Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

MOLECULAR DOCKING AND QSAR STUDIES ON CDK4 INHIBITORS USING *IN SILICO* TECHNIQUES

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DOI: 10.7897/2277-4572.032130
Published by Moksha Publishing House. Website www.mokshaph.com

Received on: 05/02/14 Revised on: 02/03/14 Accepted on: 05/04/14

ABSTRACT
Cyclin-dependent kinases are a small family of serine/threonine protein kinases which control the cell cycle progression. Literature survey revealed that CDKs, their regulators, and substrates are the targets of genetic alteration in many human cancers. The best characterized case of such alteration is the p16-CDK4, 6/cyclin D-retinoblastoma pathway found in more than half of all human cancers. Therefore, CDK4 is an attractive target for the development of a novel anticancer agent. Computer aided drug design strategy has gained much prominence due to the fast and efficient means of studying protein-ligand interactions. A molecular docking was performed using Molegro Virtual Docker 6.0 with the CDK4 protein and the selected compounds from literature as ligands. QSAR toxicity analysis has been performed using FAF Drugs ADME/tox filtering server. Considering the molecular properties of the ligands, higher inhibitory activity is associated with reduced molecular flexibility, as measured by lower polar surface area (TPSA), LogP, lower hydrogen bond counts, confirming the capability of the compounds for binding at the active site of the receptor.
Keywords: Cyclin-dependent kinases, D-type cyclins, Cyclin-dependent kinase inhibitors, Docking, Molegro Virtual Docker 6.0, QSAR

INTRODUCTION

The cyclin-dependent kinases (CDKs) are a small family of serine/threonine protein kinases and require cyclin subunits for activity. They (Cdks) play a key role in regulating cell cycle progression and govern cellular transitions from growth phases (G1 and G2) into phases associated with DNA replication (S) and mitosis (M)[1,2]. G1-S phase progression requires phosphorylation of the retinoblastoma (Rb) protein by Cdk4[3,4] or Cdk6[5,6] in complex with their activating subunits, the D-type cyclins, D1, D2, or D3[7]. Hyperphosphorylation of Rb diminishes its ability to repress gene transcription through the E2F family of transcription factors and consequently allows synthesis of several genes, the protein products of which are necessary for DNA replication[8-11]. Thus, the catalytic activity of Cdk4 or Cdk6 regulates a critical checkpoint for the G1-S transition and the commitment to cell division[12]. Alterations in the genetic control of cell division lead to cancer, resulting in an unrestrained cell proliferation. Mutations mainly occur in two classes of genes: proto-oncogenes and tumor suppressor genes. Mutated versions of proto-oncogenes or oncogenes can promote tumor growth while inactivation of tumor suppressor genes like pRb and p53 results in dysfunction of proteins that normally inhibit cell cycle progression. In cancer, mutations have been observed in genes encoding CDK, cyclins, CDK-activating enzymes, CDK inhibitors (CKI), CDK substrates, and checkpoint proteins[13,14]. For example, CDK4 over expression; that occurs as a result of amplification, has been identified in cell lines, melanoma, sarcoma and glioma[15]. Loss of CKI binding as a result of mutations in CDK4 and CDK6 genes has also been identified[16]. Cyclin D acts as a growth sensor and provides a link between mitogenic stimuli and the cell cycle. Aberrant expression of cyclin D1 has been reported in many human cancers[17,18]. Cyclin D2 and cyclin D3 have also been reported to be over expressed in some tumors. Based on the observations that CDK, their regulators and substrates are targets of genetic alteration in different types of human cancer, it stimulated the search for different therapeutic strategies to modulate CDK activity. One is indirect strategy which involves targeting the major regulators of CDK activity[14,19] and the other is direct strategy which involves inhibition of the catalytic activity of the CDK kinases. Literature survey revealed that abrogation of the G1 checkpoint or acceleration of the Cdk4/cyclin D pathway provides a distinct advantage to cancer cells for proliferation and survival. Thus cyclin D–dependent kinases have been considered for many years a prime target for cancer chemotherapy[20,21]. Natural products have historically and continually been investigated for promising new leads in pharmaceutical development. The activities of bioactive compounds and the synergistic action shown by them with other drugs make them ideal in alternative cancer therapies. The efficacy of various inhibitors reported in literature (both natural and synthetic origin) against CDK targets have been studied using computer aided drug design strategies. Molecular docking is an application, wherein molecular modeling techniques are used to predict how a protein interacts with small molecules (ligand). The concept of docking is used in the study of various properties associated with protein-ligand interactions such as binding energy, geometry complementary, electron distribution, hydrogen bond donor acceptor properties, hydrophobicity and polarizability. Thus few compounds (both natural and synthetic origin) reported in literature as possible cdk-4 inhibitors are tested computationally by docking program Molegro, their binding affinities are evaluated and QSAR toxicity analysis has been performed using FAF Drugs ADME/tox filtering server in order to design novel compounds which can act as better CDK4 inhibitor.

MATERIALS AND METHODS

Preparation of protein structure

The crystal structure of the CDK4 protein (PDB ID: 2W9Z) has been obtained from RCSB Protein Data Bank (http://www.pdb.org). All water molecules were removed and hydrogen atoms were added to the target protein molecule on the final stage.

Preparation of ligand structures

All the compounds used for docking study were selected from the literature[22-31]. Ligand structures were constructed using Chem Sketch Software (http://www.acdlabs.com), three dimensional optimizations were done and then saved in. mol file. Using Hartree-Fock (HF) calculation method by Argus Lab 4.0.1 Software, geometry optimization of the ligands were performed.

Protein ligand interaction using Molegro Virtual Docker 6.0

Docking program, Molegro Virtual Docker was used in analysis with default parameters. CDK4 protein was docked against the obtained ten ligands using Molegro Virtual Docker 6.0. Docking of the protein - ligand complex was mainly targeted only on to the predicted active site. Docking simulations were performed by selecting "Mol Dock" as the docking engine. A spacing of 0.3 Å between the grid points was used, "Mol Dock" was chosen as the calculation type, and the Mol Dock Score [GRID] was used as the scoring function. At maximum 10 poses were allowed to be analyzed. After completion of docking, the docked protein (protein - ligand complex) was analyzed to investigate the type of interactions. The docking poses saved for each compound were ranked according to their dock score function. The pose having the highest dock score was selected for further analysis.

Ligand screening and QSAR studies

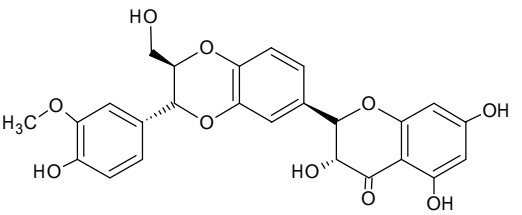
FAF-Drugs is an online absorption, distribution, metabolism, excretion and toxicity prediction tool used to calculate molecular descriptors for all the inhibitors taken for the docking analysis.

RESULTS AND DISCUSSION

In order to understand the interactions between the ligands and CDK4 protein and to explore their binding mode, docking study was performed using Molegro Virtual Docker of version 6.0. The crystal structure of the CDK4 protein (2W9Z) was derived from PDB and used as a target for docking simulation. The compounds selected from the literature were listed in Table 1. Ligands for the docking procedure were constructed using Chem Sketch and energy minimized using Argus Lab 4.0.1. The structures of the ligands obtained from the Chem Sketch were shown in Figure 1.

Docking Studies

The goal of protein-ligand docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structures. Crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein was obtained from docking studies. Inhibition was measured by the binding energy of the best ligand pose measured in kcal/mol.



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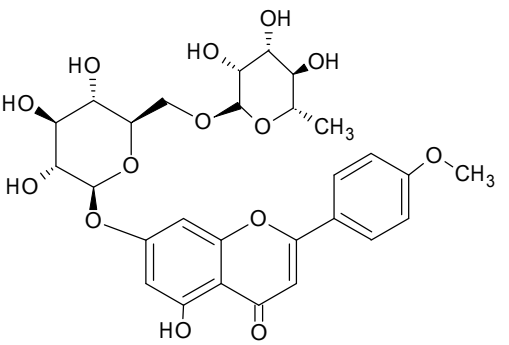

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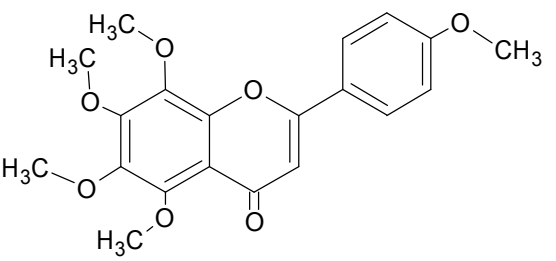

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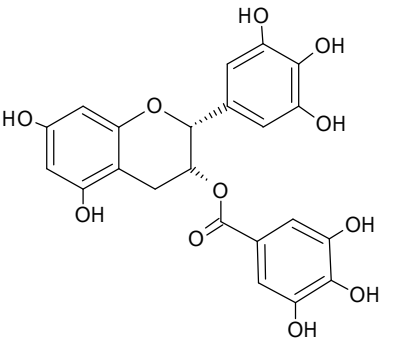

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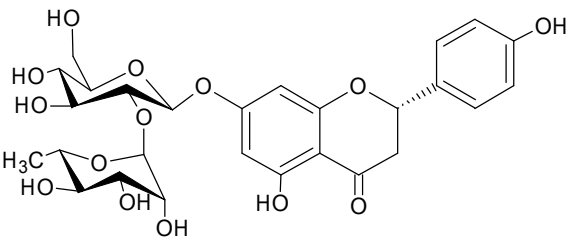

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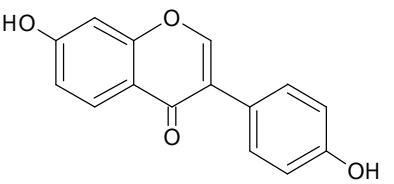

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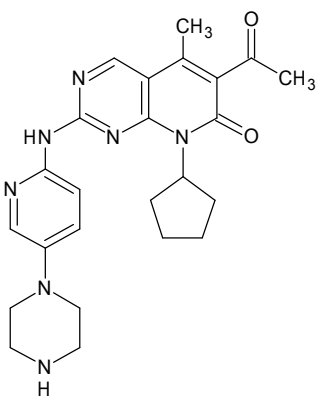

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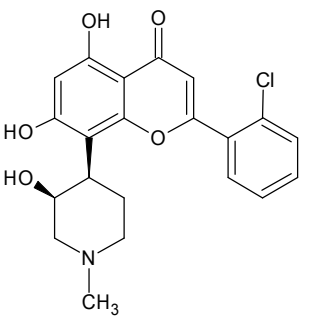

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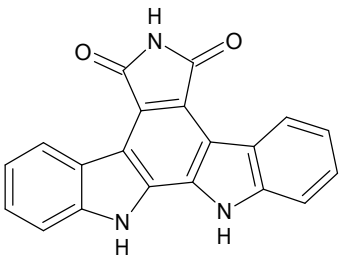

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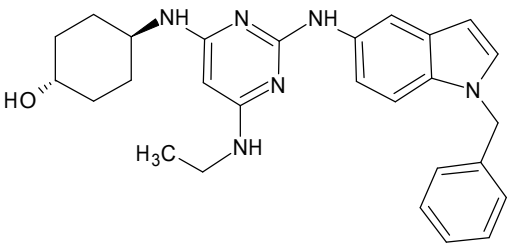

T-10

Figure 4: Ligands used for docking

Natural Origin

T-1: Silibinin; T-2: Linarin; T-3: Tangeretin; T-4: Epigallocatechin gallate; T-5: Naringin; T-6: Daidzein

Synthetic Origin

T-7: Palbociclib (PD 0332991, pyridopyrimidine-derived cyclin-dependent kinase (CDK) inhibitor); T-8: Flavopiridol; T-9: Arcyriaflavin A; T-10: CINK4 (chemical inhibitor of Cdk4, triaminopyrimidine derivative)

Table 1: List of ligands taken for docking

Compound	Reference
T-1	O. Baktiar Karim *et.al.*[22]
T-2	M. Sivashanmugham *et.al.*[23]
T-3	Jen-Kun Lin *et.al.*[24]
T-4	J. J. Chen *et.al.*[25]
T-5	D. I. Kim *et.al.*[26]
T-6	E. J. Choi *et.al.*[27]
T-7	W. David Fry *et.al.*[28]
T-8	A. Pumfery *et al.*[29]
T-9	G. Zhu *et.al.*[30]
T-10	R. Soni *et.al.*[31]

Validation of ligands by QSAR studies

In the present study, QSAR studies were performed using FAF Drugs: ADME/Tox filtering server for the determination of the inhibitor's molecular properties such as Log P (partition coefficient), TPSA (topological polar surface area), Molecular weight, hydrogen bond acceptors and donors. TPSA, captured as the Vander Waals surface area of all nitrogen and oxygen atoms and their attached hydrogen atoms, was considered as an indicator for number of HB donors and acceptors. Calculated molecular properties and docking scores of all the compounds were shown in Table 2.

Table 2: Docking score and molecular properties of the ligands

Compounds	Energy value (kcal/mol)	MW	HD (OH+NH)	HA (O+N)	Log P	TPSA
T-1	-125.311	484.44	5	10	-1.37	161.35
T-2	-132.929	594.56	8	14	-2.74	219.29
T-3	-104.307	375.39	1	7	1.04	77.68
T-4	-146.356	458.37	8	11	0.83	199.44
T-5	-137.611	580.53	8	14	-1.71	227.13
T-6	-90.599	256.25	3	4	0.56	71.99
T-7	-138.856	449.55	3	9	2.21	106.51
T-8	-132.446	404.86	4	6	1.21	95.46
T-9	-124.515	329.35	5	5	2.69	84.07
T-10	-145.804	456.58	4	7	5.40	87.03
T-11	-145.556	447.53	3	7	4.99	80.85

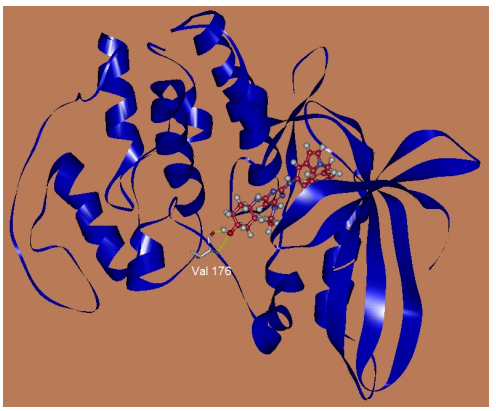

Figure 2: Crucial Interaction between compound T-10 (Red) and CDK4 protein (blue)

T-10, when analyzed showed a docking energy of -145.804 kcal/mol and very low TPSA value of 87.03. The crucial interaction of T-10 with CDK4 protein is shown in Figure 2. We have also observed that though compound **T-4** has high dock score, due to high TPSA value and low Log P values, it cannot be considered as suitable CDK4 inhibitor. We have thus designed a new chemical compound, **T-11**, based on structure of T-4, which found to possess high dock score and low TPSA value compared to T-4. The structure of T-11 and crutial interaction of T-11 with CDK4 protein is shown in Figure 3 and 4. Thus with the least binding energy, least TPSA and Log P at all ensures **T-10** and **T-11** ligands to be good lead compounds for development of better CDK4 inhibitors.

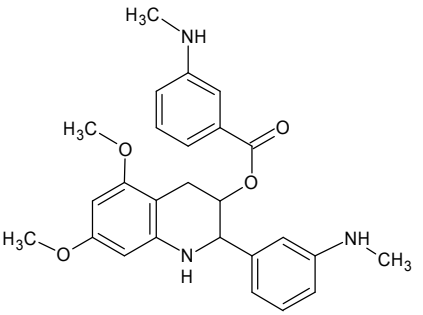

Figure 3: Structure of T-11

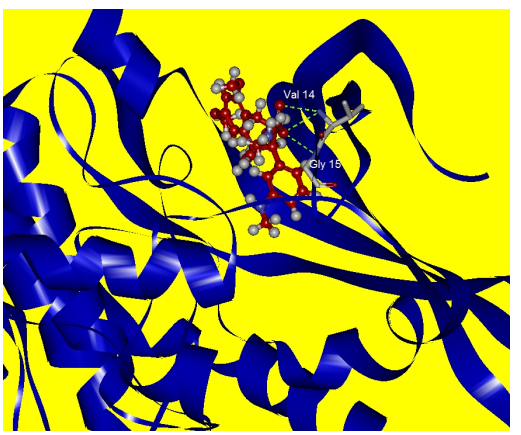

Figure 4: Crucial Interaction between compound T-11 (Red) and CDK4 protein (blue)

CONCLUSION

In this study, we tried to explore the binding mechanism by applying molecular docking and correlated its docking score with the activity of the compounds taken. The results of our present study indicated that **T-10** and **T-11** are good leads for development of novel compounds that can be used as better CDK4 inhibitors. The compound **T-10** has already been validated *in vitro* as well as *in vivo* while the compound **T-11** has to be further validated in wet lab studies for its proper function.

ACKNOWLEDGMENT

The author acknowledges Professor V. Uma, Department of Chemistry, Osmania University, for providing technical assistance in carrying out this work.

REFERENCES

1. Morgan DO, Fisher RP, Espinoza FH, *et al*. Control of eukaryotic cell cycle progression by phosphorylation of cyclin-dependent kinases. Cancer J Sci Am 1998; 4: S77–83.
2. Morgan DO. Cyclin-dependent kinases: engines, clocks, and microprocessors. Annu Rev Cell Dev Biol 1997; 13: 261–91. http://dx.doi.org/10.1146/annurev.cellbio.13.1.261
3. Harbour JW, *et al*. Cdk phosphorylation triggers sequential intra molecular interactions that progressively block Rb functions as cells move through G1. Cell 1999; 98: 859–69. http://dx.doi.org/10.1016/S0092-8674(00)81519-6
4. Lundberg AS, Weinberg RA. Functional inactivation of the retinoblastoma protein requires sequential modification by at least two distinct cyclin-cdk complexes. Mol Cell Biol 1998, 18: 753–61.
5. Chen Q, Lin J, Jinno S, Okayama H. Over expression of Cdk6-cyclin D3 highly sensitizes cells to physical and chemical transformation. Oncogene 2003, 22: 992 –1001. http://dx.doi.org/10.1038/sj.onc.1206193
6. Meyerson M, Harlow E. Identification of G1 kinase activity for cdk6, a novel cyclin D Partner. Mol Cell Biol 1994; 14: 2077–86.
7. Sherr CJ. D-type cyclins. Trends Biochem Sci 1995, 20: 187–90. http://dx.doi.org/10.1016/S0968-0004(00)89005-2
8. Trimarchi JM, Lees JA. Sibling rivalry in the E2F family. Nat Rev Mol Cell Biol 2002; 3: 11–20. http://dx.doi.org/10.1038/nrm714
9. Mundle SD, Saberwal G. Evolving intricacies and implications of E2F1 Regulation. FASEB J 2003; 17: 569 –74. http://dx.doi.org/10.1096/fj.02-0431rev
10. Harbour JW, Dean DC. The Rb/E2F pathway: expanding roles and emerging Paradigms. Genes Dev 2000; 14: 2393–409. http://dx.doi.org/10.1101/gad.813200
11. Nevins JR. The Rb/E2F pathway and cancer. Hum Mol Genet 2001; 10: 699–703. http://dx.doi.org/10.1093/hmg/10.7.699
12. Ho A, Dowdy SF. Regulation of G (1) cell-cycle progression by oncogenes and tumor suppressor genes. Curr Opin Genet Dev 2002; 12: 47–52. http://dx.doi.org/10.1016/S0959-437X(01)00263-5
13. Sherr CJ. Cancer cell cycles. Science 1996; 274: 1672. http://dx.doi.org/10.1126/science.274.5293.1672
14. McDonald III E R, el Deiry WS. Cell cycle control as a basis for cancer drug Development. Int. J. Oncol 2000; 16: 871.
15. Wolfel T, *et al*. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 1995; 269: 1281. http://dx.doi.org/10.1126/science.7652577
16. Easton J, Wei T, Lahti JM, Kidd VJ. Disruption of the cyclin D/cyclin dependent kinase/INK4/retinoblastoma protein regulatory pathway in human neuroblastoma. Cancer Res 1998; 58: 2624–2632.
17. Motokura T, *et al*. A novel cyclin encoded by a bcl1-linked candidate oncogene. Nature 1991; 350: 512. http://dx.doi.org/10.1038/350512a0
18. Hall M, Peters G. Genetic alterations of cyclins, cyclin-dependent kinases, and CDK inhibitors in human cancer. Adv Cancer Res 1996; 68: 67. http://dx.doi.org/10.1016/S0065-230X(08)60352-8
19. Senderowicz AM, Sausville EA. Preclinical and clinical development of cyclin dependent kinase modulators. J. Natl Cancer Inst 2000; 92: 376. http://dx.doi.org/10.1093/jnci/92.14.1185
20. Fry DW, Garrett MD. Inhibitors of cyclin-dependent kinases as therapeutic agents for the treatment of cancer. Curr Opin Oncol Endocr Metabol Invest Drugs 2000; 2: 40–59.
21. Garrett MD, Fattaey A. CDK inhibition and cancer therapy. Curr Opin Genet Dev 1999; 9: 104–11. http://dx.doi.org/10.1016/S0959-437X(99)80015-X
22. Baktiar Karim O. Chemoprevention utility of silibinin and Cdk4 pathway inhibition in $Apc^{-/+}$ mice. BMC Cancer 2013; 13: 157. http://dx.doi.org/10.1186/1471-2407-13-157
23. Sivashanmugam M, Raghunath C, Vetrivel U. Virtual screening studies reveals linarin as a potential natural inhibitor targeting CDK4 in retinoblastoma. J Pharmacol Pharmacother 2013; 4: 256-64. http://dx.doi.org/10.4103/0976-500X.119711
24. Jen Kun Lin. Tangeretin induces cell-cycle G1 arrest through inhibiting cyclin-dependent kinases 2 and 4 activities as well as elevating Cdk inhibitors p21 and p27 in human colorectal carcinoma cells. Carcinogenesis 2002; 23: 1677–1684. http://dx.doi.org/10.1093/carcin/23.10.1677
25. Chen JJ, Ye ZQ, Koo MW, Growth inhibition and cell cycle arrest effects of epigallocatechin gallate in the NBT-II bladder tumor cell line. BJU Int 2004; 93: 1082-6. http://dx.doi.org/10.1111/j.1464-410X.2004.04785.x
26. Kim DI, *et al*. Requirement for Ras/Raf/ERK pathway in naringin-induced G1-cell-cycle arrest via p21WAF1 expression. Carcinogenesis 2008; 29: 1701-9. http://dx.doi.org/10.1093/carcin/bgn055
27. Choi EJ, Kim GH. Daidzein causes cell cycle arrest at the G1 and G2/M phases in human breast cancer MCF-7 and MDA-MB-453 cells. Phytomedicine 2008; 15: 683-90. http://dx.doi.org/10.1016/j.phymed.2008.04.006
28. David Fry W. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Mol Cancer Ther 2004; 3: 1427-1438.
29. Adrian Senderowicz M. Small molecule modulators of cyclin-dependent kinases for cancer therapy. Oncogene 2000; 19: 6600-6606. http://dx.doi.org/10.1038/sj.onc.1204085
30. Zhu G, *et al*. Synthesis of quinolinyl/isoquinolinyl[a]pyrrolo [3, 4-c] carbazoles as cyclin D1/CDK4 inhibitors. Bio org Med Chem Lett 2003; 13: 1231-1235. http://dx.doi.org/10.1016/S0960-894X(03)00133-1
31. Soni R, *et al*. Selective *in vivo* and *in vitro* effects of a small molecule inhibitor of cyclin- dependent kinase 4. J Natl Cancer Inst 2001; 93: 436-46. http://dx.doi.org/10.1093/jnci/93.6.436

Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
Anupama Pandrangi. Molecular docking and QSAR studies on cdk4 inhibitors using In silico techniques. J Pharm Sci Innov. 2014;3(2):164-169 http://dx.doi.org/10.7897/2277-4572.032130