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Insilico docking analysis of mangrove-derived compounds against breast cancer protein (BRCA1)

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Abstract: Breast cancer is the second leading cause of cancer death in women only next to lung cancer. In normal cells, *BRCA1* and *BRCA2* (breast cancer susceptibility gene 1 and breast cancer susceptibility gene 2) ensure the stability of DNA and also prevent uncontrolled cell growth. Mutation of these genes is linked to the development of hereditary breast and ovarian cancers. Considering the lacunae of reliable and potential drugs to cure the life threatening breast cancer, the present study has focused on molecular computational analysis to identify the potential compounds, derived from mangrove ecosystem, which can block the mutated gene (*BRCA1*) responsible for the breast cancer. Six compounds were tested against the carcinogenic protein. The 3D crystal structure of the protein was retrieved from protein data bank (PDB) and the protein binding sites of the test compounds were identified. The results revealed that among six compounds, triterpenoid, stigmasterol and pyrethrin were found efficient in destroying the protein (BRCA1) responsible for breast cancer.

Keywords: Breast cancer, *BRCA1* gene, pdb, BRCA1 protein

INTRODUCTION

Breast cancer is the most common cancer in women worldwide. It is a type of cancer where cells in the breast divide and grow without normal control. The incidence of breast cancer has doubled during the past 30 years. Between 50 and 75 per cent of breast cancers begin in the ducts, 10 to 15 per cent begin in the lobules and a few begin in other breast tissues (Dillon et. al., 2010) According to American Cancer Society's Global Cancer Facts and Figures, nearly 1.4 million new cases of breast cancer occurred among women worldwide in 2008. In general, developed countries have higher rates than developing countries. Although factors that make up this difference are mysterious, lifestyle and reproductive factors play a major role.

Women who carry a mutated gene of *BRCA1* or *BRCA2* have an increased risk of both breast and ovarian cancers. Women who have a *BRCA1* or *BRCA2* mutation, have a 60 to 80 per cent chance of getting breast cancer and a 15 to 60 per cent chance of getting ovarian cancer, both by the age of 70 (American Cancer Society, 2009). *BRCA1* (breast cancer 1, early onset) is a human tumor suppressor gene that produces a protein called breast cancer type 1 susceptibility protein. Due to the presence of two domains viz. Zinc finger, C3HC4 type (RING finger) and BRCA1 C Terminus (BRCT) domain, the BRCA1 protein is also known as RING finger protein 53 (Paterson, 1998). The *BRCA1* gene is located on 17q21 and has a total length of about 100 kb. This gene consists of 24 exons, and the coding region starts at the middle of exon (Miki et. al, 1994). The gene product of *BRCA1* is a phosphorylated protein that consists of 1863 amino acids and has a molecular weight of 220kDa (Ruffner and Verma., 1997). The RING finger structure is present at the N-terminal region, and a binding site for Rad51 resides in the central part of the primary structure. Two BRCT domains, often seen in proteins that act as cell checkpoints or are involved in DNA repair, are present in the C-terminal region. The BRCT domains in *BRCA1* can remodel chromatin and activate transcription (Miyake et al., 2000). BRCA1 protein is localized in the nucleus (Wilson et al., 1999). Recent studies have shown that small deletions, insertions, nonsense mutations and splicing aberrations account for 87% of all pathogenic mutations of the *BRCA1* gene, resulting in the generation of BRCA1 protein (Couch and Weber, 1996). BRCA1 is expressed in the cells of breast and other tissues, where it helps to repair damaged DNA, or destroys cells if DNA cannot be repaired. If BRCA1 itself is damaged, damaged DNA is not repaired properly and this increases risks for cancers (Friedenson, 2007). The protein encoded by the *BRCA1* gene combines with other tumor suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex (Wang et. al., 2000). The

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BRCA1 protein associates with RNA polymerase II, and through the C-terminal domain, also interacts with histone deacetylase complexes. Thus, this protein plays a role in transcription, DNA repair of double-stranded breaks ubiquitination (Friedenson, 2007), transcriptional regulation as well as other functions (Starita and Parvin., 2003). Women with defects in either the *BRCA1* or *BRCA2* gene have a greater than 80 per cent chance of developing breast cancer. The majority of breast cancer cases are due to *BRCA1* and *BRCA2* (Thakur and Phadke, 2005). Since, the vulnerability of *BRCA1* is more than *BRCA2* gene (Ford et al., 1998), in the present study only BRCA1 protein was tested against 6 chemical inhibitors derived from coastal mangrove ecosystem through bio-computational analysis.

MATERIALS AND METHODS

Protein Structure

The 3-D crystal structure of the targeted breast cancer protein BRCA1 (ID: 3PXB) was retrieved from the protein data bank (PDB) (www.rcsb.org/pdb). Structural and active site studies of the protein were done by using CASTP (Computed Atlas of Surface Topography of Proteins) and pymol molecular visualization software. According to Lipinski's rule of five, a compound having not more than 5 hydrogen bond donors (OH an NH groups), not more than 10 hydrogen bond acceptors (notably N and O), molecular weight under 500 g/mol, partition coefficient log P of less than 5 and rotatable bonds of less than 10 is taken as drug molecules and docking procedure is carried out (Lipinski et al., 2001).

Chemicals screened

Six chemicals namely triterpenoid, stigmasterol, rubroliden, tricin and N-methylflindersine identified from the coastal mangrove ecosystems (Kathiresasn and Qasim, 2005) were screened against the breast cancer protein (BRCA1).

Amino acid binding site

The pubchem database was used for retrieving the phytochemical molecules. The selected chemical structures were generated from SMILES notation (Simplified Molecular Input Line Entry Specification) by using the Chemsketch Software (**www.acdlabs.com**). The predicted binding sites, based on the binding energy, and amino acids make up of the binding cavity. The predicted ligand binding site residues are listed in Table 1.

Table 1. Breast cancer protein BRCA1 binding site

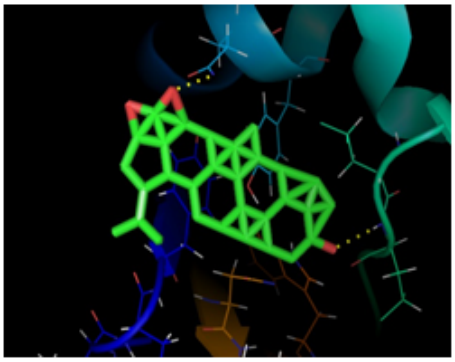
Amino acids in the binding pocket	Binding site amino acids in the structural unit
ALA1669, ARG1670, ARG1835	Alpha Helix
ALA1693, ALA1752, ASN1678	Coil
ALA1789, ARG1649	Beta strand

Docking methods

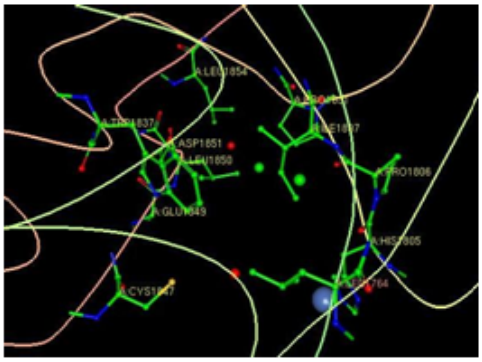
The molecular docking was performed using Argus Lab, widely distributed public domain molecular docking software. The inhibitor and target protein were geometrically optimized and docked using docking engine Argus dock.

RESULTS

Six chemicals derived from mangrove ecosystem were docked with protein responsible for breast cancer (BRCA1). The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in Table 2. Of the 6 ligand molecules, 3 showed the activation energy of greater than 10 kcal/mol and the remaining 3 molecules exhibited the values less than 10 kcal/mol. The highest activation energy (- 13.0471 Kcal/mol) was found with triterpenoid (Figure 1) followed by stigmasterol, pyrethrin, N-methylflindersin and rubroliden. While, the lowest activation energy of 6.2275 Kcal/mol was found with tricin. From the *in silico* docking results, it is quite evident that mangrove-derived compounds have the great potential against anti tumor activity of breast cancer protein BRCA1.

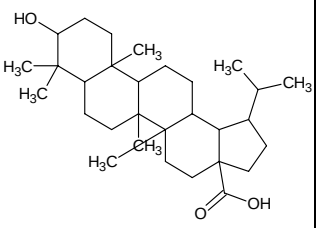
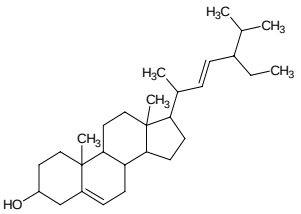
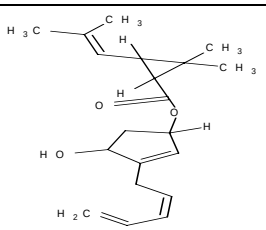
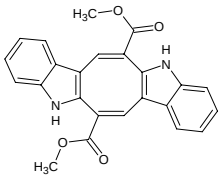
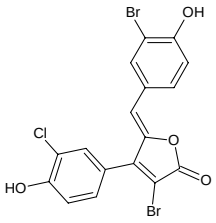
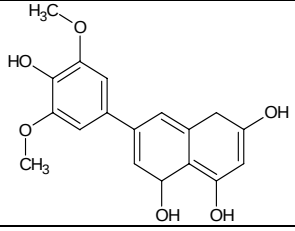


(a) Triterpenoid



(b) Hydrogen bond, Neighbor residues

Table 2. Docking results of mangrove derived compounds against BRCA1 protein

Compound Name	Pubchem ID	Compound structure	Molecular Weight [g/mol]	Hydrogen donor/ acceptor	Docking Energy Level
Triterpenoid	CID: 9804218		458.6041[g/mol]	(2,3)	*-13.0691 kcal/mol*
stigmasterol	CID: 5280794		269.082[g/mol]	(1,1)	-11.5995 kcal/mol
pyrethrin	CID:6433155		372.454 [g/mol]	(0,5)	-10.3912 k cal/mol
N-Methylflindersine	CID: 72819		241.2851[g/mol] $C_{24}H_{18}N_2O_4$	(0,2)	-9.06183 k cal/mol
rubrolide	CID 5472704		472.51196	(2,4)	-7.8583 k cal/mol
tricin	CID: 5281702		330.288[g/mol]	(3,7)	-7.31076 k cal/mol

DISCUSSION

Recent studies have shown that small deletions, insertions, nonsense mutations and splicing aberrations account for 87% of all pathogenic mutations of the *BRCA1* gene, resulted in the generation of truncated BRCA1 protein (Couch and Weber, 1996). Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. Mangrove-derived compounds such as triterpenoid and stigmasterol have already been studied for computation selection against sterol carrying protein, AeSCP-2 (Senthil and Kathiresan, 2011) and cervical viral oncoprotein, HPV16 E6 (Senthil and Kathiresan, 2011). The present study also proved that the coastal mangrove-derived compounds are capable of blocking the oncoprotein, responsible for breast cancer.

CONCLUSION

Mangroves are rich in phenolic compounds of medicinal value. These compounds are ecofriendly, safer and cheaper for application. Identification of BRCA1 will facilitate early diagnosis of breast and ovarian cancer susceptibility in some individuals as well as a better understanding of breast cancer biology. The results obtained from this study would be useful in both understanding the inhibitory mode of mangrove- derived compounds as well as in rapidly and accurately predicting the activities of newly designed inhibitors on the basis of docking scores. Here we concluded that these compounds derived from mangrove ecosystem (triterpenoid, stigmasterol and pyrethrin) could be novel chemical inhibitors for BRCA1 protein preventing the uncontrolled cell division. Further research is needed for refinement to enrich the activity of the ligands and destroying mechanism of the breast cancer protein, especially in the animal model system, and also to determine the dosage of safety levels, in order to explore this promising avenue for breast cancer control and to ensure the healthy state of women.

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