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In silico study on interaction between human polo-like kinase 1 and cyanobacterial sheath pigment scytonemin by molecular docking approach

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ABSTRACT

Cancer is one of the major causes of death throughout the globe. It is expected that the number of new reports with cancer cases will reach twenty two million in the coming two decades. Africa, Central and South America and Asia will have more than 60 percent of the world's new cancer reports and 70 percent of the world's deaths from cancer will be contributed by these continents. Hence, drug discovery for treatment of cancer is the most worked area of this century. Polo-like kinase 1 (PLK-1) is highly expressed in human tumors and is important target of anti-cancerous drugs owing to its role in cell cycle events. It is crucial in maintenance of stability of genome and during different stages of mitosis. Scytonemin is a lipid-soluble and yellow-brown pigment exclusively synthesized by several cyanobacterial species in response to ultraviolet-A radiation. It functions as a photoprotective compound and can act as non-competitive and competitive inhibitor of PLK-1. Other kinases such as Myt1, cyclin-dependent kinase 1/cyclin B, checkpoint kinase 1 and protein kinase C are also inhibited by scytonemin. In the present study, molecular docking approach has been employed for positioning the inhibitor (dimethoxyscytonemin) into the active site of PLK-1 for determining the most probable binding mode. Based on the docking studies, the models which are developed could be utilized for understanding the structure-activity relationships of the scytonemins and for the prescreening and designing of novel inhibitors of PLK-1.

Keywords: *Scytonemin; cyanobacteria; cancer; anti-cancerous activity; molecular docking; polo-like kinase 1.*

1. INTRODUCTION

According to Indian Council of Medical Research (ICMR), in India over 17.3 lakh new cases of cancer is likely to be reported and over 8.8 lakh deaths may occur due to cancer (breast, lung and cervix) by 2020 (http://www.midday.com/articles/over17lakhnewcancercasesinindiaby2020icmr/17248152).

Cyanobacteria are one of the ancient organisms and are prolific producers of value added and bioactive compounds/secondary metabolites [1]. These secondary metabolites have been presumably optimized in due course of evolution and exert enhanced affinity for their biological targets in the concerned organism but also exhibit activity in diverse biological contexts including human cells.

Several cyanobacterial secondary metabolites have been found to be potent-in-class inhibitors and serve as valuable tools for chemical biology for probing protein function, and as templates for novel drugs development [2]. Several new drugs have been developed by natural products and this remains to be the most appropriate approach for identifying molecules for the drug discovery [3]. High expression of polo-like kinase 1 (PLK-1) has been observed in human tumors and hence, is an attractive drug target owing to its role in cell cycle processes such as mitosis, assembly of the bipolar spindle, centrosome maturation, sister chromatids separation and cell's exit from mitosis [4].

Cyanobacterial sheath pigment scytonemin acts as potential sun-screen from ultraviolet (UV) radiation and have absorption maximum in the spectral range of UV-A radiation [5-11]. Scytonemin shows absorption maximum at 370 nm *in vivo* whereas purified compound shows absorption maximum at 386 nm. It also absorbs significantly at 300, 278, and 252 nm and provides protection from intense radiation to the organisms inhabiting under UV-A/B exposed harsh environments [5-6, 9]. Scytonemin exists in different forms such as reduced (Mw 546 Da), oxidized (Mw 544 Da), tetramethoxyscytonemin, dimethoxyscytonemin, scytonemin-3a-imine and scytonin in different cyanobacteria [12-14]. It has potential to cure some hyperinflammatory and hyperproliferative disorders and its structural properties such as multiple dissection points, phenolic groups and lack of chirality allow its modification/designing for the development of novel pharmacophores [15-16]. ROS production and dysfunctioning of mitochondria can be induced by reduced form of scytonemin (R-scy) resulting in programmed cell death (type II) of human T-lymphoid Jurkat cells [17]. Anti-tumor activity was also shown in murine macrophage RAW264 cells by R-scy and scytonemin which also checked the nitric oxide production induced by interferon-γ and lipopolysaccharide [18]. Stevenson et al. [15] performed a target-based screening for PLK-1 inhibitors which also yielded scytonemin. As per expectation, scytonemin inhibited the cellular proliferation which was stimulated by growth factors and caused induction of apoptosis but cells were not arrested at a specific stage, indicating multiple

cellular targets for the UV-screening pigment scytonemin [15]. Binding studies revealed that scytonemin can act as non-competitive as well as a competitive inhibitor of PLK-1. The PLK-1 inhibition *via* pharmacological modulation with a small molecule (ligand) is an effective strategy for controlling/regulating cancer as observed for the BI-2536 [4].

Alteration in the activity of other cell cycle related kinases such as protein kinase A, serine/threonine kinase, Myt1, tyrosine kinase, cyclin-dependent kinase 1/cyclin B checkpoint kinase 1 and protein kinase Cbeta2 has also been reported by scytonemin [15]. Calcium antagonistic properties have also been reported by scytonemin A, hence, it can also be utilized as blocker of calcium channels [19]. Scytonemin has the potential for the treatment of multiple myeloma because of its tendency to inhibit the PLK-1 activity [20]. For calculation of protein-ligand interactions, the most widespread approach is by molecular docking, which is a good method of predicting the ligand binding sites on the whole protein target. Here we have carried molecular docking on PLK-1 binding pockets using the dimethoxyscytonemin to explore the characteristics of the PLK-1 binding pocket and to understand the structural requirements/properties for better activities of scytonemin.

2. MATERIALS AND METHODS

Previously solved 3D structure of human PLK-1 was downloaded from protein databank (www.rcsb.org/pdb) [21] having id 3P2W and the structure of dimethoxyscytonemin were taken from PubChem database [22] (Fig. 1). Probable binding pockets of PLK-1 were predicted using CastP server [23] and the pockets having maximum area covered were selected to perform docking. Energy minimisation of protein structure was done using Swiss-PDB Viewer [24]. Docking interaction between human PLK-1 and dimethoxyscytonemin was carried out using AutoDock 4.0 [25]. Polar hydrogen atoms and kollman charges were added to the protein structure, and atoms were assigned to AD4 type during docking preparation. Flexible torsion tree was added to dimethoxyscytonemin whereas PLK-1 was kept rigid. AutoGrid 4.0 [26] was used to build grid box around one pre-selected center atom from each predicted pockets where dimension of grid box was 60×60×60 points in X, Y and Z dimension. Docking was performed based on genetic algorithm in Cygwin platform [27] and output was stored in Lamarckian genetic algorithm. Docking parameters were set in default and 100 GA runs were performed. The binding of dimethoxyscytonemin to PLK-1 was confirmed based on the lowest docking energy. Interaction between PLK-1 residues and dimethoxyscytonemin was visualized using UCSF Chimera 1.11.2 software [28].

3. RESULTS

Six different forms of scytonemin were screened for interaction with PLK-1 and the one having the least binding energy (dimethoxyscytonemin) was selected for further studies (Table 1). Binding pockets of PLK-1 having maximum area covered were predicted and selected to perform docking studies (Table 2). Summary of molecular docking of selected ligand (dimethoxyscytonemin) with target receptor (human PLK-1) has been represented in Table 3 and 4. Energy minimization of protein structure was done. The binding of dimethoxyscytonemin to PLK-1 was confirmed, based on lowest docking energy (Table 3).

Table 1. Estimated free binding energy of docking of scytonemin (different forms) against human PLK-1.

Ligand	Total energy (kcal/mol)	van der Waals interactions (kcal/mol)	H bond (kcal/mol)
Dimethoxyscytonemin	-117.86	-97.1332	-20.7270
Reduced scytonemin	-96.3451	-85.2528	-11.0923
Scytonemin	-94.7717	-82.9521	-11.8196
Scytonemin-3a-imine	-109.864	-89.5487	-20.3156
Scytonine	-103.673	-81.1294	-22.5434
Tetramethoxyscytonemin	-97.6685	-76.3749	-21.2935

From the docking studies between PLK-1 and dimethoxyscytonemin six interacting pockets were found having 23, 14, 14, 15, 11, 8 as its interacting residues in each pocket respectively as supplemented in the Table 3, from these interacting residues one central atom was selected from each pocket and docking study was performed using these central atom and minimum estimated docking energy was found in pocket 4 having central atom 512 arginine and minimum energy was found -8.84 followed by pocket one -8.50 and three -8.49 kcal/mol as supplemented in Table 3. Out of six, only three central atoms formed H-bonds which are 540 lysine, 389 proline, 512 arginine, having LEU-491, GLU-391, GLU-391 as their interacting partner as given in Table 4. Key molecular interactions between human PLK-1 and dimethoxyscytonemin after docking studies have been shown in Fig. 2.

Table 2. Binding pockets of PLK-1 having maximum area covered selected to perform docking studies.

S. No.	No. of Residues	Area	Vol	Residues
1	23	465.2	617.2	411V, 412S, 413K, 414W,490L, 491L, 492K,493A, 494G, 495A, 496N, 497I, 533N, 534F, 535F, 538H, 539T, 540K, 553I, 554D, 555E,557, 559F
2	14	262.7	444.6	8F, 371D, 374L, 435L, 439S, 441R, 456R, 510Y, 512R, 513T, 524H, 590L, 593S, 594R
3	14	242.7	260.6	405C, 406I, 407P, 408I, 409F, 527N, 529S, 542I, 544C, 546L, 547M, 549A, 550V, 551T
4	15	221.4	304.5	406I, 407P, 408I, 410W, 412S, 428C, 429N, 492K, 493A, 494G, 495A, 497I, 498T, 499P, 500R
5	11	114.6	128.1	443I, 445Y, 452Q, 460E, 462Y, 507R, 508L, 509P, 510Y, 526S, 527N

S. No.	No. of Residues	Area	Vol	Residues
6	8	116.7	109.2	385N, 388K, 389P, 390S, 391E, 577A, 580L, 581R

Table 3. Estimated free binding energy of docking between human PLK-1 and dimethoxyscytonemin.

PDB Id	Docking Energy			
	Centre atom	Minimum	Maximum	Conformation
3p2W	540 Lysine	-8.50		29
	389 Proline	-7.18	-5.90	31,4
	410 Tryptophan	-8.49	-8.83	82,58
	512 Arginine	-8.84	+0.18	68,93
	551 Threo nine	-7.95	+0.49	89,92
	460 Glutamate	-7.58	-6.91	7,43

Table 4. Hydrogen bonding between dimethoxyscytonemin and centre atoms of human PLK-1 showing their interacting residues.

Centre atom	Hydrogen Bond		
	No. of H-bond	Distance	Interacting Partner
540 Lysine	1	2.663 A°	LEU-491
389 Proline	1	2.077 A°	GLU-391
410 Tryptophan	0	-	-
512 Arginine	0	-	-
551 Threo nine	1	2.155 A°	LYS-556
460 Glutamate	0	-	-

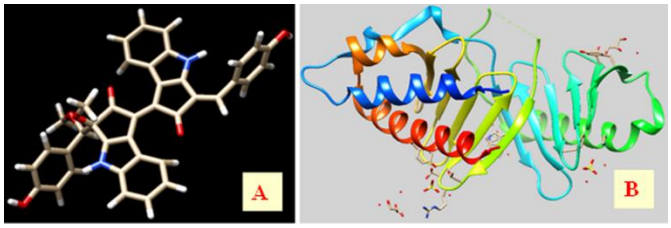

Figure 1. Molecular 3D structure of ligand, dimethoxyscytonemin; Pub-Chem CID: 10461341**(A)** and target, human polo-like kinase 1(PLK-1), 3P2W: unliganded form of PLK-1 Polo-box domain **(B).**

Discussion.

Biosynthesis of sun-screening compounds such as scytonemin is an important strategy for counteracting with harmful UV radiation as it has capacity of shielding cells from the damaging hitting photons [6]. New findings have revealed important information related to structure, genetics, biosynthesis and potential application of scytonemin as sunscreen [29-32] Scytonemin is extraordinary cyanobacterial secondary metabolite having remarkable stability, possess extraordinary photo-protection and have unique chemical [14, 33-34]. *In silico* molecular docking studies of dimethoxyscytonemin with human PLK-1 was performed for accessing anticancerous property of this form of scytonemin targeting PLK-1. All the information obtained from the docking represents that 540 lysine showed better interaction than all of the interacting central atoms because it has minimum binding energy and it has one H-bonds than other central atoms. The electrostatic bond, van der Waals, covalent bond and H-bond were involved in residues interaction which could be viewed in Fig. 2. For identification of the ligand binding pockets and prediction of interaction between a ligand and protein, as well as for predicting interactions between two proteins, molecular docking approach is preferable [35]. It is generally utilized for structure based drug designing due to its ability of highly accurate prediction of the conformation of small-molecule (ligands) within the suitable binding site of target.

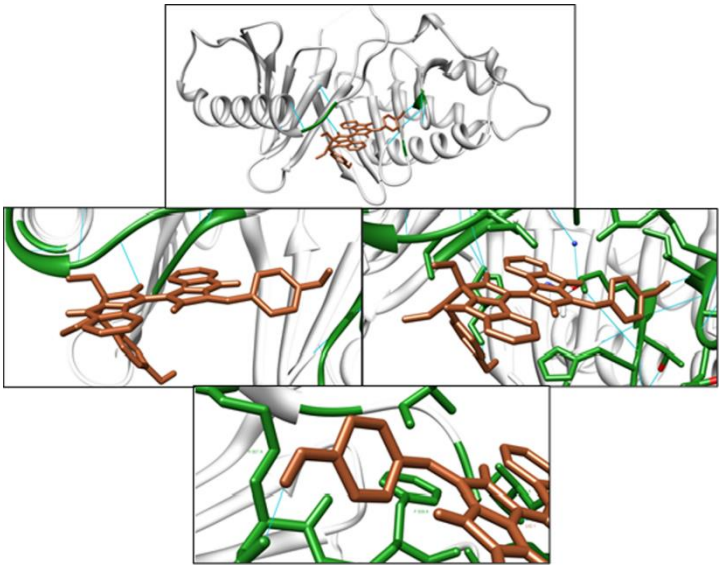

Figure 2. Key molecular interactions between human polo-like kinase 1 and dimethoxyscytonemin.

Molecular docking employs a two-step process starting with compiling different conformations of ligand in identified active sites of the protein/receptor followed by ranking them as per the binding conformation energies for each individual binding conformation [36]. By exploring the large conformational space and depiction of different binding sites, the most appropriate binding conformations are carried out leading to accurate prediction of interaction energy associated with the respective binding conformations [37-38]. During the initial part of the study we docked dimethoxyscytonemin in the identified binding sites for drawing a comparison of binding energy of the different forms of scytonemin with PLK-1. The ligand conformation of scytonemin was then ranked accordingly to their binding energy with the selected binding pocket. Our study revealed that among the different forms of scytonemin the best docking energy was exhibited by dimethoxyscytonemin with binding affinity of -117.86 kcal/mol followed by scytonemin-3a-imine (-109.864 kcal/mol), scytonine (-103.673 kcal/mol).

4. CONCLUSIONS

Six binding pockets of PLK-1 were identified on the basis of maximum area and volume (having area above 100) covered to perform docking studies. Thereafter, in all six active sites appropriate central atom was selected and docked with dimethoxyscytonemin. On the basis of above results, we found that minimum docking energy was of pocket 4 having central atom 512 arginine and out of six, only three central atoms formed H-bonds which were 540 lysine, 389 proline, 512 arginine, having LEU-491, GLU-391, GLU-391 as there interacting partner with drug (dimethoxyscytonemin). The PLK-1 causing cancer can be inhibited by dimethoxyscytonemin which can be used as a template for designing novel PLK-1 inhibitor. Further confirmation with wet lab experiments needs to be carried out for prediction of its anticancerous activity (of dimethoxyscytonemin)

and more *in silico* studies are required for various forms of scytonemin targeting different kinases involved in cancer.

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