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Research article

Molecular screening and analysis of Genistein analogues for the identification of novel therapeutic inhibitors against estrogen receptor alpha

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Abstract

Most common malignancy faced by women around the world today is breast cancer. Estrogen receptor alpha has been identified and play a major role in stimulating the growth of breast cancer. So in the present analysis, our focus has been on natural compounds which do not exhibit any toxicity and have significant estrogen receptor alpha inhibition activity. Several reports proposed that polyphenolic chemicals present in many plant foods, and hence these compounds have several chemo preventive properties against breast cancer. So by using molecular docking, we screened the selected natural compounds and investigated their binding affinity against the selected protein. As a result, we predict the novel therapeutic inhibitors against estrogen receptor alpha for the treatment of breast cancer.

Introduction

Breast cancer is the well-known cancer type caused to the females around the world and it constitutes the major cause of mortality in several developing countries including India. Present day's breast cancer is a rising public health problem [1-3]. General risk factors for breast cancer include age, hereditary, diet, family history, heavy drinking, lack of exercise, radiation exposure, life style etc [4]. Estimated new cases of breast cancer in the United States for the year 2017 is 252,710 while recorded breast cancer deaths are 40,610 [5]. More economically developed and industrialized countries, and the economical fatality differentials have been deliberate extensively screening, and low developing countries suffer the highest mortality victims as a cause of breast cancer [6]. So overall an expected 14 million new breast cancer cases occur each year [7]. Based on the above reports the life style changes are the major factors to the development of breast cancer [8]. Daily intake of fruits and raw vegetables, which are rich in antioxidants, can decrease the risk of cancer [9].

Estrogen exposure is the major risk factor for breast cancer, so overall 50-60%of breast cancers are estrogen receptor (ER) positive. Generally, estrogen is a female sex hormone and it is important for reproductive development [10-12]. The estrogen receptor originates in two subtypes: Estrogen receptor alpha (ERα) and Estrogen receptor beta (ERβ). ER alpha is mainly present in the mammary gland [13]. The targeted therapeutics of estrogen receptor has clearly been success in the medication of breast cancer [14]. Therefore the prediction of possible estrogen receptor positive drug plays a very crucial role in the future appliance. In this present analysis, our research found out the appropriate natural products with the high binding affinity with the breast cancer target protein and which can be used for the treatment of breast cancer. Natural plant products have been used to prevent various diseases and these bioactive compounds can be proved and play a positive role to decrease the breast cancer risk [15]. The most important and useful Isoflavone are genistein and it is first discovered in 1899 [16]. Genistein acts as a chemotherapeutic agent against various types of cancers and it is a major flavonoid found in legumes, particularly soya beans and soya products like textured vegetables. Isoflavones as a good therapeutic option in breast cancer chemoprevention [17]. Genistein derivatives have excellent anti-cancer isoflavone agents against breast cancer. The selected analogues have outstanding anti-tumor activity and good pharmacokinetic and ADMET properties. Based on its properties, these natural compounds were chosen for our study. And we aimed to predict the best inhibitors by using *insilico* screening and analysis of molecular docking against the active site of estrogen receptor alpha.

Experimental

Materials and methods

Preparation of protein

The three-dimensional structure of Human estrogen receptor alpha (figure 1) with PDB: 3ERT for docking studies was selected from the protein data bank [18]

(http://www.rcsb.org/pdb) serves as a target model for screening. The complexes bound to the protein molecule, the water molecules and all the heteroatoms were removed, that are not needed for docking. Hydrogen atoms were added for the receptor structure.

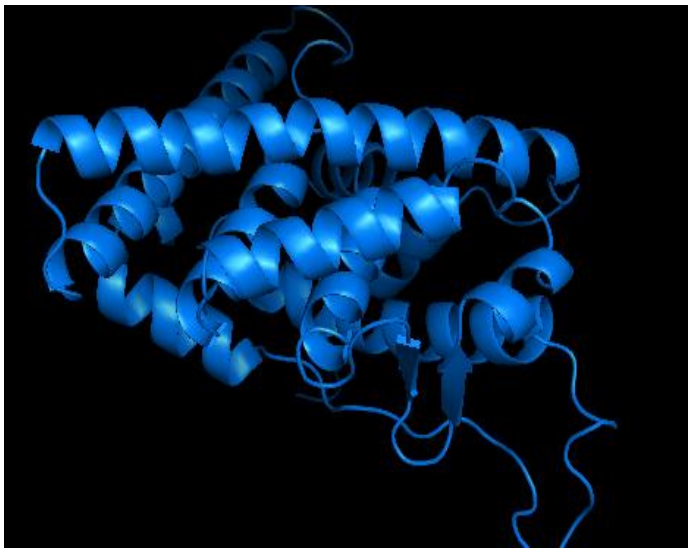

Figure 1. Three Dimensional Structure of Human Estrogen Receptor Alpha (3ERT).

Retrieval of ligands

For screening the compounds PubChem database was used. The PubChem database is a public open repository for the biological activity of small molecules. The chemicals structures were downloaded in SDF (structure data format) from PubChem database (http://www.pubchem.ncbi.nlm.nih.gov). The chemical structure of ligand is shown in figure 2. A total of 18 compounds were selected for docking studies all the selected compounds can show inhibitory effects towards breast cancer [19].

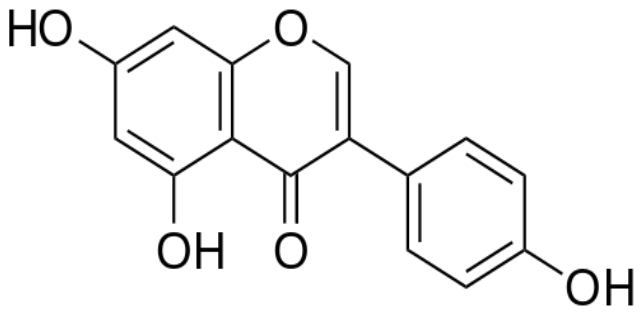

Figure 2. Chemical structure of Genistein.

Molecular docking

Molecular docking is one the most important method in structure based drug design. Docking was performed using Auto dock Vina in PyRx interface, an open source software [20]. The grid box was generated around the active site residues with grid center, X-Axis - 22.396 Å**,**Y-Axis – 5.6441 Å, Z-Axis – 21.9877Å and grid dimensions x – 48.25 Å, y - 55.54 Å, z-56.032.Å.

Drug-likeness analysis and adverse effect prediction

Lipinski's rule of five also called as Pfizers's rule of five to evaluate the drug likeness, the rule was prepared methodically by Christopher A. Lipinski in 1997 [21]. As per "Rule of 5", the drug-like molecules have the number of hydrogen bond acceptors ≤10, number of hydrogen bond donor's is not more than 5, Partition coefficient log P less than (≤5) and molecular mass less than 500 daltons [22]. The pharmacokinetic properties and ADMET (absorption, distribution, metabolism, elimination, toxicity) analysis were predicted using *insilico* methods. In this study Data Warrior is used for the calculation of drug-likeness and ADMET analysis. (http://www.openmolecules.org/datawarrior). Data Warrior is an open source visualization and data analysis program. It is a versatile tool and it calculates the various physicochemical properties directly from chemical structures [23].

Results and discussion

Receptor structure

Human estrogen receptor alpha has been used as an important therapeutic target for Breast Cancer. The 3-Dimensional Structure of Human estrogen receptor alpha retrieved from Protein Data Bank PDB Code: 3ERT determined by X-Ray crystallography at a resolution of 1.90Å as shown in figure 1.

Assessment of pharmacological properties

The selected compounds were tested for 'rule of 5'the results showed that all the compounds can satisfy Lipinski rule. The toxicity prediction and pharmacokinetic properties were predicted among all the 18 Compounds using Data Warrior. The data warrior results of toxicity analysis and pharmacokinetic properties were shown in table 1. The log P values and solubility were calculated for pharmacokinetic properties while mutagenicity, tumorigenicity, reproductive effect and irritation effect were predicted for Toxicity analysis. The results revealed that poor solubility is accompanying with bad absorption, and aqueous solubility substantially affects the absorption and distribution characteristics. The Data Warrior results displayed that among 18 compounds, PubChem CID: 5393152, CID: 5280863, CID: 5280443, CID: 11987653, have high mutagenic action, and five compounds i.e., CID:3764, CID: 10062691, CID: 656936, CID: 11987653, CID: 254171 showed high reproductive effect. Based on the results from data warrior the selected 18 compounds is predicted to have better log P, and good drug-likeness and less toxicity parameters as illustrate table 1.

Table 1. Drug likeness analysis and *In silico* ADMET Prediction.

PubChem ligand Id	Total molecular weight	C log P	C log S	H-acceptors	H-donors	Drug likeness	Mutagenic	Tumorigenic	Reproductive Effects	Irritant
5284648	270.239	1.6272	-2.727	5	3	-0.093853	None	None	None	None
10062691	272.255	2.3791	-2.889	5	3	0.085	None	None	High	None
25271556	250.304	2.7827	-5.491	4	2	-1.8789	None	None	None	None
656936	226.230	3.17	-3.961	3	2	-1.0746	None	None	High	None
688857	240.257	2.8471	-3.232	3	1	-0.22006	None	None	None	None
5320693	268.267	2.9571	-3.466	4	1	0.40331	None	None	None	None
5393152	254.240	2.5273	-3.379	4	2	-0.082832	High	None	None	None
5280863	286.238	1.8359	-2.787	6	4	-0.082832	High	None	None	None
439246	272.255	2.1557	-2.64	5	3	-0.22006	None	None	None	None
5391140	254.240	2.6814	-3.152	4	2	0.28194	None	None	None	None
5281607	254.240	2.6814	-3.152	4	2	0.28194	None	None	None	None
114829	256.256	2.5014	-2.936	4	2	-0.22006	None	None	None	None
5280443	270.239	2.3357	-2.856	5	3	0.28194	High	None	None	None
3764	268.267	2.2486	-3.337	4	1	0.036465	None	None	High	None
44201362	250.304	2.7827	-5.491	4	2	-1.8789	None	None	None	None
11987653	274.271	1.6034	-3.021	5	3	0.9234	High	None	High	None
5280373	284.266	1.9029	-3.041	5	2	0.036465	None	None	None	None
254171	224.211	0.7476	-2.037	5	2	-1.603	None	None	High	None

Table 2. Ligands and Their Corresponding Binding Affinities and Interaction Energies

PubChem Id of Ligand	Binding Affinity ΔGb (kcal/mol)	Interacting Residues
10062691	-8.9	ARG 394,GLY 420, GLU 353
5284648	-8.9	ARG 394,GLU 353
25271556	-8.6	HIS 524, GLY420
656936	-8.6	ARG 394
688857	-8.5	ARG 394, LEU 387
5320693	-8.4	ARG 394, GLU 353
5393152	-8.4	MET 522
5280863	-8.3	GLU 353, HIS 524
439246	-8.2	MET 522
5391140	-8.2	ARG 394
5281607	-8.0	MET 522
114829	-7.9	GLU 353, GLY 420
5280443	-7.8	MET 522
3764	-7.5	ARG 394
44201362	-7.3	ASP 351
11987653	-7.1	ARG 394, HIS 524, LEU 387
5280373	-6.8	LEU 536, VAL 534
254171	-6.6	LEU 387, ARG 394

Binding mode analysis

In this regard, we identified four PubChem compounds i.e., PubChem CID: 25271556, CID: 5284648, CID: 5320693, CID: 5391140 might be chosen as new inhibitors for estrogen receptor alpha and these four compounds having anti-cancer properties and have the high estrogen receptor binding capability. The structural analogues of the above mentioned compound CID: 5284648 having good binding energy value is -8.9 kcal/mol. This was shown interacts with Arg394, Glu353. CID: 25271556 contain binding energy value is -8.6 kcal/mol this was shown interact with His 524, Gly 420. CID: 5320693 contain binding energy value is -8.4 kcal/mol, this compound was found to interact with Arg 394, Glu 353. CID: 5391140 contain binding energy value is -8.2 kcal/mol this compound was found to interact with Arg 394 results will be elucidated in table 2 and the receptor and ligand interactions as shown in figure 3. These results may be used for generation of novel drugs against estrogen receptor alpha.

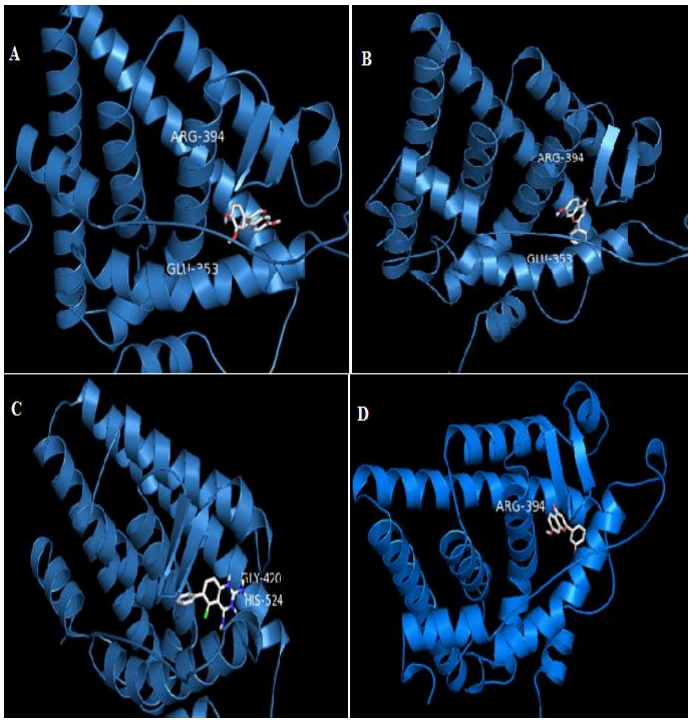

Figure 3. Docking studies of best docked derivatives with representation of binding site analysis of selected ligands with estrogen receptor alpha.
A. PUBCHEM CID: 5284648
B. PUBCHEM CID: 25271556.
C. PUBCHEM CID: 5320693
D. PUBCHEM CID: 5391140.

Conclusion

In conclusion, we suggested novel therapeutic inhibitors by using a computational method structured based virtual screening, Molecular docking. The receptor-ligand interactions play a very prominent role in Drug Designing. So in the current report, based on binding energy value we chosen the best ligand-receptor interactions. Protein for human estrogen alpha has been taken, and new potential drugs have been recognized and these newly identified drugs can be used against Breast cancer. Pharmacokinetic properties and toxicity analysis calculated in Data warrior, most of these compounds are not suitable as drug candidates. Finally four compounds i.e., PubChemCID: 5284648, CID: 25271556, CID: 5320693 and CID: 5391140shows good binding energies and in ADMET analysis these analogues have excellent pharmacokinetic and drug-likeness properties. Hence, these four compounds having anti-cancer activity and these analogues could be used for designing therapeutic lead molecules. Further analysis is needed to investigate the interactions of the above selected ligands with estrogen receptor alpha can be used for *in vivo* and *in vitro* experiments.

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Conflict of interest

The authors declare that they have no competing interests.

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