

RESEARCH ARTICLE

Prediction and Analysis of Ligands against Estrogen Related Receptor Alpha

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Abstract

Breast cancer is one of the most common malignancies in women around the world. Among the various hormonal types of breast cancer, those that are estrogen receptor (ER) positive account for the majority. Among the estrogen related receptors, estrogen related receptor α is known to have a potential role in breast cancer and is one of the therapeutic target. Hence, prediction of novel ligands interact with estrogen related receptor alpha is therapeutically important. The present study, aims at prediction and analysis of ligands from the KEGG COMPOUND database (containing 10,739 entries) able to interact against estrogen receptor alpha using a similarity search and molecular docking approach.

Keywords: Breast cancer - estrogen related receptor alpha - docking - virtual screening

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Introduction

Breast cancer is one of the common malignancies and leading causes of cancer death in women around the world. Global rise in deaths from breast cancer has been rising from about 2.5 lakhs in 1980 to 4.25 lakhs in 2010 (Choudhry et al., 2012). Its incidence rate is high in developed regions and is low in most of the developing regions of the world (Curado, 2011). In West Africa, it is a leading cause of death among women and in Western Europe the incidence rate is five times higher than that in Western Africa (Abdulrahman and Rahman, 2012). Its incidence rate is rapidly increasing even in Asian populations also. In countries like China and India, it is increased upto 30% over the last decade whereas in Japan, Korea and Singapore it was doubled or even tripled (Bhoo-Pathy et al., 2013). In general, highest incidence rates of Breast Cancer are found in Switzerland, U.S. whites, Italy, and many other European countries, whereas low rates are found in Africa, Asia, and South America. These rates are substantially higher in U.S. Hispanics and Asians compared to most cancer registries in Asia and Latin America (Jemal et al., 2010). According to National Cancer Institute (USA), estimated new cases of breast cancer in United States for the year 2013 is 232,340 (female) and 2,240 (male) whereas estimated breast cancer deaths are 39,620 (female) and 410 (male).

General risk factors for breast cancer include both genetic and non genetic. Genetic risk factor constitutes 5-10% of the Breast cancer cases. One of the major ways of defining the type of breast cancer is: Endocrine receptor (estrogen or progesterone receptor) positive; HER2 positive; Triple negative, not positive to receptors

for estrogen, progesterone, or HER2; Triple positive, positive for estrogen receptors, progesterone receptors and HER2. Majority of breast cancers are estrogen receptor (ER) positive. Breast cancer patients with tumors that are estrogen receptor (ER)-positive and/or progesterone receptor (PR)-positive have lower risks of mortality compared to women with ER- and/or PR-negative disease (Dunnwald et al., 2007) making it a powerful predictive, prognostic marker and an efficient target for the treatment of hormone-dependent breast cancer. The classical mechanism of estrogen receptor action involves estrogen binding to receptors in the nucleus, followed by receptors dimerization and binding to specific response elements known as estrogen response elements (EREs) located in the promoters of target genes (Bjornstrom and Sjoberg, 2005). Estrogen is a female hormone secreted mainly by the ovaries to proliferate the endometrium as a part of the menstrual cycle. Functions of estrogen include promotion of subcutaneous fat accumulation; mammary gland proliferation; water and sodium retention and calcium deposition (Yu et al., 2013). Before menopause, it stimulates vaginal epithelial cells to produce large amounts of glycogen and after menopause, its decreased levels causes lowering of glycogen content (Reiter, 2013). Estrogen ablation or anti-estrogen strategy is an effective means of prevention or treatment of breast cancer, especially in estrogen receptors (ERs)-dependent breast cancer. Major isoforms of ERs include ER α and ER β . ER α isoform primarily contribute to estrogen-induced growth stimulatory effects in breast cancer (Li et al., 2013).

In general, estradiol binds with high affinity to estrogen receptor alpha and this binding induces DNA synthesis, cell division, and production of growth factors and

progesterone receptor proteins (DeBruin and Josephy, 2002). Recent investigations have revealed that Estrogen receptors are highly mobile proteins continuously shuttling between cellular compartments and ligand and/or protein induced ER conformational changes regulate such movements, leading to specific responses (Leclercq et al., 2006). Although, ER α and ER β are breast cancer biomarkers of clinical course, there remains a great need to identify additional biomarkers and one such potential candidate biomarkers includes the orphan nuclear receptor Estrogen related receptor alpha (ERR α). ERR α status may be predictive of sensitivity to hormonal blockade therapy and can be a candidate target for therapeutic development (Ariazi et al., 2002). Thus, predicting the ligands that are able to show an interaction with ERR α is an important area of research. Docking various ligands to the protein of interest followed by scoring to determine the affinity of binding and to reveal the strength of interaction has become increasingly important in the context of drug discovery. Traditional way of synthesizing a series of new compounds utilizing combinatorial chemistry and high-throughput screening is expensive and also time consuming whereas screening the small molecule databases for novel compounds represents an effective alternative process in such scenario.

In the present study, we report screening a library of compounds from KEGG COMPOUND database against ERR α with bound ligand 4-(4-[(5R)-2,4-dioxo-1,3-thiazolidin-5-yl]methyl)-2-methoxyphenoxy)-3-(trifluoromethyl)benzonitrile extracted from protein data bank, by utilizing a molecular docking software Autodock which performs the docking of the ligand to a set of grids describing the target protein.

Materials and Methods

Receptor structure

The 3D coordinates of the crystal structure of Estrogen Related Receptor alpha in Complex with an Ether Based Ligand (PDB code: 3K6P) was selected from the Protein Data Bank (PDB) (Berman et al., 2007) as a receptor model for screening. For screening the compounds, KEGG COMPOUND database was used. KEGG COMPOUND database contains chemical structures of most known metabolic compounds and some pharmaceutical and environmental compounds (Kanehisa et al., 2004). Chemicals structures were downloaded in SDF format from Pubchem database (<http://pubchem.ncbi.nlm.nih.gov/>), a public information resource for archiving chemical structures and biological properties of small molecules and siRNA reagents (Bolton et al., 2008).

Molecular docking

Molecular docking was performed using the Autodock 4.2 (Morris et al., 2002; Huey et al., 2007). PyRx program (Dallakyan, 2008-2010) was employed to generate the docking input files. The empirical free energy function and Lamarckian genetic algorithm (LGA) were used for docking with the following settings: a maximum number of 2,500,000 energy evaluations, an initial population of 150 randomly placed individuals, a maximum number of

27,000 generations, a mutation rate of 0.02, a crossover rate of 0.8 and an elitism value (number of top individuals to survive to next generation) of 1. For the local search, the so-called Solis and Wets algorithm was applied with a maximum of 300 iterations per search. Default values were used for all the other parameters not mentioned.

Drug-likeness analysis and ADMET analysis

Drug-likeness was analyzed as per "Lipinski Rule of 5" (Lipinski et al., 2001) using Mol soft: Drug-Likeness and molecular property explorer (<http://www.molsoft.com/mprop/>). ADMET (absorption, distribution, metabolism, elimination, toxicity) analysis on the other hand was predicted using admetSAR, a comprehensive source and free tool for assessment of chemical ADMET properties available at (<http://www.admetexp.org/>) (Cheng et al., 2012).

Results

The receptor structure (PDB ID: 3K6P) is found to contain 4-(4-[(5R)-2,4-dioxo-1,3-thiazolidin-5-yl]methyl)-2-methoxyphenoxy)-3-(trifluoromethyl)benzonitrile (5FB) as a Ligand. The chemical structure and property of the 5FB was shown in the Figure 1, Table 1 below. Interaction analysis of 5FB showed that it forms hydrogen bond with ARG 372 and hydrophobic interactions with nearby residues. The respective bonding interactions were shown in the Figure 2 given below. We searched the KEGG COMPOUND database using structural features of 5FB and found 55 compounds similar to 5FB. Among the 55 compounds, 8 compounds were found to have a similarity score greater than 0.30 and these 8 compounds were considered for the next step. The respective compounds with similarity score greater than 0.30 were shown in the Table 2 given below. Among the 8 compounds, 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)

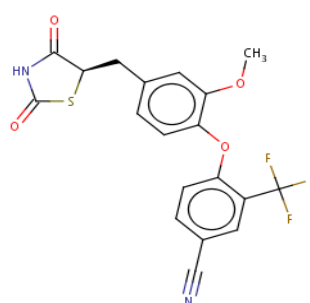


Figure 1. Chemical Structure of 5FB

Table 1. Chemical Property of 5FB

Name	4-(4-[(5R)-2,4-dioxo-1,3-thiazolidin-5-yl]methyl)-2-methoxyphenoxy)-3-(trifluoromethyl)benzonitrile
Identifiers	4-[4-[(5R)-2,4-dioxo-1,3-thiazolidin-5-yl]methyl]-2-methoxy-phenoxy]-3-(trifluoromethyl)benzenecarbonitrile
Formula	C ₁₉ H ₁₃ F ₃ N ₂ O ₄ S
Molecular Weight	422.38 g/mol
Type	non-polymer
Isomeric SMILES	COc1cc(C[C@H]2SC(=O)NC2=O)ccc1Oc(ccc1C(F)(F)F)C#N
InChi	InChI=1S/C ₁₉ H ₁₃ F ₃ N ₂ O ₄ S/c1-27-15-7-10(8-16-17(25)24-18(26)29-16(2-5-14(15)28-13-4-3-11(9-23)6-12(13)19(20,21)22/h2-7,16H,8H2,1H3(H,24,25,26)/t16-m/1/s1
InChi key	IOTPZQVXYNGZSS-MRXNPFEDSA-N

propanal has highest similarity score of 0.38. Hence, this compound was considered for docking with the receptor structure. The chemical property of the compound 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal was shown in the Table 3 given below. Before, docking the 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal to receptor structure, the docking protocol was validated by docking the 5FB into the binding pocket to obtain the docked pose. The RMSD (Root Mean Square Deviation) of all atoms between these two conformations is 0.84 Å° indicating that the parameters for docking simulation are good in reproducing the X-ray

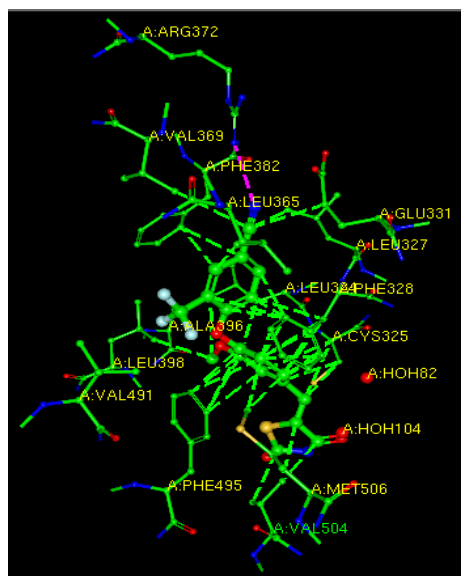


Figure 2. Interactions of 5FB with Estrogen Related Receptor Alpha. Pink color dotted lines indicate Hydrogen bonding whereas green color dotted line indicates hydrophobic interactions

Table 2. List of Compounds with Similarity Score Greater Than 0.30

Entry	Structure:	Similarity-Score	Name
C16706		0.38	4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal
C14311		0.34	4-Propylphenol
C01502		0.31	o-Methoxyphenol Guaiacol Catechol monomethyl ether
C06730		0.31	4-Methylcatechol 3,4-Dihydroxytoluene 1,2-Dihydroxy-4-methylbenzene 4-Methyl-1,2-benzenediol
C13637		0.31	4-Ethylphenol p-Ethylphenol
C14386		0.31	3-Ethylphenol
C14582		0.31	2,4-Dimethylphenol 1-Hydroxy-2,4-dimethylbenzene
C15572		0.31	Guaiacol

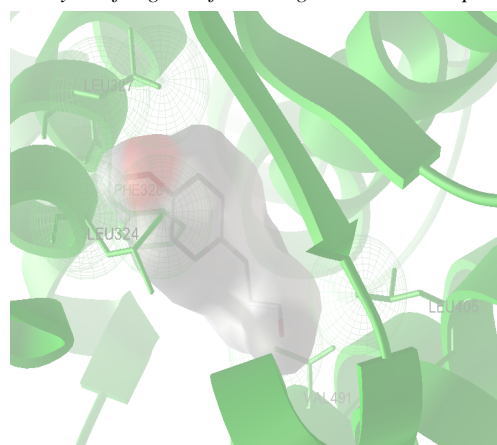


Figure 3. Interactions of 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl) Propanal with Estrogen Related Receptor alpha. Receptor was shown in Green color Ribbon; 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal was shown in the center with molecular surface

Table 3. Chemical Property of 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal

Entry Name	C16706 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal
Formula	C ₉ H ₁₀ O ₂
Exact Mass	150.0681
Mol weight	150.1745
Structure	
Pathway	Isoquinoline alkaloid biosynthesis, Biosynthesis of alkaloids derived from shikimate pathway

crystal structure. The orientation of docking pose of 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal suggests that the binding site was occupied by the residues LEU 327, PHE 328, LEU 324, LEU 405, VAL 491 with an Binding energy of -4.80 kcal/mol, Inhibition Constant K_i of 303.9 μ M (micromolar), Intermolecular Energy of -5.99 kcal/mol, Internal Energy of -0.11 kcal/mol and Torsional Energy 1.19 kcal/mol. The interaction of 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal with the receptor was shown in the Figure 3 given below. Respective Binding energies and interacting residues were shown in the Table 4 given below. As per Lipinski "Rule of 5", the "drug-like" molecules have $\log P \leq 5$, molecular weight ≤ 500 , number of hydrogen bond acceptors ≤ 10 , and number of hydrogen bond donors ≤ 5 . Molecular property analysis for 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal showed a $\log P$ value of 1.16, molecular weight 150.07, number of hydrogen bond acceptors 2 and number of hydrogen bond donors 1. Thus, satisfying the Lipinski "Rule of 5". Further, ADMET analysis showed that it is non toxic, non carcinogenic and readily biodegradable.

Discussion

A factor which predicts response or resistance to a specific therapy is called a predictive marker and the most

Table 4. Ligands and Their Corresponding Interaction Energies

Compound	AutoDock Binding Energy ΔG_b (kcal/mol)	Interacting Residues
4-(4-((5R)-2,4-dioxo-1,3-thiazolidin-5-yl)methyl)-2-methoxyphenoxy)-3-(trifluoromethyl)benzoxazole	-8.07	LEU 324, CYS 325, LEU 327, GLU331, LEU 365, VAL 369, PHE 382, LEU 398, PHE 495, VAL 504, MET 506
4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal	-4.99	LEU 324, LEU 327, PHE 328, LEU 405, VAL 491

widely used predictive marker in oncology is the estrogen receptor (ER) (Duffy, 2001). For more than a decade, estrogen receptor status was considered as a marker of different stages of tumor or different entities of the breast cancer disease. Based on the estrogen receptor (ER) level of the tumor, Breast cancer can be divided into two types i.e., ER-positive and ER-negative (Zhu et al., 1997). In mammals, two subtypes of nuclear estrogen receptors have been described i.e., estrogen receptor α (ER α or estrogen receptor 1 or ESR1) and estrogen receptor β (ER β or estrogen receptor 2 or ESR2).

Both ER α and ER β are the members of a superfamily of genes that contains nuclear receptors for diverse hydrophobic ligands such as steroid hormones (estrogens, progestins, glucocorticoids, mineralocorticoids), retinoic acids (vitamin A), vitamin D, prostaglandins, and thyroid hormones (Pavao and Traish, 2001). Among ER α and ER β , ER α plays a prominent role in the aetiology of breast cancer and with respect to the prognosis of Breast cancer, it has been extensively studied. About 70-80% of invasive breast cancers express ER α and high level of ER α is associated with tumour differentiation (Nykanen and Kuopio, 2010). In vitro study on ER α showed that, though ER α associate with chemoresistance of breast cancer, ER α itself does not mediate this resistance process (Jiang et al., 2012). It prevents p53-dependent apoptosis in breast cancer (Bailey et al., 2012) and is known to interact with other receptors like Retinoic acid receptor α which is a known estrogen target gene in breast cancer cells (Ross-Innes et al., 2010). Point mutations in ER α , may lead to hypersensitive estrogen breast hyperplasia (Abbasi et al., 2013) and several splice variants in ER α are known to found in various tumor types of Breast cancer. Among them variant in exon $\Delta 3$ of ER α (missing part of the central DNA binding domain) is known to function as a dominant-negative receptor, able to suppress estrogen induced transcriptional activity (Barone et al., 2010).

Human ER α is a 595 amino acids protein with an approximate molecular weight of 66-70 kDa (Pavao and Traish, 2001). Starting from NH₂- to COO-terminus, ER α contains a typical structure of the nuclear receptor family with a highly variable N-terminal region (A/B domain), a highly conserved DNA-binding domain (C), a hinge domain (D), a ligand-binding domain (E) and a C-terminal domain (F). ER α is a ligand inducible transcription factor which upon hormone binding, gets activated and regulates the transcription of target genes. Ligand dependent and independent activation of ER α , is done by the N-terminal A/B domain region (transactivation function-1 (AF-1)) whereas dimerization and binding to the coactivators and corepressors is done by the ligand-binding domain (transactivation function-2 (AF-2)) (Divekar et al., 2011). Estrogens have a proliferative effect on various tissues

like breast and they directly bind to the ER, which further homodimerizes and interacts with estrogen response elements to stimulate the transcription of target genes. In the absence of hormone, the ER is associated with a host of proteins that prevent it from interacting with the cellular transcription apparatus (Stoica et al., 2003).

Estrogen-related receptors (ERRs) α , β and γ on the other hand are the NR3B orphan subgroup within the nuclear receptor superfamily identified based on their sequence homology to ER α (Christina and Peggy, 2011) (highly conserved DBD among the ERRs is closely related to the ERs DBD) and they do not bind to estrogen or any other natural hormones. Several studies showed that ERR α has a potential role in cancer especially breast cancer (Ariazi et al., 2002; Suzuki et al., 2004; Barry and Giguere, 2005; Ariazi and Jordan, 2006; Stein et al., 2008; Stein et al., 2009; Dwyer et al., 2010). Several ERR α subtype-selective inverse agonists or antagonists were reported previously (Chisamore et al., 2009) and 5FB is one such lead compound co-crystallized with the ligand-binding domain structure of ERR α (Patch et al., 2011). In comparison with 5FB, 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal which is predicted as an inhibitor in this study, has more drug-likeness and binding energy with ERR α and may show similar antagonistic property against ERR α .

In conclusion, virtual screening methods are extensively used to reduce cost and time of drug discovery. The present approach utilized in this study was successful in finding an inhibitor against Estrogen Related Receptor alpha from the KEGG COMPOUND database. In particular, the present study showed that residues LEU 324 and LEU 327 interact with both 5FB and 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal. Molecular properties of 4-Hydroxydihydrocinnamaldehyde 3-(p-Hydroxyphenyl)propanal satisfied the Lipinski "Rule of 5" indicating that there is no problem in its bioavailability. Further, work should now be extended in order to study the receptor-ligand interactions experimentally and evaluation of their biological activity would help in designing new compounds.

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