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In Silico Study on Inhibitory Effect of Different Flavonoids on ERBB2 Protein Involved in Breast Cancer

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Abstract
ErbB2 plays an important role in different types of human cancers. The increased levels of expression of this erbB2 has been reported in breast cancer. The current *in silico* study aims a pharmacokinetic approach for the identification of inhibitory molecules to the ErbB2 protein involved in breast cancer. The structural analysis of ErbB2 was determined by using ProtParam (physicochemical properties) and secondary structure by SOPMA tool. The functional analysis of ErbB2 was carried out by Smart tool for domain analysis and protein-protein interactions from String database. The utilization of drug-likeness software, Molinspiration was used to analyze drug-likeness properties and bioactivity of the selected flavonoids (ligands). Tool ADMETsar was used to study pharmacokinetic properties (absorption, distribution, metabolism, excretion, and toxicity) of nine ligands. Molecular docking of five receptors (4HRL,1MFG,4HRM, 1MFL and 4HRN) with nine ligands (L1-Apigenin, L2-Curcumin, L3-Genistein, L4-Kaempferol, L5-Luteolin, L6-Narigenin, L7-Parthenolide, L8-Pterostilbene and L9-Thymoquinone) were performed individually using software Hex 8.0 . The best docked molecules were selected and Patchdock was carried out. Based upon molecular docking results and binding interaction analysis, this study represents one potential flavonoid (curcumin) with low E value ranging between to-250.04 to-240.03. This ligand has high cytochrome inhibitory effect. Therefore, Curcumin can be used as lead compound in treating breast cancer in the future.

Keywords
ERBB2, HER2, Flavonoids, Breast cancer, Hex, Patch Dock

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INTRODUCTION
In the World breast cancer is one of the leading causes of cancer deaths[1]. Gene erbB2 (HER or neu) encodes erbB2 protein, a member of epidermal growth factor receptor family along with three receptors of tyrokinase activity. Protein erbB2 contain 1255 amino acids, transmembrane glycoprotein,185kD, located at the long arm of human chromosome 17(17q12)[2].This epidermal growth factor receptor is also called as HER2, and is over expressed about 20-30% in invasive breast cancers[3].Overexpression of HER2 is allied with tumor aggressiveness[4]. The tyrokinase domain present in HER2 receptor plays significant role in breast cancer by inducing phosphorylation after HER2 homo / hetero dimerization which activates various signal transduction pathways[5, 6]. As HER2 protein has crucial role in occurrence of breast cancers, the signaling pathways are important targets of therapies. The targeted therapy drugs have drug resistance and side effects also. So, there is need for

natural anticancer drugs for the effective treatment of HER2 breast cancer patients.

Prevention of cancers by the use of dietary substances or synthetic compounds may prevent or suppress carcinogenic effect, has become principle with increasing cancer cases in the world[7]. Important phytochemicals present in fruits and vegetables as secondary metabolites include polyphenols, terpenes, carotenoids, flavonoids, isoflavones etc. Human diet contains common flavonoids with general structure consisting of O-glycosides with sugars bound at C3 position[8]. Scientific reports propose that breast cancer cases are lower in people consuming plant based diet[9]. Thus, flavonoids are considered as chemo preventive and chemotherapeutic agents for the treatment of breast cancer[8]. In the present study nine active ingredients like Luteolin, Naringenin, Apigenin, Pterostilbene, Kaempferol, Thymoquinone, Parthenolide, Curcumin and Genistein were studied.

Rational drug design is emerging in pharmaceutical industry as drug can be designed based on the identification of protein/DNA target. A tedious approach of screening and testing of natural compounds is replaced by an alternative method of rational drug design. Rational drug design is knowledge-based method, requires understanding of intermolecular forces involved and also knowing the structure and function of protein[10]. The knowledge of binding site of receptors made computational methods like docking have helped in optimizing drug like molecules since 1980[11]. Molecular docking is the widely used method in drug design, as it has ability to predict accurately of binding small ligands molecules in the target site of receptor[12].Molecular docking algorithms compute quantitative binding energies and rank the ligand-receptor complexes (docked molecules) based on scoring functions[13,14]. Docking tools are based on search algorithms like genetic, fragment-based, Monte-Carlo and molecular stimulations. Molecular methods may be either ligand-target flexible or rigid[15].Modern drug discovery process is between protein-ligand or protein-protein docking, which predicts the orientation of ligand in the receptor (protein/enzyme) when bound. In rigid type of docking, the binding of the ligand to receptor, is searched in a six-dimensional rotational/ translational space, which can act as “lead compound” in drug discovery process[16].Rigid body docking mainly works on the principle of fast Fourier transformation to compute large number of docked conformations with surface complementarity[17]. Hex uses spherical polar Fourier correlations[18, 19] for both rotational and translational space. PatchDock is freely available software for rigid docking, which predicts surface variability/flexibility totally significant through liberal intermolecular penetration. Scoring function is calculated by considering geometric fit and atomic desolvation energy[20].The aim of the present study is to perform molecular docking of erbB2 receptor with selected flavonoids using Hex and PatchDock software.

MATERIALS AND METHODS

UniProt: The protein (erbB2) P04626 sequence was retrieved from UniProt database. The Universal Protein Resource is a comprehensive, freely accessible protein sequences and annotated data.

ProtParam tool: The physicochemical properties of erbB2 protein was predicted by using ProtParam tool from ExPASy. This tool predicts the parameters like molecular weight (MW), theoretical pI, Instability Index (II), Aliphatic Index (AI) and Grand Average of Hydrophathicity (GRAVY)[21].

SOPMA tool: The secondary structure of erbB2 was predicted by using SOPMA tool. Its alternative name is Self-Optimized Prediction from Multiple Alignment. It predicts three states description of secondary structure -alpha helix, beta sheet and coil[22].

SMART: Simple Architecture Research Tool allows the identification of genetically mobile domains and prediction of domain structures [23]. The protein erbB2 was analyzed for presence of domains using SMART.

STRING database: It includes known and predicted protein-protein interactions. The interactions include physical (direct) and functional (indirect) associations by computational predictions, from knowledge transfer between organisms and interactions aggregated with primary databases [24, 25].

Molinspiration:

Lipinski rule

Lipinski’s /Pfizer’s rule was used to predict drug likeliness properties of selected ligands. This is the thumb principle considered to evaluate ligands pharmacological properties, to make orally active to humans [26].

Bioactivity Score

The bioactivity score computes GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors[27].The bioactivity score of nine ligands was calculated using Molinspiration.

Molecular Docking studies

Ligand preparation: The 3D structure of nine flavonoids acted as ligands (L1-Apigenin, L2-Curcumin, L3- Genistein, L4-Kaempferol, L5-Luteolin, L6-Narigenin, L7-Parthenolide,L8-Pterostilbene,L9-Thymoquinone) were obtained from Pubchem

database in sdf format and then converted into .pdb using OpenBabel 2.4.1 software.

Preparation of receptor: The protein erbB2 had 29, 3D structures in RCSB. From them five (4HRL,1MFG, 4HRM, 1MFL and 4HRN) 3D crystalline structures were downloaded from protein databank in .pdb format.

Hex 8.0 software: It is a first Fourier Transform (FFT) based server for protein–ligand docking with graphics[28].All the five receptors with nine ligands were docked using Hex.

The parameters used in HEX Docking were
Correlation type: Shape only
FFT mode: 3d Fast lite
Grid dimension: 0.6
Receptor range: 180
Ligand range: 180
Twist range: 360
Distance range: 40

PatchDock: This is a molecular docking algorithm based on shape complementarity principles[29].

The parameters used in patchDock were
Clustering RMSD: 4.0
Complex: Default

Results and discussion:

Primary structure analysis:

The Receptor tyrosine-protein kinase erbB-2 was studied for predicting physicochemical characteristics using ProtParam tool and results were shown in Table 1. The tool predicted that protein contains 1255 amino acids in length, molecular weight as 137910.50 Da and theoretical pI value was 5.5 which indicates protein as basic in nature. The Instability Index (II) was computed as 56.13 and classified protein as unstable. The Aliphatic Index was 82.35, considered as a positive factor for rise in thermo stability of globular proteins. The GRAVY is negative means the protein is polar. Similarly, Protparam tool was used [30] for studying Alpha 1 antitrypsin inhibitor protein**.**

Secondary structure analysis:

The secondary structure of protein is constituted of 25.10% of alpha helix, 15.78% of extended strand, 4.70% of beta turn and 54.42% of random coil as shown in Fig 1. Similarly, SOPMA tool was used [30] for studying Alpha 1 antitrypsin inhibitor protein.

Protein Interaction Study

String database was used to study erbB2 protein interacting with other proteins like EGF, GRB2, SHC1, NRG1 etc, was shown in Fig 2.

Molinspiration- All the selected ligands (L1-L9) followed Lipinski's rule of five making them potentially promising agents with biological activities. The drug likeliness properties (Table 2) and bioactivity score (Table 3) were tested using Molinspiration. The molecules (ligands) with highest active scores have the highest probability to be active.

Molecular Docking

Hex (8.0) docking

The five receptor proteins with nine ligands (L1-L9) were docked using Hex 8.0 and the E Total energies were shown in Table 4. Hex software permits the receptor molecule (protein) to rotate on Z axis [31]. The five receptors showed good binding energy with ligand 3 (Curcumin) and the increasing order of E value was 4HRL< 1MFG<4HRM < 1MFL< 4HRN. The docked five receptors with Curcumin structures were depicted in Fig 4. Receptor 4HRL showed highest binding affinity for Curcumin with least E Total energy (-250.04). Literature showed that Curcumin inhibited p185 neu tyrosine kinase which is encoded by erbB2 (HER2) gene [32]. Similarly, Hex docking was conducted on BRCA1receptor with Dibromdalcitol[33].

PatchDock result: The Table 5 showed the number of solutions as solution no, geometric shape complementary score as score, approximate interface area of the complex as ACE and 3D transformations: 3 rotational angles and 3 translational parameters. All the five receptors tested with Curcumin showed ACE values in the range of -471.94 to -323.57 with good binding energies. All the docked molecules were analyzed by using Pymol software appearance (Fig 5).

Hex and PatchDock were similarly used to study ABA receptors pyr1 & pyl1 and its analogues [34].

CONCLUSION:

From the docking results, Curcumin can act as lead compound with human epidermal growth factor 2 protein in the treatment of breast cancer. Already Curcumin was reported as anticancerous drug against colon cancer, neck squamous cell carcinoma cancer etc. Curcumin can prevent the development of breast cancer proliferation, by involving in various signaling pathways.Further investigation should be done to confirm these lead compound as "drug" molecule. So our findings will help in drug development process against breast cancer**.**

Table 1: Physicochemical characteristics by ProtoParam

Protein accession no	Protein name	No. of residues	pI	MW	Instability index	GRAVY
P04626	Receptor tyrosine-protein kinase erbB-2	1255	5.5	137910.50	56.13	-0.247

Table 2: Drug likeness properties using Molinspiration

Compound	miLogP	TPSA	natoms	MW	nOH	nOHNH	nviolations	nrotb	volume
Apigenin	2.46	90.98	20	270.24	5	3	0	1	224.05
Curcumin	2.30	93.07	27	368.38	6	2	0	8	332.18
Kaempferol	2.17	111.12	21	286.24	6	4	0	1	232.07
Lutenoiln	1.97	111.12	21	286024	6	4	0	1	232.07
Narigenin	2.12	86.99	20	272.26	5	3	0	1	230.26
Parthenoide	2.09	38.83	18	248.32	3	0	0	0	239.53
Pterostiblene	4.06	38.70	19	256.30	3	1	0	4	241.98
Thymoquninone	1.90	34.14	12	164.20	2	0	0	1	161.10
Genistein	2.27	90.98	20	270.24	5	3	0	1	224.05

Log P, logarithm of compound partition coefficient between n-octanol and water; TPSA, topological polar surface area; % ABS, percentage of absorption; MW, molecular weight HBA, number of hydrogen bond acceptors; HBD, number of hydrogen bond donors; Nrotb, number of rotatable bonds; Nvio, Number of violations

Table 3: Prediction of bioactivity by Molinspiration

Compound	GPCR	ICM	KI	NRL	PI	EI
Naringenin	0.03	-0.20	-0.26	0.42	-0.12	0.21
Apigenin	-0.07	-0.09	0.18	0.34	-0.25	0.26
Curucumin	-0.06	-0.20	-0.26	0.12	-0.14	0.08
Genistein	-0.22	-0.54	-0.06	0.23	-0.68	0.13
Kaempferol	-0.10	-0.21	0.21	0.32	-0.27	0.26
Luteolin	-0.02	-0.07	0.26	0.39	-0.22	0.28
Parthenolide	0.43	-0.07	0.56	1.16	0.04	1.10
Pterostilbene	-0.13	-0.06	-0.12	0.08	-0.33	0.01
Thymoquinone	-1.40	-0.31	-1.27	-1.47	-1.45	-0.40

GPCR = GPCR ligand, ICM = Ion channel modulator, KI = Kinase inhibitor, NRL = Nuclear receptor ligand, PI = Protease inhibitor and EI = Enzyme inhibitor.

Table 4: E total energy of docked molecules (Hex dock 8.0).

	ETOTAL ENERGY								
Proteins	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**	**L7**	**L8**	**L9**
1MFG	-188.08	-188.76	-240.03	-200.06	-190.93	-177.09	-201.12	-166.17	-194.24
1MFL	-189.87	-186.54	-230.83	-184.30	-195.10	-162.85	-193.39	-147.78	-194.90
4HRL	-194.07	-191.19	-250.04	-197.26	-198.40	-172.96	-194.87	-144.06	-191.14
4HRM	-195.87	-197.11	-236.11	-183.43	-188.91	-170.96	-206.16	-158.88	-195.40
4HRN	-193.74	-193.00	-228.82	-165.50	-172.85	-168.74	-197.44	-137.29	-186.80

L1-Narigenin, L2-Apigenin, L3-Curcumin, L4-Genistein, L5-Kaempferol, L6-Parthenolide,L7-Pterostilbene,L8-Thymoquinone,L9-Luteolin

Table 5: Results of PatchDock

Protein	Solution no	Score	Area	ACE	Transformation
1MFG	228	2218	413.80	-337.80	0.14 -0.54 -0.52 8.88 5.26 18.40
	345	1608	413.60	-395.56	-0.28 0.38 2.56 18.66 -1.05 13.65
	378	1282	390.90	-372.76	-0.32 -0.97 -0.03 6.68 -4.17 23.55
1MFL	232	2198	362.30	-288.66	0.77 0.24 -1.56 21.54 -0.80 3.18
	334	1708	375.60	-316.88	2.15 -0.68 0.07 6.15 11.61 20.60
	365	1326	356.30	-323.57	-2.28 -1.00 2.35 13.04 11.46 17.94
4HRM	262	2868	473.80	-448.75	-1.99 0.95 0.88 -10.45 -25.81 -15.90
	314	2782	475.60	-471.94	1.36 -0.94 0.60 -7.97 -22.56 -5.70
	531	2510	443.70	-456.19	-2.87 -0.48 -1.25 -7.28 -14.20 -7.00
4HRL	80	2958	402.80	-324.13	2.56 0.93 2.85 -42.40 -54.94 7.88
	196	2676	438.00	-323.50	2.04 0.60 -2.04 -40.97 -17.49 3.43
	629	1394	418.40	-382.22	-3.04 -0.82 0.24 -47.95 -20.48 9.70
4HRN	231	2632	364.10	-406.26	-1.59 0.19 1.60 15.28 -36.95 -13.95
	331	2476	407.80	-388.29	-2.35 1.30 2.10 15.58 -33.05 -21.18
	477	2264	457.30	-385.71	-1.02 -0.31 2.11 9.87 -23.75 -13.12

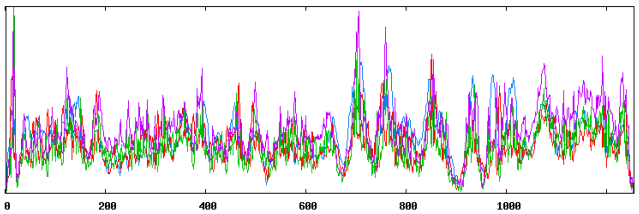

Fig 1: Secondary structure by SOPMA

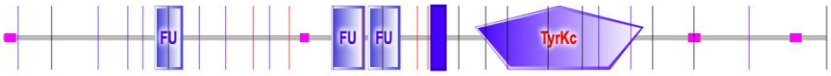

Fig 2: SMART tool Analysis

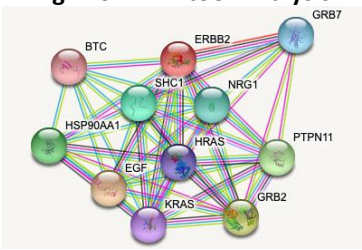

Fig 3: erbB2 protein interaction study by String database

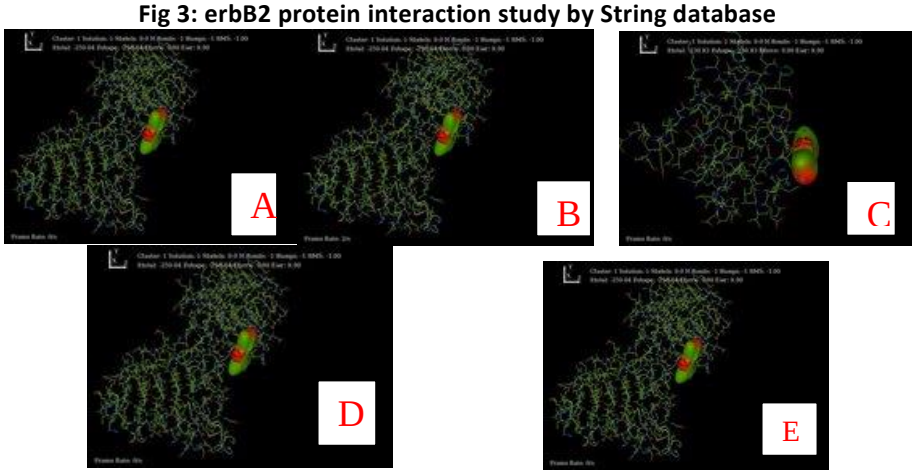

Fig 4: Docked molecules (Receptor with curcumin) using Hex 8.0
A-1MFG with curcumin; B-1MFL with curcumin; C-4HRM with curcumin; D-4HRL with curcumin; E-4HRN with curcumin

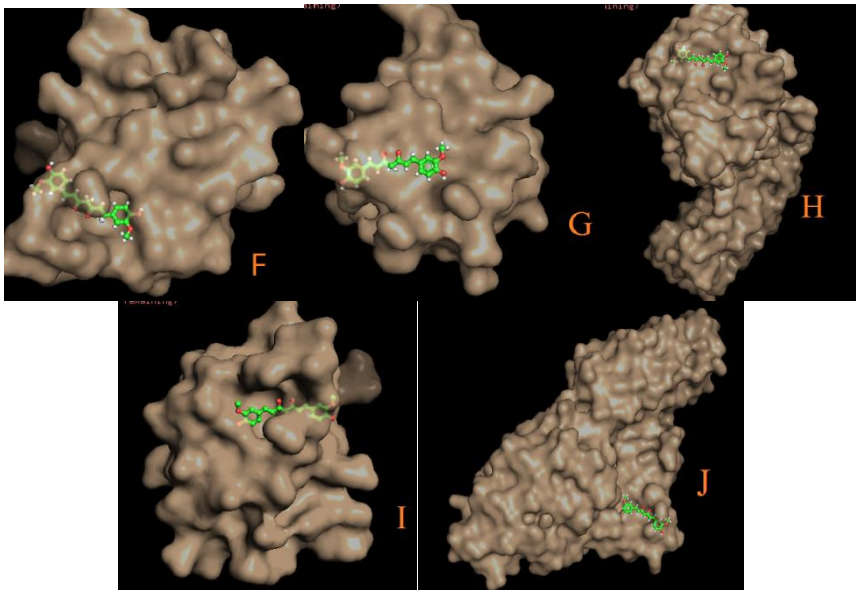

Fig 5: Docked molecules (Receptor with curcumin) using PatchDock

F-1MFG with curcumin; G-1MFL with curcumin; H-4HRM with curcumin; I-4HRL with curcumin;J-4HRN with curcumin

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