In Silico Molecular Docking Analysis of Plant Compounds Against VISTA Protein: A Novel Target in Cancer

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Abstract

V-domain immunoglobulin suppressor of T-cell activation (VISTA) is a member of B7 protein family and an important immune checkpoint that negatively regulates the T cell mediated inhibition of tumors. VISTA protein is upregulated in melanoma, pancreatic cancer, prostate cancer, colorectal cancer, ovarian cancer, Glioma and fibrosarcoma and various other cancers. Hence identifying novel drugs to target VISTA protein is utmost important in the treatment of cancer. This study involves the docking of plant phytochemicals against VISTA protein (PDB ID 6OIL) to analyze the interactions and orientation of protein ligand complex. Ligand structures obtained from Pubchem were screened for their drug likeness through Lipinski rule of five and those that cleared the Lipinski rule were chosen for further docking studies. All the docked compounds showed good docking results with Patch Dock score ranging from 2368 to 4496. Among them capsaicin showed a highest score of 4496 followed by curcumin with 4300. This shows that these plant phytochemicals could be used as potential drug molecules to treat cancer by targeting VISTA protein.

Keywords: VISTA protein, Lipinski rule, Capsaicin, Curcumin, Docking studies.

1. Introduction

VISTA (V-domain immunoglobulin suppressor of T cell activation) is a well-established immune regulatory receptor [1,2,3]. It is a member of B7 protein family and an important immune checkpoint that negatively regulates the T-cell mediated inhibition of tumors [4,5]. VISTA functions like a homeostatic regulator that actively normalizes immune responses. Thus, the regulatory role of VISTA in anti-cancer immunity remains to be fully elucidated, VISTA is primarily expressed in white blood cells and its transcription is partially controlled by p53

[6,7,8]. There is evidence that VISTA can act as both a ligand and a receptor on T cells to inhibit T cell effector function and maintain peripheral tolerance [9,10].

Chemoprevention is the use of natural, synthetic or biological agents to prevent, suppress or to reverse the initial phase of carcinogenesis or to prevent the invading potential of premalignant cells [11]. The interest in the area of chemoprevention has largely increased with growing understanding of the biology of cancer, identification of molecular targets, and success in breast, prostate, and colon cancer prevention. At the molecular level, cancer chemoprevention has been distinguished by alteration of multiple pathways, which play a critical role in the three basic steps of carcinogenesis, that is, initiation, promotion, and progression [12,13].

Phytochemicals are bioactive nutrient plant chemicals that are present in fruits, vegetables, grains, and other plant foods that may provide desirable health benefits beyond basic nutrition to reduce the risk of major chronic diseases [14,15,16]. Phytochemicals are the basis for many anticancer drugs currently in clinical use, as well as a potential source of future cancer treatments. Some phytochemicals have been found to modify the expression of checkpoint inhibitors of the immune response, as well as kill cancer cells [17]. Among the phytochemicals, the ones which can potentially provide health benefits are polyphenols, flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, and fibers [18,19].

2. Methods

2.1 Macromolecules structure retrival:

The structure of VISTA protein was retrieved from protein data bank (PDB). PDB is the database that contains the data of experimental structures of proteins and nucleic acid. The human **VISTA protein** is 279 amino acids in length, comprising a 162-amino-acid extracellular domain, a 21-amino-acid transmembrane domain, and a 96-amino-acid cytoplasmic domain. Other chains and molecules were removed using pubchem. pubchem is a useful open-source software tools to perform molecular graphics [21].

2.2 Ligand structure retrieval:

45 plant-based molecules: capsaicin, curcumin, licochalcone, gingerol,campothecins , alpinumisoflavone , nimbolide , dicumarol , balcelein , anthocynins , ellagic acid etc with potential pharmaceutical and medicinal benefits were chosen for ligand protein docking study . The docking study was performed between the vista protein and the phytochemicals. The structure of ligands and the protein were retrieved from pubchem database. Pubchem contains information about chemical compounds their structure, molecular formula, molecular weight etc . The structures were retrieved in SDF format and were changed to PDB format using online smiles translator. (https://cactus.nci.nih.gov/translate/) [22].

2.3 Drug scan:

All the ligands were tested for their drug potential based on lipsnski's rule of five. Molinspiration (https://www.molinspiration.com/) was used for calculating lipinski's properties. Lipinski's rule mainly determines the molecular attributes, such as molecular weight, logP, number of hydrogen bond acceptors, and number of hydrogen bond donars. Any ligand showing violations in Lipinski's rule were eliminated from further studies [23,24].

2.4 Docking and visualizaton:

Pathdock docking server was used for docking of ligands to the proteins. PatchDock is an algorithm for molecular docking. The input is two molecules of any type: proteins, DNA, peptides, drugs [25,26]. The output is a list of potential complexes sorted by shape complementarity criteria. The 3D visualization of docked structures was performed using graphical user interface, discovery studio ligand designing are few facilities available in discovery studio [27].

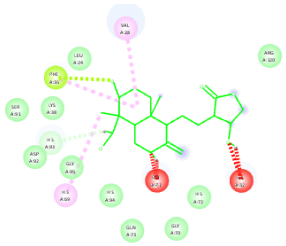
3. Results and discussion:

Many plant-based compounds are used for cancer treatments. In this study 38 plant compounds with pharmaceutical potential were chosen. For *in silico* analysis of phytochemicals the drug potential of all ligands was tested using Lipinski's rule of five with the help of molinspiration server for analysis of molecular parameters. The results include permeability of drug (Log P value), no. of hydrogen bond donors and acceptors. Out of 38 phytochemicals, 33 compounds cleared Lipinski's rule of five. Taraxerol, Betulin, Lentinan, Ginsenosides, Rosoliside showed 1,1,3,1,1 violation respectively hence they were eliminated.

3.1 Docking outcome of protein- ligand:

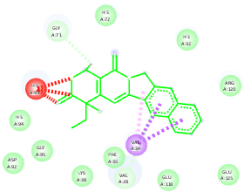
1. Capsaicin showed the highest docking score of 4496. It showed anti invasive and anti migratory activity through modulating signalling pathways involved in cell invasion and migration. Capsaicin treatment decreased the metastatic burden in transgenic adenocarcinoma of mouse prostrate (TRAMP) mice.
2. Curcumin showed the score of 4300. It has the ability to target multiple cancer cell lines. In clara cells of transgenic mice lungs, curcumin suppressed hvEGF-A_{165} overexpression to normal. It up regulates expression of E-Cadherin preventing metastasis.
3. Licochalcone showed the score of 4272. LCA suppresses the oxidation of cells and markedly inhibits proliferation of cancer cells *in vitro*.
4. Gingerol showed the docking score of 4084. It can modulate signalling pathways like nuclear factor (NF-kB) signal transducer and activator of STAT 3, proinflammatory mediators (TNFα-COX-2). Both *in vitro* and *in vivo* studies supportrole of gingerol in cancer.
5. Campothecins showed docking score of 4126. It is used as a chemotherapy drug which interferes with the activity of topoisomerase enzymes like ironotecan.
6. Alpinumisoflavone showed docking score of 3964. It is a prenylated flavanoid which has high lipophilicity and also has anti neoplastic activities *in vivo*.
7. Andrographolide showed a score of 3876. It inhibited proliferation of different tumor cell lines by cell cycle arrest at G_0/G_1 phase *in vitro*.

8. Kaempferol showed the docking score of 3514. Mechanistically, it can induce anticancer effects mainly through downregulation of the expressions of proteins involved in the cancer progression and formation alongside apoptosis induction, cell cycle arrest, and decreasing the expression for anti-inflammatory proteins.
9. Piperine showed the docking score of 3836. Deregulation of cell cycle and/or its arrest is often responsible of severe pathologies, including cancer. piperine shows the ability to control the relevant checkpoints of cell cycle in tumor, contrasting with its progression.
10. Rosmarinic acid showed the docking score of 3660.Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid commonly found in plants belonging to the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae family. It is a naturally occurring phenolic compound. The compound has been reported to have a number of interesting biological activities, e.g., antiviral, antibacterial, anticancer, anti-inflammatory and antioxidant activities.

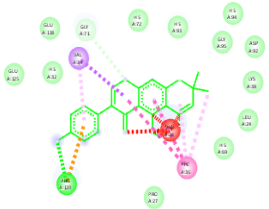


- Andrographolide

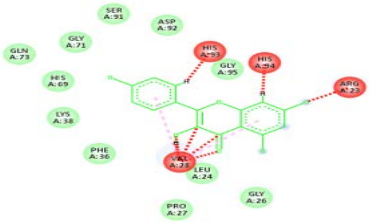
- Licochalcone



- Campothecins

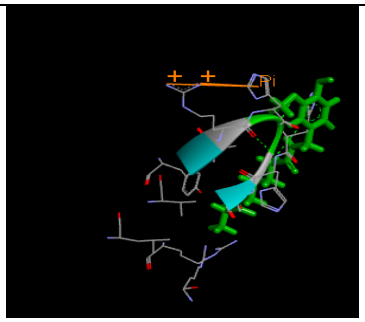
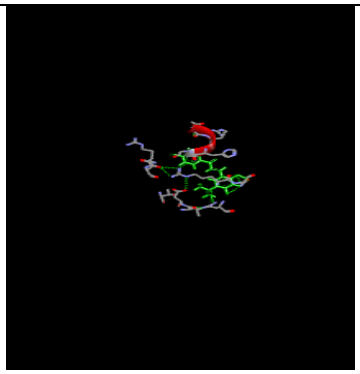
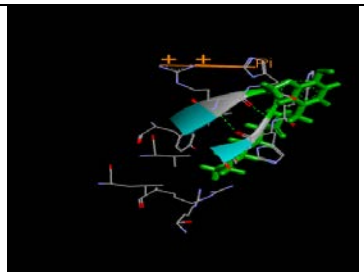


- Alpinumisoflavone



- Kaempferol

Figure-1 2-Dimensional interaction of protein and ligand

S.NO	CODE	COMPOUND NAME	SCORE	PROTEIN- LIGAND INTERACTION	AMINO ACID POSITIONS
1	C	CAPSAICIN	4496		ARG120,HIS121,HIS122,HIS123,VAL117,SER124,ARG127,ARG54,ILE119,TYR37,LEU115
2	C	CURCUMIN	4300		LEU106,ARG102, ASN103,GLY86,LEU107,GLN83,THR105,ASP108,ARG58,ARG84,HIS85,ALA82
3	L	LICOCHALCONE	4272		GLN83, HIS85, ARG84, GLY86, ASN103, ARF102, LEU107, THR105, ASP108, ARG42, ARG58, GLY59
4	C	CAMPOTHECINS	4126		GLY71, HIS72, HIS32, ARG120, GLY125, GLU118, VAL28, PHE36, VAL34, GLY95, ASP92, HIS94, HIS93
5	G	GINGEROL	4084		HIS121,ARG120,ARG127,ARG54,LEU115,HIS123,ILE119,HIS122,SER124,VAL117,TRY37

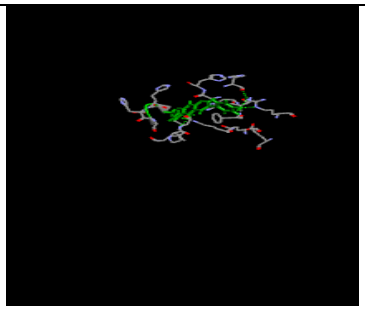
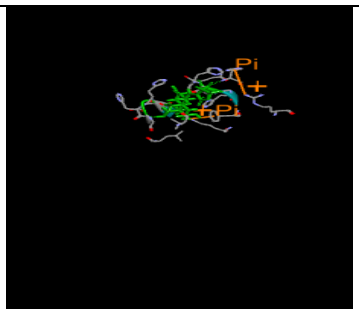
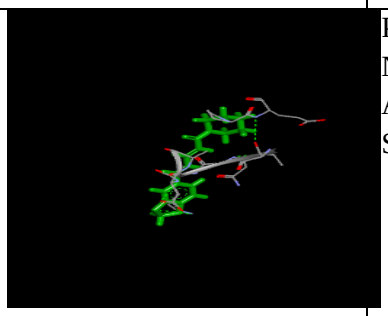
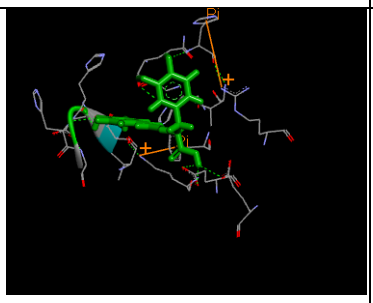
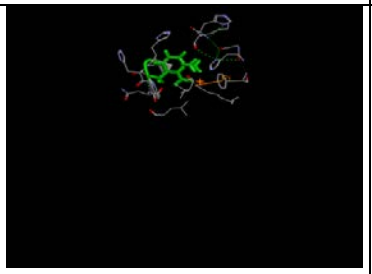
6	A	AIPINUMISOFLAVONE	3964		GLU118, GLY71, VAL34, HIS32, GLU125, HIS72, HIS93, HIS94, GLY95, ASP92, LYS38, LEU24, HIS69, PRO27, ARG120
7	A	ANDROGRAPHOLIDE	3876		PHE36, LEU24, VAL28, ARG120, HIS32, HIS72, GLY71, HIS69, GLY95, ASP92, HIS93, SER91, AYS38, PHE36, LEU24
8	P	PIPERINE	3836		PRO13,GLU14,GLN137,THR138,ALA142,GLY139,LYS140,ASP141
9	R	ROSMARINIC ACID	3660		GLU125,GLU118, VAL28,HIS94,GLY95,HIS93,HIS32, ARG120,VAL34,PHE36,GLY71,HIS69,LYS38,ASP92,SER91,GLN73
10	K	KAEMPFEROL	3514		SER91,ASP92,GLY71,GLN73,HIS93,HIS69,GLY95,HIS94,ARG23,LYS38, PHE36,VAL28,LEU24,PRO27,GLY26

Table 1. Amino acid positions and the protein – ligand reaction

3.2 LIPINSKI'S RULE OF FIVE

In modern drug discovery, the potential of a new compound is often investigated initially without making it or testing it [28,29]. A medicinal drug must have a suitable balance of solubility in water and in non-polar solvents. It should be sufficiently soluble in: water so that they can be carried around the body in the bloodstream, non-polar solvents so that it may pass through cell membranes (which consist of a phospholipid bilayer) into cells.The rule describes molecular properties important for a drug's pharmacokinetics into the human body, including their absorption, distribution, metabolism, and excretion ("**ADME**") [30].

S.NO	**COMPOUND**	**MOLECULAR WEIGHT**	**LOG P (5)**	**H BOND DONARS (5)**	**H BOND ACCEPTORS (<10)**	**NO. OF VIOLATIONS**
1	CAPSAICIN	305.42	3.10	2	4	0
2	CURCUMIN	368.38	2.30	2	6	0
3	LICOCHALCONE	338.40	4.48	2	4	0
4	CAMPOTHECINS	348.36	2.03	1	6	0
5	GINGEROL	294.39	3.22	2	4	0
6	AIPINUMISOFLAVONE	336.34	3.95	2	5	0
7	ANDROGRAPHOLIDE	350.45	1.05	3	5	0
8	PIPERINE	285.34	3.33	0	4	0
9	ROSMARINIC ACID	360.32	1.63	5	8	0
10	KAEMPFEROL	286.24	2.17	4	6	0

Table 2. Lipinski's properties of phytochemicals analysed using molinspiration

4. Conclusion:

The global cancer burden has increased to 19.3 million and accounts for 9.6 million deaths every year. Mutations in RAS genes leads to tumor development and are found in more than 30% of human cancers. In complex tumor microenvironment, tumor specific T cells have

dysfunction states due to presence of various inhibitory receptors. There are PD 1, CTLA 4, PDL 1 blockade pathways which are effective in tumor regression. VISTA in tumor cells suppressed T cell proliferation and cytokine production and decreased tumor infiltrating CD8+ T cells. Over expression of VISTA in tumor cells interferes with protective antitumor immunity *in vivo* in mice.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.
Conflicts of Interest: The authors declare no conflict of interest.

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