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Marine Pharmacology: Marine Sponges as a source for Drug Development, an *In silico* Approach

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Abstract—*Drug discovery leading to booming and possible drug candidates remain a challenging task in screening of drugs in the field of combinatorial chemistry. Breast cancer the most common cancer in women, with cells dividing and growing without normal control. Taking these into consideration the research is focused on the compounds isolated from marine sponges. Natural products and its derivatives are the sources of new therapeutic agents, these natural products compensates and overcomes the synthetic drugs used for breast cancer. The marine organisms represent a great biological history with diverse compounds and unique properties for various ailments. Among various targets in breast cancer the BRCA1, HER2 and ER positive were analyzed for protein ligand interaction with commercial drugs and natural compounds. The protein structures were downloaded from PDB and the binding energies were calculated from the docking score using Argus lab. The results showed the natural compounds stigmasterol and oleic acid to be efficient leads with better binding energy score and satisfying the ADME properties. These compounds may inhibit the breast cancer proteins by suppressing cancerous cells at different points. The lead molecules may come up as drugs or can be used as supplements against cancer therapeutics.*

1. INTRODUCTION

Marine Sponges are multicellular ancient metazoans which have existed for 800 million years, have a great biological history. They have attracted the researchers for the last five decades due to the technological advancements in various methods used for the collection of marine organisms. Around 16000 marine compounds have been isolated from marine organisms, with more than 7000 reports till date [1]. The marine life constitutes 80% of the biota [2]. The non-chordates from phylum porifera to echinoderms are found in the salt water, and halobiotic due to the metabolic activities, secretions and the adaptations. Sponges are known to be the sources of secondary metabolites produced as a defense mechanism from predators; these metabolites are of pharmaceutical importance and medical relevance. The secretions of secondary metabolites differ from one organism to the other. The bioactive metabolite of sponges ranges from steroids, terpenoids, isoprenoids, nitrogen heterocyclic, non isoprenoids, quinines and brominated compounds. Few other marine organisms like echinoderms, mollusks, cnidarians, and annelids have also attracted scientists by the biological activity like antimicrobial, antiviral, anticancer, antifungal and antihelmenthic activities [3-5]. The serendipitous discovery of spongothymidine and spongouridine isolated from the Caribbean sponge *Cryptotheca crypta* in 1950s, with anti viral activity and

the synthetic analogues from these compounds lead to the discovery of anti cancer drugs [6]. The compounds with biological activity from sponges are Manzamines, the anti malarial drug against the parasite *Plasmodium berhei* [7], discodermlide, anticancer drug from *Discodermia dissolute* [8], HTI-286, synthetic analogue of hemiasterlin from *Hemiasterella minor* , Halichondrin B from a Japanese Sponge [9] halistanol and halistanol sulfate from *Halichondria mooriei*, halistanol trisulfate from genus Topsentia tetracarbocyclic sesterterpenes from *Cacospongia* scalaris exhibited cytotoxicity against P-388 cell lines [10].

Breast cancer the second common cause for cancer death in women worldwide. The cancer which forms in the breast by the uncontrolled growth of cells. Based on the gene expression profiling the three major subtypes are Estrogen Receptor positive (ER+), Progesterone Receptor positive (PR+) and Human Epidermal Growth Factor -2 (HER2), other types include Luminal A, Luminal B, Triple negative or basal like [11].

Among various targets in breast cancer few major types are taken for the present study. HER1, HER2 and HER3 are implicated in the development of cancers, were one in 5 cases are because of HER2 overexpression. The ligand binds to the extra cellular domains of the receptor makes a conformational change or rearrangement. HER2 has no direct identified ligands and exist in open conformations with makes the dimmerization possible with other HER receptors. The inhibitors designed should suppress the over expression of HER2, the anti HER2 therapy when not given at the early stages may cause death to the patients, so HER2 are the important biomarkers [12-14]. Lapatinib, pertuzumab and

tamoxifen are given for the breast cancer cases with HER2 overexpression [15-17]. Estrogen receptors are the nuclear hormone receptors which acts as a ligand activated transcription factors [18]. The ligands bind and make a conformational change in the receptor. The drugs tamoxifen, fulvestrant and exemestane are used for the patients with breast cancer; estrogen receptor positive cases in pre menopausal women by inhibiting the estrogen biosynthesis [19].

BRCA1 is the tumor suppressor gene producing the protein called breast cancer type 1 susceptibility protein 1[20, 21]. The mutations in the BRCA1 & 2 have gradually increased the risk of breast and ovarian cancers. When there is damage in the BRCA1 gene and the DNA not repaired it increases the chances of breast cancer. The proteins coding the BRCA1 gene joins with the tumor suppressor proteins and makes a complex known as the BRCA1 associated genome surveillance complex. More than 80% chances of the breast cancer are by the defects in the BRCA1 & BRCA2 genes [22-25].

The BRCA1 is highly susceptible than BRCA2, the BRCA1 target is focused. The present study is on the three important of breast cancer targets ; HER2, estrogen receptor positive and BRCA1 with a comparative computational approach with the synthetic drugs used in breast cancer with the natural compounds isolated from marine sponges.

2. MATERIALS AND METHODS

Protein structure

The three dimensional crystal structures of breast cancer proteins HER2 (PDB ID: 1N8Z), BRCA1 (PDB ID: 1JNX) and ER (PDB ID: 1R5K) was retrieved from the protein data bank (PDB) (www.rcsb.org/pdb).

Lead molecules

The commercial drugs used in breast cancer namely tamoxifen, letrozole, raloxifene, exemestane, anastrozole, fulvestrant, olaparib, rucaparib, nivaparib and the natural compounds stigmastreol and oleic acid [26] isolated from marine sponge *Aurora globostellata* [27,28] were generated for SMILES(Simplified Molecular Input Line Entry Specification) notations using drug bank and the 3D structure downloaded from CORINA.

Docking method

The molecular docking was done using Argus lab, which is one of the widely used open source software for molecular docking analysis. The conformational analysis like protein and ligand geometry optimization was performed by docking engine Argus dock. The quantum mechanics helps to predict the energy, structures, geometrical optimization, bond length, and bond angle and vibration frequency of coordinates in atoms [29] (http://www.arguslab.com/arguslab.com/ArgusLab.html.

Molecular Visualization

The molecular visualization of the docked complexes is performed using Pymol and Discovery studio. DS visualizer is free software for the viewing and analyzing the macromolecules and small molecules, the 3D structures, SMILES notations, sequences are analyzed using the software.

3. RESULTS AND DISCUSSION

Protein-Ligand Interaction

The docking analysis was done using different drugs used for breast cancer based on the cancer types and the ligand molecules were docked to the active site of the protein target. The entire protein is treated as the binding site. The exhaustive search with $0.4A^0$ space between grid points by high precision option menu. The flexible option for ligand, dock for calculation of energy and the AScore for scoring function a maximum poses of 150 were analyzed. The binding affinities were obtained based on the energy score in Kcal/mol. The features and the binding affinities are summarized in Table 1. The higher the negative energy score indicates the stronger the binding affinity of the ligand towards the receptor, this binding stability is the main property for a good drug candidate molecule. The target 1N8Z (Crystal structure of extracellular domain of human Her2) for the HER2 overexpression docked with the commercial drugs presently given for breast cancer patients and natural compounds from sponges ranked based on the binding energy as Tamoxifen > Stigmasterol> Raloxifene> Oleic acid> Letrozole> Lapatinib.

And the target 1R5K (Human Estrogen Receptor Alpha Ligand-Binding Domain) for the estrogen receptor positive cancer docked with the ligands showed the binding energy as Stigmasterol> Tamoxifen> Exemestane> Oleic acid> Anastrozole> Letrozole>Fulvestrant.

Finally the target BRCA1 with PDB ID: 1JNX (Crystal structure of the BRCT repeat region from the breast cancer) docked with the commercial drugs Olaparib, Nivaparib, Rucaparib ranked the binding energy as Stigmasterol> Nivaparib >Oleic acid> Olaparib> Rucaparib. Among the three breast cancer targets taken in the study the natural compound stigmasterol showed higher binding energy of -16.38 Kcal/mol against the target estrogen receptor than the commercial drugs.

ADME prediction

The preclinical ADME focuses towards the elimination of weak drug candidates in the early stages of computational drug development. In order to possess a better activity a drug molecule must possess a better half life and bioavailability. It's the initial screening and accelerates the timeline of the drug under investigation.

The Lipinski rule of five is analyzed using the Lipinski filters tool summarized in Table 2. http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp[30-32]. Both the natural compounds stigmasterol and oleic acid obeyed almost all the ADME properties to satisfy as a better lead molecule, unfortunately with stigmasterol violating the LogP value alone. There are few commercial breast cancer drugs which are used for the treatment inspite of violation of few rules namely Letrozole, Lapatinib, Anastrozole by not satisfying the hydrogen bond donor value. The drugs lapatinib and fulvestrant have the molecular weight more than 500 by not satisfying the rule.

The docked complexes of the breast cancer protein 1N8Z, 1R5K, 1JNX with the ligands are depicted as the interaction map with the aminoacid residue in Fig 1-6.

Stigmasterol and oleic acid

There are various plant sterols which are said to possess anticancer effects; one of such sterol is the stigmasterol having the structure similar to cholesterol. Various research findings report stigmasterol to be effective target in tumor endothelial cells and suppress tumor growth [33, 34]. Oleic acid, the mono unsaturated fatty acid more familiar to people by olive oil .Its inhibits the over expression of HER2 protein by synergistic interaction with anti-Her-2/neu immunotherapy making the apoptosis of breast cancer cells [35, 36].

Table 1: Protein-Ligand interaction of breast cancer targets against commercial and natural drugs

Target	Ligands	Dock Energy score (Kcal/mol)	Binding residues	H bond
1N8Z	Oleic acid	-10.02	Thr 429	1
	Stigmasterol	-11.6	Thr 331	1
	Tamoxifen	-12.48	Thr 331	1
	Lapatinib	-9.51	Lys 257, Gly 256	2
	Letrozole	-9.64	Gln 253, Tyr 309, Glu 369	3
	Raloxifene	-10.11	Asn 280, Ser 441	2
1R5K	Oleic acid	-12.02	Cys 766	3
	Stigmasterol	-16.38	Ala 340	1
	Tamoxifen	-14.45	Met 867, Ser 866	2
	Letrozole	-9.99	Glu 339	1
	Exemestane	-13.38	Tyr 537, Tyr 526	2
	Anastrozole	-10.49	Leu 623, Thr 583, Thr 583	3
	Fulvestrant	-9.02	Gly 580	1
1JNX	Oleic acid	-9.51	Gln 1721	2
	Stigmasterol	-10.72	Gln 1721	1
	Olaparib	-9.87	Lys 1690, Ala 1693	2
	Rucaparib	-8.65	No poses	-
	Niraparib	-9.92	Ala 1693	1

Table 2: ADME properties of lead molecules

Ligand	Lipinski's Rule of Five			
	M. weight	H bond acceptors	H bond donors	LogP
Stigmasterol	412	1	1	7.80
Oleic acid	281	0	2	4.77
Tamoxifene	372	1	1	4.57
Raloxifene	474	4	3	4.65
Letrozole	300	2	9	-1.44
Lapatinib	567	7	6	3.02
Fulvestrant	607	3	3	8.79
Exemestane	298	2	0	4.25
Anastrozole	308	2	9	-1.47
Olaparib	436	5	3	3.188
Rucaparib	324	2	4	1.95
Niraparib	323	4	5	1.57

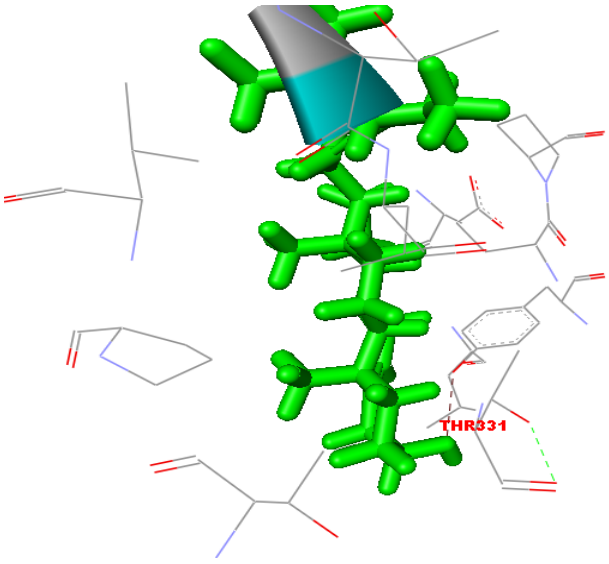

Fig. 1: 1N8Z –Stigmasterol interaction map

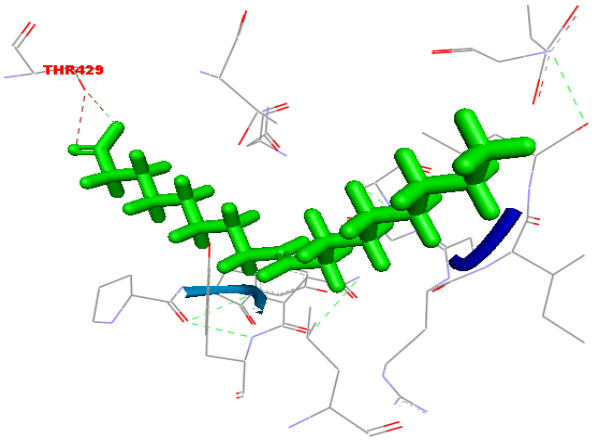

Fig. 2. 1N8Z -Oleic acid interaction map

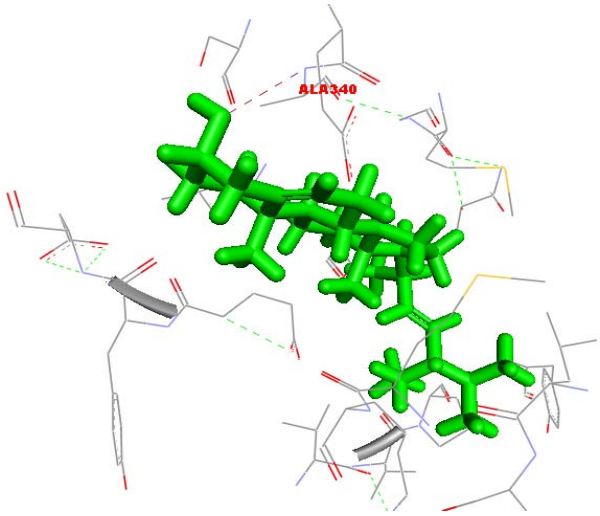

Fig 3. 1R5K- Stigmasterol interaction map

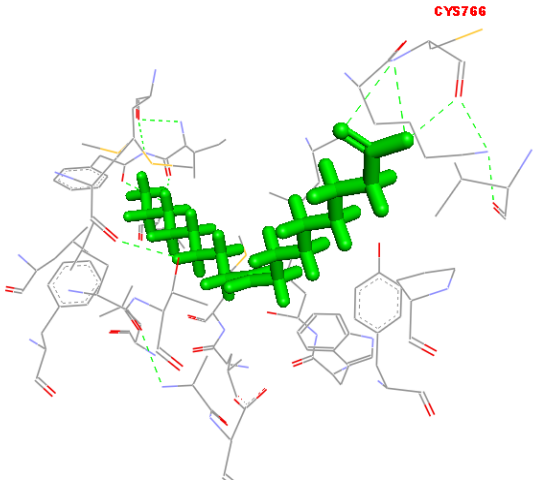

Fig 4. 1R5K- Oleic acid interaction map

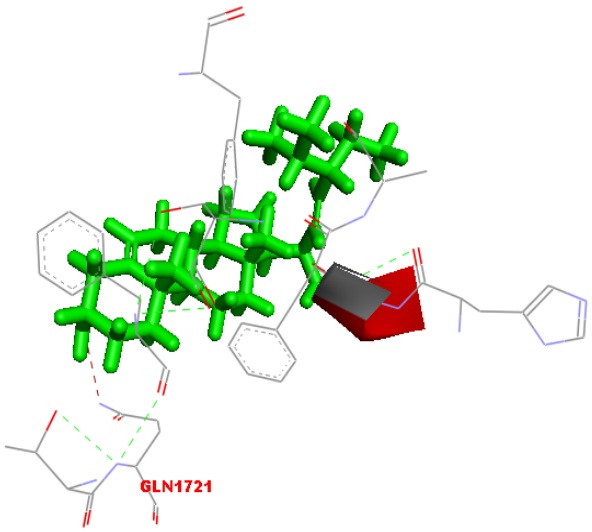

Fig 5. 1JNX- Stigmasterol interaction map

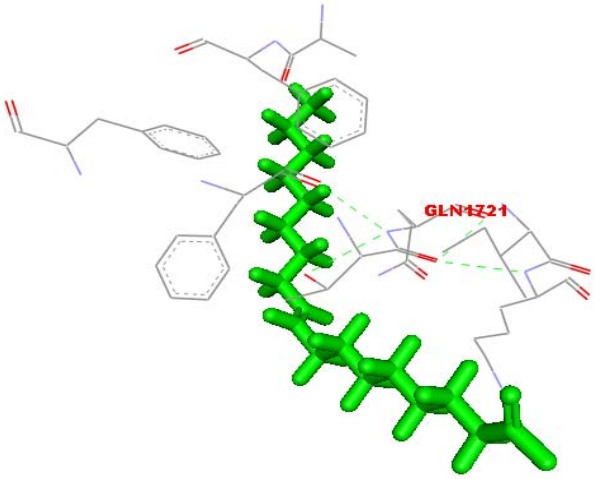

Fig 6. 1JNX- Oleic acid interaction map

4. CONCLUSION

Marine sponges are the rich sources of secondary metabolites with biological activity. Identification of the correct target with the best binding lead molecule makes a better understanding in breast cancer drug development. The results obtained from the study helps to understand the inhibitory action of stigmasterol and oleic acid based on the docking energy score. To conclude the compounds from marine sponges with long biological history would be novel inhibitors against breast cancer. Both the compounds from natural origin has a good and better activity when compared with the commercial drugs used in breast cancer treatment. Further research in vivo and in vitro may enrich the activity and the mechanism behind the apoptosis of breast cancer cells. These

explore the promising avenue to control breast cancer if these compounds when used as drugs or as supplements may ensure the healthy state of women.

5. FUTURE PERSPECTIVES

The *in silico* approach may pave the way for the *in vivo* and *in vitro* analysis to prove stigmasterol as a candidate drug molecule against breast cancer and also various other therapeutic targets. Also the structures can be altered by the QSAR studies to still increase or tweak the structure to enhance the efficiency of a molecule.

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