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Research Article

# Targeting natural compounds against HER2 kinase domain as potential anticancer drugs applying pharmacophore based molecular modelling approaches

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ABSTRACT

Human epidermal growth factor receptors are implicated in several types of cancers characterized by aberrant signal transduction. This family comprises of EGFR (ErbB1), HER2 (ErbB2, HER2/neu), HER3 (ErbB3), and HER4 (ErbB4). Amongst them, HER2 is associated with breast cancer and is one of the most valuable targets in addressing the breast cancer incidences. For the current investigation, we have performed 3D-QSAR based pharmacophore search for the identification of potential inhibitors against the kinase domain of HER2 protein. Correspondingly, a pharmacophore model, Hypo1, with four features was generated and was validated employing Fischer's randomization, test set method and the decoy test method. The validated pharmacophore was allowed to screen the colossal natural compounds database (UNPD). Subsequently, the identified 33 compounds were docked into the proteins active site along with the reference after subjecting them to ADMET and Lipinski's Rule of Five (RoF) employing the CDOCKER implemented on the Discovery Studio. The compounds that have displayed higher dock scores than the reference compound were scrutinized for interactions with the key residues and were escalated to MD simulations. Additionally, molecular dynamics simulations performed by GROMACS have rendered stable root mean square deviation values, radius of gyration and potential energy values. Eventually, based upon the molecular dock score, interactions between the ligands and the active site residues and the stable MD results, the number of Hits was culled to two identifying Hit1 and Hit2 has potential leads against HER2 breast cancers.



## 1. Introduction

Breast cancer (BC) is one of the common causes of death manifested in women worldwide (Shah and Rosso, 2014) accounting to 40,000 deaths annually in USA (Gajria and Chandarlapaty, 2011). Breast cancer incidences are relatively higher in the developed countries as compared to the under developed countries (Cleveland et al., 2012). This reflects the intrusion of the life style (Cauchi et al., 2016) in triggering the tumour development which includes physical activity (Wu et al., 2013) and obesity (Chan and Norat, 2015). Besides, exposure to radiations (Henderson et al., 2010; Ronckers et al., 2005) and family history (Tazzite et al., 2013; Melvin et al., 2016) may predominantly lead to cancer formation. Broadly cancer cells are represented by their receptors such as estrogen positive (ER+) and progesterone positive (PR+). Additionally, some breast cancers are characterized by elevated levels of growth promoting protein and are defined as HER2/neu(+) cancers. Fundamentally, human epidermal growth factor (HER) regulates the normal cell growth and its development and are comprised (Baselga, 2010) of transmembrane tyrosine kinase (TK) receptors such as epidermal growth factor receptors EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4 correspondingly (Hynes and Lane, 2005; Baselga and Swain, 2009; Gutierrez and Schiff, 2011; Baselga, 2010). Each receptor is a single glycoprotein (Iqbal and Iqbal, 2014) subunit that bears an extra cellular ligand binding domain, a transmembrane α-helix segment and intracellular tyrosine kinase domain(Li and Hristova, 2006). For proper exertion of their biological activities, receptor dimerization plays a key role which can be homodimerization or heterodimerization resulting in the

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autophosphorylation of the tyrosine residue located at the cytoplasmic domain. This mechanism leads to the initiation of a host of signalling pathways (Iqbal and Iqbal, 2014) stimulating several biochemical activities such as angiogenesis, invasion, cell differentiation, proliferation and survival (Iqbal and Iqbal, 2014). However, HER receptors generally remain inactive by avoiding dimerization (Baselga, 2010) and only specific dimers are implicated with cancers. HER2:HER3 heterodimer is regarded as being highly effective oncogenic unit because of the ligand induced tyrosine phosphorylation, interaction strength and downstream signalling (Amin et al., 2010). Additionally, HER2 demonstrates high catalytic activity and can undergo dimerization even without a ligand. Besides, HER2 confers an exposed open conformation of its dimerization domain and thus makes it an ideal partner (Gutierrez and Schiff, 2011). On the contrary, even though HER3 can bind to a ligand, its kinase domain is devoid of catalytic activity and hence relies on its partner for initiation of the signals (Garrett et al., 2003; Sierke et al., 1997; Guy et al., 1994; Dey et al., 2015). This makes the HER2:HER3 a pre-eminent dimer combination. HER2 dimerization additionally contributes to cell delocalization and the degradation of cell-cycle inhibitor $p27^{Kip1}$ resulting in cell-cycle progression (Citri and Yarden, 2006; Olayioye, 2001; Iqbal and Iqbal, 2014).

Nevertheless, HER2 displays a major role as a prime contributor to BC and its overexpression is demonstrated in 30% of early breast cancer cases (Lee-Hoeflich et al., 2008; Li et al., 2016). More specifically, the aberrant raise in the protein levels or its expression is associated with lymph node (+) and lymph node(−) breast cancers (Ross et al., 2009). Statistically, HER2 genes are elevated upto 25 ~50% and the amplified expression of HER2 is noticed in BC. Sequentially, nearly of about 2 million receptors are demonstrated at the surface of the tumour cells (Kallioniemi et al., 1992; Gutierrez and Schiff, 2011). Consequently, HER2 has been deemed trustworthy drug target in addressing HER2 (+) BCs. Besides BC, HER2 is also seen associated with ovarian (Menderes et al., 2017; Zanini et al., 2017) and gastric cancers (Rüschoff et al., 2012; Lucas and Cristovam, 2016).

Targeting HER2 has been a promising avenue to counter HER2 amplified breast cancer such as monoclonal antibodies and small molecules (Maximiano et al., 2016; Hynes and Lane, 2005; Schroeder et al., 2014). Trastuzumab, a monoclonal antibody (MB) that mechanistically acts by five different ways as reported earlier (Kute et al., 2004; Iqbal and Iqbal, 2014). Pertuzumab a humanized MB inhibits the activation of HER2 receptor duly impedes the dimerization of the receptor (Swain et al., 2015). This drug was also used in combination with trastuzumab (von Minckwitz et al., 2017) and docetaxel (Swain et al., 2015) in HER2-positive metastatic breast cancer. However, these treatments have manifested adverse effects such as infusion reactions, febrile neutropenia alopecia and diarrhea. Lapatinib a tyrosine kinase small molecule inhibitor operates by intervening with HER2 and EGFR pathways. However, it is effective when administered in combination with letrozole (Schwartzberg et al., 2010; Johnston et al., 2009). Lapatinib is credited with being the only approved orally active drug for patients with HER2-positive advanced breast cancers (Konecny et al., 2006; Li et al., 2016), while two synthetic chemical drugs namely dacomitinib and neratinib have made it to the Phase III trials (Gonzales et al., 2008; Kalous et al., 2012; López-Tarruella et al., 2012; Chan et al., 2016). However, prolonged administration of Lapatinib may induce drug resistance. This condition warrants a dire necessity for discovering new drug candidates as a majority of the incidences relapse (Li et al., 2016). Therapeutically, a small molecule hinders the process of tyrosine phosphorylation and thereby subsequent signalling events by challenging the ATP at the catalytic kinase domain and hence prevents aberrant amplification. Therefore, the objective of the current study is to identify novel potential natural compounds that can inhibit the undesirable amplification of HER2 signalling employing the 3D-QSAR based pharmacophore approach.

## 2. Materials and methods

### 2.1. Selection of the compounds

In order to generate the most reliable pharmacophore, the compounds that are involved in it play a very important role. For the current study, a total of 82 compounds have been chosen from different literatures (Hanan et al., 2016; Bryan et al., 2016; Cheng et al., 2016; Pannala et al., 2007; Liu et al., 2007; Gilson et al., 2016) that have demonstrated varied inhibitory activity values ($IC_{50}$) and were compiled into dataset. Prior to the commencement of the investigation the duplicates were removed for the explicit generation of the pharmacophore. Furthermore, the dataset was divided into the training set and the test set, correspondingly. Typically, a training set should consist of more than 16 compounds, wisely including the most active compound, should exhibit 4 order magnitudes and should be structurally distinct. Accordingly, 32 compounds were chosen as training set that demonstrated a wide range of $IC_{50}$ values spanning between 0.003 nmol/L–25,000,000 nmol/L, Fig. 1. Additionally, the training set was divided into most active compounds displaying an inhibitory activity of less than or equal to 100 nmol/L, compounds demonstrating a range between 100 nmol//L and 10,000 nmol//L were labeled as moderately active and the compounds with $IC_{50}$ above 10,000 were grouped into least active compounds. Subsequently, the 3D QSAR based pharmacophore was constructed using *HypoGen* algorithm implemented on the Discovery Studio (DS) v4.5, employing training set compounds. Likewise, the test set consists of 50 structurally diverse compounds were utilized to validate the generated pharmacophore model. The test set was further classified in the same order of magnitude as the training set compounds. Subsequently, their corresponding 2D structures were sketched using ChemSketch and were transferred to the Discovery Studio v4.5 (hereinafter DS) for processing the work further.

### 2.2. Ligand-based pharmacophore model generation

For the generation of the most potential pharmacophore models, the *Feature Mapping* protocol implemented on the DS was initiated to critically probe into the important chemical features imbibed within the training set compounds. The information rendered by the above was exploited in the generation of the pharmacophore. *3D QSAR Pharmacophore Generation* module available in the DS was employed to generate the pharmacophore using the Catalyst *HypoGen* algorithm. Additionally, the *best* algorithm was employed to generate the compounds with lower energy conformation at uncertainty value 3 having an interfeature distance of 2.97 at 95% confidence. For the generation of the pharmacophore the features such as Hydrogen Bond Acceptor (HBA), Hydrogen Bond Donor (HBD), Hydrophobic (HyP), Hydrophobic Aliphatic (HyA) and Ring Aromatic (RA) were chosen with a minimum of 0 and maximum of 5 features while retaining the remaining parameters as default. Correspondingly, the best pharmacophore from the generated was selected based upon the Debnath's analysis (Debnath, 2002; Debnath, 2003). According to Debnath's analysis, an ideal pharmacophore should essentially display a high correlation coefficient, least cost value and lowest RMSD.

### 2.3. Validation of the pharmacophore

The best pharmacophore model selected from the generated hypothesis was then subjected to validation performed by Fischer's