RESEARCH ARTICLE

In Silico Docking Studies of Selected Flavonoids - Natural Healing Agents against Breast Cancer

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Abstract

Background: Breast cancer is the serious health concern in India causing the highest mortality rate in females, which occurs due to uncontrolled cell division and can be metastasize to other parts of the human body. Interactions with estrogen receptor (ER) alpha are mainly responsible for the malignant tumors with regulation of the transcription of various genes as a transcription factor. Most of the drugs currently used for the breast cancer treatment produce various side effects and hence we focused on natural compounds which do not exhibit any toxic effect against normal human cells. Materials and Methods: Structure of human ER was retrieved from the Protein Data Bank and the structures of flavonoid compounds have been collected from PubChem database. Molecular docking and drug likeness studies were performed for those natural compounds to evaluate and analyze the anti-breast cancer activity. Results: Finally two compounds satisfying the Lipinski's rule of five were reported. The two compounds also exhibited highest binding affinity with human ER greater than 10.5 Kcal/mol. Conclusions: The results of this study can be implemented in the drug designing pipeline.

Keywords: Breast cancer - flavonoids - human estrogen receptor - argus lab - docking.

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Introduction

Breast cancer is one of the most recurring worldwide diagnosed and deadliest cancers next to lung cancer with a high number of mortality rates among females (Benson and Jatoi, 2012). At global level, it accounted for more than 1.6 million new cases in 2010. The incidence or prevalence rate of the breast cancer in India is expected to be more than 90,000 in the coming years and over 50,000 women die each year (Parveen et al., 2013).

A high level of Estrogen is linked with increased risk of breast cancer, which mediates its biological effects such as genesis, malignant progression, cell apoptosis and other important roles by binding to the Estrogen Receptor present in the breast cancer cells (Thomas and Gustafsson, 2011; Fatemeh et al., 2012). The Estrogen Receptor mainly exists in two forms: Estrogen Receptor alpha and Estrogen Receptor Beta. Estrogen receptors alpha (ER-α) is mainly expressed in the uterus, vagina, mammary gland, liver, pituitary gland (Waraphan et al., 2011). The major causes of breast cancer are identified by abnormal expression of Estrogen Receptor α-positive affecting about 70% of the primary breast cancer patients (Dickson and Stancel, 2000; Fuqua, 2001; Ariazi et al., 2006; Chao-Yang et al., 2013; Kumaraswamy and Suneetha, 2013). The Estrogen Receptor alpha plays a pivotal role in controlling transcription of nuclear DNA necessary for mammary gland development (Hayashi et al., 2003) and it is also an essential factor for breast cancer signaling network (Pantea et al., 2012). It also regulates cell proliferation and differentiation through a paracrine mechanism (Hong et al., 2013). Hence, the inhibition of Estrogen Receptor has become a major approach for preventing and treating breast cancer (Salih and Fentiman, 2001).

The drugs which are currently used, for the treatments of breast cancer were Tamoxifen, Raloxifene, Toremifene. (Fabian and Kimler, 2001). Ingestion of this drug was based on interfering with either estrogen production or estrogen action which causes so many side effects such as blood clots, strokes, uterine cancer, or cataracts (Parkkari et al., 2003; Mojgan et al., 2009; Andrew et al., 2011). The side effects of these drugs make the need for the necessity of new improved drugs.

The side effects of the currently used drug made us to explore an alternative and traditional approach to finding out new drug compound from the natural Flavonoid, compounds which are having anti breast cancer activity and also not having any side effects to human normal cell (Kawaii et al., 1999; Pouget et al., 2001). These plant compounds which are also having high binding affinity for breast cancer receptors could lead to breast cancer treatment by docking method and to determine the drug-likeness of these new molecules by estimation of the Lipinski's Rule of Five.

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Flavonoids functions as selective Estrogen Receptor modulators (SERMs) (Pritchard, 2001; Park and Jordan, 2002) changes the endogenous activities of Estrogen Receptor, which will slow down or prevent the developments of breast cancers (Limer and Speirs, 2004; Jarzabek et al., 2009). Flavonoids are large group of natural polyphenolic compounds present in fruits, vegetables, cereals, tea, red wine, legumes and exhibit antioxidant, anticarcinogenic, anti-inflammatory, antiproliferative, antiangiogenic, and antiestrogenic (or estrogenic) properties (Shuzhong et al., 2004; Paola Galluzzo and Maria, 2006; Miller and Snyder, 2012; Sanaz et al., 2012; Stapel et al., 2013). They also play a fundamental biological role in the regulation of breast cancer cell line proliferation. Several Epidemiological and in vivo studies also recommended that a high intake of Flavonoids may be associated with a reduced risk of cancer (Chang et al., 2013). Flavonoids can be further divided, according to chemical structure, into different groups. Flavonoids listed in the (Table 1), are already reported in various literatures that they possess anti breast cancer activities (Shi et al., 2011; Feng et al., 2013; Priya et al., 2013).

Materials and Methods

Protein preparation

The three dimensional crystal structure of Human Estrogen Receptor with PDB: 2IOG (Dykstra KD et al., 2007) in complex with the ligand N-[(1R)-3-(4-HYDROXYPHENYL)-1-METHYLPROPYL] - 2-[2-PHENYL-6-(2-PIPERIDIN-1-YLETHOXY)-1H-INDOL-3-YL] ACETAMIDE was retrieved from the Protein Data Bank (http://www.rscb.org/pdb) (Berman HM et al., 2002). The complexes bound to the receptor molecule, all the heteroatoms and the non-essential water molecules were removed and finally hydrogen atoms were merged to the target receptor molecule using Argus Lab.

Ligand preparation

Totally 19 flavonoids were identified from the Pubmed literatures which shows inhibitory effects towards breast cancer. The three dimensional structure of the flavonoids was downloaded in sdf format using Pubchem and converted to PDB format using Pymol and further used for docking studies.

Active site identification of human estrogen receptor

The catalytic sites of human Estrogen Receptor along with area and volume of binding pocket was carried out with Computed Atlas of Surface Topography of Proteins (Castp) program (http://cast.engr.uic.edu) (Binkowski et al., 2003).

Docking studies using argus lab

The docking analysis of Human Estrogen Receptor with 19 Flavonoids was carried out by Argus lab docking software which is most commonly available software. All the parameters used in Argus lab docking were selected by default. Calculation type was set to "dock" mode and "flexible mode" was selected for the ligand. Grid resolution was set to 0.40 Å. Least energy indicated the easy binding character of ligand and receptor (Oda et al., 2007).

Lipinski rule of five of the ligand molecule

Using the Molinspiration server (http://www.molinspiration.com/) Molecular properties and drug likeness of the compounds was examined on the basis of "Lipinski's Rule of Five" (Lipinski et al., 2001). The Lipinski's rule, formulated by Christopher A Lipinski in 1997 is a rule of thumb to evaluate drug likeness which states that an orally active drug has no more than one violation of following criteria i,e., has not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight below 500 Daltons, partition co-efficient log P less than 5.

Results and Discussion

Structure of the target protein

Human Estrogen Receptor (2IOG) has been exploited as a main therapeutic target for Breast Cancer. The three dimensional structure of Human Estrogen Receptor retrieved from the Protein Data Bank with PDB ID: 2IOG determined by X-Ray crystallography at a resolution of 1.60 (Å) was visualized in Pymol. It contains 246 amino acids and has been shown in the Figure 1.

Analysis of the catalytic site of the Human Estrogen Receptor

Human Estrogen Receptor catalytic site predictions were analyzed using Cast p program. The best ligand binding site was observed to be at pocket no. 36 of volume 1178.9Å3 and area of 901.1Å2 which consists of 36 residues, Met343, Leu346, Thr347, Leu349, Ala350,

Table 1. Different Groups of Flavonoids having Anti Breast Cancer Activities

Flavonoid group	Subgroup
Flavanols	Flavan-3-ols: Catechin, Gallocatechin, Epigallocatechin gallate
Flavones	Chrysin
	Flavonol: Myricetin,Quercetin, Fisetin
	Flavanones: Eriodictyol, Hesperitin, Naringenin
Anthocyanidins	Flavanonols: Taxifolin
Isoflavonoids	Malvidin, Peonidin, Pelargonidin
	Isoflavones: Daidzein, Genistein, Glycitein, Biochanin A
	Isoflavane: Equol

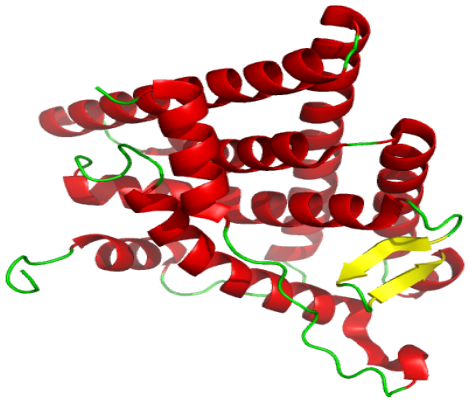
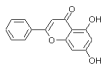
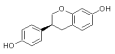
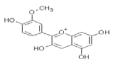
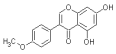
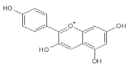
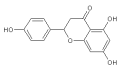
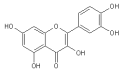
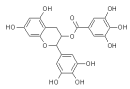
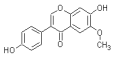
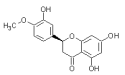
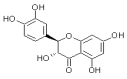
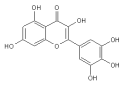
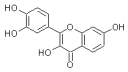
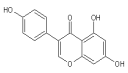
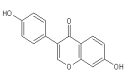
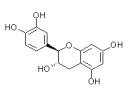
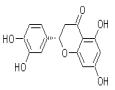
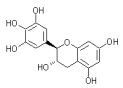
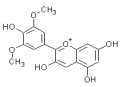


Figure 1. Three Dimensional Structure of Human Estrogen Receptor (2IOG)

Table 2. Docking Results of Human Estrogen Receptor with 19 Flavonoids

S.No	Compound	Pubchem id	Compound Structure	Energy value (kcal/mol)	Binding information	
					Bond type (DH...A)	Distance (Å)
1	Chrysin	5281607		-11.0189	F 425 (N….O) M 421 (O…O) M 421 (O…O) G 420 (O…O)	2.592309 2.792242 2.527373 2.898405
2	Equol	91469		-10.8354	L 387 (O...O)	1.93782
3	Peonidin	441773		-10.4547	E 353 (O…O) M 421 (O...O) F 425 (N...O)	2.549249 2.496559 2.220435
4	Biochanin A	5280373		-10.3986	N 532 (N...O) Y 526 (O...O)	2.844626 2.906581
5	Pelargonidin	440832		-10.0505	F 425 (N…O) M 421 (O…O) C 530 (S….O) T 347 (O…O)	2.306297 2.738949 2.726781 2.900173
6	Naringenin	932		-10.0444	F 425 (N….O) G 420 (O….O) M421 (O….O)	2.505806 2.897523 2.479408
7	Quercetin	5280343		-9.9642	M 421 (O...O) F 425 (N….O) E 353 (O….O) L 346 (O….O)	2.895519 2.922604 2.509561 2.371065
8	Epigallocatechin gallate	65064		-9.90638	G 521 (O….O) C 530 (S….O) G 420 (O....O)	2.438029 2.931772 2.900735
9	Glycitein	5317750		-9.80776	D 351 (O...O) L 536 (N….O) Y 526 (O….O) W 383 (N...O)	2.694571 2.966616 2.181222 2.460178
10	Hesperetin	72281		-9.75576	N 532 (N...O) N 532 (N...O) D 351 (O....O)	2.59341 2.959891 2.723089
11	Taxifolin	439533		-9.50215	C 530 (S….O) C 530 (S….O) G 521 (O….O) E 353 (O….O) T 347 (N….O) T 347 (O….O)	2.764527 2.683346 2.715661 2.603472 2.802442 2.899632
12	Myricetin	5281672		-9.50017	L 387 (O….O) F 425 (N….O) M 421 (O…O) L 346 (O…O) E 353 (O…O)	2.958469 2.549309 2.607202 2.717162 2.30652
13	Fisetin	5281614		-9.34624	E 353 (O….O) L 346 (O….O) G 521 (O….O)	2.766878 2.340324 2.485528
14	Genistein	5280961		-9.32563	G 521 (O….O) L 391 (N….O) L 387 (O….O)	2.102992 2.999786 2.231739
15	Diadzein	5281708		-9.21477	E 419 (O….O)	2.592063
16	Catechin	9064		-9.05333	M421 (O….O) G 420 (O…O)	2.424333 2.691531
17	Eriodictyol	440735		-9.00832	T 347 (O….O) C 530 (S….O) C 530 (S…O)	2.898798 2.461168 2.864364
18	Gallocatechol	65084		-8.76603	Y 526(O….O) N 532 (N….O) N 532 (O….O) A 350 (O….O) D 351 (O…O)	2.237829 2.856221 2.975553 2.90106 2.90009
19	Malvidin	159287		-8.70008	C 530 (S….O) C 530 (S….O) T 347 (O…O)	2.867557 2.714055 2.772607

Asp351, Glu353, Leu354, Trp383, Leu384, Leu387, Met388, Leu391, Arg394, Phe404, Val418, Glu419, Gly420, Met421, Ileu424, Phe425, Leu428, Gly521, His524, Leu525, Tyr526, Met528, Lys529, Cys530, Lys531, Asn532, Val533, Val534, Pro535, Leu536, Leu539 as shown in Figure 2.

Docking analysis

The predicted 36 active residues were used as the catalytic sites for nineteen natural Flavonoid compounds used for docking studies. The results of the interaction between the active site residues of target Human Estrogen Receptor protein and 19 Flavonoid compounds were shown in the Table 2. By analyzing the docking interactions, Chrysin and Equol were found to have the highest activation energy of -11.0189 kcal/mol (Figure 3) and -10.8354 kcal/mol (Figure 4) when compared with other compounds which are having the activation energy of < -10.5 kcal/mol. Thus the docking results were analyzed and finally reported that among the 19 plant compounds, Chrysin and Equol exhibits the best binding interaction with the human Estrogen Receptor and further it could be useful for identification and development of new preventive and therapeutic drug against breast cancer.

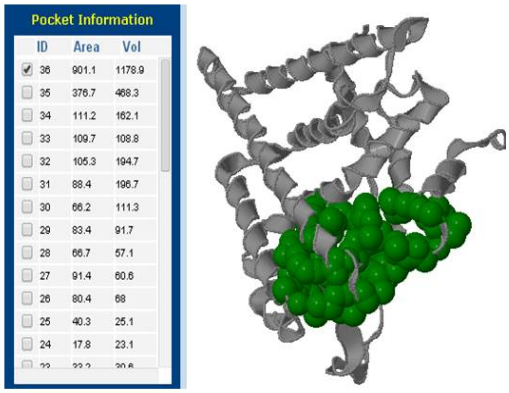


Figure 2. Active Binding Pocket no.36 of Human Estrogen Receptor Predicted using Castp

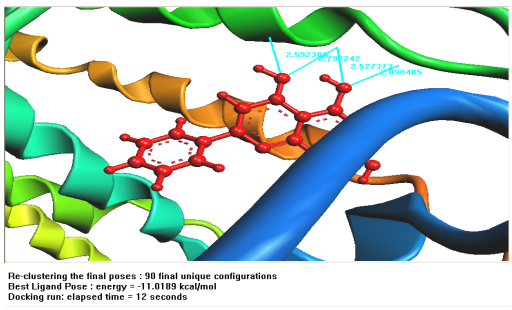


Figure 3. Docking of Chrysin with Human Estrogen Receptor (2IOG)

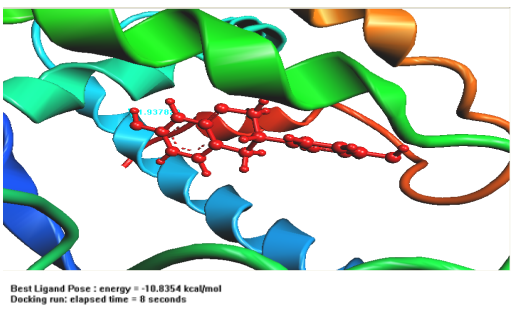


Figure 4. Docking of Equol with Human Estrogen Receptor (2IOG)

Evaluation of drug likeness

Further the 19 Flavonoid Compounds were checked for Lipinski's Rule of Five using Molinspiration software tool. The results of molecular properties and Drug likeness of the Flavonoids were shown in the Table 3 and Table 4. By evaluating the drug likeliness we conclude that all the nineteen anti breast cancer compounds have passed through the Lipinski rule's of five criteria. Three compounds namely Epigallocatechin gallate, Myricetin and Gallocatechol have acquired more than 10 hydrogen bond acceptor and more than 5 Hydrogen bond donor have failed to satisfy the Lipinski rule's of five and hence cannot be used as an oral drug. Finally two compounds Chrysin and Equol satisfies all the properties of pharmacological or biological activity with a best result when compared with other compounds .Thus we conclude that the above two compounds would be more effective as an active drug for human consumption.

The development of novel compounds with biological activity is an urgent need for developing effective drugs. In the present study, docking results revealed the binding interactions between the Human Estrogen Receptor protein and the 19 natural Flavonoid compounds. Among those compounds, Chrysin and Equol were found to have the best binding energy of greater than 10.5 kcal/mol and also satisfies the Lipinski Rule's of five which provide the strong recommendation for the compounds to be used as an oral drug. On the whole, we conclude that Flavonoids Crysin and Equol could be potent anti-breast cancer drugs against Human Estrogen Receptor which may be worth for further investigations to enrich the activity of the natural Flavonoid compounds especially in experimental studies with clinical trials to determine the dosage of safety levels.

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