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Virtual Screening of Marine Natural Antifoulant: In Silico Approach to Screen Antifouling Metabolites from Marine Sponges

D. Inbakandan[1,4*], Sumit Raj [2], C. Kumar[1], R. Venkatesan[3] & S. Ajmal Khan[4]
[1]Centre for Ocean Research, Sathyabama University, Chennai 600 119, India
[2]Department of Bioinformatics, Sathyabama University, Chennai 600 119, India
[3]National Institute of Ocean Technology, Pallikaranai, Chennai 600 100, India
[4]Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai 608 502, India
[E.Mail: inbakandan@gmail.com]

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A molecular docking analysis was carried out followed by Molecular Dynamics (MD) simulation studies on the metabolites from various marine sponges in order to identify their metabolites being potent inhibitors towards biofouling. The docking analysis reveals that the metabolites from a marine sponge *Acanthodendrilla sp*. and *Agelas cf. mauritiana* may act as potent antifouling agents with the docking scores ranging from 68 to 123 for the metabolites. Chemical structure of the most active ligand was converted into a feature-shaped query. This query was used to align molecules to generate statistically valid lead compound.

[**Keywords:** Biofouling; Barnacles; Cyprid; GPCR; Molecular Dynamics; Molecular Simulations; Marine sponges; Antifouling]

Introduction

Biofouling is the undesirable growth of microorganisms, plants and small animals on artificial structures immersed in seawater. The most visible and well-known forms of such fouling are the barnacles, limpets and seaweeds that adorn ships' hulls, seaside piers and coast defences. They lead to an increase in the drag thereby leading to increased fuel consumption, hull cleaning and removal of paint[1]. Barnacles such as *Balanus improvise* and *Balanus amphitrite* cause the most severe fouling problems. Barnacle attaches to structures immersed under water by secreting an adhesive called as adhesive proteins. After identifying a suitable surface to adhere cyprid larva secretes adhesive proteins by a pair of cement glands. Cement glands are connected by cement ducts, which widens into a muscular sac. Muscular sac is connected to an antenna by another cement duct and pours proteinaceous cement into antenna[2]. Cyprid cement flows around and embeds the attachment organs and the cyprid larva is able to attach itself to the surface and metamorphoses into a calcified adult barnacle[3]. Activation of GPCRs by the sensory cells of the antennules leads to the inducement of signal transduction pathway, which results in secretion of adhesive protein and subsequently to the settlement and metamorphosis of larvae. By blocking the biochemical pathway, it would be possible to stop the settlement and metamorphosis of larvae into adult.

Historically, development of marine natural antifoulant relied upon a sequential trial-and-error approach, testing many compounds and observing their antiofouling effects. To minimize the manpower, money and materials for searching marine natural product based antifoulant research we can mimic the methods of pharmaceutical companies which established huge chemical databases for screening large number of drugs through extensive libraries based virtual screening on peptides, nucleotides, phage-display systems and small organic molecules. Virtual screening is the computational analog of biological screening. The main objective of virtual screening is to help chemist filter out inactive from a library of compounds before going ahead for synthesis. This paper reported the preliminary studies using virtual screening approach to find out marine natural compounds based antifoulant by analog based design studies done using pharmacophore models. The designed novel marine natural compound based inhibitors will

be further subjected to docking studies to reduce the number of false positives.

Materials and Methods

The sequence for the GPCR protein of *Balanus amphitrite* was taken from Uniprot (Magrane M. and the UniProt consortium) with id Q93127. The sequence consists of a total count of 379 amino acids. Templates 3D4S[4] (identity 36%) and 3SN6[5] (identity 32.4%) were taken from PDB[6] for modeling the protein structure. BLAST[7] analysis revealed the percentage of similarity between the target sequence and the template sequence. Current studies include GPCR modeling include protein threading approach which may not lead to the elucidation of correct structure. Since the percentage identity for the templates was not too high, multi-template protein modeling approach was employed in order to derive the 3D structure of the target protein using MODELER 9v7[8].

The predicted 3D model was validated by PROCHECK[9] and ERRAT[10] to make sure that it was of reasonable quality for analyzing ligand binding. Superimposition of the modeled protein and the template was done to check the accuracy of the modeled protein. After minimization was performed to smooth out most of the steric clashes between the residues, structural validation by stereochemistry and energy distribution were commenced suing the Ramachandran Plot from SAVS server.

A set of 33 metabolites (Table 1) from various marine sponges such as *Aplysinella sp.,*[11] *Acanthodendrilla sp.,*[12] *Agelas cf. mauritiana,*[13] *Pseudoceratinaarabica sp.,*[14] *Callyspongiatruncata sp.,*[15] and *Axinyssa sp.,*[16] acting as potential antifouling agents were taken into consideration after exhaustive literature survey. Structures of all these metabolites were drawn using ACD Chemsketch[17] and saved in .mol format for the molecular docking analysis.

The LigandFit module of Accelrys Discovery Studio was used for docking analysis and the results in terms of hydrogen bonding and the scoring functions were noted. In order to investigate further details of the interactions between the protein and the ligand MD simulation was performed using the Simulation package in Discovery Studio 2.1[18] (Accelrys Inc., CA). Briefly, the complex was solvated in a water spherical boundary with harmonic restraint using an inhibitor as a centre of mass and subsequently energy-minimized by 1000 steps of steepest descent algorithm followed by 2000 steps of conjugate gradient algorithm. Temperature of the system was maintained at 300 K and 14 Å cut off for non-bonding interactions were used. All simulation steps were run with a time step of 1000 picosecond. The full MD trajectory was considered for analysis.

Results

Templates 3D4S and 3SN6 were chosen for modeling the GPCR. These templates show a total percentage identity of 36% and 32.4% with a length of 490 and 380 amino acids respectively compared to that of the target sequence of length 379 amino acids. Since the percentage identity for the chosen templates was less, a multi template modeling approach was applied to get the desired structure of GPCR.

The modeled GPCR was validated using Structural Analysis and Validation Server (SAVS) which in turn shows the total number of amino acids present in the allowed and disallowed regions according to the Ramachandran plot thereby adjudging the quality of the protein modeled as good or poor. More than 90 percent of the residues were found to be under the allowed region thereby indicating the modeled protein to be of a good quality.

The ligands 7-hydroxyceratinamine, Ceratinamine, Moloka'iamine and N,N,N-tri-methyl-3,5-dibromotyramine were taken from *Aplysinella sp*. each having a amine group in common. Similarly ligands Acanthosterolsulfate A, Acanthosterolsulfate B, Acanthosterolsulfate C, Acanthosterolsulfate D, Acanthosterolsulfate E, Acanthosterolsulfate F, Acanthosterolsulfate G, Acanthosterolsulfate H, Acanthosterolsulfate I and Acanthosterolsulfate J were taken from *Acanthodendrilla sp* .each having a sulfate group in common. Ligands Agelasine C, Agelasine B, Agelasine J and Agelasine K were obtained from *Agelas cf. mauritiana* each having a phenol group in common with each other. Ligands obtained from *Axinyssa* have phenyl isothiocyanate (NCS) group common among them.

The modeled protein was docked with the prepared ligand set of 33 metabolites (Table 1) obtained from various marine sponges. Out of these 33 metabolites docked with the *B. amphitrate* GPCR, 5 metabolites from *Acanthodendrilla sp*., and *Agelas cf. mauritiana* (Table 2) showed interaction with a dock score of 73.71, 68.08, 123.418, 72.124 and 72 respectively by forming hydrogen bonds at the arginine and threonine residue which is required for the structural stability (Fig.1-5).

Table1. List of marine sponge metabolites

S. No	Name of The Metabolites	Source (Sponge)	Reference
1.	7-hydroxyceratinamine	*Aplysinella sp.*	Schmitz FJ. 1999
2.	Ceratinamine	*Aplysinella sp.*	Schmitz FJ. 1999
3.	Moloka'iamine	*Aplysinella sp.*	Schmitz FJ. 1999
4.	N,N,N-tri-methyl-3,5-dibromotyramine	*Aplysinella sp.*	Schmitz FJ. 1999
5.	Acanthosterolsulfate A	*Acanthodendrilla sp.*	Lyndon et al., 2007
6.	Acanthosterolsulfate B	*Acanthodendrilla sp.*	Tsukamoto et al., 1998
7.	Acanthosterolsulfate C	*Acanthodendrilla sp.*	Tsukamoto et al., 1998
8.	Acanthosterolsulfate D	*Acanthodendrilla sp.*	Tsukamoto et al., 1998
9.	Acanthosterolsulfate E	*Acanthodendrilla sp.*	Tsukamoto et al., 1998
10.	Acanthosterolsulfate F	*Acanthodendrilla sp.*	Tsukamoto et al., 1998
11.	Acanthosterolsulfate G	*Acanthodendrilla sp.*	Tsukamoto et al., 1998
12.	Acanthosterolsulfate H	*Acanthodendrilla sp.*	Tsukamoto et al., 1998
13.	Acanthosterolsulfate I	*Acanthodendrilla sp.*	Tsukamoto et al., 1998
14.	Acanthosterolsulfate J	*Acanthodendrilla sp.*	Tsukamoto et al., 1998
15.	Agelasine C	*Agelas cf. mauritiana*	Appenzeller et al., 2008
16.	Agelasine B	*Agelas cf. mauritiana*	Appenzeller et al., 2008
17.	Agelasine J	*Agelas cf. mauritiana*	Appenzeller et al., 2008
18.	Agelasine K	*Agelas cf. mauritiana*	Appenzeller et al., 2008
19.	Agelasine L	*Agelas cf. mauritiana*	Appenzeller et al., 2008
20.	Hydroxymoloka'iamine	*Pseudoceratina Arabica*	Badr et al., 2008
21.	Moloka'iakitamide	*Pseudoceratina Arabica*	Badr et al., 2008
22.	5-bromo-2,3-dihydroxy-6-methoxybenzaldehyde	*Pseudoceratina Arabica*	Badr et al., 2008
23.	Ceratophenol A	*Pseudoceratina Arabica*	Badr et al., 2008
24.	CallysponginolSulfateA	*Callyspongia truncate*	Masaki Fujita et al., 2003
25.	Axinisothiocyanates A	*Axinyssa sp.*	Zubía et al. 2008
26.	Axinisothiocyanates B	*Axinyssa sp.*	Zubía et al. 2008
27.	Axinisothiocyanates C	*Axinyssa sp.*	Zubía et al. 2008
28.	Axinisothiocyanates D	*Axinyssa sp.*	Zubía et al. 2008
29.	Axinisothiocyanates G	*Axinyssa sp.*	Zubía et al. 2008
30.	Axinisothiocyanates H	*Axinyssa sp.*	Zubía et al. 2008
31.	Axinisothiocyanates J	*Axinyssa sp.*	Zubía et al. 2008
32.	Axinisothiocyanates K	*Axinyssa sp.*	Zubía et al. 2008
33.	Axinisothiocyanates L	*Axinyssa sp.*	Zubía et al. 2008

Table 2 - Dock score of top 5 marine sponge metabolites

S. No.	Name of the metabolite	Source sponge	Dock score	Number of H Bonds
1.	Acanthosterol Sulfate E	*Acanthodendrilla sp.*	62.758	9
2.	Acanthosterol Sulfate D	*Acanthodendrilla sp.*	73.71	4
3.	Acanthosterol Sulfate F	*Acanthodendrilla sp.*	68.089	4
4.	Acanthosterol Sulfate J	*Acanthodendrilla sp.*	72.124	4
5.	Agelasine K	*Agelas cf. mauritiana*	123.418	2

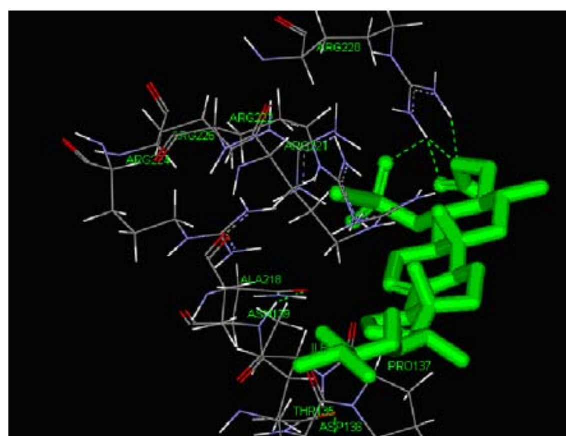

Fig.1- Interaction of GPCR with Acanthosterol sulfate E forming a total of 9 H bonds

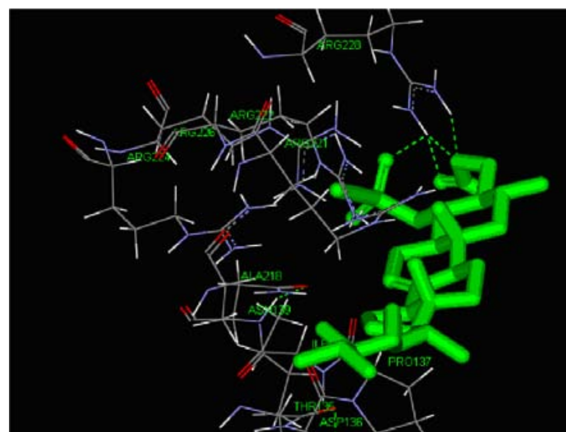

Fig.2- Interaction of GPCR with Acanthosterol sulfate D forming a total of 4 H bonds

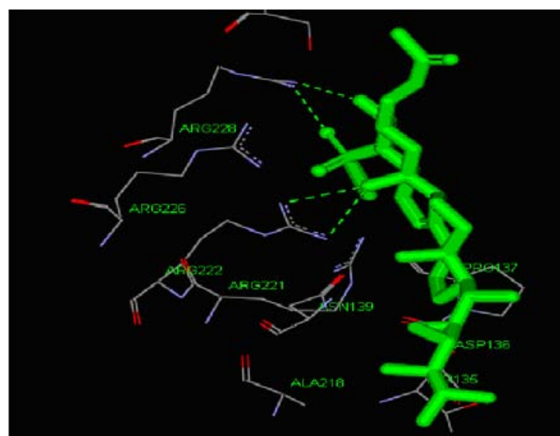

Fig.3- Interaction of GPCR with Acanthosterol sulfate F forming a total of 4 H bonds

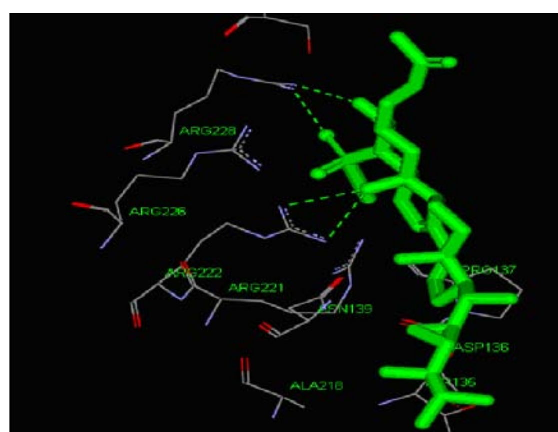

Fig.4- Interaction of GPCR with Acanthosterol sulfate J forming a total of 4 H bonds

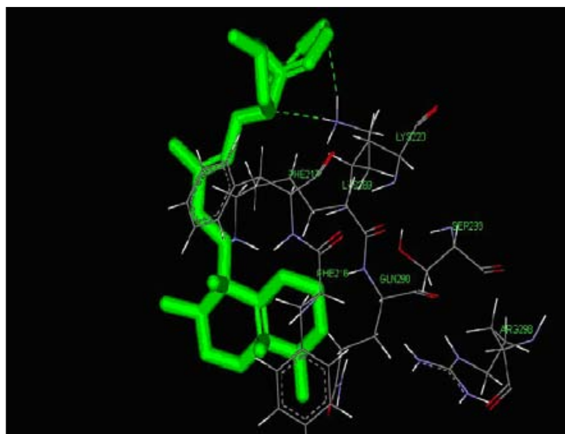

Fig.5- Interaction of GPCR with Agelasine K forming a total of 2 H bonds

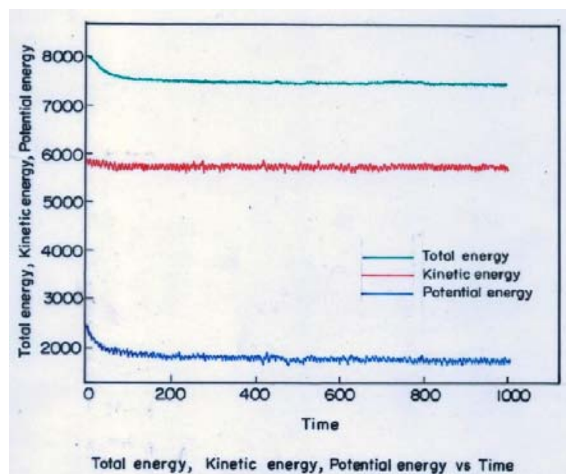

Fig.6- Total Energy, Kinetic Energy, Potential Energy Vs Time graph obtained after simulation studies. Time is in nanoseconds and energy is in kcal/mol

To ascertain the conformational variations of the GPCR-metabolites complexes, MD simulations have been carried out for the top scored receptor–ligand complexes. Analysis of the 1nanoseconds MD trajectories for each complex structure reveals that the complexes were well stabilized at the active site. It can be noted that there is not much deviation indicates that the compounds bind in a better position (Figure 6, 7). The same pattern is obtained for all the five metabolites and the graph variation was negligible. Further analyses reveals that the

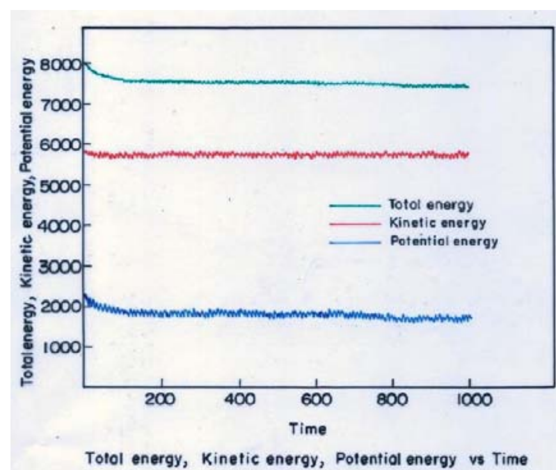

Fig.7- Total Energy, Kinetic Energy, Potential Energy Vs Time graph obtained after simulation studies (Time is in nanoseconds and energy is in kcal/mol)

binding modes of the compounds established after the MD simulation are nearly the same as that obtained of molecular docking. The compounds are stabilized by intermolecular hydrogen bonds. Interactions with the residue Arg and Thr are seen throughout the dynamics trajectory of all the complexes illustrating its pivotal role in ligand binding at the active site.

Discussion

As more information on three-dimensional protein structures become available due to X-ray crystallography, NMR spectroscopy, and homology modeling, application of software tools for virtual screening of marine natural antifoulant become novel and reliable. These software tools have to tackle the key problem while docking, namely predicting energetically favorable complexes between a protein and a putative marine natural compound or molecule, also called ligand in this context.

Our investigation revealed the complex between the protein GPCR and the experimental inhibitors (33 metabolites from various marine sponges). GPCR plays a central role in chemo-perception in larval marine invertebrates, larval settlement behavior, and signal transduction in larval development cascade and has long been an important target in antifouling research. Like many other protein, GPCR triggers a specific reaction signal transduction in larval

development cascade by forming a complex with a substrate molecule. The marine sponge metabolite experimental inhibitors were intended to bind to the same region on the protein surface, the active site, thus the substrate molecule was hypothesized to not to trigger the signal transduction of GPCR activity.

In the first step of virtual screening of marine natural antifoulant was to find a so-called lead structure, marine sponge metabolites which bind to target GPCR protein that was further developed as novel antifoulant. As the three dimensional structure of the target protein, GPCR protein of *Balanus amphitrite* was available in Uniprot (Q93127), docking algorithms such as LigandFit and CDOCKER were applied here to virtually search for potential leads. This investigation raised couple questions such as how the complex between a GPCR and marine sponge metabolites looks like and how strong the binding affinity of the lead is with respect to other candidates of larval marine invertebrates.

Quite a few features make the docking problem hard to solve such as scoring problem and calculation of binding affinity of GPCR-marine sponge metabolite complex. At the moment, specific applicable scoring function is not available for accurate prediction of binding affinity. Also, a great number of degree of freedom has to be measured during a docking calculation. The most vital degrees of freedom were the relative orientation of the two molecules and the conformation of GPCR-marine sponge metabolite complex. GPCR protein conformation, water molecules between the complex and the protonation states of the molecule were also to be considered. A small change in one variable may decide whether an overlap between the two molecules occurs or a hydrogen bond between two groups can be formed or not. This implies that the scoring function for protein- ligand stocking typically contain lots of local minima and are difficult to optimize. As no general solution of the docking problem for virtual screening of marine natural antifoulant is available, several algorithms for various kinds of docking problems have been developed and studied in future. This investigation gave a briefed out these algorithms, additional studies about software for structure based natural antifoulant design is needed further.

Conclusion

The compounds from *Acanthodendrilla sp*., and *Agelas cf. mauritiana* showed higher binding affinity with the GPCR of *B. amphitrite* and also proved by simulation studies. The docked structure fitted into the active site region of the receptor accurately. This revealed that the bioactive molecules identified from those species can be further used for designing better potent antifouling compounds. Substructure-pharmacophore studies of those top scored metabolites might help in building more specific and high affinity antifouling compounds. Such results may help to corroborate the present findings. However, wet lab experiments involving chemical synthesis and testing the *in vivo* using specific cell lines would be necessary to arrive at definite conclusions.

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