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Molecular Docking of *Catharanthus roseus* Bioactive Compounds of Targeting Bovine Leukemia Virus

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Abstract

Therapeutic potential of Catharanthus roseus bioactive compounds as the 4PHO protein inhibitors, a pathogenetic determinative factor of BLV. This work describes the use of computational drug discovery approaches in estimating the interactions conducted between 40 phytocompounds of Catharanthus roseus and the 4PHO protein. Our work has determined the presence of Pamoic acid and Vindolinine hydrochloride as the optimal choice here. Pharmacokinetic screening exhibited drug-like properties to both the highly absorbed compounds in the gastrointestinal tract and Lipinski's Rule of Five. Molecular docking screening exhibited strong binding of the compounds with 4PHO; pamoic acid exhibited binding energy of –8.7 kcal/mol and vindolinine hydrochloride exhibited –7.8 kcal/mol. VAL62, PHE222, and LEU225 were the most significant residues participating in interaction. Structural validation of 4PHO protein, for instance, through Ramachandran plot analysis confirmed its viability for docking studies. These observations form a platform for the synthesis of plant-derived antiviral drugs against BLV and offer a platform for future in vitro and in vivo studies to assess their therapeutic viability.

Keywords: Molecular docking, *Catharanthus roseus*, bovine leukemia virus (BLV), cancer, 4PHO protein, drug discovery

INTRODUCTION

Vinca rosea belongs to the family Apocynaceae and has the scientific plant name *Catharanthus roseus*. It belongs to Order Gentianales, Class Magnoliopsida, and Kingdom Magnoliophyta. "*Catharanthus roseus*" has been named with the help of Greek terminologies since Katharos signifies clean and Anthos signifies flower. It is also known as "Nayantara" or "Sadabahar" [1]. It is extensively studied to be utilized medicinally in diseases, such as cancer and diabetes and is affordable in price, as it has medicinal content. A crop so productive, flowering with extremely showy flowers and such ease of cultivation in a very broad range of climates, is cultivated as an ornamental crop also [1]. The dicotyledonous angiosperm C. roseus yields two terpene indole alkaloids, vinblastine and vincristine, which are anticancer drugs [2] and agrochemicals, food additives, flavor and fragrance chemicals, medicaments, and insecticides [3]. A deltaretrovirus, bovine leukemia virus (BLV) is a member of the Retroviridae family of viruses. Type 1 and Type 2 human T-cell leukemia viruses (HTLV-1 and -2) are related to it in close association [4, 5]. They also cause neurological or proliferative disease in human and non-human primates [6, 7]. The most prevalent neoplastic beef and dairy cattle disease, bovine leucosis, or EBL, is caused by BLV [4, 8, 9]. Seven percent of BLV-infected cattle are subclinically infected or asymptomatic. Thus, this results in a very high shedding rate of BLV in uncontrolled cow herds [10]. After a latent period of one to eight years, approximately 1–5% of BLV-infected calves develop tumors as malignant B-cell

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lymphosarcoma, and approximately 30% of BLV-infected cattle develop persistent lymphocytosis (PL) [4, 11, 12]. It has the chemotherapeutic agents vinblastine and vincristine, both of which are employed in the treatment of most malignancies [13–16].

Molecular docking at the molecular level is extremely critical in BLV protein and prospective therapeutic drug interaction research. Computer program-based molecular docking can be employed in the identification of candidate lead drugs by calculating virus protein and bioactive molecule-binding affinity. Molecular interaction is one of the areas encompassing viral replication inhibition and vinca alkaloid therapeutic use as a cancer chemotherapeutic drug caused by BLV [17].

These alkaloids biosynthesize from vindoline and catharanthine in mixture [18]. Used as anticancer drugs to treat non-small-cell lung cancer, semi-synthetic chemotherapy drug vinorelbine [15, 19] may be derived from catharanthine and vindoline [15, 20] or the vinca alkaloid leurosine [21], both methods employing anhydrovinblastine [19]. The plant contains the insulin-raising vincoline [22, 23]. Sensitive and specific serological assays, such as agar gel immunodiffusion (AGID), radioimmunoprecipitation assays (RIA), or enzyme-linked immunosorbent assays (ELISA), and proviral DNA detection methods, such as single, semi-nested, nested, or real-time polymerase chain reaction (PCR) tests are sensitive and specific for the diagnosis of BLV infection [24].

Despite the existence of a BLV vaccine does not present on the market for use in prevention of EBL, vaccination would be ideal disease control. This is because when tested, all the control measures under investigation were found to have host immune response initiation but with limited duration or not fully [25–28].

The present research study attempts to assess the drug likeness of *Catharanthus roseus* against BLV diseases, define the molecular interaction of vinca alkaloids with BLV proteins using molecular docking methods, examine the vinca alkaloids activity as a novel antitumor agent against cancer induced by BLV, and explore the possibility of devising new antiviral protocols employing vinca alkaloids against BLV infection (Figure 1).

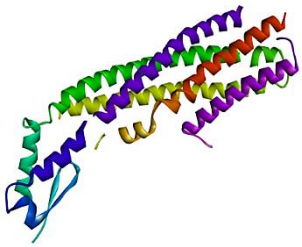

Figure 1. Purified structure of protein 4PHO.

METHODS

The aim of the molecular docking and virtual screening experiments in this study was to look for the probable pharmacological molecules of *Catharanthus roseus* to act as potential drugs in inhibiting the 4PHO protein, which is the etiological protein of bovine leukaemia virus for animals.

Ligand Retrieval

A potential ligand was recovered and isolated from the OSADHI database (https://osadhidb.com/) in the current study. 46 canonical SMILES were isolated and published [29] for analysis in the follow-up by virtue of computational models. The compounds were derived from *Catharanthus roseus*. PubChem IDs as candidate compounds and 2D structures were recovered from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Ligands were also stored in SDF format to perform virtual screening experiments and molecular docking. Recovery of ligands will be investigated for therapeutic drugs [30].

Protein Retrieval

Crystal structure of 4PHO protein was retrieved from RCSB PDB database (https://www.rcsb.org/structure/4PHO) as ClyA CC6/264 ox (2-303). It is Escherichia coli K-12 and was solved by X-ray diffraction at 2.123 Å resolution. The protein was stored in PDB format, and missing residues were later modelled with SWISS-MODEL (https://swissmodel.expasy.org/). The resulting structure was further used for computational studies and molecular docking [31].

Protein Purification

4PHO protein purification involved the elimination of water molecules since their free energy is not crystallography-dependent and can affect docking outcomes. Chain A was kept following the elimination of the other chains through the deletion of the other chains. Prebound heteroatoms and ligands like complex GOL and PEG were deleted to create room for favorable ligand binding. Missing residues were modeled, and polar hydrogen atoms were added with the assistance of SWISS-MODEL to make the structure stable. Ramachandran plot validation and secondary structure prediction by PDBsum and hydrophobicity profile prediction by BIOVIA Discovery Studio were utilized in purification structure validation. The structure was now ready to move further for computer-aided docking and analysis [32–34].

Pharmacological Study

SwissADME (http://www.swissadme.ch/) was used to evaluate the pharmacological characteristics of the chosen ligands to determine their oral bioavailability and drug-likeness. Lipophilicity, polarity, insolubility, size, flexibility, and saturation were among the physicochemical characteristics evaluated. The ligands were assessed using Lipinski's Rule of Five, which indicates favorable pharmacokinetic properties when they meet the requirements with zero violations. Ligands were also effective in GI tract absorption orally and systemically distributed as well. Oral availability and systemic distribution were attained if the availability is ensured. The bioavailability score was ≤ 0.55, i.e., the drug was relatively available when given. The discovery finds that drug-like ligands and good ligands are selected for drug improvement and drug designing [35].

Molecular Docking

Virtual screening was performed using PyRx (https://pyrx.sourceforge.io/), a widely utilized computational software utilized in drug discovery computing, to execute the molecular docking analysis [36]. The 4PHO protein was minimized by removing water molecules, heteroatoms, and complex ligands (GOL and PEG) for efficient ligand interaction, and it was converted to PDBQT format [37]. With the assistance of the incorporation of Kollman charges and AutoDock 4 atom types in the protein structure [38], docking was performed.

Using the Open Babel Universal Force Field (UFF) (http://openbabel.org/wiki/Main_Page), a selection of selected ligands was downloaded in SDF files and processed through an energy minimization step [39]. Following torsional calculation for all ligands, docked ligands in PDBQT format were achieved [40]. For the best ligand binding conformation retrieval, the docking grid was set using appropriate dimensions of X = 51.8099 Å, Y = 41.9341 Å, and Z = 97.9662 Å to cover the active site of the 4PHO protein [41]. Nine conformations of each ligand were separately docked into 4PHO to determine the best pose of binding [42]. The binding affinities were estimated by docking scores (kcal/mol), and low energy scores indicated strong interaction between the ligand and protein [43]. Non-bonded interaction analysis was performed in BIOVIA Discovery Studio, where van der Waals forces, hydrophobic contacts, and hydrogen bonds were analyzed [44]. 2D/3D visualization of the docked complexes indicated the major interacting residues and the likely mechanism of binding [45].

Visualization

Target protein and chosen ligand docking interaction were explored using BIOVIA Discovery Studio (https://www.3ds.com/products-services/biovia/). 3D interaction plot presents good amino acid interaction and indicates the exact placement of the ligand in the pocket. Molecular other interactions

were also analyzed by using a 2D interaction diagram and delineated the inner structure of the ligand with interaction against residues LEU225, PHE222, VAL62, LEU47, ARG49, VAL218, LYS214, and GLU42. Such other interactions as hydrogen bonding, that may be an important contribution to the ligand binding affinity, are also taken into consideration. The data act as a good input to the structure-based drug design process by providing ligand binding activity data.

RESULTS

Protein and Ligand Recovery

The 4PHO protein crystal structure was retrieved from the RCSB PDB databank and was ClyA CC6/264 ox (2-303). It is an Escherichia coli K-12 structure solved by X-ray diffraction with a resolution of 2.123 Å. The protein was retrieved in the PDB format, and then SWISS-MODEL was used to model the missing residues. The downloaded structure was used for the rest of the computational experiments and molecular docking. Accepted ligands were searched and obtained from the OSADHI database for this study. 46 canonical SMILES were downloaded and noted for future computational study. The ligands were isolated from *Catharanthus roseus*. 2D structures and corresponding PubChem IDs of chosen compounds were downloaded from the PubChem database. Ligands were also downloaded as SDF for performing virtual screening experiments and molecular docking. Recovery of these ligands was investigated to identify those with medicinal activity.

Ramachandran Plot Analysis

The Ramachandran plot (Figures 2 and 3) will be the most convenient stereochemical quality control procedure for a protein structure, because it is used to determine the phi (φ) and psi (ψ) dihedral angles of amino acid residues. SAVES server and PROCHECK (PDBsum) were used to check modelled protein structure in the present study. In the present study, it was discovered that modeled protein structure was satisfactory. An exceptionally good percentage of 96.8% of the residues occur in the most favorable regions. Moreover, 3.2% of the residues occur in the permissible regions but with slight movements and within acceptable tolerances. Surprisingly, there are no residues present in the prohibited regions, hence not containing any gross steric clashes or backbone strains in the structure. The outcome was over 90% residues in the optimal positions. The outcome justified the justification for the model's suitability for future computational research and experimental applications (Tables 1 and 2).

G-Factor Analysis

And the G-factor scores measure how deviant the protein structure is, and those are compared against structures of known proteins. Average G-factor for the entire is 0.37. Abnormal G-factors are below −0.5, and highly abnormal ones are below −1.0. All the values are above −0.5, and thus the structure is definite and flawless in conformation. Ramachandran plot analysis shows a good quality model with 96.8% residues in the most favored areas.

Further indication of structural stability of the protein is that 3.2% of residues lie in the additional allowed regions and no residue lies in the disallowed regions. Further, precision and stability of the structure are indicated by the fact that G-factor scores for various parameters, i.e., main-chain bond lengths and bond angles, lie within acceptable bounds. Apart from the absence of residues in the off-limits regions, these findings confirm that the model is of good quality and can be used in subsequent computational experiments and research. Calculation of G-factor and Ramachandran plot statistics show minimal departure from the mean of the structure (Table 3).

Phytochemical Selection and Pharmacological Screening

A Total of 46 phytocompounds that were extracted from Osadhi database, along with the SID that corresponded to each of them. These compounds were evaluated for drug-likeness based on Lipinski's Rule of Five through pharmacokinetic screening utilising the SwissAdme instrument. Numerous phytocompounds were eliminated from the 40 compounds because they failed to satisfy the selection criteria. The two other phytocompounds were pamoic acid (SID:8546) and vindolinine hydrochloride

(SID:419377), which were both showing good bioavailability with good GI absorption. Pamoic acid and vindolinine hydrochloride both passed the Lipinski criteria of molecular weights (MW) below 500 Da, favorable MLOG values, and an appropriate number of H-bond donors and acceptors (Table 4).

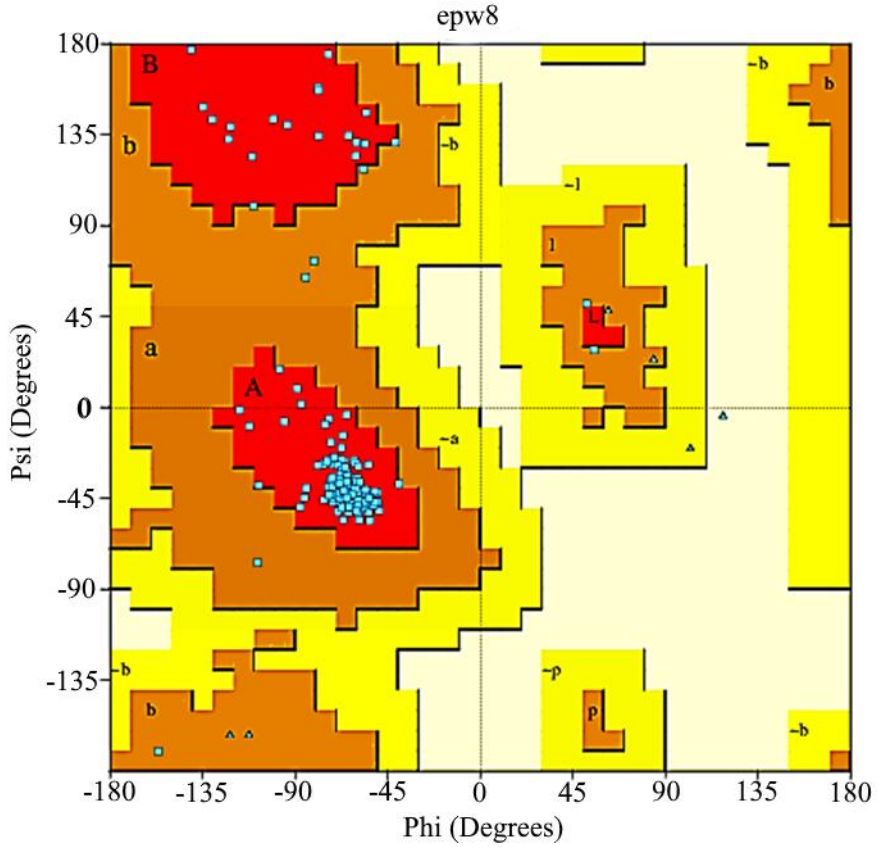

Figure 2. Saves server Ramachandran plot.

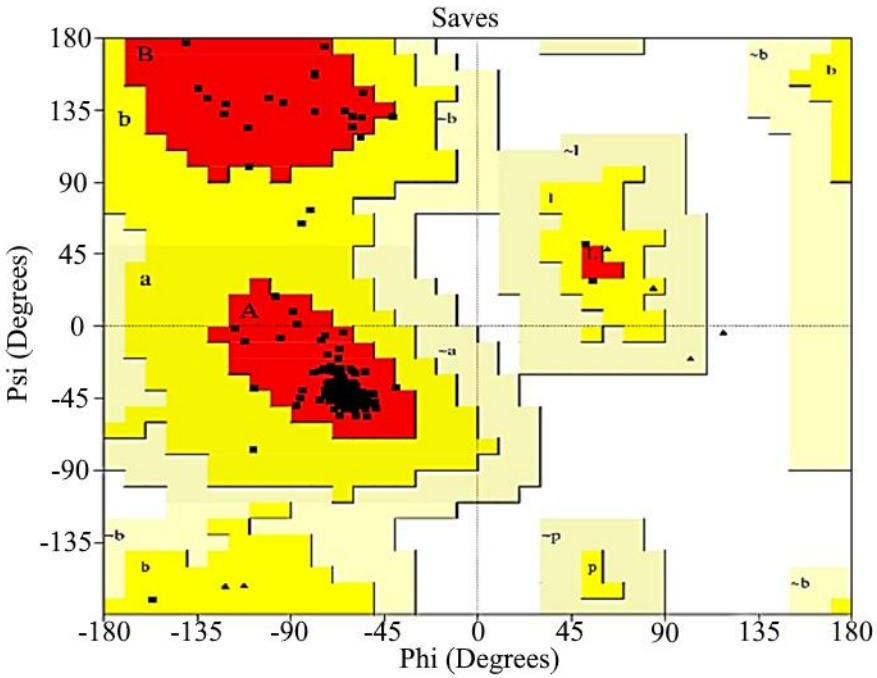

Figure 3. PDBSum generate Ramachandran.

Table 1. Residue distributions.

Region	No of Residues	Percentage
Residues in most favoured regions [A, B, L]	240	96.8%
Residues in additional allowed regions [a, b, l, p]	8	3.2%
Residues in generously allowed regions [~a, ~b, ~l, ~p]	0	0.0%
Residues in disallowed regions [XX]	0	100.0%

Table 2. Special residues analysis.

Residue Type	No of Residues
Number of Non-glycine & Non-proline Residues	284
Number of End-Residues (Excluding Glycine & Proline)	18
Number of Glycine Residues (Triangles in Plot)	12
Number of Proline Residues	3
Total Residues	281

Table 3. G-factor analysis.

Dihedral Angle G-Factors	Score
Phi-Psi Distribution	0.59
Chi1-Chi2 Distribution	0.04
Chi1 Only	0.07
Chi3 & Chi4	0.56
Omega	-0.13
Average Score	0.22
Main-Chain Covalent Forces G-Factors	
Main-Chain Bond Lengths	0.64
Main-Chain Bond Angles	0.55
Average Main-Chain Score	0.59
Main-Chain Bond Lengths	0.37

All these attributes indicate that the chemical is good at oral bioavailability. The physiochemical attributes include the % Csp3 (0.2) of pamoic acid, which is lower than that of vindolinine hydrochloride (0.89) and demonstrates the variation in their saturation values. There are more ratable bonds in pamoic acid (2) than in vindolinine hydrochloride (0) that can be involved in the two molecules' ability to form and bind with the target (Table 5).

ADME characteristics showed that both molecules have good GI absorption with a Bioavailability of 0.55, which is an excellent sign of systematic distribution capability. Both molecules also showed BBB permeability, i.e., both molecules would be able to cross the blood-brain barrier (Table 6). These findings show the potential of vindolinine hydrochloride and pamoic acid as better drugs for further computational and experimental work (Figure 4).

Table 4. Data for the properties of Lipinski rule obtained using SwissADME.

Ligand	MW	MLOGP	H-Bond Acceptor	H-Bond Donor	MR
Pamoic acid	239.11	1.96	1	2	58.48
Vindolinine Hydrochloride	140.22	2	1	0	43.2

Table 5. Physicochemical properties of the ligand molecules.

Ligand	Formula	Fraction Csp3	#Rotatable Bonds
Pamoic acid	$C_{10}H_{11}BrN_2$	0.2	2
Vindolinine Hydrochloride	$C_9H_{16}O$	0.89	0

Table 6. ADME data obtained using SwissADME.

Ligand	GI Absorption	BBB Permeant	Pgp Substrate	Bioavailability Score	Silicos-IT LogSw
Pamoic acid	High	Yes	No	0.55	–4.59
Vindolinine Hydrochloride	High	Yes	No	0.55	–2.33

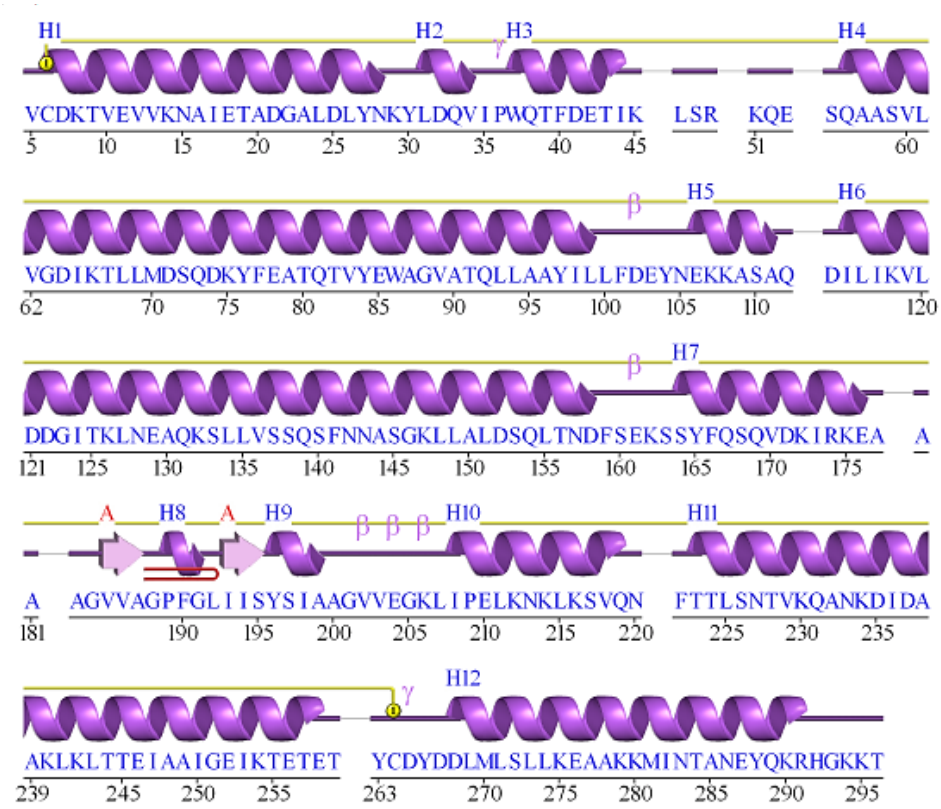

Figure 4. Secondary structure of protein 4PHO using PDBsum.

Molecular Docking Analysis

The molecular docking calculation of PyRx provided the binding affinity scores of the chosen ligands, pamoic acid, vindolinin hydrochloride, against the target protein. Pamoic acid had a very good interaction with the protein and obtained a binding affinity of –8.7 kcal/mol. Additionally, vindolinine hydrochloride obtained a very close binding affinity of –7.8 kcal/mol, weaker but very favorable compared to pamoic acid. These findings indicated towards the fact that both the ligands can bind with the pamoic acid target protein with varying affinities. In pamoic acid, this greater affinity could be for binding in greater numbers and in the process would generate more inhibitory or therapeutic effects (Table 7).

Table 7. Binding affinity of the ligands with protein.

Ligand	Binding Affinity
Pamoic acid	–8.7
Vindolinine Hydrochloride	–7.8

Visualization

The two top docked ligands were 3D and 2D modeled and downloaded through BIOVIA Discovery Studio. Evaluations based on bond type, bond distance, non-bond atoms, and interaction type were done. The 3D and 2D interaction diagrams depicted binding interactions of ligand with protein 4PHO as Figures 5 and 6.

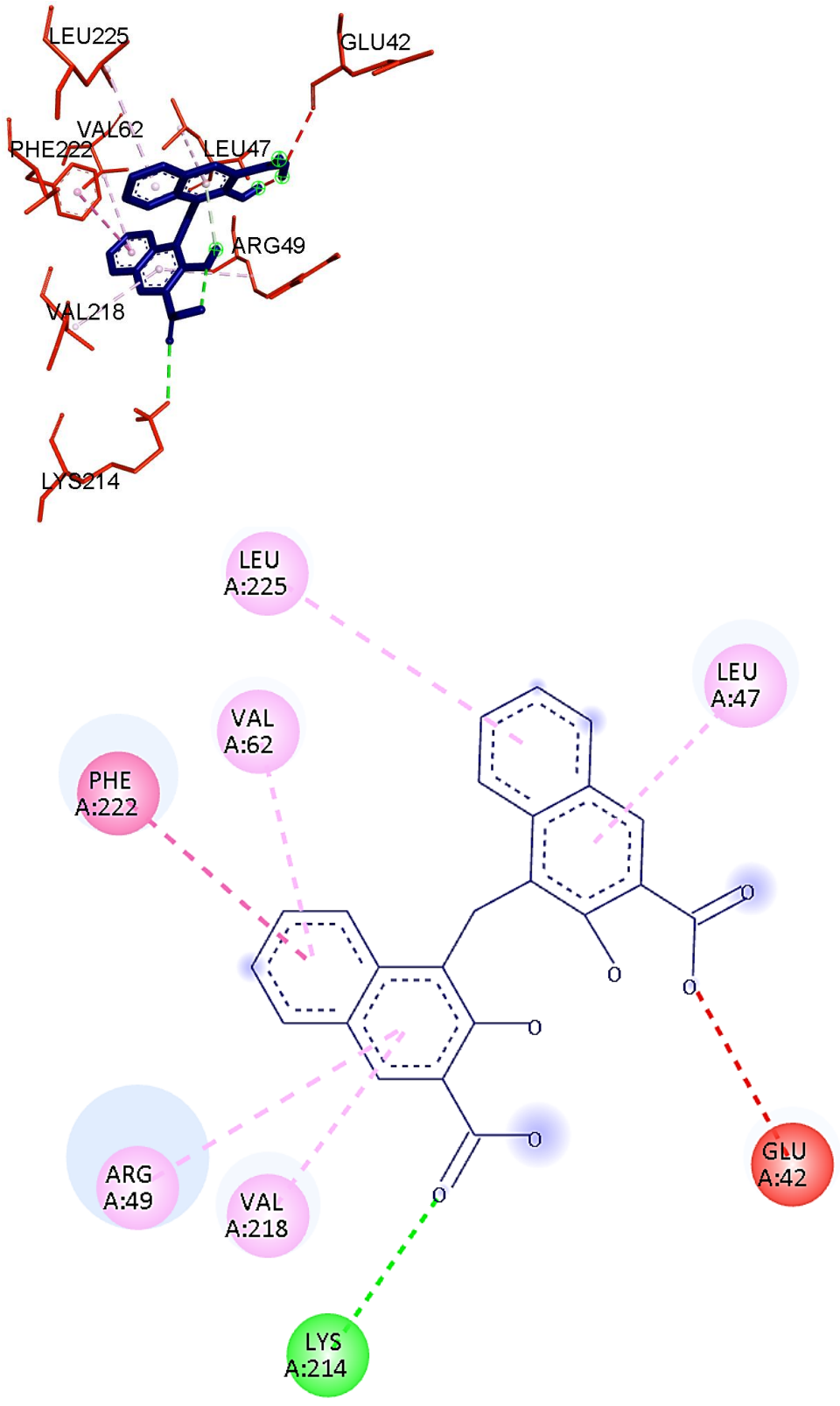

Figure 5. 2D and 3D interaction of pamoic acid with 4PHO.

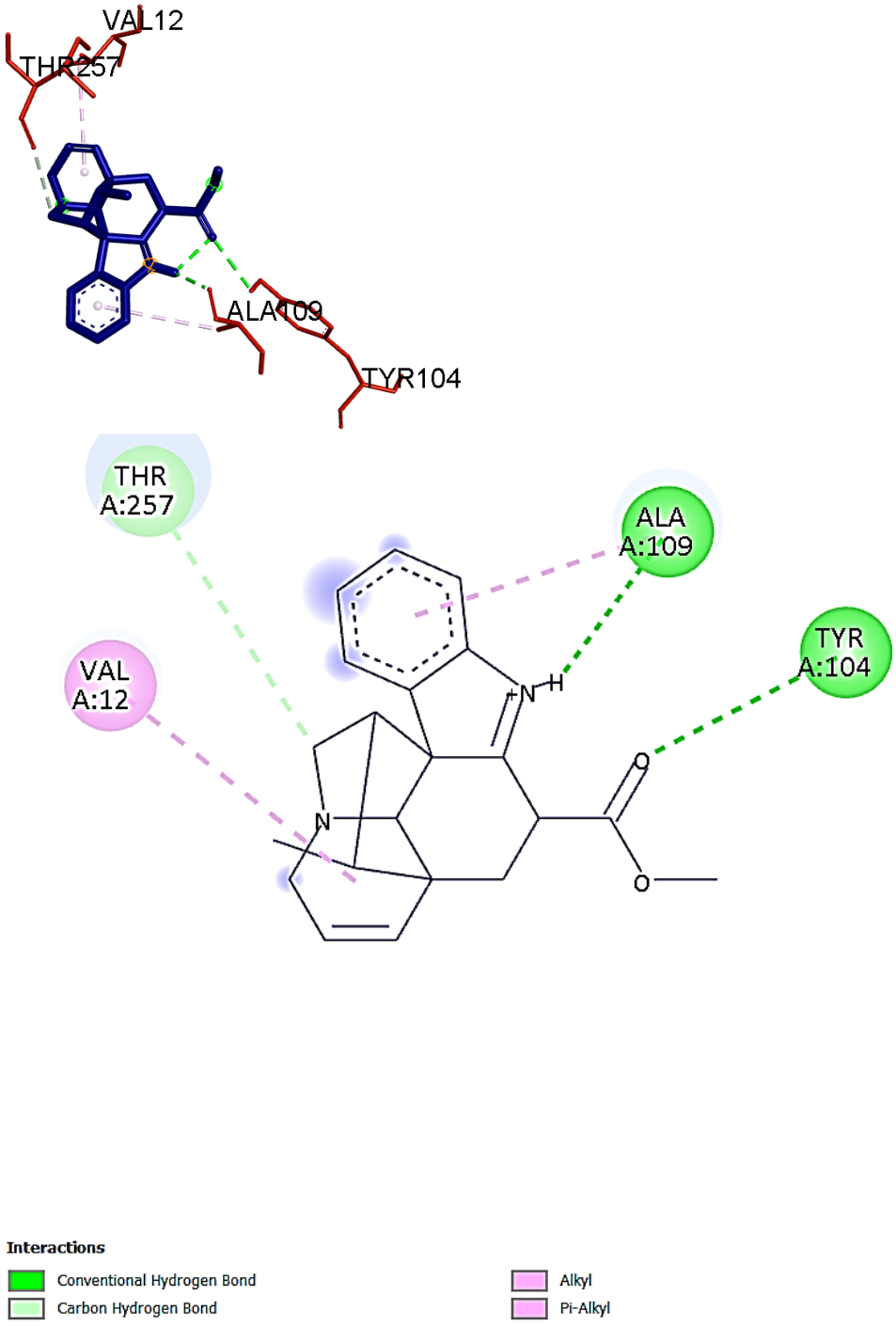

Figure 6. 2D and 3D interaction of vindolinine hydrochloride with 4PHO.

DISCUSSION

Bovine Leukemia Virus (BLV) is probably the most significant cattle pathogen inducing enzootic bovine leucosis (EBL), a neoplastic syndrome that inflicts immense economic losses on beef and milk producers. BLV is complicated, as the carrier rate is high and no useful vaccines are available. Molecular docking and virtual screening procedures in the present study were aimed at screening and selection of certain therapeutic compounds of Vinca rosea (*Catharanthus roseus*) with the potential to inhibit 4PHO protein, the target protein of BLV pathogenesis. Ramachandran plot of the 4PHO protein was found to be of good stereochemical quality with 96.8% residues in the favored region and none in the disallowed region. This construction quality was also supported by a mean value of 0.37 of the G-factor, as proof

of workability in the molecular docking study. PDBsum secondary structure prediction revealed several putative ligand-binding sites efficient in finding good inhibitors.

The first two phytocompounds of 40 OSADHI database leads were pamoic acid and vindolinine hydrochloride. Both the molecules were of good drug-like nature, as they followed Lipinski's Rule of Five, high gastrointestinal tract permeability, and 0.55 bioavailability ratio. Both the molecules were also BBB-permeable, which will prove to be useful while treating the neurological symptoms of BLV, but all side effects achieved will need to be explored thoroughly. The optimal binding energy of pamoic acid was determined to be –8.7 kcal/mol and represents hydrogen bond, hydrophobic, and van der Waals contacts with the crucial residues, such as LEU225, PHE222, VAL62, LEU47, ARG49, VAL218, LYS214, and GLU42. The contacts significantly increase the binding energy to an extremely large level and exhibit a possible inhibitory action on the 4PHO protein. Vindolinine hydrochlorides also showed tight binding to the 4PHO protein with a binding energy of –7.8 kcal/mol, and acceptable interactions with residues, such as LEU225, PHE222, VAL62, and LYS214. Hydrophobic interaction and hydrogen bonding were established by the compound to stabilize its high binding energy.

2D and 3D interaction models prepared with BIOVIA Discovery Studio presented ample information regarding the binding modes of the compounds. The hydrogen bonding, hydrophobic contacts, and van der Waals interaction of the ligands with the 4PHO protein pocket are common to the efficacy of pamoic acid and vindolinine hydrochloride in inhibiting the 4PHO protein and its possible viral load inhibition and EBL development inhibition. The ADME study also confirmed the drug efficacy of the compounds. Pamoic acid and vindolinine hydrochloride both had proper pharmacokinetic parameters, such as molecular weight, lipophilicity, and saturation capacity at proper levels. This work is incomplete in many respects, though. Calculated work is the basis of this work, while experimentally derived facts should rationalize computationally calculated drug action and binding interaction. The future research will include in vitro assays for inhibitory activity screening of the compounds against BLV, in vivo studies to determine therapeutic activity and toxicity of the compounds, and molecular dynamics simulations to study long-term stability of the ligand-protein complex.

Taken together, the study validates the activity of two bioactive compounds of *Catharanthus roseus*, vindolinine hydrochlorides and pamoic acid, as effective inhibitors of BLV 4PHO protein. Their unique molecular binding, strong binding affinity, and drug-like characteristics render them the most suitable ones to be fabricated into natural antiviral drugs against BLV and associated illnesses. These observations provide a good foundation for more research, which can lead to promising therapies against BLV with the medicinal value of plant medicines.

CONCLUSIONS

The present work introduces the potential of Vindolinine Hydrochloride and Pamoic acid, the lead phytochemicals of *Catharanthus roseus*, as inhibitors of 4PHO protein, an important etiological factor in bovine leukemia virus (BLV). The molecular docking study based on the in silico approach showed good binding energies of –8.7 kcal/mol and –7.8 kcal/mol for pamoic acid and vindolinine hydrochloride, respectively, as therapeutic agents against BLV and other diseases, such as enzootic bovine leucosis. The results provide molecular evidence for the ancient ethnomedicinal use of *Catharanthus roseus*, especially for the treatment of diseases, such as cancer, because of its alkaloid content. Some of the 4PHO protein interactions of the isolated compounds provided what the probable mechanism of antiviral activity of the compounds is. Both are drug-like properties that are good, with good gastrointestinal absorption and bioavailability, and hence good leads to be developed. Experimentation, however, must be done to validate the in-silico predictions, for example, cellular antiviral efficacy assays, in vitro binding assays to guarantee inhibition of 4PHO protein, and in vivo assays to guarantee efficacy and safety. Moreover, structure-activity relationship and synergistic effect studies of the compounds may lead to identification of more active analogs. Finally, this current study forms the basis for further research on *Catharanthus roseus* as a natural source of antiviral drug compounds against BLV and other diseases.

List of Abbreviations

BLV	Bovine Leukemia Virus
EBL	Enzootic Bovine Leucosis
HTLV-1	Human T-cell Leukemia Virus Type 1
HTLV-2	Human T-cell Leukemia Virus Type 2
AGID	Agar Gel Immunodiffusion
RIA	Radioimmunoprecipitation Assay
ELISA	Enzyme-Linked Immunosorbent Assay
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
SDF	Structure Data File
SMILES	Simplified Molecular Input Line Entry System
ADME	Absorption, Distribution, Metabolism, and Excretion
BBB	Blood–Brain Barrier
P-gp	P-glycoprotein
MW	Molecular Weight
MLOGP	Moriguchi Octanol–Water Partition Coefficient
MR	Molar Refractivity
GI	Gastrointestinal
2D	Two-Dimensional
3D	Three-Dimensional
UFF	Universal Force Field
GOL	Glycerol (PDB ligand)
PEG	Polyethylene Glycol (PDB ligand)
SID	Substance ID (from PubChem)
PDBQT	Protein Data Bank, Partial Charge, and Atom Type (dock file format)
G-Factor	Geometric Factor (protein structure validation)
IT LogSw	Intrinsic Thermodynamic Solubility (log scale)
Csp3	Carbon Saturation (fraction of sp3 hybridized carbons)
OSADHI	Online Structural and Analytics-Based Database of India's Herbs

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