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MOLECULAR DOCKING STUDIES OF TETRACYCLIC TRITERPENES (CUCURBITACIN DERIVATIVES) WITH REFERENCE TO THEIR ANTI-CANCER ACTIVITIES AGAINST BCL2 & CYCLIN D3 RECEPTORS USING *IN SILICO* DRUG DESIGNING TOOLS

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Abstract

Computational chemistry (also called molecular modelling) is a set of techniques for investigating chemical problems on a computer. Computational chemistry is used in a number of different ways. One particularly important way is to model a molecular system prior to synthesizing that molecule in the laboratory. Computational models are very useful information because synthesizing a single compound could require months of raw materials & generate toxic waste. A second use of computational chemistry is in understanding a problem more completely. There are some properties of a molecule that can be obtained computationally more easily than by experimental means. There are also insights into molecular bonding, which can be obtained from the results of computations that cannot be obtained from any experimental method. Computational chemistry has been of great importance to develop fast & accurate target identification and prediction method for the discovery of targeted drugs, construction of drug-target interaction as well as the analysis of small molecule. BCL2 (B-cell lymphoma 2) & CYCLIN D3 are critical receptors that control cell growth. However, mutational changes in these receptors leading to uncontrolled cellular proliferation or cell death. In humans, mutations in BCL2 & CYCLIN D3 is responsible for nearly 50% of lung cancers. In this paper, molecular docking was performed of natural tetracyclic triterpenes (Cucurbitacin derivatives) & various standard compounds that are thought to have potential to inhibit mutated BCL2 & CYCLIN D3 receptor. Out of 17 Cucurbitacin derivatives & various standard compounds, Cucurbitacin D had higher inhibition activity against BCL2 & CYCLIN D3 receptor. From this study, it was observed that Cucurbitacin D had promising inhibitory effect against lung cancer than other cucurbitacin derivatives & various standard compounds.

Keywords: Cancer, Lung Cancer, Cucurbitacin derivatives, BCL2 receptor, CYCLIN D3 receptor, Apoptosis, Cell cycle, Preparation of receptor (protein), Docking, Lipinski's rule, Prediction of ADMET (Absorption, Distribution, Metabolism, Excretion & Toxicity) properties

Introduction

Cancer arises from a loss of normal growth control

- In normal tissues, the rates of new cell growth & old cell death are kept in balance [(1)].
- In cancer, this balance is disrupted. This disruption can result from uncontrolled cell growth or loss of a cell's ability to undergo 'apoptosis' [(1)].
- Apoptosis or 'cell suicide' is the mechanism by which old or damaged cells normally self destruct.

When cancer develops due to exposure to toxic chemicals (Figure 1), a toxic chemical is introduced to the cells of the body and causes mutation which is a reversible stage by either immune system attack to the abnormal cell or DNA repair process [(1)]. The second step is the promotion and cell division of mutated cells. Continuation of the cell division leads to more cells which will end up forming a noncancerous tumor in early stages, if the tumor is not treated in time it becomes a cancerous tumor with mutated cell proliferation. Cancerous tumor initially cannot be transported to other

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areas of the body but with the development of stem cells, cancer cells will start by infecting neighboring organs & spread even further to far cells [1]. According to the cancer hypothesis, a tumor needs the stem cells which are responsible in giving rise to new cells, growth of those cells, cell proliferation & transport of the cells to various organs [2].

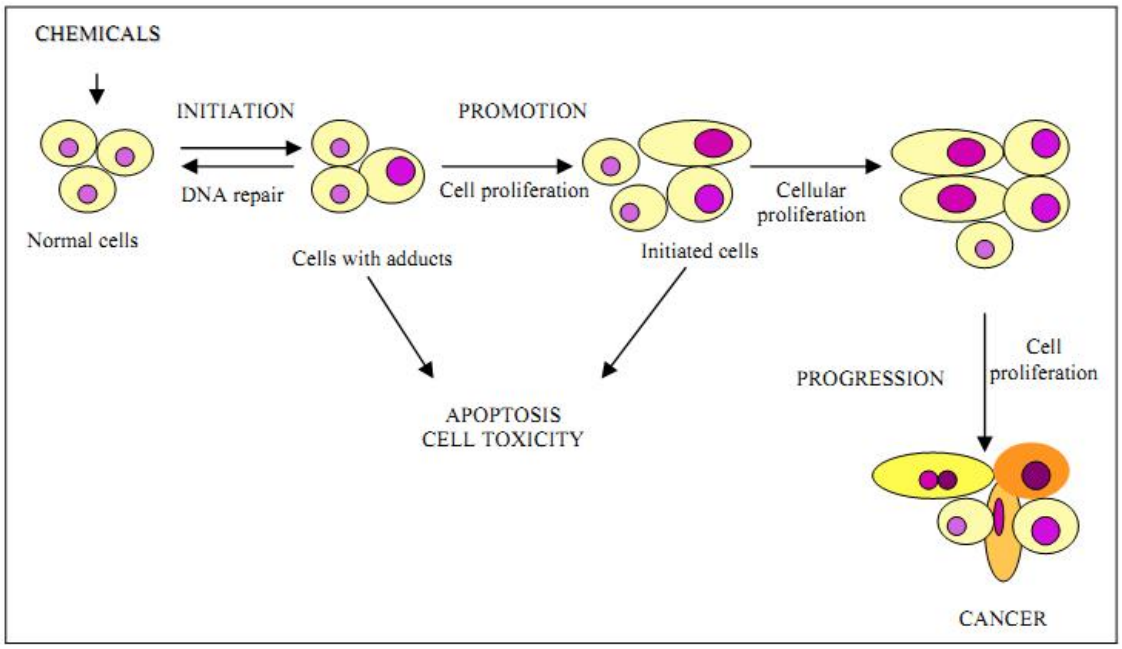

Figure 1: Illustration of chemical carcinogenesis [1]

According to the American Cancer Society, Lung cancer is the most common cause of death in the world & accounts for nearly 1 or 2 of every 4 deaths related to cancer [1]. The World Health Organisation (WHO) estimates that worldwide, there were 1.59 million lung cancer-related deaths in 2012 [1].

Multi-drug resistance (MDR) has major barrier to the success of lung cancer treatment [2]. So, the discovery of new potent drug is important for treatment of lung cancer.

Cucurbitacin is a group of tetracyclic triterpenes derived from plants related cucurbitaceae family including the pumpkins, citrus & gourds such as *Luffao perculata, Citrullus colocynthis* etc [3]. At room temperature, Cucurbitacin derivatives are generally crystalline substances & having poor water solubility [3].

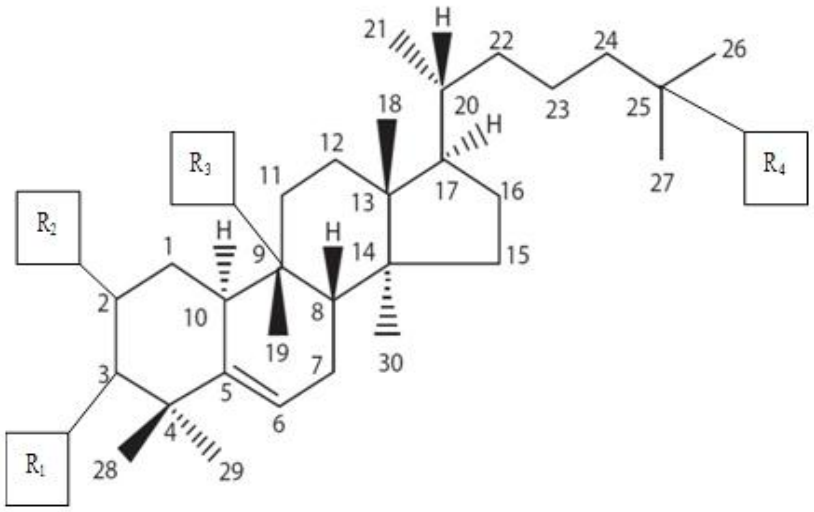

Figure 2: General structure of Cucurbitacin skeleton [3]

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Sl. No.	Name of compound	R_1	R_2	R_3	R_4
1	Cucurbitacin A	OH	OH	CH_3	$OCOCH_3$
2	Cucurbitacin B	OH	OH	H	$OCOCH_3$
3	Cucurbitacin C	OH	OCH_3	OH	$OCOCH_3$
4	Cucurbitacin D	OH	OH	H	OH
5	Cucurbitacin E	OH	CH_3	CH_3	$OCOCH_3$
6	Cucurbitacin F	OH	OCH_3	CH_3	OH
7	Cucurbitacin H	OCH_3	OCH_3	H	$OCOCH_3$
8	Cucurbitacin I	OH	OH	CH_3	OH
9	Cucurbitacin J	OF	CH_3	OH	OH
10	Cucurbitacin K	OCH_3	CH_3	OH	OH
11	Cucurbitacin L	$OCOCH_3$	OH	H	OCH_3
12	Cucurbitacin O	OH	OCH_3	OH	CH_3
13	Cucurbitacin P	OH	OH	CH_3	OH
14	Cucurbitacin Q	OH	OH	CH_3	$OCOCH_3$
15	Cucurbitacin R	OCH_3	$OCOCH_3$	OH	OH
16	Cucurbitacin S	OH	$OCOCH_3$	H	OH
17	Dihydro Cucurbitacin B	OH	OH	H	OCH_3

Table 1: Different substitutions at different sites on chemical structures of different types of Cucurbitacin derivatives [3] .

Sl. No.	Types of different cucurbitacins	Sources of different cucurbitacins
1	Cucurbitacin A	*Trichosanthes Cucumerina*
2	Cucurbitacin B	*Luffao perculata*
3	Cucurbitacin C	*Citrullus colocynthis*
4	Cucurbitacin D	*Trichosanthes kirilowii*
5	Cucurbitacin E	*Citrullus colocynthis*
6	Cucurbitacin F	*Trichosanthes kirilowii*
7	Cucurbitacin H	*Cayaponia tayuya*
8	Cucurbitacin I	*Cayapo niatayuya*
9	Cucurbitacin J	*Cayapo niatayuya*
10	Cucurbitacin K	*Trichosanthes kirilowii*
11	Cucurbitacin L	*Citrullus colocynthis*
12	Cucurbitacin O	*Trichosanthes kirilowii*
13	Cucurbitacin P	*Trichosanthes kirilowii*
14	Cucurbitacin Q	*Citrullus colocynthis*
15	Cucurbitacin R	*Cayaponia tayuya*
16	Cucurbitacin S	*Cayaponia tayuya*
17	Dihydro Cucurbitacin B	*Cayapo niatayuya*

Table 2: Different types & sources of Cucurbitacin derivatives [3]

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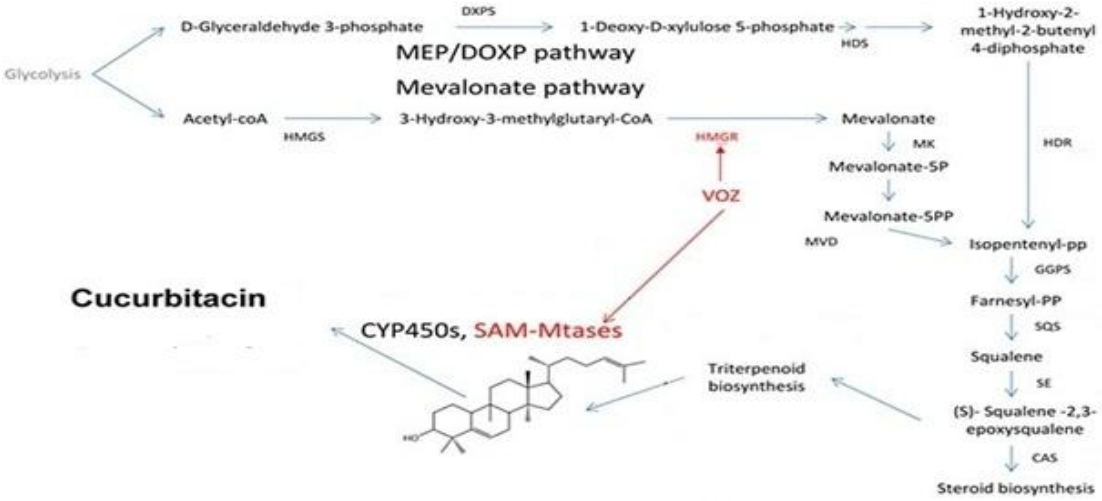

Figure 3: A schematical illustration shows Mevalonate pathways for biosynthesis of Cucurbitacin derivatives in *Aquilaria agallocha* [3]

Enzymes identified in the Cucurbitacin derivatives pathway & their change in gene expression is shown below:

(DXPS: 1-deoxy-D-xylulose-5-phosphate synthase
HDS: 4-hydroxy-3-methylbut-2-enyl diphosphate synthase
HDR: 4-hydroxy-3-methylbut-2-enyl diphosphate reductase
HMGS: hydroxyl methyl glutaryl-CoA synthase
HMGR: 3-hydroxy-3-methyl glutaryl-CoA reductase
MK: -mevalonate kinase
MVD: diphospho mevalonate decarboxylase
GGPS: geranyl geranyl pyrophosphate synthetase
CYP450s: cytochrome P450s;
SAM-Mtases: S-adenosyl-L-methionine-dependent methyl transferases
SQS: squalene synthetase
CAS: cyclo artenol synthase
SE: squalene monooxygenase
VOZ: vascular plant one-zinc-finger transcription factor)

Selection of various targets: Cucurbitacin derivatives are hypothesized to activate caspase-3 as well as reduce in various downstream BCL2 & CYCLIN D3 all of which are implicated in apoptosis & the cell cycle control [4, 5].

a.BCL2: BCL2 (B-cell lymphoma 2) is encoded in humans by the BCL2 gene. The major function of BCL2 is related with cell death (apoptosis). In cancer, activated BCL2 inhibits apoptosis. The activator of BCL2 protein (receptor) is mainly different types of cytokines including interleukins & interferons [4].

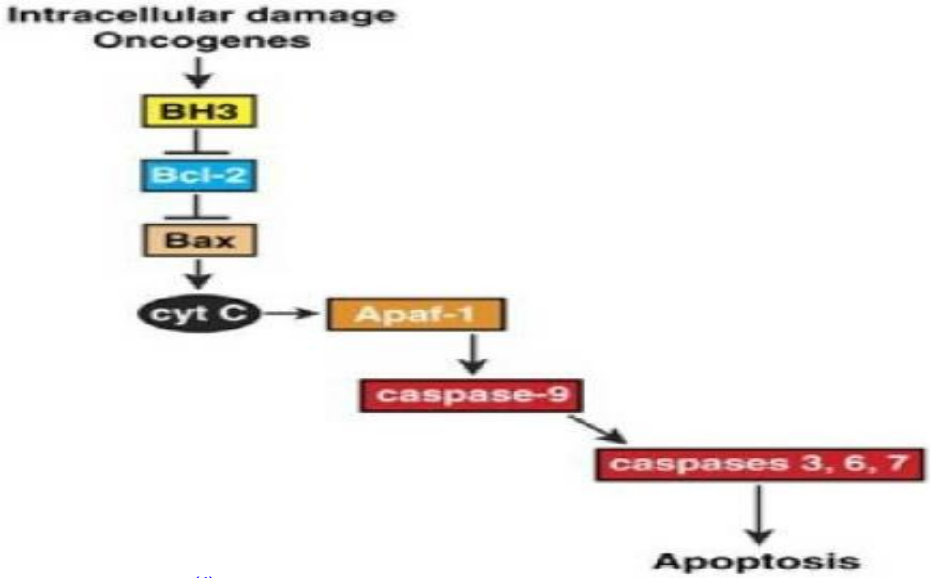

Figure 4: Pathways to cell death [4] [Figure 4]: The pathway is initiated by BH3 proteins which inactivate the BCL2 proteins, keeping them from restraining BAX or BAK. BAX or BAK can permeabilize the mitochondrial outer membrane, releasing cytochrome c which provokes APAF-1 (apoptotic protease activating factor 1) to activate caspase-9, 3, 6, 7 & apoptosis.

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- **CYCLIN D3:** CYCLIN D3 is a protein that in humans is encoded by the CCND3 gene [5].
- In normal cell, they are involved in conducting the cell cycle, mRNA processing & the differentiation of nerve cells. They are present in all known eukaryotes [5].
- They are relatively small proteins with molecular weights ranging from 34 to 40 kDa [5] & contain little more than the kinase domain.
- They phosphorylate their substrates on serines & threonines, so they are serine-threonine kinases.
- The consensus sequence for the phosphorylation site in the amino acid sequence of a CYCLIN D3 substrate is the phosphorylated serine or threonine or proline, lysine & arginine as an activator [5].

- **The cell cycle:** The cell cycle [6] is an ordered series of events consisting of several sequential phases.

These are:

1. G_1: preparation for DNA synthesis
2. S: DNA synthesis & chromosome duplication
3. G_2: preparation for division
4. Mitosis (M): division into two daughter cells

In cancer, during G_1, the concentration of CYCLIN D3 increases & the CYCLIN D3 phosphorylates & activates the necessary proteins. In mid-G_1, the CYCLIN D3 phosphorylates the Rb (retinoblastoma) protein, releases a transcription factor that activates the genes for the components essential for the next phase-DNA synthesis [5].

So, to prevent cancer the CYCLIN D3 is blocked to also block cell cycle especially G_1 phase.

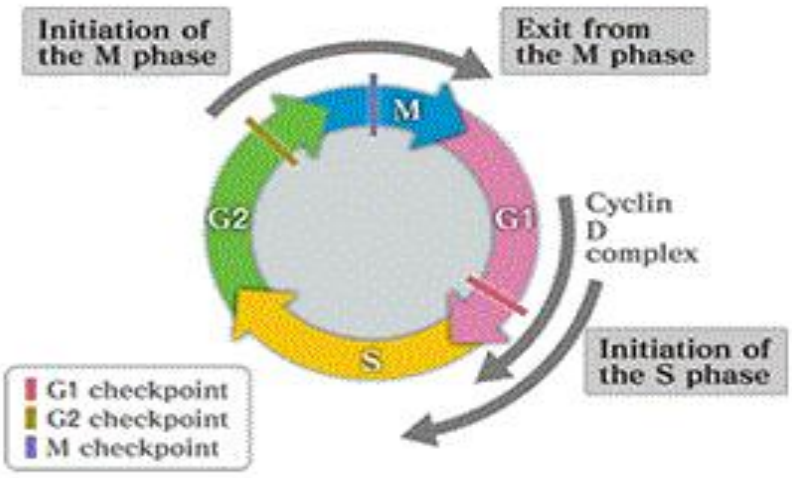

Figure 5: Schematic diagram of various phases of cell cycle [5] [Figure 5] including the role of the CYCLIN D complexes in particular phase of cell cycle.

Bioinformatics tools:

A. Softwares: Auto dock tools**,** Accelrys Discovery studio 3.5 visualizer**,** UCSF Chimera 1.10**,** Rasmol**,** Microsoft office excel 2007, Padel-descriptor.

B.Web servers:

1. Pubchem: (http://www.pubchem.ncbi.nim.nih.gov/)
2. Chemspider: (http://www.chemspider.com/)
3. RCSB protein data bank: (http://www.rcsb.org/)
4. PDBsum: (http://www.ebi.ac.uk/pdbsum/)
5. ALOGPS: (www.vcclab.org/lab/alogps/)
6. E-dragon: (www.vcclab.org/lab/e-dragon/)

Methodology:

1. From previously published articles, disease (specially lung cancer) was selected according to 'WHO' [1] based on current global data on percentage of death records in 2012 on all cancers in all developing countries.
2. From previously published papers, selection of various targets (proteins or receptors) due to which these disease was occurred.
3. From previously published articles, various standard compounds (activators or inhibitors) were selected for targets (BCL2 & CYCLIN D3). The 3D (three-dimensional) structures of these compounds were downloaded from pubchem web server.

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4. From previously published papers, various test compounds (different Cucurbitacin derivatives) were selected based on their activation against the targets [BCL2 & CYCLIN D3]. The 3D structures (three-dimensional) of selected targets were downloaded from the official web server of protein data bank (PDB).
5. Conformations of downloaded ligands (standard & test compounds) & conformations of downloaded proteins were generated.
6. Docking sites of BCL2 & CYCLIN D3 receptors were identified by using the protein variant files from which the amino acid residues & coordinates were obtained for crystal proteins.
7. Docking of standard compounds were done to the active site of BCL2 & CYCLIN D3 receptors.
8. Docking of test compounds were done to check the binding of different test compounds (Cucurbitacin derivatives) to the activator attachment site of BCL2 & CYCLIN D3 receptors.
9. Binding energies (kcal/mol) of various standard & test compounds were calculated by following formula, Binding energy (kcal/mol) = A+B+C-D, in where A = vanderwal energy + hydrogen bond energy + intermolecular interactions + desolvation energy + electrostatic energy, B = total internal energy, C = torsion energy & D = unbounded energy [6].
10. Best docked Cucurbitacin derivatives (with lowest maximum binding energy against BCL2 & CYCLIN D3 receptor) was selected for prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity properties), carcinogenicity, mutagenicity & components for fulfilling Lipinski's [7, 8] rule.

Lipinski's [7, 8] rule: Good absorption are more likely when:

a. There are not greater than 5 H-bond donors.
b. The molecular weight is within 500 daltons or 800gms.
c. The LogP is within 5.
d. There are not greater than 10 H-bond acceptors.

Results and Discussion:

Structure of downloaded BCL2 (1G5M) receptor (protein) from official website of protein data bank (PDB):

Receptor (protein) code of BCL2: 1G5M
Classification:- Apoptosis
Structural weight: 19227.50 kDa
Source organism: *Homo sapiens*
Type: Crystal structure

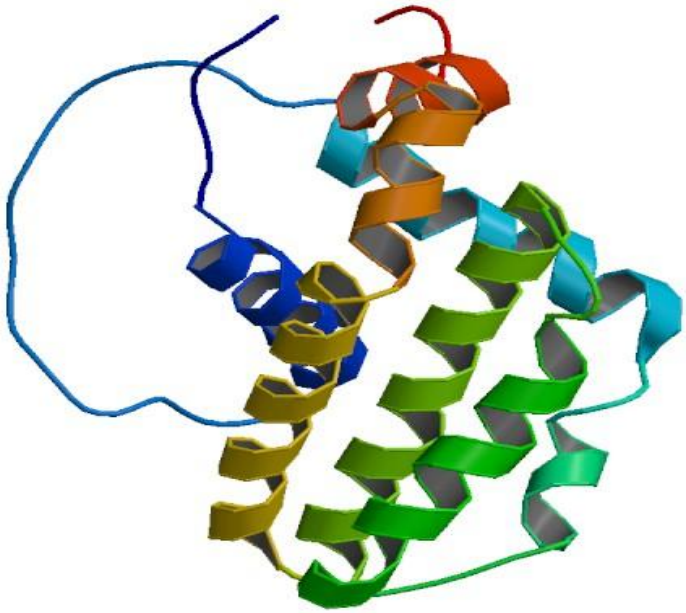

Figure 6: Downloaded structure of BCL2 (1G5M) receptor (ribbon structure) from website of protein data bank (PDB)

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Structure of prepared BCL2 (1G5M) receptor (protein):

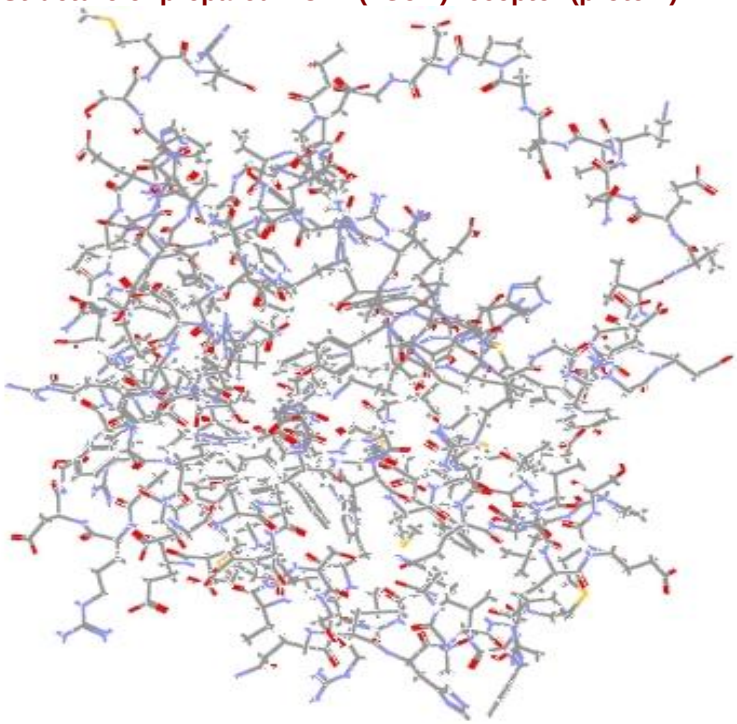

Figure 7: Structure of prepared receptor BCL2 (1G5M)

Structure of active site of prepared BCL2 (1G5M) receptor (protein):

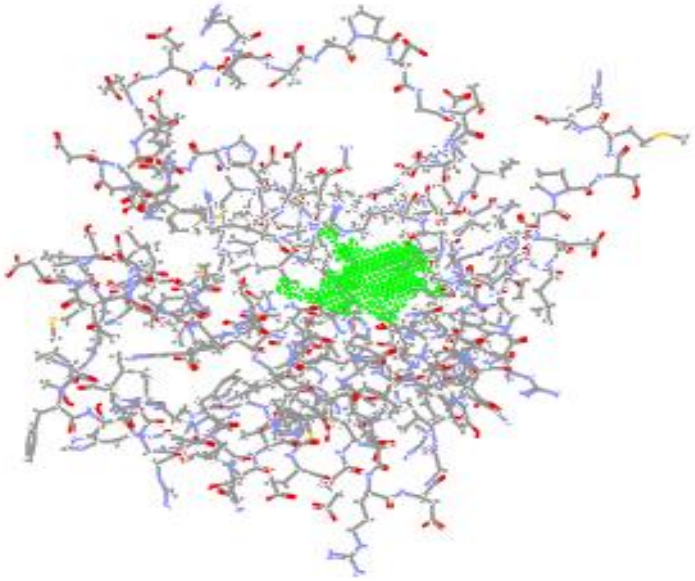

Figure 8: Structure of active site of prepared receptor BCL2 (1G5M) [In this figure, the green portion represents active site for BCL2 (1G5M) receptor]

Coordinates (X, Y & Z) & volume or size of the active site of BCL2 (1G5M) receptor (protein) for binding of each ligand:

Name of the receptor (protein)	Code	X	Y	Z	Volume or size of the active site (Å)
BCL2	1G5M	12.515	0.962	-13.712	648.870

Table 3: Coordinate & volume or size of active site of BCL2 (1G5M) receptor (protein) [The volume or size of active site of BCL2 (1G5M) receptor must be greater than the volume of the each ligand or compound]

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Docking of various standard compounds to the active site of BCL2 (1G5M) receptor (Figure 9):

Sl. No.	Name of the compound	Binding energy (kcal / mol)	Volume (Å)
1.	Alvespimycin (Standard inhibitor)	-2.110	394.4
2.	Bortezomib (Standard inhibitor)	-3.971	374.5
3.	Entinostat (Standard inhibitor)	-3.423	447.8
4.	Fenretinide (Standard inhibitor)	-2.154	412.5
5.	Gambogic acid (Standard inhibitor)	-2.178	331.2
6.	Gimatecan (Standard inhibitor)	-3.710	397.2
7.	Gossypol (Standard inhibitor)	-2.788	369.6
8.	Indibulin (Standard inhibitor)	-3.621	402.3
9.	Navitoclax (Standard inhibitor)	-2.180	336.3
10.	Obatoclax (Standard inhibitor)	-3.518	399.6
11.	Omipalisib (Standard inhibitor)	-4.879	396.3
12.	Romidepsin (Standard inhibitor)	-2.198	321.3
13.	Tamoxifen (Standard inhibitor)	-3.803	421.3
14.	Teniposide (Standard inhibitor)	-2.095	414.6
15.	Tosedostat (Standard inhibitor)	-3.871	407.8
16.	Triciribine (Standard inhibitor)	-3.978	417.3
17.	Interferon alpha 2b (Standard activator)	-1.792	320.0
18.	Interleukin 2 human (Standard activator)	-1.511	414.2

Table 4: Docking of various standard compounds to the active site of BCL2 (1G5M) receptor [In this table, the deep black colour row represents lowest maximum binding energy of ligands (for both standard compounds & test compounds) for BCL2 (1G5M) receptor]

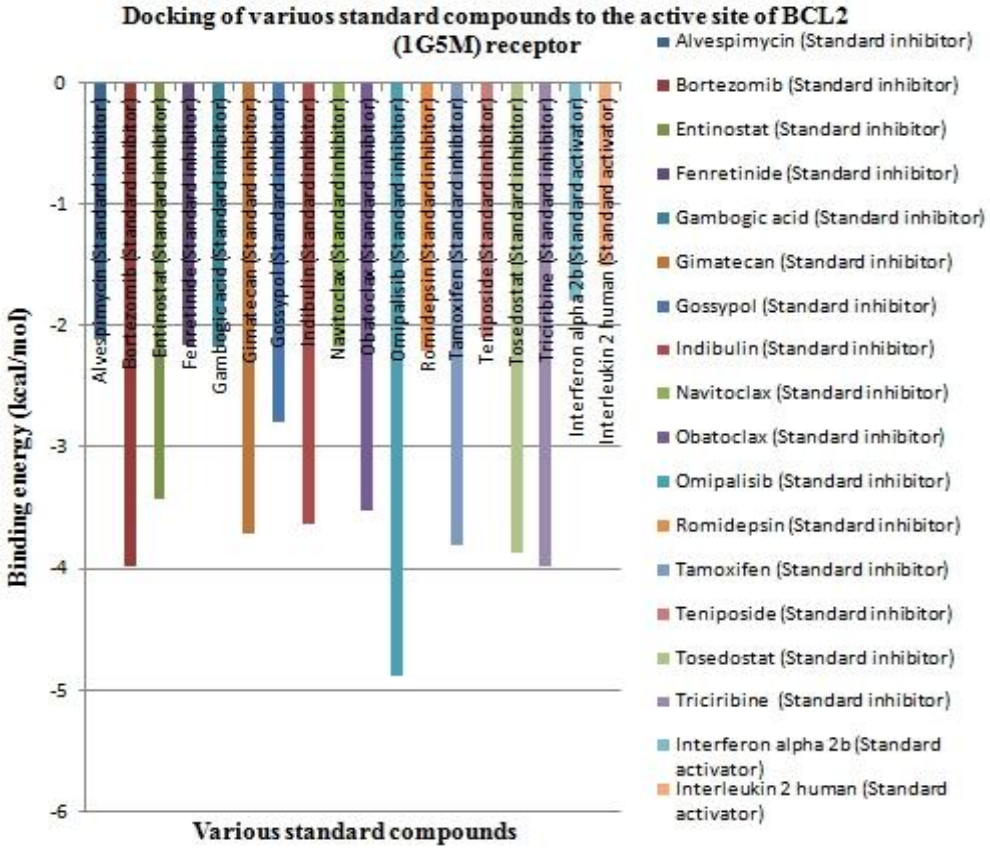

Figure 9: Graphical representation for docking of various standard compounds to the active site of BCL2 (1G5M) receptor [Omipalisib has lowest maximum binding energy for stable binding to the active site of BCL2 [1G5M] receptor]

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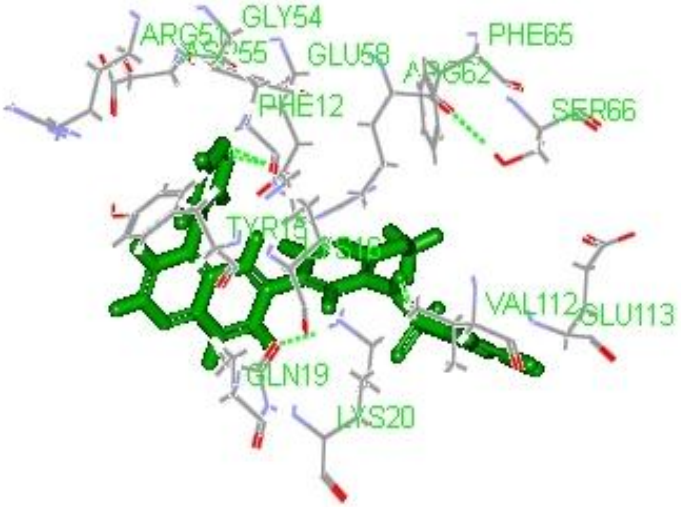

Figure 10: Docking of standard compound (Omipalisib has lowest maximum binding energy for stable binding to the active site of BCL2 [1G5M] receptor) to the active site of BCL2 (1G5M) receptor. [Total 3 Hydrogen-bonds: A: LYS16: HZ3 - OMIPALISIB: N10, A: LYS16: HZ3 - OMIPALISIB: N11, A: LYS16: HZ3 - OMIPALISIB: N12].

Docking of various test compounds (Cucurbitacin derivatives) to the active site of BCL2 (1G5M) receptor (Figure 11):

Sl. No.	Name of the compound	Binding energy (kcal / mol)	Volume (Å)
1.	Cucurbitacin A (Test inhibitor)	-3.738	428.0
2.	Cucurbitacin B (Test inhibitor)	-4.821	399.8
3.	Cucurbitacin C (Test inhibitor)	-4.615	407.4
4.	Cucurbitacin D (Test inhibitor)	-5.941	427.1
5.	Cucurbitacin E (Test inhibitor)	-4.897	411.5
6.	Cucurbitacin F (Test inhibitor)	-3.499	473.5
7.	Cucurbitacin H (Test inhibitor)	-3.311	416.3
8.	Cucurbitacin I (Test inhibitor)	-3.589	411.4
9.	Cucurbitacin J (Test inhibitor)	-3.991	378.3
10.	Cucurbitacin K (Test inhibitor)	-3.990	387.8
11.	Cucurbitacin L (Test inhibitor)	-3.851	473.2
12.	Cucurbitacin O (Test inhibitor)	-4.647	402.6
13.	Cucurbitacin P (Test inhibitor)	-3.225	456.2
14.	Cucurbitacin Q (Test inhibitor)	-3.627	383.2
15.	Cucurbitacin R (Test inhibitor)	-3.944	394.5
16.	Cucurbitacin S (Test inhibitor)	-3.600	385.0
17.	Dihydro Cucurbitacin B (Test inhibitor)	-3.678	403.6

Table 5: Docking of various test (Cucurbitacin) compounds to the active site of BCL2 (1G5M) receptor [In this table, the deep black colour row represents lowest maximum binding energy of ligands (for both standard compounds & test compounds) for BCL2 (1G5M) receptor]

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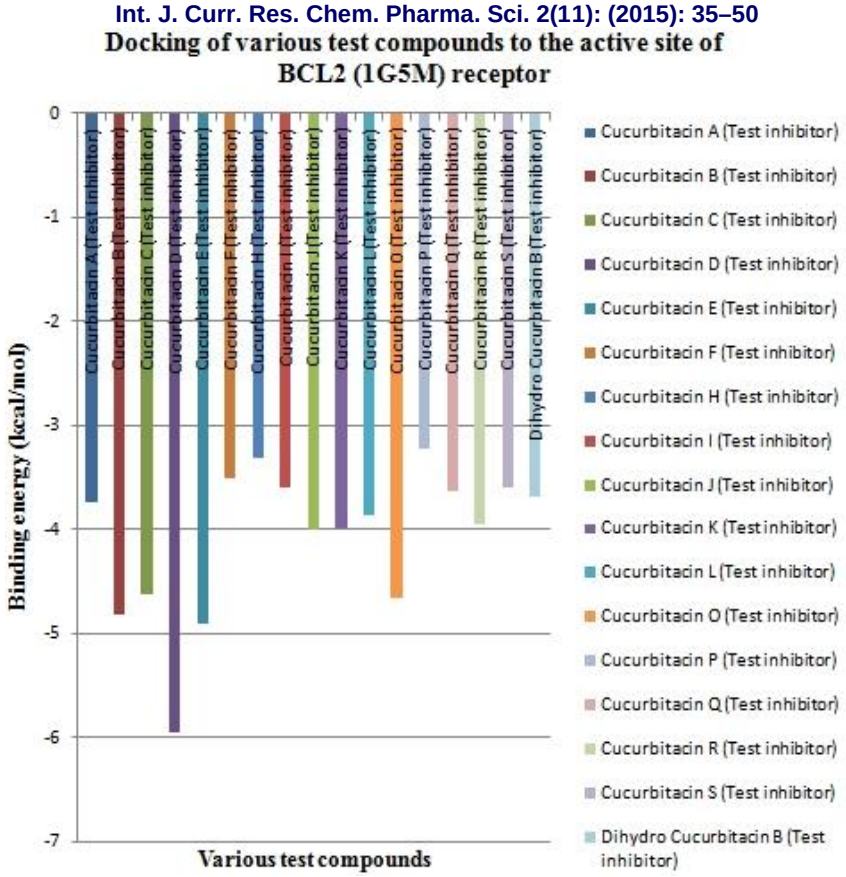

Figure 11: Graphical representation for docking of various test (Cucurbitacin) compounds to the active site of BCL2 (1G5M) receptor [Cucurbitacin D has lowest maximum binding energy for stable binding to the active site of BCL2 [1G5M] receptor]

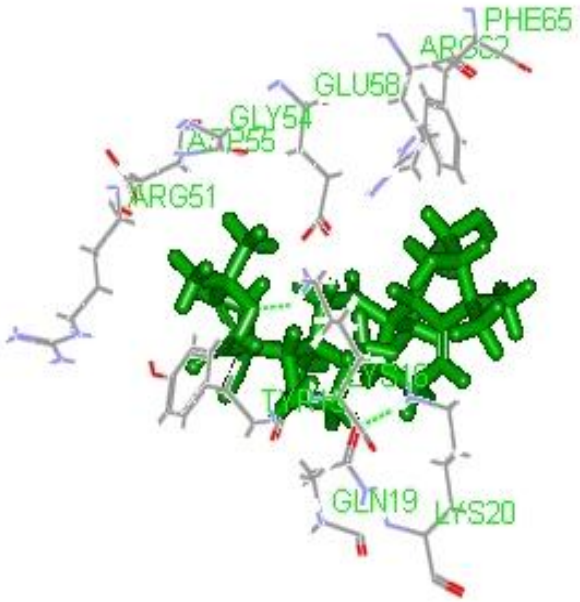

Figure 12: Docking of test compound (Cucurbitacin D has lowest maximum binding energy for stable binding to the active site of BCL2 [1G5M] receptor) to the active site of BCL2 (1G5M) receptor [Total 5 Hydrogen-bonds: A: LYS16: HZ1 - CUCURBITACIN D: O7, A: LYS16: HZ2 - CUCURBITACIN D: O5, A: LYS20: HZ2 - CUCURBITACIN D: OE1, A: ASP55: HN - CUCURBITACIN D: O6, A: LYS16: HZ1 - CUCURBITACIN D: OE2]

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Structure of downloaded CYCLIN D3 (3G33) receptor (protein) from official website of protein data bank (PDB):

Structure weight: 136482.00 kDa
Source organism: *Homo sapiens*
Type: Crystal structure

Receptor (protein) code of CYCLIN D3: 3G33
Classification: Cell cycle

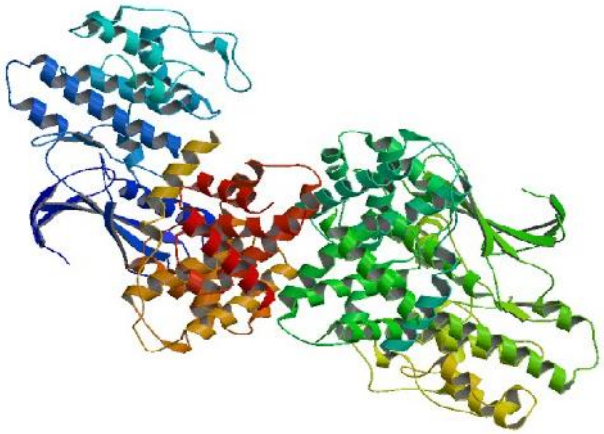

Figure 13: Downloaded structure of CYCLIN D3 (3G33) receptor (ribbon structure) from website of protein data bank (PDB)

Structure of prepared CYCLIN D3 (3G33) receptor (protein):

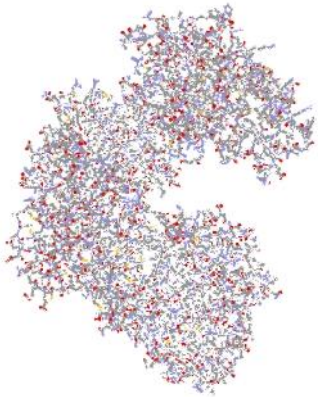

Figure 14: Structure of prepared receptor CYCLIN D3 (3G33)

Structure of active site of prepared CYCLIN D3 (3G33) receptor (protein):

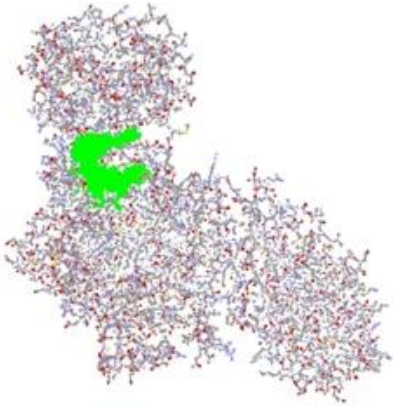

Figure 15: Structure of active site of prepared receptor CYCLIN D3 (3G33) [In this figure, the green portion represents active site for CYCLIN D3 (3G33) receptor]

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Coordinates (X, Y & Z) & volume or size of the active site of CYCLIN D3 (3G33) receptor (protein) for binding of each ligand:

Name of the receptor (protein)	Code	X	Y	Z	Volume or size of the active site (Å)
CYCLIN D3	3G33	14.917	-28.074	-54.974	610.214

Table 6: Coordinate & volume or size of active site of CYCLIN D3 (3G33) receptor (protein) [The volume or size of active site of CYCLIN D3 (3G33) receptor must be greater than the volume of the each ligand or compound]

Docking of various standard compounds to the active site of CYCLIN D3 (3G33) receptor (Figure 16):

Sl. No.	Name of the compound	Binding energy (kcal / mol)	Volume (Å)
1.	Abemaciclib (Standard inhibitor)	-3.687	381.4
2.	Amsilarotene (Standard inhibitor)	-3.652	401.2
3.	Dinaciclib (Standard inhibitor)	-3.022	343.5
4.	Docetaxel (Standard inhibitor)	-3.289	356.8
5.	Etalocib (Standard inhibitor)	-3.142	402.0
6.	Flavopiridol (Standard inhibitor)	-3.414	326.3
7.	Idronoxil (Standard inhibitor)	-2.265	385.2
8.	Indirubin (Standard inhibitor)	-3.097	463.9
9.	Indisulam (Standard inhibitor)	-3.731	418.2
10.	Olomoucine (Standard inhibitor)	-3.969	489.7
11.	Palbociclib (Standard inhibitor)	-3.725	436.6
12.	Purvalanol A (Standard inhibitor)	-3.705	463.2
13.	Purvalanol B (Standard inhibitor)	-3.398	456.3
14.	Roniciclib (Standard inhibitor)	-4.687	427.8
15.	Roscovitine (Standard inhibitor)	-4.794	489.3
16.	Voruciclib (Standard inhibitor)	-4.632	363.6
17.	L-lysine (Standard activator)	-1.275	130.5
18.	L-arginine (Standard activator)	-1.418	148.8
19.	L-proline (Standard activator)	-1.874	95.16
20.	L-threonine (Standard activator)	-1.056	98.97

Table 7: Docking of various standard compounds to the active site of CYCLIN D3 (3G33) receptor [In this table, the deep black colour row represents lowest maximum binding energy of ligands (for both standard compounds & test compounds) for CYCLIN D3 (3G33) receptor]

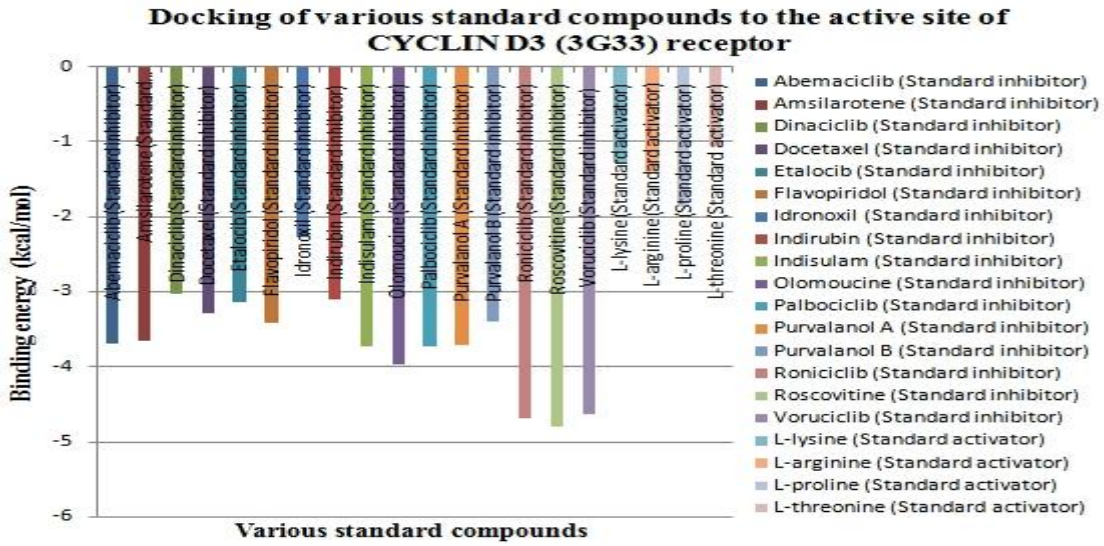

Figure 16: Graphical representation for docking of various standard compounds to the active site of CYCLIN D3 (3G33) receptor [Roscovitine has lowest maximum binding energy for stable binding to the active site of CYCLIN D3 (3G33) receptor]

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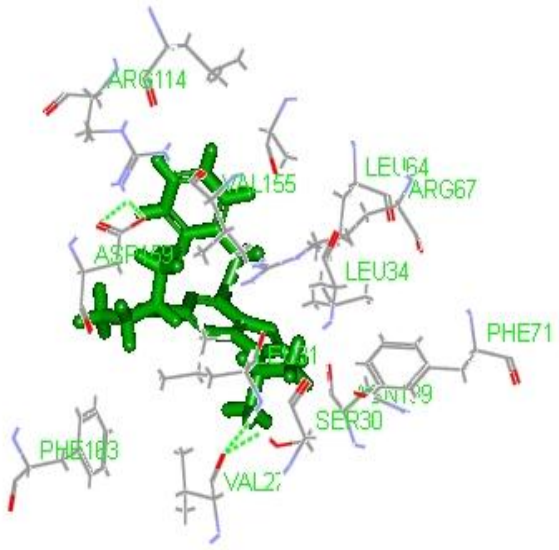

Figure 17: Docking of standard compound (Roscovitine has lowest maximum binding energy for stable binding to the active site of CYCLIN D3 [3G33] receptor) to the active site of CYCLIN D3 (3G33) receptor. [Total 3 Hydrogen-bonds: ROSCOVITINE: HH12 - A: ASP159: OD1, ROSCOVITINE: HH12 - A: VAL27: O, ROSCOVITINE: N1 - A: ASP159: OD2]

Docking of various test compounds (Cucurbitacin derivatives) to the active site of CYCLIN D3 (3G33) receptor (Figure 18):

Sl. No.	Name of the compound	Binding energy (kcal / mol)	Volume (Å)
1.	Cucurbitacin A (Test inhibitor)	-3.821	428.0
2.	Cucurbitacin B (Test inhibitor)	-3.283	399.8
3.	Cucurbitacin C (Test inhibitor)	-3.459	407.4
4.	Cucurbitacin D (Test inhibitor)	-5.955	427.1
5.	Cucurbitacin E (Test inhibitor)	-3.576	411.5
6.	Cucurbitacin F (Test inhibitor)	-3.845	473.5
7.	Cucurbitacin H (Test inhibitor)	-3.586	416.3
8.	Cucurbitacin I (Test inhibitor)	-2.862	411.4
9.	Cucurbitacin J (Test inhibitor)	-2.992	378.3
10.	Cucurbitacin K (Test inhibitor)	-2.417	387.8
11.	Cucurbitacin L (Test inhibitor)	-3.492	473.2
12.	Cucurbitacin O (Test inhibitor)	-3.710	402.6
13.	Cucurbitacin P (Test inhibitor)	-3.017	456.2
14.	Cucurbitacin Q (Test inhibitor)	-3.049	383.2
15.	Cucurbitacin R (Test inhibitor)	-3.119	394.5
16.	Cucurbitacin S (Test inhibitor)	-2.721	385.0
17.	Dihydro Cucurbitacin B (Test inhibitor)	-3.615	403.6

Table 8: Docking of various test (Cucurbitacin) compounds to the active site of CYCLIN D3 (3G33) receptor [In this table, the deep black colour row represents lowest maximum binding energy of ligands (for both standard compounds & test compounds) for CYCLIN D3 (3G33) receptor]

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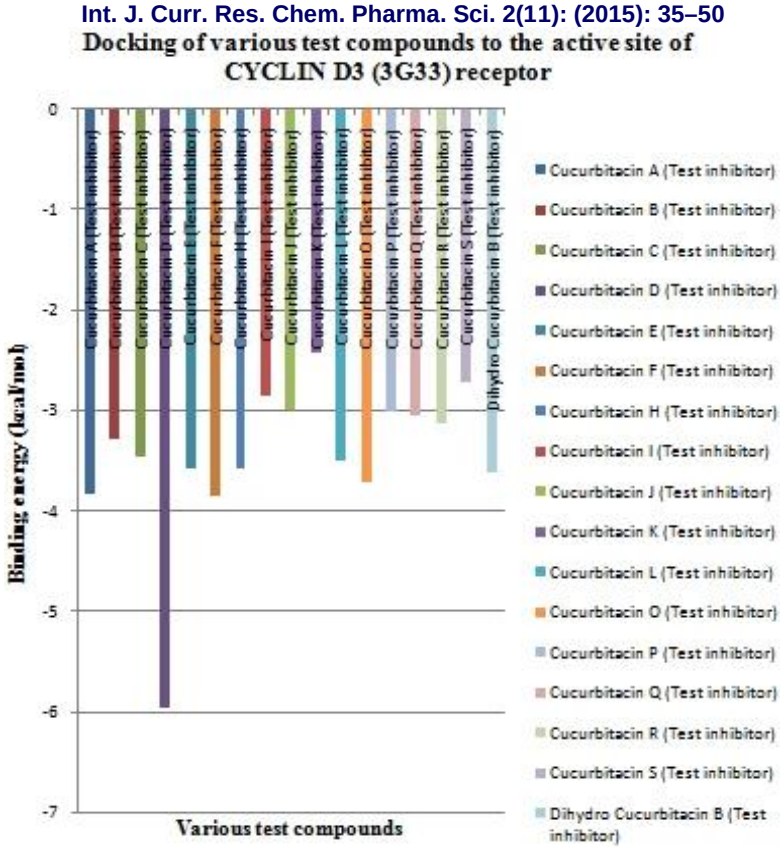

Figure 18: Graphical representation for docking of various test (Cucurbitacin) compounds to the active site of CYCLIN D3 (3G33) receptor [Cucurbitacin D has lowest maximum binding energy for stable binding to the active site of CYCLIN D3 [3G33] receptor]

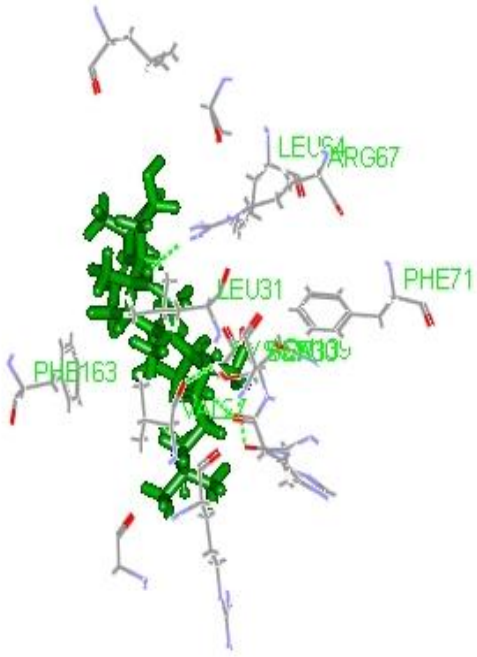

Figure 19: Docking of test compound (Cucurbitacin D has lowest maximum binding energy for stable binding to the active site of CYCLIN D3 [3G33] receptor) to the active site of CYCLIN D3 (3G33) receptor [Total 5 Hydrogen-bonds: CUCURBITACIN D: H60 - A: VAL27: O, A: ARG67: HH22 – CUCURBITACIN D: O2, A: CUCURBITACIN D: HN - A: HIS137: O, CUCURBITACIN D: H61 - A: ALA138: O, CUCURBITACIN D: H63 - A: ALA138: O]

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Prediction of ADMET (Absorption, Distribution, Metabolism, Excretion & Toxicity) properties:

A. Prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity) properties of best docked test molecule (Cucurbitacin D) against BCL2 & CYCLIN D3 receptor:

Name of compound	BBB (Blood brain barrier) level	Absorption level	Solubility level	Hepatotoxicity level	LogP (Must be less than 5)
Cucurbitacin D	Undefined	Good	Good	Non-toxic	1.933

Table 9: Prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity) properties of best docked test molecule (Cucurbitacin D) against BCL2 & CYCLIN D3 receptor

B. Prediction of FDA (Food & drug administration) male & female rat carcinogenicity of best docked test molecule (Cucurbitacin D) against BCL2 & CYCLIN D3 receptor:

Name of compound	Prediction of carcinogenicity for male rat	Prediction of carcinogenicity for female rat	Prediction of Mutagenicity
Cucurbitacin D	Non-carcinogen	Non-carcinogen	Non-mutagen

Table 10: Prediction of male & female rat carcinogenicity of best docked test molecule (Cucurbitacin D) against BCL2 & CYCLIN D3 receptor by FDA (Food & drug administration)

C. Prediction of NTP (National toxicology programme) male & female rat carcinogenicity of best docked test molecule (Cucurbitacin D) against BCL2 & CYCLIN D3 receptor:

Name of compound	Prediction of carcinogenicity for male rat	Prediction of carcinogenicity for female rat	Prediction of Mutagenicity
Cucurbitacin D	Non-carcinogen	Non-carcinogen	Non-mutagen

Table 11: Prediction of male & female rat carcinogenicity of best docked test molecule (Cucurbitacin D) against BCL2 & CYCLIN D3 receptor by NTP (National toxicology programme)

D. Prediction of all components for fulfilling Lipinski's rule of best docked test molecule (Cucurbitacin D) against BCL2 & CYCLIN D3 receptor:

Name of compound	Molecular weight [gms] (not greater than 500 daltons or 800 gms)	Partition coefficient [LogP] (not greater than 5)	Number of total hydrogen bond donors (not greater than 5)	Number of total hydrogen bond acceptors (not greater than 10)
Cucurbitacin D	540.45 gms	1.933	3	6

Table 12: Prediction of all components for fulfilling Lipinski's rule of best docked test molecule (Cucurbitacin D) against BCL2 & CYCLIN D3 receptor

Conclusion

Cucurbitacin D (test compound) had lowest maximum binding energy (-5.941) [Table 5] with total 5 hydrogen-bonds than best docked standard compounds Omipalisib [Table 4] {binding energy (-4.879) with total 3 hydrogen-bonds} for stable binding of Cucurbitacin D to the active site of BCL2 receptor & was highly active against BCL2 receptor than various standard compounds. Cucurbitacin D (test compound) also had lowest maximum binding energy (-5.955) [Table 8] with total 5 hydrogen-bonds than best docked standard compounds Roscovitine [Table 7] {binding energy (-4.794) with total 3 hydrogen-bonds} for stable binding of Cucurbitacin D to the active site of CYCLIN D3 receptor & was highly active against CYCLIN D3 receptor than various standard compounds. Cucurbitacin D against BCL2 & CYCLIN D3 receptor was non-toxic (Table 9), non-carcinogenic (Table 10 & Table 11) for both male & female rat, non-mutagenic (Table 10 & Table 11) after prediction of ADMET (absorption, distribution, metabolism, excretion & toxicity) properties & was fulfilled all components of Lipinski's rule (Table 12).

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