Journal of
Computer Science & Systems Biology

Ganatra and Suchak, J Comput Sci Syst Biol 2012, 5:2
DOI: 10.4172/jcsb.1000092

Research Article Open Access

Inhibition Studies of Naturally Occurring Terpene based Compounds with Cyclin-Dependent Kinase 2 Enzyme

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Abstract

The knowledge of the molecular basis of carcinogenesis has helped to discover new, less toxic chemotherapy agents. At present, considerable attention has been focused on identifying the molecular level interactions of naturally occurring Terpene based substances, capable of inhibiting target enzymes. CDKs enzymes are known as cell regulators in eukaryotic cell cycle. In finding new anti-cancer agents, CDKs are used as target enzymes, particular among them are CDK2 enzymes.

Computer based Chem-office and Autodock molecular modeling tools used to understand the ways with which Terpene based natural products interacts with Cyclin-dependent kinase 2 (CDK2). Using in-silico techniques, the binding energy between ligands and receptor enzyme are calculated in the form of ΔG in Kcal.mol^{-1}. The reported binding energies for series of molecules are ranging from -7.96 to -16.62 Kcal.mol^{-1}. The negative docking energies and a few hydrogen bonds between ligand and receptor enzyme support the affinity of Terpene based compounds with selected enzyme. Number of hydroxyl groups present in ligand enhances the interaction strength and stability of complex. The finding confirms the affinity of Terpene based natural products as CDK2 inhibitor.

Keywords: Anti-cancer agent; Natural product; Terpene; Drug discovery; CDK protein; Cancer; In-silico; Molecular modeling; Docking

Introduction

Natural products played most vital role in human disease treatment [1,2]. It is reported that 60% of drugs used for treating cancer are born from natural products [3]. Presently, more than 23000 known natural products, Terpene based compounds are the largest class of natural products [4,5]. Among this group, many interesting compounds show biological activities and used as a medicine for treating various diseases including cancer [5,6]. For example, Paclitaxel (Taxol®) is used in treating breast cancer. These compounds are also used in varieties of diseases including cancer chemopreventive effects, anti-microbial, anti-fungal, anti-viral, anti-hyperglycemic, anti-inflammatory, and anti-parasitic activities [7-9].

The drug discovery process activates from the selection of target enzyme. The drug molecule interacts with target enzyme and inhibits it. The successful inhibition of enzyme with small drug molecule stops the normal functioning of enzyme. In major cases of cancer treatment the cell regulator enzymes are target enzymes [8]. A family of conserved serine/threonine kinase known as cyclin-dependent kinases (CDKs) drives orderly cell cycle progression in eukaryotic cell. The cyclin-dependent protein kinases [10] are regulators of the timing and coordination of eukaryotic cell cycle events [11]. Prior studies have suggested that CDK2 regulates S-phase entry and progression, and frequently shows increased activity in a wide spectrum of human tumors [12].

CDKs are inactive as monomers and their activation requires binding to cyclins with phosphorylation by CDK-activating kinase on a specific threonine residue. The cyclin belongs to diverse family of proteins and their level oscillates during cell cycle [13,14]. It is difficult to design the inhibitor specific to a particular CDKs due to the structural homology among number of CDKs (CDK2, CDK4 etc.) [15]. CDK2 activity is necessary for normal mammalian cell cycle progression and it is suggested that CDK2 might be a useful therapeutic target for treating cancer [12].

The crystal structure of CDK2 is available and one among many available structures is having PDB reference 2BHH. This crystal structure contains natural inhibitor *(2e,3s)-3-Hydroxy-5'-[(4-Hydroxypiperidin-1-Yl) Sulfonyl]-3-Methyl-1,3-Dihydro-2,3'- Biindol-2'(1'h)-One.*

Majority of plant origin compounds are tested against CDKs and found active but still the interactions involved between these compounds and CDKs are not studied in detail.

Availabilities of various computer processing based drug discovery tools help in providing the insight of interactions between small molecules and target enzymes. The importance of these tools is due to their ability to show possible interactions between ligand and receptor enzyme at atomic level. They also calculate the probable binding energy between them and explore the numerous possibilities of ligand conformations inside enzyme active sites. These tools provide the binding energy (ΔG_{bind}) in kcal.mol^{-1} between ligand and receptor enzyme (or of complex formed). This technique is used for screening the library of molecules showing better interactions for further drug discovery processes. The techniques save time and cost accounted for drug discovery process.

Terpene based natural products can be made more effective as a drug if their interactions with enzyme at molecular level are known. This is possible by using computer based molecular modeling techniques. There are numerous computer based drug discovery programs available in market which help in mimicking the chemical compound

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Received April 21, 2012; **Accepted** July 07, 2012; **Published** July 10, 2012

Citation: Ganatra SH, Suchak AS (2012) Inhibition Studies of Naturally Occurring Terpene based Compounds with Cyclin-Dependent Kinase 2 Enzyme. J Comput Sci Syst Biol 5: 068-073. doi:10.4172/jcsb.1000092

interactions with enzyme and provides valuable information related to types of interactions, possibilities of bonding and conformations. The most prominent technique is docking of small molecule (ligand) with target enzyme. Gold, Autodock, Dock, ArgusLab etc. are a few computer based docking programs presently available in market. Binding energy in kcal.mol^{-1} is the major parameter investigated using these programs along with steric, hydrophobic and electrostatic interactions. It also provides the list of possible hydrogen bonding between ligand and enzyme. The state of art graphics shows the ligand-enzyme complex from various angles.

In present study, twenty five naturally occurring Terpenes are selected and tested for their inhibition possibilities with CDK2 (PDB reference 2BHH) enzyme using molecular docking techniques. The aim is to investigate the possible binding energies, various interaction poses, and possible hydrogen bonding and hence understanding the effectiveness of these molecules as a CDKs inhibitor, specifically CDK2 inhibitor.

Materials and Methods

Design of small molecules (Ligand)

To study inhibition of enzyme with designed small molecules (called as ligand), twenty five Terpene based known natural products are selected as listed in Table 1.

Ligand preparation

The structures of 25 Terpene based plant-derived compounds are designed in-silico using Chem-office software [16]. Initially 2-D structures were designed. The 2-D compounds converted to 3-D using Molecular Mechanics (MM2) method with the help of Chem-office software [16]. The designed molecules are checked for its conformation by ascertaining achievement of global minima. The list of compounds designed along with molecular formula is listed in Table 1.

Molecule No.	Phytochemical Name	Molecular Formula
1	Abietane	$C_{20}H_{36}$
2	Abscisic acid	$C_{15}H_{20}O_4$
3	Aconitine	$C_{34}H_{47}NO_{11}$
4	Aphidicolin	$C_{22}H_{36}O_5$
5	Arjunolic acid	$C_{30}H_{48}O_5$
6	Betulin	$C_{30}H_{50}O_2$
7	Cannabinol	$C_{21}H_{26}O_2$
8	Gingerol	$C_{17}H_{26}O_4$
9	Ginsenoide	$C_{30}H_{52}O_2$
10	Glaucarubin	$C_{25}H_{36}O_{10}$
11	Kaurane	$C_{20}H_{34}$
12	Labdane	$C_{20}H_{38}$
13	Limonene	$C_{10}H_{16}$
14	Lupeol	$C_{31}H_{52}O$
15	Lutein	$C_{40}H_{56}O_2$
16	Lycopene	$C_{40}H_{56}$
17	Maslinic acid	$C_{30}H_{48}O_4$
18	Neurosporene	$C_{40}H_{58}$
19	Oleanolic acid	$C_{30}H_{48}O_3$
20	Phytofluene	$C_{40}H_{62}$
21	Sapogenin	$C_{27}H_{42}O_4$
22	Taraxosterol	$C_{30}H_{50}O$
23	Tetrahydrocannabinol	$C_{21}H_{30}O_2$
24	Ursolic acid	$C_{30}H_{48}O_3$
25	Zeaxanthin	$C_{40}H_{56}O_2$

Table 1: List of Terpene-Based Phytochemicals.

Non-genetical docking parameters
Grid Resolution=0.4 Number Of Steps=50
Genetical docking parameters
The population size=100 Maximum Generation=5000 Elitism Number=5 Crossover Rate=0.8 Mutation Rate=0.2 Local Search Rate=0.06 Local Search Maximum Iteration=20 Converged when RMSD Population Fitness <1 kcal.mole^{-1} Grid Dimensions=67x77x61 Total Number Of Grid Points=314699 Grid Resolution=0.4

Table 2: Selected Parameters for Non-Geneticaland Genetical Docking.

Receptor enzyme

Electronic structure of CDK2 is selected as a target protein having PDB reference 2BHH. The protein file procured from online data base having *(2E,3S)-3-HYDROXY-5'-[(4-HYDROXYPIPERIDIN-1-YL) SULFONYL]-3-METHYL-1,3-DIHYDRO-2,3'-BIINDOL-2'(1'H)-ONE* as a natural inhibitor [17]. The selected enzyme structure was prepared in such a way that it has no ambiguities in the form of missing atoms or amino acids. All the heteroatoms (i.e. non-receptor atoms such as water, ions, etc.) were removed followed by assigning Kollmann charges. The Solvation parameters were added to the final macromolecule structure using the Addsol utility of AutoDock [18].

The place of natural inhibitor in enzyme is treated as active site of selected enzyme and used as it is without any further processing.

Docking

Autodock 4.0 [18] is used for docking process. Initially protein grid was designed using grid design tool of Autodock. Dockings were performed using both genetic (GA) and non-genetic (Non-GA) algorithm techniques. The genetic algorithm (GA) is the newly adopted conformational search techniques and searches the best possible conformations of ligand inside the active site of enzyme. For each conformational position, it also reports the possible binding energy in the form of ΔG in kcal.mol^{-1}. The selected parameters and settings, which were used for docking, are listed in Table 2.

The docking algorithm makes use of force field equations and parameters to calculate the binding energy between ligand and enzyme [19-25]. The binding free energy is the total of van der Waals interactions, H-bond interactions, electrostatic interactions and the internal static energy of the ligand as shown in Equation 1 [26-33].

$$\Delta G_{bind} = \Delta G_{vdw} + \Delta G_{hydrophobic} + \Delta G_{H\text{-}bond} + \Delta G_{H\text{-}bond(chg)} + \Delta G_{deformation} + \Delta G \quad (1)$$

The obtained results of binding energy for Non-GA and GA Dockings for each set of experiments are listed in Table 3. The negative values of docking energies favour the interaction among ligand and enzyme. Though there are chances of non-favourable interactions, the non-favourable results are marked as '*'.

Results

There are number of CDK2 electronic structures available in protein databank [17]. The selection of 2BHH is due to presence of natural inhibitor located in one of the main active sties. This structure is also error free and complete one.

Table 3 lists obtained binding energies for all docked molecules. The reported values of docking energies are between –8.89 to -17.20 kcal.mol^{-1} and -8.58 to -16.62 kcal.mol^{-1} for Non-GA and GA docking respectively. Both types of docking give nearly same results. The binding energy is not reported for a few compounds in non-GA docking. Hence for further analysis and comparisons only GA docking were used. All compounds report negative binding energy and hence possibilities of stable complex formation. The stability is also enhancing in few compounds due to the formation of hydrogen bonding (HB).

Hydrogen bonding analysis

The strength of the HB is evaluated from the bond distance. Autodock provides the possibilities of HBs between ligand and protein. Though software reports nearly all possibilities of HBs, only those bonds having length less than 2.5Å are counted and others are discarded. However, it is possible to have less binding energy and higher HBs; this may be due to lack of other types of interactions.

Depending on the binding energy of GA docking and HB, out of 25 studied molecules, 5 molecules showing better interactions are selected for further analysis. The selected molecules are Cannabinol (Molecule No. 7), Lutein (Molecule No. 15), Neurosporene (Molecule No. 18), Oleanolic Acid (Molecule No. 19) and Zeaxanthin (Molecule No. 25). The binding energy values for the selected five molecules are ranging from -13.71 kcal.mol^{-1} to -16.62 kcal.mol^{-1}. Table 4 shows the binding energy values and possible HB whereas, Table 5 shows the docking images in Wire-frame and CPK modes for best five selected compounds.

Molecule Number.	Phytochemical Name	Molecular Formula	Docking Energy (ΔG) in kcal mol^{-1}	
			Non-GA Docking	GA Docking
1	Abietane	$C_{21}H_{38}$	-12.90	-12.42
2	Abscisic acid	$C_{15}H_{20}O_4$	-10.70	-9.55
3	Aconitine	$C_{34}H_{47}NO_{11}$	*	-8.58
4	Aphidicolin	$C_{22}H_{36}O_5$	-10.27	-11.10
5	Arjunolic acid	$C_{30}H_{48}O_5$	-11.19	-12.09
6	Betulin	$C_{30}H_{50}O_2$	-12.27	-12.77
7	Cannabinol	$C_{21}H_{26}O_2$	-11.91	-13.71
8	Gingerol	$C_{17}H_{26}O_4$	-10.97	-8.65
9	Ginsenoside	$C_{30}H_{52}O_2$	-13.84	-12.10
10	Glaucarubin	$C_{25}H_{36}O_{10}$	*	-9.24
11	Kaurane	$C_{20}H_{34}$	-12.35	-13.02
12	Labdane	$C_{20}H_{38}$	-12.30	-11.67
13	Limonene	$C_{10}H_{16}$	-10.65	-9.85
14	Lupeol	$C_{31}H_{52}O$	-13.49	-13.75
15	Lutein	$C_{40}H_{56}O_2$	-15.72	-14.78
16	Lycopene	$C_{40}H_{56}$	*	-7.96
17	Maslinic acid	$C_{30}H_{48}O_4$	-9.98	-11.32
18	Neurosporene	$C_{40}H_{58}$	*	-16.05
19	Oleanolic acid	$C_{30}H_{48}O_3$	-11.02	-14.65
20	Phytofluene	$C_{40}H_{62}$	*	-10.61
21	Sapogenin	$C_{30}H_{50}O_3$	-8.89	-13.02
22	Taraxosterol	$C_{30}H_{50}O$	-13.09	-12.60
23	Tetrahydrocannabinol	$C_{21}H_{30}O_2$	-10.69	-12.75
24	Ursolic acid	$C_{30}H_{48}O_3$	-12.89	-12.74
25	Zeaxanthin	$C_{40}H_{56}O_2$	-17.20	-16.62

* Unable to dock.

Table 3: List of Observed Binding Energy of Terpene-Based Molecules with CDK2 Enzyme (PDB Ref. 2BHH).

Molecule No.	Molecular Formula	Binding Energy ΔG in Kcal.Mol-1	Total Hydrogen Bonding	Amino Acids Involved in Hydrogen Bonding	Hydrogen Bonding Distance in Á
7	$C_{21}H_{26}O_2$	-13.71	1	81GLU	2.027404
15	$C_{40}H_{56}O_2$	-14.78	*	*	*
18	$C_{40}H_{58}$	-16.05	*	*	*
19	$C_{30}H_{48}O_3$	-14.65	1	145ASP	2.654306
25	$C_{40}H_{56}O_2$	-16.62	*	*	*

Table 4: Binding Energy Values Of Cannabino, Lutein, Neurosporene, Oleanoic Acid, Ziaxenthin Compounds Complex with CDK2 (Pdb Ref.: 2BHH) Enzyme.

To understand the stability of ligand-protein complex and possibilities of various conformations of ligand in active site, cluster studies for best five molecules were performed.

Cluster analysis

The cluster analysis helps in understanding the conformation of ligand molecules in docking site at the time of flexible interactions. Higher the cluster number along with higher binding energy shows the possibilities of best fitting of ligand and hence higher ligand-protein complex stability. Autodock provides number of cluster values along with possible binding energies. Top rank three such values along with corresponding binding energies were analyzed and reported in Table 6. In this set of molecules the highest number of cluster 31 is reported for Oleanolic acid (molecule number 19) having binding energy -14.66 kcal.mol^{-1}.

Conclusion

The molecular docking studies of twenty five Terpene based natural compounds report negative binding energies and compact inhibition. A few among them also report the possibilities of hydrogen bonding. Cannabinol, Lutein, Neurosporene, Oleanolic acid and Zeaxanthin are reported as the best inhibitors as they show better ligand-enzyme interactions and stability. Therefore they show potency to be anti-CDKs agents. Their reported binding energies ranging from -13.0 kcal.mol^{-1} to 16.50 kcal.mol^{-1} are reported in Table 7.

Molecule no. 7 and 19 having smaller surface areas compared to molecule no. 15, 18 and 25. Molecule no. 7 and 19 bind in active site and remain within the boundary of selected active site, whereas, molecule no. 15, 18 and 25 are taking more space due to long chain compounds. This can be confirmed from the docking pictures displayed in Table 5. It is also reported that molecule having higher surface area and fits in active site of receptor enzyme gives better binding energy value compared to molecule having lower surface area. Molecule no. 7 and 19 shows better binding energy values due to the presence of

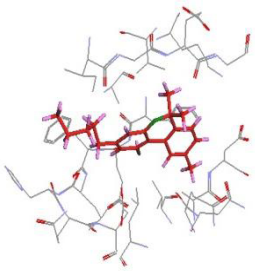
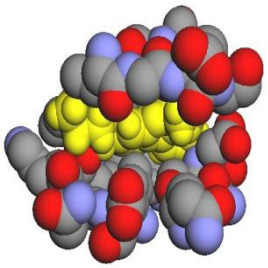
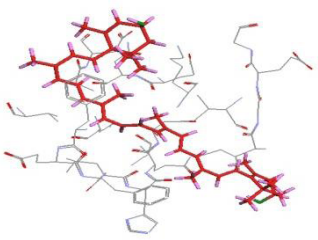
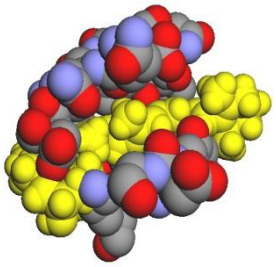
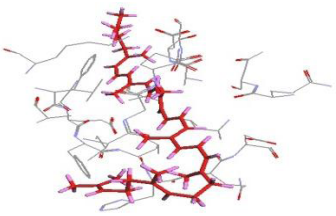
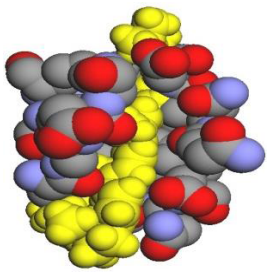
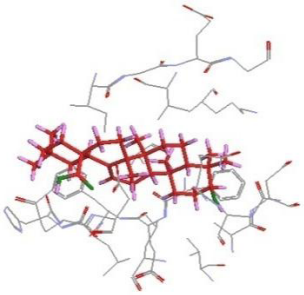
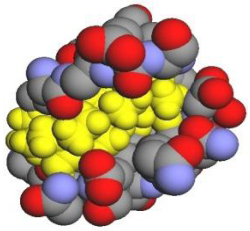
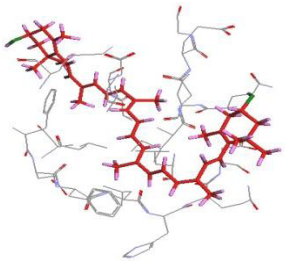
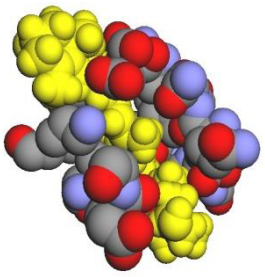
Molecule No.	Phytochemical Name	Molecular Formula	Wire-Frame Mode	CPK Mode
7	Cannabinol	$C_{21}H_{26}O_2$		
15	Lutein	$C_{40}H_{56}O_2$		
18	Neurosporene	$C_{40}H_{58}$		
19	Oleanolic acid	$C_{30}H_{48}O_3$		
25	Zeaxanthin	$C_{40}H_{56}O_2$		

Table 5: The Interactions of Cannabino, Lutein, Neurosporene, Oleanoic Acid, Ziaxenthin Compounds with CDK2 (Pdb Ref. 2BHH) Enzyme.

one hydrogen bonding. Figures 1 and 2 show the docking pictures of Cannabinol and Oleanolic acid (Molecule Number 7 and 19) with receptor enzyme along with one hydrogen bond respectively.

81 Glutamine and *145 Aspartic acid* of receptor enzyme are the most prone amino acids participating in making hydrogen bonds with the ligand atoms.

Cluster study represents number of possible conformations of ligand in active site of enzyme. Nearly all selected molecules show higher cluster size, except Neurosporene. Cannabinol and Oleanolic acid are two small molecules and show higher cluster size as per expectation. It also confirms the procedural correctness of docking work and validation of model.

Molecule Number	Binding energy (B. E) ΔG in kcal/mol/Cluster Size					
	Ist Highest		IInd Highest		IIIrd Highest	
	B. E.	Number of Conformations	B. E.	Number of Conformations	B. E.	Number of Conformations
7	-13.71	27	-10.93	3	-10.10	2
15	-14.79	11	-14.41	9	-14.50	3
18	-16.05	7	-13.90	2	-13.52	2
19	-14.66	31	-11.61	3	-10.94	3
25	-16.62	21	-8.56	4	-9.14	3

Table 6: Cluster (Number of Conformations) for Terpene Based Ligand Compounds with CDK2 (PDB Ref. 2BHH) Enzyme.

Class of Compound	CDK2 (PDB Ref. : 2BHH) Enzyme	
Terpene	Minimum ΔG kcal·mol^{-1}	Maximum ΔG Kcal·mol^{-1}
	-7.96	**-16.62**

Table 7: Minimum and Maximum Binding Energy (ΔG in Kcal.Mol-1) of Terpene-Based Compounds with CDK2 (PDB Reference 2BHH) Enzyme.

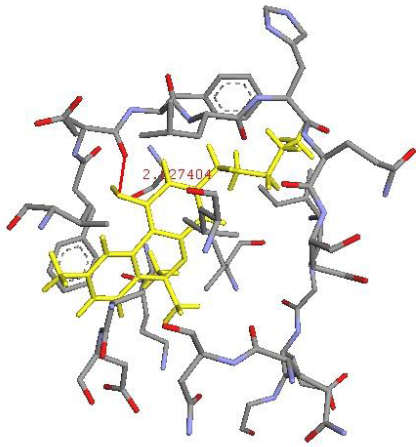


Figure 1: Docking picture of Cannabinol (Molecule Number 7) with CDK2 (PDB Reference 2BHH) along with one hydrogen bond. (Bond distance 2.42 Å).

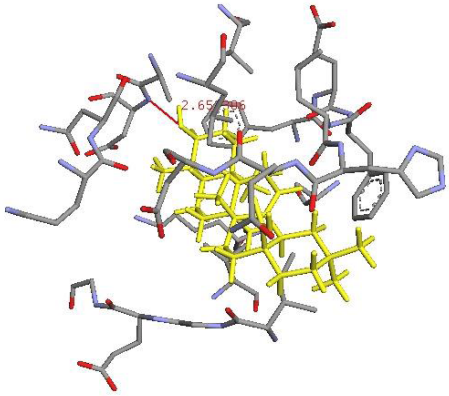


Figure 2: Docking picture of Oleanolic acid (Molecule Number 19) with CDK2 (PDB Reference 2BHH) along with one hydrogen bond. (Bond Distance 2.65 Å).

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