ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Insilico docking studies for investigating the anticancer properties of plant based compounds against cervical cancer.

Aishwarya Deshpande[1], Keerthi Kesavan[1], and Jayanthi Sivaraman[1]*

[1]Computational Drug Design Lab, Department of Biotechnology, School of Bio-Sciences and Technology, VIT University, Vellore-632 014, Tamil Nadu, India.

[1*]Assistant Professor (Senior),Computational Drug design Lab, Department of Biotechnology, School of Bio Sciences and Technology, VIT University, Vellore 632014, Tamil Nadu, India.

ABSTRACT

Plant based compounds have been used as potential drugs because of their advantage over synthetic drugs. Hence much importance has been given to find suitable plant based lead molecules for inhibiting cancer. Cervical cancer is one of the leading causes of death among the female population and is considered as one of the major health problems in the world. It is being treated through various methods but the re-emergence of the tumors is said to be a reason of concern. In this study we selected around 116 plant derived compounds having anticancer property from NPACT to test for their binding against CDKN3 vital target in cervical cancer through *in silico* docking studies. Screening for the inhibitor against the selected target protein was performed and the compound Cis-Zeatin bound with the greater affinity to our target protein.

Keywords: Plant compounds; cervical cancer; target protein; docking; cis-zeatin .

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cervical cancer accounts for about 275,100 deaths each year. It is the second most common cancer among women after breast cancer (Petignat and Roy 2007). Cervical cancer, or the cancer of cervix, is mostly caused by the prolonged infection of human papillomavirus (HPV). HPV type 16 is the major causative agent in half of the cases while HPV 18, HPV 45, HPV 31 cover the rest 50% of the cases. Being the second most common cancer in females, research has shown that a total of 2% to more than 20% of the world's female population have detectable levels of HPV-DNA in their cervix at all times (Bosch et al. 1995). The Papanicolaou (Pap) test is a vital screening procedure for the detection of cervical cancer which has decreased the incidence of the disease in the past few years (McCrory et al. 1999). Cervical cancer is currently being treated by surgery, chemotherapy and radiotherapy, though the patient's survival reduces with the stage of the disease (Andrae et al. 2012). But there are numerous cases of treatment failure due to invasion, metastasis, and local recurrence of the disease. Additionally, there are various drawbacks of the present treatment procedures. Surgery cannot be used in all complex stages of the disease and side effects are a major concern in the use of radiotherapy (Cadron et al. 2007). Paying heed to this problem, *in silico* methods is used to seek new and efficient compounds through important markers and therapeutic targets (Li et al. 2015). Therefore, understanding important key mechanisms in the proliferation and growth of tumor and finding novel therapeutic targets will aid in curbing tumorigenesis (Vogelstein and Kinzler 2004). This can be achieved by identifying the genes whose expressions are altered and targeting them through novel treatments (Thomas et al. 2013). One such gene is the Cyclin-Dependent Kinase Inhibitor 3 (CDKN3), which encodes a protein belonging to the phosphatase family. The CDKN3 gene, mapped to chromosome 14q22 (Demetrick et al. 1995) encodes a dual specificity phosphatase at G1/S transition, which interacts with CDK2, dephosphorylates Thr161 when cyclin is dissociated or degraded (Nalepa et al. 2013), and prevents its cyclin dependent kinase activity (Zang et al. 2015). In fact, it acts as a tumor suppressor in many cancer types. However, it is upregulated in cervical cancer as compared to that in normal condition (Barrón et al. 2015). As inhibition of mitosis can prove to be a strategy to treat cancers, this makes it a potential therapeutic target for treating cervical cancer. For the inhibition of CDKN3, plant based compounds were chosen as ligands because of their herbal properties and widespread use in treating many diseases. Currently, some secondary metabolites produced by plants are being investigated for their anticancer activities, which can potentially help in the discovery of new and better drugs (Greenwell and Rahman 2015). *In silico* analysis has immense use in drug discovery; efficiently helping in reducing the time in screening of novel effective inhibitors and designing new drugs through computational drug design (Gschwend et al. 1996). One of the techniques is docking and the main objective is to identify the binding site and binding energy of ligands (plant based compounds) to a particular target protein (Auxilla et al. 2013), which, in this case is CDKN3. This helps in the discovery of new drugs by recognizing small molecular scaffolds with high binding energy and their subsequent binding with the target protein (Sengupta et al. 2008). Thus, molecular docking is a promising approach in computer aided drug design in which screening of small molecules or ligands is done by scoring and orienting them in the binding site of receptor protein (Shoichet et al. 2002). In this study, we have found a novel biologically active plant compound for inhibiting CDKN3 through molecular docking methods which can further be used for designing newer effective drugs.

RESULTS AND DISCUSSION

An improvement in the procedures to screen and treat cervical cancer is needed as the impact of the preventive HPV vaccine on the reduction of occurrence of cervical cancer will remain unknown for another three decades (Espinosa et al. 2013). One way to deal with this problem is to find out potential targets for cervical cancer which can be docked against ligands for potential drug discovery. In the present study, the anticancer compounds from NPACT shown to be active for cervical cancer were selected and all the 116 compounds were computationally prepared using Ligprep (LigPrep, version 2.3, Schrödinger, LLC, New York, NY, 2009), a ligand preparation tool. CDKN3 protein crystal structure was obtained from Protein Databank (PDB ID: 1FPZ) (Song et al. 2001) shown in Figure 1, and prepared using the protein preparation wizard Preparation Wizard of Schrodinger Maestro suite 2015. GLIDE (Halgren et al. 2004) was used for molecular docking (Kawatkar et al. 2009). As it is necessary in GLIDE molecular docking for the ligand to bind with X-ray crystal structure of protein, the active site receptor grid was determined using the SiteMap tool (SiteMap, version 2.4, Schrödinger, LLC, New York, NY, 2010). The receptor grid based molecular docking made the ligands bind in various possible conformations. A systematic computational simulation method was applied through GLIDE to

evaluate poses and ligand flexibility. All the 116 compounds were docked against the crystal structure of CDKN3 at the computationally determined active site. The output was in the form of GScore, an empirical score which is an amalgamation of different parameters. The unit of GScore is kcal/mol it includes ligand–protein interaction energies, hydrophobic interactions, hydrogen bonds, internal energy, p–p stacking interactions, and root mean square deviation (RMSD) and desolvation (Singh and Bast 2014).

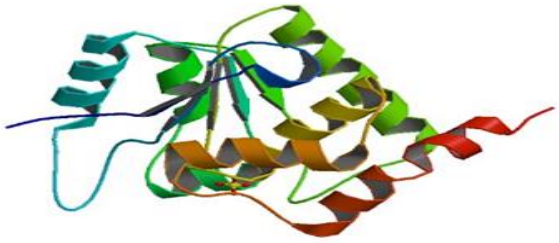

Figure1: 3D structure of target protein CDKN3

It was observed that out of 116 ligands, Cis-Zeatin, with the molecular formula $C_{10}H_{13}N_5O$, binds the best and fits into the active site as shown in Figure 2 . It has a binding energy of -6.331, which is the least when compared to other 115 ligands. The top best ligands based on their docking score are listed in Table 1. The ligand Cis-Zeatin interacts with GLY143, TYR141, GLY142, VAL56, PHE53, CYS51, ALA47, LEU48, PRO49, GLY50, ILE27, ARG58, THR25, GLU24, ASP63, HIS28 as illustrated in Figure 3. It forms two hydrogen bonds with LEU48 and HIS28. Hydrogen bonding is a vital requirement for many drug-receptor interactions (Bolton et al. 2008). The more the number of hydrogen bonds, more stable is the interaction. CDKN3 can be either tumor-suppressive or oncogenic depending on different molecular contexts in different cancer types (Zang et al. 2015). In cervical cancer, CDKN3 activity is upregulated. In our study, the compound Cis-Zeatin (Figure 4) showed the highest activity. HIS28 and the LEU48 are directly involved in the binding of the compound. Zeatin is a phytohormone which belongs to the family cytokinins. It was first discovered in corn from the genus zea. Cis-Zeatin and its derivatives are found in various plants such as potato, *Mercurialis*, hops, rice, wheat, oats, and chickpeas (Martin et al. 2000). CDKN3 permits cells to exit from M phase and start a new cycle. It induces DNA synthesis, thus promoting tumorigenicity. Also, an aberrant splicing leads to a dominant-positive CDKN3 variant that enhances proliferation (Berumen et al. 2014).

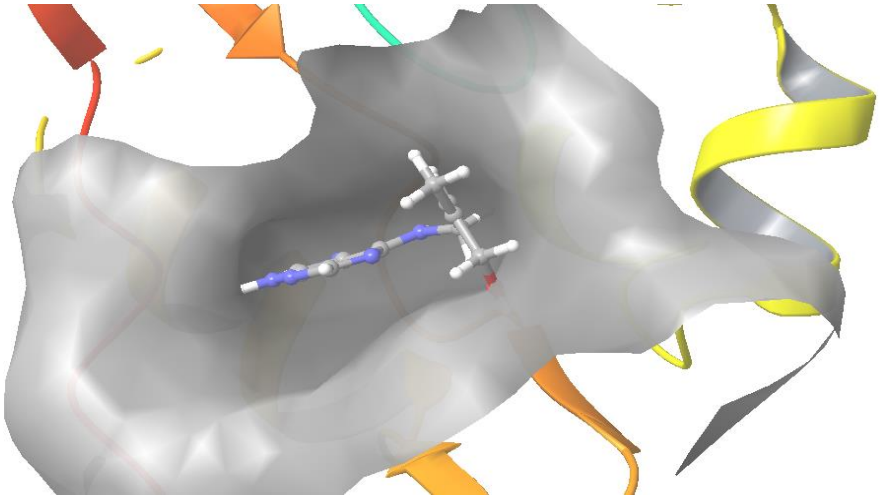

Figure 2: Ligand binding in the active site of protein CDKN3

ISSN: 0975-8585

Table 1: Docking and Glide GScore of top 10 compounds to CDKN3 obtained from GLIDE

Sr. no	Compound	NPACT ID	Docking score	Glide GScore
1.	Cis-Zeatin	NPACT00430	-6.331	-6.352
2.	Naringenin	NPACT00798	-6.261	-6.261
3.	Carvacrol	NPACT00397	-6.228	-6.228
4.	(2R)-7,4'-Dihydroxy-5-methoxy-8-methylflavan	NPACT01434	-6.214	-6.214
5.	N6-isopentenyladenine	NPACT00795	-6.082	-6.102
6.	7-hydroxycadallin	NPACT01390	-6.079	-6.079
7.	7,4'-dihydroxy-8-methylflavan	NPACT01103	-6.070	-6.071
8.	7,4'-dihydroxyhomoisoflavan	NPACT01430	-5.945	-5.945
9.	5,7-dihydroxyflavanone	NPACT01095	-5.872	-5.872
10.	6.4'-dihydroxy-7-methoxyhomoisoflavan	NPACT01464	-5.872	-5.872

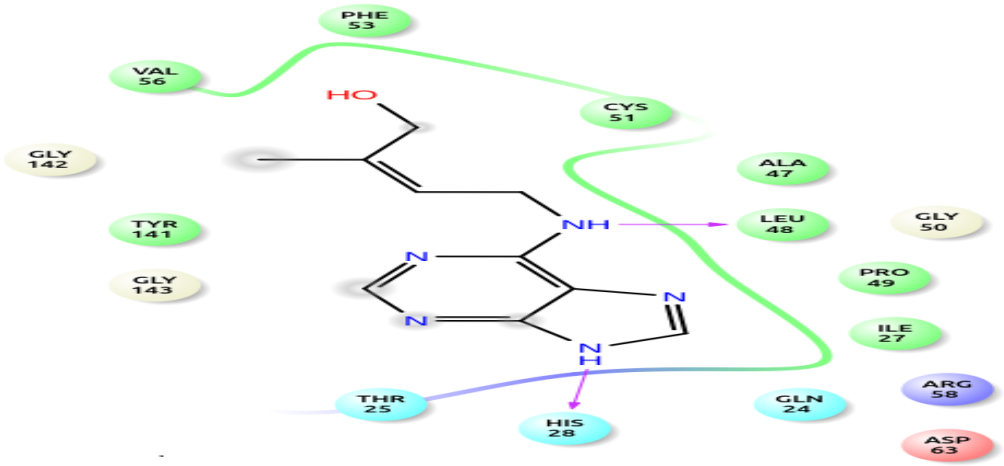

Figure 3: Binding interactions (hydrogen bonds) of cis-zeatin with amino acids in the receptor protein CDKN3

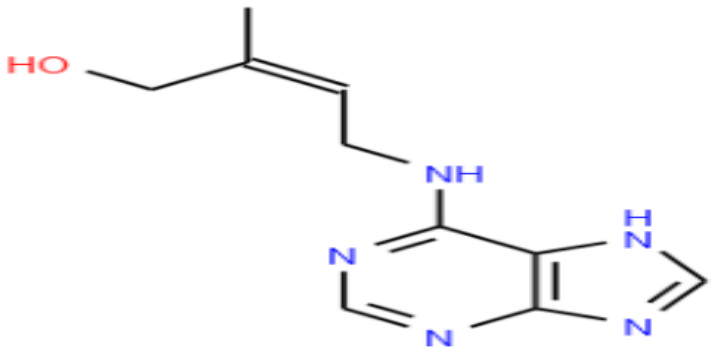

Figure 4: Cis-Zeatin showed high binding activity towards CDKN3 protein

A variety of plant based natural compounds like polyphenols have been identified previously to have an inhibitory activity against tumorigenesis. They can be used as potential chemotherapeutic agents (Pellecchia et al. 2004). On similar lines, our results show that Cis-Zeatin can be a promising ligand for inhibiting the activity of CDKN3. Cis-Zeatin causes apoptosis, G1 or G2/M block. Thus, its binding in the active site of CDKN3 can potentially block the cells at M phase, thus preventing them to start a new cell cycle, which can be used in further drug development research for cervical cancer (Voller et al. 2010).

CONCLUSION

In this study, natural plant based compounds with anti-cancerous property were used as ligands to dock against Cyclin-Dependent Kinase Inhibitor 3. Out of 116 compounds Cis-Zeatin, present in common foods such potato, rice, wheat etc., showed the highest binding in to the active site of CDKN3. No such work has been performed earlier and an *in vitro* study into this inhibitory activity can help us to develop better drugs. Virtual screening is thus a necessary step in drug development for reducing the time and money on testing. In future studies, Cis-Zeatin and its derivatives obtained from natural sources can be a promising approach to develop better drugs for the efficient treatment of cervical cancer.

ACKNOWLEDGEMENTS

The authors would like to thank VIT University for the facilities and carrying out this work.

REFERENCES

[1] Andrae B, Andersson TM, Lambert PC, Kemetli L, Silfverdal L, Strander B, Ryd W, Dillner J, Törnberg S, Sparén P. 2012. Screening and cervical cancer cure: population based cohort study. Bmj. 344: e900.

[2] Auxilia LR, Sundari TM, and Daniel RR. 2013. Molecular Docking Studies of Dolichin A and B, Pterocarpans from Horsegram (Macrotylomauniflorum) against HIV Replication Enzymes. Int J Comput Appl. 75(14).

[3] Barrón EV, Roman-Bassaure E, Sánchez-Sandoval AL, Espinosa AM, Guardado-Estrada M, Medina I, Juárez E, Alfaro A, Bermúdez M, Zamora R, García-Ruiz C. 2015. CDKN3 mRNA as a biomarker for survival and therapeutic target in cervical cancer. PloS one. 10: e0137397.

[4] Berumen J, Espinosa AM, Medina I. 2014. Targeting CDKN3 in cervical cancer. Expert Opin Ther Targets.18:1149-1162.

[5] Bolton EE, Wang Y, Thiessen PA, Bryant SH. 2008. PubChem: Integrated platform of small molecules and biological activities. Annu Rep Comput Chem. 4:217–241.

[6] Bosch FX, Manos MM, Muñoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shan KV. 1995. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst. 87: 796-802.

[7] Cadron I, Van Gorp T, Amant F, Leunen K, Neven P, Vergote I. 2007. Chemotherapy for recurrent cervical cancer. Gynecol Oncol. 107: S113-S118.

[8] Demetrick DJ, Matsumoto S, Hannon GJ, Okamoto K, Xiong Y, Zhang H, Beach DH. 1995. Chromosomal mapping of the genes for the human cell cycle proteins cyclin C (CCNC), cyclin E (CCNE), p21 (CDKN1) and KAP (CDKN3). Cytogenet Cell Genet. 69: 190-192.

[9] Espinosa AM, Alfaro A, Roman-Basaure E, Guardado-Estrada M, Palma Í, Serralde C, Medina I, Juárez E, Bermúdez M, Márquez E, Borges-Ibáñez, M. 2013. Mitosis is a source of potential markers for screening and survival and therapeutic targets in cervical cancer. PLoS One. 8: e55975.

[10] Greenwell M, Rahman PKSM. 2015. Medicinal Plants: Their Use in Anticancer Treatment. IJPSR. 6: 4103–4112.

[11] Gschwend DA, Good AC. and Kuntz ID, 1996.Molecular docking towards drug discovery. J Mol Recogn. 9: 175-186.

[12] Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL. 2004. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment Factors in Database Screening. J Med Chem. 47: 1750–1759.

[13] Kawatkar S, Wang H, Czerminski R, Joseph-McCarthy D. 2009. Virtual fragment screening: an exploration of various docking and scoring protocols for fragments using Glide. J Comput Aided Mol Des. 23: 527–539.

[14] Li XW, Tuergan M, Abulizi G. 2015. Expression of MAPK1 in cervical cancer and effect of MAPK1 gene silencing on epithelial-mesenchymal transition, invasion and metastasis. Asian Pac J Trop Med. 8: 937–943.

[15] Martin RC, Mok MC, Habben JE, Mok DW. 2001. A maize cytokinin gene encoding an O-glucosyltransferase specific to Cis-Zeatin. Proc Natl Acad Sci USA. 98: 5922-5926.

[16] McCrory DC, Matchar DB, Bastian L, Datta S, Hasselblad V. 2009. Evaluation of cervical cytology. Evid Rep Technol Assess (Summ). 1999: 1–6.

[17] Nalepa G, Barnholtz-Sloan J, Enzor R, Dey D, He Y, Gehlhausen JR, Clapp W. 2013. The tumor suppressor CDKN3 controls mitosis. J Cell Biol. 717: 997-1012.
[18] Pellecchia M, Reed JC. 2004. Inhibition of Anti-Apoptotic Bcl-2 Family Proteins by Natural Polyphenols New Avenues for Cancer Chemoprevention and Chemotherapy. Curr Pharm Des. 10: 1387-1398.
[19] Petignat P, Roy M. 2007. Diagnosis and management of cervical cancer. BMJ. 335: 765–768.
[20] Schrödinger Release 2015-2: LigPrep, version 2.3, Schrödinger, LLC, New York, NY, 2009.
[21] Schrödinger Release 2015-2: SiteMap, version 3.9, Schrödinger, LLC, New York, NY, 2016.
[22] Sengupta D, Verma D, Nair PK. 2008. Docking – MM- GB/SA and ADME screening of HIV-1 NNRTI inhibitor: Nevirapine and its analogues. In Silico Biol. 8: 275-289.
[23] Shoichet BK, McGovern SL, Wei B, Irwin JJ. 2002. Lead discovery using molecular docking. Curr Opin Chem Biol. 6: 439-446.
[24] Singh P, Bast F. 2014. *In silico* molecular docking study of natural compounds on wild and mutated epidermal growth factor receptor. Med Chem Res. 23: 5074–5085.
[25] Song H, Hanlon N, Brown NR, Noble ME, Johnson LN, Barford D. 2001. Phosphoprotein–protein interactions revealed by the crystal structure of kinase-associated phosphatase in complex with phosphor CDK2. Mol Cell. 7: 615-626.
[26] Thomas A, Mahant shetty U, Kannan S, Deodhar K, Shrivastava SK, Kumar-Sinha C, Mulherkar R. 2013. Expression profiling of cervical cancers in Indian women at different stages to identify gene signatures during progression of the disease. Cancer Med. 2: 836-848.
[27] Vogelstein B, Kinzler KW. 2004. Cancer genes and the pathways they control. Nat Med. 10: 789–799.
[28] Voller J, Zatloukal M, Lenobel R, Doležal K, Béreš T, Kryštof V, Spíchal L, Niemann P, Džubák P, Hajdúch M, Strnad M. 2010. Anticancer activity of natural cytokinins: A structure–activity relationship study. Phytochemistry, 71: 1350-1359.
[29] Zang X, Chen M, Zhou Y, Xiao G, Xie Y, Wang X. 2015. Identifying CDKN3 gene expression as a prognostic biomarker in lung adenocarcinoma via meta-analysis. Cancer Inform. 14: 183–191.