Research Article

Molecular Docking of Flavonoids for the Treatment of Breast Cancer Using *In Silico* Approaches

Sudeshna Sasmal*[1], Mithun Bhowmick[2], Somenath Bhattacharya[3]

[1]Ex-Student, Department of Pharmaceutical Technology, Bengal College of Pharmaceutical Sciences and Research, Durgapur, West Bengal, India
[2]Principal, Department of Pharmaceutical Technology, Bengal College of Pharmaceutical Sciences and Research, Durgapur, West Bengal, India
[3]Assistant Professor, Department of Pharmaceutical Technology, Global College of Pharmaceutical Technology, Nadia, West Bengal, India
***Corresponding author's E-mail:** sona190696@gmail.com

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ABSTRACT

The ultimate goal is to find out the potential inhibitor of breast cancer against caspase 3 which is the principal component of the cysteine-aspartic acid protease (caspase) family that play a presiding role in the apoptotic signaling pathway and to control cellular apoptosis. During the apoptotic cell death process caspase 3 is a crucial executioner. Caspase 3 is usually essentially found in the cytoplasm and during the apoptotic process, it is transferred into the nucleus to combine with its nuclear substrates. This is a study with ChemSketch, Protein Data Bank (PDB), LigPlot, AutoDock Tools, ALOGPS, E-Dragon, PaDEL Descriptors, PubChem to find the potential inhibitors of breast cancer against caspase 3 and to calculate different parameters of test sample with its biological activity and finally to predict ADMET of final series of compound. After this study we have found the receptor structure and visualize it with PDB software after that we prepare conformation of this receptor with autodock tool and then we predict the active site with ligplot and found the coordinates (X,Y,Z coordinates) for drug-receptor interaction. Then we bind the standard drugs as well as test drug with this receptor. Our study shows that this compound has good absorption, no mutagenecity, no hepatotoxicity and also has very poor blood brain barrier crossing level. Beside this compound was non-toxic, non-carcinogenic after in silico predictions of ADMET.

Keywords: Breast Cancer, Types, Treatment, Receptors, Docking, ADMET Prediction.

INTRODUCTION

Now a day, breast cancer is the most traditional cancers (invasive) in women globally. Following lung cancer, breast cancer is the second major cause of cancer death in women. Breast cancer attributes to cancer which is beginning from breast tissue, most prevailing from the inner lining of milk ducts that supply ducts with milk[1]. According to the survey of august 2019, the chances of women decaying from breast cancer is all over 1 in 38 (2.6%). Almost 268600 women will obtain a diagnosis of intrusive breast cancer and almost 62930 people will obtain a diagnosis of non-invasive breast cancer in 2019 according to the ACS (American Cancer Society). In United States, the number of breast cancer survivors is about 3.1 million. Approximately there are 570.000 passing in 2015. Throughout the universe the number of women who are diagnosed with breast cancer every year is about 1.5 million. America was predicted that 30% of newer cancer cases (252,710) among women are completely breast cancer in 2017. Breast cancer is ecstatic cancer and can move to distant organs such as lung, liver, brain and bone. Early diagnosis of breast cancer can be a good prognosis which have high survival rate [2,3,4,5,6].

Breast cancer unfavorably affects women physical and physiological well-being. Almost 1.7 million newly determined cases and 521900 deaths appeared on a global span in 2012. It is necessary to identify a convenient biomarker that used to decrease the disease mortality. Caspase 3 is a crucial executioner particle during the apoptotic cell death process. Forecasting value of caspase 3 declaration for patient with breast cancer remains undetermined. Growing expression of caspase 3 had adverse influence on breast cancer. Caspase 3 is the principal member of the cysteine-aspartic acid protease (caspase) family, play a governing role in the apoptotic signaling pathway and to control cellular apoptosis. Caspase 3 is usually essentially found in the cytoplasm and during the apoptotic process, Caspase 3 is transported into the nucleus to do interaction with its nuclear foundations. Stimulated caspase 3 plays an important role in tumor cell repopulation and the expanded rate of tumor recurrence. After irradiation, Caspase 3 was involved in angiogenesis promotion in dying tumor cells [7].

Table 1: Age-specific probabilities of developing female breast cancer [8]

Current Age	The Probability of Developing Breast Cancer
20	0.1%
30	0.5%
40	1.5%
50	2.3%
60	3.4%
70	3.9%
Lifetime risk	12.4%

Classification of Breast Cancer [9]:

Invasive breast cancer: It is the most common type of breast cancer where the cells break over the duct and lobular wall which occupy the surrounding fatty and connective tissues of breast. It can be interfere without being metastatic to other organs and to the lymph nodes.

Non-Invasive Breast Cancer: In this type of breast cancer, cells are restricted only to ducts and do not interfere surrounding fatty and connective tissues of breast. The most confirmed form (90%) of non-invasive breast cancer is Ductal carcinoma in situ (DCIS) and less confirmed is Lobular carcinoma in situ (LCIS) which is considered as an indicator for increased risk of breast cancer.

Ductal Carcinoma in situ (DCIS): It is the most accepted non-invasive breast cancer which is restricted to ducts of breast. Ductal comedocarcinoma is an example of such case.

Lobular Carcinoma in situ (LCIS, Lobular neoplasia): It is a pointe raise in the number of cells within lobules (milk glands) of the breast. The word "in situ" means which has not spread past the region where it initially developed.

Infiltrating Lobular Carcinoma (ILC): It popularly recognized as invasive lobular carcinoma which prepares in the lobules (milk ducts) though often metastatizes (escalation) to the another parts of the body and it accounts for 10-15 % of breast cancers.

Infiltrating Ductal Carcinoma (IDC): It is also recognized as invasive lobular carcinoma that prepares in the milk ducts and penetrates the surface of the duct, invading the tissues (fatty tissues) of breast and another region of the body. IDC (Infiltrating ductal carcinoma) is accounting for 80% of breast cancer diagnoses.

Tubular Carcinoma: It is a special category of invasive breast carcinoma.Tubular carcinoma accounts for 2% of breast cancer diagnoses and mostly have better prognosis than other prevalent type of invasive carcinoma.

Medullary Carcinoma: It is a special category of invasive breast carcinoma which forms a definite boundary between normal tissue and tumor tissue. Medullary carcinoma accounts for only 5% of breast cancer.

Mutinous Carcinoma: It is an infrequent breast cancer developed by mucus-producing cancer cells. Colloidal carcinoma is another name for it. Women with this type of carcinoma have advance prognosis than any other types of invasive carcinoma.

Inflammatory Breast Cancer: It is an infrequent (about 1% of breast cancer) and excessively rapid growing cancer. There is appearance of aroused breasts (warm and red) with dimples or/and thick ridges produced by cancer cells that obstruct lymph arteries or channels in the breast skin.

Paget's Diseases of the Nipple: This category of cancer begins in milk ducts and develops to the skin of nipple and areola. It is an infrequent form and accounts for 1% of breast cancer.

Phylloides Tumor: This perhaps either benign (non-cancerous) or malignant (cancerous). It develops in the connective tissues of the breast and can be treated by surgical removal.

Complications of Breast Cancer [1,9]:

- Pain
- Fatigue
- Difficulty breathing
- Nausea
- Diarrhea/constipation
- Weight loss
- Chemical changes in body
- Brain and nervous system problems.

Possible Symptoms of Breast Cancer [10]:

- Inflammation of all part of a breast
- Skin irritability or dimpling
- Pain in breast or nipple
- Nipple retraction
- Redness or thickening, scaliness of nipple or breast skin
- Discharge (other than breast milk) from nipple
- Formation of lump

Treatment of Breast Cancer [11]: The main treatment of breast cancer is radiation therapy, surgery, chemotherapy, targeted therapy and hormone (endocrine) therapy.

1. **Adjuvant Therapy:** This treatment should be depending on (i) to predict awareness to specific methods of treatment and to get benefitted from their use (ii) there is a liability of relapse.
2. **Endocrine Therapy:** Blocking hormone or balancing hormone is the main purpose of endocrine therapy.
3. **Chemotherapy:** In case of estrogen-negative tumors, the benefit from chemotherapy is more pronounced.
4. **Neoadjuvant Therapy:** It may allow for accomplishing operability or reducing the extent of surgery.

Liability Aspects of Breast Cancer [11]:

A. Non-Preventable Liability Aspects:

1. Gender: Woman is more prone to have breast cancer and about 100 times more familiar amongst women than men.

2. Age: Possible risk factors of growing breast cancer increases with age. Mainly breast cancer found in women with age of fifty five or older.

3. Hereditary Risk Factors: The two autosomal dominant genes, BRCA1 & BRCA2 are responsible for maximum cases of genetic breast cancer. BRCA mutations have 65-85% risk of growing breast cancer.

4. Family History: The risk increases if woman's mother, father, child or sister has been diagnosed with ovarian or breast cancer.

B. Preventable Liability Aspects:

1. Overweight: A corpulent woman is at greater risk of being diagnosed with breast cancer than women with healthy weight. If a woman is overweight there is growing risk of recurrence breast cancer.

2. Hormone Replacement Theory: Estrogen replacement therapy is used to relieve symptoms of osteoporosis and menopause but also cause more liability of breast cancer.

3. Alcoholic: There is linear and dose dependent relationship with alcohol consumption which rises the risk of developing breast cancer.

4. Smoking: Younger and premenopausal women are at greater risk of developing breast cancer if they have habit of smoking.

Diagnosis of Breast Cancer [12]:

Table 2: Conventional Breast Screening Methods [12]

Type	Use
Mammography	At the same time mass screening, soft tissue and blood vessels, image bone are done
Ultrasound	It evaluates lumps found in mammography
MRI	It images small particulars of soft tissues
CT	It is a single exam to diagnose and image distant metastasis
PET	It is functional imaging of biological process

MATERIALS AND METHODS

1. ChemSketch
2. Protein Data Bank (PDB)
3. LigPlot (PDBsum Web Server)
4. AutoDock Tools
5. ALOGPS
6. E-Dragon
7. PaDEL Descriptors
8. PubChem Web Server

1. **Introduction to ChemSketch:** ChemSketch is a 3D modeling software which is designed by chemistry programming. It is a freeware package of tools that makes it simple to generate 3D model as well as Lewis structure, space filling model, ball and stick models. It is available for the PC as well as Mac platform. By using this software we designed the sample and standard molecule structure [13,14].
2. **Protein Data Bank (PDB):** The single worldwide collection of structural statistics of biological macromolecules is the Protein Data Bank (PDB) [15,16].
3. **LigPlot:** LigPlot is a computer program which necessarily generates schematic diagrams of protein - ligand interaction for a certain PDB file. Hydrogen bonds are marked by dashed lines & hydrophobic contacts are represented by an arc [17,18,19,20].
4. **AutoDock Tools:** Autodock is a suite of computerized docking tool. It is created to anticipate small molecules such as substrates bind to a receptor of well-known 3D structure [21,22].
5. **ALOGPS:** ALOGPS is the program tool that can find the logP value, pka and water solubility of compounds for prediction of ADMET [23,24].
6. **E-Dragon:** E-Dragon is an electronic remote version of well known version of dragon which can predict ADMET of a molecule [25].
7. **PaDEL Descriptors:** PaDEL Descriptor is the important tool for detecting different types of molecular descriptors present in molecule structure [26].
8. **PubChem Web Server:** This program is used for identifying and designing the molecule structure [27,28,29,30].

RESULTS AND DISCUSSION

1. **Visualization of Receptor & Preparation of Conformation of Receptor:**

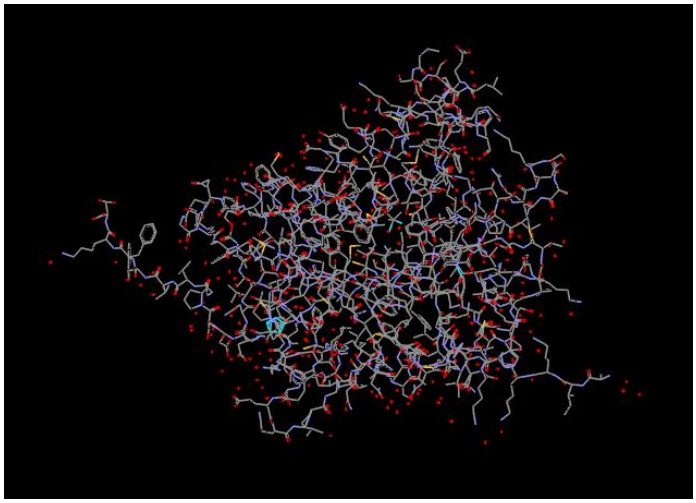


Figure 1: Receptor Structure (2HBQ)

Source: Protein Data Bank (PDB) & AutoDock Tools

2. Prediction of Active Site:

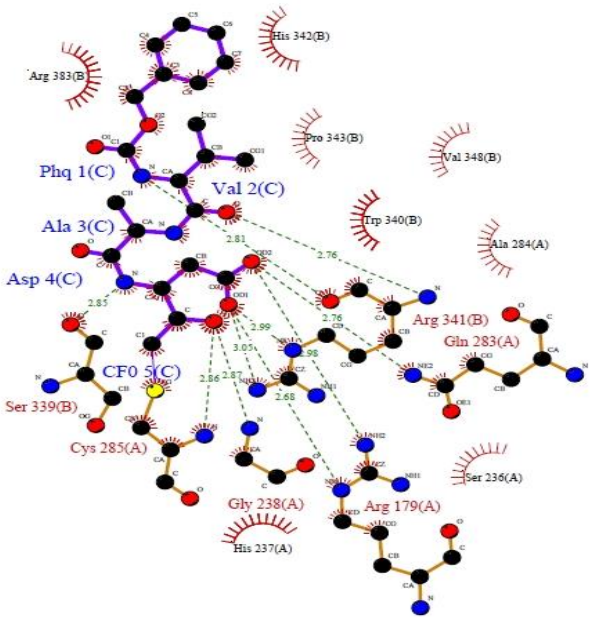


Figure 2: Ligplot of 2HBQ (by using PDBsum Web Server)

3. Prediction of Active Site:

Table 3: Prediction of Active Site

Receptor	X Coordinate	Y Coordinate	Z Coordinate
2HBQ	34.75	62.01	2.50

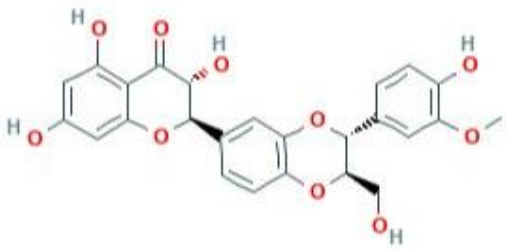
4. Binding of Standard Drugs to 2HBQ:

Table 4: Docking of Standard Molecules to 2HBQ

S. No.	Name of Drugs	Binding Energy (kcal/mol)
1	Apigenin	-3.62
2	Catechin	-3.56
3	Chrysin	-3.25
4	Daidzein	-3.29
5	Genistein	-3.54

5. Binding of Test Drug to 2HBQ:

Table 5: Docking of Test Molecules to 2HBQ

Name of Test Drug	Structure of Silibinin (Source: PubChem Web Server)	Binding Energy (kcal/mol)
Silibinin		-4.06

6. ADMET Prediction:

Table 6: ADMET Prediction

Name of Compound	Absorption	Carcinogenicity	Hepatotoxicity	BBB Level	Mutagenicity	logP (Partition Co-Efficient)
Silibinin	Good	Nil	Nil	Poor	Nil	2.35

CONCLUSION

Flavonoids are a family of phytonutrient compounds which have two phenyl ring attached to a heterocyclic ring. In the current research, we are focusing on flavonoid compounds which are the part of polyphenol class of phytonutrient. Due to their activity, flavonoids have drawn enough consideration towards their application in anti-cancer therapies.

In the current study, we focused five flavonoids on the ground of their best docking scores against breast cancer. Breast cancer inception and advancement is triggered by certain cellular downstream signaling pathways. According to the reports, silibinin shows binding energy against caspase 3 receptor through caspase 3 dependent mechanism.

Flavonoids are bountiful flavonoids which are found normally in vegetables and fruits like onions, tea, grapes, strawberries, citrus fruits.

Based upon the binding affinities as displayed by the docking application supported by the computational strategies, the current research shows that silibinin (flavonoid) can be studied as an effective anti-breast cancer. Also, this compound can be further examined by accomplishing *in-vitro* and *in-vivo* research on breast cancer models for the management and prevention of breast cancer.

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