Prediction of antiviral drug targeting VP1 protein of Blue Tongue Virus

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ABSTRACT

Blue Tongue is a vector mediated viral disease of cattle that is prevalent in tropical and subtropical countries. It has been listed as a 'notifiable disease' by the Office International des Epizooties (OIE) because it causes heavy losses in the form of productivity loss (weight loss, wool break, reduced milk and meat yield), high morbidity, mortality, abortion. The disease is caused by a virus belonging to family Reoviridae and species of the genus Orbivirus called Blue Tongue Virus (BTV). It is an intricate non-enveloped virus with seven structural proteins which are arranged in two capsids forming outer and inner shell of virus and a genome of ten double-stranded RNA (ds RNA) segments contained in the inner shell. Shortly after gaining entry into the cell the outer capsid is degraded to release an inner core (inner capsid) which helps in synthesis of capped mRNAs from each one of the genomic segment contained inside the core, expelling them into the cytoplasm. Twenty six serotypes of the Blue Tongue virus have been discovered till date. The viral genome encodes for seven structural proteins (VP1 to VP7) and also for four non-structural proteins (NS1, NS2, NS3/NS3A and NS4), out of which structural protein VP1 is highly conserved and is present in transcription complex and act as RNA dependent RNA polymerase (RdRP). Targeting of VP1 is an effective way to treat Bluetongue Disease. In the present study, docking of various anti-viral drugs with VP1 was studied using bioinformatics tools and two effective candidate drug molecules, namely Favipiravir and BAS 01884755, were selected based on the docking studies.

CHAPTER 1

INTRODUCTION

Blue Tongue Virus (BTV) belongs to the genus *Orbivirus*. *Orbivirus* is largest among the 15 genera of family *Reoviridae* and can infect camelids, ruminants, and occasionally large carnivores (*Meyer et al., 2009; Attoui et al., 2009*). In mammals it is mainly transmitted by certain species of *Culicoides* (biting midges)but it can also be transmitted transplacentally and transovarian (*Menzies et al., 2008*). Office International des Epizooties (OIE) has listed Bluetongue (BT) as a 'notifiable disease', because it causes heavy losses in the form of productivity loss (reduced milk, weight loss, low meat yield and wool break), high morbidity, mortality, abortion, foetal abnormality and still birth *(Osburn, 1994)*. The major indirect losses include trade losses which are caused animal movement restrictions, prohibition on the import and export of cattle semen and the cost of enforcing control measures, including diagnostic tests *(Osburn et al., 1981).* Clinical symptoms of bluetongue disease are fever, swelling of the lips and tongue of infected cattle, coronitis (lameness) and death.

The BTV particles are complex particles which are non-enveloped and arranged in an icosahedral manner in two capsids. The inner core having a diameter of 75 nm and outer capsid is of approximately 90 nm in size *(Grimes et al., 1998; Huismans et al., 1981; Nason et al., 2004)*. There are 10 segments of dsRNA in the viral genome, each of which encodes seven structural[Viral protein(VP)1 to Viral protein(VP)7] and four non-structural [NS1, NS2, NS3/NS3A and NS4] proteins *(Verwored et al., 1972; Mertens et al., 1984; Wu et al., 1992; Belhouchet et al., 2011)*. Segment one is the largest (~3900 bp) and segment ten is the smallest in size (~822 bp).

Segment 1 of the viral genome encode for the VP1 protein, which is found in the transcription complex and serve as RNA dependent RNA polymerase (RdRP) *(Roy et al., 1988; Urakava et al., 1989).* Inside the transcription complex, VP6 and VP4 are also found along with VP1. The confirmation of VP1 as the Blue Tongue Virus polymerase protein was further supported by generation of a structural model. As most of the RNA dependent RNA polymerases are similar to each other as compared to other polymerases, it is possible to predict a 3D model for the VP1structure based on many available crystal structure of RNA dependent RNA polymerases

submitted in several structural databases *(Wehrfritz et al., 2007).* All RNA dependent RNA polymerase proteins possesses a typical structure resembling to the right hand, complete with having sub-domains resembling ‘Palm’, ‘Fingers’ and ‘Thumb’.

Since the crystal structure of the protein is still unknown so it is difficult to predict an accurate structure for whole protein. But Bluetongue virus has a GDD (Gly-Asp-Asp) motif at position 763–765 on its VP1 protein sequence which is flanked by the other sequence motifs which characterize polymerase proteins (*Roy et al., 1988; Bruenn, 1991; 2003*), the presence of these motifs on VP1 sequence indicates that VP1 most probably have a single, central polymerase domain as found in reovirusλ3. 300 amino acid residues (aa 581-880) in the central region of VP1 that include the GDD motif sequence have been shown to align with the RdRP of the two ssRNA viruses, poliovirus (PV) and rabbit haemorrhagic disease virus (RHDV) *(Wehrfritz et al., 2007).*

In the present study, targeting the central core region of VP1 having the polymerase activity, screening of chemical compounds was carried out (considering only those which have RdRP inhibition activity). Usually RdRP inhibiting antiviral drugs (e.g. Ribavirin, Favipiravir, Triazavirin, BCX 4430, BAS 01884755 etc.) works by misincorporation into the nascent RNA as false bases or they may bind to the RNA polymerase itself, blocking the process of RNA replication of virus *(Yousuke Furuta1 et al., 2003).*

Thus, docking of various anti-viral drugs exhibiting RdRP inhibition with VP1 was studied using bioinformatics tools.

CHAPTER 2

REVIEW OF LITERATURE

Blue Tongue Virus (BTV) belongs to the genus Orbivirus. Blue Tongue virus can infect camelids, ruminants and rarely large carnivores. The BT virus is transmitted by Culicoides (biting midges) inside which it replicates further. The virus can also be transmitted sometimes via an oral route in cattle and sheep. BT virus enters inside the mammalian cells by adhering its outer capsid to a receptor already present on the surface of plasma membrane of the mammalian cells *(Eaton and Hyatt, 1989).* Through confocal microscopy and biochemical studies, along with RNA interference and specific inhibitors, it was shown that BT virus gain entry inside the cells by pH-dependent penetration and clathrin-mediated endocytosis *(Forzan et al., 2007).* Bluetongue (BT) is an economically important, Office International des Epizooties (OIE) listed disease. The disease is prevalent in all continents except Antarctica and is widely distributed worldwide. Therefore, to control the disease, studies regarding virus, the nature of vector, environment and the types of interactions between them are needed. In the recent years with the advanced molecular techniques, a lot of information has been generated on BTV molecular biology, immunology, diagnostics, ultrastructure, evolution and epizootiology. The studies have created a huge amount of literature on bluetongue. It is not possible to review all the literature on this virus due to space limitation. Hence the most relevant work related to the present study is reviewed here.

2.1 Bluetongue disease

It was first reported in 1881 by Hutcheon with the introduction of European breeds of sheep into Southern Africa (*Hutcheon, 1902*). In 1905 Spurell suggested the name 'Bluetongue' based on cyanosed tongue in sheep (*Spreull, 1905*), later the viral aetiology of the disease was demonstrated in 1906. In 1948 the disease was reported from North America as sore muzzle (*Hardy and Price, 1952*), in 1958 from west Pakistan in Rambouillet sheep imported from USA (Howell, 1963), in 1977 from Australia in insects (*St. George et al., 1978*) and from China (*Zhang et al., 1989*).

Recently the disease has spread across Europe and Mediterranean basin (*OIE, 2006; Rodriguez-Sanchez et al., 2008; Meroc et al., 2009*). In 1961 the disease was first time reported in India in Maharashtra region in sheep and goat (*Sapre, 1964*).

It has been found that biting midges of the genus Culicoides, family Ceratopogonidae transmits BT virus (*DuToit, 1944*). This virus replicates in salivary glands of Culicoides (*Browne and Jones, 1966*). There are more than 1000 species of Culicoides but few are considered as competent vectors of bluetongue virus (*Meiswinkel et al., 2008*). In 2012 Burgin investigated bluetongue virus incursions using long-distance culicoides biting midge dispersal as a model (*Burgin et al., 2012*). The disease may also be transmitted transplacentally and transovarian in cattle (*Wilson et al., 2009; Santman-Berends et al., 2008*). Blue Tongue Virus enters into the mammalian cells by attaching its outer capsid to a receptor present on the surface of plasma membrane of the mammalian cells *(Eaton and Hyatt, 1989).* Through confocal microscopy and biochemical studies, along with RNA interference and specific inhibitors, it was shown that BTV gain entry inside the cells by pH-dependent penetration and clathrin-mediated endocytosis *(Forzan et al., 2007).*

2.2 Bluetongue virus

On the basis of serological cross reactivity, more than 21 serogroups can be found within the genus Orbivirus (*Mertens et al., 2004*).

In 1971, Borden proposed the name ‘Orbi’(Latin word meaning ring or circle), for many groups of viruses on the basis of appearance of large doughnut shaped capsomeres of the negatively stained viral particles under electron microscope (*Borden et al., 1971*). International Committee on Taxonomy of Viruses (ICTV) approved the group ‘Bluetongue virus’ in Orbivirus genus in 1976 (*Fenner, 1976; Murphy et al., 1995*).

In case of serogroup BTV, 26 different serotypes have been characterized worldwide on the basis of serum neutralization test (SNT) (*Hofmann et al., 2008; Maan et al., 2011*). Due to the variation in viral protein in different serotypes, all these serotypes can be determined by the specific

interaction of viral outermost VP2 protein of a particular serotype with corresponding neutralizing antibodies in serum neutralization assays (*Eaton et al., 1990; Maan et al., 2007*).

2.3 Bluetongue virus structure

Blue Tongue Virus is a non-enveloped having 850-Å-diameter and structurally complex virus particle having 10 dsRNA segments surrounded by three concentric layers of protein shells (Verwoerd, 1969). The outer shell consists of two structural proteins, VP2 and VP5 (responsible for serotype determination). Cryo-electron microscopic studies revealed that, it is composed of 180 copies of VP2 protein (snail-shaped), arranged as trimeric structures, together with 360 copies of an underlying VP5 protein (inter-dispersed), and appears to be arranged as 120 trimers. Because of the protruding spike like structure, VP2 protein project4nm beyond the globular VP5 protein molecules, thus making it the outermost protein of BT virus.

The outermost capsid is degraded as virus enters into the cell, releasing the transcriptionally competent inner nucleocapsid known as core. The outer layer of the inner capsid (core particle) consists of 260 trimers of VP7 organized on icosahedral lattice. The VP3 layer forms a ring which houses the segmented genome along with 3 minor structural proteins (VP1, VP4 and VP6). VP1 and VP4 jointly make a flower-shaped structure linked to the underside of VP3 (*Nason et al., 2004*). The VP7 layer is a crucial structural protein which interact with the underlying innermost layer (‘sub core’ consists 120 copies of VP3) arranged as 60 dimers inside the icosahedral lattice (*Verwoerd et al., 1970, 1972*). These minor proteins are present in small number (10-12 copies) and act as transcriptase complex comprising of the polymerase VP1, capping enzyme VP4 and helicase VP6 protein. In addition to seven structural proteins, the inner core protein VP3 and VP7 and non-structural NS1 proteins are the major serogroup specific antigens.

The ten RNA genomic segments are numbered as 1-10 and designated as large (L1-L3), medium (M4-M6) and small (S7-S10) according to their size and migration pattern in RNA-PAGE *(Sugiyama et al., 1981*). All the ten genomic segments of BT virus have conserved 3' and 5' terminal sequences (*Rao et al., 1983*).

2.3.1 Coding assignments of BT virus

The 10 dsRNA segments encode seven structural and four non-structural proteins, where non-structural proteins are produced only in virus infected cells and take part in virus replication, assembly and release. The structural proteins (VP1-VP7) form the part of virus particle (*Mertens et al., 1984; Roy, 2008*) (Fig. 2.1). The sizes, locations, functions and other properties of the gene products of ten genome segments of BT virus are summarized in the Table 2.1.

Table 2.1: Genome segments, functions and properties of bluetongue virus 10

Genome segments	No. of bp	Protein	No. of amino acids	Copy no. per virion	Location	Function and properties
1	3954	VP1	1302	10	Within Inner core	RNA polymerase
2	2926	VP2	956	180	Outer capsid	Serotype specific antigen
3	2770	VP3	901	120	Inner core	Forms scaffold for VP7
4	1981	VP4	644	10	Within Inner core at 5 fold axis	Capping and methyl transferase activity
5	1769	NS1	552	120	Cytoplasm	Tubule formation
6	1638	VP5	526	NA	Outer capsid	Outer capsid protein,
7	1156	VP7	349	780	Core surface	Major core protein
8	1124	NS2	357	NA	Cytoplasm of BTV infected cells	Forms viral inclusion bodies, binds ssRNA
9	1046	VP6	329	40	Inner core	Binds ssRNA and dsRNA
10	822 216	NS3 NS3A	229 216	NA NA	Not abundant in mammalian cells	Virus egress Unknown

2.3.2 Outer capsid proteins

The outer capsid of the virus core is surrounded by two proteins VP2 and VP5. The VP2 is present on outermost side, is a haemagglutinin responsible for receptor binding, entry in vertebrate host cell, virulence and virus egress (*Forzan et al., 2007; Eaton and Crameri, 1989; Bhattacharya et al., 2007*). The haemagglutination of sheep RBCs, binding capacity and infectivity of virus for vertebrate cells gets reduced significantly when VP2 protein is removed (*Huismans et al., 1983*) but the viruses with cleaved VP2 products after partial digestion with chymotrypsin are still fully infectious (*Mertens et al., 1987*), indicating that the site for cell attachment and haemagglutination activity on VP2 protein may not be the same. However the virus can infect the Culicoides species without VP2 protein (*Mellor, 1990*). After entry into the cell via clathrin mediated endocytosis, the VP2 protein is degraded in acidic medium of endosome exposing the VP5 protein of the virus (*Forzan et al., 2004, 2007*). The denatured VP2 protein fails to induce immune response indicating that its conformation plays a crucial role in eliciting the neutralization antibodies production (*Huismans et al., 1987; Hwang and Li, 1993*). VP2 protein is the most variable among serotypes, having different variable regions in different serotypes and thus responsible for the serotype specificity (*Fukusho et al., 1987; Roy et al., 1990; Roy, 1992*).

VP5 is the second most variable protein of BT virus which is indirectly involved in induction of immune response. The protective neutralizing activity of VP2 protein is greatly enhanced by VP5 protein (*Huismans et al., 1983; Roy et al., 1990*).

2.3.3 Core proteins

The core or inner capsid of the virus consists of two major VP3 and VP7proteins along with three minor VP1, VP4 and VP6 structural proteins (*Verwoerd et al., 1970, 1972*). Genomic segment S7 of the BT virus encodes VP7 protein which is 36% of total core proteins. There are 260 trimers or knobs of VP7 proteins which are organized along with 132 aqueous channels (*Huismans et al., 1987; Nason et al., 2004*). These channels are for transportation of mRNA and metabolites during the core transcription *(Roy, 2005).* Second major core protein VP3 encoded by segment 3 (vp3 gene), exists as dimers and forms the scaffold for the deposition of VP7 protein. VP3 protein also interacts with VP1 and VP4 proteins which are encoded by L1 and M4 genomic segments

respectively. VP1 is the largest protein of 150 kDa having RNA dependent RNA polymerase (RdRP) activity (*Roy et al., 1988; Roy, 2008*). The capping and methyl transferase activities of VP4 proteins enable it to cap the nascent mRNA as it emerges out of polymerase (*Roy, 2005; Sutton et al., 2007*). The dsRNA segment S9 encodes the ssRNA and dsRNA binding VP6 protein, which performs the helicase and nucleoside triphosphatase (NTPase) activities of the virus.

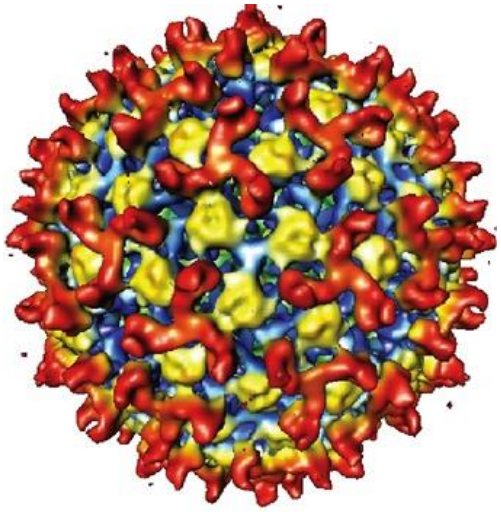


Figure 2.1: Arrangement of VP2 and VP5 on outer cpasid of BTV (http://www.horizonpress.com)

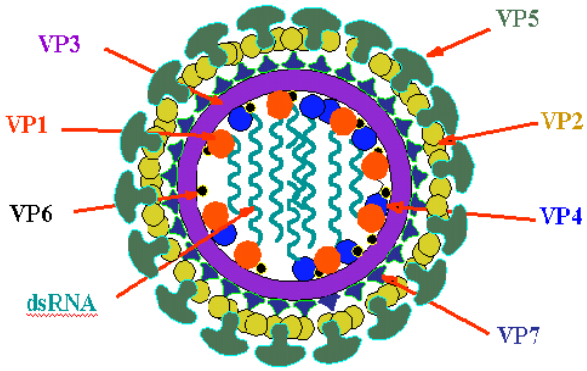


Figure 2.2: Schematic structure of bluetongue virus showing location of all ten dsRNA and organization of seven structural proteins (indiaveterinarycommunity.com)

2.3.4 Non-structural proteins

Genomic segment M6 of the BT virus encodes the major non-structural 1 (NS1) protein which forms characteristic tubules in perinuclear region of BT virus infected cells (*Eaton et al., 1988; Roy, 2005, 2008*). However, widely held view is that segment 6 encodes VP5 protein and segment 5 encodes NS1 protein. NS2 protein encoded by dsRNA segment S8 forms the cytoplasmic inclusion bodies after phosphorylation (*Modrof et al., 2005*) and binds to ssRNA segments of BTV before the viral genome encapsidation (*Mumtsidu et al., 2007; Roy, 2008*). Genomic segment S10 is transcribed into two non-structural NS3 and NS3a proteins which are membrane bound and form a component of ESCRT (The endosomal sorting complexes required for transport) pathway thus playing a role in virus egress by interacting with VP2 and VP5 protein (*Bhattacharya and Roy, 2008; Celma and Roy, 2009*).

2.3.5 Characterization of BTV

In the last few years lot of data is generated regarding the sequences of BTV. In 2008 first full genome of BTV 8 was sequenced by Maan *et al*. (*Maan et al. 2008*). Before it full gene of vp2 and vp5 of all 24 serotype were sequenced, analysed and phylogenetically compared (*Maan et al., 2004; Singh et al., 2004; Maan et al. 2007*). Nomikou performed the phylogenetic and evolutionary analysis of full-length VP3 genes in 2009 on Eastern Mediterranean BTV isolates (*Nomikou et al. 2009*). In 2010 the full genome characterisation of BTV serotype 6 from the Netherlands and comparison of it with vaccine strains was done by Maan (*Maan et al., 2010*). Mann *et al.* sequenced full genome sequence of BTV1, 2, 3, 10, 23 and 26 and also determined reassortment between eastern and western topotypes (*Maan et al. 2011 and 2012*). Yang *et al.* completed genomic sequencing of BTV serotype 1 and 16 from China (*Yang et al., 2011*). Rao *et al*. genetically characterized BTV serotype 9 isolates from India (*Rao et al., 2012*). Anshul *et al*. and Mukhtar *et al*. amplified and sequenced full length segment 2 and segment 6 of BTV9, 10, 16 and 21 (*Anshul et al., 2012; Mukhtar et al., 2012*).

2.4 Anti-viral drug targeting

The chemical compounds which are used specifically for treating viral infections are grouped under a class of medication known as antiviral drugs. Specific antiviral drugs are used for specific viruses. Antiviral drugs inhibit the pathogen development instead of destroying their target pathogen.

The main concept behind modern antiviral drug design is to identify those viral proteins, or parts of proteins, that can be disabled. The targets should be conserved across many different strains of a virus, or different species of the virus in the same family, so that a single drug may have broad effectiveness.

Once the target proteins are identified, candidate drugs can be screened, either from drugs already known, or by designing the drug at the molecular level using computationally available tools or drug designing programs.

In the present study, the target was the central core region of VP1 having the RNA-dependent RNA polymerase activity (RdRP). Usually RdRP inhibiting antiviral drugs (e.g. Ribavirin, Favipiravir, Triazavirin, BCX 4430, BAS 01884755 etc.) works either by misincorporation into the nascent RNA as false nucleoside bases or they may bind to the RNA polymerase itself, blocking the process of RNA replication of virus *(Yousuke Furuta1 et al., 2003).*

CHAPTER 3

METHODOLOGY

A working computer system with internet connection was required to carry out the research work. Online tools and software used in our research work are discussed below:

3.1 National Centre for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov)

NCBI has been assigned with creating automated system for sorting and analyzing information about molecular biology, genetics, biochemistry etc. The NCBI interlinks various databases which are related to biotechnology and biomedicine related research works. Major databases include PubMed (database for exploring the biomedical literature). GenBank (for DNA sequences) and other databases include the NCBI Epigenomics database (for whole-genome epigenetics data sets), PubChem database (as central repository for small chemical compounds) etc. All these databases are available online through the Entrez search engine. Entrez permits a user to access and retrieve specific data from a single database and access integrated data by exploring many interconnected NCBI databases.

Using NCBI, following were explored in the present study:

PubMed: was used to explore and review pertaining literature.

Protein database: was used to download and access the amino acid sequence of the VP1 protein of the Blue Tongue Virus.

PubChem: was used to screen and select drug like lead chemical compounds. PubChem contains small molecules with their substance descriptors.

3.2 ZINC database

Especially for virtual screening ZINC database holds a vast curated collection chemical compounds that are commercially available. ZINC is used by research Universities, biotech companies, biologists and chemists working in pharmaceutical companies. ZINC represent the three dimensional biologically relevant form of the molecule.

3.3 Phyre/Phyre 2: (Protein Homology/Analogy Recognition Engine)

The crystal structure of VP1 protein is not yet available. Thus the first step was to predict the structure of VP1. Phyre/Phyre 2, a web-based server was used for protein structure prediction.

It works on the principle and techniques of homology modeling to predict the three-dimensional structure of a protein sequence. As the structure of a protein is more conserved during evolution than its sequence, a query sequence (target) can be modeled on a very distantly related sequence of known structure called the template.

Currently the most accurate and powerful method for aligning and detecting remotely related sequences is based on Hidden Markov Models (HMMs). These models are employed in the prediction algorithms of Phre/Phyre 2.

3.4 Open Babel

Open Babel is file format conversion software; it is mainly used for converting file formats of chemical compounds (e.g. from PDB format to MOL 2 and vice versa). It is mainly used in the field of chemo-informatics. Inter-conversion of many chemical file formats can also be performed by this programme.

3.5 Swiss Dock

Swiss Dock is a web-based server which is used to predict the molecular interactions between a target protein and a ligand (small molecule). It is an online available tool used for docking related studies. In the present study Swiss Dock was used for docking various small chemical drug-like compounds obtained from the chemical databases with our target protein VP1 of BTV.

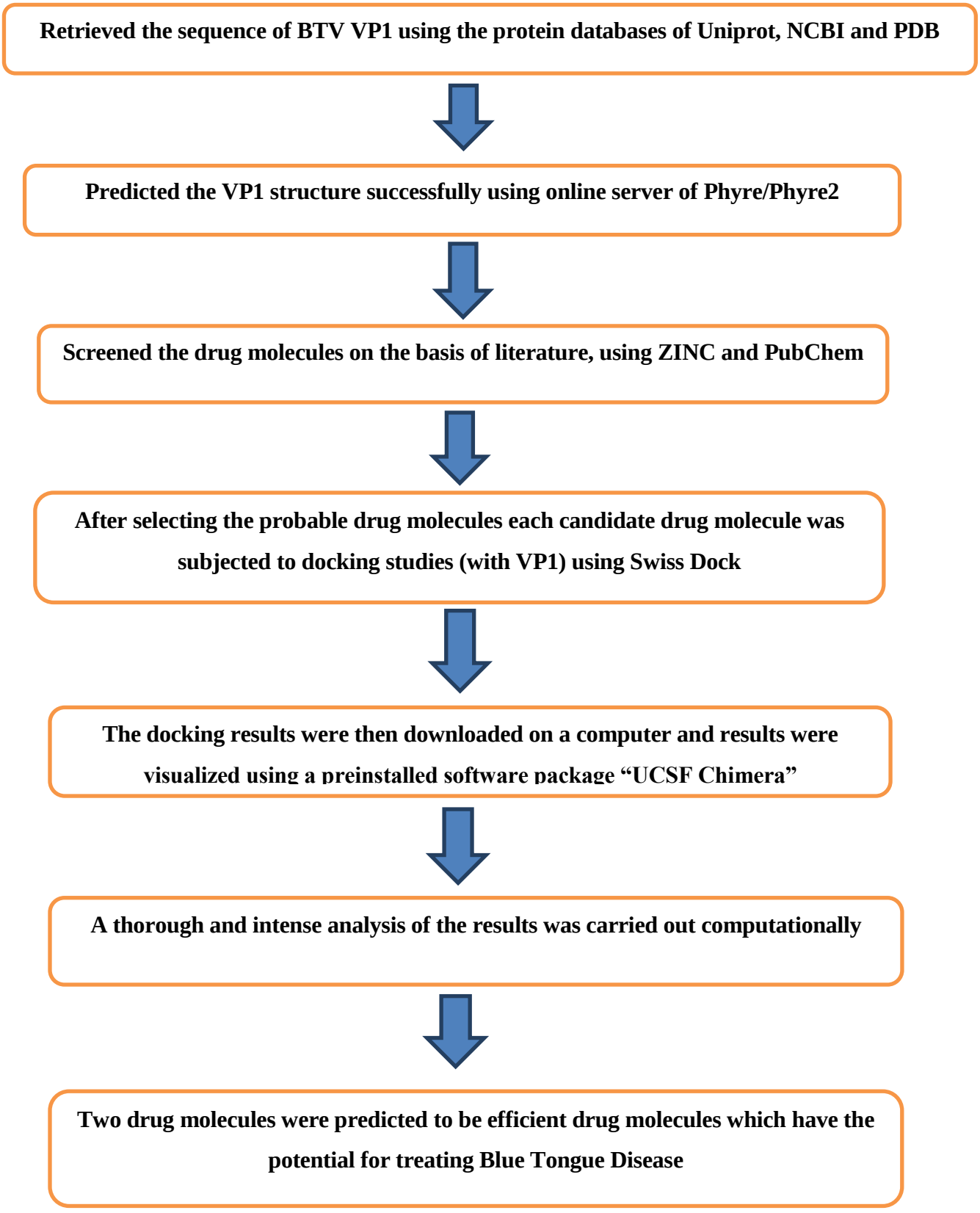
Swiss Dock is based on *EADock DSS* (docking software); algorithm of Swiss Dock consists of:

1. Generation of many binding modes either in local docking (in a box) or blind docking (in the vicinity of all target cavities).
2. Estimation of their CHARMM (Chemistry at Harvard Macromolecular Mechanics) energies on a grid. The CHARMM is a very popular and frequently used MS (molecular simulation) tool. It was developed with a primary objective of the study of molecules of biological interest mainly proteins, peptides, prosthetic groups, small chemical ligands, carbohydrates and lipids, as these are found mainly in membrane environments, solution, and crystals forms. CHARMM provides a large group of computational tools which contains numerous path sampling and conformational methods. It also encompasses analysis techniques along with model-building capabilities with free energy estimates. Molecular minimization and dynamics are also performed by using CHARMM. It can be supplemented with various energy functions and models.
3. The binding modes with the most favorable energies are evaluated by using a more advanced programme FACTS (Fast Analytical Continuum Treatment of Solvation). FACTS is a highly accurate and efficient generalized model. It is mainly used for Molecular Dynamics simulations because of its speed and accuracy. It works by evaluating the volume and spatial symmetry of the solvent which is displaced by a solute atom along with its neighboring atoms.
4. Most highly ranked and favorable clusters can be visualized online or can be downloaded on computer for visualizing later.

3.6 UCSF Chimera

It is a software tool which is used for interactive analysis and visualization of molecular structures and docking related data, including density maps, sequence alignments, supramolecular assemblies, trajectories, and conformational ensembles. High-definition images and movies can be created using Chimera. It is developed by the Resource for Biocomputing, Visualization, and Informatics (RBVI) at the University of California, San Francisco. All development related cost is funded by the National Institutes of Health.

3.7 Flow chart depicting methodology used



RESULTS

4.1 VP1 structure determination

Protein sequence of BTV VP1 was successfully retrieved from NCBI Protein Database

GI: 496629143

>gi|496629143|gb|AGL45574.1| VP1 [Bluetongue virus]

MVAITVQGAQLIKRVVERFYPGIEFRNDEGACYIYKFSDHIRRIRMKHGTKYQRQAEEIM
RNISLRKEKLHGIPVLDEVEWKYVFDGQTFQSYAFEVYINSILPWSELDPEEEFLRNYRV
SREMTEVEKFIEFRAKNEMQIYGDIPIKVWCCFINELSAEVNHTPLGMQVMADFVNRFG
SPFHQGNRDLSNLEDFQVAYSTPLLFEMCCMESILEFNIKMRMREEDISALEFGDTRIDPI
GLLREFFVLCLPHPKKINNVLRAPYSWFVKMWGVGADPIVVLQSAAGDDRNSKDVFYD
KFRTEANHYKGLFRSSFYSESRRMNVEKVLEAVKYSQELGSHDHRLPIFEKMLKTVYTT
PFYSHKSSNMILASFLLSIQTITGYGRAWVKNVSTEFDKQLKPNPNNLVQDVSDLTREFF
KQAYVEAKERREEIVKPEDLYTSMLRLARNTSSGFSTEIFVKKKFGPRLKDKDLIKINSRI
KALVIFTKGHTVFTDEELRKKYNSVELYQTKGSRDVPIKATRTIYSINLSVLVPQLIVTLP
LNEYFSRVGGITSPDYRKIGGKVIVGDLEATGSRVMDAADCFRNSADRDIFTIAIDYSEY
DTHLTRYNFRTGMLQGIREAMTPYRDLRYEGYTLEQIIDFGYGEGRVANTLWNGKRRL
FRTTFDAYLRLDESERDKGTFKIPKGVLPVSNIDVAKRIAVDKGFDTLVAATDGSDLALI
DTHLSGENSTLIANSMHNMAIGTLIQREVGKEEPGVLTFLSEQYVGDDTLFYAKLHTTD
TKVFDRIATSIFNTVAKCGHEASPSKTMMTPYSVEKTQTHAKQGCYVPQDRMMIISSER
RKDIEDVQGYVRSQVQTMITKVSRGFCHDLAQLILMLKAAFIGAWKMKRTIKEDATYR
DRKFDSNDEDGFTLIQIRNPLALYVPIGWNGYGAHPAALNIVMTEEMYVDSIMISKLDEI
MAPIRKIVHDIPPCWNETQGDKRGLISATKMSFFSKMARPAVQAALSDPQVMNLVEELP
LGEFSPGRISRTMMHSALLKESSARTLLSSGYELEYQKALNNWISQVSMRLGEESGVIST
SYAKLFDVYFEGELDSAPYMFPDQNLSPQFYIQKMMIGPRVSSRVRNSYVDRIDVILRK
DVVMRGFITANTILNVIEKLGTNHSVGDLVTVFTLMNIETRVAEELAEYMTSEKIRFDA
LKLLKKGIAGDEFTMSLNVATQDFIDTYLAYPYQLTKTEVDAVSLYCTQMIMLRAAIGL
PKKKMRIAVTDDAKKRYKVRLQRFRTHVPKIKVLKKLIDPNRMTVRNLENQFV

To predict the 3-D structure of the polymerase subunit of VP1, aa581-880 sequence was submitted to Phyre/Phyre 2 server. The predicted structure showed a polymerase like structure of VP1 similar to a right hand structure of RNA polymerase with sub domains namely: thumb, palm and fingers (Fig. 4.1). The fingers subdomain has four β strands and three α helices. The palm subdomain predicted for VP1 consisted of 4 antiparallel β sheets surrounded by 3 α helices. Universally this arrangement is found in most of the polymerases.Predicted structure of VP1 (central region sequence aa 581-880) using Phyre/Phyre2 (Fig. 4.2)

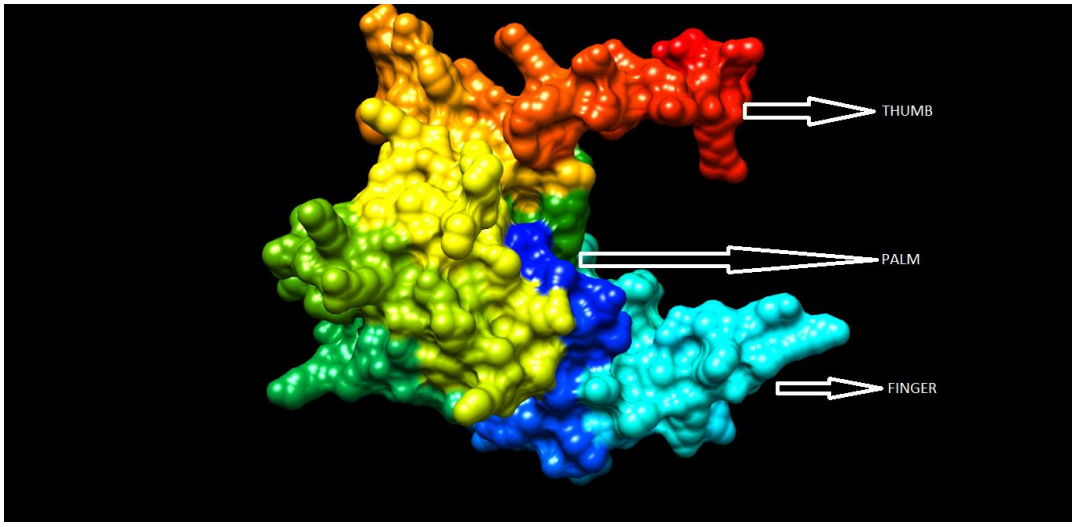


Figure 4.1: Predicted structure of VP1 showing 'Thumb', 'Palm' and 'Finger' subdomains (Solid surface topology)

Figure 4.2: Predicted structure of VP1 (central region sequence aa 581-880)

4.2 Favipiravir structure determination

As the target protein was a RNA polymerase it was decided to screen only those compounds which are potent drug compounds targeting RNA polymerases of other similar viruses. While screening such chemical compounds using ZINC and PubChem databases, Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide), an antiviral drug that selectively inhibits the RNA dependent RNA polymerase (RdRP) of influenza virus was selected.

Favipiraviris an experimental drug developed by Toyama Chemical of Japan with RdRP inhibition activity against many RNA viruses. Like other experimental antiviral drugs (T-1105 and T-1106), it is a pyrazinecarboxamidederivative. It is active against influenza viruses, foot-and-mouth disease virus, West Nile virus, virus, as well as other alphaviruses, flaviviruses, bunyaviruses and arenaviruses.

Favipiravir may be introduced in nascent viral RNA (misincorporation), or it may also act by binding to conserved polymerase domains further preventing incorporation of nucleotides for viral RNA replication and transcription *(Yousuke Furuta1 et al., 2003).*

The 2-D structure of Favipiravir was downloaded from PubChem database in SDF file format. SDF format was converted into PDB format by using Open Babel software. Favipiravir structure in PDB format can be viewed using UCSF Chimera (PubChem CID: 492405) (Fig. 4.3). Force fields ofFavipiravir in mesh topology (Fig. 4.4) or in solid surface topology (Fig. 4.5) were generated by using UCSF Chimera.

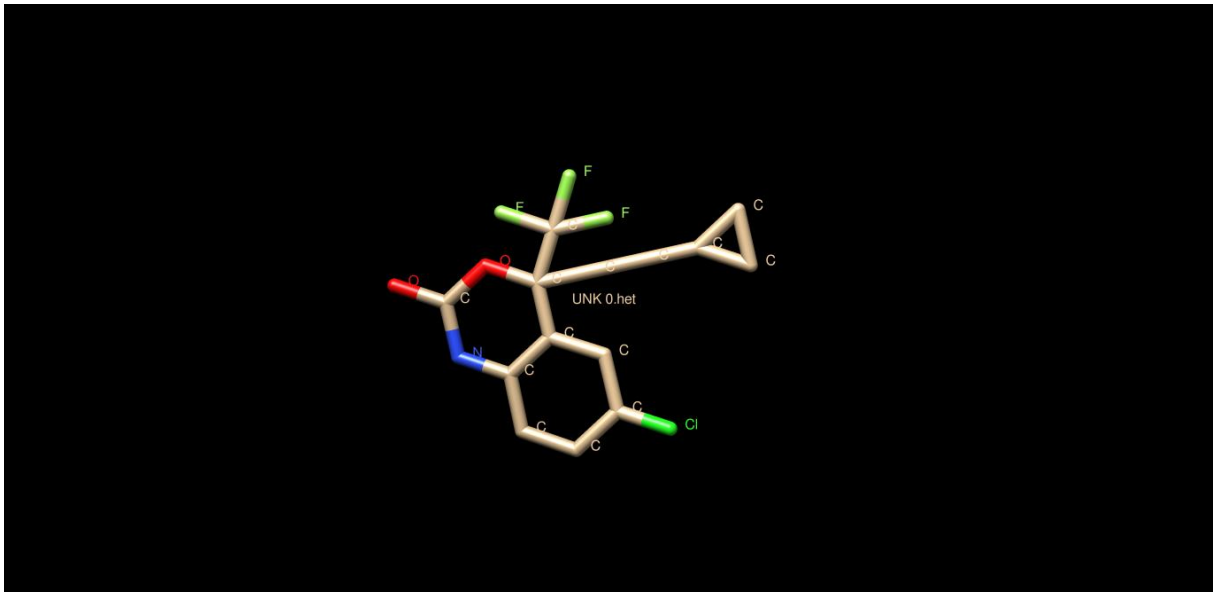


Figure 4.3: Two dimensional structure of Favipiravir

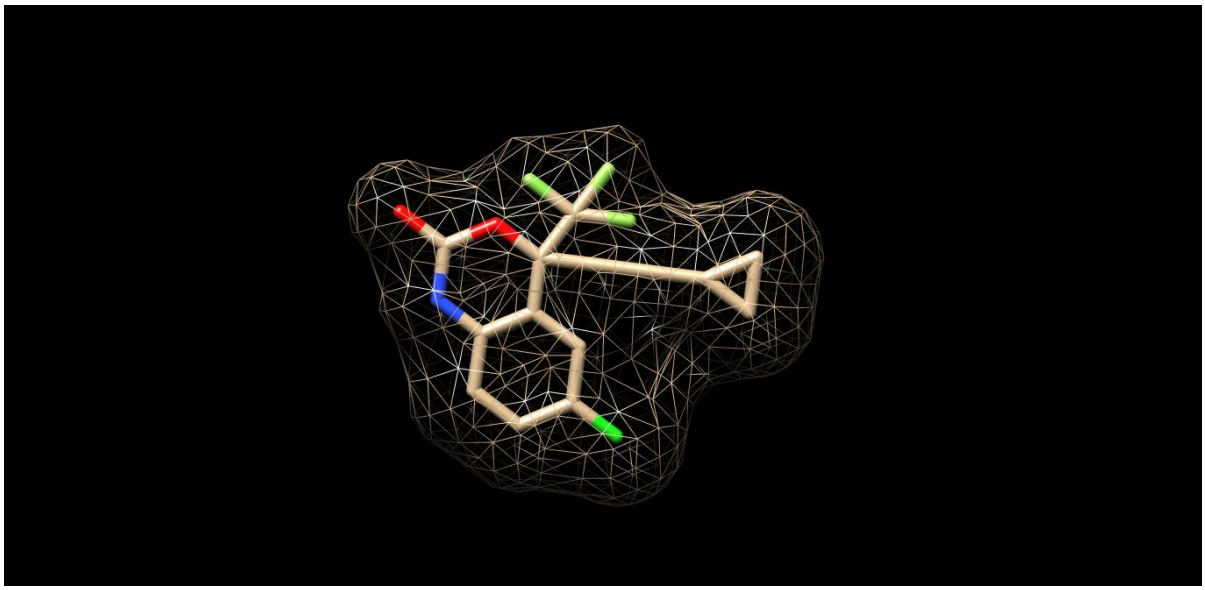


Figure 4.4: Force fields of Favipiravir shown in mesh topology

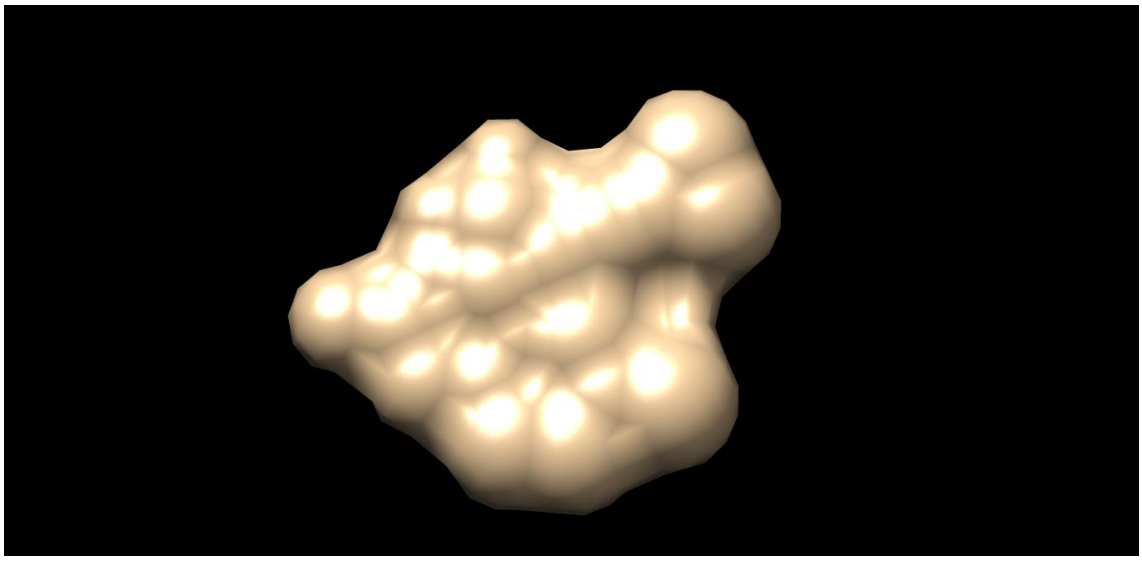


Figure 4.5: Solid surface topology of Favipiravir

4.3 Docking of Favipiravir with VP1

After screening of chemical compounds and selecting Favipiravir as the ligand against target protein VP1, Swiss Dock (online web-based docking server) was used for docking studies. The structure of the target protein was given in PDB format and that of ligand was given in MOL2 format. After giving the input files in required format the docking results were mailed directly to E-mail ID. After receiving docking results the UCSF Chimera software was used for further structural analysis of binding clusters.

In case of Favipiravir the binding energy, i.e., Δ **G was found to be: -6.473245**(Table 4.1)

Table 4.1: Docking energies of Favipiravir:

Energy: 9.13592	Simple Fitness: 9.13592	Full Fitness: -1561.9479
InterFull: -39.2251	IntraFull: 51.3073	solvFull: -1777.36
surfFull: 203.33	extraFull: 0.0	ΔGcompsolvpol:-1777.36
ΔGcompsolvnonpol: 203.33	Δ Gprotsolvpol:-1793.56	Δ Gprotsolvnonpol: 204.942
Δ Gligsolvpol: -13.3645	Δ Gligsolvnonpol: 4.53919	Δ Gvdw: -39.2251
Δ Gelec: 0.0	**deltaG: -6.473245**	Cluster: 18
Cluster Rank: 5		

The docking results downloaded from the Swiss Dock server were viewed in UCSF Chimera, showing ligand and target separately before docking (Fig. 4.6), and ligand bound to the target in 'Palm' subdomain after docking (Fig. 4.7; 4.8).A separate window was generated in Chimera showing docking energies of ligand (Favipiravir) in numeric values (Fig. 4.9).

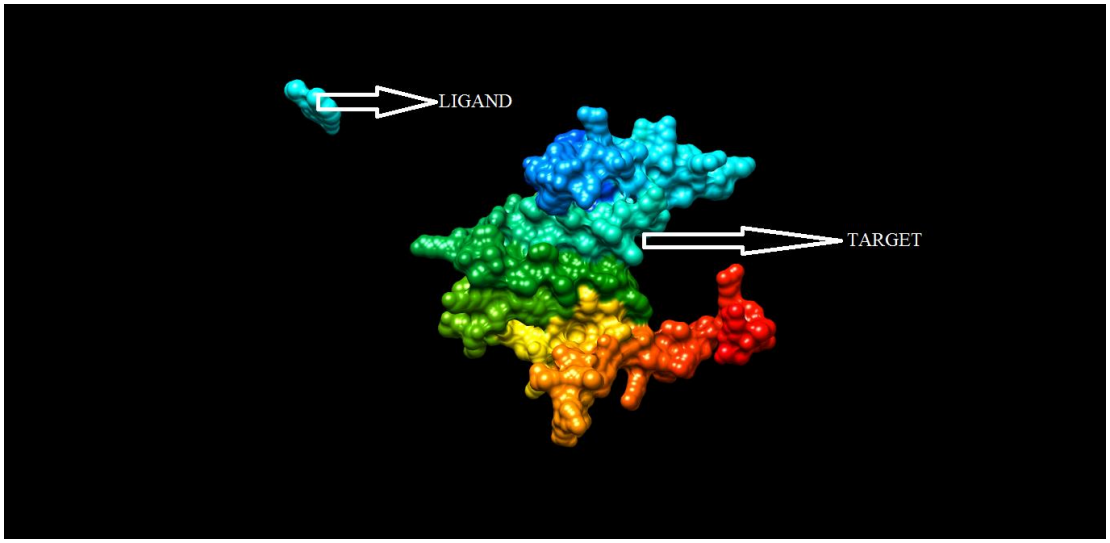


Figure 4.6:Chimera window showing ligand and target separately before docking

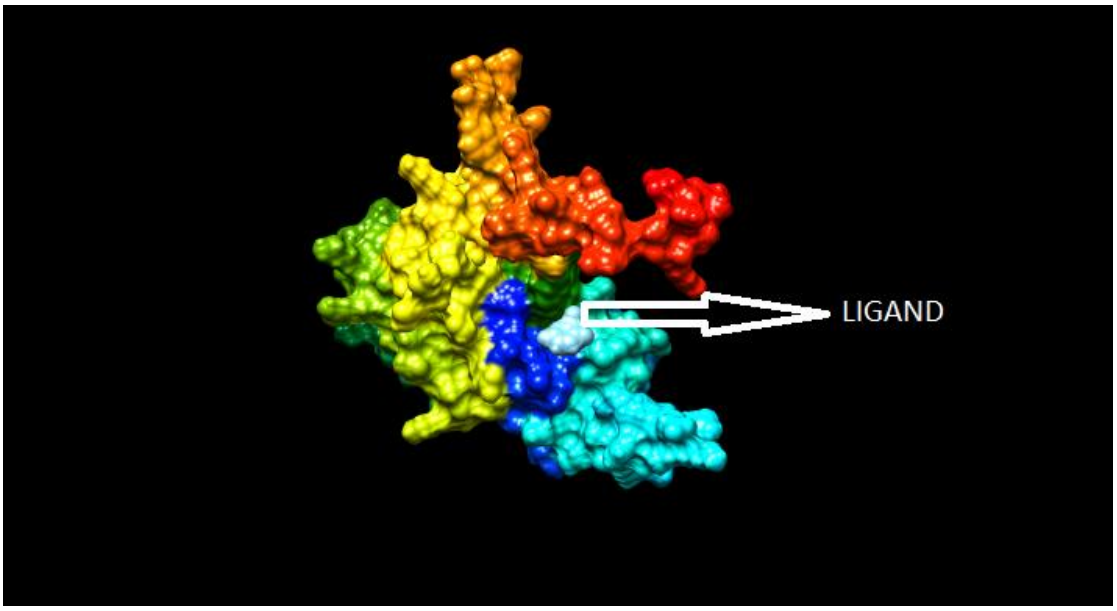


Figure 4.7: Ligand bound to target in 'Palm' subdomain after docking (top view)

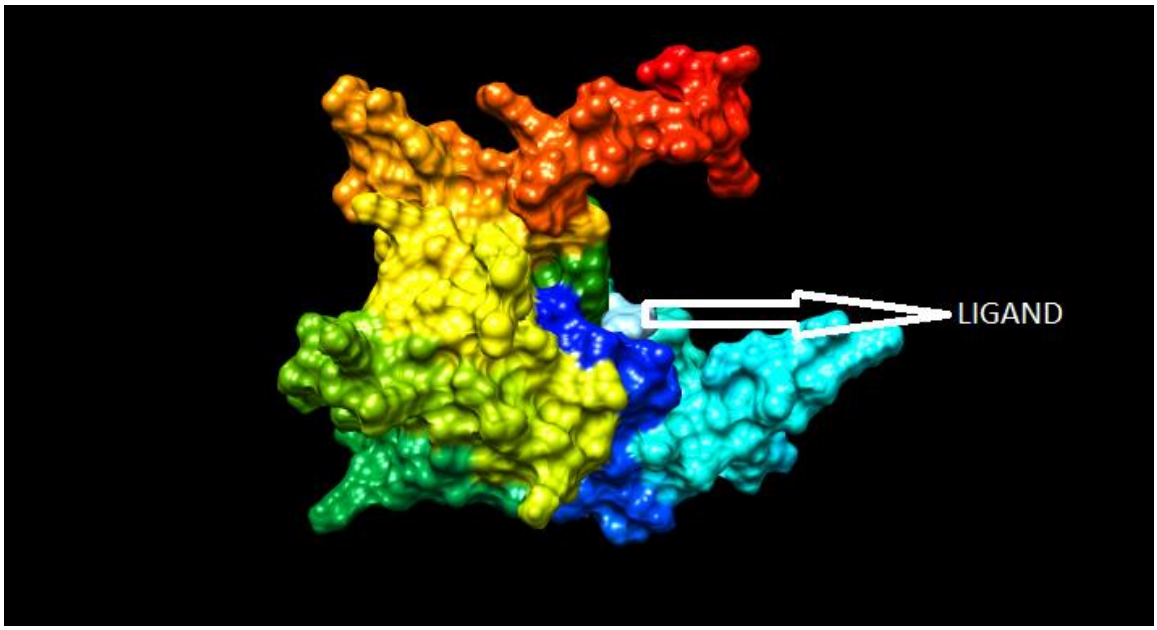


Figure 4.8: Ligand bound to target in 'Palm' subdomain after docking (lateral view)

```
Energy: 9.13592
SimpleFitness: 9.13592
FullFitness: -1561.9479
InterFull: -39.2251
IntraFull: 51.3073
solvFull: -1777.36
surfFull: 203.33
extraFull: 0.0
deltaGcompsolvpol: -1777.36
deltaGcompsolvnonpol: 203.33
deltaGprotsolvpol: -1793.56
deltaGprotsolvnonpol: 204.942
deltaGligsolvpol: -13.3645
deltaGligsolvnonpol: 4.53919
deltaGvdw: -39.2251
deltaGelec: 0.0
deltaG: -6.473245
Cluster: 18
ClusterRank: 5
```

Figure 4.9: Docking energies for ligand (Favipiravir) in their numeric values

4.4 BAS 01884755 structure determination and docking

After obtaining positive results from docking of Favipiravir with VP1, a similarity search was carried out to find analogs similar to Favipiravir in ZINC and PubChem databases. An extensive work of docking with the target was carried out with various analogs, but the binding energy was not found at par with Favipiravir except for few compounds. BAS 01884755 (methyl 5-acetyl-6-methyl-2-oxo-1H pyridine-3-carboxylate) PubChem CID: 2289888 was one compound whose binding energy was found to be more than that of the Favipiravir.

2-D structure ofBAS 01884755 (methyl 5-acetyl-6-methyl-2-oxo-1H pyridine-3-carboxylate) PubChem CID: 2289888, was viewed in Chimera (Fig. 4.10). Force fields of BAS 01884755in mesh topology (Fig. 4.11)or in solid surface topology (Fig. 4.12)were also generated by using UCSF Chimera.

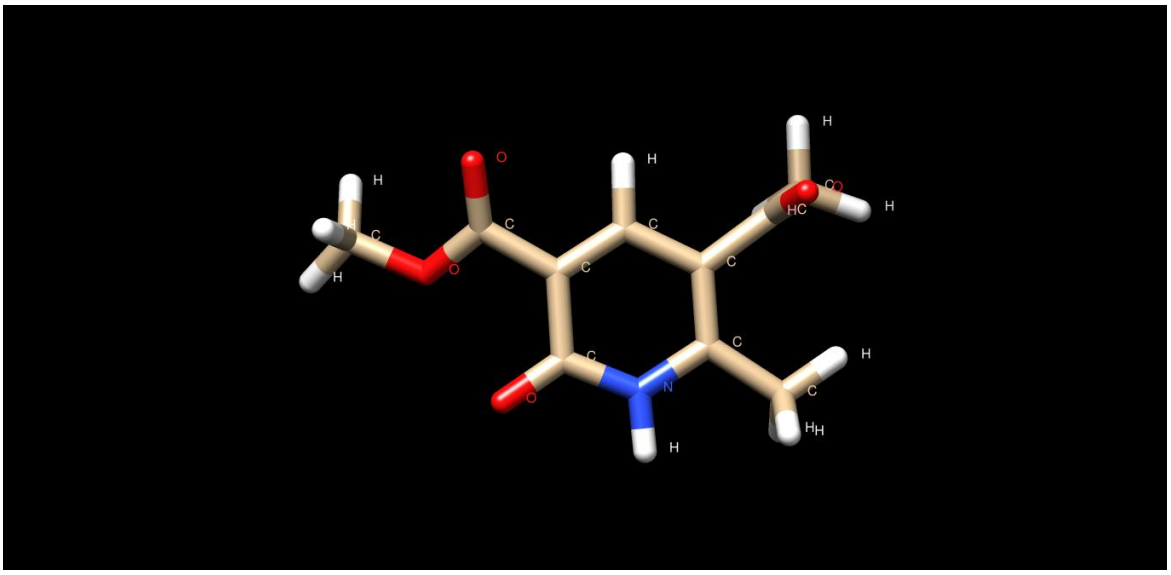


Figure 4.10: Two-dimensional structure of BAS 01884755 (methyl 5-acetyl-6-methyl-2-oxo-1H pyridine-3-carboxylate) PubChem CID: 2289888

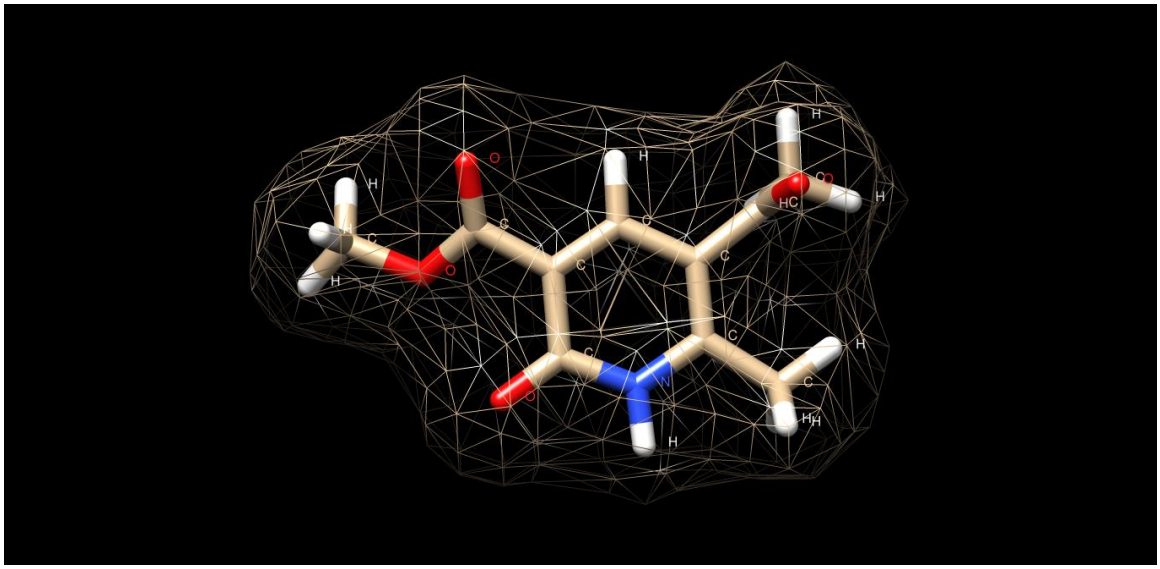


Figure 4.11: Force fields of BAS 01884755 shown in mesh topology

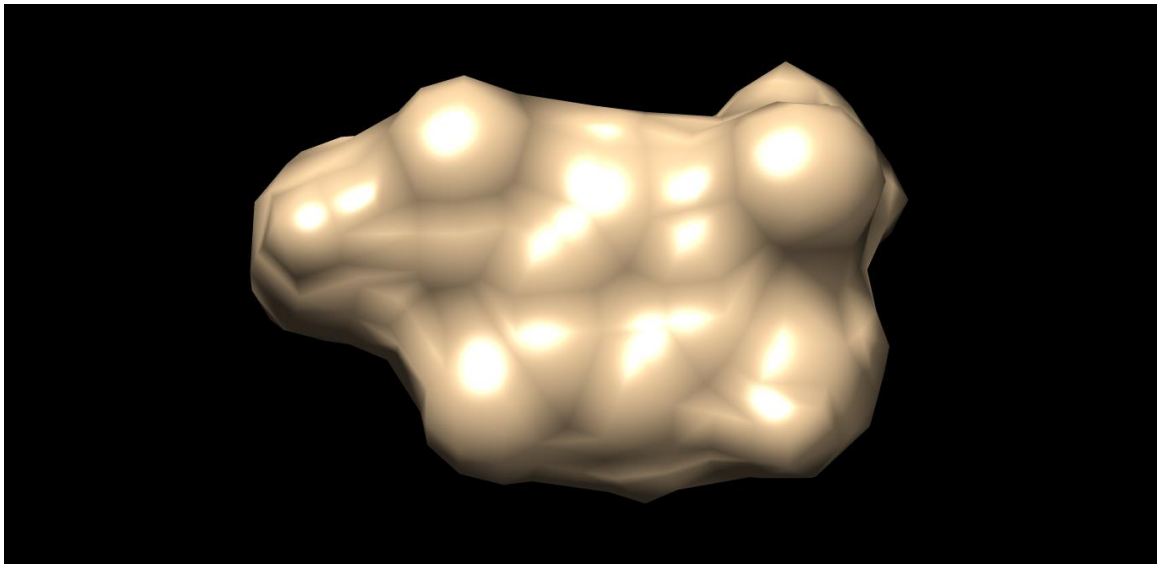


Figure 4.12: Solid surface topology of BAS 01884755.

In case of BAS 01884755the binding energy i.e. Δ **G was found to be: -6.9730735**(Table 4.2)

Table 4.2:Docking energies of BAS 01884755

Energy: 7.21061	Simple Fitness: 7.21061	Full Fitness: -1600.0167
InterFull: -33.3203	IntraFull: 21.3995	solvFull: -1791.94
surfFull: 203.844	extraFull: 0.0	
ΔGcompsolvpol: -1791.94	ΔGcompsolvnonpol: 203.844	ΔGprotsolvpol: -1793.56
ΔGprotsolvnonpol: 204.942	ΔGligsolvpol: -11.7679	ΔGligsolvnonpol: 5.77641
ΔGvdw: -33.3203	ΔGelec: 0.0	Δ **G: -6.9730735**
Cluster: 0	Cluster Rank: 0	

The docking results for BAS 01884755 downloaded from the Swiss Dock were viewed using UCSF Chimera, showing ligand bound to the target in 'Fingers' subdomain after docking (Fig. 4.13).A separate window was generated in Chimera showing docking energies of ligand (BAS 01884755) in numeric values (Fig. 4.14).

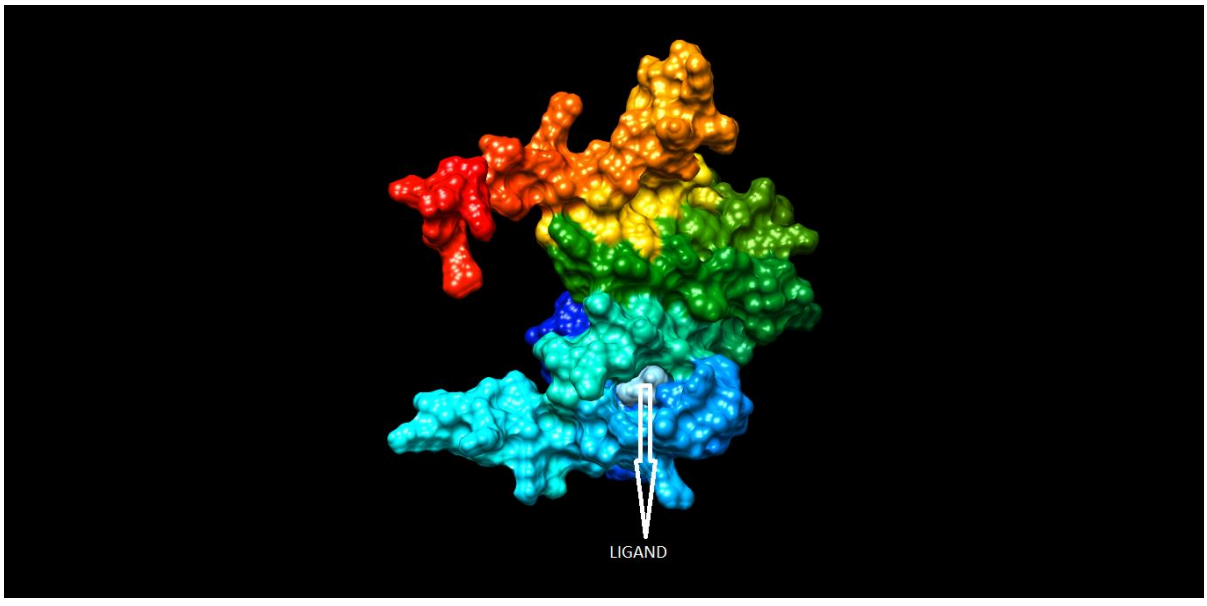


Figure 4.13: Ligand (BAS 01884755) bound to target in 'Finger' subdomain after docking

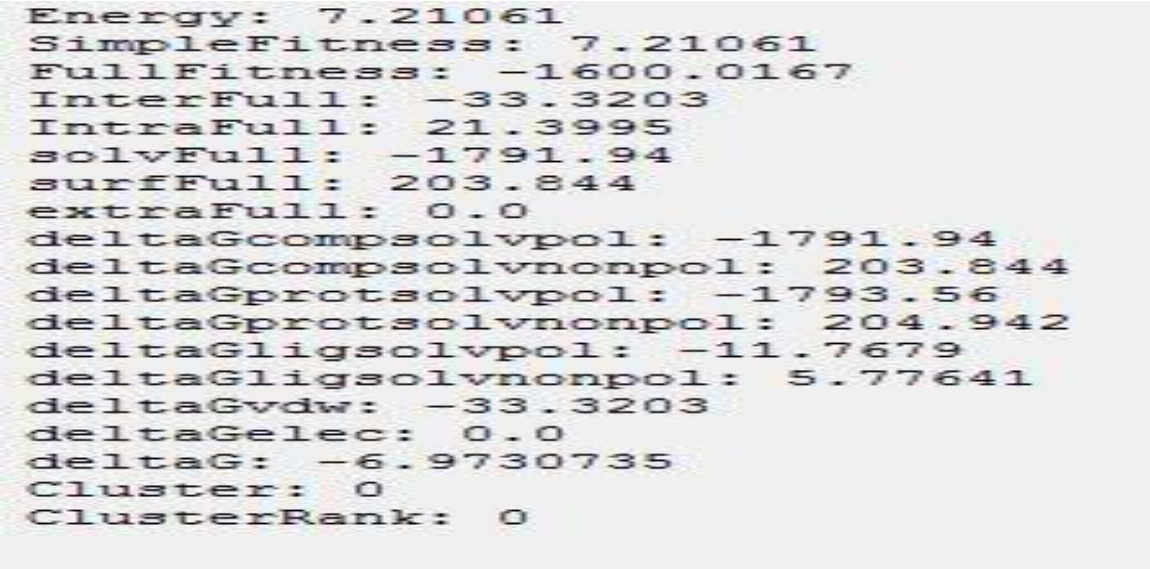


Figure 4.14: Docking energies of ligand (BAS 01884755) in their numeric values

CHAPTER 5

DISCUSSION AND FUTURE PERSPECTIVE

Bluetongue disease is highly infectious, vector-borne viral disease of ruminants having a serious economic impact mainly due to high morbidity, mortality and mandatory trade barrier on the movement of bluetongue infected livestock and germplasm. BT is gaining more importance and posing newer challenges day by day. Unnatural host like canines also are contracting bluetongue infections. Many species of *Culicoides* have been reported to spread infections. Recently bluetongue has been categorized as 'multispecies disease' by OIE (2011) while earlier it was listed as 'notifiable' disease of sheep by OIE.

In the present study, an intensive computational biology approach to predict suitable drug compounds for treating Blue Tongue disease caused by Blue Tongue virus in cattle is presented. In the whole research work the main task was the targeting of VP1 protein which is not only a structural protein for the virus but also acts as its RNA polymerase. For this purpose various drug compounds (ligands) were used to carry out docking studies and estimating their Gibbs free energies (ΔG) against VP1.

To get an idea which of the ligands might bind the receptor stronger, it is required to compare the lowest ΔG values for different ligands. The lower ΔG value indicates the stronger probability of a ligand to bind its target receptor. After screening various chemical compounds followed by their docking with target protein (VP1), two chemical compounds namely: Favipiravir having binding energy (ΔG) = -6.473245 and BAS 01884755 having binding energy (ΔG) = -6.9730735, have been found to efficiently bind with the target protein in the crucial 'palm' and 'fingers' subdomains. These subdomains are responsible to carry out divalent cation co-ordination and binding of NTPs in RNA to carry out its replication and transcription. Binding of any ligand or chemical compounds in these subdomains may result in induction of conformational changes in the structure of RNA polymerase making it non-functional or blocking of the NTPs incorporation into nascent RNA. Both these possibilities make VP1 of BTV non-functional and results in the containment of Bluetongue disease.

It is suggested that the selective targeting of the RdRP may provide an effective way of treating Blue Tongue disease. The findings of the present study may, however, be confirmed by experimental validation.

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