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CONSTITUTIVELY ACTIVATED TYROSINE KINASE INHIBITOR DRUG DESIGN: HOMOLOGY MODELING AND DOCKING STUDIES ON CHRONIC MYELOGENOUS LEUKEMIA BCR-ABL FUSION PROTEIN

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ABSTRACT

Constitutively activated tyrosine kinase is a sole reason for chronic myelogenous leukemia (CML) in humans. Normal tyrosine kinase is responsible for phosphorylation, which support division of white blood cell .After reciprocal translocation between 9th and 22nd chromosome a fusion protein called constitutively activated tyrosine kinase are formed which increase the number of white blood cell and cause leukemia. Currently this constitutively activated tyrosine kinase are inhibited by tyrosine kinase inhibitor such as imatinib and dasatinib but a low remission rate force to search for a novel tyrosine kinase inhibitor. In the present work homology modeling of the fusion protein was done and actively inhibited by phytoligands. Docking analysis of various phytoligands on the active sites of modelled fusion protein were performed and the phytoligand which shows minimum energy hence maximum stability was selected and considered as a novel inhibitor for constitutively activated tyrosine kinase. Docking of modeled protein with the previously used drug was also performed to confirm the maximum stability of chosen phytoligand 1, 3-Diacetylvilasinin with (molDocscore -163.768) with the fusion protein. The results reveal that virtual screening of phytoligands is a very promising area which could lead to the discovery of the potent drug compound. This computational predicted data could be further validated using suitable assays for further consideration.

KEYWORDS: ***Tyrosine kinase, Molagro Virtual Docker, Docking complex, Fusion protein.***

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INTRODUCTION

Chronic myelogenous leukemia(CML) is a cancer of white blood cells in which number of white blood cells rise exponentially[1]. Reciprocal translocation between 9th and 22nd chromosome leads to the formation of Philadelphia chromosome, which codes for the constitutively activated tyrosine kinase enzyme which continuously carried out the phosphorylation and promotes division of white blood cells[2][3]. Generally chronic myelogenous leukemia is characterized by three phases, namely-chronic, accelerated phase and blasticphase[4][5]. In chronic phase less than 10% of blood and bone marrow are blast cells. The accelerated phase often consists of 10%-19% blast cells and is the terminal phase of CML in which approximately 20% cells in the blood and bone marrow are blast cells[6][7]. At present chronic myelogenous leukemia are targeted by tyrosine kinase inhibitor namely imatinib mesylate, dasatinib and nilotinib but complete remission with these tyrosine kinase inhibitor is a big challenge due to the side effects of these drugs on nearby cells, which increases the need for a search of natural constitutively activated tyrosine kinase inhibitor which is more effective in nature and having negligible mutagenic effect on nearby cells[8][9]. Monitoring of chronic myelogenous leukemia is mostly done by Real Time PCR and Fluorescence in situ hybridization technique[10][11]. Conventional chemotherapy with busulfan is effective to achieve hematologic control, but for a small extent and unable to modify natural disease course[12]. Introduction of stem cell transplant for leukemic patient proved significant resolution for the treatment of chronic myelogenous leukemia but donar availability and age restriction again posed a great challenge in the path of treatment of this lethal disease[13][14]. Present work in this study based on the virtual screening of ligands from plant source and search for a phytoligand which reduces the chances of mutation and other side effects and act as a potent constitutively activated tyrosine kinase inhibitor. Plant contains various bioactive compounds which can be act as a potent drug for the treatment of various diseases[15]. BCR-ABL fusion protein has been modeled by using swiss modeler program and Docking analysis were performed using Molagro virtual docker. The results indicate that 1,3-Diacetylvilasinin can be a more effective drug for the treatment of CML.

MATERIALS AND METHODS

I. SEQUENCE AND STRUCTURE ANALYSIS

Protein sequence of BCR-ABL Fusion protein was retrieved from NCBI>gi|179389|gb|AAA35594.1| BCR-ABL protein [Homo sapiens]. Physical parameters were predicted by using Protparam tool. Using BLASTP and PDB Sum, a similarity search of the protein was done selecting the PDB database. A sequence similarity search was done in order to find out the structure availability of the fusion protein and their similarity to other solved structures. Secondary structure of the fusion protein was predicted by utilizing GOR and SOPMA tools.

II. HOMOLOGY MODELING OF THE RECEPTOR

Homology modeling was done by the Swiss modeler tool. Suitable template was chosen from BLASTP search for homology modeling of BCR-ABL fusion protein and sequence in alignment mode was submitted to the Swiss modeler program.

III. MODEL VALIDATION

Stability of 3-D model of BCR-ABL fusion protein was validated by construction of Ramachandran plot. It shows the possible conformations of ψ and φ angles for a modeled BCR-ABL fusion protein. For Ramachandran plot SAVES server was utilized in which Procheck program validates the structure of the predicted fusion protein.

IV. ACTIVE SITE PREDICTION

Molagro Virtual Docker was used for predicting the active sites of the receptor molecule. Detect cavities option in molagro virtual docker have been utilized to detect the active sites.

V. LEAD COMPOUND GENERATION

Five drugs related to the treatment of Chronic myelogenousleukemia ; BCR-ABL fusion protein inhibitor, were selected and their analogous structures were searched from the PubChem and Drug Bank database. A list of drugs which were available for treatment was prepared and drugs with approved structure were selected. All ligands in this study are derived from plant source so that the chances of mutagenic behavior of the ligand can be minimized.

VI. LEAD OPTIMIZATION

The ORISIS Property Explorer have been used to draw chemical structure and to calculate on the server itself various drug relevant properties whenever a structure is valid . A red color depicts having a high risk of undesired effects or poor intestinal absorption, whereas a green color indicated drug conform behavior.

VII. VIRTUAL SECREENING

Selected target constitutively activated BCR-ABL fusion protein was structurally characterized through molagro virtual docker which is a docking software. MVD performs flexible ligand docking which helps in recognizing the optimal geometry of the molecule. The interactions shown are those mediated by hydrogen bonds and by hydrophobic contacts.

VIII. DOCKING ANALYSIS

Screening of different docked complex was done by molagro virtual docker on the basis of energy as a important constraint of stability. The ligand molecule which shows minimum energy with the receptor molecule was chosen as best drug for the respective target constitutively activated tyrosine kinase.

RESULTS

Physiochemical characterization of the BCR-ABL fusion protein sequence was done by using protparam tool details are provided in Table 1. Isoelectric point (P.I) parameter shows that modeled protein is neutral in nature. Extinction coefficient is an important parameter which tells how much light a protein can absorb at specific wavelength. From the knowledge of amino acid composition molar extinction coefficient can be easily calculated. Instability coefficient parameter confirms the stability of modeled protein. A protein whose instability index is smaller than 40 is predicted as stable. Aliphatic index of a modeled protein is regarded as a positive factor for the increase of thermo stability of globular proteins.

Table 1
Physicochemical characterization of BCR-ABL fusion protein

Sequence length	Molecular weight	Isoelectric point (P.I)	Extinction coefficient	Instability coefficient	aliphatic index
690	76296.6	6.09	65820	38.50	59.58

Secondary structure of protein was predicted using GOR &SOPMA. Present study predicts the protein model as a helix-sheet-random coil structure. Details of the prediction are depicted in Table 2.

Table 2
Secondary structure analysis of BCR-ABL fusion protein from GOR and SOPMA tool

	Sequence length	Alpha helix	Beta sheets	Turns	Random coil
SOPMA	690aa	38.12%	0.00%	3.04%	51.16%
GOR	690aa	34.93%	0.00%	0.00	56.23%

Homology modeling of BCR-ABL fusion protein was done by using swiss modeler tool and Qmean score in swiss model workspace shows value 0.8 which confirms the 80% reliability of the fusion protein 3D model. 2hzi is annotated as MONOMER. The following biological unit was used to build the template structure: 2hzi.pdb1.gz. Model was succesfully built as monomer as depicted in Fig.1. The modeled proteins are validated using saves. Saves gives the details of ramachandran percentage, core regions and disallowed regions. It also gives the ramachandran figure which displays the allowed regions and disallowed regions. The percentage of ramachandran should be more than 85% and the disallowed regions should be less than 1 as depicted in Fig.2.

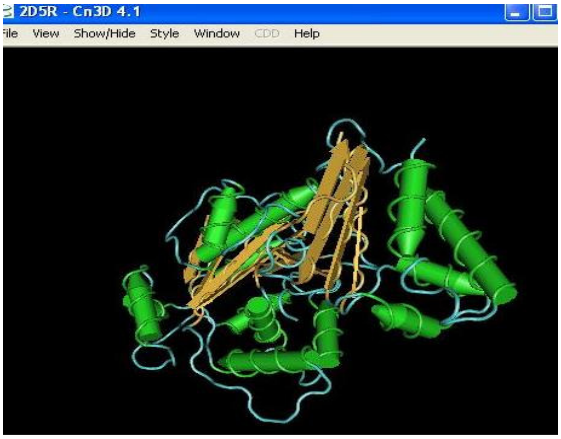
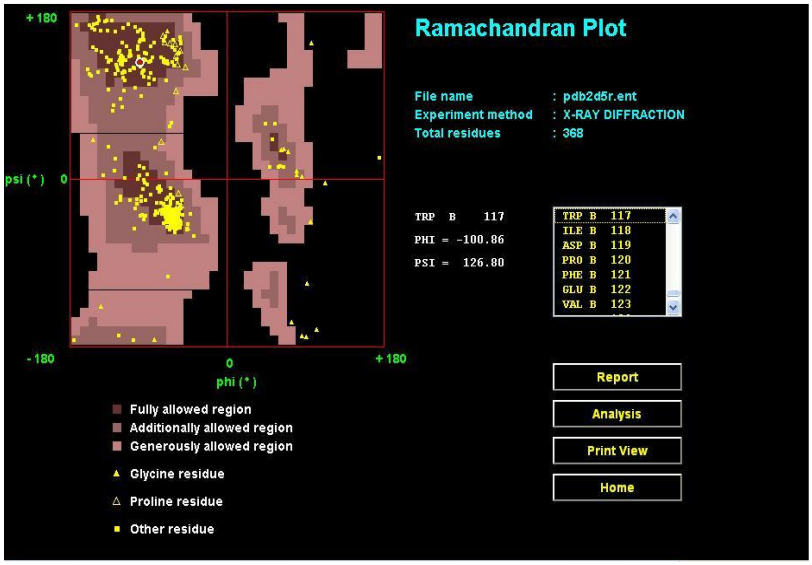


Figure 1
CN3D view of protein



Fully Allowed Region (285 residues)	:	78.30 %
Additionally Allowed Region (63 residues)	:	17.31 %
Generously Allowed Region (8 residues)	:	2.20 %
*Outside Region (8 residues)	:	2.20 %
		100.00 %

Figure 2
Ramachandran Plot

Size of cavities in the receptor molecule was predicted with the help of docking software Molagro Virtual Docker (MVD) as shown in Fig.3

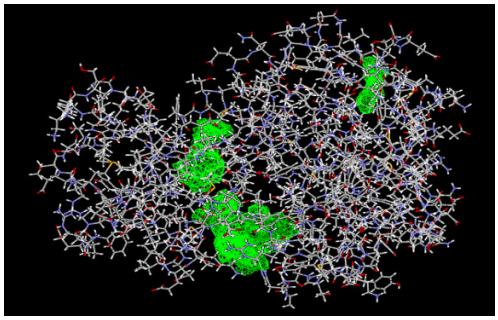


Figure 3
Cavities representation within the receptor or target molecule using Molagro Virtual Docker (MVD)

The proteins are searched for the drugs using drug bank. The modeled sequence is submitted to the drug bank. It gives the list of ligand related templates and the details of the drugs are also given to the particular templates. All ligands have been chosen from plant sources for easy availability and effective drug like behavior. The analogues of the drug are searched through PUBCHEM by giving name of the drug in the PubChem. Identification of ligands was carried out using

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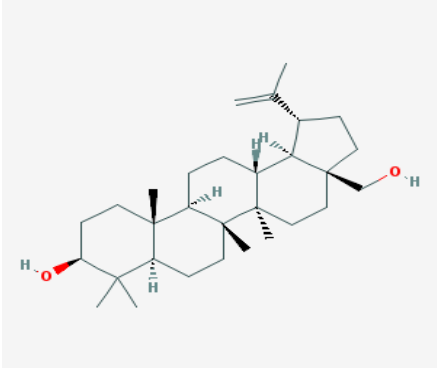
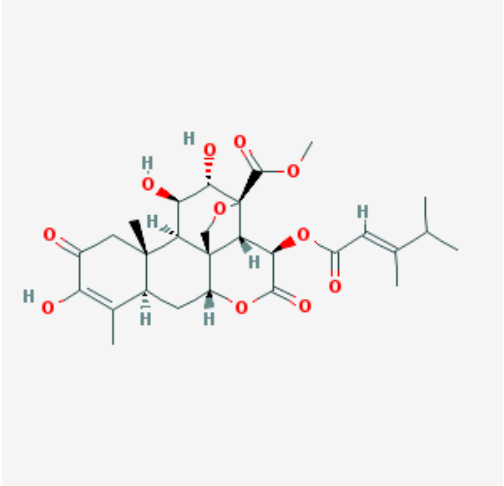
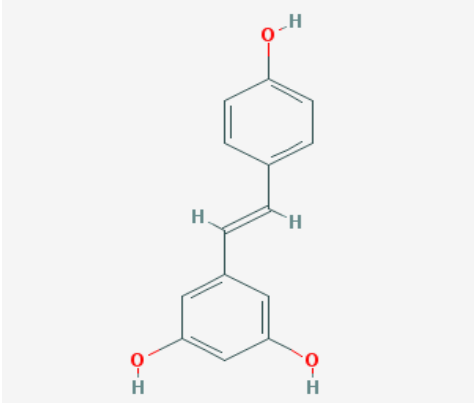
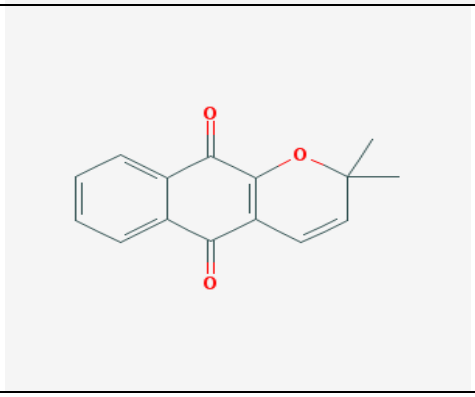
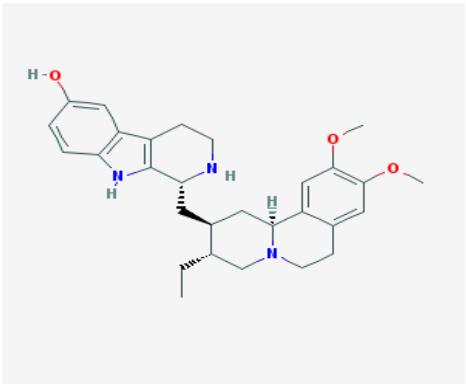
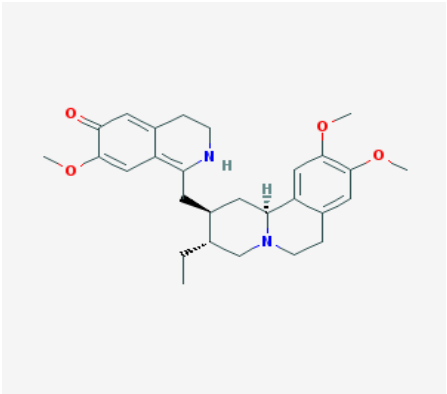
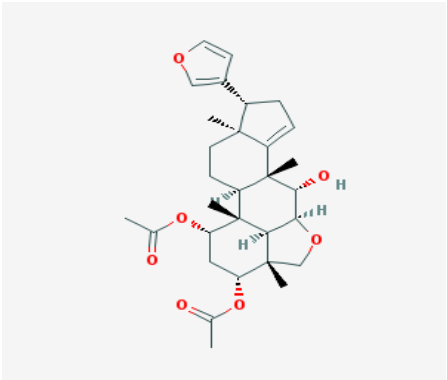
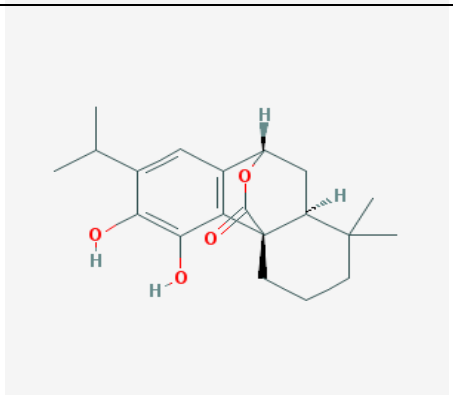
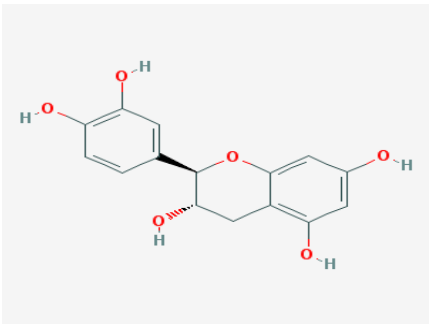
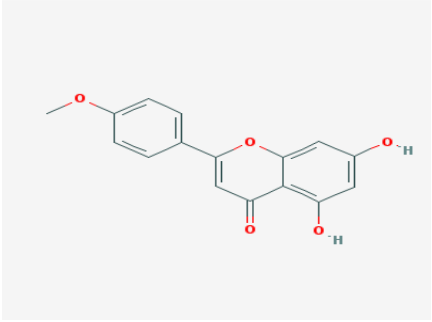
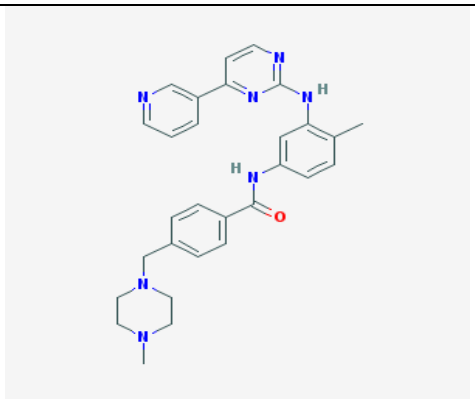
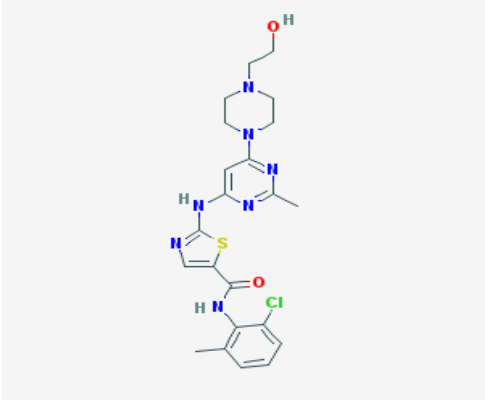
Table 3
Shows the list of ligands utilized for docking studies.

S.No	PUBCHEM .Id	LIGAND NAME	STRUCTURE OF LIGAND
1.	CID_72326	Betulin	

2.	CID_5281304	Bruceantine	
3.	CID_445154	Resveratrol	
4.	CID_72734	Alph - lapachone	
5.	CID_72341	Tubulosine	

6.	CID_5462438	Psychotrine	
7.	CID_44566526	1,3-Diacetylvilasinin	
8.	CID_442009	Carnosol	
9.	CID_72276	Catechin	

10.	CID_5280442	Acacetin	
11.	CID_ 5291	Imatinib	
12.	CID 3062316	Dasatinib	

1,3- Diacetylvilasinin shows effective binding energy as compared to previously used drugs which is depicted in Fig.4

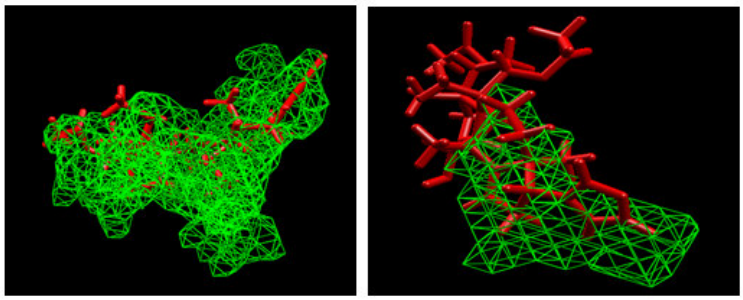
1,3- Diacetylvilasinin

Poses

Name	Ligand	MolDockScore	Rerank Score	HBond
[00] 4456...	44566526	-128.462	-56.2374	-4.33943
[03] 4456...	44566526	-119.926	-82.5101	-2.01338
[02] 4456...	44566526	-119.828	-58.0822	-5.11456
[04] 4456...	44566526	-117.184	-72.5649	-5.94266
[01] 4456...	44566526	-115.605	-39.5087	0

Poses

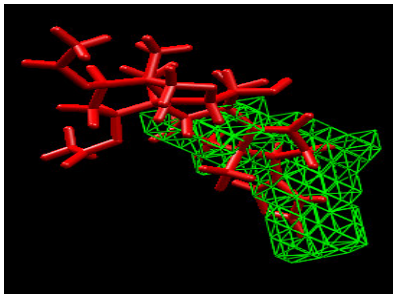
Name	Ligand	MolDockScore	Rerank Score	HBond
[00] 4456...	44566526	-163.768	100.186	-8.91955
[01] 4456...	44566526	-152.596	82.0881	-8.5259
[02] 4456...	44566526	-148.369	58.8516	-2.94773
[03] 4456...	44566526	-131.821	173.625	-4.35767
[04] 4456...	44566526	-126.068	131.939	-4.3337



Cavity -1 **Cavity -2**

Poses

Name	Ligand	MolDockScore	Rerank Score	HBond
☑ [00] 4456...	44566526	-148.439	1.75386	-9.77433
☐ [01] 4456...	44566526	-122	6.56043	0
☐ [03] 4456...	44566526	-120.929	44.1909	-2.944
☐ [02] 4456...	44566526	-120.207	22.1406	-1.77969
☐ [04] 4456...	44566526	-119.328	-11.1016	-7.53913



Cavity -3

Figure 4
Binding to the ligand

Docking was carried out by using Molagro Virtual Docker. Table-4 shows the result of docking process. The table has the description of the docking complex, the cavity number, Moldoc score, Rerank score and hydrogen bonding value. As outlined in the table, the target protein-1,3-Diacetylvilasinin complex has the best score of -163.768.

Table 4
shows Docking parameter and cavity with minimum energy and maximum interaction affinity

S.No	Docking complex	Cavity	MolDoc score	Rerank score	H Bond
1.	Target protein + Betulin	Cavity-2	-111.985	37.426	0
2.	Target protein + Bruceantine	Cavity-1	-115.366	92.0911	-3.95907
3.	Target protein + Resveratrol	Cavity-1	-98.8343	-79.2158	-10.8867
4.	Target protein + Alpha -lapachone	Cavity-1	-121.8131	-76.0762	-2.5
5.	Target protein + Tubulosine	Cavity-3	-110.8461	-53.5064	-0.5189
6.	Target protein + Psychotrine	Cavity-2	-153.126	-108.67	-0.4742
7.	Target protein + 1,3-Diacetylvilasinin	Cavity-2	-163.768	100.186	-8.919
8.	Target protein + Carnosol	Cavity-2	-95.8159	-25.9703	-8.02829
9.	Target protein + Catechin	Cavity -2	-107.758	-99.2777	-9.44999
10	Target protein + acacetin	Cavity -2	-110.867	-95.4278	-5.52961

Drug which are conventionally used shows less MolDoc score and rerank score as compared to the 1,3-Diacetylvilasinin ligand selected in this study which justifies that 1,3-Diacetylvilasinin can be act as a potent drug molecule in the future for the treatment of chronic myelogenous leukemia. Two conventionally used drug for the treatment of CML have also been selected and their docking parameter are listed in the table 5.

Table 5
shows binding energy in terms of MolDoc score and affinity between conventionally used drug molecule and target protein

S.No	Docking complex	Cavity	MolDoc score	Rerank score	H Bond
1.	Target protein + Imatinib	Cavity-3	-138.667	-60.1878	-3.88082
2.	Target protein + Dasatinib	Cavity-2	-144.127	-102.62	-2.41947

The comparative analysis between the traditional drugs and the chosen ligand 1,3Diacetylvilasinin proves that 1,3-Diacetylvilasinin can be a more effective drug for the treatment of CML because 1-3,diacetylvilasinin shows effective binding energy as compared to previously used drugs such as imatinib and dasatinib.

DISCUSSION

The result of molecular modeling favorably shows that predicted BCR-ABL protein is stable in nature. Tertiary structure prediction of BCR-ABL fusion protein has been carried out by using swiss modeler tools and Qmean score in swiss model workspace shows value 0.8 which confirms the 80% reliability of the fusion protein 3D model. Virtual screening of the phytoligands with modeled target protein has been carried out using Molagro Virtual Docker(MVD)[16]. Docking analysis shows 1,3-Diacetylvilasinin possess maximum binding affinity, minimum interaction energy and hence maximum stability with the target fusion protein and could be act as a potent drug for inhibiting constitutively activated tyrosine kinase in the chronic myelogenous leukemic patients.

CONCLUSION

In this study BCR-ABL fusion protein was targeted which codes for constitutively activated tyrosine kinase and is a sole agent of chronic myelogenous leukemia in humans. Homology modeling of the fusion protein was done and modeled fusion protein structure utilized for docking analysis. For docking analysis phytoligand was chosen and docking studies were done with the help of molagro virtual docker. In Molagro virtual docker (MVD) binding energy of the ligand and target macromolecule is important in describing how well drug bind to its target protein. Docking analysis shows 1,3-Diacetylvilasinin as a potent constitutively tyrosine kinase inhibitor on the basis of binding energy between lead and receptor molecules. Various investigations reveal that virtual screening of phytoligands is a very promising area which could lead to the discovery of the potent drug compound. In silico analysis and docking studies used in virtual screening saves large amount of expenditure and time, which are needed when thousands of phytoligands screened clinically. Virtual screening analysis reduces the number of phytoligands which need to be tested in the final validation step. So instead of choosing random bioactive compound we can select bioactive compound which has already been shown some of the desired affinity with the target in the biological system.

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