doi.org/10.36721/PJPS.2020.33.1.SUP.287-294.1

Insilico modeling and analysis of small molecules binding to the PHLPP1 protein by molecular dynamics simulation

Tehreem Anwar[1#], Mirza Jawad Ul Hasnain[1#], Amina Sarwar[1], Hajra Kanwal[1], Bakhtawar Afzal[2], Masroor Ellahi Babar[3] and Muhammad Tariq Parvez[1]*

[1]Department of Bioinformatics and Computational Biology, Virtual University of Pakistan, Lahore, Pakistan
[2]Department of Biosciences, COMSATS University, Islamabad, Pakistan
[3]Department of Genetics, Virtual University of Pakistan, Lahore, Pakistan

Abstract: The PHLPP (Pleckstrin homology domain leucine-rich repeat protein phosphatases) is a newly discovered group of genes which includes PHLPP1 and PHLPP2 and plays an integral part in several cellular processes like apoptosis, cell signaling cell survival, and cell proliferation etc. Both the activation and deactivation of these genes can have vital role in several ailments like heart diseases, circadian rhythm and most importantly the cancer, hence encouraging the growth of novel therapeutic elements. To give new directions into the development of PHLPP1-targeting drugs, the interaction mechanism between PHLPP1 and five important ligands 4IP, B39, 635, ATP and GTA were investigated through docking and Molecular Dynamics Simulation. It is also noteworthy to be mentioned here that there is no previous crystal structure of PHLPP1 available. The in-silico results can provide potential base for advancements in development of new therapeutic elements targeting different diseases, mainly cancer. In this study, we employed homology modeling technique to develop a high-quality structure model of PHLPP1. The PHLPP1 model was then used in docking interaction analysis and Molecular Dynamics Simulation, to study binding pockets and interactions of PHLPP1 ligands and finding actively contributing residues in binding pocket. In final step, Free Energy Estimation was performed to observe ligand binding's quantitative characteristics.

Keywords: PHLPP1, cancer, molecular docking, homology modeling, molecular dynamics, phosphorylation.

INTRODUCTION

The PHLPP (Pleckstrin homology domain leucine-rich repeat protein phosphatase) family composed of two members: PHLPP1 and PHLPP2 which belong to a novel Ser/Thr phosphatases family that plays an integral role in maintaining cell signaling (Brognard *et al*., 2007; Gao *et al*., 2005). Both PHLPP1 and PHLPP2 mapped to chromosome 18q21.33 and 16q22.3-16q23.1 respectively and have almost similar domain structure, containing N-terminus Ras association domain followed by LRR, PH domain, PP2C domain and PDZ binding motif (Brognard and Newton, 2008). PHLPP family is involved in various cellular processes like cell migration, apoptosis, cell survival, cell proliferation and quiescence and helps in making their identity as tumor suppressor genes (Brognard *et al*., 2007; Gao *et al*., 2005; Li *et al*., 2013). Downfall of PHLPPs expression is involved in several human cancers like colon cancer (Liu *et al*., 2009), breast cancer (Karnoub *et al*., 2007), prostate cancer (Chen *et al*., 2011), melanoma (Talantoy *et al*., 2005), Wilms tumor (Rakha *et al*., 2006) and ovarian cancer (Patael-Karasik *et al*., 2000).

Akt (v-akt marine thymoma viral oncogene homolog) has been identified as main target of PHLPPs and aberrant PI3K (phosphoinositide 3-kinase) pathway signaling is important in many cancers as it holds great therapeutic potential due to the specific targeting (Majumder *et al*., 2003; Taylor *et al*., 2010; Wong *et al*., 2010). Accurate balance maintenance is necessary between protein kinases that causes protein phosphorylation and protein phosphatases results in protein dephosphorylation for balanced cellular homeostasis (Yang *et al*., 2002). Any imbalance in these pathways can lead to diseased states such as cancer, diabetes and heart diseases. Previously the most studied gene in these pathways is PTEN (phosphatases and tensing homolog) which dephosphorylates PIP3 (lipid second messenger) thus preventing AKT kinase activation (Maehama and Dixon, 1998). After discovery of PHLPP in 2005, the fact that they dephosphorylate AKT directly and results in its inactivation, PHLPPs were introduced as new negative regulator of PI3K pathway (Gao *et al*., 2005). PHLPP is hence a novel drug target for the suppression of tumorigenic pathways and is latent candidate gene to study its regulation for suitable development of novel therapeutic compounds.

In order to better understand mechanism of PHLPP1 and association with small particles and hence provide potential platform for the production of novel drug targets and new therapeutic elements, we predicted three-dimensional structure of PHLPP1, evaluated the structure

**Corresponding author:* e-mail: m.tariq@vu.edu.pk

[#]Authors have equal contribution

and then five compounds were docked with it. Docking provided us interaction of ligands with PHLPP1. Once binding positions were revealed we performed Molecular Dynamics Simulation for the equilibration of the model. Lastly, to get favorable ranks based on free energy, we performed free energy estimation calculation to validate the competence of our predicted structure and furnishing the quantitative indication of importance of each residue interaction and recognition.

MATERIALS AND METHODS

Experimental section comprises of four steps: Prediction of three-dimensional structure of PHLPP1, Docking interaction analysis, Molecular dynamics Simulation and Free Energy Estimation. Overall depiction of methodology is presented in the flow chart given in fig. 1.

Homology modeling

To find molecular interactions of PHLPP1, modeling was necessary. No crystal structure of PHLPP1 is available, so *insilico* procedure was adopted, and we used Swiss Model (Waterhouse *et al*., 2018) for this purpose. A PDB structure 4U09. A was used as template. The refinement of structure was then performed by ModRefiner (Dong and Yang, 2011) which uses minimization process to refine the structure. The quality of structure was then evaluated by using two types of software: Error evaluation was done with ERRAT (Colovos and Yeates, 1993) and structural evaluation was performed with PROCHECK.

Molecular docking

Molecular docking was performed in order to find ligand protein interactions and for finding potential ligands. For this purpose, we docked all selected ligands with PHLPP1 using PyRx AutoDock Vina Wizard (Dallakyan and Olson, 2015). When we compared binding energies of ligands and protein interaction, five compounds showed greater binding energy namely 4IP, B39, 635, GTA and ATP. These ligands were then selected for ligand-protein interactions. We used LigPlus (Wallace *et al*., 1995) and created ligplots for these five dockings which showed interactive residues.

Molecular dynamics simulation

We used VMD, NAMD and QwikMD (Humphrey *et al*., 1996) to run molecular dynamics simulation on our newly modeled protein. PSF structures were created from PDB file and this PSF structure was then solvated. We put a water box around the protein and make solvated PSF and PDB structures. These solvated structures were then used for performing minimization by using NAMD. After that QwikMD a plugin of VMD was used for molecular dynamics simulation. We used explicit solvent model, set the temperature to 300K and simulation time to 0.1 ns to see results in realistic time. RMSD, Energy, temperature and pressure plots were then plotted.

Free energy minimization

Binding free energies were estimated using Kdeep online tool (Jimenez *et al*., 2018). It took docked structures of TLR9 protein and ligands and estimated free binding energies. It is a crucial measure in drug designing approach as it estimates affinity between target and ligand. It is also necessary for verifying the capability of our model and furnishing the quantitative indication of importance of each residue interaction and recognition.

RESULTS

Homology modeling

PHLPP1 is a 1717 residues long protein. 4U09 was taken as template with a highly conserved residue zone. New minimized model is reported in fig. 2a. QMEAN scores graph is represented in fig. 2b which shows model is somehow in stream of non-redundant protein structures. Structural evaluation performed through ERRAT and PROCHECK which is presented in table 1. ERRAT quality factor is comparable to both holo-structure and minimized structure of PHLPP1. PROCHHECK results indicated that wrong amounts of ψ and ϕ were presented by only a few amino acids (0.6%) and a major part of amino acid residues (81% after optimization) were found in theoretically correct part of Ramachandran plot. These results represent our model as a good quality model.

Molecular docking

Total of 25 ligands were retrieved from RCSB Protein Data Bank that are related to PHLPP1 protein for analysis of docking interactions. All 25 ligands were docked with PHLPP1 protein structure using PyRx. Five ligands with strong binding affinities were selected for further analysis. Ligand binding affinities of all five ligands were as follows: 4IP (-5.4), 635(-6.7), B39 (-7.3), ATP (-6.8), GTA (-7.6). Lesser values indicate a stronger affinity between the ligands and protein. Structures of the ligands and their IDs are shown in fig 3. The docked compounds were analyzed using Ligplot and the docking interactions are shown in fig 4. Table 2 represents the Ligplot interactions. PHLPP1 forms 7 hydrogen bonds with 4IP ligand at residues Glu860, Ser902, His926, Asp968, Arg903, Asp923, Lys944, 3 hydrogen bonds with ligand B39 at residues Gln970, Ser902, Arg903, 2 hydrogen bonds with ligand 635 at residues Lys944, Ser902, 3 hydrogen bonds with ATP at residues Ser902, His926, Arg903 and 7 hydrogen bonds with GTA ligand at residues Gln1015, Gln970, His949, Arg903, Glu860, Asp968, His926. Four residues within binding pocket namely Gly925, Leu946, Ser902 and Arg903 have been actively contributing to protein-ligand interactions (fig. 4).

Molecular dynamics simulation

The MDS of PHLPP1 predicted structure was performed that allowed observation of energy, temperature, pressure and Root Mean Square Distance (RMSD) of the protein. First, we observed RMSD tendency that stretched to a

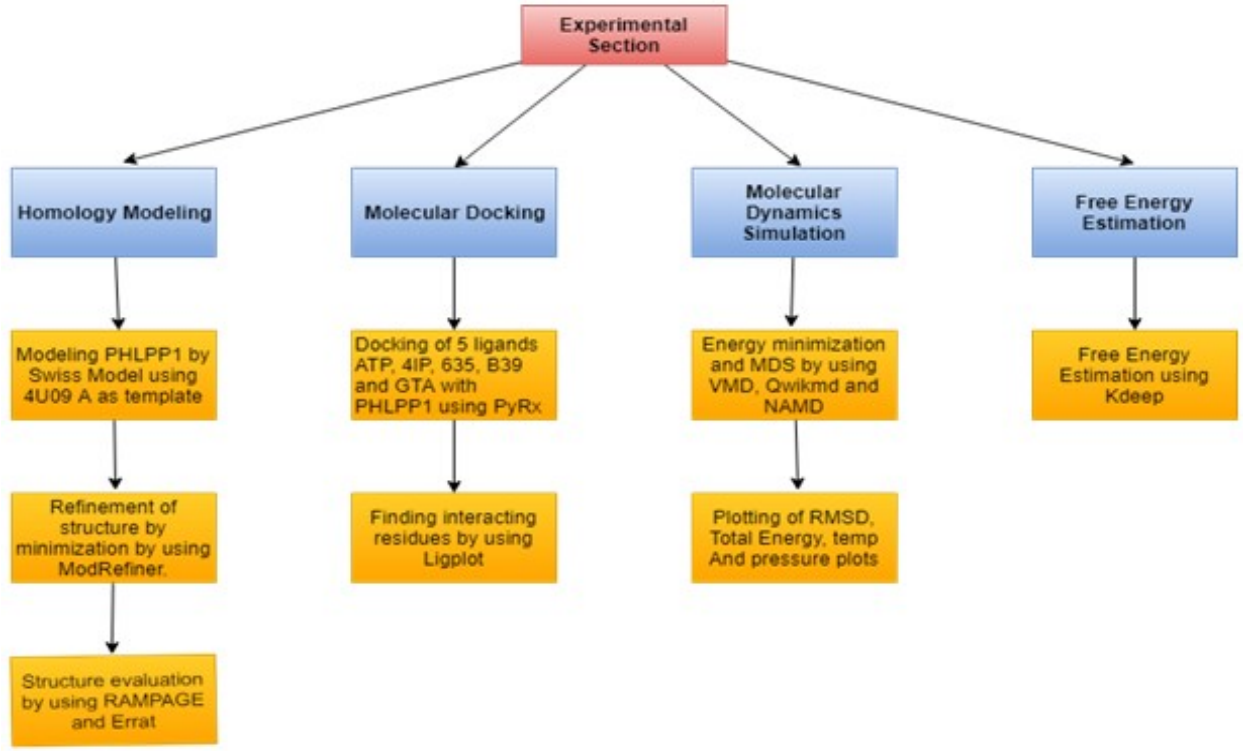

Fig. 1: Overall methodology followed in the current study.

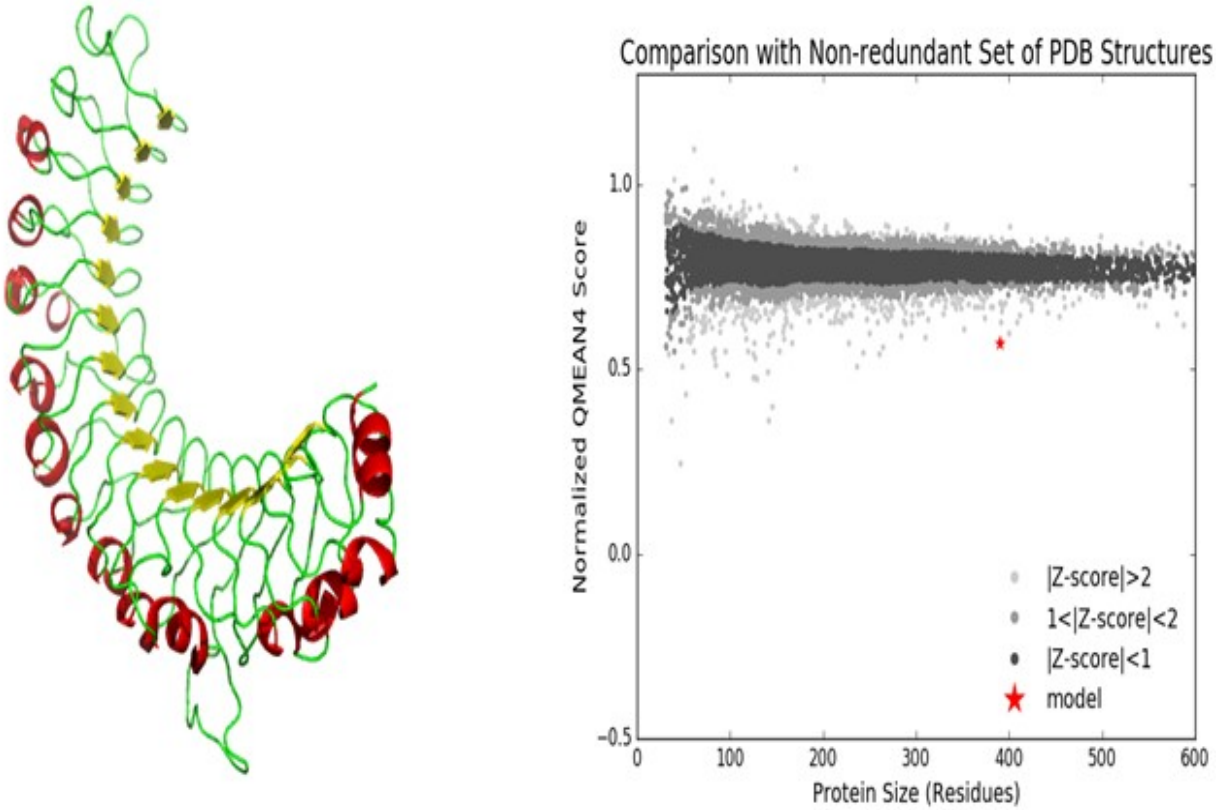

Fig. 2: A) New minimized model B) QMEAN score of non-redundant proteins. Our model somehow lies between the streams of non-redundant protein structures.

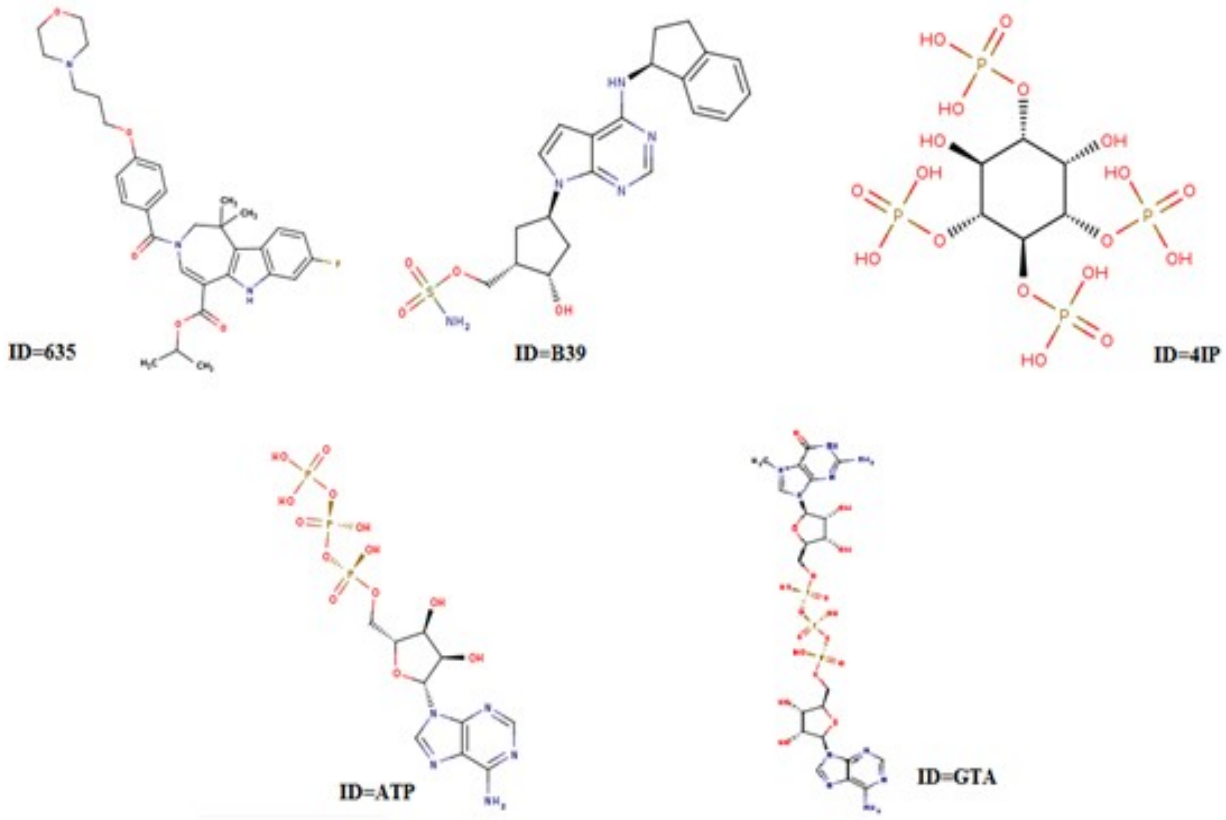

Fig. 3: Structures of ligands docked with PHLPP1

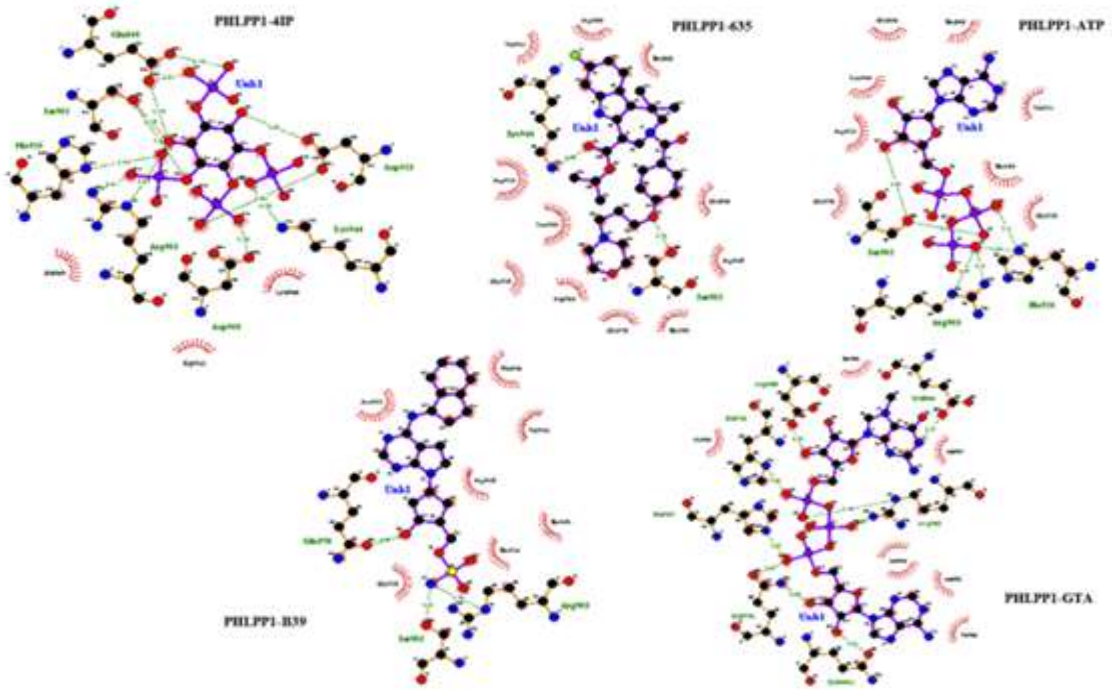

Fig. 4: Ligplot of PHLPP1 and five ligands 4IP, 635, ATP, B39 and GTA showing interacting residues. Olive green residues are Protein's hydrogen bond interactions, Blue residues are ligand hydrogen bond interactions and black residues are protein's hydrophobic interactions

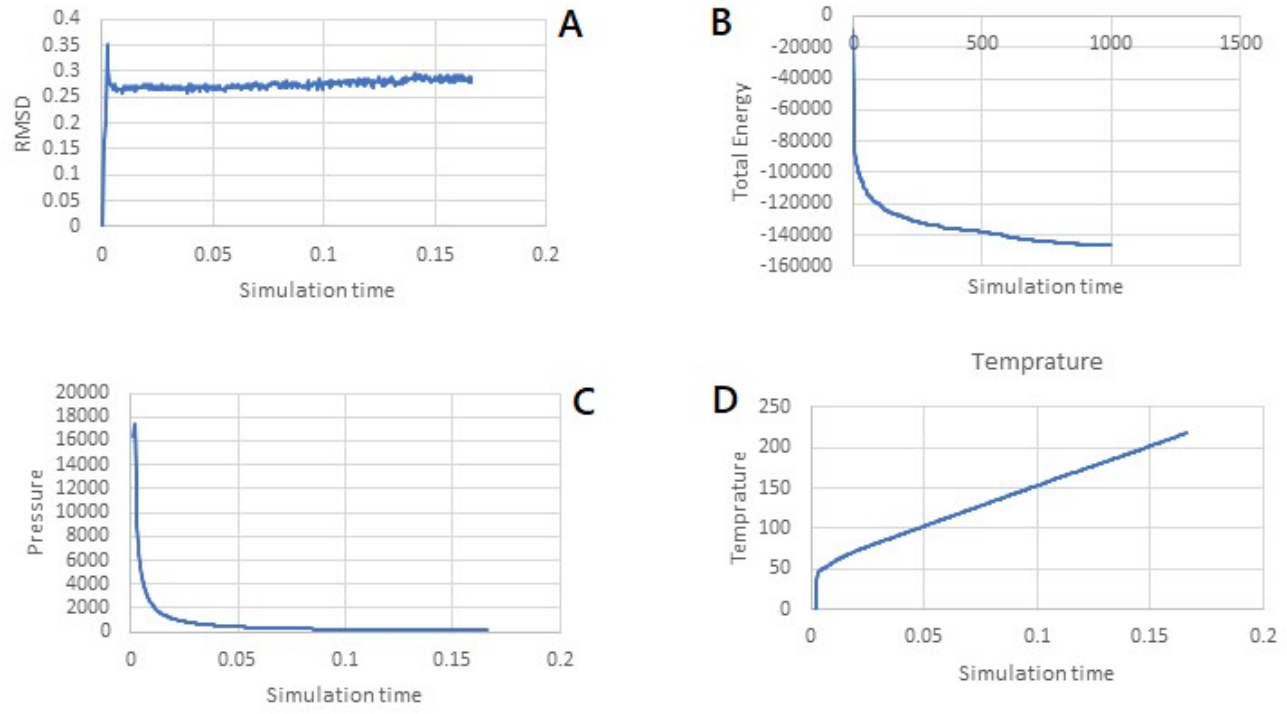

Fig. 5: Molecular Dynamics Simulation results: a) RMSD trends for PHLPP1 backbone plotted against time b) Total Energy of the system plotted against simulation time c) Temperature plotted against simulation time d) Pressure plotted against simulation time.

Table 1: Structural evaluation performed for PHLPP1 newly modeled structure and its minimized form. ERRAT quality factor refers to that % of proteins whose error value falls below 95% of rejection limit. PROCHECK results show that which amino acid residues angles lie in which area of Ramachandran plot. Both result of PHLPP1 and its minimized form are presented.

Structure	ERRAT Quality Factor	PROCHECK			
		Core	Additional	Generously	Disallowed
PHLPP1	73.03	72.3%	26.0%	1.4%	0.3%
Minimized PHLPP1	82.46	81%	17.6%	0.3%	0.6%

Table 2: Description of ligplot for protein-ligand interactions in tabular form Olive green residues are Protein's hydrogen bond interactions, Blue residues are ligand hydrogen bond interactions and black residues are protein's hydrophobic interactions.

Protein-Ligand	Hydrogen bond interactions		Hydrophobic Interactions	
	Receptor	Ligand	Receptor	Ligand
PHLPP1-4IP	Glu860, Ser902, His926, Asp968, Arg903, Asp923, Lys944	Unk1	Gly925, Leu946, His949	---
PHLPP1-B39	Gln970, Ser902, Arg903	Unk1	Gly925, Asn992, Phe990, Val966, Asp968, His926, His949	---
PHLPP1-635	Lys944, Ser902	Unk1	Asp900, Val921, His858, Asp923, Leu946, Gly925, Arg903, Gln970, His949, Asp968, Glu860	---
PHLPP1-ATP	Ser902, His926, Arg903	Unk1	Glu860, His858, Leu946, Asp923, Gln970, Val921, His949, Gly925	---
PHLPP1-GTA	Gln1015, Gln970, His949, Arg903, Glu860, Asp968, His926	Unk1	Leu946, Asn992, Val966, Asp923, Gly925, Ser902	---

plateau around 0.35 Å in starting and become stable after that at 0.27-0.29 Å (fig. 5a). Trends show relative stability after equilibration during whole simulation except the initial period where conditions were not so favorable. Moreover, the total energy came to a stable state after instability due to release of harmonic restraints and heating (fig. 5b). Temperature during simulation was plotted (fig. 5c) a steady rise in temperature die to heating up of system was reported. Pressure after the initial instability showed a stable low value and its almost minimized (fig. 5d). MD simulations in QwikMD are performed in isothermal-isobaric ensemble (NpT) where N is number of atoms, p is pressure and T is temperature and all these parameters are kept constant by implying barostat and thermostat. However, small observations of T and p values are observed under user defined values.

Table 3: Results of free energy estimations of proteins and Ligand performed by KDEEP

Protein-Ligand	Mol. Weight (g/mol)	pKd	ΔG (Kcal/mol)	Ligand Efficiency
PHLPP1-4IP	507.99	6.19	-8.35	-0.3
PHLPP1-635	565.3	6.42	-8.67	-0.21
PHLPP1-ATP	507.181	5.86	-7.92	-0.26
PHLPP1-B39	445.18	4.45	-6.0	-0.19
PHLPP1-GTA	793.14	6.59	-8.89	-0.17

Free energy minimization

SDF format docked protein and ligand files were provided as input and following results were collected. PHLPP1-4IP binding resulted in Mol. Weight (g/mol) =507.99 = pKd =6.19, ΔG (Kcal/mol) =-8.35 and Ligand efficiency =-0.3. PHLPP1-635 binding resulted in Mol. Weight (g/mol) =565.3, pKd =6.42, ΔG (Kcal/mol) =-8.67 and Ligand efficiency =-0.21. PHLPP1-ATP binding resulted in Mol. Weight (g/mol) =507.181, pKd =5.86, ΔG (Kcal/mol) =-7.92 and Ligand efficiency =-0.26. PHLPP1-B39 binding resulted in Mol. Weight (g/mol) = 445.18, pKd =4.45, ΔG (Kcal/mol) =-6.0 and Ligand efficiency =-0.19 and PHLPP1-GTA binding resulted in Mol. Weight (g/mol) =793.14, pKd =6.59, ΔG (Kcal/mol) = -8.89 and Ligand efficiency= -0.17. Results are depicted in tabular format in table 3.

DISCUSSION

This study focused on the computational analysis of PHLPP1 structure modeling, small molecule binding and activation of PHLPP1 due to interactions with many known ligands. It made use of ligands and activators, of which the mode of action and molecular mechanism on the target has not been previously studied. Our study performed virtual screening and elucidated the detail mechanism of action of PHLPP1 and its interactions with ligands at atomistic level.

Swiss model was used to model 3D structure of PHLPP1. The absence of 3D structure of PHLPP1 was considered as an obstacle for performing computational procedures and precisely measuring protein ligand interactions which then ultimately lead to drug discovery. So, we make use of homology modeling; a tool which speed up this process of finding protein ligand interactions (Vyas *et al*., 2012). Structure of PHLPP1 was not available anywhere, as it is a newly discovered gene, so modeling its structure was a challenge. Our structure evaluation by PROCHECK confirmed that our predicted structure was of good quality based on percentage of residues lying in favorable region (Laskowski *et al*., 1993). Many inactive molecules and ligands with known structures were docked with the gene based on their relation with PHLPP1 found with the help of RCSB Protein Data Bank (Burley, 2018). Molecular modeling and simulation techniques were employed for understanding biological function and physical basis of protein's structure referring the study performed by (Gentile *et al*., 2015). Binding affinities calculated for the protein ligand interaction allowed us to achieve a relative rank between compounds and GTA ligand showed best binding energy. Gly925, Leu946, Ser902, and Arg903 have been found mainly to contribute to protein ligand interactions as they are forming the binding pocket responsible for protein ligand interaction and leading to changes in pathways in which phlpp1 is involved like counter-regulation of STAT1-mediated inflammatory signaling, targeting of Akt inhibitors and quality control of PKC; all the mechanisms involved in cancer (Katsenelson *et al*., 2019; Yan *et al*., 2015; Baffy *et al*., 2019).

The above-mentioned results not only present our predicted structure as a potential base for discovering novel drug targets but also depicted straight relation amid ligand activity and binding energies. The results we presented clearly showed that the docking technique, AutoDock Vina wizard of PyRx implements, is a powerful tool to analyze protein-ligand interactions efficiently with identification of the best possible confirmation of a ligand within a protein. Though, some issues were encountered docking mainly related with protein rigidity, inefficient scoring function during simulation, but these issues were solved by running MD runs and using free energy estimations. In conclusion, our study not only presented powerful platform for performing virtual screening on possible PHLPP1 small molecules via a consistent and comprehensive procedure, but also revealed the molecular platform for acknowledgment of major ligands and small molecules.

CONCLUSION

PHLPP1 is an important pharmaceutical target for therapy of several severe pathologies and diseases including cancer; however, a main hindrance in the advancement of therapeutic elements that targets PHLPP1 is absence of three-dimensional structure of this protein. In present study, homology modeling technique was used to develop a high-quality three-dimensional structure of PHLPP1. The predicted PHLPP1 model is then used for Docking interaction analysis and Molecular Dynamics Simulation, to observe binding pockets and interactions of PHLPP1 ligands and finding actively contributing residues in binding pocket. In final step, Free Energy Estimation has been performed to observe ligand binding's quantitative characteristics. Four residues within binding pocket namely Gly925, Leu946, Ser902 and Arg903 have been actively contributing to protein-ligand interactions. The precise description of binding of ligands to PHLPP1 can be a beginning of design and development of new therapeutic compounds targeting phosphatases and their pathways for the treatment of vast range of diseases mainly cancer.

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