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SCREENING OF NOVEL INHIBITOR FOR HER2 INDUCED BREAST CANCER-AN *INSILICO* APPROACH

Amit R. Rupani*[1] and Biplabh Bhattacharjee [2]

The Oxford College of Engineering [1], Bangalore, Karnataka, India
Institute of Computational Biology [2], Bangalore, Karnataka, India

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Correspondence to Author:

Amit R. Rupani

Royal Residency, Flat No-202, House No. 5-9-209, Chiragali Lane, Abids, Hyderabad, Andhra Pradesh, India

ABSTRACT

HER2 stands for Human Epidermal growth factor Receptor 2. Each normal breast cell contains copies of the HER2 gene coding for HER2 protein found on the cell surface, which helps normal cells grow.HER2+ breast cancer is characterized by over expression of this gene in breast cells which fosters the cell growth giving rise to breast tumors. Studies show that 20-25% of breast cancer patients have tumors that are HER2+ making it a significant target for breast cancer studies. Identification of effective, well tolerated HER2 inhibitors represents a rational chemo preventive strategy. Here I present a study on the 27 herbal HER2 targeted lead compounds and their potential binding affinity to HER2. Iressa and Lapatinib served as reference drugs. Lipinski's rule of five was applied to all the herbal compounds to evaluate their drug likeness, pharmacological or biological activity. Depending on Lipenski's rule, the molecules which were following the criteria for the same were subjected to energy minimization using MARVIN SKETCH and receptor-ligand interaction study using QUANTUM docking tool. Reference drugs were also subjected to the same studies. Silymarin showed good docking score in comparison with the reference drugs. After the docking step, Silymarin was subjected to binding site analysis in Q site Finder where the binding interaction with HER2 was clearly observed. Using ADME TOX analysis it was found that silymarin was showing lower Ames test value than the reference drugs. The high ligand binding affinity of silymarin to HER2 receptor introduce the prospects of its use in chemo preventive applications. Further animal studies needs to be done to confirm the exact role and mechanism of Silymarin as a cancer chemo preventive agent for breast cancer.

INTRODUCTION: Breast cancer is a cancer that arises in the breast tissue, mostly from the inner lining of the milk ducts or the lobules that supply the milk ducts. It is the second most common type of non-skin cancer after lung cancer and the fifth most common cause of cancer death [1]. Several factors leading to breast cancer have been reported for instance sex, age, hormonal causes, genetic causes, environmental causes, etc [1-3].

In this study, focus mainly lies on the genetic causes. Mutations and abnormal functioning of certain genes have lead to aggressive breast cancers [5, 7]. Mutations in genes like BRCA1, BRAC2 and p53 have been extensively studied for their breast cancer inducing properties [5, 6]. Inheritance of the altered forms of these genes will confer a high life time risks and a high relative risks [4]. Over expression of genes like HER2 will consequently lead to over expression of corresponding cell growth controlling protein, which will expedite the cell growth and proliferation leading to formation of tumors. Therefore, targeting the HER2 protein may bring back the cell growth under control [7, 12]. In this work an attempt has been made to target HER2 with naturally occurring molecules that are suspected to inhibit it (based on evidence).

Human epidermal growth factor receptor (HER)2 is a transmembrane tyrosine kinase protein that is encoded by a proto-oncogene located on chromosome 17q21 [8]. The HER2 protein is found on the surface of some normal cells in the body [11]. In normal cells, HER2 proteins help send growth signals from outside the cell to the inside of the cell. These signals tell the cell to grow and divide. HER2 receptors exist as monomers but dimerize on ligand binding. HER ligands are bivalent growth factor molecules whose low-affinity site binds to HER2 to send the growth signal. Under conditions where HER2 is over expressed, it acts as a networking receptor that mediates signaling to cancer cells, causing them to proliferate [12]. The HER2 proto-oncogene is over-expressed in approximately 20% to 25% of invasive primary breast cancers. Positive HER2 status has been linked with aggressive tumor behavior [8]. Therefore, inhibition of this receptor becomes crucial in breast cancer treatment[12]. Drugs that are commercially used to inhibit HER2 and suppress the growth of over expressing tumor cells are Iressa and Lapatinib [9, 10]. These drugs have been used as standard reference drugs in this study for comparative analysis. In this study an in silico approach has been followed to find a drug molecule, that targets HER2, having properties better than the reference drugs.

MATERIALS AND METHODS:

A. Data mining and Lead Identification: *In silico* approach towards finding out the lead compounds began with the literature survey. An attempt was made to find all the compounds which have been proven to inhibit HER2. Wide range of books and journals were referred to gather the information about the compounds. Compound Structures were retrieved using pubchem[13]. All the compounds were then subjected to Lipenski's Rule of Five to evaluate their absorption and permeability as shown in fig. 1. For any compound to act as a drug, it has to meet a certain criteria. That criterion is given by Lipenski's Rule of Five. Compounds satisfying this criteria show better prospects of acting as drugs due to their high druglikeness. Therefore, only the compounds meeting the above criteria were considered for further computational analysis [14].

B. Docking studies: Filtered compounds were then subjected to energy minimization using MarvinSketch. MarvinSketch is bioinformatics software used for drawing and editing chemical structures. Apart from drawing and editing it can

also perform several tasks such as energy minimization, structure visualization, changing the file types, etc[15]. Energy Minimization is an essential step in computational approach towards drug discovery. This will lower the overall energy of the molecule making it flexible enough to fit in the active sites of the protein molecule with greater compatibility during the protein-ligand docking [16]. Each Lipenski cleared compound was opened in MarvinSketch and different energy conformers were obtained for the same. The least energy conformer of each compound was saved for performing docking studies [17].

Drugs generally exhibit their action by binding to certain target receptor[17]. The energy required for the drug-receptor binding directly correlates to the effective binding of the drug to the target receptor. This energy at the expense of which protein-Ligand interaction occurs is denoted as G-bind value[21, 20]. The energy minimized compounds were ready to get docked with the target protein molecule (HER2). Quantum 3.3 was used as a docking tool to carry out the docking operations with greater accuracy[18]. Energy value (g-bind) for each ligand docked with HER2 was noted down. Compounds requiring minimum energy to bind to HER2 were analyzed based on the energy values. For comparative analysis, reference drugs (Iressa and lapatinib) were also docked with the same receptor and their energy values were noted down as well. Lead compound was identified based on the comparative analysis[20]. This lead compound was then subjected to ADME-Tox analysis[19].

C. **Binding Site Analysis:** Identification and evaluation of surface binding-pockets and occluded cavities are initial steps in protein structure based drug design. Characterizing the active site's shape as well as the distribution of surrounding residues plays an important role for a variety of applications such as automated ligand docking or *in situ* modeling. After the docking step, the protein was subjected to binding site analysis in Q site Finder [13, 14].

D. **ADME-Tox and Property evaluation Studies:** ADME-Tox drug properties viz. absorption, distribution, metabolism, elimination and toxicity, are properties that decide overall efficacy of the drug molecule. It is possible to study these properties in silico using ADME-TOX web server. It is an online server giving information about twelve major pharmacokinetics and pharmacodynamics features of the molecule. Toxicity of the molecule can also be investigated using this server which includes acute toxicity, genotoxicity and organ specific health effects. Genotoxicity result is in the form of Ames test value. It correlates to the ability of the compound to act as a mutagen. Features like bioavailability, solubility, drug plasma binding protein, volume of distribution and Ames test were considered for the comparison studies. A comparison was made with commercially used drugs for hepatitis treatment [19].

Molecular property evaluation of the lead compound was done using Osiris property explorer which is an online tool. The chemical structure of the lead compound was drawn and various drug-relevant properties like druglikeness, drug score, etc are calculated. Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red. Whereas a green color indicates drug-conform behavior [22, 23, 24].

RESULTS AND DISCUSSION: The literature survey resulted in 27 HER2 inhibiting compounds which includes Curcumin, Biochanin A, Epigallocatechin 3-gallate, Quercetin, Osthole, Indole-3-carbinol, etc. Out of these 27 compounds only 14 followed the Lipenski's criteria for druglikeness. These 14 compounds were taken as targeting agents which are suspected (based on evidence) to be responsible for inhibiting the biological processes important in causing HER2 induced breast cancer. Compounds were prepared for docking analysis through their energy minimization in MarvinSketch. Different energy conformers obtained for a compound in MarvinSketch are shown in **fig 1**.

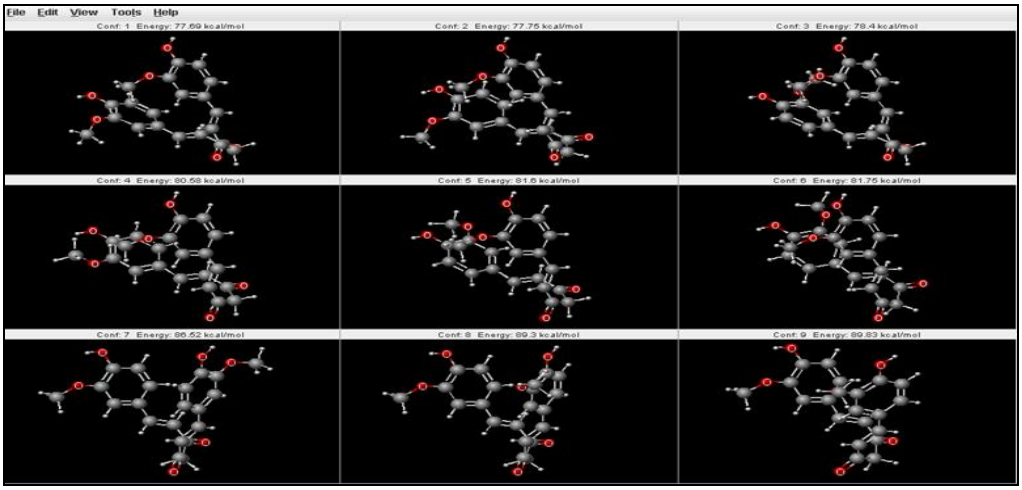

FIG. 1: DIFFERENT ENERGY CONFORMERS FOR A COMPOUND IN MARVINSKETCH

The docking studies were carried out in Quantum. It was found that the compounds namely Silymarin, Curcumin, Apigenin and 3,3Diindolylmethane showed relatively better interaction with HER2 receptor requiring less energy to bind to it [**table 1**]. Reference drugs (Iressa and Lapatinib) were also docked with the same receptor and their energy values were noted. It was found that energy required by Silymarin to dock with HER2 was significantly lower than that required by reference drugs [table 1]. A screenshot of running docking operation in Quantum 3.3 is shown in **fig. 2**.

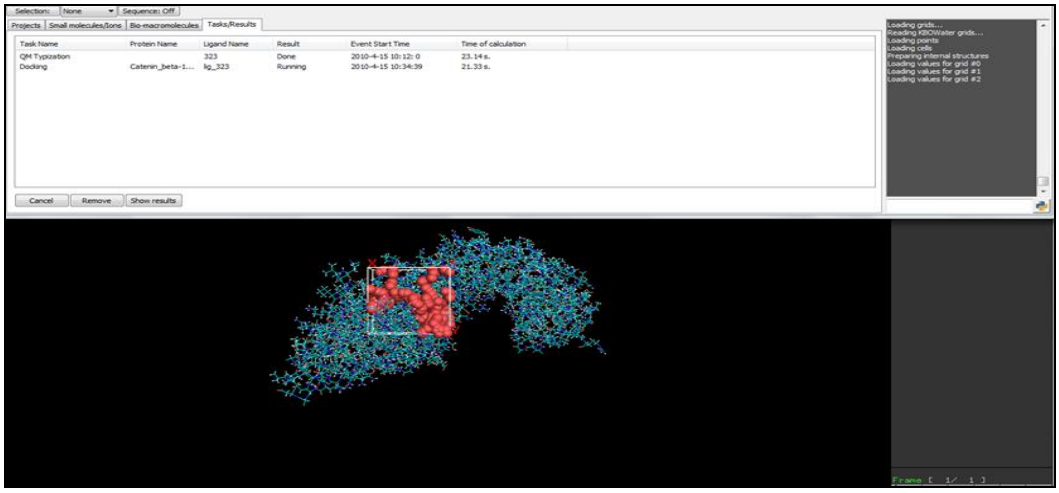

FIG. 2: RUNNING DOCKING OPERATION IN QUANTUM 3.3

TABLE 1: COMPOUNDS HAVING MINIMUM ENERGY VALUES

Name of the Compound	Compound Type (lead/drug)	ΔG kcal/mol
Silymarin	lead	**-33.17**
Curcumin	lead	**-23.40**
Apigenin	lead	**-22.28**
3, 3 diindolylmethane	lead	**-22.68**
Iressa	drug	**-27.31**
Lapatinib	drug	**-25.89**

An attempt was also made to retrieve the active site sequence of HER2 receptor for silymarin using Q site Finder. But, unfortunately, Q site Finder did not reveal its active site sequence nonetheless, the binding interaction was clearly observed as shown in **fig. 3**.

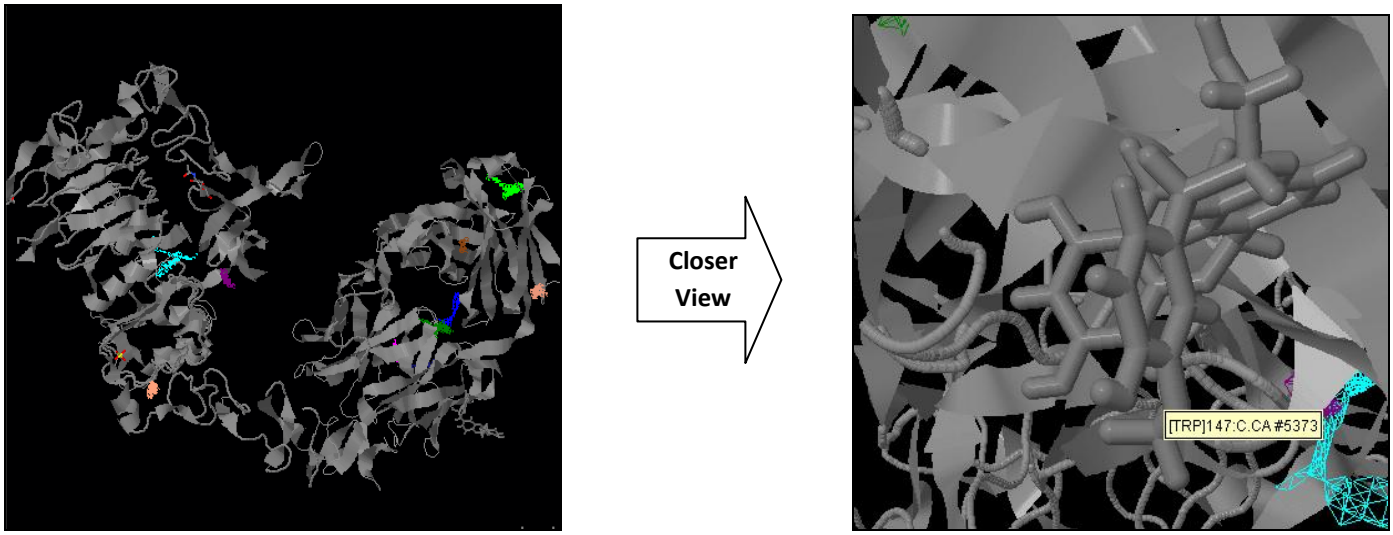

FIG 3: PREDICTION OF ACTIVE SITE TO WHICH SILYMARIN BINDS WITH ITS CLOSER VIEW

ADME-TOX analysis of this compound showed reliable pharmacokinetics and pharmacodynamics features. Toxicity box results showed the compound has lower ames test value when compared to reference drugs [**table 2**]. Ames test result for Silymarin in ADME-TOX web is shown in **fig. 4**.

TABLE 2: COMPARISION OF AMES TEST VALUES OF LEAD COMPOUND FOUND THROUGH INSILICO ANALYSIS WITH COMMERTIALLY USED DRUGS TO TREAT BREAST CANCER

Compound Name	Compound Type (lead/drug)	Ames Test Value
Silymarin	Lead	0.113
Iressa	Drug	0.133
Lapatinib	Drug	0.883

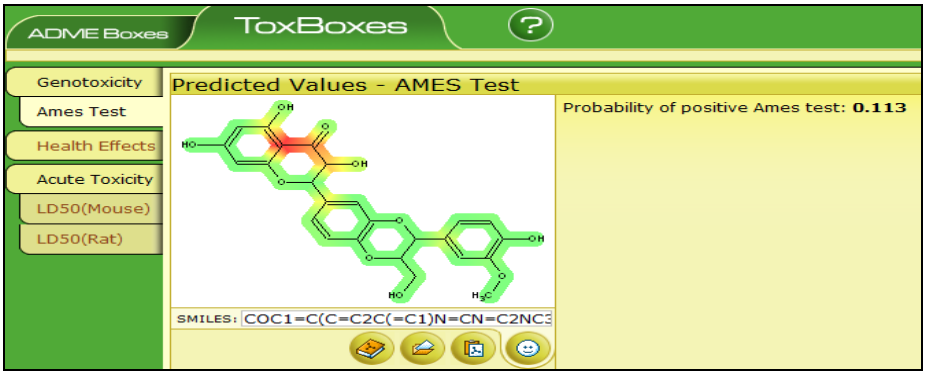

FIG. 4: AMES TEST RESULT FOR SILYMARIN IN ADME-TOX WEB

Property evaluation of Silymarin in Osiris Property Explorer gave a positive drug score and druglikeness. It also showed that Silymarin had lower toxicity risks which confirmed the drug-conform behavior of Silymarin. Prediction results for silymarin in Osiris property explorer is given by **fig. 5.**

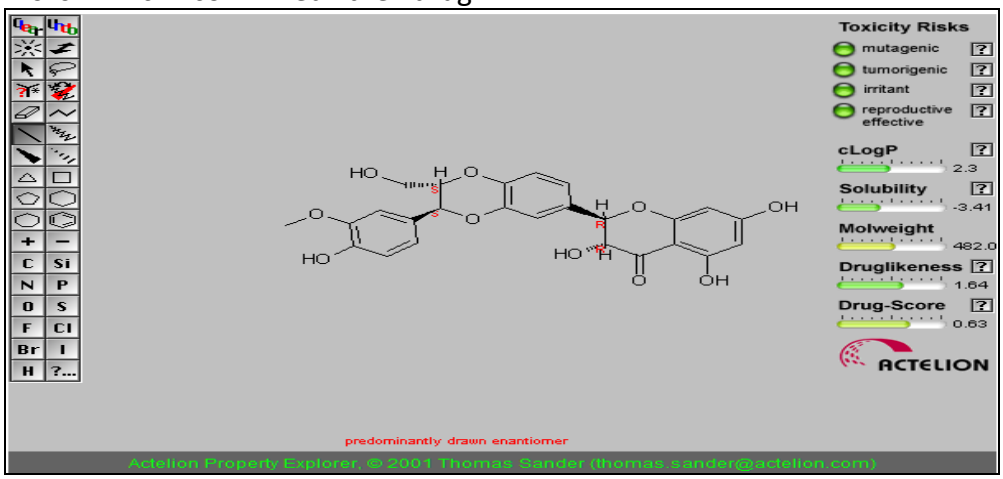

FIG. 5: PROPERTY EVALUATION OF SILYMARIN IN OSIRIS PROPERTY EXPLORER

CONCLUSION: It can be concluded that Silymarin holds a strong potential for acting as drug candidate that target HER2 to inhibit the biological processes leading to breast cancer. In silico analysis renders it a stronger candidate, than the drugs in use such as Iressa and Lapatinib and the other HER2 targeting molecules, for curing HER2 induced breast cancer. Further animal studies needs to be done to confirm the exact role and mechanism of this compound as a chemo preventive agent.

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