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In Silico Screening of Receptors Over Expressed in Breast Cancer for the Docking of Amygdalin

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Abstract—*The 3D chemical structure of amygdalin was docked against protein macromolecules on breast cancer cell surface in-silico to screen whether the drug might deliver some pharmacological activity in cancer or not. To serve the purpose, breast cancer surface receptors were selected so that the pharmacological function could be established by the binding drug to these receptors. In present study a new aspect of molecular interaction has been employed that is in silico docking of amygdalin against breast cancer cell surface receptors in Autodock vina. On the basis of calculated drug interaction scores, we propose that amygdalin might impart anticancer activity.*

1. INTRODUCTION

The chemical name of amygdalin 'D-mandelonitrile-β-D-gentiobioside' well represents the two molecular building blocks: gentiobiose and D-mandelonitrile [1]. The D-mandelonitrile unit contains nitrile functional group [2]. It occurs naturally as R-amygdalin which converts into S-amygdalin during extraction process from natural resources [3]. Prunus armeniaca, commonly known as Apricot, is a natural and well-known source of amygdalin. The plantations are distributed all over the world in countries like India, America, China, North Africa, Mediterranian regions, etc. [4].

Amygdalin performs a number of other pharmacological activities such as a reduction in inflammation, asthma symptoms, and pain [5]. It has also been found to be helpful in treating atherosclerosis, gastric ulcer, arthritis, and in healing wounds [6]. Over the years, researchers have debated the efficacy of amygdalin in cancer therapeutics. It's been reported that it increases apoptotic cell death by caspase-3 activation as a result of down-regulation of Bcl-2 and up-regulation of Bax in DU 145 and LN CaP prostate cancer cell lines [7]. It had also been observed to down-regulate genes which controlled cell cycle in SNU-C4 colon cancer cell lines [8]. It mimics the actions of peptide T which makes it useful in the treatment of psoriasis [9] [10]. Breast cancer is highly lethal to women in this modern age of medicine. It has been estimated that the numbers will cross 3 million by 2050 [11]. The catastrophe of this magnitude requires thorough endeavor from researchers [12] [13]. In the present study, the elucidated structure has been subjected to In Silico molecular docking. Binding energies have been calculated for amygdalin against 3D structure of conventional breast cancer surface receptors through Autodock vina for the first time. On the basis of these calculated drug interaction scores, we propose that amygdalin might have the potential to impart anticancer activity.

2. LIGAND AND MACROMOLECULE FOR MOLECULAR DOCKING

Docking requires two entities: the ligand structure and macromolecule 3D model. The structure of isolated natural drug amygdalin as ligand was edited in Chemsketch. 3D structure of the ligand was prepared with UCSF Chimera using canonical smiles retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/ compound/656516). The 3D structures of receptors on breast cancer cell surface were retrieved from the archives of protein data bank RCSB PDB (https://www.rcsb.org/). The databank holds approximately 151955 X-ray diffraction crystal structures of biological entities. Each macromolecule is tagged with a specific PDB ID on submission. The surface receptors chosen were Estrogen receptor alpha (ERα: 3ERT), Human epidermal growth factor receptor (HER2: 3RCD, FGFR1: 1AGW), and G protein-coupled receptor (GPER: 1F88). These receptors are responsible for physiological malfunctions leading to breast cancer development. Autodock vina had been used to perform docking of ligand to macromolecular binding sites [17]. Information on active site residues was identified from previously reported literature. Before docking, polar hydrogen and Gasteiger charges were assigned. The ligand torsion was also detected and chosen. Grid box parameters which clustered the binding site residues were set and saved with x, y, and z coordinates in grid file preparation. Docking parameters were initialized with the help of Genetic algorithms while Lamarckian genetic algorithm generated the docking parameter files with bound conformations [18]. The grid and docking algorithms were run through UCSF Chimera and after successful completion of 10 cycles provided with the RMSD table (root mean squared deviation of atomic coordinates) with docking scores [19]. After running the simulations the receptors least binding energies were compared for potential

binding receptors to the drug. The docking interactions were visualized also in UCSF chimera (http://www.rbvi.ucsf.edu/chimera).

3. ANALYSIS OF INTERACTION BETWEEN DOCKED LIGAND RECEPTOR

The pathophysiology of breast cancer depends upon the type of receptor that is malfunctioning out of the following; estrogen receptor alpha (ERα), progesterone receptor, human epidermal growth factor receptors, etc. The function of estrogen receptor alpha is cell proliferation in the endometrium of uterus, breast and ovary cancer. Estrogen hormone, produced in the ovaries, binds to these receptors and initiates growth as well as differentiation in the mammary gland. It has been observed clinically that patients with ERα positive cancer have better survival rate than ERα negative cancer. Drugs like tamoxifen bind to ERα receptor and inhibit further channelization of cancer [29]. In present study ligand based docking of this receptor, 3D structure with PDB ID - 3ERT was selected. The binding site residues sit in a hydrophobic cavity which contains the helices 3, 6, 7, 8, 11, and 12 [30]. The human epidermal growth factor receptor (EGFR) dysfunction is associated with more than half cases of triple-negative breast cancers. Two representative members of this family had been chosen for docking. The first member of EGFR family is HER1. This receptor's antagonists have been reported to inhibit metastasis and induce chemo-sensitivity. The binding of ligands to the transmembrane portions of the receptor leads to phosphorylation of tyrosine residues which then interacts with intracellular docking proteins. This signal initiates cellular proliferation and also protects cells against apoptosis [31] [32]. The second member of EGFR family is HER2. This receptor is reported to be involved in early development and progression of breast cancer [32]. The overexpression of these receptor also causes cancer in ovaries and gastrointestinal tracts. As a result of overexpression of HER2, the cancer cells may develop resistance to certain hormone therapy, propagate more extensively to brain, and even show significant sensitivity to certain chemotherapy drugs with higher cytotoxicity [33]. The 3D structure of HER2 with PDB ID – 3RCD was chosen to carry out the docking analysis. Fibroblast growth factor receptor (FGFR) also works on receptor tyrosine kinase activation principal like EGFRs. It's found in 4 isoforms FGFR1, FGFR2, FGFR3, and FGFR4. Functions of FGFRs are to control cell differentiation, proliferation and migration [34]. FGFR1 is known to be commonly associated with breast cancer [35]. The 3D structure of FGFR1 with PDB ID – 1AGW was chosen to carry out the docking analysis. G protein coupled receptors function as a mediator in estrogen hormone signalling pathway in cancer propagation. The GPCR is a congregation of seven transmembrane helices from I-VII. These helices are interconnected with intracellular and extracellular loops [36] [37]. The 3D structure of GPCR with PDB ID - 1F88 was chosen to carry out the docking analysis.

Table 1 summarizes post docking analysis based on H bonding between ligand and receptors. The amino acid residues of ERα receptor that binds to amygdalin is Cys530. The number of H bonds visualised is 1 and the docking score was found to be -7.1kcal/mol. In order to form stable H bonds with ERα the ligand needs to bind with Arg394 but amygdalin didn't interact with it. So the H bond formed cannot be considered stable [30]. The drug ligand formed 3 H bonds with Thr862, Arg849, and Lys753amino acid residues of HER2 receptors which is similar to peptidomimetic drugs docking [33]. So peptidomimetic activity of amygdalin can be studied in future with in vivo experimentation. Binding score was also found to be highest -8.4kcal/mol among cancer surface receptors chosen for the study. Amygdalin formed only one H bond with Leu484 residue of FGFR1 receptor. The overall interaction is weak with binding energy of -7.3kcal/mol. The GPER also had weak interacting bonding with it's residue Arg314. Docking score of -5.0kcal/mlol is the least among chosen receptors. The visualization of docking interactions is illustrated in Fig.1-4.

Table 1: Binding energy and molecular docking analysis of primary breast cancer cell surface receptors.

Receptors	Receptor class	Interacting amino acids	Hydrogen bonds	Binding Energy (kcal/mol)
HER2	Human epidermal growth factor receptor (3RCD)	Thr862, Arg849, Lys753	3	-8.4
ERα	Estrogen receptor (3ERT)	Cys530	1	-7.1
FGFR1	Human epidermal growth factor receptor (1AGW)	Leu484	1	-7.3
GPER	G protein coupled receptor (1F88)	Arg314	2	-5.0

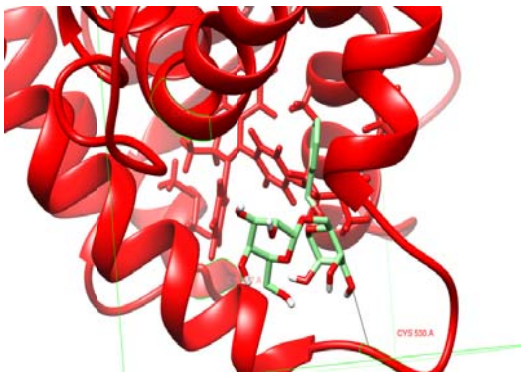

Figure 1: 3D interactions of amygdalin with estrogen receptor ERα, black line represents the H bond between ligand and macromolecule

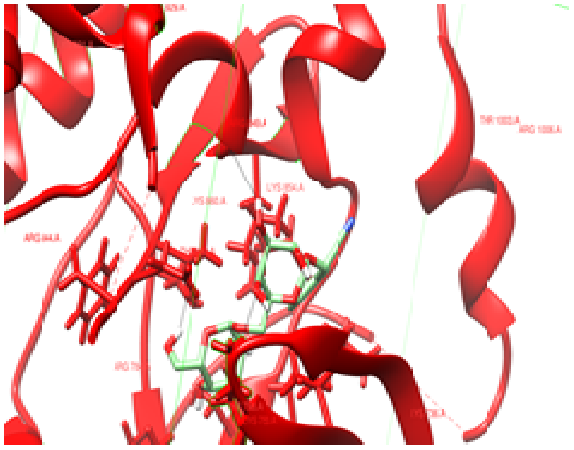

Figure 2: 3D interactions of Amygdalin with Human epidermal growth factor receptor HER2

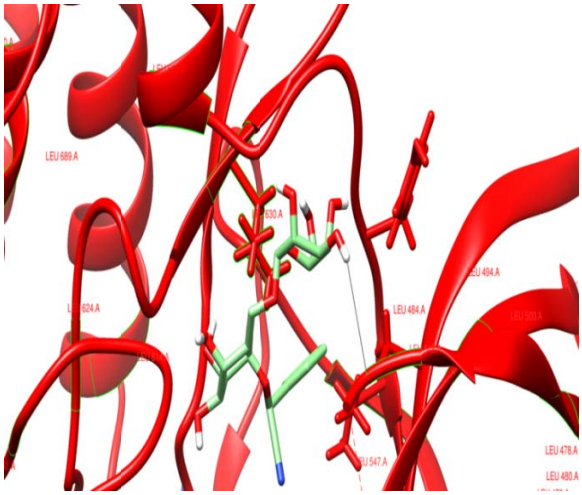

Figure 3: 3D interactions of Amygdalin with Human epidermal growth factor receptor FGFR1

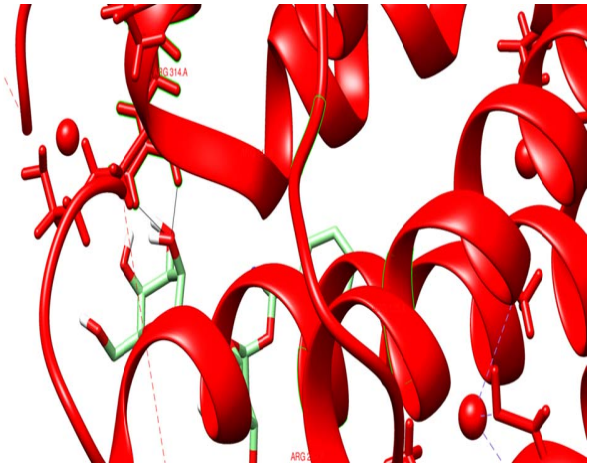

Figure 4: 3D interactions of Amygdalin with G protein coupled receptor GPER

4. CONCLUSION

The necessary role of nutraceuticals in healthcare has led to its evolution as formulation drugs at an industrial scale. Thus, the in-silico investigation gives an idea about its pharmacological applicability in breast cancer. An initial bonding to HER2 receptor has been observed which suggests the need of further more comprehensive In Silico investigation. Also peptidomimetic behaviour should also be analysed in future studies through in vivo experimentation.

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