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Potential Phytocompounds to Inhibit Human Polo-Like Kinase 1 in Cancer Therapy.

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ABSTRACT

Cancer is a potential fatal disease characterized by the uncontrolled growth and spread of abnormal cells. Polo-like kinases (Plks) are serine/threonine protein kinases which play critical role in regulating cell cycle. Deregulated expression of Plks is detected in many types of cancer and is associated with oncogenesis. Though there are five Plk homologous, Plk 1 is identified as the best target to defend uncontrolled cell growth. Natural compounds from plant resource are being exploited recently on a large scale for treating various diseases. The aim of this study is to identify the inhibitory activity of selected phytochemicals on Plk 1 protein so as to develop effective drugs based on natural compounds. *In silico* docking analysis is a successful way of screening molecules so as to confirm their inhibitory activity against respective targets. About 574 phytocompounds of various conformations were docked with the target protein Plk 1 using the software Discovery Studio version 4.0. The most effective ones were identified based on interaction energy and docking score. Phytocompounds such as ferulic acid, caffeic acid and N-methyltyramine from the common medicinal plants like *Curcuma longa*, *Pseudarthria viscida* and *Wrightia tinctoria* showed strong anticancer activity through the suppression of Plk 1.

Keywords: Discovery Studio 4.0, Human polo-like kinases, Molecular docking, Phytocompounds.

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INTRODUCTION

Cancer is a leading cause of death worldwide and about 70 percent more new cases is expected in the next two decades. [1]. Polo-like kinases (Plks) are of serine family or threonine protein kinases which are key regulators of mitosis, meiosis and cytokinesis [2]. In humans, five Plk homologs, viz. Plk1 to Plk 5 have been identified. Out of these, Polo-like kinase 1 (Plk1) is associated with oncogeniesis and detected in many types of cancer [3]. This plays a significant role in cell division and represents a promising target for the development of specific inhibitors to selectively treat cancer. During mitosis, Polo-like kinases phosphorylate multiple proteins, there by regulating cellular proliferation. It is expressed only in dividing cells and an increased level can be observed during late G2 and M phase of mitosis. Mutated or over expressed Plk1 alters the mitotic cell cycle and promotes the proliferation of normal cells resulting in progression of cancer. The Plk1 is overexpressed in many cancers such as uterine, ovarian, breast, stomach, skin etc. compared to normal cells. Due to its involvement in carcinogenesis, Plk1 is considered as a valid target for cancer therapy [3, 4].

Herbal medicines are widely accepted due to its compatibility with metabolism and lesser side-effects. Phytocompounds are increasingly becoming popular in the modern world as natural alternative to synthetic chemicals [5]. The important phytocompounds being exploited are alkaloids, flavonoids, tannins and phenolics [6].

Computational techniques have substantially enhanced the success rate in the designing of potential lead compounds and in drug discovery. Molecular docking is a successful method to predict the binding mode of small molecules such as substrates or drug candidates to a receptor [7]. This process helps in obtaining the best geometry of receptor- ligand complex and to design more effective drug molecule by computing the interaction energy. The present study is an *in silico* approach to identify the inhibitory activity of bioactive compounds against Polo-like kinase 1 so as to exploit them for anti-cancerous drug discovery

MATERIALS AND METHODS

Preparation of protein

The three dimensional crystal structure of Plk1 with PDB ID 3KB7 was retrieved from the Protein Data Bank (PDB). The retrieved protein structure was prepared using 'prepare protein' protocol of Discovery Studio 4.0. Crystallographic water molecules and heteroatoms were removed and hydrogen atoms were added to correct the chemistry of protein. Energy minimization was performed by applying CHARMm force field to avoid steric overlap and to relax the confirmation [8]**.** The optimized and energy minimized protein was further used for docking analysis.

Active site identification

Active sites of the protein were predicted using discovery studio based on the receptor cavity method [9]. The active site suitable for the current interaction study was selected by identifying the key amino acid residues present in the binding pocket. The active site identified was used for understanding the interaction between the ligand compounds and the protein.

Identification of ligands

About 574 pharmacologically active compounds from 15 medicinal plants such as *Allium sativum, Curcuma longa, Murraya koenigii, Piper nigrum, Zingiber officinale, Pseudarthria viscida, Pterocarpus marsupium, Wrightiatinctoria, Trigonella foenum-graecum, Sida rhombifolia, Catharanthus roseus, Aegle marmelos, Phyllanthus niruri, Boerhavia diffusa* and *Aloe vera* were selected as ligands. The molecular structures of these phytocompounds were retrieved from PubChem database. The ligands were then prepared using 'prepare ligand' protocol of Discovery studio 4.0. [10].

Evaluation of drug likeliness

Drug likeliness properties of the selected phytochemicals were examined using Lipinski and Veber rule through Discovery studio version 4.0. The phytocompounds which satisfied the Lipinski's rule of 5 [11] which

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include molecular weight < 500 daltons, number of hydrogen bond donors < 5, number of hydrogen bond acceptors <10, calculated water partition coefficient (ALOGP) < 5; were screened out for docking studies .

Molecular Docking

Molecular docking is a method to predict the binding orientation of small molecule candidates to their protein targets and in turn predict the binding affinity and strength of association between the target and ligand. Molecular docking was performed between the active binding region of the energy minimized structure of 3KB7 and the screened phytocompounds using CDOCKER docking protocol of Discovery studio 4.0. CDOCKER is a molecular dynamics based docking algorithm which uses the CHARMm force field and offers full flexibility to ligands including dihedrals, angles and bonds [12].The efficiency and accuracy of compounds in the binding process depend on scoring functions. The strength of interaction between the protein and the phytochemicals was evaluated using various scoring functions such as protein-ligand binding energy, CDOCKER energy, CDOCKER interaction energy and hydrogen bond interaction. Binding poses with lowest binding energy and least energy difference between CDOCKER energy and CDOCKER interaction energy were selected as good interacting compounds with Plkl.

ADME and Toxicity detection

ADMET refers to absorption, distribution, excretion, metabolism and toxicity. The pharmacokinetics and toxicity of selected phytocompounds was evaluated using ADME toxicity suite of Discovery Studio 4.0. Phytocompounds with acceptable ADMET properties were considered for finding the best inhibitory compounds.

RESULTS AND DISCUSSION

Protein preparation

Based on extensive literature review, the three dimensional structure of Polo-like kinase 1 (Plk1) complexed with pyrazoloquinazoline inhibitor (PDB ID: 3KB7) was selected and retrieved from PDB. Pyrazoloquinazoline is a selective Polo-like kinase inhibitor which has shown 82 percent tumor growth inhibition after oral administration [13]. The retrieved 3D structure of Plk1 (PDB ID: 3KB7) was prepared and then energy minimized to stabilize the structure for performing docking analysis. The optimized and energy minimized structure of Plk1 is shown as Fig 1.

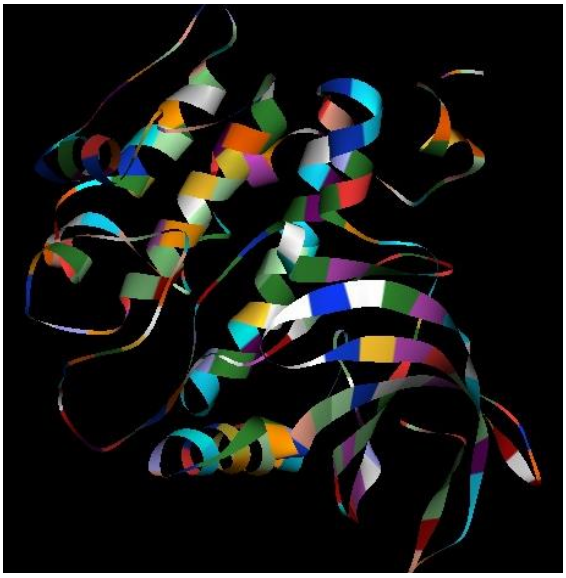

Figure 1: Energy minimized structure of Plk1

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Active site identification

Three active sites were predicted based on the receptor cavity method. Of these predicted active sites, active site 1 was selected as the binding site for the study. The amino acid residues in the selected active site included Glu131, Cys133, Leu59, Cys67, Arg136, Asp194, Lys82, Glu101, Phe195, His105, Leu132, Ser137, Glu140 and Phe183 (Fig 2). These amino acids are of great importance for the binding activities and most of these amino acids interacted with the inhibitor, pyrazoloquinazoline and the interaction can be seen in the retrieved PDB structure.

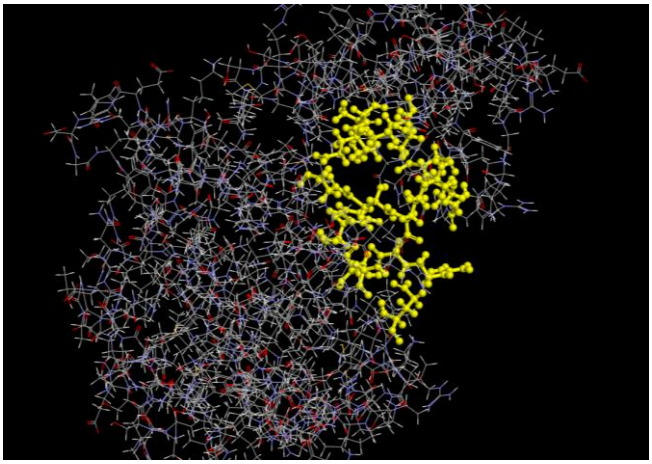

Figure 2: Structure of Plk1 with active site highlighted in yellow colour

Ligand Preparation and drug likeness study

574 phytochemicals from 15 medicinal plants were used as ligands for the interaction study. The 3D structure of these compounds were retrieved from PubChem database and prepared using Discovery Studio version 4.0. Preparation process included cleaning up compounds, calculating 3D coordinates and generating possible isomers. After the ligand preparation process, 477 ligands with various conformations were obtained.

The molecular properties associated with drug-likeness and the bioactivities of these compounds were evaluated by satisfying Lipinski and Veber rule. The information from the filter process helps to find out whether the chemical compounds possess biological or pharmacological activity that would make them acceptable as orally active drug for consumption. Out of the 477 conformations filtered, 97 satisfied the rule based on molecular weight, number of hydrogen bond donors, number of H bond acceptors and water partition coefficient (ALOGP) and were further utilized for molecular docking.

Molecular Docking

The comparative and automated docking studies with 97 phytocompounds on polo-like kinase (PDB ID: 3KB7) was performed with the CDOCKER algorithm in the docking program, Discovery Studio 4.0. The better interacting compounds were selected based on the binding compatibility of the compound. The strength of the protein-ligand interaction was analyzed based on dock score, number of hydrogen bonds and binding energy. The top-ranked compounds with lowest docked binding affinities and high docking scores were selected and summarized in Table 1. The conformation with lowest binding energy was considered as the most favorable docking pose. Binding energy represents the sum of the total intermolecular energy, total internal energy and torsional free energy minus the energy of the unbound system [14]. Those compounds with binding energy less than -100 and with minimum deviation between CDOCKER and CDOCKER interaction energy were selected and listed in the table 1.

The protein-ligand complex is well stabilized mainly by hydrogen bonds and hydrophobic interactions. All the generated top docked poses exhibited bonds with one or more amino acids in the binding pocket of Polo-like kinase. The dock score analysis and binding energy computed indicated that 2'-Hydroxygenistein,Dalbergioidin, Leucopelargonidin, methyl-4-tyramine, Ferulic acid, Caffeic acid and Gallic acid had good binding efficiency with Plk1 with binding energies ranging between -224.39 kcal/mol and -102.97

kcal/mol. 2'-Hydroxygenistein recorded the lowest binding energy while Boeravinone F recorded the best CDOCKER and CDOCKER interaction energy. All the selected compounds recorded very little energy difference between CDOCKER and CDOCKER interaction energies. Amino acid residues that showed interaction with the ligand compounds included LYS82, CYS133 and ASP194.

Sl No.	Compound	Source	(-) CDOCKER energy) (kcal/mol)	(-) CDOCKER interaction energy (kcal/mol)	Active site residue	No. of hydrogen bonds	Binding energy (kcal/mol)
1	2'-Hydroxygenistein 5282074	*Pseudarthria viscida*	48.97	51.46	CYS133 ASP194	2	-224.39
2	Dalbergioidin 181994	*Pseudarthria viscida*	47.90	50.64	LYS82	1	-216.27
3	3,7,4'-trihydroxyflavone 5281611	*Pterocarpus marsupium*	41.23	52.19	LYS82 CYS133	2	-194.32
4	Boeravinone F 12004175	*Boerhavia diffusa*	56.42	56.66	LYS82	1	-192.78
5	4',7-dihydroxyflavone 5282073	*Pterocarpus marsupium*	53.79	51.27	LYS82 CYS133	2	-192.57
6	Leucopelargonidin 3286789	*Pseudarthria viscida*	36.00	43.02	LYS82	1	-156.26
7	methyl-4-tyramine 9727	*Pseudarthria viscida*	42.32	41.45	LYS82	1	-150.71
8	Ferulic acid 445858	*Pseudarthria viscida* *Wrightia tinctoria* *Curcuma longa* *Trigonella foenum graecum*	36.91	40.75	LYS82 CYS133 ASP194	3	-146.97
9	Caffeic acid 689043	*Pseudarthria viscida*	36.48	37.41	LYS82 ASP194 CYS133	3	-122.15
10	Gallic acid 370	*Pseudarthria viscida* *Phyllanthus amarus*	35.23	32.85	CYS133	1	-102.97

Table 1: Docking scores of Plk 1 -protein interaction with selected phytocompounds

Sl No.	Compound	Solubility level (2-4)	BBB level (2-3)	Hepatotoxicity (FALSE)	Absorbtion level (0-1)	AlogP8 (<4)
1	2'-Hydroxygenistein	3	3	TRUE	0	1.898
2	Dalbergioidin	3	4	TRUE	0	2.014
3	3,7,4'-trihydroxyflavone	3	3	TRUE	0	2.114
4	Boeravinone F	2	4	TRUE	0	2.458
5	4',7-dihydroxyflavone	3	2	TRUE	0	2.652
6	Leucopelargonidin	4	4	TRUE	0	1.432
7	methyl-4-tyramine	4	2	FALSE	0	1.446
8	Ferulic acid	4	3	FALSE	0	1.669
9	Caffeic acid	4	3	FALSE	0	1.443
10	Gallic acid	4	3	TRUE	0	0.733

Table 2: ADMET properties of selected active phytocompounds (desirable values given in parenthesis)

The better interacting compounds were further screened based on their toxicity. The ADME and Toxicity studies provide insights into the pharmacokinetic property of the compounds. The ADME/T properties of the compounds which showed highest interaction are shown in Table 2. The hydrogen bond interaction between N-methyltyramine, ferulic acid, and caffeic acid with the receptor 3KB7 are shown in Fig.3.

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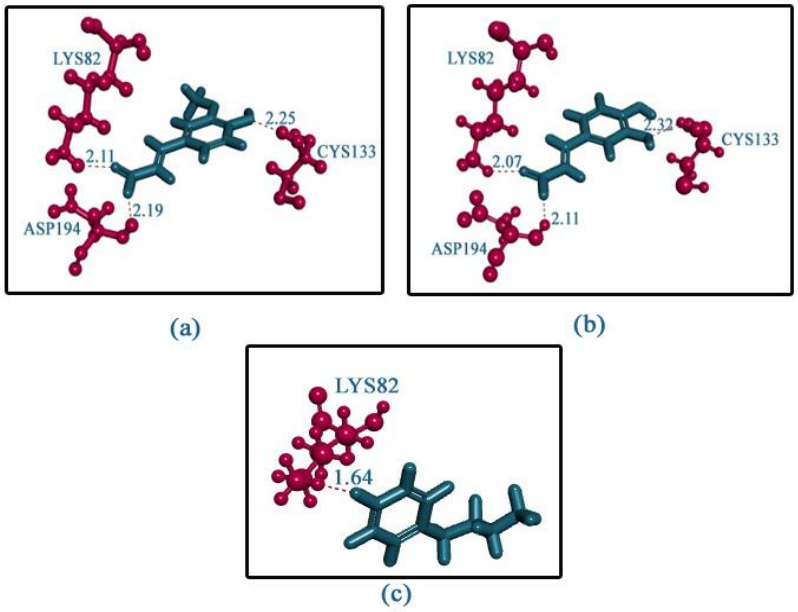

Figure 3: Predicted H bond interactions (red dotted lines) with Plk1 (A) interaction with ferulic acid (B) interaction with caffeic acid (C) interaction with N-methyltyramine .H-bond distances are indicated in Å unit

It was observed that the best interacting compounds like 2'-Hydroxygenistein and 3,7,4'-trihydroxyflavone were hepatotoxic while the others like Dalbergioidin and Boeravinone F were both hepatotoxic and with undesirable BBB level. Only three compounds namely methyl-4-tyramine, caffeic acid and ferulic acid qualified all the prescribed properties in ADMET analysis. However the ranking of these three compounds with respect to binding energy, CDOCKER and CDOCKER interaction energy were poor compared to other compounds. The compounds with unsatisfactory ADMET parameters cannot be directly forwarded for drug development. However better conformations with different functional group for such compounds could be identified through *in silico* methods such as combinatorial library design so as to reduce the side effects.

Among the different medicinal plants evaluated in the study, *Pseudarthria viscida*, was found superior to others in possessing seven out of ten compounds (Table 1) identified for suppressing Plk 1. *Pseudarthria viscida*, is an important plant used in many Ayurvedic preparations for diseases like fever, rheumatism, bronchial asthma, hemorrhoids and diabetes mellitus. Other properties like antihypertensive, antioxidant, antiulcer, antifungal, antidiarrheal, and antitumor have also been reported [15]. The present study highlights the superiority of *Pseudarthria viscida* for exploiting its phytocompounds in developing anticancer drugs through suppressing Plk1.

CONCLUSION

Computer aided drug designing and molecular docking analysis is one of the highly effective methodologies in identifying and analyzing new candidate drug molecules. The present study revealed that compounds such as 2'-Hydroxygenistein,Dalbergioidin,3,7,4'-trihydroxyflavone, Boeravinone F, 4',7-dihydroxyflavone and Leucopelargonidin are highly potent in inhibiting Plk1 but with little side effect like hepatotoxicity. Few other compounds like Methyl-4-tyramine, Caffeic acid and Ferulic acid, though with low dock score, are also identified as efficient inhibitors of Plk1 with no side effects, qualifying all the ADMET properties. Among the 15 medicinal plants studied, *Pseudarthria viscida,* was found superior by possessing seven out of ten compounds identified for inhibiting Plk1.

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