

Drug Based Computational Analysis For Initial Stages Breast Cancer with Compounds Obtained from *Acorus Calamus*

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ABSTRACT

The present research on drug development, this study progressed to find the drug target for breast cancer from the plant compound *Acorus calamus*. Analysis by GCMS of the extract revealed 14 compounds that were consequently taken in for docking studies using highly influential proteins such as BRCA1, BRCA2, PTEN, HER2, CHEK2, ERBb1, ATM in order to check the potential of the binding affinity of the compound. Out of 98 complexes docked with Schrodinger Glide module, four complexes such as ERBb1 interacted with [(2R)-2-[(1S)-1-hexadecanoyloxy-2-hydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-yl] hexadecanoate, PTEN interacted with Tetradecanoic Acid, ATM interacted with Tetradecanoic acid and CHEK2 interacted with 2-hydroxy-6-undecylbenzoic acid showed highest Glide score of -7.17, -5.92, -4.56, -5.21 correspondingly. Further, Molecular Dynamics Simulation for the protein complex of ERBb2 done using Macromodel module for 1 nanosecond revealed the protein is feasible deviation and fluctuation map from Root mean square calculations. Based on this study, we can conclude that the ErBB1 protein can be a good target for breast cancer diseased pathway and can be considered that [(2R)-2-[(1S)-1-hexadecanoyloxy-2-hydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-yl] as a ligand can act as a good inhibitor for breast cancer.

Keywords: Breast cancer, *Acorus calamus*, Docking, Molecular dynamics, herb

INTRODUCTION

Breast cancer is a malignant tumour that occurs predominantly in females, although men also account for approximately 1% of cases. This is higher in urban areas where 1 in every 22 persons in a lifetime has been reported compared to rural areas where this risk is relatively 1 in 60 women¹. In India the average age of the high risk group in India is 43-46 years unlike in the West where women aged 53-57 years are more prone to breast cancer. Several well-established factors such as family history, nulliparity, early menarche, advanced age, and a personal history of breast cancer (in situ or invasive) have been associated with an increased risk of breast cancer. The mortality rate due to breast cancer is directly linked to metastatic spread by a cell that exhibits a complex phenotype and its capacity to escape from the primary tumour mass, invade the surrounding normal tissue, and penetrate into the circulation before proliferating in the parenchyma of distant organs to produce a metastasis further leads to associative disease conditions.

Despite the common belief that phytochemicals are safe, they all have inherent risks similar to synthetic compounds. Thus it is within the scope of the phytosciences to elucidate side-effects, appropriate doses, identify bioactive phytochemicals and ways of extraction and conservation. With this regard, the plant extract which contains 14 molecules from *Acorus calamus* have been taken for this study to inhibit breast

cancer diseased protein such as BRCA1, BRCA2, PTEN, HER2, BRBb2, ATM and CHEK2. These proteins are detailed in Fig 1 indicating their importance in triggering cancerous pathway.

BRCA1 and BRCA2 are human genes that produce Tumor Suppressor Proteins by repairing the damaged DNA and play a role in ensuring the stability of the cell's genetic material. When either of these genes is mutated, or altered DNA damage may not be repaired properly resulting in cellular development of additional genetic alterations that can lead to cancer². In preclinical studies, drugs called BRCA inhibitors, which block the repair of DNA damage, have been found to arrest the growth of cancer cells that have BRCA1 or BRCA2 mutations. These drugs have shown some activity in cancer patients with BRCA1 or BRCA2 mutations therefore leading these compounds to further testing stages.

The PTEN protein is a lipid phosphatase with putative tumor suppressing abilities, including inhibition of the PI3K/Akt signaling pathway. Inactivating mutations or deletions of the PTEN gene resulting in hyper-activation of the PI3K/Akt signaling pathway are increasingly being reported in breast cancer and have been related to features of poor prognosis and resistance to chemotherapy and hormone therapy. Prior studies in different tumor models have shown that, under conditions of PTEN deficiency, the PI3K/Akt signaling pathway becomes a fundamental proliferative and survival pathway with its

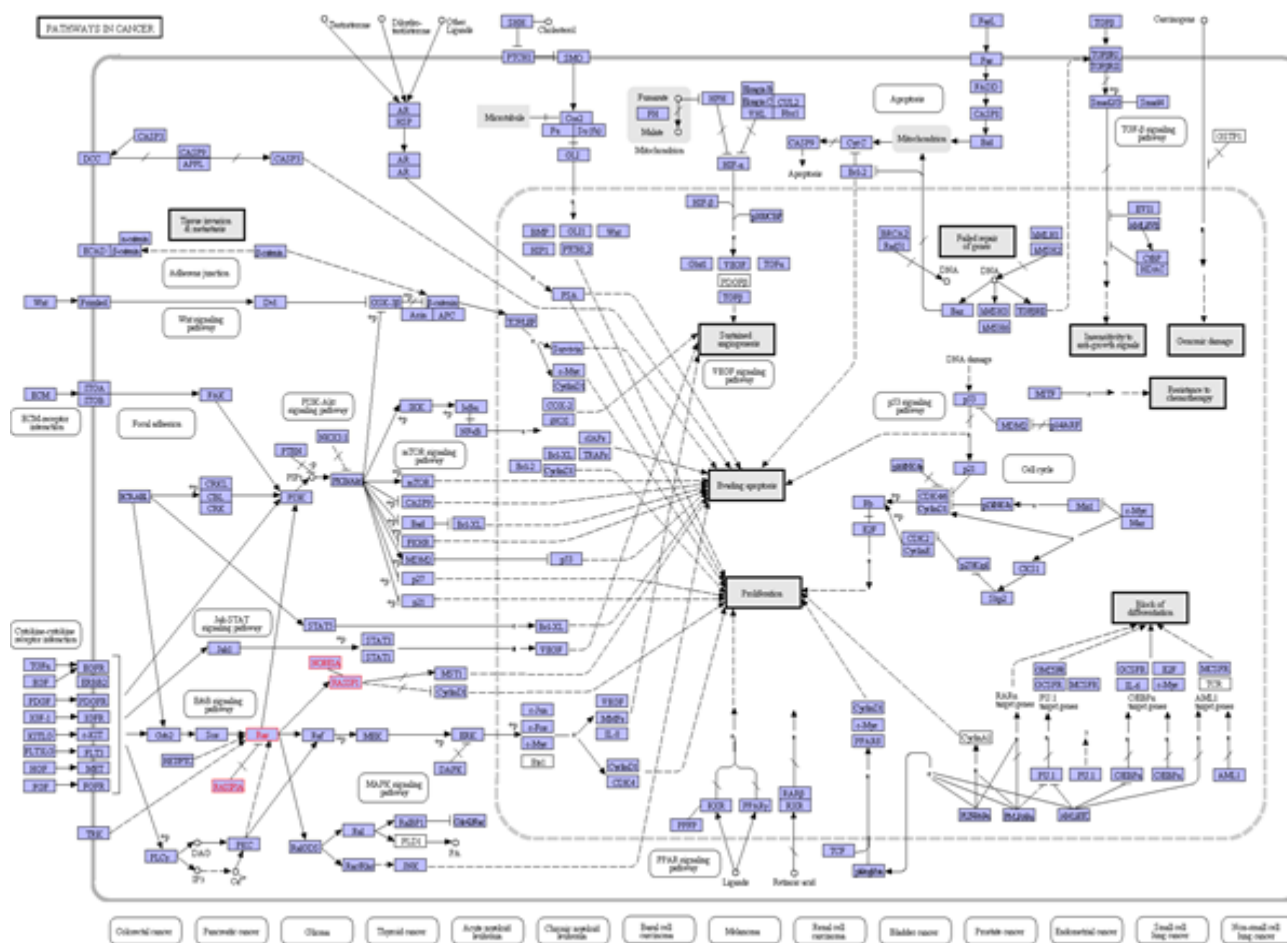


Figure 1: The network of signaling molecules those act as potential receptor proteins for cancer therapy

pharmacological inhibition resulting in tumor growth inhibition³.

Human epidermal growth factor receptor 2 (HER2)-positive breast cancer is highly aggressive and has higher risk of recurrence than HER2-negative cancer. With few treatment options available, new drug targets specific for HER2-positive breast cancer are needed. Pharmacological profiling can be targeted selectively on inhibited growth of HER2-positive breast cancer cells⁴.

CHK2 is a multiorgan tumor susceptibility gene that encodes for a serine/threonine protein kinase involved in the response to cellular DNA damage. In the basis of its role during DNA damage response, Chk2 has been suggested as an anticancer therapy target, but given its recently discovered new function and its role as a tumor suppressor, it is questionable whether inhibition of Chk2 is indeed beneficial for anticancer treatment⁵. However, investigations may able to exploit the of CHK2 in human tumors to develop novel therapies based on synthetic lethal inhibitor interactions.

ATM, the protein kinase mutated in the rare human disease Ataxia Telangiectasia (A-T), has been the focus of intense scrutiny over the past two decades. Initially this was because of the unusual radiosensitive phenotype of cells from A-T patients, and recently as investigating ATM signaling has yielded valuable insights into the DNA damage response, redox signaling and cancer, ATM

alterations have been revealed both in the germ line as a predisposing factor for cancer target⁶.

ErbB2-overexpressing cancer cells derived from a primary mouse ErbB2 tumor also show HSF1 inactivation and HSP90 client destabilization in response to ErbB2 inhibition. ErbB2-positive breast cancer is characterized by highly aggressive phenotypes and reduced responsiveness to standard therapies. Although specific ErbB2-targeted therapies have been designed, only a small percentage of patients respond to these treatments and most of them eventually relapse. The existence of this population of particularly aggressive and non-responding or relapsing patients urges the search for novel therapies⁷.

In this present study, ligand based drug designing for breast cancer target was analysed from isolated *A. calamus* plant compounds. Further the study was progressed for Interaction profiling, Post Docking Validation and MD Simulation of protein ligand complex to analyse the protein with higher influence to target and design the drug to find the criteria of derivative groups that interacted with the protein as the isolated compounds have irrelevant derived source.

MATERIAL AND METHODS

Protein Data bank⁸ was used to find out the structure of BRCA1 [PDB ID: 3KOH], BRCA2 [PDB ID: 1IYJ],

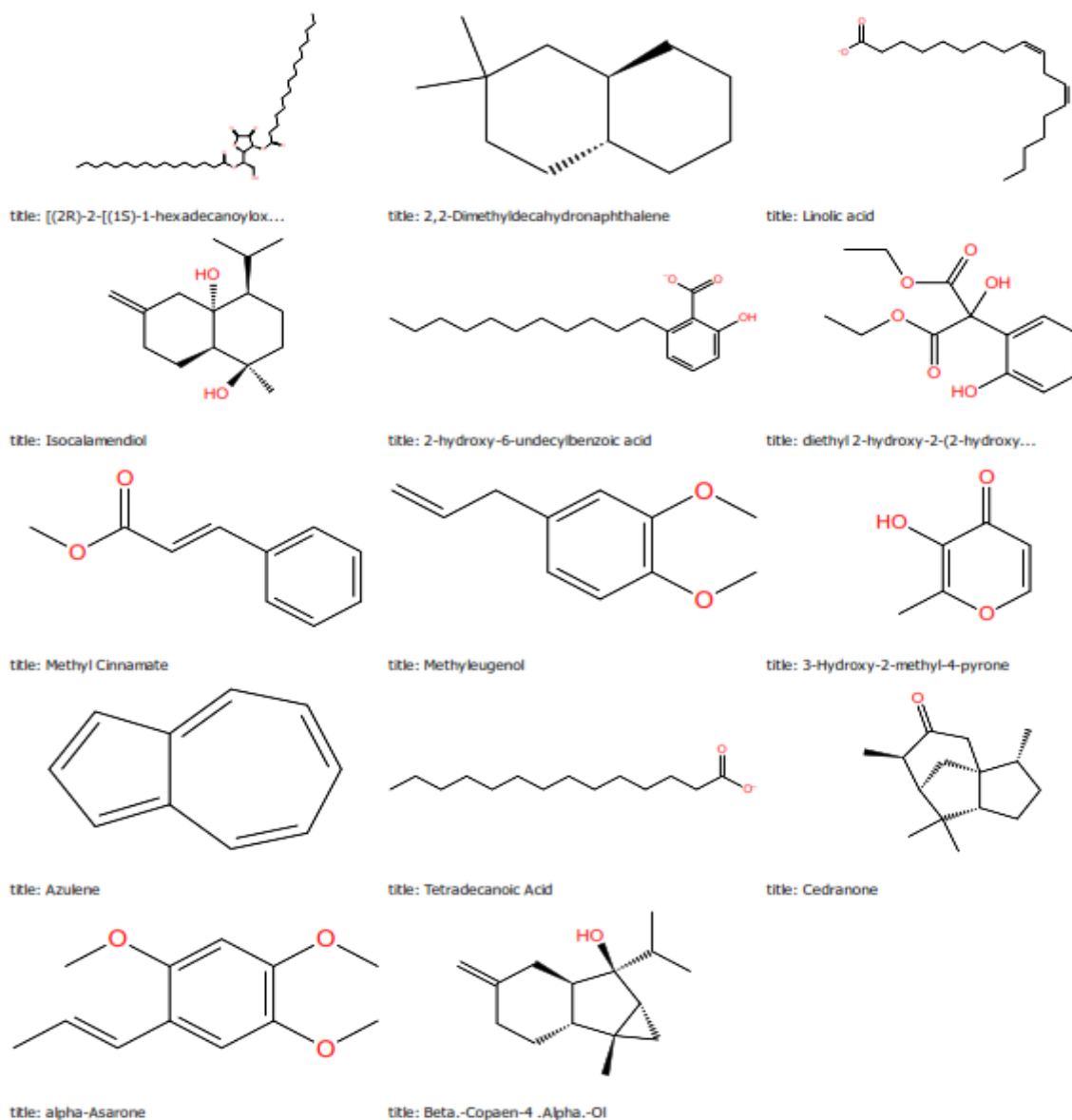


Figure 2: Structures of the Proteins used for this study

PTEN [PDB ID: 1D5R], ATM [PDB ID: 4HDO], HER2 [PDB ID : IN8Z], CHEK2 [PDB ID: 2CN5], ERBb2 [PDB ID:1S78] protein. Three parameters such as <4 Å resolution of crystal studied protein, monomer from the complex and exclusion of complex ligand was used to select the protein from PDB hits. 14 Isolated Compounds from *A. calamus* were drawn using ChemsSketch (<http://www.acdlabs.com/download>) and optimized in 3-dimensional way to view and import into the Schrodinger Suite (Figure 2).

Docking of targeted protein and ligand

Maestro is the graphical user interface for the products of Schrödinger - CombiGlide, ConfGen, Desmond, Epik, Glide, Impact, Jaguar, Liaison, LigPrep, Macro-Model, Phase, Prime, PrimeX, QikProp, QSite, SiteMap, Strike,

and WaterMap. It contains tools for building, displaying, and manipulating chemical structures; for organizing, loading, and storing these structures and associated data;

and for setting up, monitoring, and visualizing the results of calculations on these structures. For this study, Glide is used to generate grid and interact the isolated ligand where as MacroModel is used to simulate the complex protein.

Preparation of Protein and Ligand

Retrieved structures were subjected to removal of Water upto 5 Å distances, conversion of selenomethionine into methionine, selection of monomer to interact, assigning bond order in both polar and non polar amino acids, assigning lone pair electron atom, created disulfide bridges, filled side chains as the norms of crystallized protein may have missing side chains, filled loop within the active regions in the protein and addition of hydrogen bond in the hydrophobic and hydrophilic amino

acids. These parameters processed from Schrodinger Suite 2013-Protein Preparation Wizard and were made

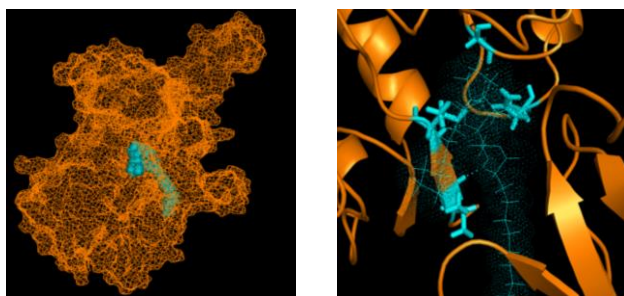


Figure 3: Interaction profile of [(2R)-2-[(1S)-1-hexadecanoyloxy-2-hydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-yl] complexed to ERBb2 protein

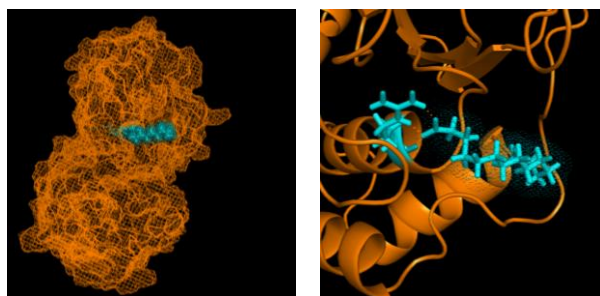


Figure 4: Interaction profile of Tetradecanoic Acid complexed to PTEN protein

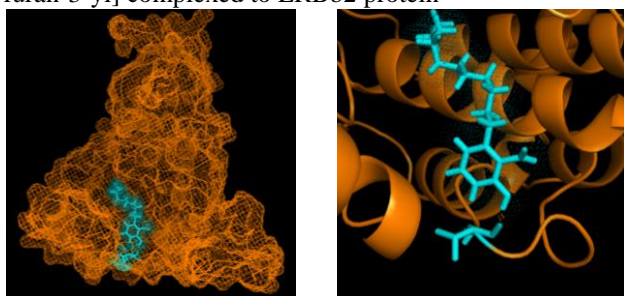


Figure 5: Interaction profile of 2-hydroxy-6-undecylbenzoic acid complexed to Check Point kinase 2

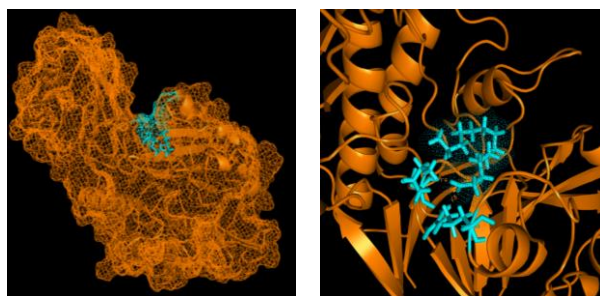


Figure 6: Interaction profile of Linolic Acid complexed to ATM protein-co-crystal ligand complex

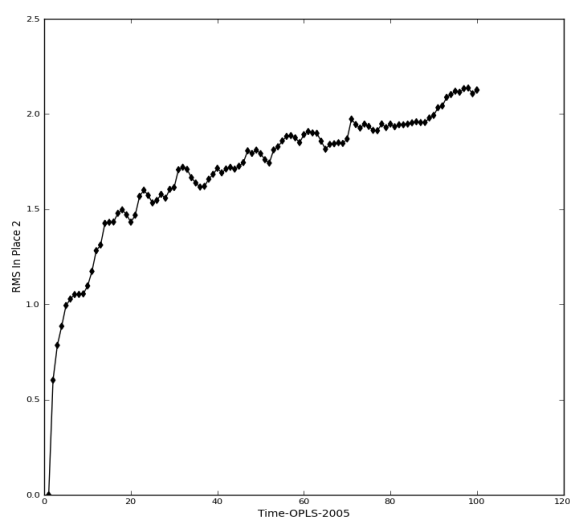


Figure 7: RMSD plot of protein-ligand complex superpositioned structure after 1 ns molecular dynamics simulation run

viable to interact with derived group. pH of 14 isolated compounds was between 7.0 to +/- 2.0 and retained chirality and original binding state of the compound.

Interaction of Targeted Protein and Isolated Ligand

Glide ligand docking jobs⁹ were organized with 7 calculated protein grids and 14 ligand structures. Corrected Lewis structure was generated for ligand. Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. Conformational flexibility is handled in

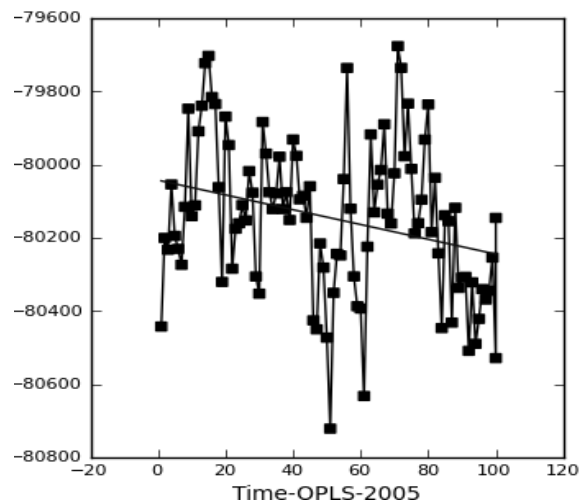


Figure 8: The potential energy of the system on MD simulation

Glide by an extensive conformational search, augmented by a heuristic screen that rapidly eliminates unsuitable conformations, such as conformations that have long-range internal hydrogen bonds. Each rotamer group is attached to the core by a rotatable bond, but does not contain additional rotatable bonds. The core is what remains when each terminus of the ligand is severed at the "last" rotatable bond. Carbon and nitrogen end groups terminated with hydrogen ($-\text{CH}_3$, $-\text{NH}_2$, $-\text{NH}_3^+$) are not considered rotatable because their conformational variation is of little significance. Schrödinger's proprietary GlideScore multi-ligand scoring function is used to score the poses.

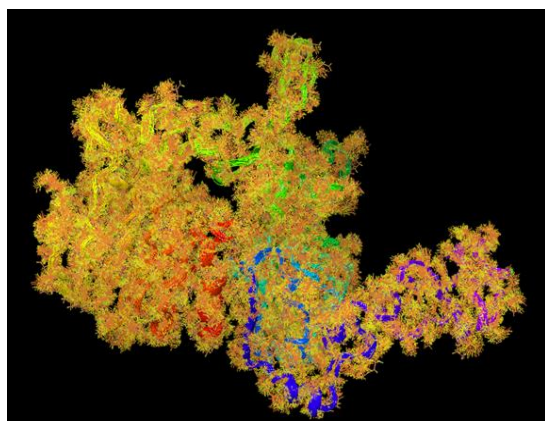


Figure 9: Super Position of first and last complex of the Protein and Ligand in 100 Picoseconds.

Molecular Dynamics Simulation of Protein and Ligand Complex

Protein conformational stability was checked using Schrodinger MacroModel. Protein Solvation effects can be accounted for using the efficient continuum solvation models employed by MacroModel. Additional advanced features include molecular dynamics simulations, free energy perturbation simulations, and pure and mixed methods for ensemble sampling. MacroModel provides this study with multiple advanced methods to aid the understanding of protein structure, energetic, and dynamics using OPLS_2010 as the force field. Simulation was set to run for 1 nano second with 1.5 pico second equilibrium time and 100 samples intervals in the total time period. Temperature of 310 K was maintained during the equilibrium of Simulation period. Water environment was chosen to run the Dynamics along with vander waal energy of 8.0, electrostatic potential of 2.0 and Hydrogen bond limit till 4.

RESULTS AND DISCUSSION

Molecular Interaction Observation of Proteins and Isolated Compound

Out of Seven protein interaction profiling, ERBP2, PTEN, CHEK2, ATM proteins interacted with a higher Glide score *i.e* greater than >-4 .

G Score Ranked 1 Complex - ERBb2 target protein

Canonical ranking indicated [(2R)-2-[(1S)-1-hexadecanoyloxy-2-hydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-yl] complexed to ERBb2 protein with G Score of -7.99. The protein residues such as VAL 3, THR 5, ASN 466, GLY 442 interacted in the bond distance of 2.01A, 2.10A, 2.09A and 2.12A correspondingly with a lipophilic interaction score of -5.43, Lipophilic pair term with ligand atom score of -3.18 and 0.62 score for Rotatable bond Penalty from Ligand atom (Figure 3).

G Score Ranked 2 Complex - PTEN target protein

Docking analysis revealed tetradecanoic Acid complexed to PTEN protein with G Score of -5.98. Interactions with the protein residues of Arg 130, Cys 124 were observed at the bond distance of 2.29A [couple of H Bond] and 1.85A correspondingly. Lipophilic interaction score of -2.43 and Lipophilic pair term with ligand atom score of -

3.65 and 0.10 score for Rotatable bond Penalty from Ligand atom was observed (Figure 4).

G Score Ranked 3 Complex - CHEK2 target protein

2-hydroxy-6-undecylbenzoic acid when complexed to Check Point kinase 2 indicated a G Score of -5.27. In the protein residues such as LYS 209, ARG 183 interacted in the bond distance of 1.63A, 1.85A correspondingly with a Lipophilic interaction score of -1.43, Lipophilic pair term with ligand atom score is -3.65 and 0.81 score for Rotatable bond Penalty from Ligand atom (Figure 5).

G Score Ranked 4 Complex - ATM target protein

Linolic Acid complexed to ATM was observed with G Score of -4.26. The protein residues such as ARG 474, VAL 469 interacted in the bond distance of 1.79A, 2.03A [Couple of H Bond] and 1.62A correspondingly. Lipophilic interaction score is -1.43, Lipophilic pair term with ligand atom score is -2.94 and 0.11 score for Rotatable bond Penalty from Ligand atom (Figure 6).

MD Simulation of Best Interacted Complex

Molecular dynamics of protein-ligand complex was performed by applying Berenson's Temperature (301K) coupling method and Parrinello-Rahman Pressure coupling method. The protein-ligand complex was analysed for its stability from the molecular trajectories obtained after 1 ns equilibration run. Following observables were determined. The potential energy of the system was found to be stable at 79 J/mol with mean fluctuation of ± 8 J/mol (Fig. 8).

The RMSD analysis predicts stability of protein and its structural variation while evolving with time. The RMSD of protein-ligand complex for the trajectories written for 1 ns production run was analysed to identify the stability of the system at each time interval. It was observed that the complex is stable within early 500 ps production run (Fig. 7). Between 750 to 900 ps, the complex was observed to fluctuate from equilibrium with minor deviation of 0.1 nm, thereafter *i.e.*, from 140.1 ps to 200 ps the system again attained equilibrium. On an average, system was observed to be stable with RMSD of 3.3 nm. The system achieves equilibrium early at 10-15 ps and remains stable thereafter for 1 ns simulation period. The complex stability shows that there is no major structural variation in protein after binding with ligand.

This state of observation of protein structure stability in the TIP3P (water) environment observed through gyration of complexity of each trajectories in super imposed manner and the relational difference of each complex was notified for protein structure stability (Fig 9).

CONCLUSION

In this study, designing of drug for Breast cancer has been approached with isolated compounds from *A.calamus* plant. As the cancer pathway has many higher and lower expressed protein and DNA damaging enzyme, this study was targeted to design an 'inhibitor' for diseased proteins of Breast cancer which has been higher expression during the initial stage of cancer disease. Out of the seven targeted proteins, ERBb2 protein has higher interaction profile and more protein conformational stability with [(2R)-2-[(1S)-1-hexadecanoyloxy-2-

hydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-yl]. Henceforth this research using ligand based drug designing on the Breast cancer drug target suggests [(2R)-2-[(1S)-1-hexadecanoyloxy-2-hydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-yl] ligand can act as potential drug in the early stages of cancer detection. However, further validation will be necessary using clinical trials.

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