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In silico prediction of D-HSCDK2 structure and its docking study with Flavopiridol analogues

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ABSTRACT

In order to develop a specific and effective anti cancer drug, various analogues of flavopiridol have been taken from Pubchem database and docking studies were performed to identify best suited analogue. Homology modeling of D-HSCDK2 was performed in silico using known protein crystal structure. Its 3-D structure was evaluated and validated using PROCHECK comprising 90.5% amino acid residues in favored region of Ramachandran plot. Stability of the structure was confirmed by the program ERRAT having the overall quality factor as 80.08 and Verify_3D with 85.28% residues having 3D-1D score > 0.2. With the structure of D-HSCDK2 generated, flexible docking was performed using GLIDE. Results indicate that among fifteen flavopiridol analogues, CID 24867231 was found as best interacting with D-HSCDK2 having lowest dope score of -8.82. In Particular LEU83 and ILE144 residues of D-HSCDK2 form hydrogen bonds and showed strong nonbonding interaction with analogue (CID 24867231). Further the generation of different derivatives can be made by the modification in the moieties of CID 24867231. These derivatives can be used to develop effective drugs against cancer. Further these results are subject to clinical verification before use.

Keywords: Flavopiridol, D-HSCDK2, homology modeling, Modeler, docking.

INTRODUCTION

Abnormalities in cell cycle are the root cause of cancer and its progression. Its progression is tightly controlled by the activity of cyclin-dependent kinases (CDKs) [1]. CDKs activation requires binding to cyclins, whose levels oscillate during the cell cycle and phosphorylation by CDK-activating kinase (CAK) on a specific threonine residue [2]. In addition to the positive regulatory role of cyclins and CAK, many negative regulatory proteins (CDK inhibitors, CKIs) have been discovered [3]. Flavopiridol is one such antitumor agent in clinical trials.

Flavopiridol is a semi-synthetic flavonoid derived from rohitukine, an alkaloid isolated from a plant indigenous to India *Dysoxylum binectariferum* [4]. It is an experimental, investigational and small molecule type drug with molecular weight 401.84. It comes under the drug category of anti neoplastic agents, glycogen phosphorylase inhibitor, growth inhibitors and protein kinase inhibitors [5]. It is a potential anti-cancer therapeutic agent against breast cancer, ovarian cancer, chronic lymphocytic leukemia, lymphomas, non-small-cell lung cancer, non-Hodgkin lymphoma, prostate, colon, gastric and kidney carcinomas [6]. In addition it also blocks cell cycle progression, promotes differentiation and induces apoptosis in various types of cancerous cells [7]. Importantly, this antitumor activity is p53 independent [8, 9]. Another interesting aspect of flavopiridol's cell cycle regulatory properties is the depletion of cyclin D1 an oncogene that is upregulated in many human neoplasias [10-12].

The structural information on the Human Delta cyclin dependent kinase 2 (D-HSCDK2), required for design of an effective drug against different types of cancer, is not known. Cyclin-dependent kinase 2 belongs to protein kinase superfamily .This protein has 268 amino acids and molecular weight of 30 kDa. It is a catalytic subunit of the cyclin dependent protein kinase complex, whose activity is restricted to the G1-S phase, and essential for cell cycle G1/S phase transition. This protein associates with and is regulated by the regulatory subunits of the complex including cyclin A or E. Activity of D-HSCDK2 is maximal during S phase and G2. Phosphorylation at Thr-14 or Tyr-15 inactivates the enzyme, while phosphorylation at Thr-160 activates it. D-HSCDK2, involved in cell cycle regulation (G1 to S transition), make complex with cyclin E for the regulation of chromosome stability [5, 13].

Structure of the drug target D-HSCDK2 (Target ID 01742) for the drug Flavopiridol is not available till date [5]. The present study is aimed to explore the structure of the said drug target and its interaction study with different flavopiridol analogues which may provide a better understanding in drug-target interaction for preventing different types of cancer. In this study we have used the comparative homology modeling to construct an atomic-resolution model of the "target" protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein (the "template"). The quality of the homology model is dependent on the quality of the sequence alignment, template structure and the extent of identity between the template and target sequences [14]. The homology modeling had been used for generation of 3-D structures of vaccine related kinase 1 (VRK1) protein, [15] Cyclin Dependent Kinase 4 (CDK4) protein, [16] Tubulin β-1 protein, [17] and CDCP2 protein [18]. Further docking was done using the program GLIDE to show the interaction between D-HSCDK2 target protein and flavopiridol analogues.

EXPERIMENTAL SECTION

Information about Flavopiridol and its various targets were retrieved from Drug bank (http://www.drugbank.ca/drugs/DB03496).

Collection of sequences and Selection of template

The complete protein sequence of human D-HSCDK2 was retrieved from Gene Bank (http://www.ncbi.nlm.nih.gov/protein/3551191?report=genpept). Template was selected by homology search of query protein (D-HSCDK2) sequence against the databases available on PDB (http://www.rcsb.org) through BlastP [19]. Homologous structure of sequence having the lowest E-value, higher identity and higher resolution was selected as template.

Generation of 3-D structure through homology modeling

Homology modeling was done using Modeler 9v7 [14, 20, 21]. This requires one sequence of known 3D structure with significant similarity with the target sequence and Python 2.5 script files containing Modeler commands. The co-ordinate file of template from PDB was used as such;

Evaluation and validation of the 3-D structure

All predicted 5 models were evaluated by Procheck [22], Whatif check [23], Errata [24], and Verify_3D. Ramachandran plot statistics was used to evaluate the stability of the model. Gnuplot was finally used to plot the profiles generated by Modeler (http://www.gnuplot.info) to validate substantially the structure.

Virtual screening of flavopiridol analogues through molecular docking

Flavopiridol and its analogues were taken from NCBI Pub-chem in SDF (2D) format and converted to PDB (3D) format structure using open-babel and web-lab viewer lite program. The 3-D structure of D-HSCDK2 and flavopiridol 3-D analogues was used for molecular docking using GLIDE program.

Protein structure accession number

The refined homology model of 3D structure of human D-HSCDK2 protein was submitted to PMDB (http://mi.caspur.it/PMDB/) and the same was assigned the identifier PM0076253.

RESULTS AND DISCUSSION

The complete protein sequence of human D-HSCDK2 (gi 3551191) was used in the study. This consists of total 268 amino acid residues with expected molecular weight of 30.061 kDa and isoelectric point (pI) 9.67. Search for template on Protein Data Bank through blastP has generated 1492 homologous structures. Among them 3EZR was selected on the basis of higher resolution (1.90 Å) and identity (85%) with lowest E-Value (1.6E-142) (Table 1).

Table No. 1 – List of templates and related information for D-HSCDK2

Sl. No.	PDB ID	Resolution	E-value	Identity
1.	**3EZR**	**1.90 Å**	**1.6E-142**	**85%**
2.	3EZV	1.99 Å	1.6E-142	85%
3.	3BHT	2.00 Å	1.6E-142	85%
4.	3BHV	2.10 Å	1.6E-142	87%
5.	1OIU	2.00 Å	2.3E-142	85%

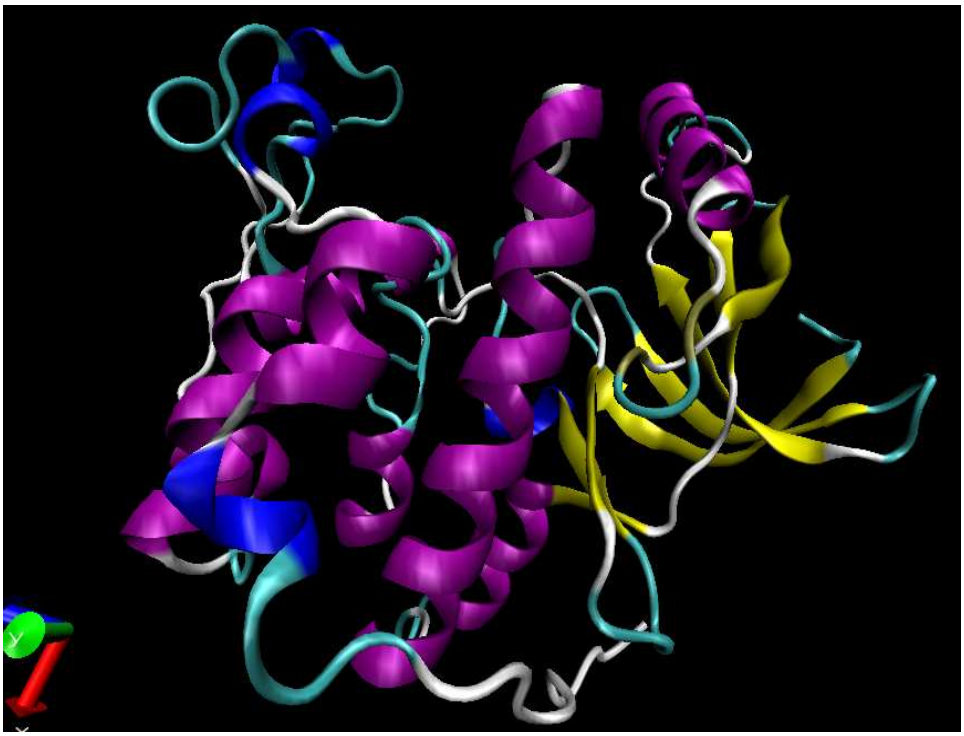

Figure 1 Model of Delta human Cell division protein kinase 2

By using 3EZR as a template Modeler 9v7 predicted five 3D models for D-HSCDK2 protein. The 3D structure of best model D-HSCDK2.B99990002.pdb (Figure 1) was selected on the basis of its lowest Dope score (Table 2). The presence of maximum numbers of H-bonds, strands and turns in the selected model confirms that this structure is more compact than others (Table 2). Further validation program, Procheck was used to perform full geometric analysis as well as stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry. After running Procheck, Ramachandran plot (Fig. 2) shows that for the model D-HSCDK2.B99990002.pdb, 90.4% residues were in favored region, 8.3% in the additional allowed region, 0.4% in the generously allowed region and only 0.9% of the residues in the disallowed region, which made this model more acceptable as compared to other predicted models. The overall quality factor, derived from the program ERRAT was 79.930 and the structure got passed in Verify_3D result with 89.80% residues having 3D-1D score > 0.2 in 3-D structure of D-HSCDK2. The assessments mentioned above for the best predicted model, indicate that the predicted structure is of good and acceptable quality.

Table 2 - Dope Scores and number H-bonds, Helices, strands and turns for different models of D-HSCDK2 generated through modeler

Protein Structures	Dope score	H-bonds	Helices	Strands	Turns
D-HSCDK2.B99990001.pdb	-29153.02148	163	12	12	22
D-HSCDK2.B99990002.pdb	**-29196.10742**	**163**	**11**	**13**	**25**
D-HSCDK2.B99990003.pdb	-29123.47070	159	11	13	23
D-HSCDK2.B99990004.pdb	-28806.05273	161	11	13	25
D-HSCDK2.B99990005.pdb	-28787.34375	163	12	13	24

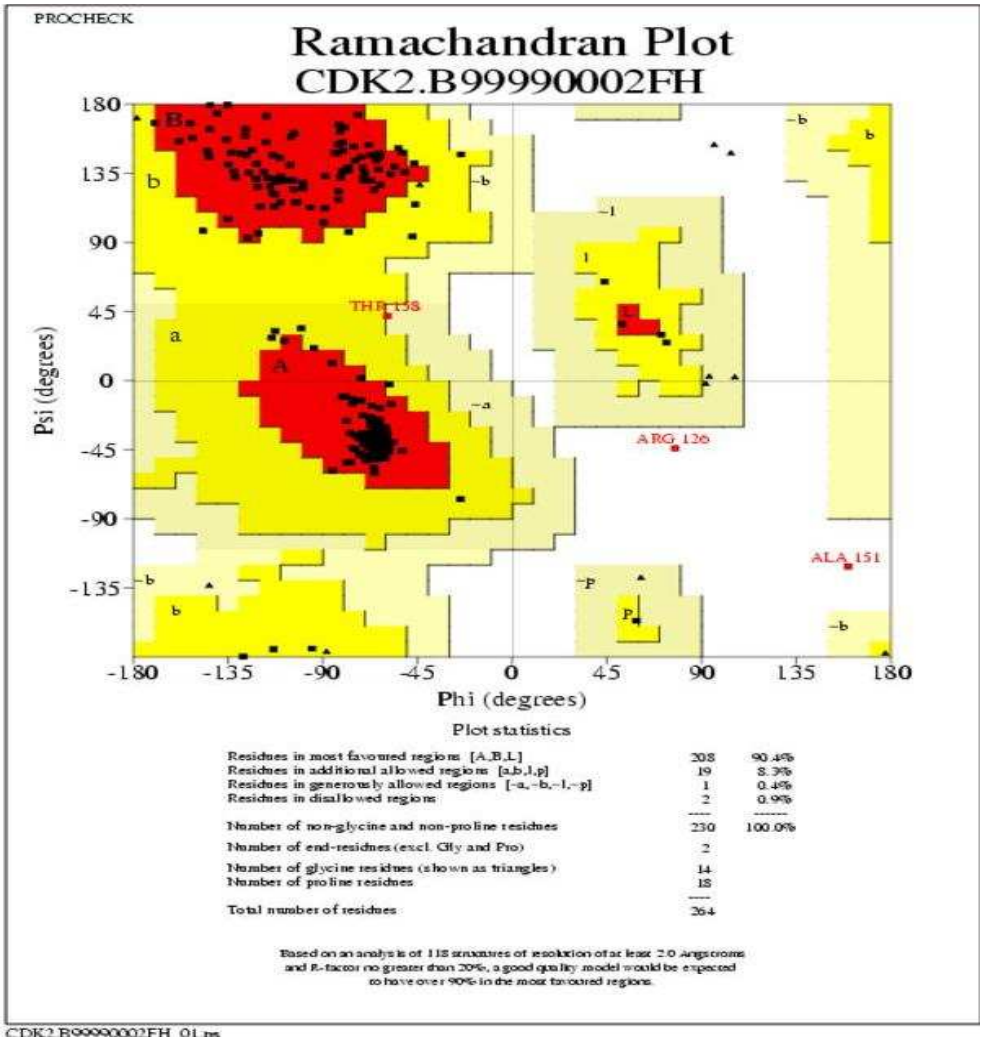

Figure 2 Ramachandran plot for D-HSCDK2 shows that out of 264 amino acid residues of the predicted model of D-HSCDK2.B99990002.pdb, 208 residues were present in favored, 19 in additionally allowed, 1 in generously allowed and only 2 residues lie in disallowed region

Further docking study of the modeled protein of D-HSCDK2 with 15 analogues of flavopiridol was performed. Results showed that analogue number 23 having CID24867231 got rank one with the minimum glide score of -8.82 in comparison to other analogues (Figure 3). Other

binding paramenters like Van der Waals interaction energy (-25.1), Coulomb interaction energy (-12.2), Emodel (-34.5) and cumulative Van der Waals interaction energy (-37.3) of CID5459219 was found higher than the other analogue, which shows the greater binding affinity of the said analogues than others (Figure 3).

Rank	Title	GScore	vdW	Coul	Emodel	CvdW	Intern	Conf#	Pose#
1	24867231_23	-8.82	-25.1	-12.2	-34.5	-37.3	5.9	1	1
2	5329713_1	-8.70	-36.1	-7.5	-50.0	-43.6	7.9	1	3
3	5459219_12	-8.48	-31.8	-9.7	-58.0	-41.5	1.6	1	1
4	5459219_12	-7.99	-35.6	-7.8	-54.5	-43.4	1.7	1	8
5	5459219_12	-7.94	-33.6	-7.8	-56.3	-41.4	2.6	1	3
6	5287969_2	-7.87	-33.3	-6.0	-55.0	-39.3	1.7	1	12
7	458005_3	-7.54	-38.4	-10.2	-57.4	-48.6	10.3	1	3
8	2091179_25	-7.47	-29.7	-8.5	-49.2	-38.3	7.6	1	1
9	5329714_4	-7.36	-37.4	-10.2	-59.7	-47.5	11.3	1	6
10	5329713_1	-7.35	-36.6	-6.0	-52.3	-42.7	7.6	1	1
11	458005_3	-7.08	-32.4	-8.8	-63.4	-41.2	5.5	1	12
12	5329713_1	-6.98	-35.9	-5.5	-51.6	-41.4	4.2	1	5
13	2091179_25	-6.78	-26.9	-10.4	-51.0	-37.2	2.2	1	2
14	14624081_14	-6.66	-32.8	-6.5	-56.6	-39.3	4.4	1	2
15	5329721_11	-6.44	-33.5	-11.7	-59.6	-45.2	11.8	1	8
16	5329717_7	-6.38	-27.3	-8.5	-53.4	-35.8	5.1	1	2
17	5329716_6	-6.37	-37.8	-10.1	-61.8	-47.9	3.5	1	18
18	2122413_21	-6.33	-26.6	-11.1	-48.6	-37.8	2.8	1	8
19	5287969_2	-6.29	-31.2	-11.2	-60.2	-42.3	1.3	1	1
20	5329717_7	-6.26	-33.5	-11.3	-57.1	-44.8	2.3	1	9
21	1000591_24	-6.20	-24.1	-7.3	-38.8	-31.4	6.0	1	16
22	5329721_11	-6.15	-34.7	-10.4	-64.0	-45.1	1.8	1	16
23	9841370_13	-6.14	-36.5	-8.8	-60.7	-45.2	3.0	1	2
24	5329713_1	-6.02	-33.9	0.2	-50.0	-33.8	5.8	1	8
25	5329720_10	-6.02	-33.0	-12.4	-65.5	-45.4	0.7	1	1
26	5329714_4	-5.80	-36.0	-8.6	-54.5	-44.6	14.2	1	17
27	5329719_9	-5.77	-34.9	-11.6	-62.3	-46.6	8.1	1	1
28	5329718_8	-5.75	-37.6	-7.8	-57.7	-45.4	6.8	1	2
29	9910986_19	-5.64	-32.5	-3.2	-36.5	-35.7	2.4	1	8
30	24867231_23	-5.55	-24.7	-1.8	-34.4	-26.5	4.1	1	9
31	2094025_22	-5.53	-30.6	-5.8	-48.9	-36.3	6.1	1	1
32	24835312_20	-5.52	-21.4	-4.8	-37.4	-26.3	2.8	1	9
33	1000591_24	-5.36	-23.0	-9.0	-39.3	-32.0	6.0	1	7
34	5329718_8	-5.03	-31.7	-7.5	-54.5	-39.2	1.9	1	3
35	6419859_17	-4.93	-26.1	-4.2	-38.4	-30.3	7.8	1	5
36	24867231_23	-4.91	-25.7	-4.9	-40.0	-30.6	4.7	1	2
37	24835312_20	-4.90	-24.3	-12.0	-31.9	-36.3	5.7	1	14

Sun *et al.* in 2005 [25], in study on cell dependent protein kinases, found that 3-D structure of CDK10 created by homology modeling based on the crystal structures of cyclin-dependent kinase 2 (CDK2) (PDB code 1AQ1). The modeled structure was further used to perform the

docking of ATP. Through the docking studies, the model structures of the ligand–receptor complex were obtained. The docking results indicated that conserved amino acid residues in CDK10 played an important role in maintaining a functional conformation and are directly involved in binding to donor and acceptor substrates. In the present study we performed flexible docking study between modeled CDK4 protein and its fifteen analogues to find out the better interaction.

Figure 3 Print-screen of detailed result of docking of flavopiridol analogues with D-HSCDK2 protein model

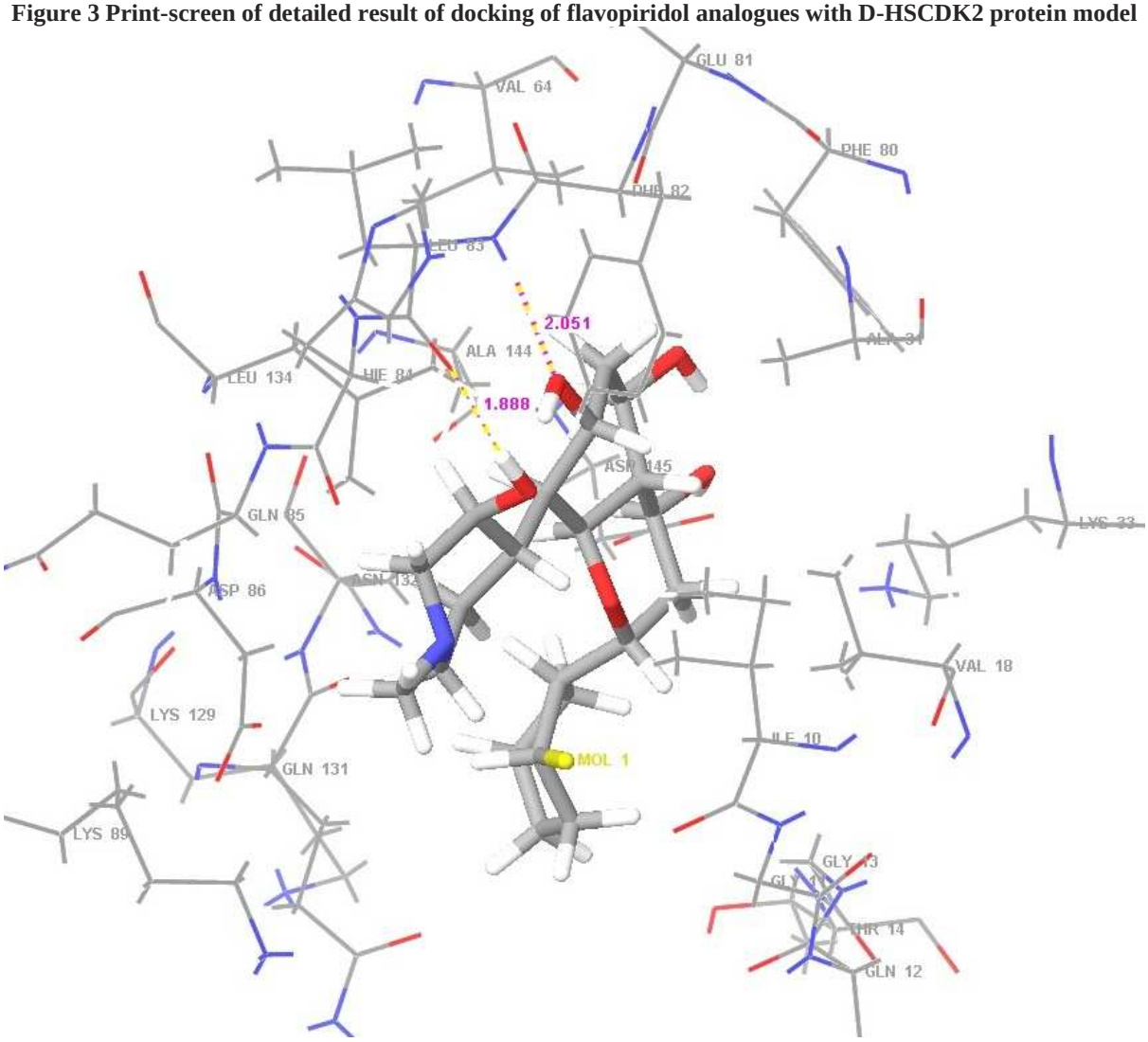

Figure 4 Binding mode of D-HSCDK2 with ligand CID 24867231

Hydrogen bonds play an important role for structure and function of biological molecules, especially for the enzyme catalysis. In binding mode D-HSCDK2 with the ligand having lowest dope score CID 24867231 makes two hydrogen bonds of length 1.888Å between Leu83:(o) and hydrogen of ligand and other of length 2.051Å between Leu83:(H) with oxygen of ligand (Figure 4).

Predicting energy of protein-ligand binding and searching space of possible poses and conformations are the major challenges in finding new drugs. We can design the various variants of analogue CID 24867231 by modifying different components to find out better drug than the existing one. This ligand has diverse and interesting biological activities, and it should be particularly useful as lead compound for drug development. Better understanding of the interactions between D-HSCDK2 and ligand CID 24867231 will be crucial for the treatment of different types of cancers. Hence the selected analogue CID_5370577 can be used as a replacement of the existing drug flavopiridol and may be subject to clinical trial.

CONCLUSION

The result of comparative structural analysis shows that model-3 (D-HSCDK2.B99990002.pdb) is the best structural model for human cell dependent kinase 2, a drug target of Flavopiridol based on its lowest Dope score, maximum residues (98.7%) in the favored and allowed region with overall quality factor of 79.93. Further flexible docking study with GLIDE was performed to see the interaction between this model and 15 analogues of Flavopiridol, a potent drug for cancer treatment. The study shows that the analogue CID 24867231 was the best among 15 having minimum GLIDE score. This result can be utilized for the designing of effective drug against cancer. This study would also initiate the research on discovery and design of more effective drug by modifying the structure of analogues CID 5459219 to find out more effective drug than the existing one. These finding are the subject of clinical verification to establish this analogue as a potent drug.

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