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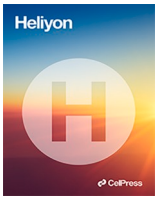

Research article

# In-silico characterization of LSDV132 protein divulged its BCL-2-like nature

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ABSTRACT

Lumpy skin disease virus (LSDV) belongs to *Poxviridae* family. This virus possesses various proteins which impart potential functions to it including assembly of newly synthesized viruses in the replication cycle and forming their structure. LSDV132 protein is also one of such proteins. Its key characteristics were unknown because, no any relevant study was reported about it. This study aimed to investigate its characteristic features and essential functions using several bioinformatics techniques. These analyses included physiochemical characterization and exploring the crucial functional and structural perspectives. Upon analysis of the physiochemical properties, the instability index was computed to be 30.89% which proposed LSDV132 protein to be a stable protein. Afterwards, the phosphorylation sites were explored. Several sites were found in this regard which led to the hypothesis that it might be involved in the regulation of apoptosis and cell signaling, among other cellular processes. Furthermore, the KEGG analysis and the analysis of protein family classification confirmed that the LSDV132 protein possessed Poxvirus-BCL-2-like motifs, indicating that it might be responsible in modulating the apoptosis of host cells. This crucial finding suggested that the protein under study possessed BCL-2-like features. Proceeding this very important finding, the molecular docking analysis was performed. In this context, various viral BCL-2 inhibitors were retrieved from the ChEMBL database for docking purpose. The docking results revealed that pelcitoclax exhibited best docking scores i.e., −9.1841 kcal/mol, among all of the other docked complexes. This fact signified that this compound might serve as an inhibitor of LSDV132 protein.

## 1. Introduction

Lumpy skin disease virus (LSDV), is a causative agent of Lumpy skin disease (LSD) [1]. This pathogen infects eukaryotic hosts,

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including cattle, sheep, and goats [2]. It belongs to the *Poxviridae* family. *Capripoxviruses* (CaPV) include sheep pox virus (SPPV) and goat pox virus (GTPV), known to be antigenically similar to LSDV, infecting sheep and goats [3]. LSDV entails 156 ORFs as putative genes, having a coding density of 95% and about 53–2025 amino acid sequences that encode their respective proteins [4]. The functions of most of the proteins are known which include the involvement in nucleic acid biogenesis, virion assembly, and formation of the virion structure [5]. Although all such putative genes have well-known functions but there are exceptions as well [6]. One of these genes named, 'Lumpy skin disease virus 132 (LSDV132)' encodes LSDV132 protein whose characteristic features are unknown [7]. LSDV132 was the first to be identified and isolated from Neethling 2490 (NI-2490) [8]. This protein is currently proposed to be an uncharacterized protein with 159 amino acids [9,10]. Furthermore, no study was reported which demonstrated the key features of this protein. Our research aimed to explore those crucial properties of LSDV132 protein which may assist in defining its characteristic features.

This research work was carried out using bioinformatics or *in-silico* approaches [11]. In this perspective, we explored multi-dimensional features of LSDV132 protein including the prediction of a three-dimensional (3D) and physiochemical characterization. The analysis of physiochemical properties indicated that LSDV132 was a stable protein. Whereas, to explore the functional perspectives of this protein, we incorporated KEGG analysis and protein family prediction by Pfam (http://pfam.xfam.org/), which is now hosted by InterPro (https://www.ebi.ac.uk/interpro/) [12,13]. The results of these analyses suggested that the given protein possessed a beta cell lymphoma-2 (BCL-2) like motifs [14]. This finding proposed that it may immunomodulate the apoptosis in the host cells by activating the mitochondrial pathway known as the intrinsic apoptosis pathway [15]. While, the apoptosis is the host cell's response to tackle such viral infections. It includes either the deprivation of the host cell's death or its activation [16]. Keeping in view this important fact, LSDV132 protein was further subjected to molecular docking analysis through virtual screening approach to screen out the potential inhibitors. In this regard, a library of 'viral BCL-2' inhibitors was constructed locally, in molecular operating environment (MOE) software [17] after their retrieval from ChEMBL database (https://www.ebi.ac.uk/chembl/). Our results indicated that LSDV132 showed the best docking interaction with pelcitoclax which was one of the interacted inhibitors [18]. Due to good docking results and the possession of anti-BCL-2 activity, this compound may be proceeded for computer-aided drug design for further validations. This will assist to particularly design a potent drug against LSDV132 protein which may inhibit the immunoregulation characteristic of this protein in the host cells.

## 2. Materials and methods

### 2.1. Sequence retrieval

The sequence of LSDV132 protein was retrieved from National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). The reference sequence i.d of this protein was NP_150435. This protein comprised of 159 amino acids which was submitted as a hypothetical protein (HP) of Lumpy skin disease virus, isolated from Neethling 2490 (NI-2490).

### 2.2. Analysis of physiochemical properties

The ExPASy ProtParam online tool (http://expasy.org/tools/protparam.html) was employed for physiochemical characterization of this protein. Various crucial parameters were evaluated in this regard including, theoretical isoelectric point (PI), extinction coefficient, instability index, aliphatic index, grand average of hydropathy (GRAVY) and a total number of positive and negative residues. The aliphatic index, which was one of the computed properties, was highly significant since, it is related to those amino acids which contain aliphatic side chains (A, V, I, and L). Moreover, it also defines the thermostability of the proteins [19].

### 2.3. Secondary structure prediction

Self-Optimized Prediction Method with Alignment (SOPMA) (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) tool was incorporated for the prediction of the secondary structure of LSDV132 protein [20]. It predicted the total number of alpha helices, beta sheets, coils, and turns in the amino acid sequence of the protein under study. The computed results were represented with various colors so that their respective percentages could be assessed conveniently. SOPMA incorporates various parameters in order to predict the secondary structure of the given protein. These parameters included the width of the window, similarity threshold and the number of states, while, their values were set to 17, 8 and 4, respectively.

### 2.4. Three dimensional (3D) structure prediction

To predict the 3D structure of LSDV132 protein, SWISS-MODEL server was applied (http://swissmodel.expasy.org/qmean/cgi/index.cgi) [21]. It is a fully automated online server in which a 3D protein structure is predicted through a homology-modelling approach. This particular server subjects the given amino acid sequence to the target-template sequence alignment. On the basis of the alignment scores, various models are predicted. Different parameters are considered in this context including sequence identity, GMQE score and coverage. In the current study, the model with the required GMQE score and highest sequence identity was selected to proceed for the subsequent analyses.

### 2.5. Model refinement

The predicted protein models often contain extra rotamers, which hinder their ability to bind with other interacting molecules. In such scenarios, model refinement is always required. The DeepRefiner server (http://watson.cse.eng.auburn.edu/DeepRefiner/) was employed to accomplish this task. This online tool process the given.pdb format file by applying the deep learning models, especially the deep neural network models for model refinement.

### 2.6. Model validation

The predicted three dimensional (3D) model of LSDV132 protein was further stereochemically analyzed after the refinement process so that its quality could be estimated. In this context, the Ramachandran plot was incorporated which is available at PROCHECK online server (https://saves.mbi.ucla.edu/). This particular method assessed the given structure, based on its stereo-chemical properties. On the basis of these properties of amino acid residues of the given protein model, one of the four regions are allocated. These regions include favored, additionally allowed, generously allowed and disallowed regions. The residues with least or no steric hindrances are allocated in allowed region while, the ones with the most steric hindrances are allotted with the disallowed region. There are four main quadrants in Ramachandran plot in which red color indicates the most favorable regions, the yellow region corresponds to the allowed areas and white indicates the disallowed region. In our study, most of the amino acid residues were placed in the favored region which signifies the good quality of the predicted model.

### 2.7. Pockets prediction

For the prediction of pockets in LSDV132 protein, the Computed Atlas of Surface Topography of proteins (CASTp), an online web server (http://sts.bioe.uic.edu/castp/) [22] was used. The pockets on the surface of protein are concavities on which solvent (probe sphere 1.4A) can have its access and where the residues interact with the probe. Various pockets/active sites were predicted which comprised of different area ($Å^2$) and volume ($Å^3$) sizes. Moreover, different colors were opted for all of the pockets for discrimination.

### 2.8. Prediction of phosphorylation sites and transmembrane helices

The prediction of the phosphorylation sites and transmembrane helices was achieved by utilizing the NetPhos 3.1 server https://services.healthtech.dtu.dk/services/NetPhos-3.1/ [23] and TMHMM- 2.0 server https://services.healthtech.dtu.dk/service.php?DeepTMHMM [24], respectively. Various phosphorylation sites were predicted while, no transmembrane helices were found in the protein under study.

### 2.9. KEGG analysis and protein family prediction

Since, LSDV132 protein is an uncharacterized protein and there was no annotated data found regarding it on any of the databases, therefore, to predict its key functions, KEGG (https://www.genome.jp/kegg/pathway.html) analysis was executed. Moreover, in order to get to know the relevant protein family of LSDV132 protein, InterPro database (https://www.ebi.ac.uk/interpro/) was employed. KEGG analyzed the given name of the required gene or protein to search for the relevant entries in its database to produce necessary results. While, InterPro required the FASTA amino acid sequence to generate the desired results.

### 2.10. Molecular docking analysis and virtual screening

The docking analysis was performed by utilizing the molecular operating environment (MOE) software which is a well-known platform for docking and virtual screening analyses. A library comprising of viral BCL-2-like inhibitors/ligands was constructed in MOE for virtual screening purpose. The individual files of all these ligands were retrieved from ChEMBL database (https://www.ebi.ac.uk/chembl/) in structure data format (.sdf). Furthermore, the protein was initially annotated to prepare for docking analysis. In this regard, the energy of the receptor protein (LSDV132) was minimized by using the energy minimization tool of MOE. Afterwards, the polar hydrogen atoms were added followed by identifying different active sites through the 'site finder' function of MOE. Consequently, different sites having variable number of amino acid residues were found while the largest one which comprised of 113 amino acids, was selected. This particular selection was made in order to provide an enough room to the interacting ligands for their different poses so that the best docked orientation could be achieved. As far as the other parameters of docking are concerned, the induce-fit refinement model was opted for flexible docking. Moreover, the number of poses of ligands was set to more than one whereas, the London dG scoring method was selected for this particular analysis.

### 2.11. Post-processing of the selected docked complex

The docking interactions were inferred by incorporating BIOVIA Discovery Studio Visualizer (*ver. 2021*). In this particular processing, the two dimensional (2D) and three dimensional (3D) docking interactions were inferred.

The overall methodology which is followed in this study is also graphically demonstrated in Fig. 1.

## 3. Results

### 3.1. Physiochemical properties analysis

The computed physiochemical properties of LSDV132 protein prophesied the theoretical (isoelectric point) pI = 6.58, which showed that the protein was acidic. Further, its molecular weight, amino acid residues, and estimated half-life were also calculated to be 17857.53 Da, 159, and 30 h (mammalian reticulocytes in vitro), respectively. Other important computed parameters are summarized in the following Table 1. The stability index was found to be 30.89% which suggested that LSDV132 protein was structurally stable.

### 3.2. Secondary structure prediction

The predicted secondary structure exhibited an interesting blend of the basic components with different percentages. The results represented that LSDV132 protein possessed alpha helices and coils with comparatively higher percentages of 50.31% and 28.30%, respectively. Moreover, the extended strands and beta turns were also found but with comparatively lower percentages i.e., 12.58% and 8.81% respectively. In Fig. 2, the respective colors represent the location of all of the secondary structure components in two different forms, including bars and peaks at their respective sites.

### 3.3. Homology modelling and refinement of the predicted model

The results of predicted three-dimensional (3D) model of LSDV132 proteins suggested that it showed homology with 3 models. Hence, to choose the best structure, the selection was made on the basis of sequence identity and global model quality estimate (GMQE) score. The first model with a GMQE score of 0.57 was selected for further analyses. It also exhibited a sequence identity 20.27%, with the highest sequence coverage among all of the predicted models. Furthermore, the predicted protein structure of LSDV132 protein was refined by incorporating the DeepRefiner server. This online tool works on the principle of deep neural network models. Hence, it processed the.pdb format protein structure and presented the final results [25]. The results of this analysis are presented in Fig. 3. Afterwards, this model was subjected for further validation.

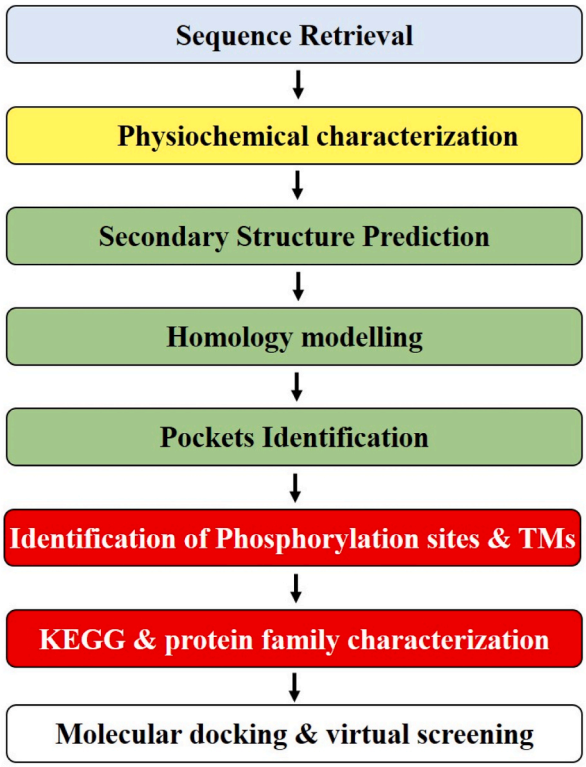



**Fig. 1.** A schematic workflow of this study which illustrates the analysis performed to explore the major attributes of LSDV132 protein.

**Table 1**
Important attributes regarding the physiochemical properties of LSDV132 protein.

| | |
|---|---|
| Aliphatic index | 101.13 |
| Ext. coefficient | 18575 |
| Grand average of hydropathicity (GRAVY) | 0.077 |
| **Instability index** | 30.89% |

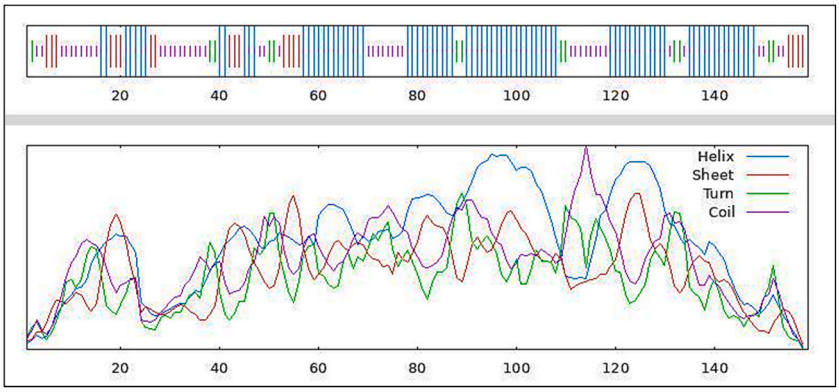



**Fig. 2.** Secondary structure of LSDV132 protein.

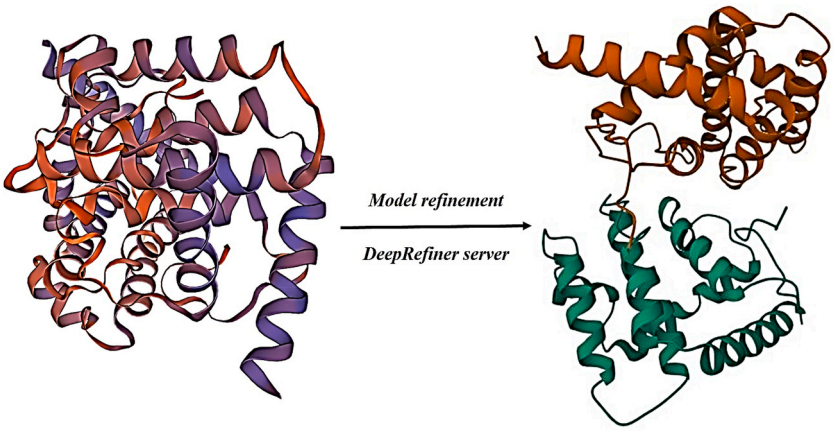



**Fig. 3a.** 3D predicted model of LSDV132 protein; **2b.** The refined model which was produced by the DeepRefiner server.

### *3.4. Model validation*

The validation of the predicted model was one of the crucial factors in this regard. This process imparted confidence in terms of the quality of the 3D model of LSDV132 protein. For this purpose, the Ramachandran plot which is available, on PROCHECK server, was employed [26]. The results recommended that most of the residues in the predicted and refined structure of LSDV132 protein were lying in the most favorable region constituting 93.6% of the total residues. These scores were sufficient in ensuring the good quality of protein model. Moreover, some of the amino acid residues were allocated in the additionally allowed regions, i.e., 5.7%. This analysis supported the fact that the predicted model was qualitatively good and can be used in the subsequent analyses in this study. The combined results of Ramachandran plot are illustrated in Fig. 4.

### *3.5. Protein pockets prediction*

Furthermore, the predicted model was then proceeded for the identification of pockets or active sites. We found various actives sites upon this analysis which featured different area and volume sizes. The data regarding their respective area and volume sizes is summarized in Table 2. While, the amino acid residues in all of the chosen pockets/sites involved serine, glycine, asparagine, tyrosine,

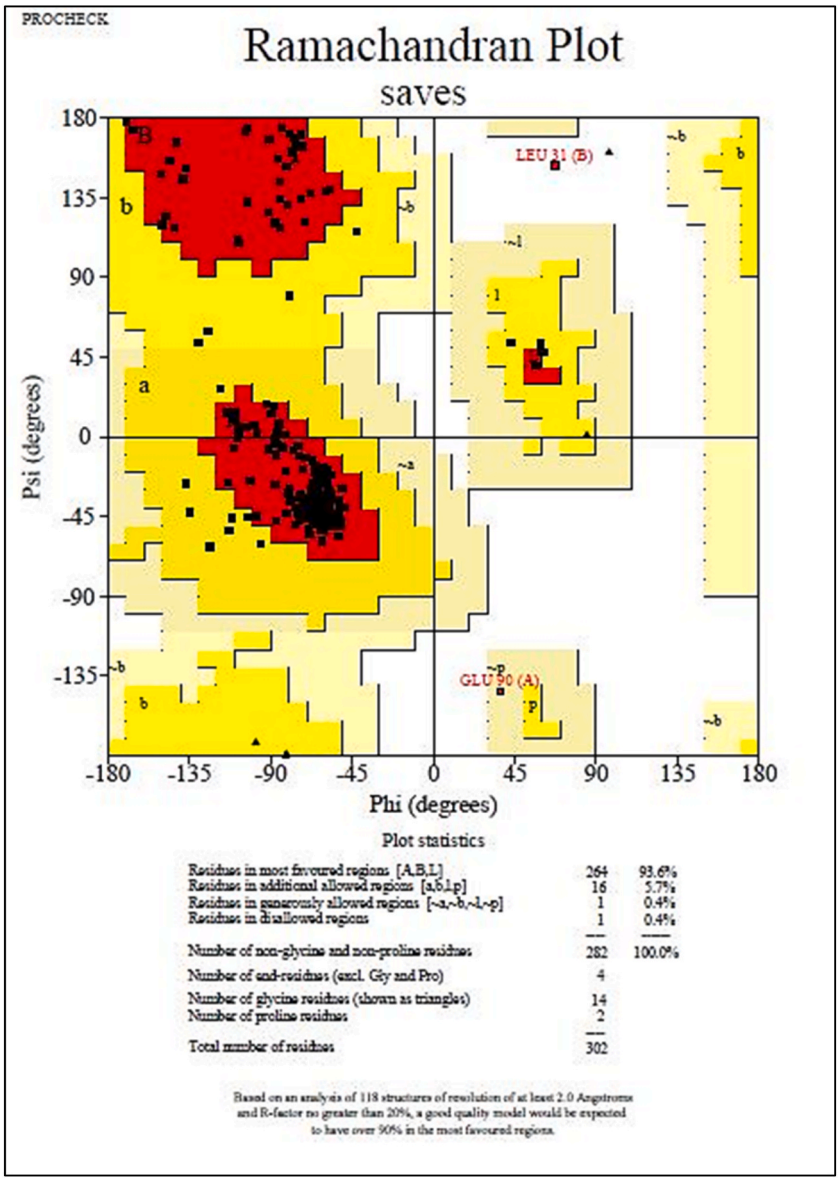



**Fig. 4.** Ramachandran plot showing the respective percentages of residues in favored regions, allowed and disallowed regions. 93.6% of LSDV132 protein's residues lie in favored region.

**Table 2**
Area and volume sizes in angstroms (Å) of all the selected pockets in LSDV132 protein.

| Pocket | Area | Volume |
|---|---|---|
| a | 614.352 $Å^2$ | 330.452 $Å^3$ |
| b | 189.471 $Å^2$ | 311.730 $Å^3$ |
| c | 173.571 $Å^2$ | 277.565 $Å^3$ |
| d | 138.317 $Å^2$ | 126.519 $Å^3$ |
| e | 121.319 $Å^2$ | 78.407 $Å^3$ |

valine, threonine, aspartate, phenylalanine, tryptophan, leucine, proline, isoleucine, glutamate, cysteine, lysine, and arginine. The largest among all of these pockets would usually be selected when intended to proceed the given protein for molecular docking. The composite Fig. 5 demonstrates all of the pockets (a: largest, b: second largest, c: third largest, d: fourth largest, e: smallest) in LSDV132 protein's structure, each is being represented with distinct colors.

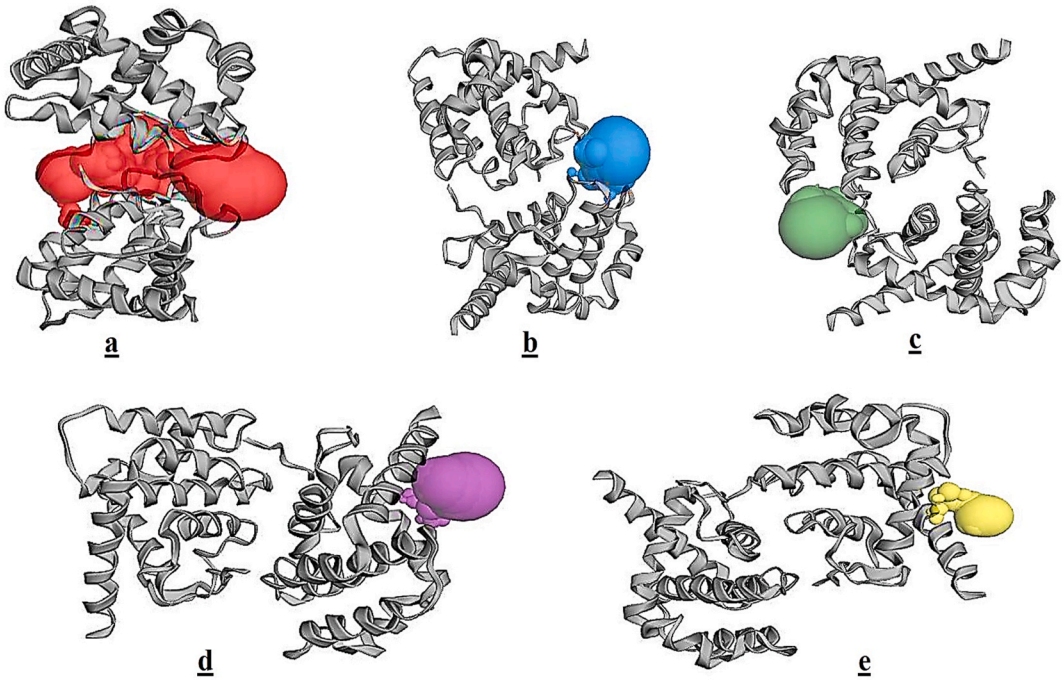



**Fig. 5.** Different pockets in 3D structure of LSDV132 protein, highlighted with respective colors. The figure 'a' indicate the largest pocket among all the selected ones while, figure 'e' represents the smallest one in terms of number of residues, area (Å$^2$) and volume (Å$^3$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

### *3.6. Phosphorylation sites and transmembrane (TM) helices*

The analysis of phosphorylation sites prediction exhibited various sites in the given amino acid sequence with relevance to a score of more than 0.5, predicting positive phosphorylation with some specific kinases such as PKA, unsp, CKII, cdc2, DNAPK, PKC. While, the results suggested that LSDV132 protein possessed 19 phosphorylation sites which are represented with different colors in Fig. 6. Since, the phosphorylation sites are involved in different cellular activities, including the regulation of apoptosis [27], therefore, it was hypothesized that LSDV132 protein might be responsible for the regulation of apoptosis in host cells. This fact was later validated in KEGG analysis and protein family prediction analysis, as well. Furthermore, no transmembrane helices found in this protein in the respective analysis. This fact suggested that the protein under consideration does not play any role in signal transduction or any other relevant processes during the viral replication process. The plot in Fig. 7 shows the posterior probabilities of the given protein sequence.

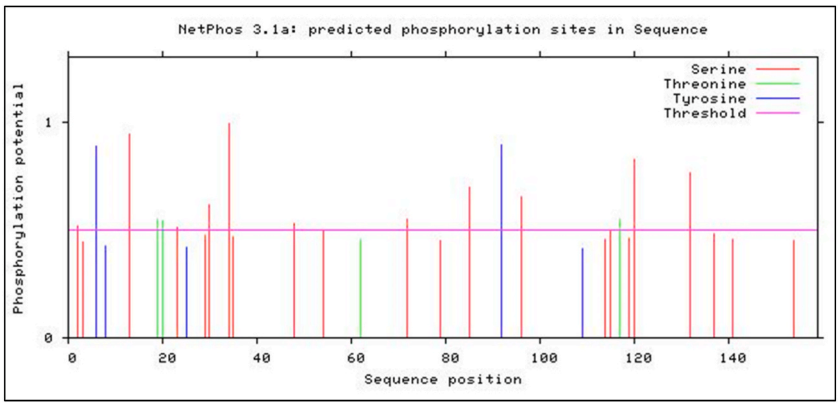



**Fig. 6.** Phosphorylation sites in different residues of LSDV132 protein.

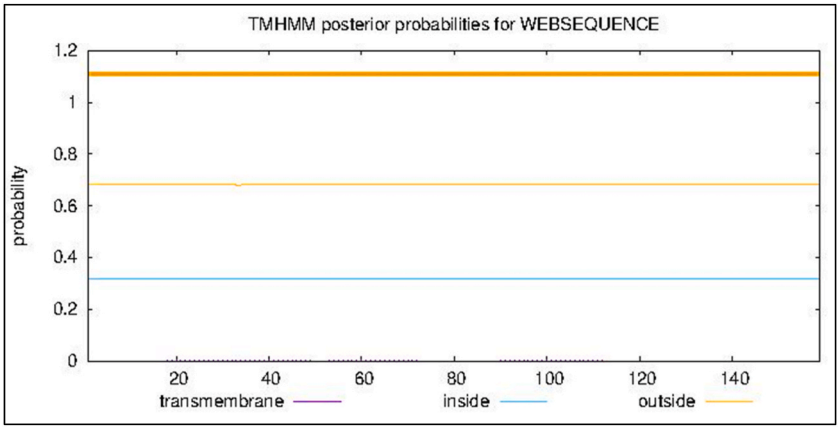



**Fig. 7.** The plot represents that there were zero TMs found in LSDV132 protein.

### *3.7. KEGG analysis and protein family characterization*

KEGG is a database encompassing the functions of genes and genomes. Moreover, it also focuses on representing various crucial biological pathways regarding any query e.g. an enzyme, a protein, etc. [28]. In the current perspective, the KEGG was incorporated to predict the characteristic function of our protein of interest. The results of these analyses suggested that LSDV132 protein possessed Poxvirus BCL-2-like motifs. These were the crucial findings in this study because the presence of beta cell lymphoma-2 (BCL-2) like motifs indicates that this protein may function as immunomodulator of apoptosis in host cells [29]. In various perspectives, it has been studied that the host/infected cells incorporate cell death as a defensive strategy to overcome a particular infection. Relevant to this specific fact, it has been reported that BCL-2 proteins can function in both ways i.e., either the activation of apoptosis or may also act as anti-apoptotic agents [30,31]. On such grounds, it was then deduced from these results that LSDV132 protein might function like a BCL-2 protein by either triggering or declining the apoptotic activity in host cells. The results of KEGG analysis and InterPro can be observed in Figs. 8 and 9, respectively in which the relevant parameters regarding LSDV132 protein are demonstrated.

KEGG **Viruses: 921665** Help

| | |
|---|---|
| Entry | 921665 CDS T40000 |
| Symbol | LSDV001, LSDVgp001 |
| Name | (RefSeq) Lumpy skin disease virus NI-2490; hypothetical protein |
| Virus | 376849 Lumpy skin disease virus NI-2490 [Taxonomy] |
| SSDB | [Ortholog] [Paralog] [GFIT] [Voc] |
| Motif | Pfam: Poxv_Bcl-2-like [Motif] |
| Other DBs | NCBI-GeneID: 921665<br>NCBI-ProteinID: NP_150435<br>RS: NC_003027<br>UniProt: Q910J8 |
| Position | NC_003027:complement(234..713) |
| AA seq | 159 aa [AA seq] [DB search]<br>MSSGNYVYRNNFSDDDDITTAISDYLFWSSLAFSSREVAGKVFLVFESFKKDASLVFGND<br>LTAFVKNMFLDSKIGFEQSKIMINSMLKKENYIRESCAVIGILARAAEYWGGESSPTCSS<br>VKVLVLLRDLVSDNDISLVKSALIIRLKRLNEKSIHLQV |
| NT seq | 480 nt [NT seq]<br>atgtcttccggcaactatgtctaccgaaacaacttttcagatgatgatgatatcacaacc<br>gccatctctgattatttgttttggtcatcactggcattttcctccagggaggttgctgga<br>aaggtgttcttagtttttgaatctttcaaaaaggatgcctcacttgtatttggaaatgat<br>ctaacagcttttgtcaaaaacatgtttttggattctaaaattggatttgaacaatcaaaa<br>attatgattaattccatgttaaaaaaagaaaattacattagggaatcatgtgcagtgatt<br>ggcattttagcaagagcagcagaatattggggtggtgaatcatctccaacttgttcttct<br>gtgaaagtgttggtattgcttcgagacctcgtttctgacaacgatatttcgctagtgaaa<br>tcagcactaataattagacttaaaagattgaatgaaaaaagtatccatttacaggtttaa |

**Fig. 8.** KEGG results showing various resultant parameters regarding LSDV132 protein. It can also be visualized that this protein possesses the *Poxv_Bcl-2-like* motifs.

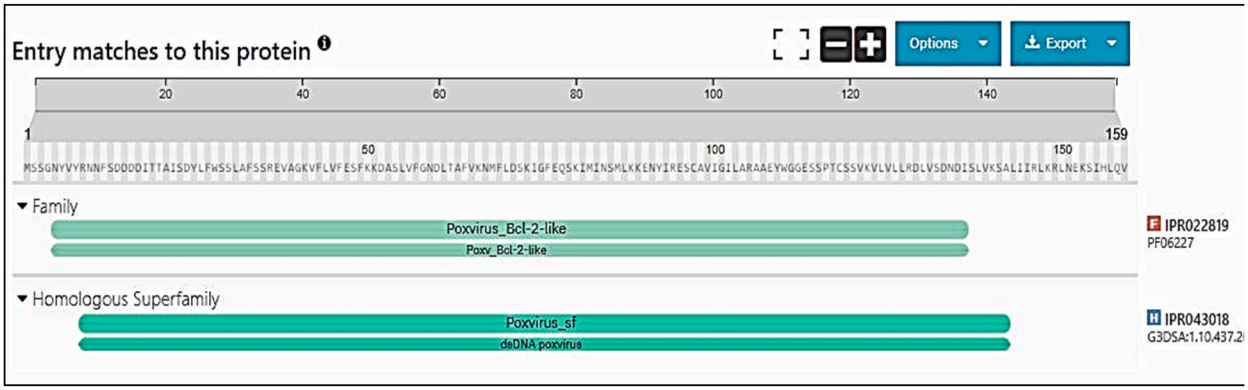



**Fig. 9.** InterPro results, which represented that LSDV132 protein possessed Poxvirus-BCL-2-like motifs.

### *3.8. Molecular docking and virtual screening of inhibitors*

Proceeding further the findings of KEGG analysis which suggested that LSDV132 was a BCL-2 like protein, the molecular docking analysis was incorporated. In this context, it should be considered that LSDV132 is a viral BCL-2 like protein which means that this protein may not function exactly like a eukaryotic BCL-2 protein. Therefore, only viral BCL-2 inhibitors were specifically retrieved from ChEMBL database (https://www.ebi.ac.uk/chembl/) [32]. All of these ligands/inhibitors are collectively summarized in Table 3, along their ChEMBL i.d's and names.

Before performing the analysis, MOE predicted various sites and the one with a maximum number of residues i.e., 113, was preferred to dock with inhibitors. Both chains (A & B) of the protein were incorporated to form the respective active site. The results of docking study revealed that pelcitoclax exhibited good docking energy score (S-score), i.e., −9.1841 kcal/mol. Moreover, the root mean square deviation (RMSD) value of LSDV132 protein with this inhibitor was also considerably good i.e., 2.74 Å. As shown in Fig. 11, this compound was observed to be quite occupied within the selected site of receptor protein. This signified that pelcitocalx may be a potential inhibitor against this protein, presenting that it could block its BCL-2 activity i.e., the immunoregulation of apoptosis in host cells. However, further relevant validations would be required to support this fact. Fig. 10 demonstrates the two-dimensional (2D) interaction between the selected active site and pelcitoclax. While, it can be more clearly visualized in Fig. 11a and b in which two different layouts are displayed to indicate the docking interactions. Fig. 11a showed the ribbons format of LSDV132 protein being docked with the pelcitoclax while, 11b presented the specific interacting atoms between the receptor (LSDV132 protein) and ligand (pelcitoclax).

## 4. Discussion

LSDV132 protein is one of the proteins in the lumpy skin disease virus (LSDV). The key features of this protein were unknown yet, because there was no reported data available about it. This study aimed to explore LSDV132 protein from various perspectives so that its core characteristics could be assessed. Initially, the physiochemical properties were analyzed which computed its pI value 6.58, suggesting that it possessed acidic nature. The instability index, calculated in this analysis was 30.89%, indicating that LSDV132 protein was structurally stable. A similar approach was followed in previous findings in which the physiochemical properties of hypothetical proteins (HPs) were assessed through the similar approach by using ProtParam tool [33].

Proceeding this protein further, the prediction of secondary structure prediction was performed by SOPMA which is a web-based online tool. Several other tools also exist to achieve the same task, but this particular tool computes more comprehendible and presentable results. For this purpose, the FASTA amino acid sequence was subjected to generate the required results. This particular analysis revealed that the protein being studied predominantly possessed alpha helices with 50.31%, compared to the rest of the components in its secondary structure. Other relevant studies have also reported similar approach to perform this task, specifically the one, in which Dengue 4 NS protease was investigated. It was described that the protease contained random coils with a higher percentage i.e., 41.26% [34]. Moreover, the TANK-Binding Kinase 1-Binding Protein 1 was also analyzed in a similar fashion which exhibited that it comprised mostly the alpha helices (45.37%), following the random coils with 43.41% in secondary structure, among other elements [35].

The next objective of this research was the prediction of its three-dimensional (3D) model so that its structural insights could be unveiled. Homology modelling approach was incorporated for this purpose. Whereas, the SWISS-MODEL online tool was employed to perform this analysis. This server applies the comparative modelling or homology modelling method for developing the 3D models of the given protein [36]. The results of this analysis enlisted three models with different scores of various parameters. We selected the first model on the basis of GMQE score and the percentage of sequence identity. It was found that GMQE and sequence identity scores of the selected model were 0.57 and 20.27%, respectively. The predicted 3D model of LSDV132 protein was comprised of two chains that can be designated as A-chain and B-chain. The envelope protein of Zika virus was also studied using the bioinformatics tools but specifically, utilizing the immunoinformatics approaches. As part of that research work, the 3D model of that envelope protein was also

**Table 3**
List of inhibitors found in ChEMBL database specifically for viral BCL-2 like protein.

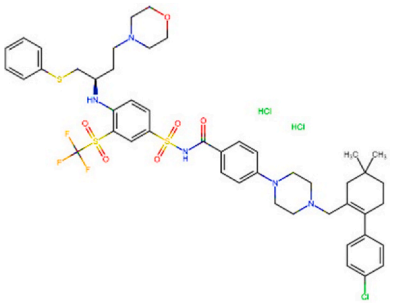
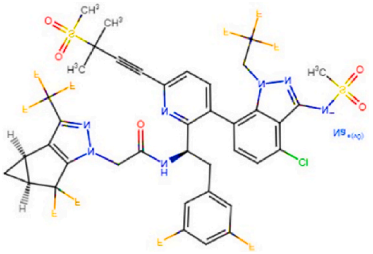
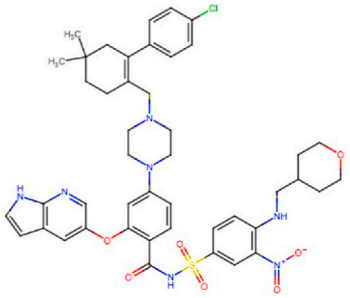
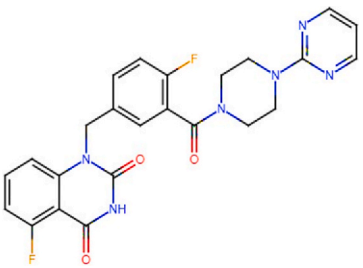
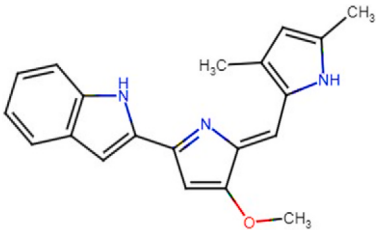
| Sr. no. | Molecular structure | ChEMBL_ID | ChEMBL_pref_name |
|---|---|---|---|
| 1. |  | ChEMBL2105690 | NAVITOCLAX DIHYDROCHLORIC |
| 2. |  | ChEMBL4802249 | LENACAPAVIR SODIUM |
| 3. |  | ChEMBL3137309 | VENETOCLAX |
| 4. |  | ChEMBL4802152 | PELCITOCLAX |
| 5. |  | ChEMBL408194 | OBATOCLAX |

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**Table 3** (*continued*)

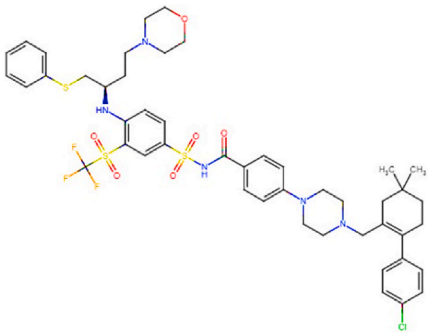
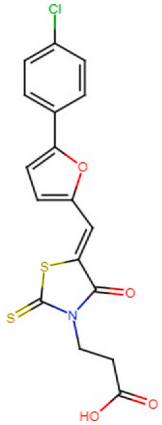
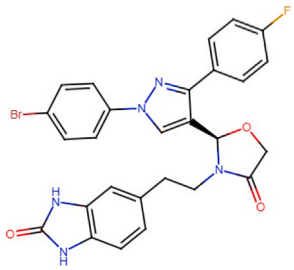
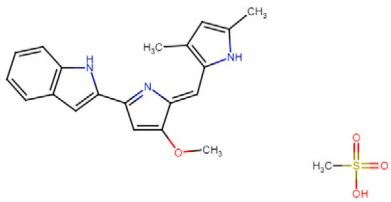
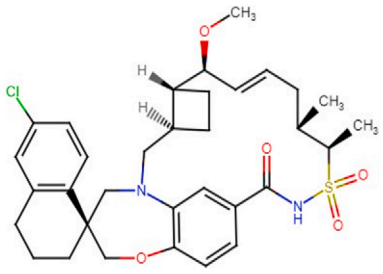
| Sr. no. | Molecular structure | ChEMBL_ID | ChEMBL_pref_name |
|---|---|---|---|
| **6.** |  | ChEMBL443684 | NAVITOCLAX |
| **7.** |  | ChEMBL200022 | CLAFICAPAVIR |
| **8.** |  | ChEMBL5095093 | CANOCAPAVIR |
| **9.** |  | ChEMBL2107358 | OBATOCLAX MESYLATE |
| **10.** |  | ChEMBL4446378 | TAPOTOCLX |

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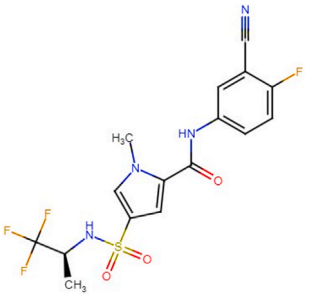
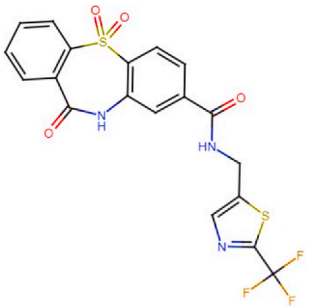
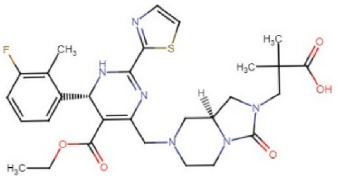
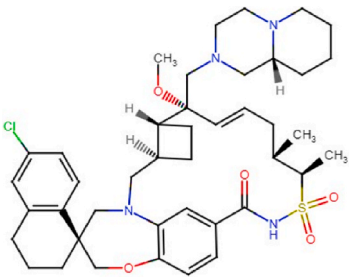
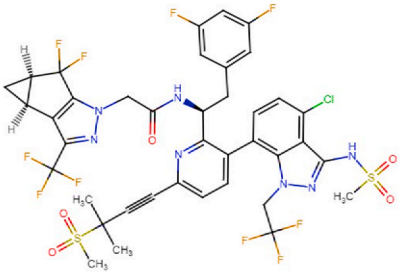
**Table 3** (*continued*)

| Sr. no. | Molecular structure | ChEMBL_ID | ChEMBL_pref_name |
|---|---|---|---|
| **11.** |  | ChEMBL4744254 | BERSACAPAVIR |
| **12.** |  | ChEMBL4650324 | VEBICORVIR |
| **13.** |  | ChEMBL5095071 | LINVENCORVIR |
| **14.** |  | ChEMBL4650225 | MURIZATOCLAX |
| **15.** |  | ChEMBL4594438 | LENACAPAVIR |

built using the SWISS-MODEL server. In that scenario, the selection of the model was made based on query coverage and sequence identity percentages that were 100% and 99.80%, respectively [37].

All of these analyses were some of the exploratory structural analyses of LSDV132 protein, which were done to feature its physical attributes. To learn about its characteristic functions, we analyzed it by exploring some of its functional sites. It included the prediction of phosphorylation sites and the identification of transmembrane (TM) helices. The results of both of these processes suggested that LSDV132 protein contained the phosphorylation sites but there were no TM helices found. It established the perception that due to the

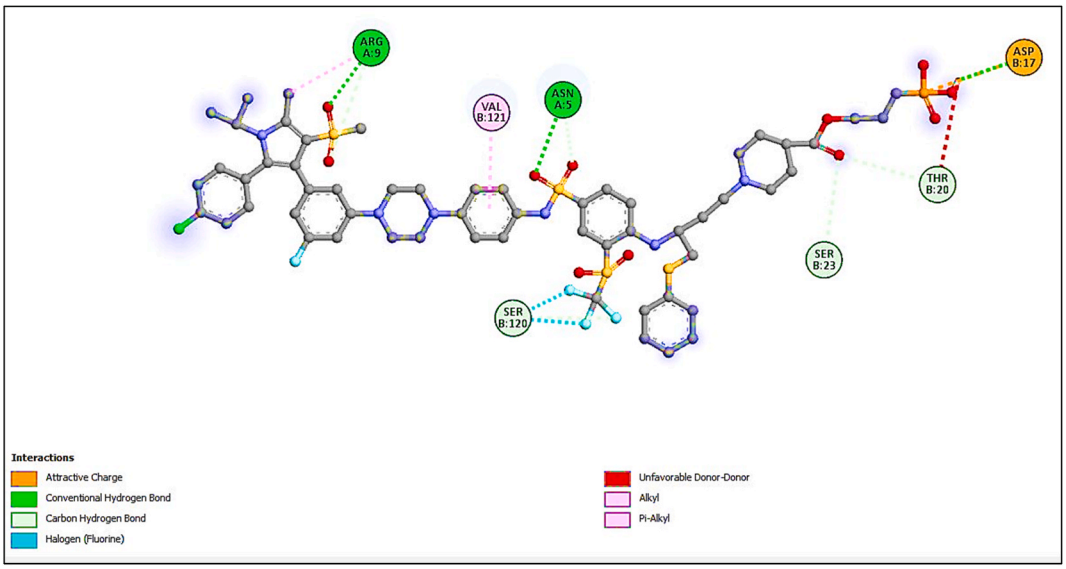



**Fig. 10.** 2D docking view, showing the co-crystallization of the pelcitoclax inhibitor within pocket of LSDV132 protein.

presence of phosphorylation sites, it may be involved in the regulation of various cellular activities in host. According to the reported studies, these phosphorylation sites play a vital role in the modulation of apoptosis, cell metabolism, gene expression, and differentiation [38,39]. On the contrary, the absence of TMs recommended that it could not be involved in modulating the transit of molecules, in and out of the host cells.

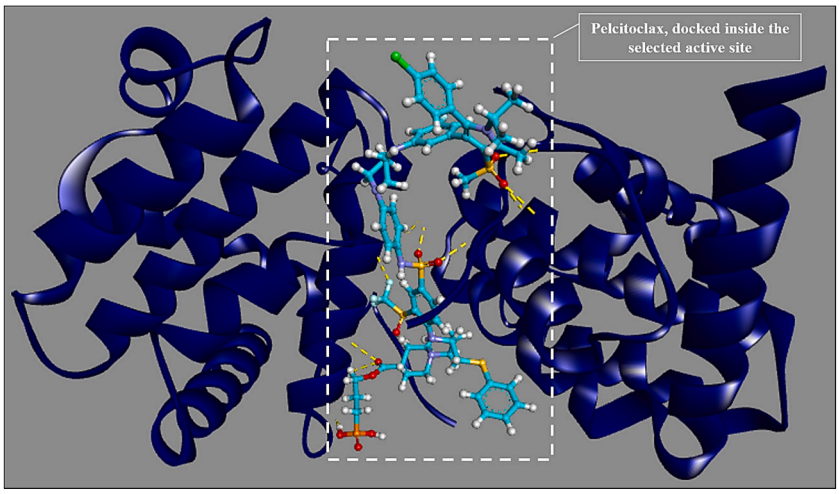
Afterwards, the KEGG analysis and Pfam analysis which is hosted by InterPro, were performed for protein's family prediction. The significance of this identification was to discover the key feature of this protein. The results of KEGG analysis revealed that this protein possessed Poxvirus-BCL-2-like motifs, thus, it might be involved in immunomodulation of apoptosis in host cells. The infected cells embody the apoptotic cell death to evade the spread of viruses to the neighboring cells [40]. To avoid this evasion, the viral BCL-2 protein regulates this activity in the individual host, accordingly. Subsequently, the InterPro also validated this fact, which suggested that LSDV132 was a BCL-2-like protein. This was one of the key findings of this study because it encompassed the characteristic nature of LSDV132 protein that was previously hypothesized in the prediction of phosphorylation sites.

Furthermore, the molecular docking analysis and virtual screening approach was employed to dock this protein with viral BCL-2 inhibitors, retrieved from ChEMBL database. In this regard, viral BCL-2 inhibitors were retrieved collectively as a library, in.sdf format. Molecular operating environment (MOE) was utilized for this particular purpose. MOE-assisted multiple active sites of LSDV132 protein were found. The largest among all of them were opted to furnish the interacting compound. This facilitation was provided so that every inhibitor could bind appropriately at the active site and the best docking orientation could be attained. The final results suggested that pelcitoclax, a member from the 'clax' category of chemical compounds, showed best score of −9.1841 kcal/mol. Multiple studies have reported that pelcitoclax tend to inhibit the BCL-2 activity [41–43]. Another scientific investigation also reported screening out the potentially pressing plant-based compounds such as Naringin, Quercetin, Capsaicin, Psychotrine, and Gallic acid through an analogous approach. It was reported that these compounds could be tested against Coronavirus for the development of effective antiviral drugs [44].

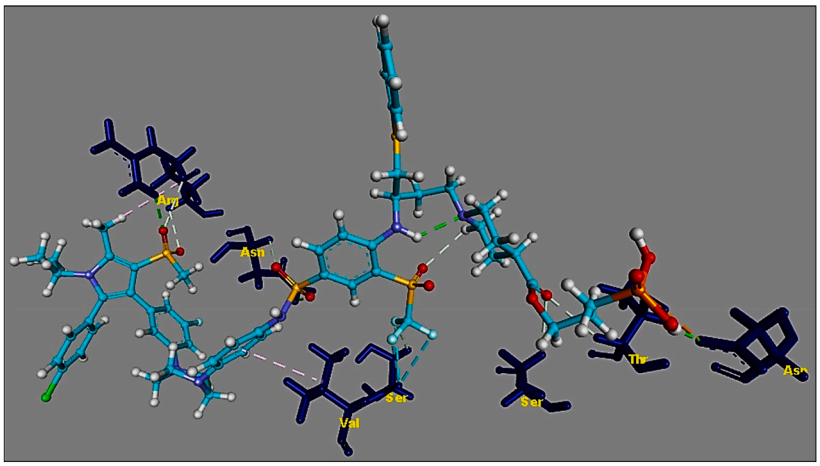
According to our work, it can be proposed that pelcitoclax may be proceeded further for computer added drug designing (CADD) approach to evaluate its drug-like properties. Moreover, the results of this study also concluded that LSDV132 protein is a BCL-2-like protein that may immunoregulate the apoptosis in host cells upon infection [45]. As far as future perspectives are concerned, the findings of this research work may be helpful to study its other attributes and explore more crucial functions. The limitation of this study is that the *in-vivo/in-vitro* validations would be necessary to strengthen the findings of the study. Moreover, the validation of plecitoclax inhibition against LSDV132 protein could also be estimated by the in vitro enzyme kinetic assays and the testing in model animals.

## 5. Conclusion

LSDV132 protein belongs to lumpy skin disease virus (LSDV), the causative agent of lumpy skin disease (LSD). Like other members of its group i.e., poxviruses, this virus possesses different proteins encoded by their respective genes. Each of these proteins is determined for specified functions, including virion assembly. LSDV132 protein is one of these proteins, but its key features were unknown. In this study, we have employed various bioinformatics tools to unveil its different attributes. Physiochemical properties revealed that this protein was acidic and structurally stable because of low instability index, i.e., 30.89%. Alpha helices were abundantly found with 50.31% among other components in its secondary structure, upon respective analysis. This protein also possessed




(a)




(b)

**Fig. 11. a:** 3D layout with ribbons format, showing the binding of pelcitoclax with the active site between the two chains of LSDV132 protein; **11b.** The 3D illustration of interacting residues of the LSDV132 protein with the atoms of pelcitoclax.

various phosphorylation sites but no transmembrane helices. KEGG analysis and InterPro family prediction were found to play a key role in this study because they prophesied the possession of Pox-BCL-2-like motifs in this protein. This finding suggested that LSDV132 protein may be responsible for the immunomodulation of apoptosis in host. Therefore, we subjected LSDV132 to interact with various viral BCL-2 inhibitors to identify a potential ligand/compound through molecular docking and virtual screening approach. Binding affinities and S-scores (energy values), among other parameters, were computed in this regard. The overall docking scores suggested that the pelcitoclax exhibited comparatively good docking scores, indicating that it could be a potential inhibitor against this protein. Therefore, it was concluded that this compound can be proceeded further for computational drug designing for better evaluation. As far as the future perspectives of this study are concerned, this study can provide future directions to validate the findings of this study *in-vivo*. And, it can also assist in developing a potential inhibitor against this protein through conventional pharmacological approaches.

## Data availability statement

All of the data produced during study has been given in the manuscript.

## CRediT authorship contribution statement

**Muhammad Farhan Sarwar:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Qurat ul Ain Waseem:** Writing – original draft, Software, Resources, Methodology, Investigation, Conceptualization. **Mudassar Fareed Awan:** Supervision, Resources, Formal analysis, Conceptualization. **Sajed Ali:**

Validation, Resources, Methodology, Investigation. **Ajaz Ahmad:** Writing – review & editing, Resources, Project administration, Conceptualization. **Saif ul Malook:** Supervision, Software, Investigation, Conceptualization. **Qurban Ali:** Writing – review & editing, Software, Resources, Formal analysis.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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