"In silico analysis on plant determined flavonoids compounds with HER2 and Estrogen Receptor to limit the utilization of synthetic medications that aided in breast cancer growth"

To be submitted as Major Project in partial fulfillment of the requirement for the degree of

Master of technology

In

Bio-Informatics

Submitted By

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(2k15/BIO/05)

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DECLARATION

I Garima Singhal, hereby declare that the work entitled **"In silico analysis on plant determined flavonoids compounds with HER2 and Estrogen Receptor to limit the utilization of synthetic medications that aided in breast cancer growth"** submitted to Department of Biotechnology, Delhi Technological University as a result of the work carried out by me as a major project.

Date:

Garima Singhal

2K15/BIO/05

CERTIFICATE

This is to certify that the dissertation entitled **"In silico analysis on plant determined flavonoids compounds with HER2 and Estrogen Receptor to limit the utilization of synthetic medications that aided in breast cancer growth"** in the partial fulfillment of the requirements for the award of the degree of Masters of Engineering, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by her under my guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honoring of any other degree.

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ACKNOWLEDGEMENT

I would like to express my gratitude to **Delhi Technological University, New Delhi** for giving me opportunity to be a part of it. I would like to sincerely thank **Prof. D. Kumar** Head of Department for his kind leadership.

Most of all I am very highly obliged to my project advisor **Dr. Navneeta Bharadvaja**, **Assistant Professor, Department Of Biotechnology**, Delhi Technological University, New Delhi for her exemplary guidance, monitoring and constant encouragement. I would also like to thank her for sparing the efforts in compiling the work presented here.

I would also like to thank my parents and my friends and non- technical staff of Department of Biotechnology for giving me support.

Garima Singhal

2K15/BIO/05

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"In silico analysis on plant determined flavonoids compounds with HER2 and Estrogen Receptor to limit the utilization of synthetic medications that aided in breast cancer growth"

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ABSTRACT

Breast cancer disease development is one of the veritable prosperity stresses in India bringing over the most critical passing rate in females, which happens in view of uncontrolled cell division and can be metastasize to different parts of the human body, and different medications are accessible to its cure. Drugs like Tamoxifen and Herceptin can cure breast cancer disease however these medications have their unsafe impacts on human body. The research here manages docking, toxicity, bioactivity and ADME analysis of flavonoids compounds with HER2 and estrogen receptor, to limit the utilization of destructive medications. Lipinski's channel is utilized to screen the flavonoids compounds on the premise of five guidelines. Out of 200 flavonoids compounds 15 compounds were screened on the premise of Lipinski's channel. The outcomes uncovered that the top positioning screened flavonoids indicates most extreme docking and least binding energies with the HER2 and ER receptor when contrasted and the accessible medications. The above analysis demonstrated the compounds ST026594 (7-hydroxyflavone), ST070967 (2-(- 4-fluorophenyl)- 4n-chromen-4-one), ST086622 (3-hydroxyflavone) and ST055369 (8-methylflavone) were the best compounds indicating minimum binding energies in examination with medication Tamoxifen with Estrogen receptor and compounds ST060160 (4-hydroxyflavone) and ST058442 (6,3-dimethylflavone) were the best compounds demonstrating minimum binding energies in correlation with medication Herceptin with HER2 receptor, were additionally bioactive and non harmful in nature with great pharmokinetics properties and drug likeliness.

INTRODUCTION

Breast cancer is one of the lethal maladies that bringing about higher death rates in females around the world. Breast development causes when cells in the breast tumor begin to end up plainly wild and can be metastasize to various parts of the human body. The breast cancer can be estrogen positive and HER2 (human epidermal growth factor receptor) positive. There are different sorts additionally, however here we are managing ER and HER2 positive breast cancer.

In estrogen positive breast cancer the cells develop because of hormone estrogen. Around 80% of the breast cancer are of estrogen positive cancer, and about20% of breast cancer, the cells make excessively of protein known as human epidermal growth factor receptor 2 protein (Waraphan *et al.,* 2011). The HER2 quality makes HER2 protein, HER2 proteins are receptors on breast cells. In 25% of breast cancer the HER2 quality doesn't work suitably and makes an extreme number of copies of it known as HER2 gene amplification. This makes breast cells create and segregate in an uncontrolled way. This kind of cancer is called HER2 positive. While in estrogen receptor positive cancer the cancerous cell are getting development signals from estrogen proteins. The most well-known receptor is estrogen receptor-α include in ER positive cancer. (Dickson and Stancel, 2000).

In spite of the fact that these sorts of cancer are reparable if recognized at early stage, different medications are accessible for the treatment of ER positive and HER2 positive breast cancer. The medications like Tamoxifen, Raloxifene, Toremifene are at present being used to help ER positive cancer and Herceptin is utilized as a part of treating HER2 breast cancer (Fabian and Kimler, 2001). Ingestion of these medications causes many reactions, for example, blood clumps, strokes, uterine cancer (Parkkari *et al.,* 2003; Mojgan *et al.,* 2009).

The symptoms of these medications made us to investigate an option and customary way to deal with discovering new medication compound from the regular Flavonoid, compounds which are having hostile to breast cancer action and furthermore not having any reactions to human typical cell (Kawaii *et al.,* 1999; Pouget *et al.,* 2001). Flavonoids are plant compounds have high restricting partiality for breast cancer receptors, flavonoids are polymers of common polyphenolic compounds found in organic products, tea, red wine, oats, vegetables and show hostile to oxidant, against cancer-causing, calming, against proliferative properties.(Paola

Galluzzo and Maria, 2006). The objective of this research is to clarify the auxiliary elements of flavonoid derivatives against the receptor proteins by insilico analysis i.e. by performing docking, by bioactivity prediction, by toxicity quality checking, ADME examination. The examination should be possible by the different bioinformatics tools and to discover the drug likeliness. This strategy for finding new medication by insilico approach known as Computer Aided Drug Discovery (CADD).

Insilico CADD systems can be directed as key to create and screen the compounds or medications to make successful leads for treatment of various maladies. Structure based drug designing strategies includes the 3-D structure of protein on which docking analysis of a few particular little moleculs have been have been approved in order to calculate their docking score and binding affinity of ligands and their cooperating residues. The virtual screening and molecular docking of the medication competitors on stamp protein could find to finalize the lead. (Rajamani *et al.,*2007). The research manages the docking of receptor with flavonoid derivatives taken after by bioactivity determination by Molinsipiration online tool, Toxicity checking by lazar online tool and by toxicity checker and ADME examination.

OBJECTIVES

1. Drug target identification
2. Active site prediction of protein receptor
3. Screening of flavonoid compounds by lipinski's channel
4. Docking of screened compounds and receptor protein
5. Bioactivity prediction, lethality checking and ADME investigation of screened flavonoid compounds.

REVIEW OF LITERATURE

Breast cancer is a standout amongst the most widely recognized cancers in ladies everywhere throughout the world. It is the main source of death from cancer consistently among ladies. The recurrence of breast cancer has been expanding comprehensively, because of increment future, increment urbanization and taking up of western ways of life.

Estrogen receptor is a ligand-actuated translation figure made out of DNA restricting space; estrogen and its receptor are critical for sexual improvement yet over creation of estrogen upgrade unnatural development and cause cancer (Salih AK *et al.,* 2001).

Estrogen and its receptor (ER) assume imperative parts in creating threatening sort of breast cancer. The interpretation of different qualities as a translation variable is controlled by ER-α, which ties to an upstream component known as estrogen response elements (ERE). ER-α expression is firmly related with breast cancer science, the advancement of tumors particularly; for instance, breast carcinomas which need articulation of ER alpha frequently uncover more forceful phenotypes. Concentrate the components that manage the translation of the ER-α quality may in this manner give knowledge into the comprehension of breast carcinogenesis (S.I Hayashi *et al.,* 2003). Human epidermal growth factor receptor 2 (HER2) is over communicated in around 20–30% of breast cancer tumors. It is connected with more forceful malady, expanded mortality, higher repeat rate; and. Herceptin is a HER2 receptor blocker that has turned into the treatment of HER2 positive breast cancer. (Zahi Mitri *et al.,* 2012). Herceptin has been appeared to be viable in blend with chemotherapy, for the treatment of early stage and metastatic HER2 positive breast cancer.

Many mechanisms of action and literature have been identified, that medications which are utilized for treating breast cancer have symptoms, including strokes, uterine diseases, blood clumps. The poisonous quality of Tamoxifen and Herceptin has been examined in the human retinal color epithelium in vitro, the medication lessen the movement of chemicals in retinal pigment cells (Parkkari *et al.,* 2003).

The side effects of the currently used medication made us to analyze an option and customary way to deal with discovering new medication compound from the regular Flavonoid, compounds

which are having anti breast cancer activity and furthermore not having any symptoms to human typical cell (Kawaii *et al.,* 1999; Pouget *et al.,* 2001).

Some plant sustenance's contain compounds that may have long haul consequences for human and animals wellbeing other than being a wellspring of compounds important for human nutrients. Among the most critical are the plant optional metabolites flavonoids. Flavonoid most ordinarily isoflavones have a long history in science. Alluded regularly to as powerless estrogens and they were blended synthetically before the ring structure of the mammalian steroids was resolved in the 1920's and 1930's (Barnes, 2004). Flavonoids and isoflavonoids are presents in food glycosidic conjugates as or, for the most part, as aglycone, however isoflavone glycosides have not been identified in human blood plasma or pee (Xu *et al.,* 1994). It polyphenols once eaten, then it enter a complex pathway of bio-transformation so that, the molecular forms reaching the peripheral circulation and tissues to be excreted are usually different from those present in foods (Manach *et al*., 2004).The majority of the classes of flavonoids (flavones, flavanones, 2′-hydroxychalcones and flavan-4-ols) were examined for their anti-proliferative action against MCF-7 human breast cancer cells. Structure–activity relationships of these compounds were examined. 2′-hydroxychalcones and methoxylated flavanones were observed to be intense inhibitors of MCF-7 cells development though flavones and flavan-4-ols gave off an impression of being frail inhibitory operators with the exception of 7, 8-dihydroxyflavone. (Pouget C *et al.,* 2001).

More than 4000 flavonoid compounds have been distinguished till date and a number of which have against tumor activity (Thomas *et al.,* 2012). Therapeutic outcomes indicate that the regular intake of fruit and vegetables can decrease the frequency of cancer.

There are a few medications accessible for the treatment of the breast cancer, most usually utilized medications are herceptin and tamoxifen yet these medications have their symptoms on ordinary human cell, the medication does not diminish the rate of ER and HER certain cancer yet molecular abnormities were likewise seen in the ladies who were expending these medications. (Fabian and Kimler, 2001).

The insilico approach of the compounds on target protein could discover the lead like compounds. This approach drives us to discover new medications and decides the medication

resemblance of these new particles by estimation of the Lipinski's Rule of Five, trailed by docking, bioactivity checking of compounds, danger checking and ADME analysis. Configuration based drug designing approaches incorporates the 3-D structure of protein on which docking studies of a few individual small molecules have been affirmed keeping in mind the end goal to process docking score and binding energy by utilizing a progression of scoring function.(Rajamani R *et al.,* 2007). Insilico strategies are for the most part conveyed and put to use to lessen the time, cost and hazard related with Drug Discovery. Molecular docking is a technique which predicts the more favorable orientation of one molecule to a second when bound to each other to form a stable complex (Lengauer T *et al.,* 1996). In this study, we took flavonoids as blocker for estrogen receptor and alongside the medication tamoxifen and flavonoid as inhibitor for HER2 receptor to upset the gene amplification. Further we have done bioactivity, toxicity and ADME analysis and found that these flavonoids could go about as better medications in examination with known medications. Lipinski's lead is utilized to channel the compounds taken after by docking.

The Computer-supported drug discovery (CADD) includes two primary systems, right off the bat target-based and also, ligand based methodologies. Target based drug discovery (TBDD) relies on upon the structure of the objective and its collaborations with the ligands. While ligand-based drug discovery (LBDD) is depends on the basic data and molecular properties of known ligands as it were. It doesn't require the information of target structure or its cooperations with ligands. (Vilar and Costanzi, 2012).

Auto Dock is molecular modeling simulation software. It is particularly successful for Protein-ligand docking. Auto Dock 4 is accessible under the GNU General Public License. Auto Dock Vina is accessible under the Apache permit. Auto Dock includes two fundamental projects: Auto Dock for docking of the ligand to a set of grids representing the target protein; Auto Grid for pre-calculating these networks. (B.Revathi Mani *et al.,* 2014). Docking is a procedure by which the best configuration of binding molecules is resolved. In this procedure, a complex structure is acquired having stable structure (Jun KY *et al.,* 2011). Information of desirable orientation thus can be utilized as a part of foreseeing quality of relationship between the two particles and binding energy can be measured regarding scoring function.

Docking is often used for predicting the binding of ligand candidates to their protein target to predict the activity and binding affinity of the ligand compound. Therefore, docking perform a very significant character in the rational drug design (Trott O et al., 2010).

Docking programs utilize a scoring function that can be viewed as a effort to surmise the standard substance possibilities of the framework. At the point when the externally material science based terms like the 6–12 van-der Waals interactions and Coulomb energies are utilized as a part of the scoring function, they should be altogether experimentally weighted, to a limited extent, to represent this distinction amongst energies and free energies (Gilson M *et al.,* 1997).

Docking approach incorporate two methodologies which are prominent in molecular docking group. To begin with approach manages a strategy for coordinating where protein and ligand molecular surfaces are accounted for as corresponding to each other. The second approach utilizes docking process that happens and the interaction energy of protein-ligand complex is resolved (Morris G *et al.,* 1998).

MATERIALS AND METHDOLOGY

1. NCBI

NCBI is one of the parts of National Institute of Health Branch (United States National Library of Medicine). There are number of database accessible in NCBI which are valuable for biomedicine and biotechnology. Real databases are: Genbank for DNAsequencing, Pubmed for literature and so forth. The NCBI here is utilized here to get all the writing identified with breast cancer malignancy, to think about the current reviews that are going on breast cancer growth.

2. PubCem Database

It is part of NCBI and is a database of chemical molecules and their activity against biological essays. The flavonoids compounds 3D structures were downloaded from this database**.**

3. PDB

The protein data bank is a repository for 3D biological molecules (proteins and nucleotides) structural data. The structural data found experimentally by NMR (Nuclear magnetic resonance) spectroscopy or X-ray crystallography and put in by the biochemist, scientists or biologists and are accessible freely on internet. It helps student, teachers and researchers to understand all aspects of biomedicine. Users can search protein molecule by PDB ID, by macromolecule name and download the required file in different format.

4. SCF-IITD

Supercomputing facility at IIT-Delhi has various online tools and softwares which can be used for ligand screening and ligand optimization. One can predict active site of a protein or ligand structure also this tools is used to check lipinski's filter, easily accessible online.

5. Molinspiration Chemoinformatics Software

Molinspiration software is used to predict the bioactivity score of the compounds. The online tool supports the SMILE or SDF file format to run the software. The results include

bioactivity of compound and drug likeliness towards GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitor and other enzyme target.

Compound should be Active when score lies (bioactivity score>0), moderately active(-5.0-0.0) and inactive(<-0.5).

6. **Lazar toxicity prediction tool**

   Lazar toxicity prediction tool takes a chemical structure or SMILE format as input and provides predictions for a variety of toxic properties mainly to check ability of a compound to cross blood brain barrier and carcinogenicity.

7. **Toxicity Checker**

   As the name suggests, toxicity checker aims to identify whether any toxic substructure of the query compound is available or not and it also calculates the different properties of the compounds. It is available freely to the users, easily accessible. This tool checks toxicity of a compound either by input of chemical structure or by input of SMILE format.

8. **SwissADME**

   The SwissADME web tool presented here is freely accessible at http://www.swissadme.ch and meant for user-friendly submission and easy analysis of the results, also for nonexpert in CADD. This tool is use to predict physiochemical properties of a compound as well as to predict ADME(absorption, distribution, metabolism and excretion) parameters, pharmokinetics and drug like nature to support drug discovery.

9. **Discovery studio 4.1**

   Discovery Studio is software for simulating small molecule and macromolecule systems. It is developed and distributed by Accelrys. Here discovery studio is used to generate separate PDB format files of both ligands and receptors for further docking.

10. **AutoDock 4.2.5**

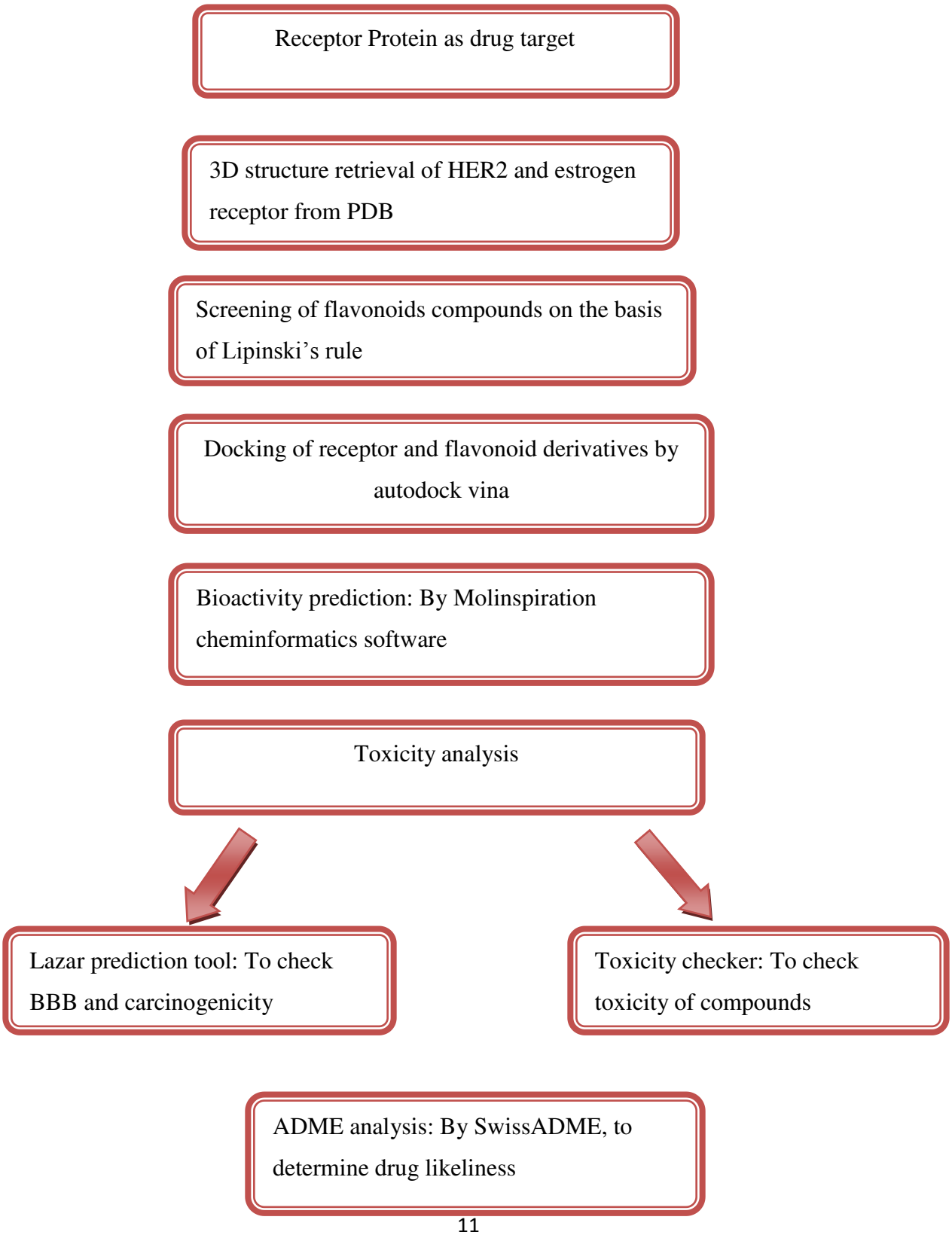
    AutoDock 4.2.5 is software used for the purpose of molecular docking of ligand to macromolecules like proteins. There are two main programs in AutoDock:

- AutoGrid program for the identification of pre-computing grids, and
- AutoDock program for docking ligand molecule to a number of grids of the target protein. Binding energy calculated is the combination of intermolecular and torsional energies.

11. Pymol

Pymol is protein molecule visualization software. This software is used for visualization of docked compounds with protein structure shows overlapping of ligands in multiple conformations; it displays binding of ligand at protein surface.

STEPS:


Receptor Protein as drug target
3D structure retrieval of HER2 and estrogen receptor from PDB
Screening of flavonoids compounds on the basis of Lipinski's rule
Docking of receptor and flavonoid derivatives by autodock vina
Bioactivity prediction: By Molinspiration cheminformatics software
Toxicity analysis
Lazar prediction tool: To check BBB and carcinogenicity
Toxicity checker: To check toxicity of compounds
ADME analysis: By SwissADME, to determine drug likeliness

1. **Receptor protein as target**

   Breast cancer mainly of two types one Estrogen positive and other is HER2 positive, Estrogen positive is associated with hormone estrogen and estrogen receptor while HER2 gene encodes HER2 proteins which are receptors on breast cells, in cancerous cell this gene makes too many copies of itself, HER2 gene tell breast cell to make HER2 receptor. Thus these two receptors were used as target protein with flavonoids in comparison with existing drugs.

2. **3D structure retrieval of target protein**

   The three dimensional protein crystal structure of Human Estrogen Receptor and Human Epidermal growth factor2 (HER2) receptor were retrieved from PDB (http://www.rscb.org/pdb). The complex molecules associated with protein and non essential waters were removed by using discovery studio.

3. **Ligand preparation**

   The flavonoids were downloaded in 3D format from PubChem database and downloaded flavonoids screened by using Lipinski's filter on the basis of Lipinski's rule of five 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**.**

4. **Docking of protein receptor with the screened flavonoids derivative.**

   The proteins prepared in above step were used for docking by using AutoDock 4.2.5 with the flavonoids along with the drugs Tamoxifen and herceptin and compared the results. Those flavonoids which were showing less binding energy similar to binding energy of with drugs were considered to be more drugs like compounds.

5. Bioactivity prediction of compounds by using Molinspiration tool

The docked flavonoids which showed low binding energy with the protein receptors were analyzed with molinspiration tool for predicting bioactivity of the compounds to check whether the compounds were bioactive or not.

The bioactivity scores of compounds indicated the probability of good to moderate activity towards GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets. Active (bioactivity score>0), moderately active (-5.0-0.0) and inactive (<-0.5).

6. Toxicity analysis of bioactive compounds

The compounds which are found to be bioactive are analyzed further to check toxicity of compounds on human cells. To determine toxicity there were two tools used; first tool was Lazar prediction tool which determined the capability of compounds to cross Blood Brain Barrier (BBB) and to check the carcinogenicity of the compounds and second tool was Toxicity checker which uses SMILES sequence of the compounds or ligands and molecular structure was drawn and was checked for toxic substructure.

7. ADME analysis

The ADME (absorption, distribution, metabolism and excretion) analysis was done by using SwissADME tool available online which determines the liphophilicity, water solubility, pharmokinetics properties like GI absorption and drug likeliness of compounds.

RESULTS AND DISCUSSION

1. 3D structural view of estrogen receptor(PDB ID 5T92) and HER2 receptor protein(PDB ID 5JIH)

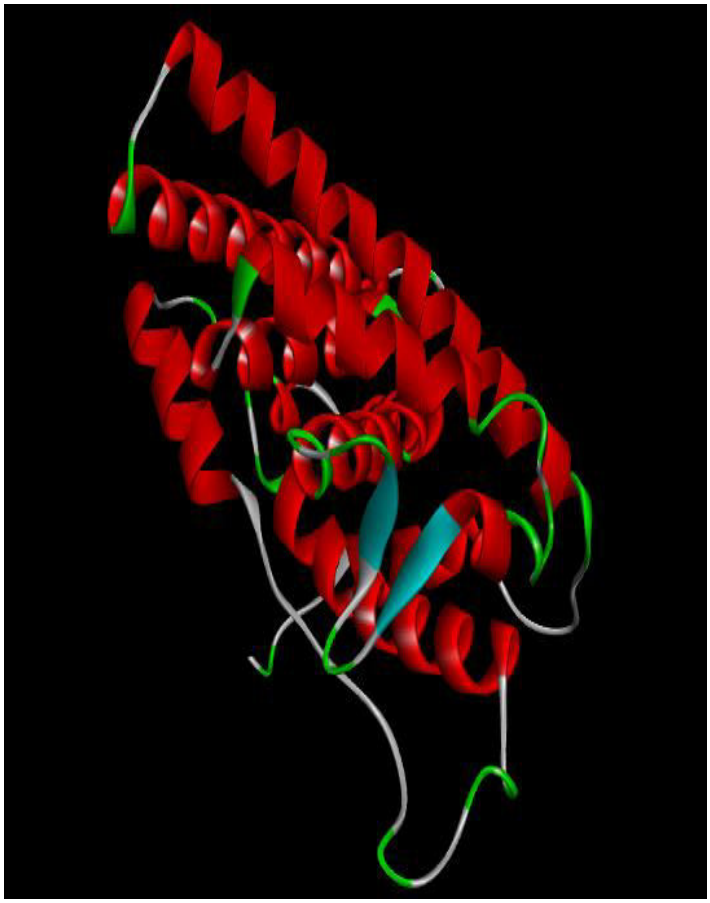


Fig1 (a). 3D structure of Estrogen receptor **Fig1 (b). 3D structure of HER2**

2. **Screening of ligand on the basis of Lipinski's rule of Five**

   The Fifteen flavonoids compounds were screened on the premise of lipinski's rule of five criteria i.e., a compound has not more than 5 hydrogen bond givers, not more than 10 hydrogen bond acceptors, molecular weight beneath 500 Daltons, partial co-efficient log P under 5, which is appeared in underneath table (I).

S.NO	Ligands (timtec ID)	Chemical Formula	Molecular Mass	Hydrogen Bond donors	Hydrogen Bond acceptors	LogP
1.	ST001473	C15H11ClO	242.71	0	1	3.891119
2.	ST019933	C17H16O	236.32	0	1	4.199539
3.	ST026594	C15H10O3	238.25	1	3	3.008399
4.	ST055369	C16H12O2	236.27	0	2	3.611218
5.	ST055369	C15H12O3	240.26	1	3	3.098699
6.	ST059082	C15H10O3	238.25	1	3	3.008399
7.	ST059919	C16H14O2	238.29	0	2	3.591299
8.	ST060160	C15H10O3	238.25	1	3	3.008399
9.	ST069348	C16H12O2	236.27	0	2	3.611218
10.	ST070967	C15H9FO2	240.24	2	3	2.993899
11.	ST072640	C15H12O3	240.26	2	3	2.993899
12.	ST079153	C15H12O3	240.26	1	3	3.098699
13.	ST086622	C15H10O3	238.25	1	3	3.188499
14.	ST019949	C18H18O	250.34	0	1	4.507959
15.	ST058442	C17H14O2	250.3	0	2	3.919639

Table (I). Flavonoids compounds screened by using Lipinski's filter

3. Visualization of docking results of receptors and flavonoids by PYMOL.

The Docking of compounds or ligands with that of receptors (HER2 and estrogen receptor) is completed by utilizing AutoDock 4.2.5 and the outcomes were envisioned by utilizing pymol. The over fifteen compounds have least binding energy scores in examination with the accessible medications.

Visualized result of docking: flavonoids derivatives with estrogen receptor(ER)

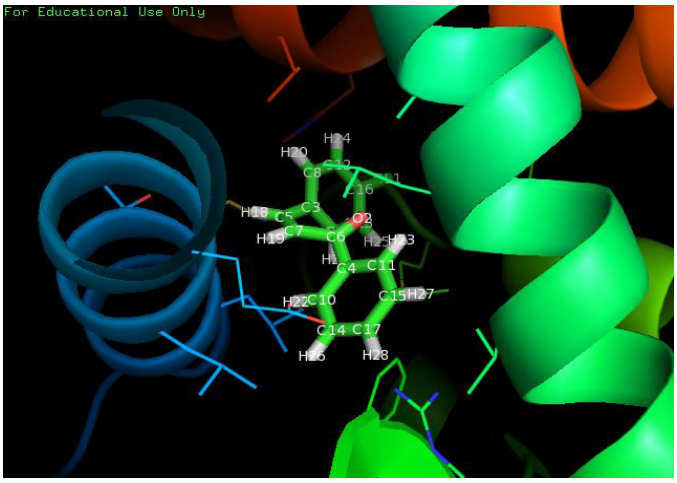


Fig.2 (a). ST001473 with ER

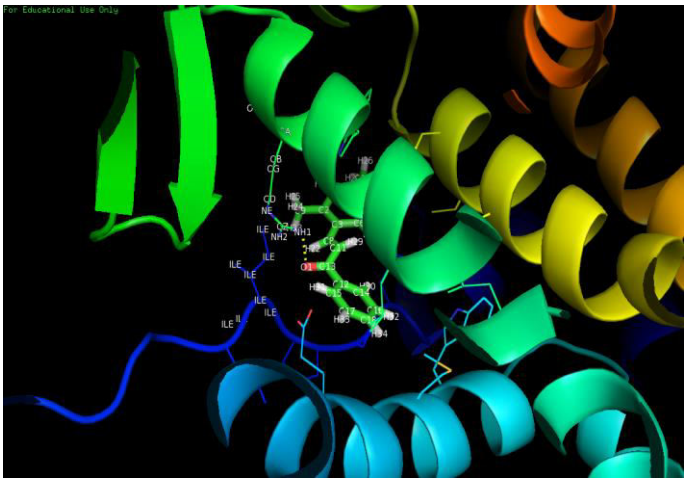


Fig.2 (b). ST019933 with ER

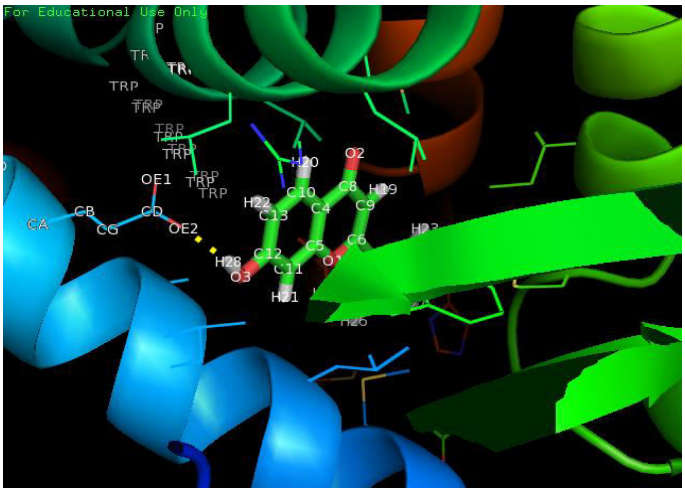


Fig.2 (c). ST026594 with ER

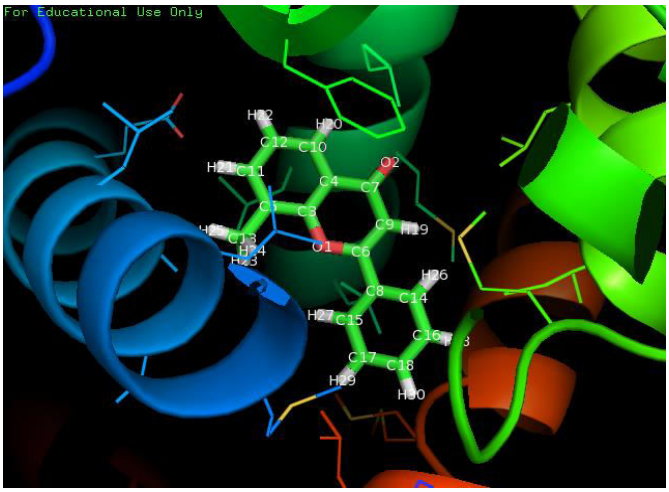


Fig.2 (d) ST055369 with ER

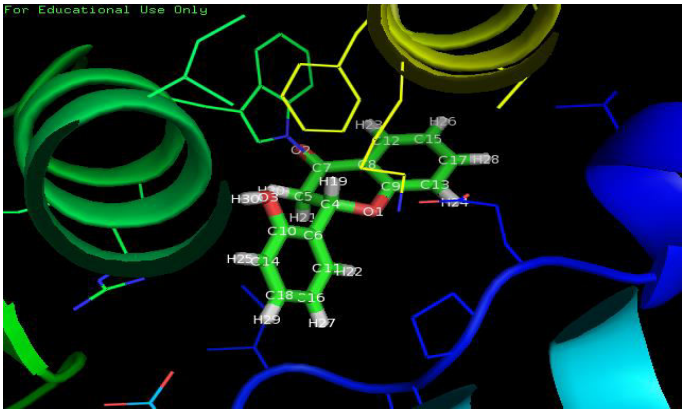


Fig.2 (e). ST059080 with ER

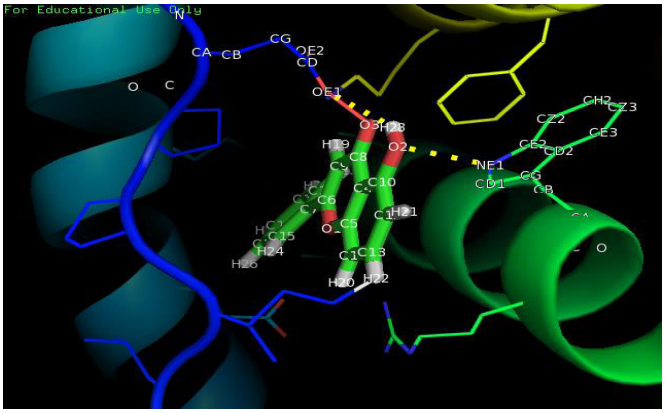


Fig.2 (f) ST059082 with ER

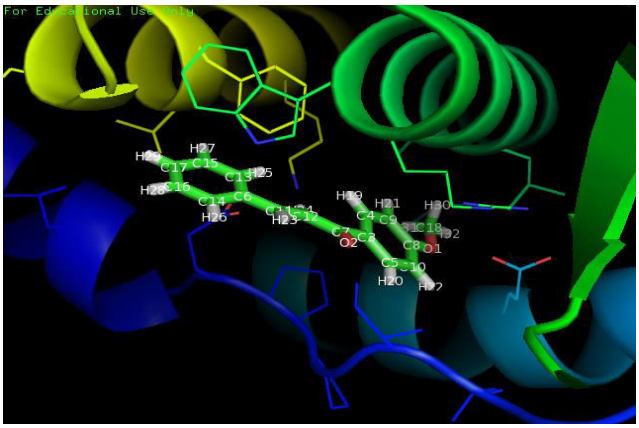


Fig.2 (g) ST059919 with ER

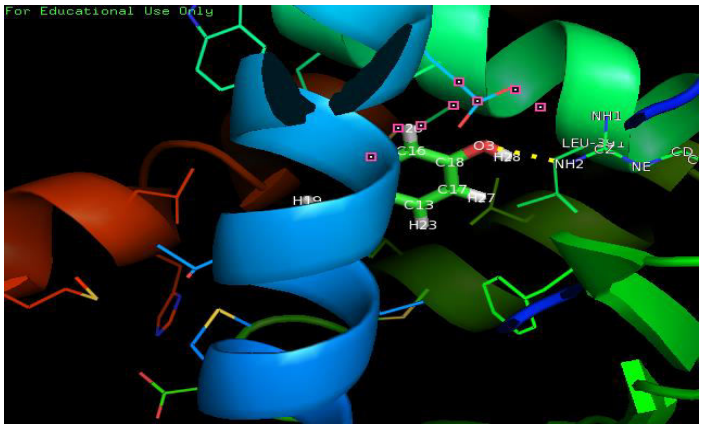


Fig.2 (h) ST060160 with ER

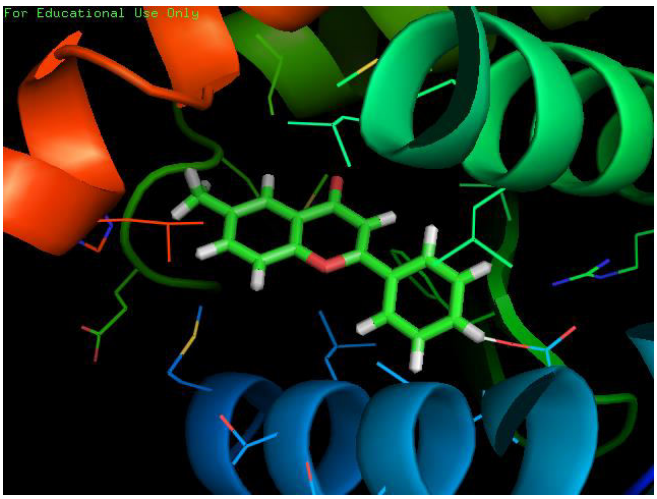


Fig.2 (i) ST069348 with ER

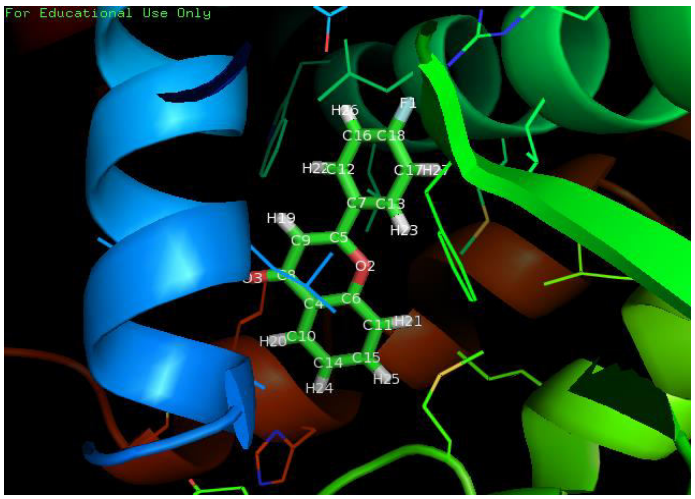


Fig.2 (j) ST070967 with ER

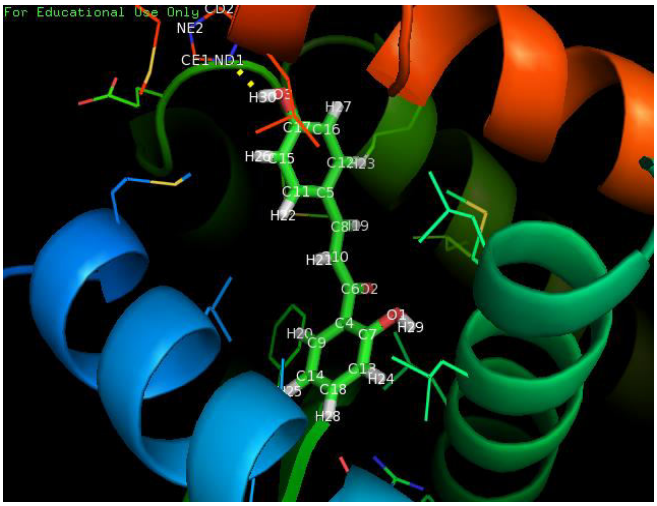


Fig.2 (k) ST072640 with ER

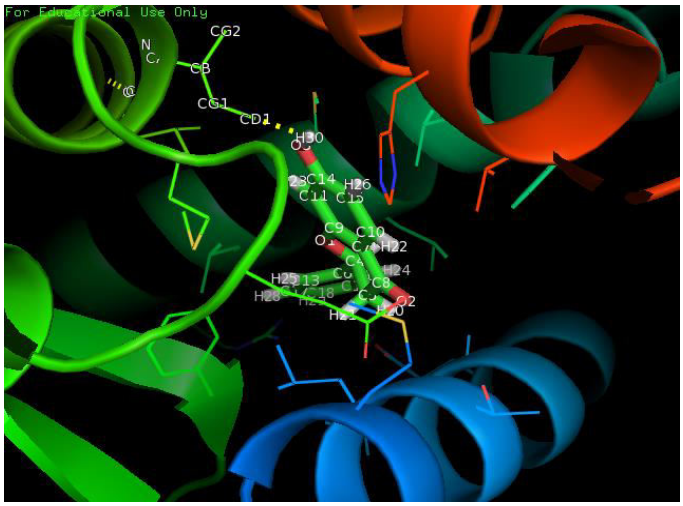


Fig.2 (l) ST079153 with ER

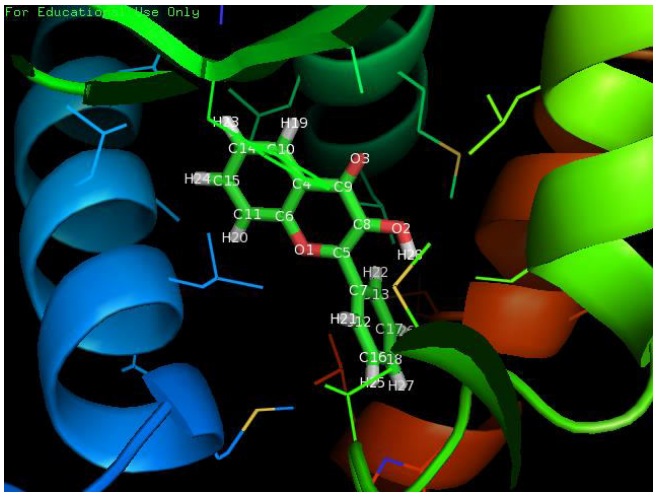


Fig.2 (m) ST086622 with ER

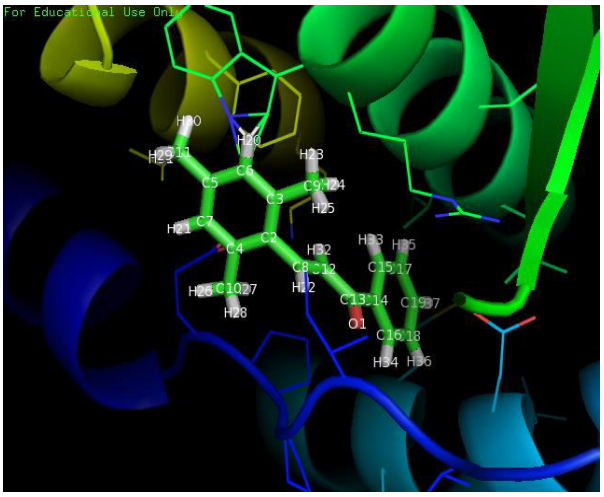


Fig.2 (n) ST019949 with ER

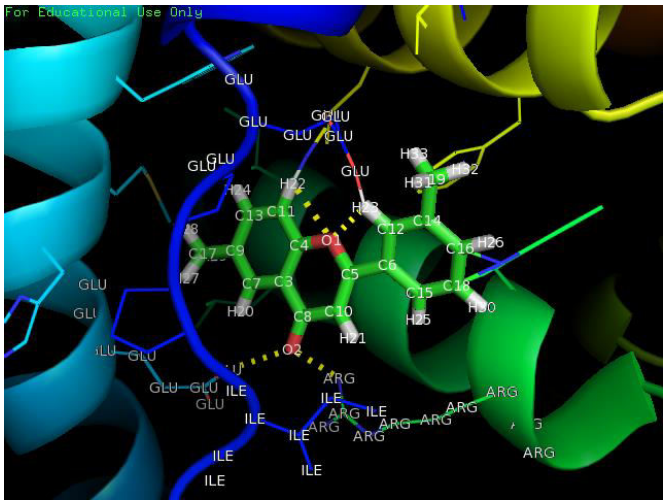


Fig.2 (o) ST058442 with ER

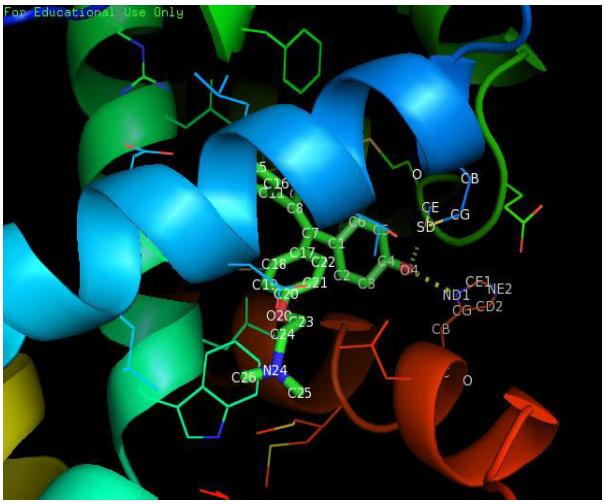


Fig.2 (p) Drug Tamoxifen with ER

Visualized result of docking: flavonoids derivatives with Human Epidermal growth factor 2 (HER2) Receptor

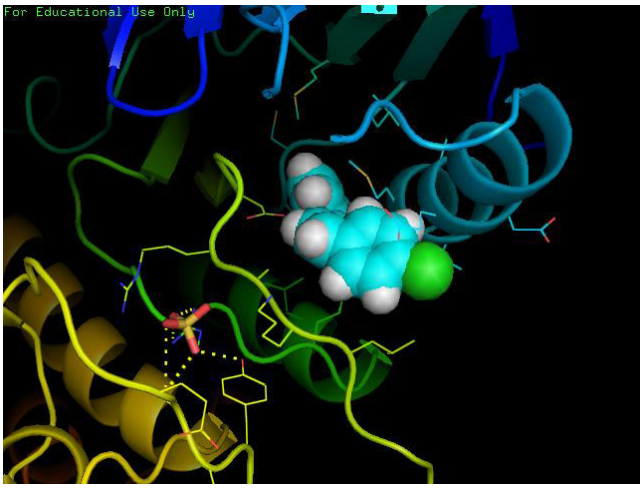


Fig.3 (a) ST001473 with HER2

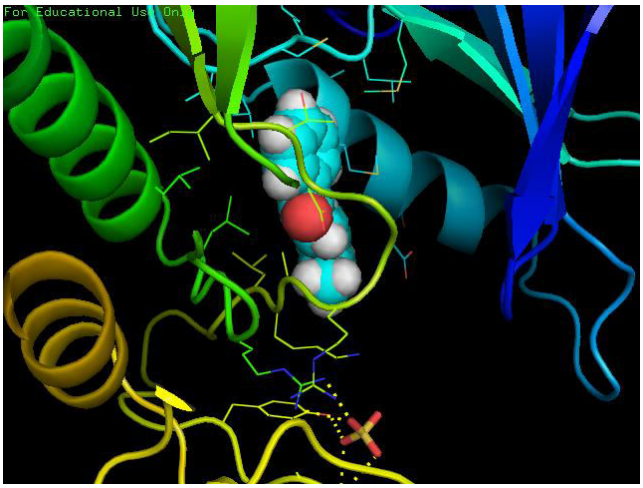


Fig.3 (b) ST019933 with HER2

Fig.3 (c) ST026594 with HER2

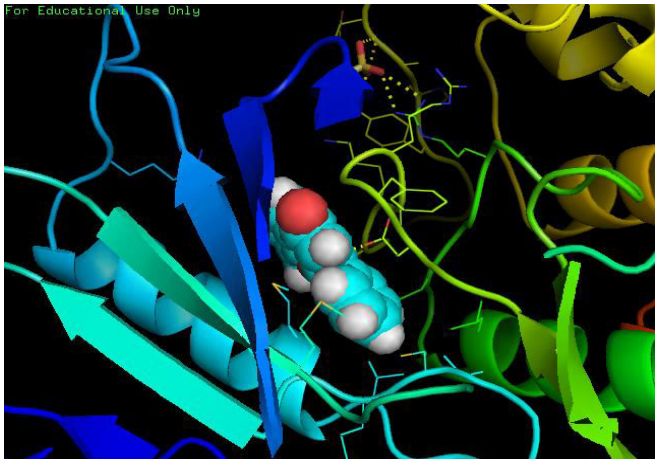


Fig.3 (d) ST055369 with HER2

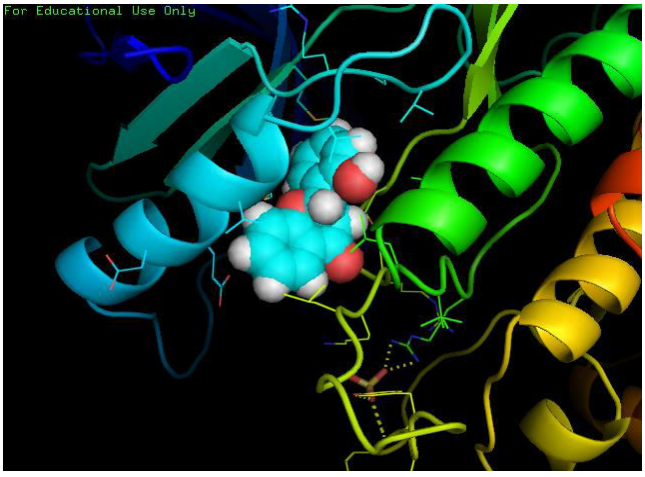


Fig.3 (e) ST059080 with HER2

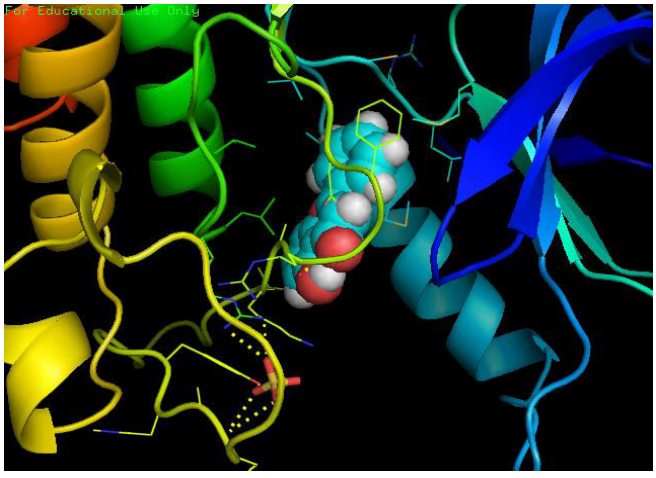


Fig.3 (f) ST059082 with HER2

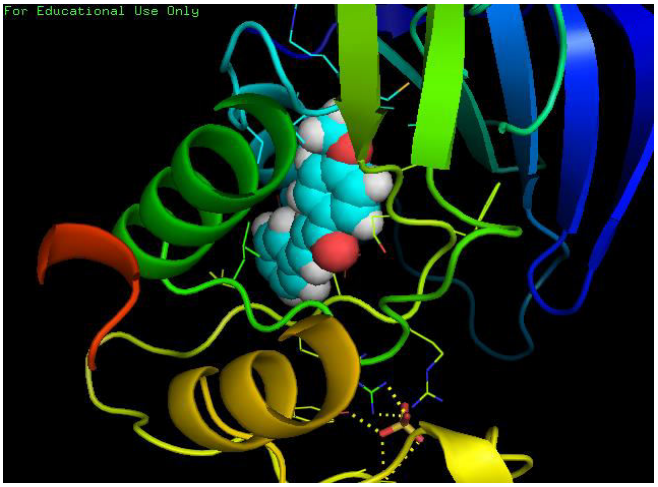


Fig.3 (g) ST059919 with HER2

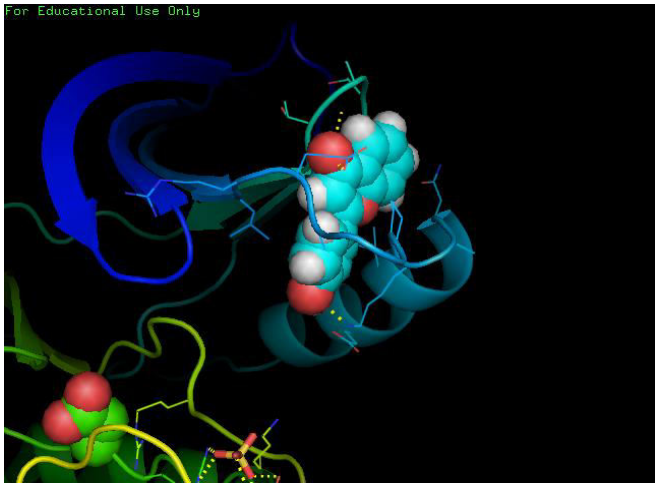


Fig.3 (h) ST060160 with HER2

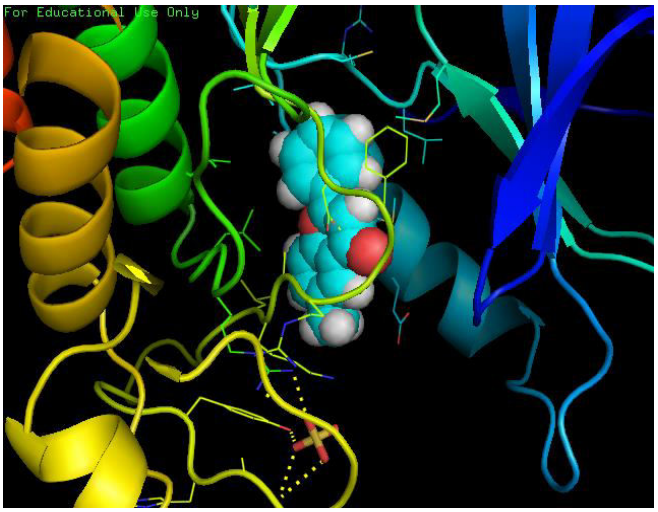


Fig.3 (i) ST069348 with HER2

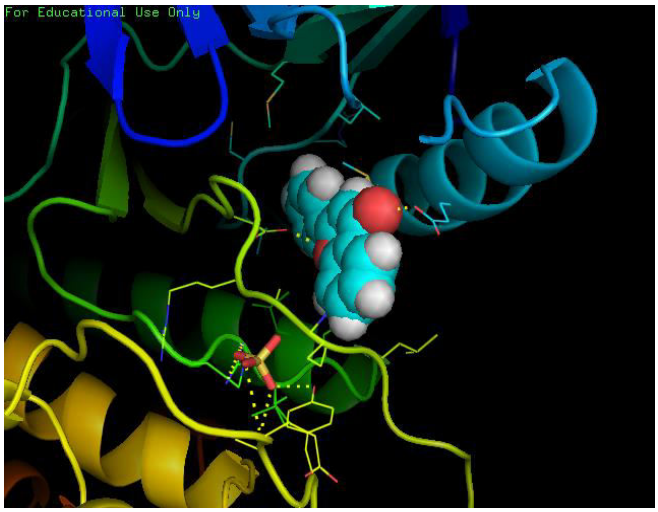


Fig.3 (j) ST070967 with HER2

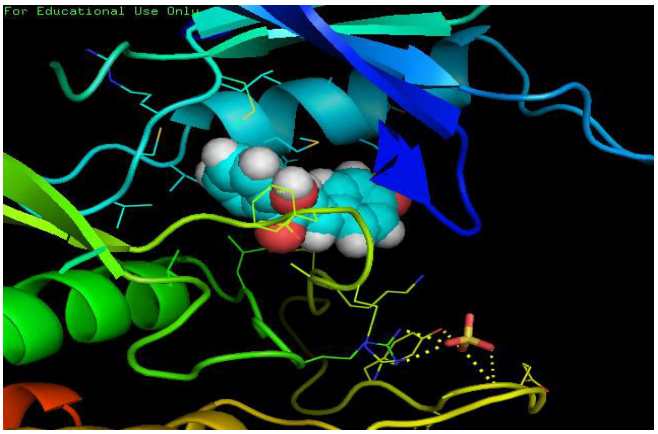


Fig.3 (k) ST072640 with HER2

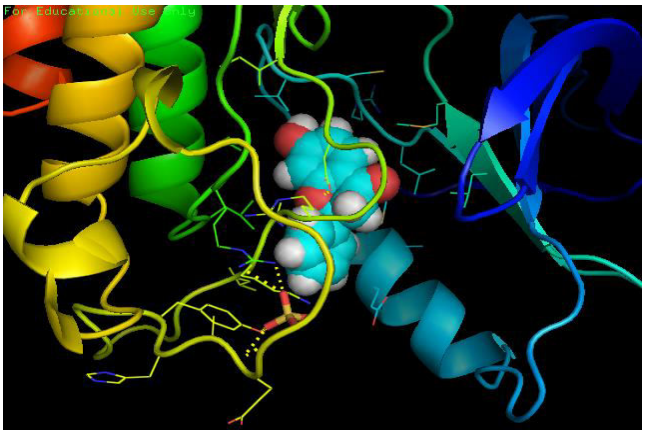


Fig.3 (l) ST079153 with HER2

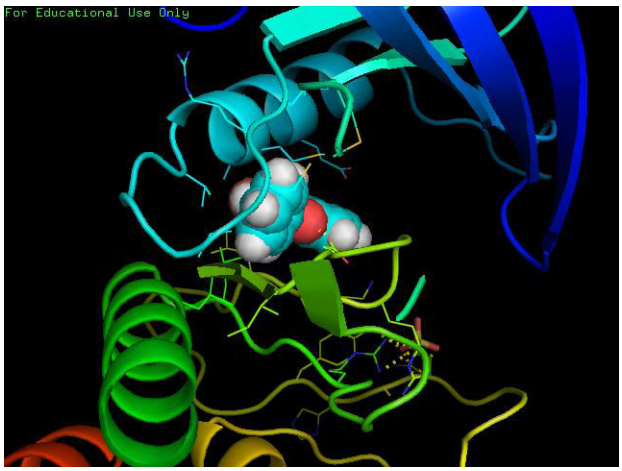


Fig.3 (m) ST086622 with HER2

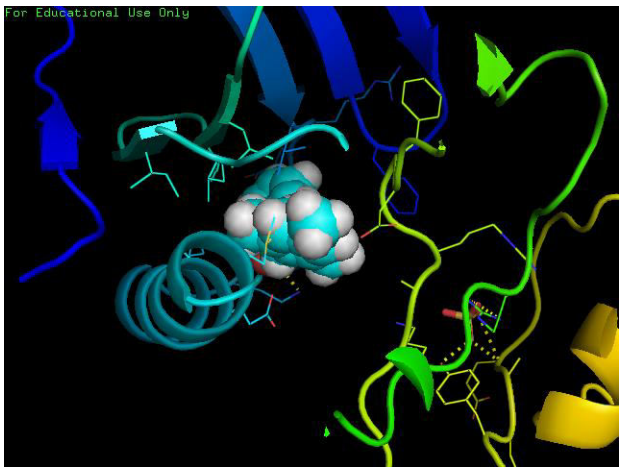


Fig.3 (n) ST019949 with HER2

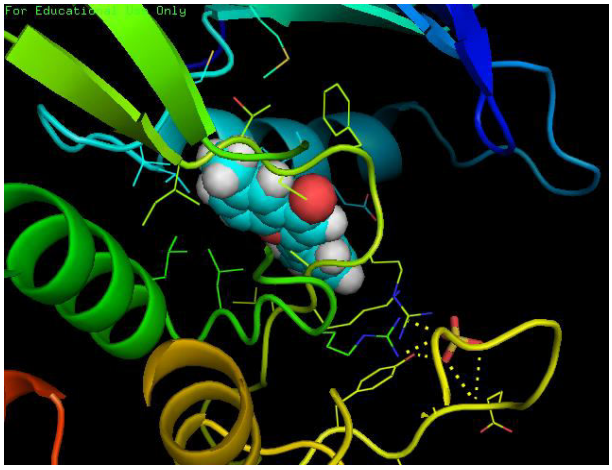


Fig.3 (o) ST058442 with HER2

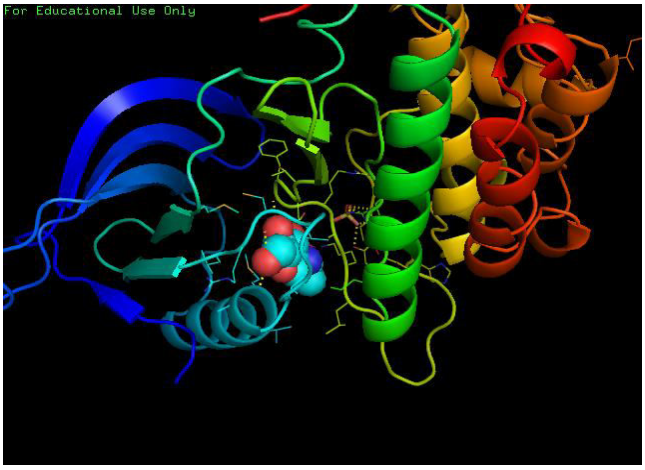


Fig.3 (p) Drug Herceptin with HER2

S.No.	Compound ID	Binding energy with ER	Binding Energy with HER2
1.	Tamoxifen	-8.92	-
2.	Herceptin	-	-8.98
3.	ST001473	-8.5	-7.5
4.	ST019933	-8.3	-7.3
5.	ST026594	-8.7	-6.1
6.	ST055369	-8.9	-6.01
7.	ST059080	-7.5	-7.3
8.	ST059082	-7.4	-7.5
9.	ST059919	-8.7	-8.8
10.	ST060160	-6.7	-8.9
11.	ST069348	-7.4	-7
12.	ST070967	-8.87	-7.3
13.	ST072640	-7.7	-8.72
14.	ST079153	-6.8	-7.2
15.	ST086622	-8.9	-8
16.	ST019949	-8.89	-9
17.	ST058442	-6.3	-8.83

Table (II). Binding energy of flavonoids compounds with ER and HER.

Flavonoids compounds those demonstrating least binding energy scores in correlation with the medications are test assist for Bioactivity. Those compounds which are observed to be bioactive are test advance for toxicity and for ADME analysis.

4. Results of Bioactivity prediction by Molinsipiration

I.D	GPCR ligand	Ion-channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
ST001473	-0.34	0.16	-0.51	0.45	0.5	0.13
ST019933	0.29	0.28	-0.1	0.37	-0.3	0.15
ST026594	-0.20	-0.17	0.0	0.10	-0.45	0.14
ST055369	0.12	0.21	0.18	0.23	-0.1	-0.21
ST059919	0.23	-0.24	0.45	0.3	-0.32	0.2
ST070967	0.22	0.0	-0.12	0.46	-0.02	0.23
ST060160	-0.17	-0.13	0.00	-0.08	0.2	0.12
ST072640	0.32	-0.01	0.11	0.39	-0.2	0.4
ST086622	0.05	0.30	0.02	0.37	-0.13	-0.21
ST019949	0.02	-0.21	0.0	0.32	0.5	-0.19
ST058442	-0.19	-0.29	-0.19	0.0	0.37	0.2
Tamoxifen	0.3	0.0	-0.01	0.57	0.0	0.32
Herceptin	0.32	0.12	-0.12	0.49	0.0	-0.23

Table (III) Shows Bioactivity of Selected flavonoids compounds

5. Toxicity Checking of Ligand molecules

a. Lazar toxicity prediction of flavonoids.

Compound ID	Blood Brain Barrier	Carcinogenicity
ST001473	Penetrating	Active
ST019933	Penetrating	Active
ST026594	Penetrating	Inactive
ST055369	Penetrating	Inactive
ST059919	Penetrating	Active
ST060160	Penetrating	Inactive
ST070967	Penetrating	Inactive
ST072640	Penetrating	Active
ST086622	Penetrating	Inactive
ST019949	Penetrating	Inactive
ST058442	Penetrating	Inactive
HERCEPTIN	Penetrating	Inactive
TAMOXIFEN	Penetrating	Inactive

Table (IV). Shows Blood brain carrier capacity and carcinogenicity of the compounds.

Above table demonstrates that flavonoids compounds with ID ST070967 and ST086622 are Carcinogenic in Nature. Subsequently not appropriate for Drug designing.

b. Toxicity checking by toxicity checker.

Nearness of any Toxic substructure was checked by the online device Toxicity checker mcule by putting SMILES Sequence of the compounds. Results are demonstrated as follows**.**

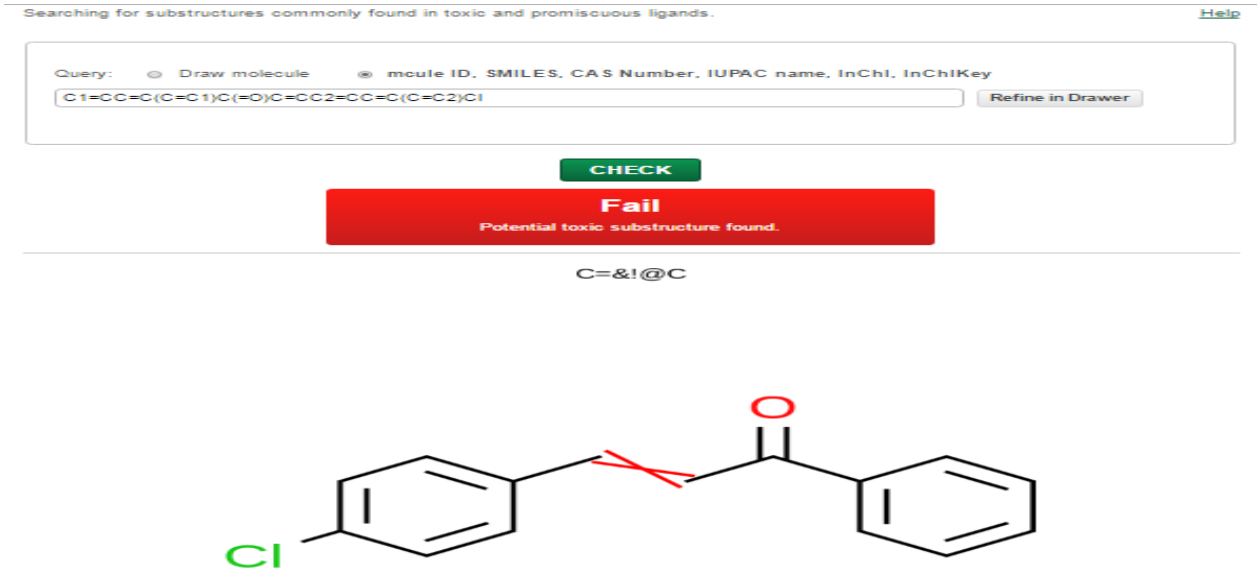


Fig.4 (a) Result of toxicity of Compound ST001473

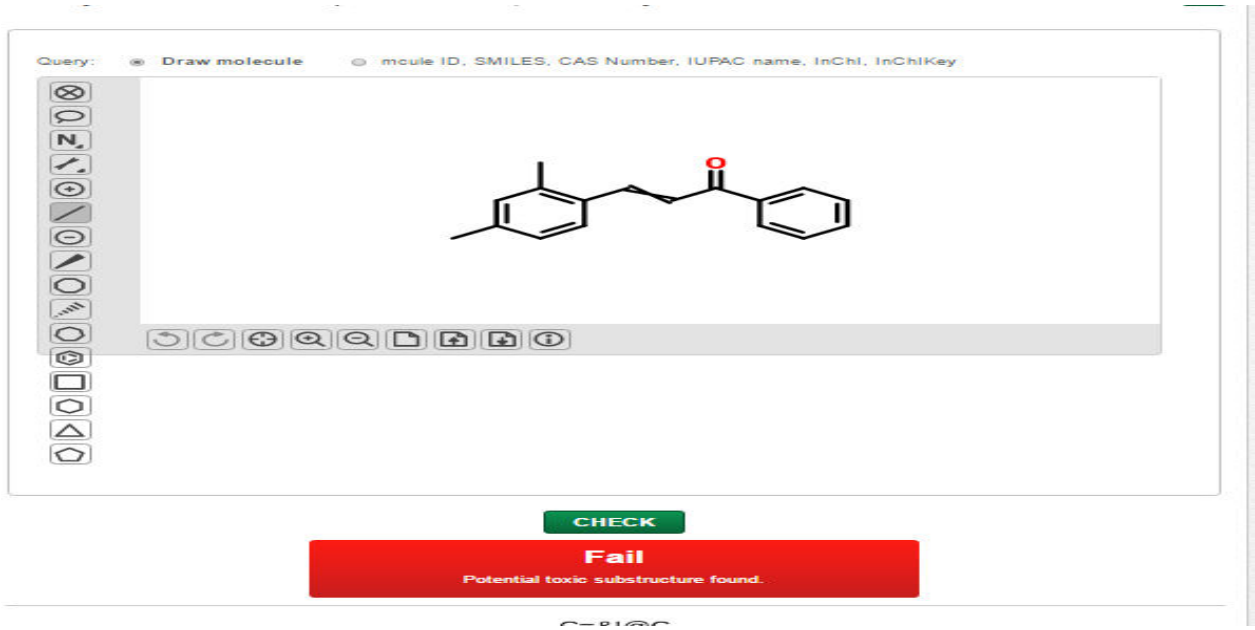


Fig.4 (b) Result of toxicity of compound ST019933

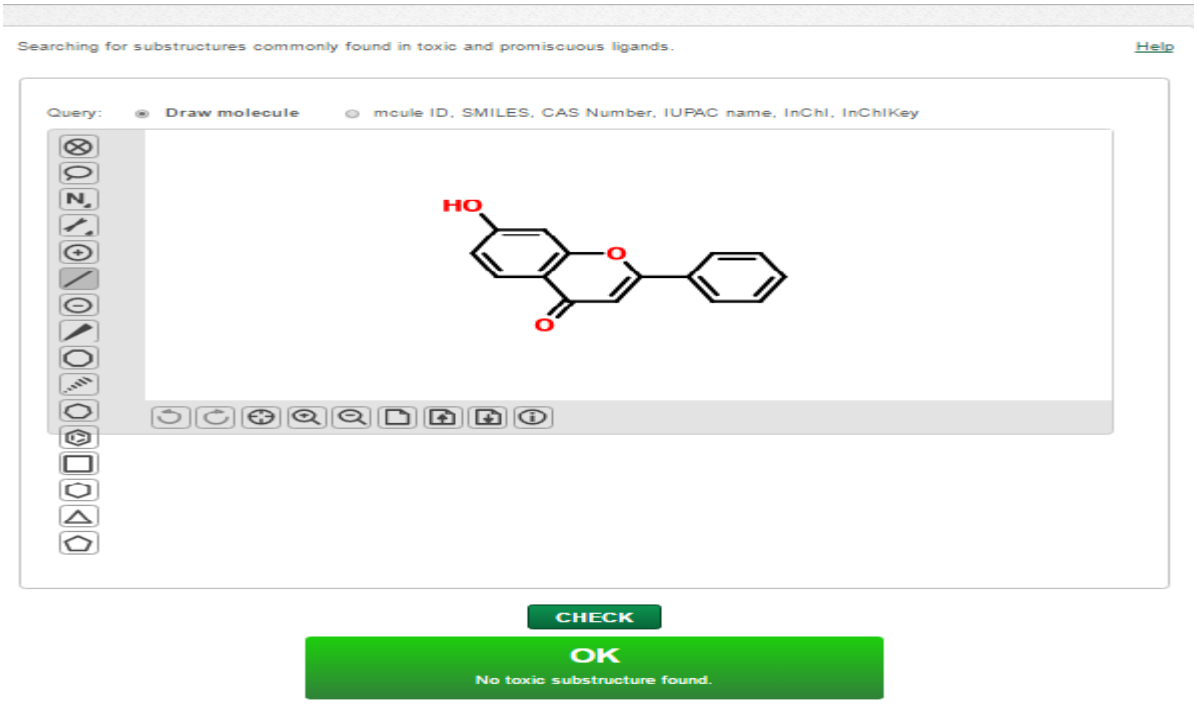


Fig.4 (c) Result of toxicity of compound ST026594

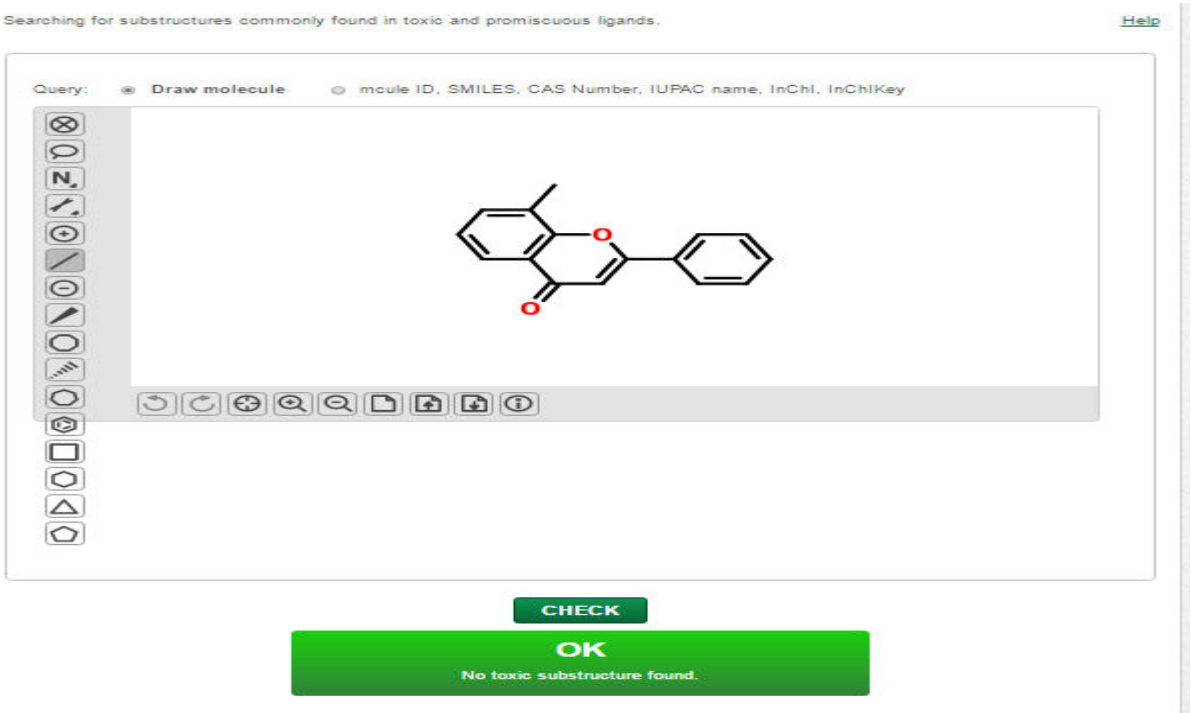


Fig.4 (d) Result of toxicity of compound ST055369

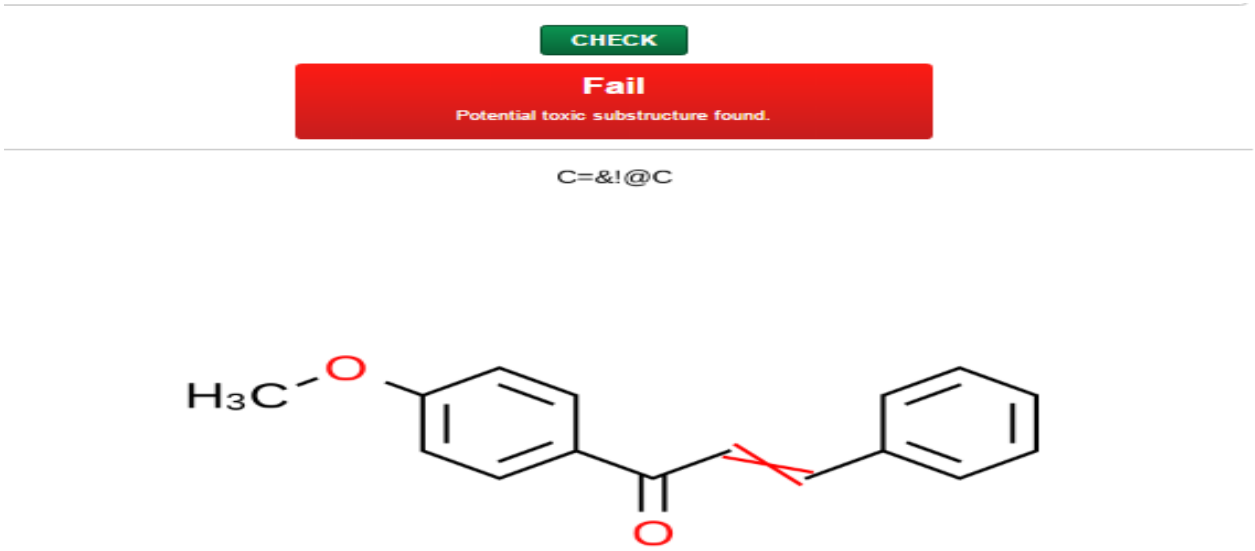


Fig.4 (e) Result of toxicity of compound ST059919

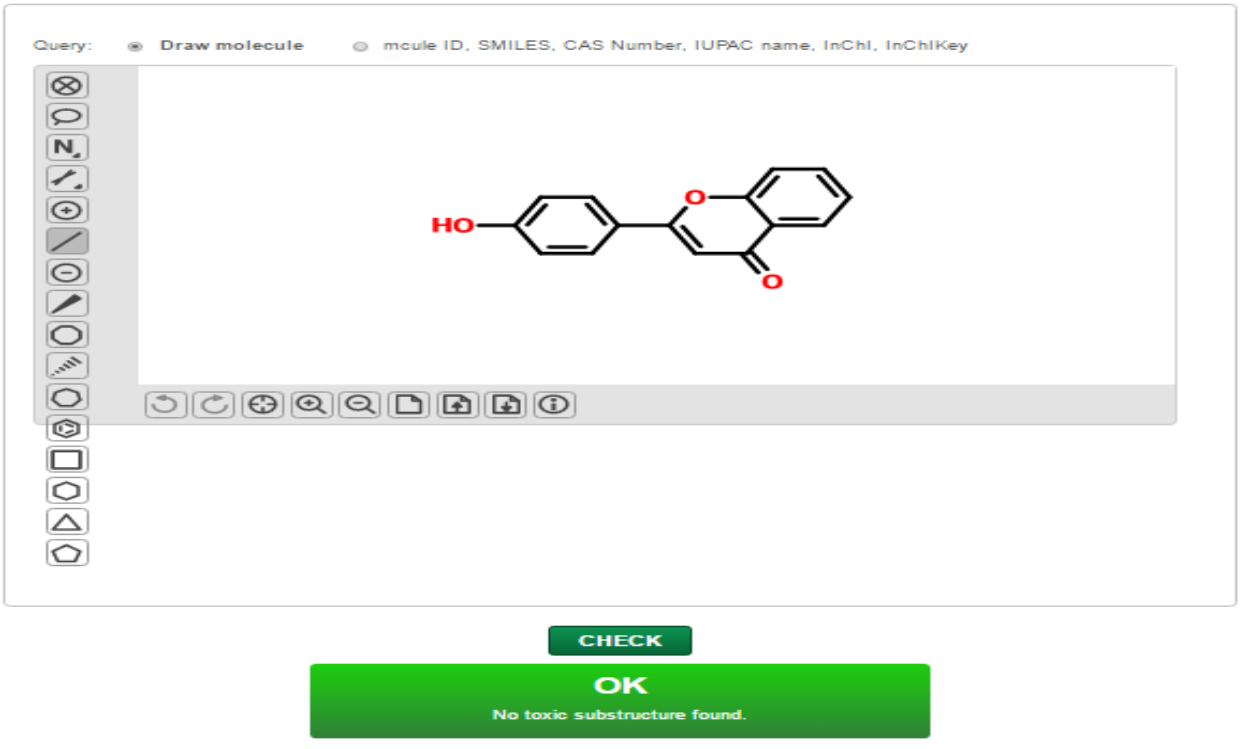


Fig.4 (f) Result of toxicity of compound ST060160

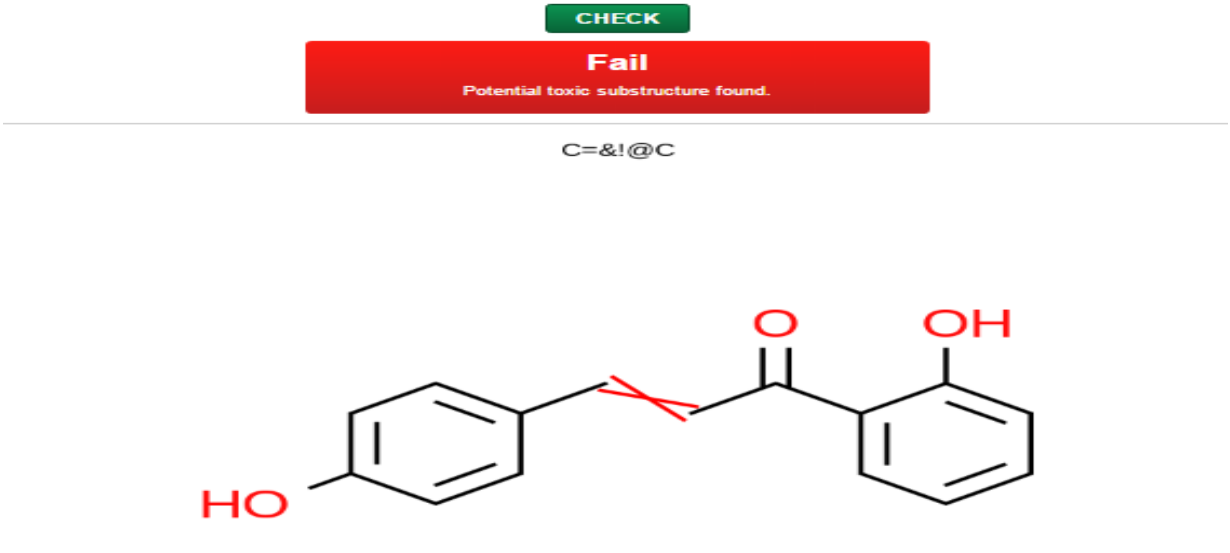


Fig.4 (g) Result of toxicity of compound ST072640

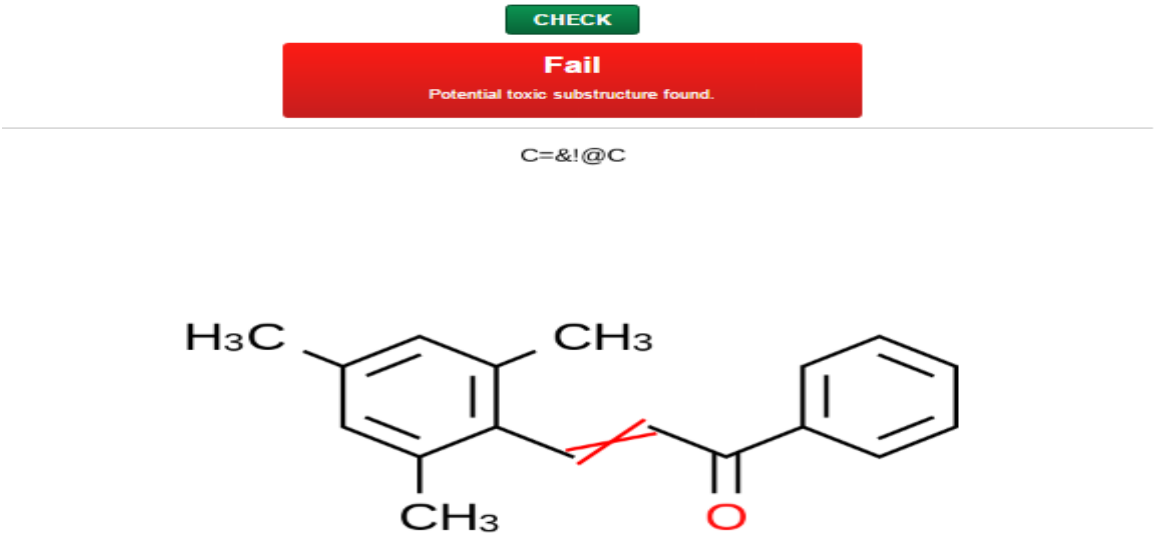


Fig.4 (h) Result of toxicity of compound ST019949

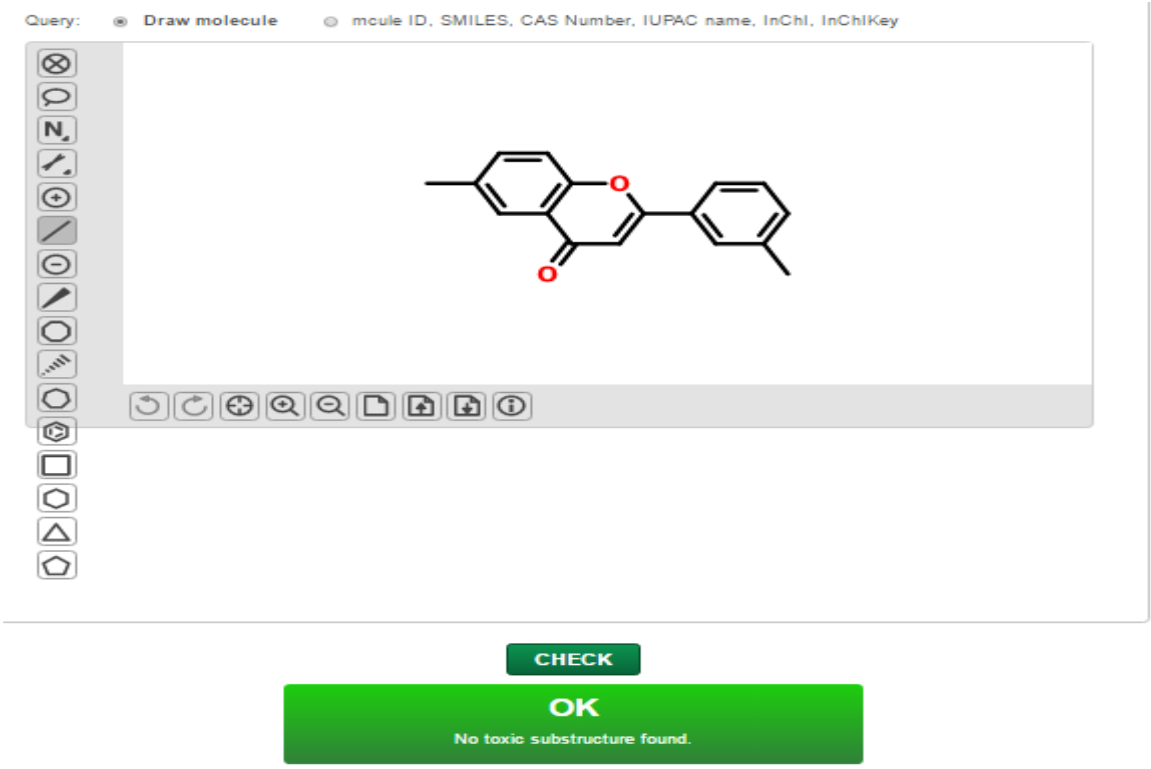


Fig.4 (i) Result of toxicity of compound ST058442

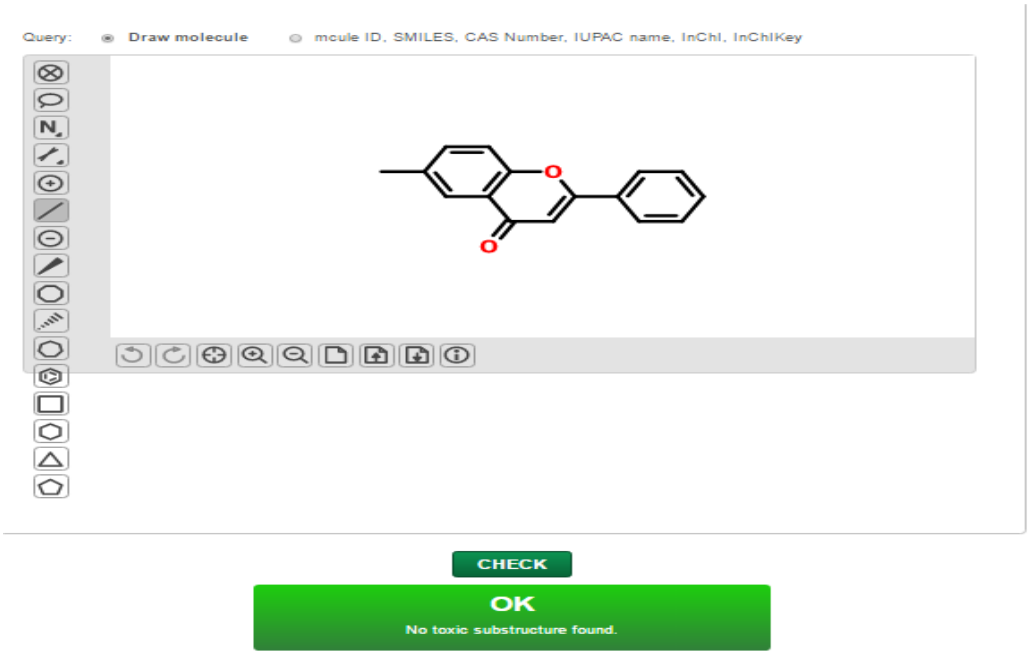


Fig.4 (j) Result of toxicity of compound ST070967

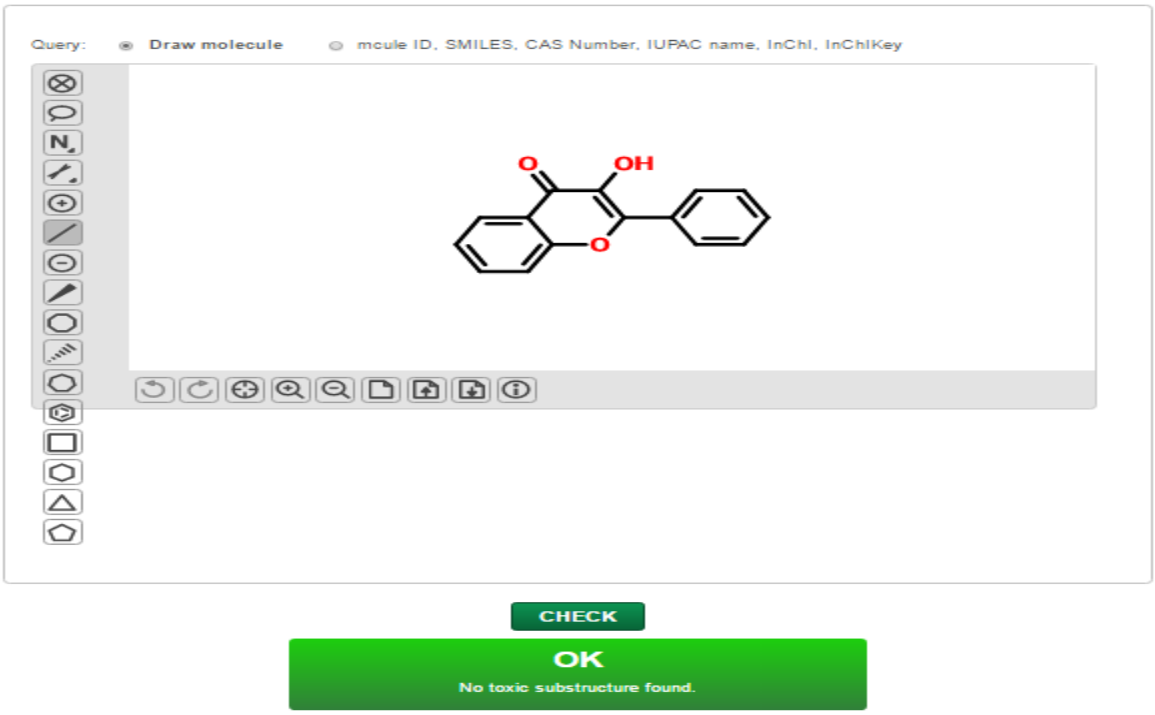


Fig.4 (k) Result of toxicity of compound ST086622

Above Toxicity results showed that compounds with ID ST026594, ST055369, ST060160, ST058442, ST058442, ST070967 are non toxic in nature while compounds with ID ST001473, ST019933, ST059919, ST072640, and ST019949 are toxic in nature.

6. Results of ADME analysis

Ligand ID	Liphophilicity	Water solubility	Pharmokinectics		Drug likeliness
			GI absorption	Blood brain barrier	
ST001473	2.82	-4.01(moderately soluble)	HIGH	YES	YES
ST019933	2.96	-4.39(moderately soluble)	HIGH	YES	YES
ST026594	2.22	-4.19(Partially soluble)	HIGH	YES	YES
ST055369	2.73	-4.37(partially soluble)	HIGH	YES	YES
ST059919	2.20	-3.92(soluble)	HIGH	YES	YES
ST060160	2.65	-4.25(moderately soluble)	HIGH	YES	YES
ST070967	2.19	-4.23(partially soluble)	HIGH	YES	YES
ST072640	1.91	-3.85(soluble)	HIGH	YES	YES
ST086622	2.44	-4.05(partially soluble)	HIGH	YES	YES
ST019949	3.18	-4.7(moderately soluble)	HIGH	YES	YES
ST058442	3.07	-4.65(partially soluble)	HIGH	YES	YES
HERCEPTIN	2.19	-3.92(soluble)	HIGH	YES	YES
TAMOXIFEN	2.22	-3.8(soluble)	HIGH	YES	YES

Table (V). Shows ADME pharmokinetics properties of the compounds.

DISCUSSION

The anticipated 15 regular Flavonoid compounds utilized for docking studies. The after effects of the interaction between the catalytic site deposits of target Human Estrogen Receptor protein and Human epidermal growth factor receptor protein and 15 Flavonoid compounds were appeared in the Table 2. By analyzing the docking interaction, ST026594 (7-hydroxyflavone), ST070967 (2-(- 4-fluorophenyl)- 4n-chromen-4-one), ST086622 (3-hydroxyflavone) and ST055369 (8-methylflavone) were found to have the most noteworthy initiation binding energy scores in kcal/mol of -8.7, -8,87, -8.9 and -8.9 with estrogen receptor in comparison with drug tamoxifen which has binding energy score of -8.92, which is almost equal to the binding energies of the above flavonoids compounds similarly with HER2 receptor the flavonoids compounds with ID ST060160 (4-hydroxyflavone) and ST058442 (6,3-dimethylflavone) were demonstrating minimum binding energies scores of -8.9 and -8.83 in correlation with drug Herceptin which has score of -8.98 which is similar as scores of above two flavonoids compounds. In this manner the docking results were analysed lastly revealed that among the six plant compounds, displays the best restricting interaction with the human Estrogen Receptor and HER2 and further it could be helpful for distinguishing proof and advancement of new preventive and restorative medication against breast cancer.

CONCLUSION

The present work is an endeavor to recognize potential medication focuses against the receptor protein (ER and HER2) as a characteristic solution for cure breast cancer. The study uncovers the flavonoids can go about as medication to cure cancer. The primary intention behind this study is to limit the utilization of accessible medications as these medications illustrate hurtful symptoms to ordinary human cell. In this study we screened potential flavonoids compound initially on the premise of lipinski's rule of five, then these flavonoids compounds are utilized for docking with the receptors and the docking comes about shows few compounds with minimum binding energy and taken for the further examination including bioactivity expectation: the compounds which are observed to be bioactive are analysed with toxicity checking and ADME analysis.

The above analysis demonstrated the compounds ST026594 (7-hydroxyflavone), ST070967 (2-(- 4-fluorophenyl)- 4n-chromen-4-one), ST086622 (3-hydroxyflavone) and ST055369 (8-methylflavone) were the best compounds indicating minimum binding energy in examination with medication Tamoxifen with Estrogen receptor and compounds ST060160 (4-hydroxyflavone) and ST058442 (6,3-dimethylflavone) were the best compounds demonstrating minimum binding energy in correlation with medication Herceptin with HER2 receptor, were additionally bioactive and non poisonous in nature with great Physiochemical properties and drug likeliness. In this way, in future one can go for drug outlining of these compounds including clinical trials.

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