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Research Article

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Computational screening of the active compounds isolated from the macro algae *Valaniopsis Pachynema*, against AKT1, a cancer drug target protein

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ABSTRACT

Drug target identification and metabolic pathway reconstruction are the significant components in the present cancer research. Kinase protein target is one of the niche domains in the cancer studies because the cascade of protein plays a wide role in cell progression. In this study, an attempt has been made on protein kinase B, also known as AKT or AKT1 in human, coded by AKT1 gene with different synonyms. The compounds such as Hydnocarpic and Pristane isolated from Valoniopsis pachynema, a green algae, are targeted against AKT1.Theoretical results were found for the synonym Hydnocarpic acid and Drug target protein(AKT1) which showed three interactions with estimated free energy of binding of -5.50 kcal/mol. On the other hand another synonym Pristane with Drug target protein(AKT1), had eight interactions with an estimated free energy of binding of -6.40 kcal/mol. Since for both the compounds the free energy binding was found to be negative, these compounds could be well expected to serve as effective drug candidates and may be used for the treatment of cancer after carrying out the required clinical trials.

Keywords: Kinase, AKT1, Hydnocarpic acid, Pristane, cancer.

INTRODUCTION

Cancer is the generic term for a group of diseases that can affect any part of the body. All cancers begin when one or more genes in a cell are mutated (changed), creating an abnormal protein or no protein at all. The information provided by an abnormal protein is different from that of a normal protein, which can cause cells to multiply uncontrollably and become cancerous.

Cancer continues to be a big threat to our society, in spite of the advancements achieved both in diagnosis and treatment. This is the second most common disease after cardiovascular disorders for maximum deaths in the world (Jemal et al, 2007). Worldwide, there are over 10 million new cases and more than 6 million deaths of cancer every year(Tomatis *et al*., 1990). In India its prevalence is estimated to be around 2.5 million, with about 8, 00,000 new cases and 5, 50,000 deaths per annum (Nandakumar, 1990-96). India has the highest number of oral cancers in the world with 75,000 to 80,000 new cases every year. Still more alarming is the prediction that the Indian figures are likely to double in 20 years, according to the International Agency for Research on Cancer (IARC), based in France.

Targeted therapy for Cancer:
Researchers have learned more about the gene changes in cells that lead to cancer and have developed drugs that could target these changes. Treatment with these drugs is often called targeted therapy. This blocks the proliferation

of cancer cells by interfering with specific molecules required for tumor development and growth. This newer type of treatment usually causes little damage to normal cells and has become a major focus of current cancer research.

Protein kinases as a primary drug target:
Protein kinases have been identified as important cellular regulatory proteins in many diseases. They are enzymes that covalently transfer the gamma phosphate group of ATP to specific tyrosine, serine, or threonine residues in proteins, thereby changing the activity of key signaling proteins or expediting the formation of multi-enzyme complexes. Kinases directly or indirectly control most cellular processes including metabolism, transcription, cell cycle progression,cytoskeletal rearrangement, cell movement, apoptosis, and differentiation(Blume-Jensen.,*et al*.,2001 ; Hanahan, *et al*,,2000).

It has been estimated that there are around 500 protein kinases encoded within the human genome representing approximately 1.7% of all human genes (Manning,*et al*.,2002). A majority of the more than 30 known tumor suppressor genes and more than 100 dominant oncogenes are protein kinases (Futreal *et al.*,2001). Thus protein kinases offer an abundant source of potential drug targets to be intervened in cancer treatment.

Among the signalling proteins that respond to a large variety of signals, protein kinase B (PKB, also known as Akt) appears to be a central player in regulation of metabolism, cell survival, motility, transcription and cell-cycle progression. Conserved from primitive metazoans to humans, PKB belongs to the AGC subfamily of the protein kinase super family, which consists of 518 members in humans (Manning *et al*., 2002).

Recent evidences indicate that PKB/AKT is frequently constitutively active in many types of human cancer. Constitutive PKB/AKT activation can occur due to amplification of PKB/Akt genes or as a result of mutations in components of the signaling pathway that activates PKB/AKT. Although the mechanisms have not yet been fully characterised, constitutive PKB/AKT signaling is believed to promote proliferation and increased cell survival and thereby contributing to cancer progression.

Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases that play central role in regulation of cell cycle, apoptosis, DNA repair, senescence, angiogenesis, cellular metabolism, and motility (Cantley,2002). PI3Ks transmit signals from the cell surface to the cytoplasm by generating second messengers – phosphorylated phosphatidylinositols – which in turn activate multiple effectors kinase pathways, and ultimately result in survival and growth of normal cells (Osaki,2003; Akinleye,2013). It has been recognized that deregulation of the PI3K signaling pathway is associated with development in one-third of human cancers (Arteaga, 2010).

Aberrantly activated PI3K pathway promotes carcinogenesis and tumor angiogenesis (Osaki, 2003; Patel,2013). In addition, dysregulated PI3K pathway signaling has been implicated in conferring resistance to conventional therapies and cytotoxics in breast cancer, glioblastoma, and non-small cell lung cancer (Miller, 2011; Burris, 2013). PI3K has become recognized as a viable target for novel anti-cancer therapy.

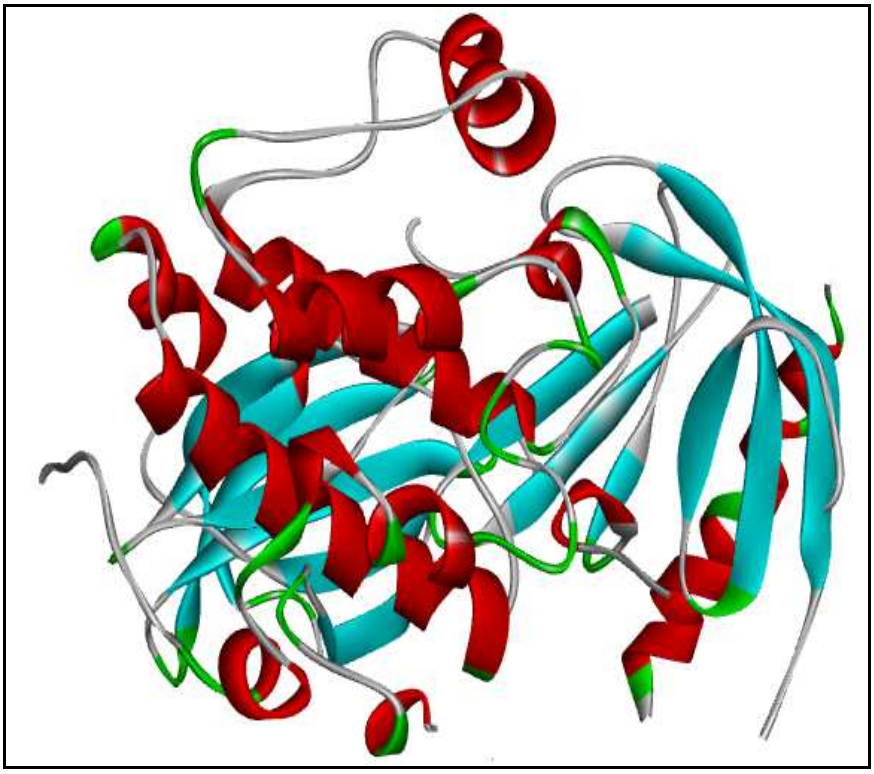
PI3K/AKT/mTOR Pathway:
Aberrations in various cellular signaling pathways are instrumental in regulating cellular metabolism, tumor development, growth, proliferation, metastasis and cytoskeletal reorganization. The fundamental cellular signaling cascade involved in these processes, the phosphatidylinositol 3-kinase/protein kinase-B/mammalian target of rapamycin (PI3K/AKT/mTOR), is a crucial and intensively explored intracellular signaling pathway in tumor genesis. Various activating mutations in oncogenes together with the inactivation of tumor suppressor genes are found in diverse malignancies across almost all members of the pathway. Substantial progress in uncovering PI3K/AKT/mTOR alterations and their roles in tumorigenesis have enabled the development of novel targeted molecules

In Silico studies on natural compounds such as triphala constituents gallic acid was targeted for casein kinase II enzyme (Divya et al.,2013). Similar studies have reported that epicatechin and similar pharmacophore can also be served as drug candidates for treating the protein kinase C-alpha in colon cancer, The natural compound pristane resembles like synthetic ethylene glycol compounds and can also used for targeting the important cancer drug target protein nuclear Factor κB with potential for developing efficacious anticancer treatment. (Dhivya, 2012. Srinivasan, 2013)

This study is mainly aimed on the efficiency of the compounds Hydnocarpic and Pristane which are readily isolated from *Valoniopsis pachynema*, a marine algae, in targeting against the kinase. *Valoniopsis pachynema* is filamentous Astro-turf green algae (Chlorophyceae) forming stiff, spongy mats of tangled filaments grows throughout the year and is very often seen washed ashore in Palk Bay and Gulf of Mannar regions of South India.

The secondary structure of the protein is shown in the figure (1).

Figure 1: Secondary structure of AKT1



EXPERIMENTAL SECTION

Ligand preparation:
The ligands were initially drawn using chemsketch tool and the chemical language format is converted in to protein data bank format for the docking using the online smiles translator tool available in website of http://cactus.nci.nih.gov/translate/. Each ligand was prepared individually for the docking optioning with detect root, root expansion and torsions respectively. Finally the ligands were saved individually as per auto dock format.

Drug target identification and retrieval :
The drug target protein human akt1 bound with binding allosteric inhibitor of id 3O96 and its X-ray crystallographic structure with 2.70Å resolution with UniProtKB unique identification number is P31749 with amino acid sequence of 367 lengths was retrieved from PDB database. (http://www.rcsb.org/pdb/home/home.do).

Protein preparation:
Water and ligand that were bound to the protein (AKT1) were removed. Initially the hydrogen's were added along with kollmann charges and the respective drug target protein was saved in current mode of protein data bank.

Auto Grid calculation:
Precalculating of grid map interaction for various atoms such as oxygen, nitrogen, aromatic carbon, aliphatic carbons present in the biomolecules were calculated using the auto grid. Sum of total ligand interaction energy for a

ligand with a macromolecule was used as an input source for auto dock calculation during the time of docking. The figure (2) shows the grid before and after calculation.

Molecular docking

The liagnds such as Hydnocarpic acid and pristane were docked with drug target protein AKT1 and interaction between ligand and protein active site was theoretically analyzed using the computational tools and software's. In this study Molecular, docking was performed using the structure based drug-designing concept in autodock 4.01v.

Figure 2: A: Before grid calculation, B: After grid calculation, C&D: Grid in 3Dimesional

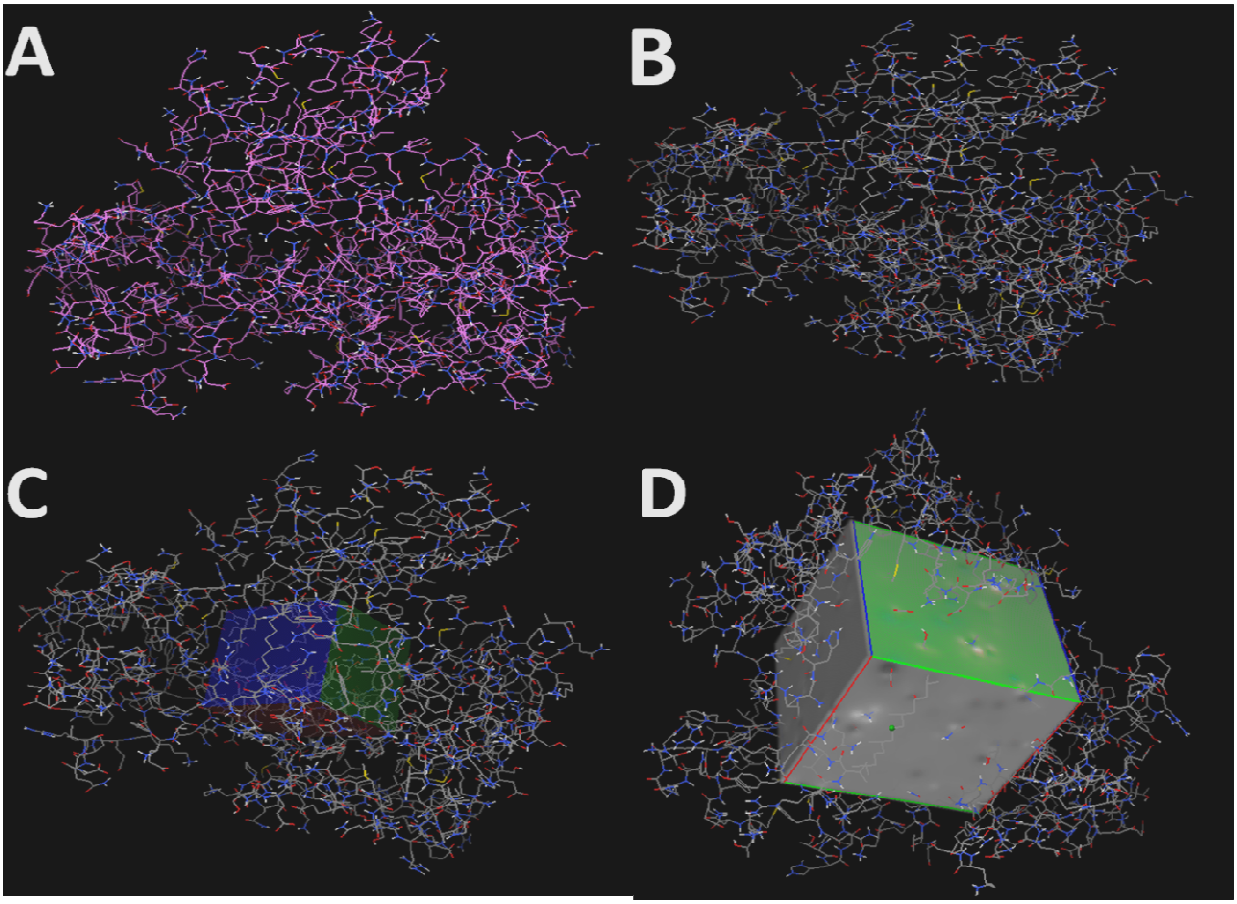
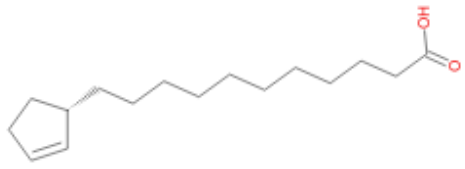
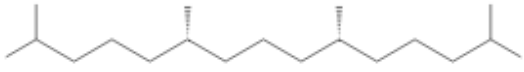


Table 1: Molecular properties of ligand

Structure	No Of Atoms	Molecular Composition	Molecular Formula	Molecular weight
 **Hydnocarpic acid**	46	C16 H28 O2	C: 0.761, H: 0.112, O: 0.127	252.4
 **Pristane**	59	C19 H40	C: 0.850, H: 0.150	268.529

RESULTS AND DISCUSSION

Molecular property calculation:

The general molecular properties of the ligand were calculated, the chemical composition of the compounds plays an important role in pharmacophore prediction, the 2D property will state about the nature of the compounds before proceeding in to docking. The table (1) shows the general molecular properties of the ligands.

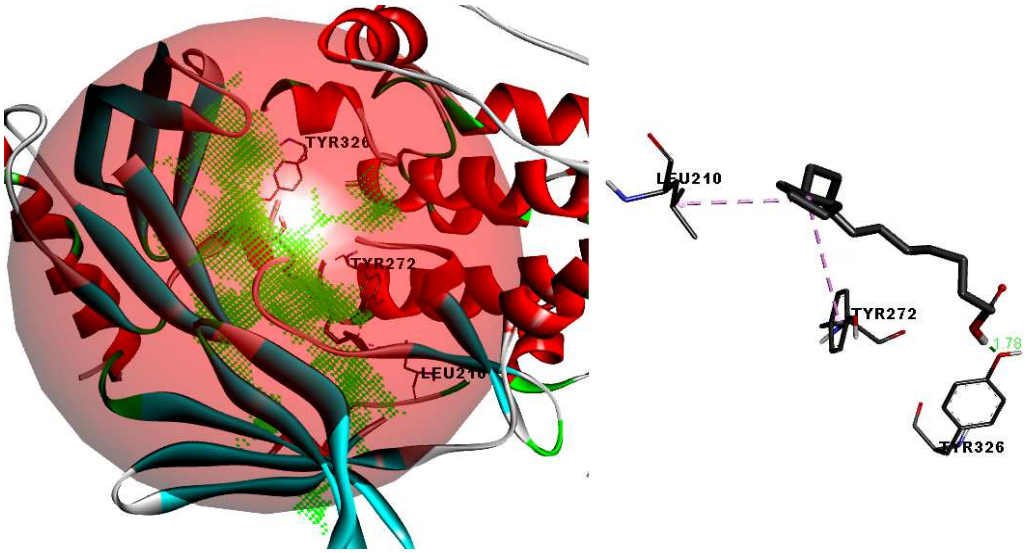
Receptor ligand interaction

Binding affinity between the most ligand with active site amino acid of protein generally determines the efficacy of the ligand. High affinity results good intermolecular force of attraction between the ligand and receptor, where as low-affinity ligand binding involves less intermolecular force between the ligand and its receptor. The crucial amino acids in active site were defined as rigid residues in auto dock 4.01v, with grid points of 40 (X), 40 (Y), 40 (Z) in 3D direction respectively with 0.375Å grid spacing for receptor types A C HD N OA SA respectively along with grid centre of 6.29 (X), -7.943(Y), 17.261(Z). The genetic algorithm calculates for 150-population size with 2500000 maximum evaluations for 27000 generations for 10 runs. After docking the results were visualized using the discover studio visualizer 4v. The table (2) shows the free energy binding of ligand with drug target protein AKT1,along with inhibition constant Ki values.

Table 2: Free energy binding of ligand with drug target protein AKT1

Energy parameters	Hydnocarpic acid	Pristane
Estimated Free Energy of Binding	-5.50 kcal/mol	-6.04 kcal/mol
Estimated Inhibition Constant, Ki	92.35 uM	37.51 uM
Final Intermolecular Energy	-9.08 kcal/mol	-9.62 kcal/mol
Electrostatic Energy	-8.52 kcal/mol	-9.61 kcal/mol
vdW + Hbond + desolv Energy	-0.56 kcal/mol	-0.00 kcal/mol
Final Total Internal Energy	-0.67 kcal/mol	-1.53 kcal/mol
Torsional Free Energy	+3.58 kcal/mol	+3.58 kcal/mol
Unbound System's Energy	-0.67 kcal/mol	-1.53 kcal/mol

Figure 3: Hydnocarpic acid binding with active site residues of AKT1 receptor



Binding interaction between ligand and active site amino acid was shown in the figure(3) and table (3) for Hydnocarpic acid. Similarly, the Binding interaction between pristine and active site amino acid was stated in figure (4) and table (3) respectively. Over all potency of the drug not only depends on the binding affinity but also with the ligand efficacy to produce the biological responses. This responses may me antagonist or agonist that depends on the physiological responses [Kenakin,2006). In the present study ,through computational biology studies attempts have

been made to find a better alternative to allosteric inhibitor from natural sources to minimize the side effects caused by synthetic compounds.

Figure 4: Pristane binding with active site residues of AKT1 receptor

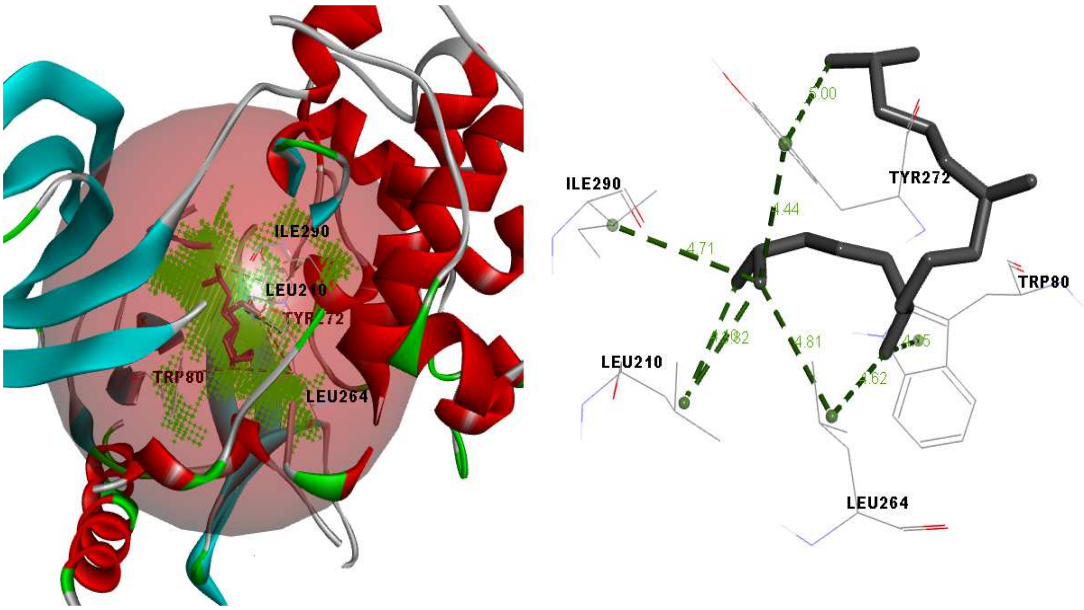


Table 3: Interaction analysis of ligand and receptor along with distance

Ligands	Receptor active site Amino acid Binding	Distance in Å
Hydnocarpic acid	LEU210	4.85
	TYR 272	5.09
	TYR326	1.78
Pristane	LEU210	4.10
		3.82
	LEU264	4.81
		4.62
	LEU290	4.71
	TYR 272	4.44
		5.00
	TRP80	4.15

CONCLUSION

Dysregulation of the AKT pathway has been identified in multiple human cancers. Several clinical trials are in progress to test the efficacy of the AKT pathway inhibitors in treating cancer with Hydno carpic acid on Drug target protein (AKT1). It shows three interactions with estimated free energy of binding of -5.50 kcal/mol. In the case of Pristane binding with Drug target protein(AKT1),It shows eight interactions with estimated free energy of binding of -6.40 kcal/mol but the active site of the drug target protein is rich in Leu and Tyr residues also. These isolated compounds shows more affinity to binding with Leu and Tyr amino acid residues. Estimated Inhibition Constant, Ki of Hydnocarpic acid is 92.35 µM (micromolar) at temperature 298.15 K and Pristane is 37.51 µM (micromolar) at temperature 298.15 K. It could be concluded that these compounds can serve as effective drug candidates and may be used for the treatment of cancer.

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