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Insilico Docking Studies of Phytomolecules as Anti-Breast Cancer Agents

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ABSTRACT

Breast cancer is the most frequent cancer among women. The present study focuses on the comparative in-silico based investigation of the plant natural compounds namely Cubenin, Curcumin, Delta Cadiene, Eugenol, Terpene and Quercetin binding efficacy towards HER2 and their intermolecular interactions were compared with the commercial drugs:Cyclophosphamide, Doxorubin, Letrozole, Methotrexate and Tamoxifen.The comparative molecular docking was performed with the natural compounds and the synthetic drugs used in breast cancer treatment against the target HER2. The molecular docking analysis was done using Discovery Studio. The ADME properties were also studied. The observation of the common binding site for all the ligands confirms the binding pocket; where the isolated compound agrees well with the binding residues and thus can be optimized further to arrive at a molecule that has a high binding affinity and low binding constant. The results of the docking studies carried out on HER2 provide an insight for the natural compounds having druggable properties. These results are supportive to confirm the natural compounds from plants as a better lead for cancer therapeutics. Thus, the results of the docking studies carried out on HER2 corroborate to the findings that the most suitable drug like properties are possessed by the compound. In comparison with other compounds natural compounds are better and it is druggable. This provides evidence of how a natural compounds from plants can be a source of potential anti- cancer agent. The preclinical studies will pave way for a potential anti-cancer compound.

KEY WORDS: DOCKING; ADME; HER2; DISCOVERY STUDIO.

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INTRODUCTION

Cancer is the leading causes of death globally where 1 in 6 die. Low economic prosperity and lack of awareness of prevention strategies are noted to be vital factors contributing to the burden and incidence for cancer ERBB is abbreviated from erythroblastic oncogene B, a gene isolated from avian genome. Heterodimerization of this receptor with other members of the EGFR family, typically owing to HER2 overexpression, results in the autophosphorylation of tyrosine residues within the cytoplasmic domain of the heterodimer and initiates a variety of signalling pathways leading to cellular proliferation and tumorigenesis (Yarden, et al.,2001)).Amplification or over-expression of this oncogene has been shown to play an important role in the development and progression of certain aggressive types of breast cancer. According to TCGA (The cancer Genome Atlas) data portal HER2 aberration studied in various solid tumours associated with cervical, bladder and were have limited therapeutic options (Cancer Genome Atlas,2013). Approximately, 70% of death due to cancer occurs in low- and middle-income countries. Receptor tyrosine-protein kinase erbB-2, otherwise known as HER2, a significant member in the EGFR family of receptor tyrosine kinases family, (Pegram et al.,2020 Siegel et al.,2020).

So, outspreading the HER2-based therapeutic option beyond breast cancer to other solid tumours with HER2 overexpression will be beneficial to the affected individuals. In recent years, the protein has become an important biomarker and target of therapy for approximately 30% of breast cancer patients. In this study, the natural compounds from plant source is considered based on their pharmacological properties. The compounds have been characterized in detail for breast cancer. The natural source would overcome the existing synthetic drugs in mode of action and also reduce the side effects caused by the commercial compounds (Christy and Swetha 2019). The present study focuses on the comparative in-silico based investigation of the plant natural compounds namely Cubenin, Curcumin, Delta Cadiene, Eugenol, Terpene and Quercetin binding efficacy towards HER2 and their intermolecular interactions were compared with the commercial drugs:Cyclophosphamide, Doxorubin, Letrozole, Methotrexate and Tamoxifen. The in-silico approach enables one to screen for ADMET properties of vast number of molecules within short span thus reducing the time and is a non- expensive and non-tedious process with great accuracy, which is not possible in standard experimental methods.

MATERIAL AND METHODS

HER2/ERBB2 expression datamining: The cancer genome data repository was used to assess the HER2 positive cancer types and the Figure 1 revealed that HER2 expression was seen in other solid tumors associated with bladder, uterine, cervical regions also (TCGA GDC portal).Human pathology atlas module of human protein atlas also revealed the HER2 varied expression with breast, colorectal, cervical, renal and urothelial cancers and dataset related information's listed in Figure 2. (Uhlen et al.,2017)

Figure 1: HER2 aberrations in various cancer types

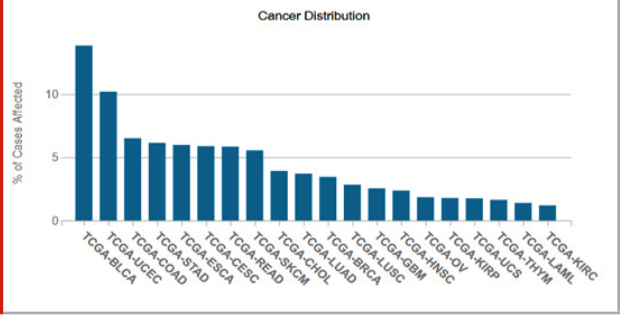
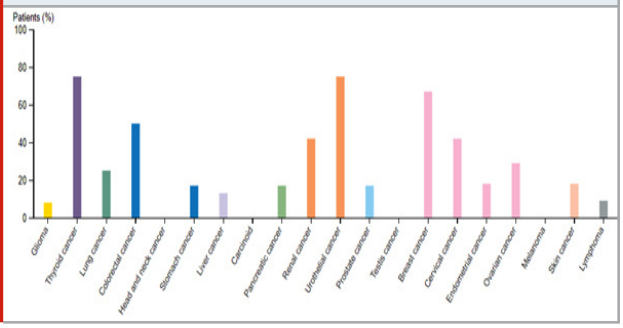


Figure 2: HER2 profile based on human pathology atlas



HER2 protein structure quality assessment: The target is retrieved from the Protein Data Bank, RCSB-PDB (H.M Berman et al.,2000). Upon searching the protein structure for the HER2 protein, the structure 3RCD has been chosen the fitting protein model (Ishikawa et al.,2011). Stereochemical properties based assessment revealed that nearly 90% of residues are residing in the allowed region( ).Before proceeding with the docking the protein structure has been energy minimized to find an arrangement in space of a collection of atoms where, the net inter-atomic force on each atom is acceptably close to zero and the position on the potential energy surface (PES) is a stationary point . the energy value of the protein molecule after Energy minimization is-44093.590. The energy minimisation was done in Swiss PDB viewer, it is a downloadable software (Swiss PDB viewer). Then after this the protein structure was uploaded in the Discovery Studio, where the force field CHARMM was applied and then receptor binding pockets where determined so as the ligand fits in.

Preparation of Ligand: The commercial compounds Cyclophosphamide, Doxorubin, Letrozole, Methotrexate and Tamoxifen and the natural compounds Cubenin, Curcumin, Delta Cadiene, Eugenol, Terpene and Quercetin three dimensional structures were retrieved from Pubchem database (Kim et al.,2016). Then the ligands were analysed if they are druggable or not using the online tool, which is based on the Lipinski's rule of 5 (Jayaraman et al.,2012).

ADMET Profiling: The ADME profiling was done using the Accelrys Discovery Studio 2.5 software. The lead compounds from natural resources fail to enter the market due to the poor pharmacokinetic properties. So, designing ligands satisfying the Adsorption, Distribution, Metabolism Elimination and Toxicity (ADMET) properties will go through the market as a good drug. The drugs should be orally absorbed and distributed to the site of action and eliminated from the body without leaving any traces, which produces adverse effects. Hence, the tools and computer-aided methods, nowadays, have become popular in identifying good drug candidate molecule.

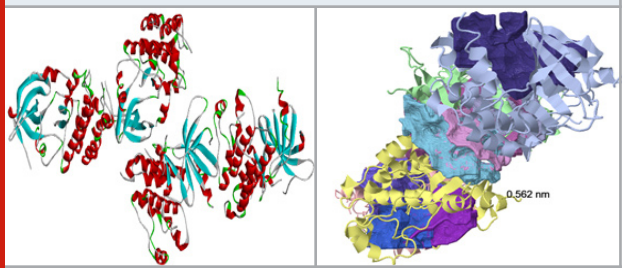
Molecular Docking: Molecular docking is a key tool in structural molecular biology and computer-assisted drug design (Venkatachalam et al.,2003). The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. The receptor-ligand interaction uses Ligand Fit protocol, which docks the ligands into the binding site of receptors using shape-based searching and Monte Carlo sampling of ligands (Gangopadhyay et al,2017).

The parameters used are PLP1 algorithm for energy grid and conjugate gradient for energy minimization. The scores for docked poses are obtained by LigScore1, LigScore which predicts the binding affinities, -PLP1, -PLP2 known as Piecewise Linear Potential scoring function calculates both the shape and hydrogen bond complementarity of poses to the active site and Jain scoring function which scores the non-covalent protein-ligand interactions(Krammer et al.,2005;Jain 1996).

RESULTS AND DISCUSSION

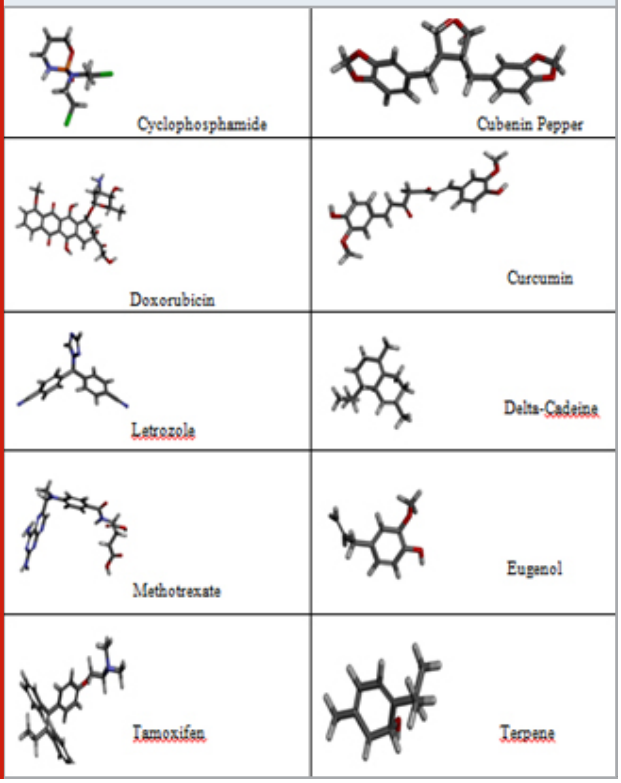
The protein structure retrieved from RCSB-PDB: There were eight druggable cavities populated in the chains of HER2 three-dimensional structure and were ranked based on the cavity score and drug score. In general The ligandability score used to assess the possible small ligands affinity to the specified cavity, whereas druggability scores reveal and rank the good target for HER2 binding .Specified caities of HER2 were predisposed based on cavity volume, pocket lip size, hydrophobic volume, cavity surface area, and hydrogen-bond-forming surface area of the chosen HER2 cavity.

Figure 1a: Three-dimensional structure of HER2 and druggable cavities



The Ligand Structures retrieved from Pubchem: PubChem an open source data repository for chemical substances and their related biological activities. This structure based search module compiled with variety of chemical structure format like SMILES, SMARTS, InChI, CID, molecular formula and SDF format. Since our ligandfit module accepts the SDF format we have downloaded all the Plant based natural compounds and synthetic compounds in SDF format. HER2 specific synthetic drugs as well natural compounds were listed in the Figure 2.

Figure 2: Natural compounds and synthetic compounds three dimensional structures



ADME Studies: ADME profiling of the natural compounds aids in proving the compounds are less toxic and it passes through various barriers.

Lipinski Rule: Lipinski rule of 5 helps in distinguishing between drug like and nondrug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules:

Molecular docking studies using ligandfit module: The docking studies where done to find the Receptor-Ligand Interactions LigandFit assess the small molecules affiinity into the HER2 protein active site by applyinng its shape complementary screening. In general docking process list the top conformations of ligand after the energy minimization method based on steepest descent method and conjugate gradient method. Ligandfit uses the consensus scoring to minimize the false positive. Proposed consensus scoring method was depends on LigScore1, LigScore2, piecewise linear potential 1 (PLP1),

piecewise linear potential 2, potential mean force (PMF), Jain score.

The above are the docking 2D & 3D images of the receptor with the ligand, with their docked positions and the respective amino acids. The dock scores are evaluated based on the parameters of lig score, PLP1, PLP2,JAIN score and PMF score.

Table 1. ADME Profiling of Natural Compounds

Compound	ADMET-BBB	Solubility	Hepatotoxicity Probability	CYP2D6 Probability	AlogP98	ADMET_PSA_2D
Cubenin	-0.203	-4.448	0.708	0.227	3.194	65.466
Curcumin	-0.544	-3.537	0.887	0.376	3.554	94.092
Delta Cadien	1.373	-5.713	0.258	0.178	4.939	0
Eugenol	0.172	-2.416	0.35	0.029	2.579	29.745
Terpene	0.242	-2.285	0.37	0.029	2.346	20.815

Table 2. ADME Profiling of Synthetic Compounds

Compound	ADMET-BBB	Solubility	Hepatotoxicity Probability	CYP2D6 Probability	AlogP98	ADMET_PSA_2D
Cyclophosphamide	-0.723	-1.296	0.589	0.059	0.33	42.393
Doxorubicin		-4.798	0.9	0.455	-0.044	209.31
Letrozole	-0.471	-3.828	0.841	0.663	2.749	73.74
Methotrexate		-4.018	0.741	0.178	0.376	207.82
Tamoxifen	1.605	-6.711	0.894	0.702	6.319	12.282

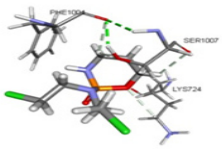
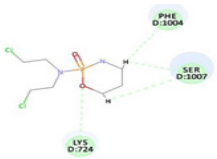
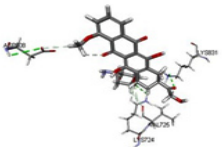
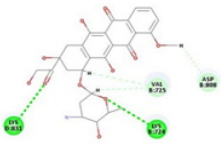
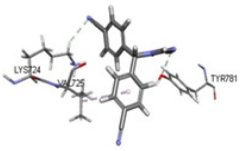
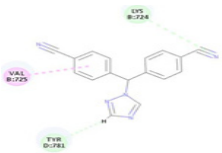
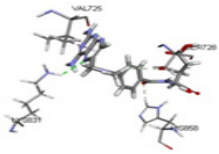
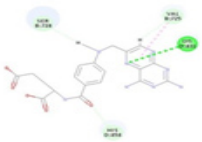
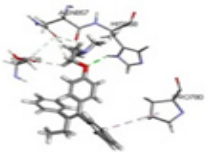
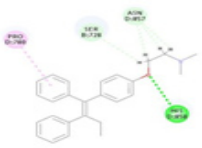
Table 3. Lipinski Rule of Druggability for Synthetic Compounds

	MASS	HBD	HD A	Log P	MR
Cyclophosphamide	356	1	6	2.5102	91.47678
Doxorubicin	368	2	6	3.369898	102.0166
Letrozole	204	0	0	4.725199	66.74298
Methotrexate	164	1	2	2.1293	48.55979
Tamoxifen	152	1	1	2.2797	47.30179

Table 4. Lipinski Rule of Druggability for Natural Compounds

	MASS	HBD	HD A	Log P	MR
Cubenin	356	1	6	2.5102	91.47678
Curcumin	368	2	6	3.369898	102.0166
Delta Cadeine	204	0	0	4.725199	66.74298
Eugenol	164	1	2	2.1293	48.55979
Terpene	152	1	1	2.2797	47.30179

Table

Synthetic compounds	Intermolecular interaction of HER 2 and synthetic drug compounds	
Cyclophosamide Doxorubicin		
Letrozole		
Methotrexate		
Tamoxifen		
		

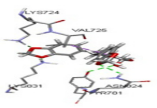
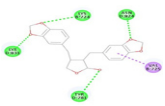
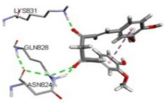
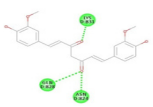
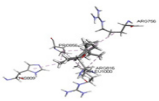
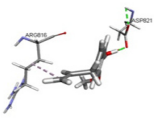
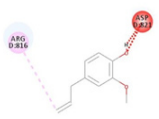
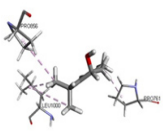
Natural compounds	Intermolecular interaction of HER2 with plant-based compounds	
Cubenin		
Curcumin		
Delta cadeine		
Eugenol		
Terpene		

Table 5. Dock scores of Synthetic compounds

Compounds	Lig score 1	Lig score 2	PLP1	PLP 2	JAIN	PMF
Cyclophosphamide	-0.168	1.652	30.288	32.14	1.035	13.01
Doxorubin	2.961	4.52	45.541	41.176	1.873	41.452
Letrozole	1.375	3.749	50.645	46.388	-0.093	19.141
Methotrexate	2.883	4.105	38.079	30.442	-2.452	34.579
Tamoxifen	1.878	3.238	34.473	32.632	-1.709	41.811

Table 6. Dock scores of natural compounds

Compounds	Lig score 1	Lig score 2	PLP1	PLP 2	JAIN	PMF
Cubenin	3.563	4.065	47.011	48.088	0.271	37.173
Curcumin	3.235	4.174	45.657	44.984	-1.815	50.533
Delta Cadiene	6.009	2.93	42.196	41.498	1.496	39.294
Eugenol	1.321	2.519	19.224	20.169	1.367	30.905
Terpene	1.368	2.549	26.964	29.576	-0.044	33.962

CONCLUSION

Thus, the results of the docking studies carried out on HER2 corroborate to the findings that the most suitable drug like properties are possessed by the compound. In comparison with other compounds natural compounds are better and it is druggable. This provides evidence of how a natural compounds from plants can be a source of potential anti- cancer agent. The preclinical studies will pave way for a potential anti-cancer compound. The study aimed in finding the compatible natural lead molecules from plants and it shows that the compounds are druggable based on the Lipinski's rule and the Insilico toxicity study studies are also positive, adding advantage for the compounds to be druggable. With the docking studies , the compounds that dock with the target has been found. The dock scores are also supporting for the further study. The work will be continued on breast cancer cell line study invitro and then in vivo studies.

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