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RESEARCH ARTICLE

IN SILICO DOCKING STUDIES OF FLAVOPIRIDOL AND ITS ANALOGUES WITH HUMAN CDK9/CYCLINT COMPLEX

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ABSTRACT

Breast cancer is a malignant disease most commonly occurring in women. Mortality is high due to the fact that breast cancer in later stages metastases to other parts of the body causing multiple carcinoma and death. Many therapeutic targets have been identified for breast cancer of whichCDK9 is emerging as target for cancerous cells due to its role in cell proliferation. CDK9 is a component of multiprotien complex P-TEFb which plays a vital role as an elongation factor for RNA pol2 mediated transcription. Down regulation of CDK9 leads to disruption of P-TEFb complex thereby inducing apoptosis. Flavopiridol is a semisynthetic compound based on an extract from an Indian tree. Flavopiridol is a nonspecific inhibitor of CDK's showing higher selectivity towards CDK9. Flavopiridol acts by competing for ATP binding site thereby disrupting CDK9/Cyclin t complex. In addition, flavopiridol is found to work synergistically with several other compounds which act on other targets like HER2 receptor, Androgen receptor, Oestrogen receptor, etc., which are extensively studied for their role in breast cancer. In this study we focus on the molecular interactions of flavopiridol and its analogues using Insilco docking methods. The results provide convincing proof to establish that using flavopiridol and its analogues might prove beneficial in treatment of breast cancer whether as a sole drug or in combination with other drugs to work synergistically.

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INTRODUCTION

Breast cancer is the most common malignant disease in Western women. In these patients, the secondary metastases results in death than the primary tumour.Current treatment options for breast cancer patientsinclude surgery, hormonal therapy, radiation, and chemotherapy. Targeted therapies include administration of inhibitors to targetslike CDK4/6, Src, JAK/STAT, PARP, HDAC and PI3K/AKT/mTOR pathways, etc.(Sarah *et al*., 2014). Recent studies suggest that inhibitors of CDK9 as a novel therapeutic targeted treatment for breast cancer. CDK9 is a major kinase which is involvedin and responsible for the phosphorylation of serine-2 of RNA polymerase II (Saunders *et al.,* 2006), (Shim *et al*., 2002).CDK9 is one of a family of 13 protein kinases that share sequence homology and depends upon the binding of a cyclin subunit for its activation. Other members of this CDK protein family are also transcriptional regulators. Recent work suggests that targeting CDK9 might be critical for their anticancer activity (Cai *et al*., 2006; Gojo *et al*., 2002; MacCallum *et al*., 2005; Raje *et al*., 2005; Santo *et al*., 2010).

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CDK9 is a basal transcription factor important for transcription elongation. CDK9 activity is highly involved in pathological cellular processes than in normal cellular processes (Werner *et al*., 1997). CDK9 interacts also with the Androgen Receptor (AR) to amplify transcription activity (Lee *et al*., 2001). CDK9 inhibition leads to the down regulation of transcriptionally inducible genes with short half-lives which include cell cycle regulators and anti-apoptoticfactors (Lam *et al*., 2001). CDK9 presents as a possible therapeutic target. Inhibition of CDK4 by flavopiridol was an initially plausible basis for synergy with trastuzumab; recent studies and reports have implicated that the Cdk9 component of the transcriptional elongation factor P-TEFb is a more sensitive target of flavopiridol (Nahta *et al*., 2003; Price *et al*., 2000). Recent work suggests that targeting CDK9 might be critical in enhancing their anticancer activity as it is involved in cellular transcription regulation (Wang *et al*., 2008).

Flavopiridol is a synthetic flavonoid based on an extract from an Indian plant for its potential in the treatment of cancer. It functions by inhibiting cyclin-dependent kinases, arresting cell division and causing apoptosis in non-small lung cancer cells. Flavopiridol[5,7-dihydroxy-8-(4-N-methyl-2-hydroxypyridyl)-

69-chloroflavone hydrochloride] is a flavone that inhibits several cyclin-dependent kinases and exhibits potent growth inhibitory activity against a number of human tumour cell lines, both *in vitro* and when grown as xenografts in mice (Carlson *et al*., 1996; Sedlacek *et al*., 1996; Jager *et al*., 1998; Stinson *et al*., 1998; Arguello *et al*., 1998). Flavopiridol has 16 analogues in PubChem of which 14 are human and follow Lipinski's rule of 5. Flavopiridol mainly inhibits transcriptional elongation in vitroby targeting CDK9 at an IC50 5–10 fold lower than required for its effect on any other CDK (Chao *et al*., 2001). Inhibition is by interacting with P-TEFb. The Cdk9 component of the transcriptional elongation factor P-TEFb is a more sensitive target of flavopiridol than other CDK's (Nahta *et al*., 2003; Price *et al*., 2000). Administration of flavopiridol with trastuzumab was shown to enhance apoptosis (Nahta *et al*., 2003). Flavopiridol at nanomolar concentrations greatly enhances the induction of apoptosis by mitomycin C and paclitaxel in gastric and breast cancer cell lines (Werner *et al*., 1997; Schwartz *et al*., 1997). Flavopiridol has been shown to inhibit tumour growth by blocking cell cycle progression at g1and g2 phase (Kaur *et al*., 1992). Also, flavopiridolis found to induce apoptosis and inhibit c-erb-2 in breast cancer cell lines (Li Y *et al*., 2000). Synergism between flavopiridol and paclitaxel has been observed against A549 non-small cell lung cancer cells. When administered after paclitaxel the effect of paclitaxel is increased by flavopiridol (Werner *et al*., 1997; (Bible, K.C., Kaufmann, S. H., 1997). X-Ray crystallographic structure of CDK9 associated with CyclinT (3BLR) (Baumli *et al*., 2008) is available in and was downloaded from PubChem for this study.

Arguslab is an electronic structure program that is based on the quantum mechanics. It predicts the potential energies, molecular structures; geometry optimization of structure, vibration frequencies of coordinates of atoms, bond length, and bond angle (Thompson, 2004). The Argusdock docking engine, implemented in ArgusLab4.0, approximates an exhaustive search method, with similarities to DOCK and Glide. Flexible ligand docking is possible with Arguslab, where the ligand is described as a torsion tree and grids are constructed that overlay the binding site. Ligand's root node (group of bonded atoms that do not have rotatable bonds) is placed on a search point in the binding site and a set of diverse and energetically favourable rotations is created. For each rotation, torsions in breadth-first order are constructed and those poses that survive the torsion search are scored. The N-lowest energy poses are retained and the final set of poses undergoes coarse minimization, re-clustering and ranking. The advantage of using Arguslab is that it is quick and reliable results are obtained. Also Arguslab only displays those poses which are able to dock and does not display poses which cannot interact with the active site.

This articles focuses on the docking of flavopiridol and its analogues present in PubChem with the target protein CDK9 (3BLR). Using Marvin Bean (ChemAxon) and Discovery Visualizer (Accelry's now BIOVIA) we designed 10 analogues of flavopiridol. These were also docked with the target protein (3BLR) and the results tabulated. It was seen that some of the designed analogues docked with similar binding energy like those of analogues already present in PubChem. We also evaluate the drug likeness of these analogues

MATERIALS AND METHODS

Software tools used ArgusLab 4.0.1(Thompson, 2004), Discovery studio Visualizer 4.1, Marvin Sketch 5.6.0.1

Ligand preparation

Totally 14 compounds including flavopiridol and its analogues were identified from the PubMed literatures which showed inhibitory effects towards breast cancer. The three dimensional structure of the analogues was downloaded in ".sdf" format using PubChem and converted to PDB format using discovery visualizer 4.1 and further used for docking studies. 10 structures were again synthesized by changing the atoms that showed hydrogen bonding from the docking of the analogues. Analogue modification was done using Marvin bean (Chemaxon) and Arguslab. The modified drugs were tested for their drug likeness based on Lipinski's rule of 5.

Receptor x-ray structure

Protein Data Bank was searched for the presence of Cyclin-dependent kinase-9 targets, which resulted in 16 hits. Required filters were applied and the resultant 14 hits were downloaded in pdb format. Based on literature reports the X-ray crystal structure of 3BLR was chosen in which flavopiridol was already bound.

Arguslab software

The target protein structure (3BLR) was loaded cleaned using the clean geometry feature of Arguslab. The Flavopiridol binding site was recognised and defined as the binding site. The Binding site box parameters were 20.735 x 20.518 x 15.698 angstroms and grid resolution was 0.4 angstroms. Geometry of Flavopiridol and its analogues were cleaned using clean geometry feature of Arguslab. The analogues were loaded individually and docked with cdk9 binding site that was defined. The Docking parameters were set to default docking parameters i.e.;

Dockengine: Exhaustive Search
Precision: Regular Precision
Augment root node: false

The results obtained were compared and tabulated. The docking was scored based on Ascore.

RESULTS AND DISCUSSION

Evaluation drug likeness

Analogues of flavopiridol that were downloaded from PubChem had already passed Lipinski's rule of 5. The analogues that were designed using Marvin bean (chemaxon) were evaluated for their drug likeness from Lipinski filters of Supercomputing Facility for Bioinformatics & Computational

Biology, IIT Delhi website. A total of 8 compounds passed the Lipinski filter, the analogue FAS005 had one exception, in which it showed more than 5 hydrogen donors (6 donors found in FAS005). FAS003, 008 and 009 had 5 hydrogen donors each. The results of the selected analogues are shown in

The predicted active site was used as catalytic site for 23 analogues of Flavopiridol used for docking studies. The results of the interaction between human CDK9 and flavopiridol and its analogues were tabulated.

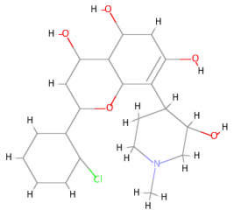
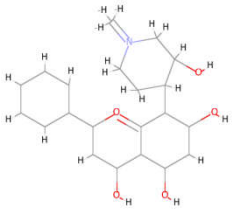
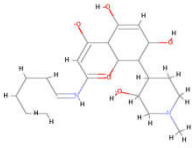
Table 1. The selected compounds showed drug likeness Docking analysis

Compound name	Mass (Da)	Hydrogen bond donor	Hydrogen bond acceptor	log P	Molar refractivity	pH
FAS003	439	5	5	1.25626	104.331863	7.4
FAS004	402.5	4	4	1.42197	101.799072	7.4
FAS005	403.5	6	5	0.117981	103.275467	7.4
FAS006	414.5	3	5	1.06528	104.936264	7.4
FAS007	386	4	5	1.216	99.092567	7.4
FAS008	384	5	5	0.75642	102.36787	7.4
FAS009	377	5	5	0.432301	97.332878	7.4
FAS010	403.5	4	7	2.03318	98.101662	7.4

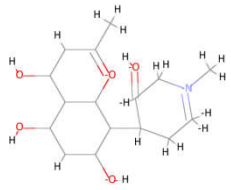
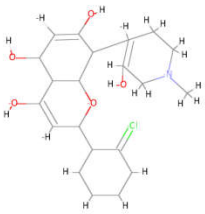
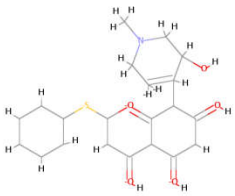
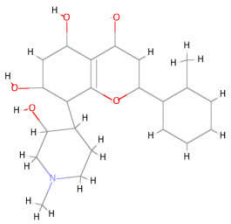
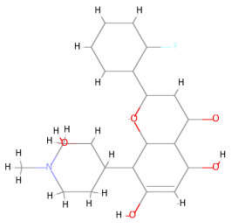
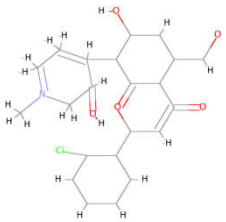
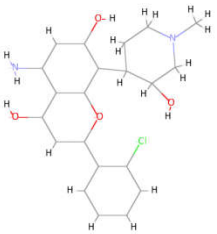
Table 2. Analogues of flavopiridinol and interaction between amino acids

Flavopiridol	
Deschloro	ASP167; ALA153
FA25	ALA153; ASP167; ASP109
ROHITUKINE	GLU107; ASP109
FAHCl	ASP109; GLU107
FA22	GLU107
FASCHEM	ASP109; GLY112; GLU107
FA19	GLU107; HIS108
+-FA	ALA153
FA16	GLU107
FA24	ASP109
FAS003	ASP109; GLY112
FAS004	ASP109; GLU107; GLY112
FAS005	CYS106; ASP109
FAS006	ASP109; GLU107
FAS007	CYS106
FAS008	GLU107

Table 3. Docking results of flavopiridol and analogues of flavopiridol using Arguslab

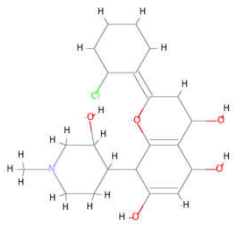
Compound	Structure	Energy value	Max score	Min score
Flavopiridol		-12.74 kcal/mol	-12.74 kcal/mol	-6.38 kcal/mol
Deschloro		-12.83 kcal/mol	-12.83 kcal/mol	-6.61 kcal/mol
FA25		-9.68 kcal/mol	-9.68 kcal/mol	-2.29 kcal/mol

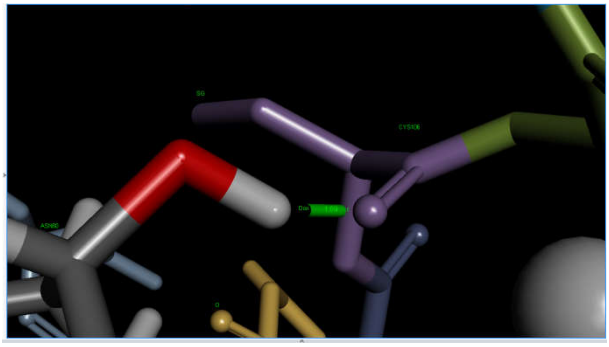
Continue……..

Rohitukine				
FAHCL		-8.75 kcal/mol	-8.75 kcal/mol	-0.70 kcal/mol
FA22		-8.29 kcal/mol	-8.29 kcal/mol	-0.70 kcal/mol
FAS008		-8.29 kcal/mol	-8.29 kcal/mol	-1.14 kcal/mol
FAS006		-8.16 kcal/mol	-8.16 kcal/mol	-0.46 kcal/mol
FAS007		-7.78 kcal/mol	-7.78 kcal/mol	-1.61 kcal/mol
FAS005		-9.61 kcal/mol	-9.61 kcal/mol	-1.57 kcal/mol

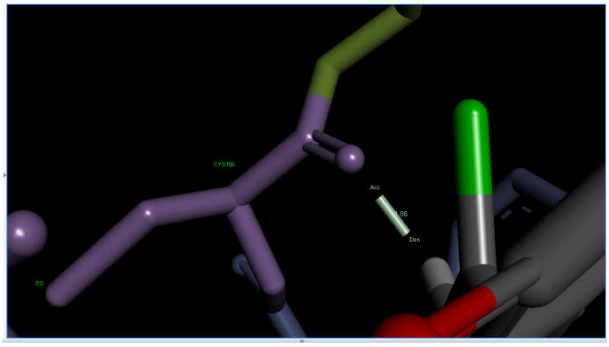
Continue……..

FASCHEM

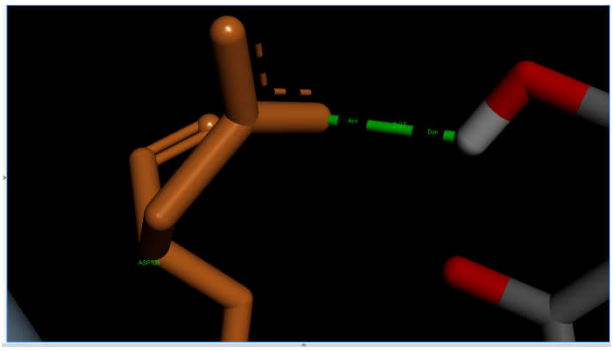




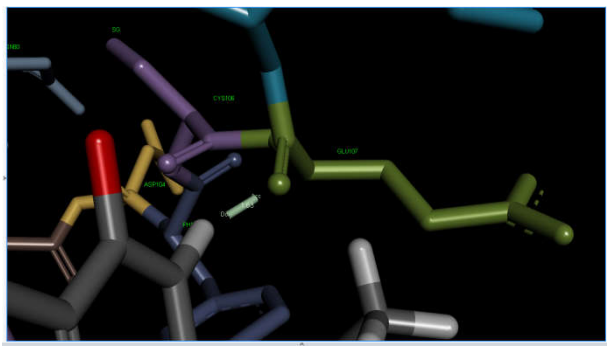
a) FAS007



b) FAS008



c) FAS007



d) FAS008

(a) FAS005 interacts with CYS106 residue with a conventional hydrogen bond of length 1.69 angstroms
(c) FAS006 interacts with ASP109 residue with a conventional hydrogen bond of length 2.07 angstroms
(b) FAS007 interacts with CYS106 residue with a carbon hydrogen bond of length 1.86 angstroms
(c) FAS008 interacts with GLU107 residue with a carbon hydrogen bond of length 1.63 angstroms

Figure 1. Bonding results of flavopiridol and synthesized analogues of flavopiridol (FAS###) with target protein (3BLR)

Of these 24 analogues 10 docked successfully and were shown to interact mainly with Asp167, Asp104, Cys106, Glu107, Asp109, Ala153 and Gly112 suggesting that these amino acid residues were present in the active site (Table 2). Based on Ascore the analogues were compared to flavopiridol. Flavopiridol had a binding energy in the range of -12.74kcal/mol to -6.38kcal/mol. In comparison deschloroflavopiridol (DESCHLORO) had a slightly lower binding energy range (-12.83 kcal/mol to-6.61 kcal/mol). The modified analogues had binding energy that were in the range of -9.61kcal\mol to -7.6kcal/mol. The results are tabulated In Table 3.

Analysis of docking interactions showed that although flavopiridol had a low activation energy, there are other analogues which can be used as drug to substitute flavopiridol. Five of the total 10 synthesized compounds docked with Human CDK9 active site. These may further be evaluated as Flavopiridol analogues (shown in Figure 1).

Conclusion

Molecular docking study is novel way of visualizing protein ligand interactions and has proven to be a useful tool in drug designing. The present study visualized the interactions between CDK9 a targeted protein for cancer therapy with a ligand Flavopiridol an anticancer drug. This study evaluates the capacity of flavopiridol and analogues of Flavopiridol to interact with the target protein (CDK9). Also we designed 10 analogues of Flavopiridol. Docking analysis and drug likeness of these novel ligands show that newly designed ligands dock similarly like the analogues of Flavopiridol. Invitro studies of

these drugs are needed to establish their biological function and activity and to establish them as novel drugs for breast cancer treatment.

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