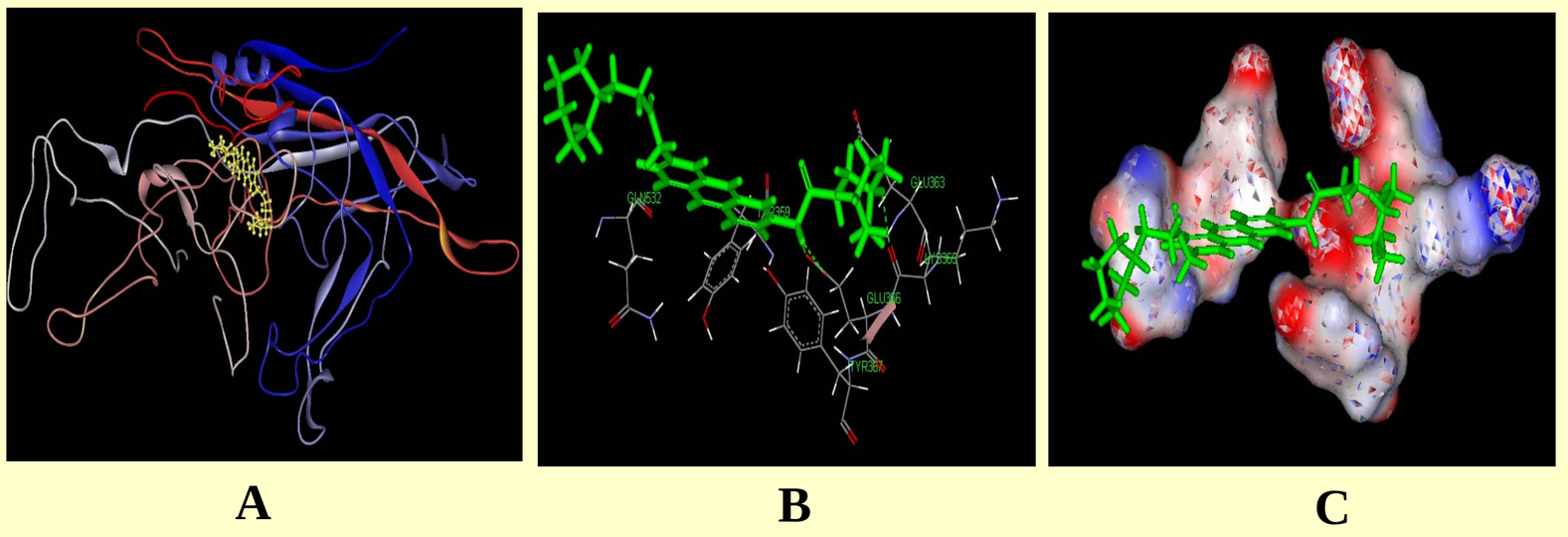
POTENTIAL INHIBITOR IDENTIFICATION BASED ON LIGANDFIT SCORING FUNCTIONS AND VISUAL OBSERVATION OF THE DOCKING COMPLEXES



A **B** **C**

Fig.1 : A) Telomerase inhibitor (viii) – human *Parvovirus B19* VP2 protein docking complex. B) The docking complex showing VP 2 protein residues blocked by Telomerase inhibitor (viii). Three hydrogen bonds formed were shown in green dotted lines. C) Interacting residues of VP2 protein with Telomerase inhibitor (viii) in Surface view.

ANTIGENIC SITES	LIGANDS BINDING WITH RESIDUES	RESIDUES	SCORE
QPGVYPPHAAGHLPYVLYDP	2ndligand (ALA514) 4thligand(ALA514) 8thligand (ALA515, HIS513,PRO 512)	506->525	1.196
TTLVQYAVGIM	-	476->486	1.166
SMLVDHEYKYPYVLGQ	-	150->165	1.159
TLAPELPIWVYFPPQYAYLTVGDV	10thligand (VAL178,TRP177,PRO172,GLU173,LEU170,PHE180)	169->192	1.142
SAFYVLEHSSFQLLG	-	209->223	1.129
LHQPPPQIFLKILPQSG	-	449->465	1.127
SLRPGPVSQPYHH	-	327->339	1.121
ERPLMVGSV	-	406->414	1.113
ANSVTCTFSRQFLI-PYDPEHHYKVFSPAASSCHNA	-	33->67	1.112
DFNALNLFFSPLEFQHLIEN	10thligand (ALA94)	91->110	1.102
SIAPDALTVTISEIAVKDV	-	113->131	1.098
LEGCSQHFY	-	242->250	1.096
YNPLYGSRLGVPDT	-	253->266	1.085
DKYVPGINAI	2ndligand (TYR345) 5thligand(TYR345)	343->352	1.068

Table1: List of antigenic peptides of VP2 protein and ligands

S.No	Ligand molecule	Molecular weight	ALog P	Docking score	H-Bond donars	Ligscore1 & 2	-PLP1	-PLP2	JAIN	-PMF
1	Telomerase inhibitor (viii)	492.68	1.129	169.303	5	-999.9	4.49	10.85	0.02	41.4
2	Diethylspermine (N,N)	262.48	-4.866	165.713	4	-999.9	20.09	20.85	-2.52	25.41
3	Dequalinium	458.69	4.946	128.613	2	-999.9	10.95	15.26	-4.26	0.69
4	Sti-571	496.63	2.591	126.219	4	-999.9	13.88`	15.49	-1.88	44.5
5	Sildenafil	476.59	0.583	111.956	3	-999.9	3.98	5.76	-1.61	31.33
6	Nizatidine	334.47	-3.379	109.133	3	-999.9	-12.1	-6.11	-4.23	0.87
7	Diethylnorspermine (N,N)	248.45	-5.446	106.377	4	-999.9	8.72	11.59	-3.25	7.15
8	Ranitidine	316.42	-2.983	106.32	3	-999.9	8.41	11.09	-2.29	17.85
9	Bisindolylmaleimide (vii)	454.55	1.583	102.913	3	-999.9	-5.9	0.95	-2.67	11.6
10	Raloxifene	474.59	3.396	101.978	3	-999.9	35.87	47.4	3.59	74.8

Table2: Summary of docking information of the ten Top ranked poses of VP2 protein with ligands (values copied from the table browser window of Discovery studio 2.0).

Results and Discussion

- VP2 is the major structural protein, accounting for 96% of total capsid protein. The VP2 protein is encoded by sequences from nucleotides 3125 to 4786, having molecular mass of 58 kDa with 554 amino acid length.
- The VP2 protein belongs to different protein function families like Zinc-binding (91.3%), Coat protein (68.5%), Outer membrane (58.6%), Chlorophyll biosynthesis (58.6%), DNA repair (58.6%) and Calcium-binding (58.6%). The SVM-Prot functional annotation results had confirmed that VP2 protein is essential for constant parvovirus B19 infection and pathogenicity.
- Jemboss antigenic server predicted presence of potentially 14 antigenic regions in human *parvovirus B19* (Table 1). Presence of 14 antigenic sites in VP2 proteins refers to its high degree of pathogenicity.
- Homology search against human genome had revealed that VP2 protein was not having significant similarity with host genome. Thus, VP2 protein would be highly useful as molecular target for designing antiviral drug against *parvovirus B19* infection.
- VP2 protein crystal structure was retrieved from protein data bank (PDB ID 1S58).
- The VP2 protein crystal structure was imported to Discovery studio visualizer. 22 Binding sites were predicted and largest binding site was expanded to 20Å in x, y and z axis direction from its centroid and used for virtual screening.
- The prepare ligand protocol helps to prepare ligands for input into other protocols. Harvard's ChemBank is the database of Food and Drug Administration approved drugs. The database having 2344 small molecule entries was downloaded. Tautomers and isomers were generated for each ligand conformations with a pH range of 6.5 to 8.5. Duplicate structures were removed, Lipinski's filter was applied and the 3D structure was generated for the final set of 3253 filtered ligands.
- Through VHTS method, using LigandFit 410 ligands were found binding favorably to the predicted binding site of VP2 protein. The ligands were ranked based on DockScore.
- Careful visual observations of the docking complexes were made to propose 10 lead molecules based on DockScore. These lead molecules were further evaluated using various LigandFit scoring functions such as Ligscore1&2 PLP1, PLP2, JAIN and PMF (Table 2).
- The docking result had revealed that the residues such as ALA514, ALA515, HIS513, PRO512, VAL178, TRP177, PRO172, GLU173, LEU170, PHE180, ALA94 and TYR345 are involved in the docking complex of the ten potential leads (Table 1). These residues are also predicted as important for antigenicity resulted due to parvoviral infection.
- Telomerase inhibitor (viii) was found as the best probable lead against parvovirus VP2 protein (Fig. 1A, Fig. 1B and Fig. 1C).These ten potential inhibitors (Table 2) would be highly useful against parvoviral infection. However, *in vitro* binding assays are necessary for confirming the activity of predicted lead molecules.

Conclusion

- The SVM-Prot functional annotation of VP2 protein had shown its Zinc binding, Coat and envelope protein like and Calcium-binding properties. Higher Zinc binding property of the protein refers to its critical role in maintaining viral stability (Funk *et al.*, 1992). Coat and envelope protein like properties signifies presence of the protein in viral outer membrane. Calcium ions appear to play a major role in maintaining the structural integrity and are likely involved in the process of viral uncoating (Rebecca *et al.*, 1996).
- Presence of 14 antigenic sites in VP2 protein refers to its high degree of antigenicity and its non homology with human confirmed it as potential drug target for discovery of potent inhibitor against parvovirus.
- Through virtual screening ten potential inhibitors targeting VP2 protein of human parvovirus was identified. Telomerase inhibitor (viii) was found as the best probable lead among the ten. However, *in vitro* binding assays are necessary to confirm the inhibition ability of the proposed lead molecules.

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