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PHARMACOPHORE MAPPING AND DOCKING STUDIES OF SOME BIOACTIVE COMPONENTS FOR ANTICANCER ACTIVITY TARGETING HER2

Rupanjali Bhattacharjya Sharma*[1], Mainu Borah[2] and Surabhi Johari[2,3]

[1]Women Scientist-A (Ex), Dept. of Science and Technology Govt. of India. Dept.of Pharmaceutical Sciences, Dibrugarh University.

[2]Centre for Bioinformatics Studies, Dibrugarh University, Dibrugarh Assam, India.

[3]Institute of Management Studies (University Courses Campus) Ghaziabad, UP, India.

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***Corresponding Author**
Rupanjali Bhattacharjya Sharma
Women Scientist-A (Ex), Dept. of Science and Technology Govt. of India. Dept.of Pharmaceutical Sciences, Dibrugarh University.

ABSTRACT

The natural plant products are commonly occurring bioactive components used for drug discovery. The current approach reveals the use of natural plants derivatives in treating breast cancer targeting Human Epidermal growth Receptor (HER2). In order to obtain most potent inhibitors, different computer aided designing technologies like pharmacophore modelling, virtual screening and molecular docking studies were utilised. In order to identify the necessary chemical features of HER2 inhibitors, pharmacophore models were generated using PharmaGistThe screened molecules were filtered by applying Lipinski's rule of five and molecular docking study using HER 6.3 package to refine the retrieved hits.The bioactive components were virtually screened in order to classify them as drug like molecules. The datasets of 7 components revealed that all of them although satisfy Lipsinki's rule of five but not ADME/TOX rule. The detailed analysis predicted that the compound 3, Daucosterol satisfy L-rule as well as ADME/TOX prediction with low risk to HER2 inhibition. Also when the particular molecule was allowed to interact with HER2 protein, it showed binding energy of-257.34 kcal/mol which was quiet acceptable. Whereas other plant components also showed minimum binding energy but they were violation ADME/TOX rules so they were excluded from the current study. From the phytochemical and virtual screening, ligand-based pharmacophore modelling and docking studies, we concluded that our pharmacophore model, Model-3 can be used in order to identify new

potent compounds from any chemical databases that may act as good leads against 4HRL. Docking of 7 bioactive anticancerous compounds was carried out and three compounds namely, comp1, comp 3 and comp7 exhibited minimum energy values and also followed all criteria of Lipinski's properties but not ADME/TOX rule. And it was also observed that the compound 3, Daucosterol satisfy Lipinski's-rule as well as ADME/TOX prediction with low risk to HER2 inhibition and therefore found to be highly potent inhibitors against 4HRL causing breast cancer. Hence, we concluded that the above mentioned compound can be used as inhibitors for treating breast cancer.

KEYWORDS: Cancer, HER2, pharmacophore, docking, inhibitor.

INTRODUCTION

Breast cancer is the most frequent carcinoma and second most common cause of cancer-related mortality in women Human epidermal growth factor receptor (HER) pathways play a critical role in cancer biology and are an area of intense research at Genentech, a member of the Roche Group. In the year 2000, S. Ménard et.al said that dysregulation of HER-mediated signaling pathways resultsin the growth and spread of cancer cells.[1,2,3] The HER family consists of 4 structurally related receptors: HER1 (EGFR), HER2, HER3, and HER4.[1,4] Cancer is the main cause of death worldwide, approximately 7.6 million deaths (around 13% of all deaths) in 2008, with an expected 13.1 million deaths in 2030.[3,5,6,7,8] And breast cancer covers 22.9% of all cancers in women. In 2008, breast cancer caused 13.7% of cancer deaths in women all over the world.[4] There is a lifetime risk of developing breast cancer. The occurrence of breast cancer in India is on the escalation and is quickly becoming the number one cancer in females. The importance of the situation is apparent after working through data from Indian Council of Medical Research (ICMR). It is testified that one in 22 women in India is expected to suffer from breast cancer during her lifespan.

MATERIALS AND METHODS

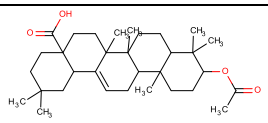
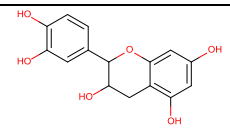
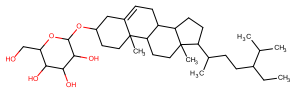
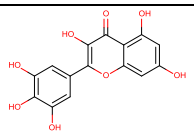
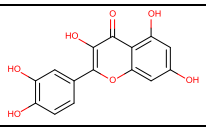
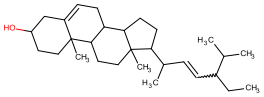
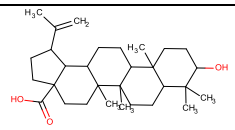
Table 1: List of Software used in insilico experiment.

Software	Source	Utility
Marvin sketch	Open source	Drawing of 2D structure of ligands.
Chem office	Paid software from Cambridge soft	Chem3D generates 3D models. ChemFinder is a chemically-intelligent personal database system used to organize the compounds and to search and correlate structures with properties.
UCSF Chimera	Under Academic license	Viewing of docking results.

ArgusLab (4.0.1)	Open source	The Argus Lab contains tools for building and visualising molecules as well as looking at the output from calculations.
SPDBV	Open source	Swiss-Pdb Viewer is an application that provides a user friendly interface allowing analyzing several proteins at the same time.
Hex (version 6.3)	Free for academic use	Docking software.
Pymol	Free for academic use	Pymol is used to visualise the interactions.
PharmaGist	Open source	PharmaGist is used for pharmacophore modelling.
Swiss Dock	Open source	Docking software.

A set of 7 bioactive anticancer compounds were taken and their structures were retrieved from the PubChem database for ligand based molecular docking as shown in the Table 2.

Table 2: Ligands and their properties.

Compound	Structure	Comp ID	M Wt (g/mol)	XLogP3	HBD	HBA	RB	TSA (A^2)	IC_{50} (μ)
Ligand 1		21337502	498.7370	7	2	4	2	74.6	3.4
Ligand 2		1203	290.2681	0.4	5	6	1	110	2.42
Ligand 3		5742590	576.8473	7.7	4	6	9	99.4	30
Ligand 4		5281672	318.2351	1.2	6	8	1	148	15.003
Ligand 5		5280343	302.2357	1.5	5	7	1	127	34.5
Ligand 6		5280794	412.6908	8.6	1	1	5	20.2	50
Ligand 7		64971	456.7003	8.2	2	3	2	57.5	13.09

Virtual Screening

Virtual screening is a technique which is performed to retrieve new molecules from databases for further biological testing. The main purpose of our study was to identify new novel inhibitors of 4HRL, a cancer causing protein. The 3D structure of bioactive plant components

was retrieved from PubChem. The hit molecules obtained from database were filtered by applying Lipinski's rule of five.[9,10,11 &12]

Generation of Ligand-Based Pharmacophore Model

From the information obtained after virtual screening the data sets of 7 screened bioactive compounds of anticancerous plants was considered for pharmacophore analysis. Based on the conformations for each compounds, online tool PharmaGist was used to construct possible pharmacophore models. Five pharmacophore models were generated with different scores using PharmaGist which showed different feature patterns and pharmacophore-Fit scores of each compound. The pharmacophore model with the highest score was considered as the best model.[13-14]

Generation of Molecular Docking

Docking study is used in order to find the binding affinity between the protein-ligand complexes. The components were considered for docking study was performed in order to understand how these compounds bind to its target, the hit molecules, which retrieved from the virtual screening technique and the training set compounds which showed maximum necessary features were further filtered by the molecular docking studies using Hex (version 6.3) [15-16]. We found 3 compounds out of 7 compounds, which showed the higher docking values. The docking studies was performed between receptor Human Epidermal growth Receptor 2 (PDB code: 4HRL) and ligands (Anticancerous bioactive components). Hex 6.3 is an Interactive Molecular graphics program developed by Dave Ritchie for estimating docking calculations and displaying docking modes of pairs of protein and ligand molecules[17]. HEX is used as the docking tool which calculates intermolecular "energies" by adding up all intermolecular interactions that occur between a ligand and protein target. 4HRL **(Fig 1)** was retrieved from RCSB and prepared by UCSF Chimera.

The compound 1, 3 & 7 shows the best docking values which signify higher affinity of binding of proteins to the ligands. As the structures of more potential drug target are elucidated the opportunity for computers to perform initial binding studies is increasing. By computationally docking a ligand to a protein, one limits concerns about assay complication such as compound solubility and the needs to maintain extensive physical compound libraries. The objective of computational docking is to determine how the molecules of known structure will interact. The molecule may bind to receptor and modify their function[18] and further after docking, the binding interactions were visualised using Pymol.

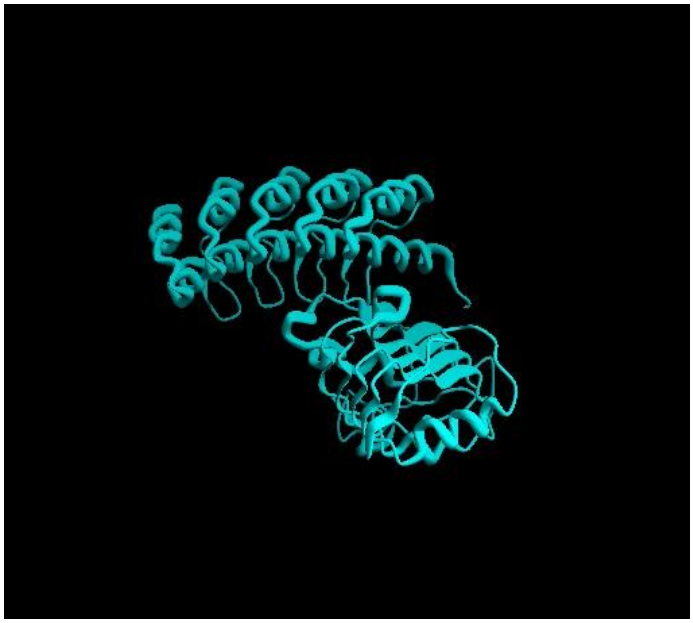


Fig. 1: HER2 protein (PDB id- 4HRL).

For the Her2 protein, the structure was retrieved from RCSB and the complete model analysis with different evaluations had been performed. Here, the stereo chemical quality, main chain and side chain parameters were analysed with Ramachandran plot. The model bears 92.9% residues in the core region of Ramachandran plot making it quiet acceptable. Thus based on PROCHECK analysis of 118 strutcures of resolution of at least 2.0 Å and R- factor greater than 20% indicates that the quality of the model 2 is better. [18, 19] It was also observed in the 3D structure of the predicted protein that the ratio of the bond length/ bond angle cluster around the value of 3.4 with no bad contacts.

The stereochemical quality check for the main chain parameters gives the overall averade G-factor for 4HRL retrieved from the RCSB PDB which shows that the overall quality of model is better.

On the further analysis, the stereochemical qualiity of the side chain parameter of the generated model as phi-psi, Chi-1 standard deviation distribution, H-bond energy, standard deviation, and all the side chain parameters were in better range.[19]

Thus considering Morris et.al classification model is reproduced with good accuracy. The model thus was used for investigation of their interactions with novel inhibitors**.**

RESULTS AND DISCUSSION

LIGAND-BASED PHARMACOPHORE MODEL GENERATION

Table 3: Results obtained from ligand-based pharmacophore modelling using Pharma Gist.

S. No	Comp Id	Features pattern	Pharmacophore e-fit score	Mutagenic	Carcinogenic	herG inhibition
1	21337502	3H, AR, 5HBD, 5HBA	20.55	–	+	Low risk
2	1203	24H, 2AR, HBA	14.9306	+	–	Medium risk
3	5742590	2H, AR, 3HBD, 4HBA	14.1601	–	–	Low risk
4	5281672	3H, AR, 6HBD, 7HBA	23.57	+	–	Low risk
5	5280343	2H, AR, 6HBD, 7HBA	23.26	+	–	Medium risk
6	5280794	9H, 2AR, HBD, HBA	11.7727	–	+	Low risk
7	64971	3H, AR, 6HBD, 7HBA	23.57	+	–	Low risk

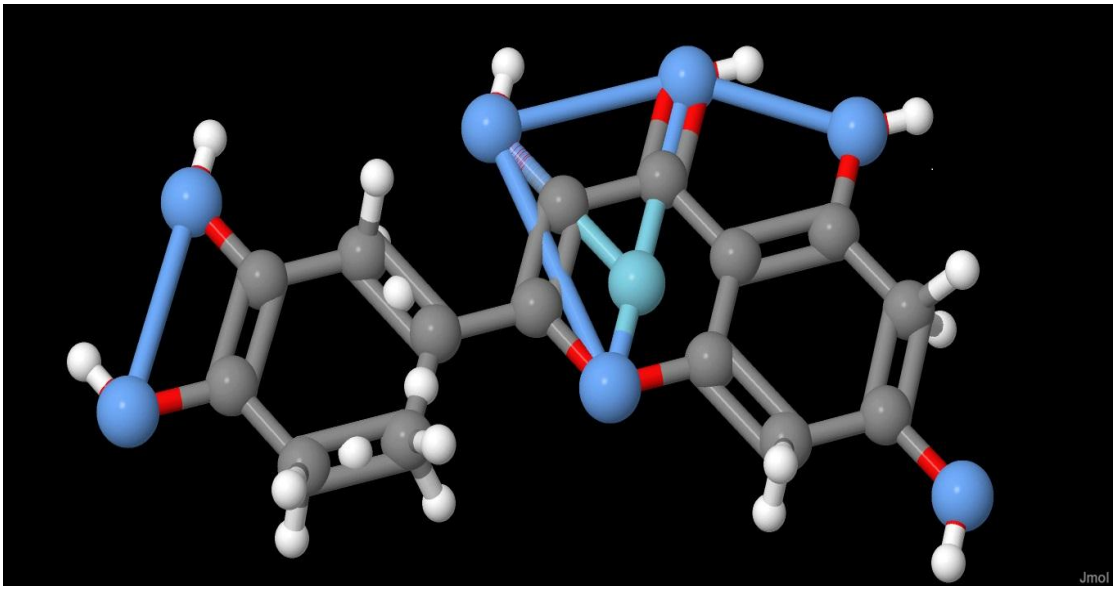


Fig. 2: The snap shot of the chemical features of best pharmacophore model (Model-3). Red color represents hydrogen bond acceptor, blue represents aromatic,white represents the hydrogens and grey represents the carbons.

Secondary Structural Assessment of Protein HER2 (PDB id 4HRL)

The Ramachandran plot analysis is presented in (**Fig 3)**. The Ramachandran Plot statistics and G-factors are presented in **Table 4** and **Table 5**. The G factor provides a measure of how unusual, or out-of-the-ordinary, a property is. The values of G factor below -0.5 indicates, unusual; and values below -1.0 indicate highly unusual. In this study, the value of G factor was found to be 0.51.Thus making it a suitable model for protein ligand interaction studies.

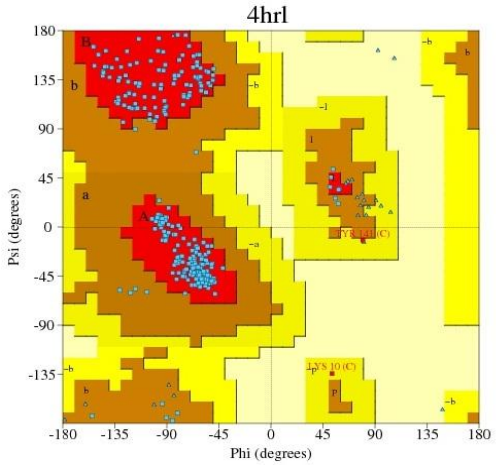


Fig. 3: It Shows Ramchandran Plot of 4HRL receptor.

Table 4: It Shows Ramachandran Plot statistics.

	No. of residues	Percentage
Most favoured regions[A,B,L]	263	92.9%
Additional allowed regions[a,b,l,p]	18	6.4%
Generouslyallowed regions[~a,~b,~l,~p]	2	0.7%
Disallowed regions [XX]	0	0.0%*
Non-glycine and non-proline residues	283	100.0%
End-residues (excl. Gly and Pro)	11	
Glycine residues	25	
Proline residues	12	
Total number of residues	331	

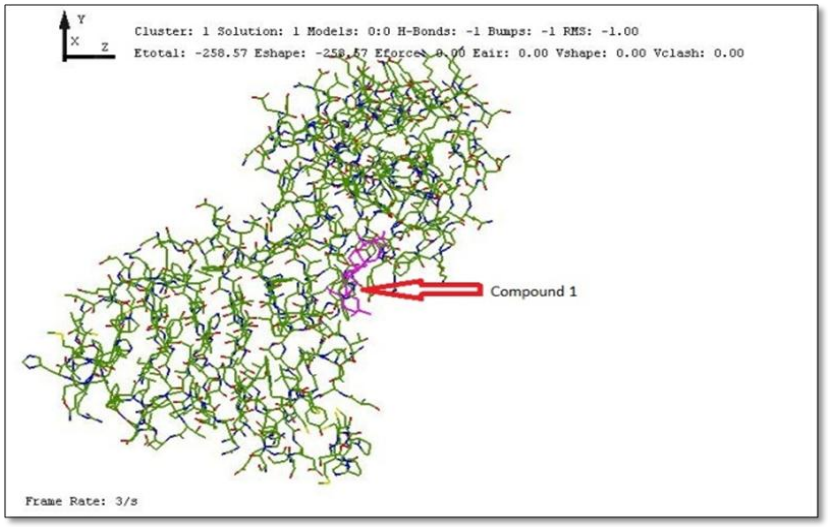
Table 5: It shows the G-Factors.

Parameter	Score	Average Score
Dihedral angles:-		
Phi-psi distribution	--0.08	
Chi1-chi2 distribution	- -0.03	
Chi1 only	-0.10	
Chi3 & chi4	0.49	
Omega	-0.49	
		-0.15
Main-chain covalent forces:-		

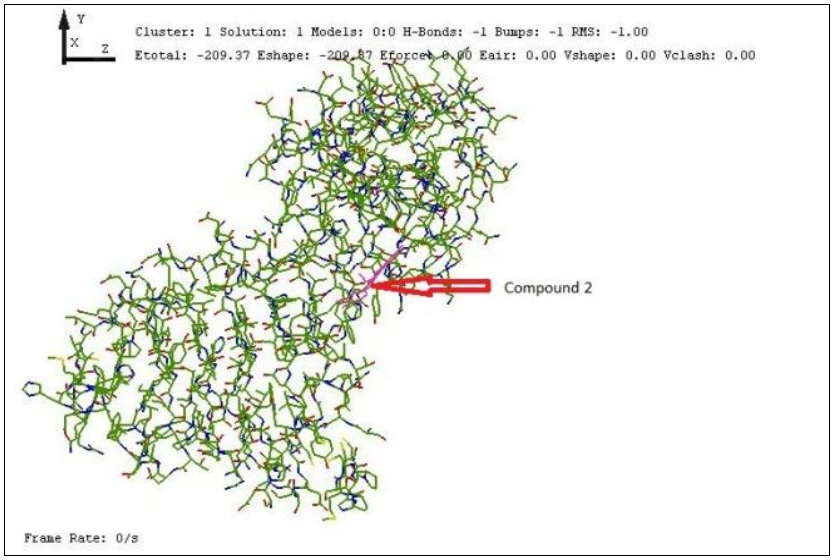
Main-chain bond lengths	0.62	
Main-chain bond angles	0.41	
		0.50
OVERALL AVERAGE		0.12

Ligand-Based Molecular Docking

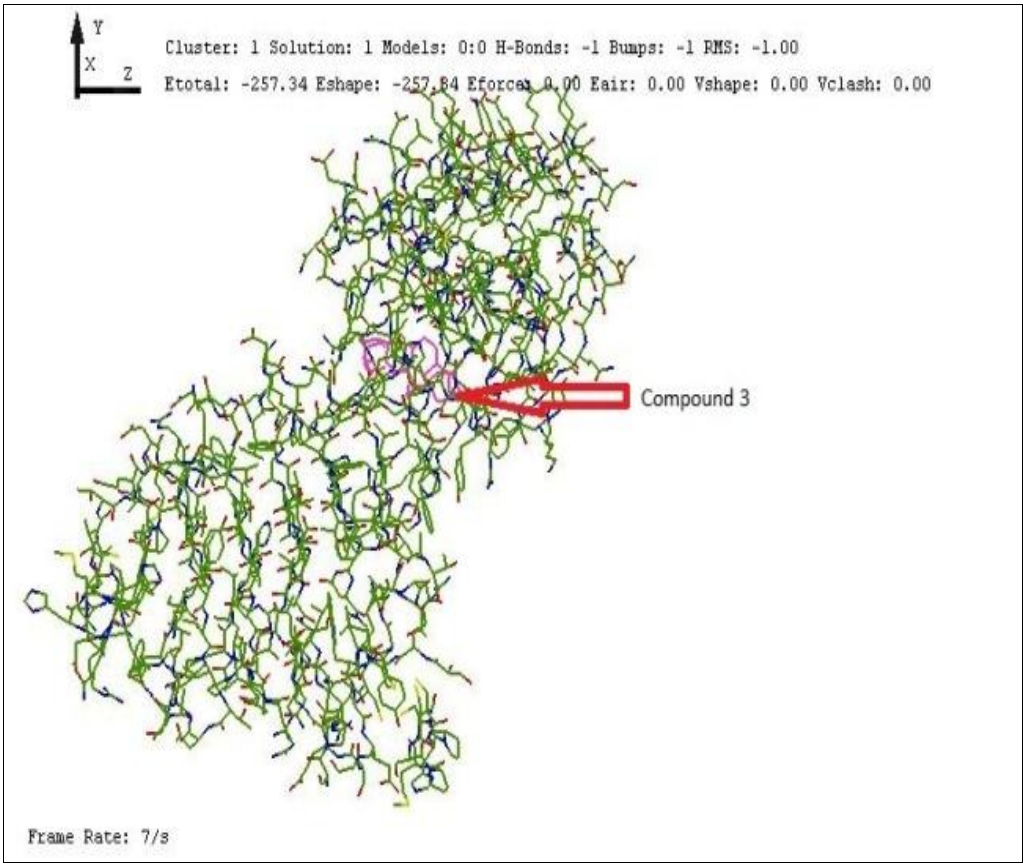
The anticancerous bioactive components were docked to the receptor 4HRL and the energy values were computed using Hex 6.3 version. All the 7 docking compounds are shown here. The outcome of docking studies and Lipinski's properties are presented in **Table 6**. The energy values obtained were ranged from **-159.07 to -258.57**Kcal.mol^{-1}. The results indicate that ligand1, ligand3 and ligand7 exhibited promising inhibitory activity in comparison to other compounds which are presented in **Fig4 (a-g).**



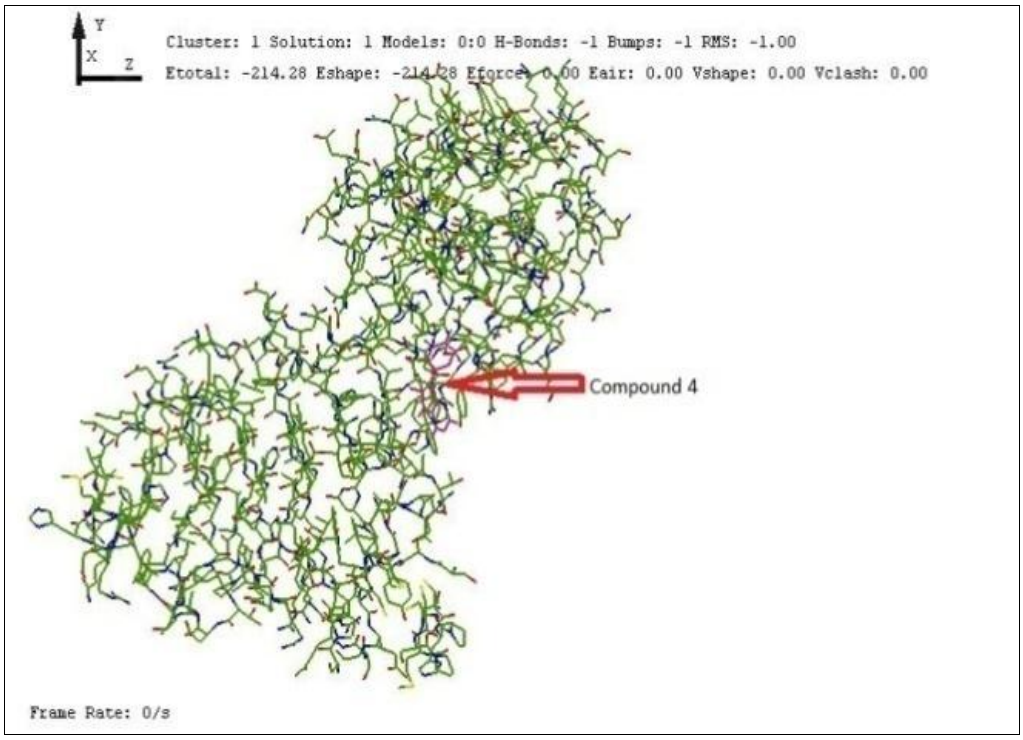
(a)



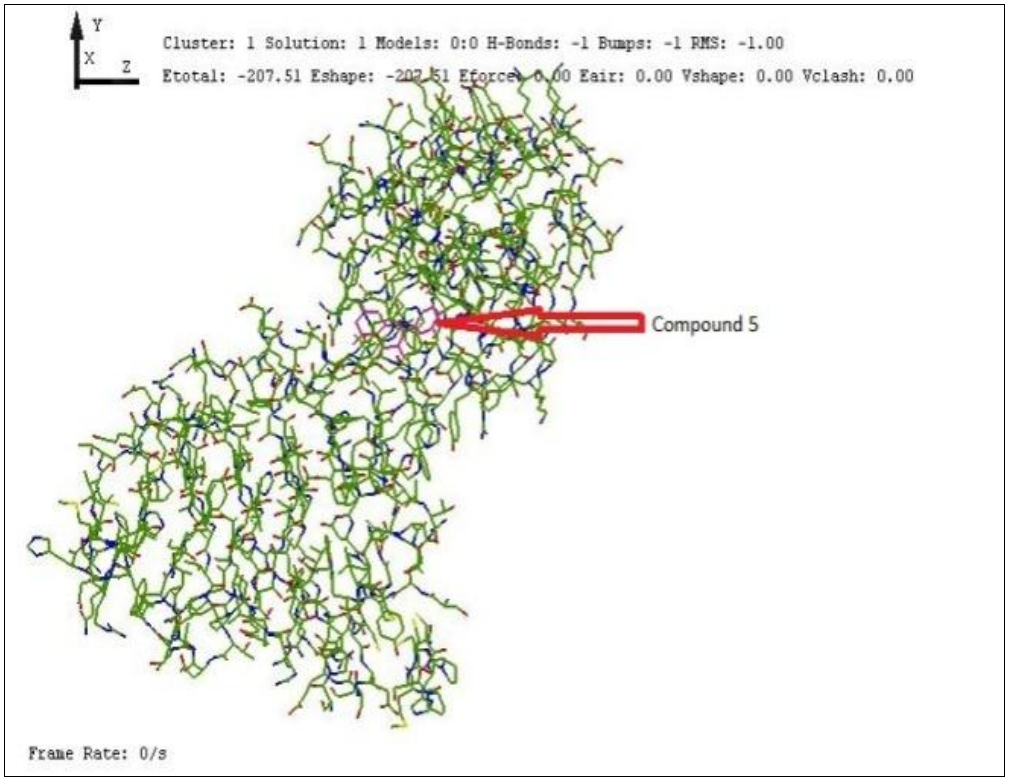
(b)


Y
X
Z
Cluster: 1 Solution: 1 Models: 0:0 H-Bonds: -1 Bumps: -1 RMS: -1.00
Etotal: -257.34 Eshape: -257.84 Eforces: 0.00 Eair: 0.00 Vshape: 0.00 Vclash: 0.00
Compound 3
Frame Rate: 7/s

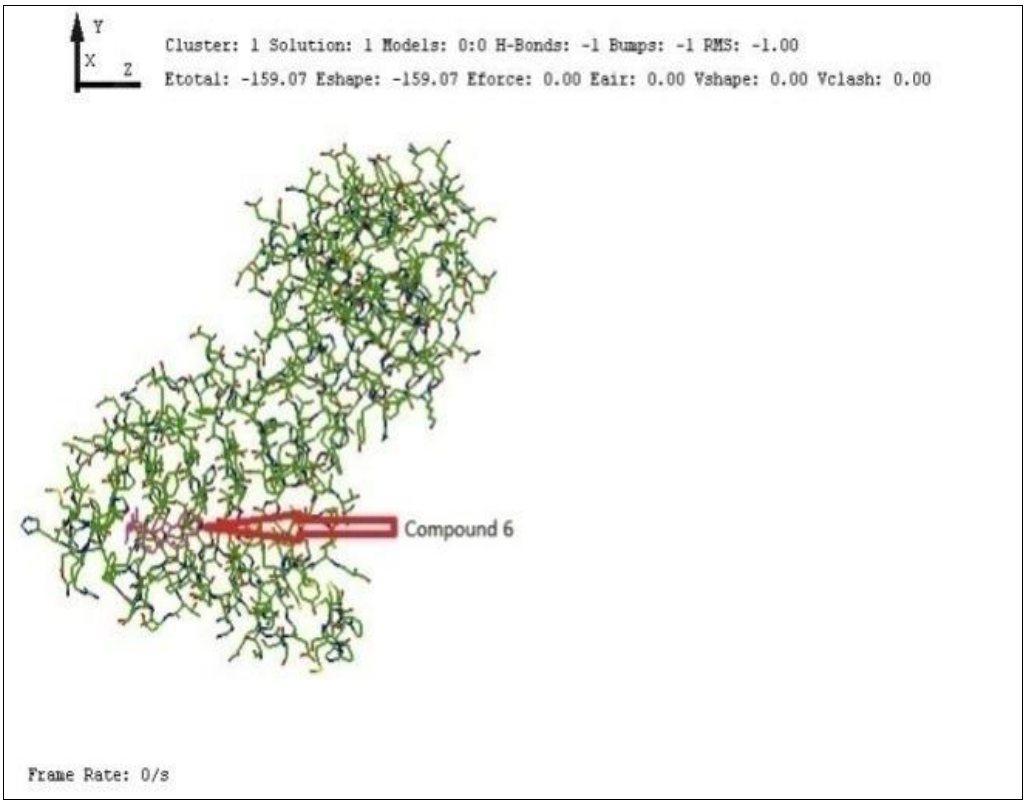
(c)


Y
X
Z
Cluster: 1 Solution: 1 Models: 0:0 H-Bonds: -1 Bumps: -1 RMS: -1.00
Etotal: -214.28 Eshape: -214.28 Eforces: 0.00 Eair: 0.00 Vshape: 0.00 Vclash: 0.00
Compound 4
Frame Rate: 0/s

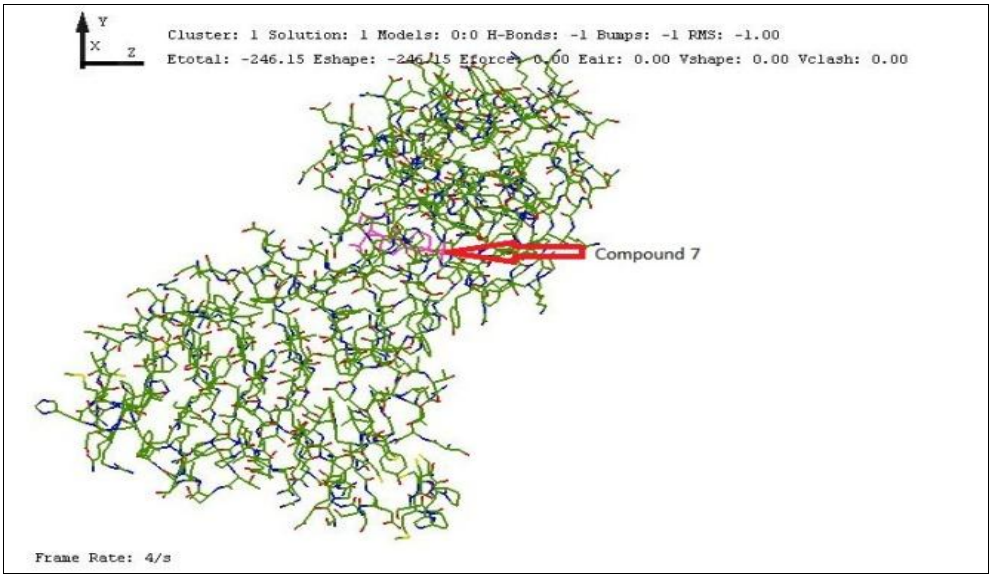
(d)


Y
X
Z
Cluster: 1 Solution: 1 Models: 0:0 H-Bonds: -1 Bumps: -1 RMS: -1.00
Etotal: -207.51 Eshape: -207.51 Eforce: 0.00 Eair: 0.00 Vshape: 0.00 Vclash: 0.00
Compound 5
Frame Rate: 0/s

(e)


Y
X
Z
Cluster: 1 Solution: 1 Models: 0:0 H-Bonds: -1 Bumps: -1 RMS: -1.00
Etotal: -159.07 Eshape: -159.07 Eforce: 0.00 Eair: 0.00 Vshape: 0.00 Vclash: 0.00
Compound 6
Frame Rate: 0/s

(f)



(g)

Fig 4: (a- g) Ligand inside the cavity of Protein HER2 with their Binding energy.

Table6: Showing the binding energy of 7 anticancerous bioactive components with 4HRL.

Compound	E value (Kcal.mol^{-1})
Ligand 1	-258.57
Ligand 2	-209.37
Ligand 3	-257.34
Ligand 4	-214.28
Ligand 5	-207.51
Ligand 6	-159.07
Ligand 7	-246.15

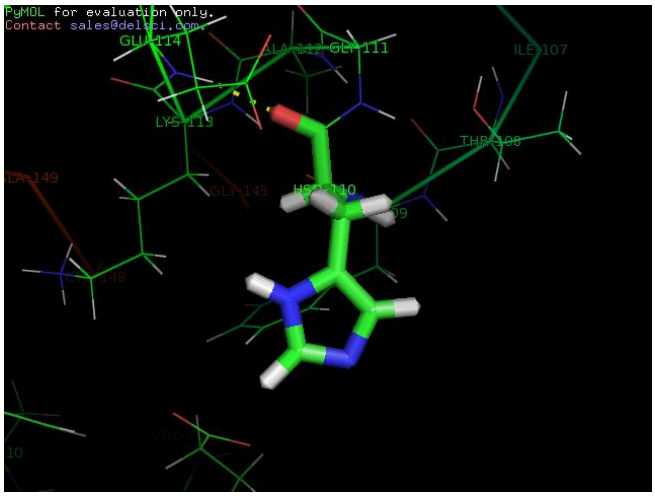


Fig 5: The snap shot of interaction of 4HRL with the most potent compound, visualised using Pymol.

DISCUSSION

The objective of our study was to identify novel potential inhibitors for breast cancer infections. Breast cancer is a major health threat because it is one of the most prevalent women associated disorder resulting into mortality. From literature it was observed that there are a number of plants like *Camellia sinensis*, *Delonix regia*, *Terminalia chebula*, *Syzygium cumini* found North east region which contain natural plants products having best anticancerous medicinal values. So, for our study we had taken a data set of bioactive compounds from the anticancerous plants.

The bioactive components of *S. Cumini* were virtually screened in order to classify them as drug like molecules. The datasets of 7 components revealed that all of them although satisfy Lipsinki's rule of five but not ADME/TOX rule. The detailed analysis predicted that the compound 3, Daucosterol satisfy L-rule as well as ADME/TOX prediction with low risk to HER2 inhibition **(Table 3).** Also when the particular molecule was allowed to interact with HER2 protein, it showed binding energy of-257.34 kcal/mol, which was quiet acceptable. Whereas other plant components also showed minimum binding energy but they were violation ADME/TOX rules so they were excluded from the current study.

Then we performed ligand-based pharmacophore modelling of the data set to generate a model that defines the chemical features (pharmacophores) or necessary structural characteristics that they possess to bind to its target. From this analysis, we obtained the pharmacophore models having different feature patterns and different Pharmacophore-Fit scores. The Feature Patterns indicate which features are met by which compound and Pharmacophore-Fit score indicates how well the features in the pharmacophore overlap the chemical features in the compound .Maximum Pharmacophore-Fit score is given to that compound which shows the maximum necessary features required in order to bind with its target. The model (Model-3) having highest score was selected as best model and taken for further analysis.

Finally to verify the results, the compounds having maximum common features obtained from pharmacophore modelling and the hits found from virtual screening were docked into receptor of 4HRL protein and the energy values were computed using Hex 6.3. The outcome of docking studies followed all criteria of Lipinski's properties. The energy value obtained ranged from **-159.07 to -258.57**Kcal.mol^{-1}. The results indicate that compound1, compound3 and compound7 exhibited minimum binding energy in comparisons to the other compounds.

From all the above studies it was observed that the above mentioned compound 3was found to be highly potent inhibitors against 4HRL protein causing breast cancer. Hence, we concluded that compound 3, Daucosterol can be used as inhibitors for treating breast cancer for further studies can be carried out.

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