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Research Article

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Bioactivity of isolated compounds from *Gracillaria corticata* and it's associated endophytic fungus against HIV virus-A computational study

B. Edhaya Naveena and K. P. Vinod Kumar

Department of Chemistry, University College of Engineering, Nagercoil, Konam, Nagercoil, Kanyakumari, Tamilnadu, India

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ABSTRACT

Recently many chemical drugs are capable of causing the side effects to the humans , in order to prevent this side effects herbal based drugs is necessary for the better alternative. Most promisingly the algal based drug molecules have been explored for their interesting physicochemical and biological properties. The amine functionality is present in many natural products and due to its interesting physiological activity it is an extremely important, pharmacophore in many biologically active compounds. Same time HIV is an inevitable one of the most studied and unsolved human diseases. So far six million HIV positive people are on treatment, a majority of them in countries like India, Brazil, Thailand and South Africa. Thus Patents on lifesaving drugs can end access for them and so activists across the world are fighting against multinationals from stopping the production of generics recently most of the scientist and doctors are continuously working to resolve this diseases by new effective drugs for this Anti HIV treatment. At present study we have isolated a natural compound from Gracilaria corticata and another compound from its associated endophytic fungus . Most of the compounds reveal that some Pharmacophores are indeed essential to impact desired therapeutic effect in the molecules. The significant Pharmacophores like halogen, Phenolic $–NH_2$,$-CH_2OH$,$-CH=N-$,$-C_6H_5$ and chiral centre in the molecules could exhibit broad spectrum activities. Some of the FDA approved Anti-HIV active compounds are lopinavir, Ampenavir and Indinavir etc.

Keywords: Molecules, endophytic fungus, molecular docking, Envelope protein of HIV.

INTRODUCTION

The drug discovery process pursued by major pharmaceutical companies begins with target identification and validation, assay development and high-throughput screening, the aim being to identify new leads. [1]. The need for a rapid search for small molecules that may bind to targets of biological interest is of crucial importance in the drug discovery process. One way of achieving this is the insilico or virtual screening (VS) of large compound collections to identify a subset of compounds that contains relatively many hits against the target, compared to a random selection from the collection. That in turn helps find new and better drug targets [2]. This is essentially the essence of using Bioinformatics in drug discovery; identifying and validating targets. In cases where the target is a protein, the drugs themselves are primarily small chemical molecules or, in some cases, small proteins, such as hormones, that bind to a larger protein in the body. [3]. Structure based drug design has already yielded several drugs currently on the market. It is a now growing rapidly in research field in which many successes have reported in recent years. [4-7).

Their ecological position at the base of the aquatic food chain and their essential roles in nitrogen and phosphorus cycling are critical to aquatic ecosystems [8]. Moreover, the alternation of species composition in an aquatic community because of toxic stress may affect the structure and function of the aquatic ecosystem [9].The diversity of life in the terrestrial environment is extraordinary; the greatest biodiversity is in the world's oceans, with 34 of the 36 phyla of life represented. The oceans cover more than 70% of the earth's surface and contain more than 300,000 described species of plants and animals [10-11]. The marine environment represents a treasure of useful products awaiting discovery for the treatment of infectious diseases. Ecological pressures, including competition for space, the fouling of the surface, and predation have led to the evolution of unique secondary metabolites with various biological activities [12]. Additionally, natural products that are biologically active in assays are generally small molecules with drug-like properties. That is, they are capable of being absorbed and metabolized by the body. 13-14].

For the pharmaceutical industry, the number of years to bring a drug from discovery to market is approximately 12-14 years and costing up to $1.2 - $1.4 billion dollars [15-16]. They can also be used to analyze the target structures for possible binding/ active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics. [17].

Molecular docking is powerful tool to identify the lock and key model. Molecular docking provides useful information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Docking plays an important role in the rational design of drugs [18-19]. The design of novel ligands for a given binding site is of high importance in current drug research, in such a way that the given ligand try to modify with the interactions of the target protein which are to be improved or optimized [20].

The HIV viral protein gp120 induces apoptosis of neuronal cells by inhibiting levels of furin and tissue plasminogen activator, enzymes responsible for converting pBDNF to mBDNF[21]. Different retroviruses vary widely in N-linked glycosylation sites: HIV-1can have as many as 30 sites glycosylated, 25 of which reside in gp120. gp120 induces mitochondrial-death proteins like caspases which may influence the upregulation of the death receptor Fas leading to apoptosis of neuronal cells[22-23] Gp120 is anchored to the viral membrane, or envelope, via non-covalent bonds with the transmembrane glycoprotein, gp41. Three gp120s and gp41s combine in a trimer of heterodimers to form the envelope spike, [24] that mediates attachment to and entry into the host cell.

In this current study, the theory of structure, based drug designing was employed, since the structure of the protein and natural compounds are known. Natural compounds were isolated from marine source and targeted against the envelope protein of HIV.

EXPERIMENTAL SECTION

Retrieval of the natural compound

The chemical structure of carrageenans and Thailandolide B was retrieved from pubchem (http://pubchem.ncbi.nlm.nih.gov/). Canonical SMILES of this compound was taken as input to calculate the general molecular properties.

Methodology :

Drawing the chemical structure

The structure of revised compounds were drawn in chem. sketch available through ACDLABS. Converted to MOL and again transformed to tripos MOL2 format for autodock docking.

Molecular property calculation

Molecular properties of chemical compounds plays an vital role in biological process AlopP, Molecular weight, hydrogen bond acceptors(HBA),hydrogen bond donors (HBD) are four important drug likeness property of Lipinski's rule of 5[25].other property such as TPSA(Total polar surface area) , no of rotatable bonds(nrotb) and volume[26] are important property for drug transportation in invivo system.

1. ADME prediction
Pharmacokinetic of drug was calculated with five -letter word called ADMET absorption, distribution, metabolism, excretion, and toxicity. [27].

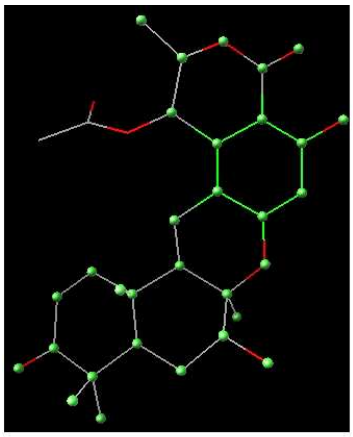
2. Structure based drug designing
In this docking both receptor and ligand is known followed by performing the respective steps for docking using the receptor ligand protocol.

3. Molecular docking
The grid calculations were set up with the utility and maps were calculated with the program AutoGrid

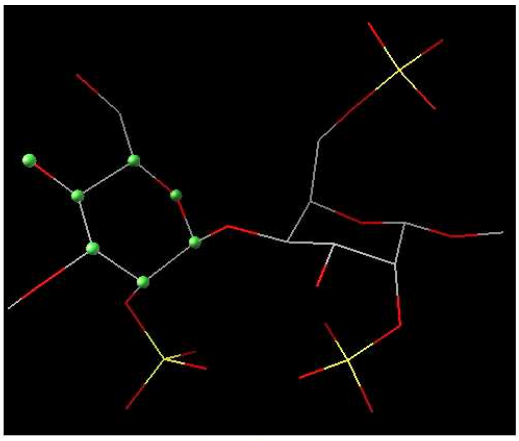
RESULTS

The ligands which are taken for docking studies is shown in figure 1 and the protein before and after grid preparation is shown in the figure 2 respectively, compounds and its smiles were tabulated in table 1.

Figure 1: Compounds isolate from Marine algae



Thailandolide B



Sulfated Polysaccharide –carrageenans

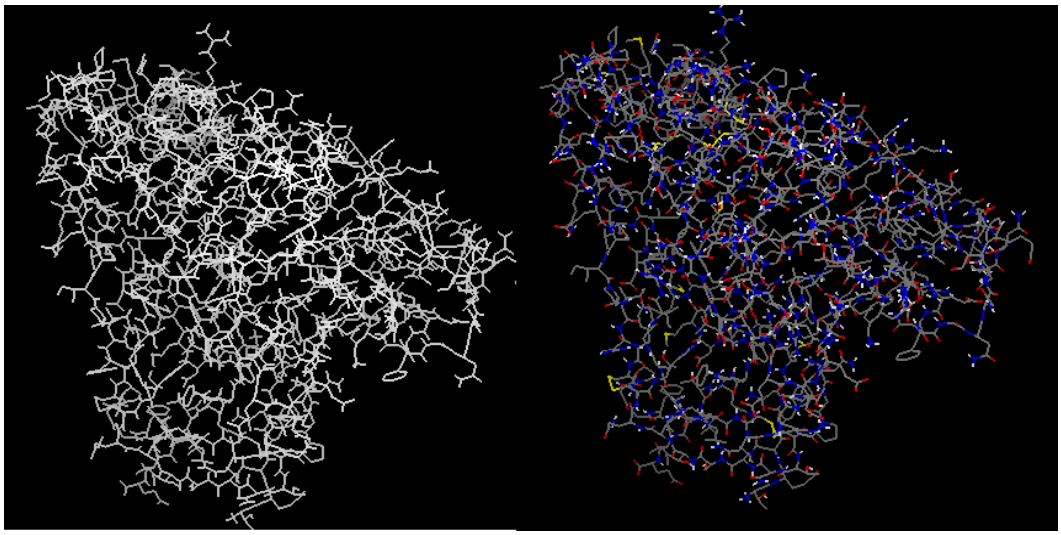


Figure 2: The Protein Before and After Grid Preparation

Table 1: Marie algae's isolates SMILES

Name of the Compound	Smiles notation
Sulfated Polysaccharide – carrageenans	CO[C@H]2OC(COS([O])(=O)=O)[C@H](O[C@@H]1OC(CO)[C@H](O)C(OC)[C@@H]1OS([O-])(=O)=O)[C@@H](O)C2OS([O-])(=O)=O
Thailandolide B	CC(=O)OC2c1c3CC4C5(C)C=CC(=O)C(C)(C)C5CC(O)C4(C)Oc3cc(O)c1C(=O)OC2C

Table 2: Calculated molecular properties

Property	Value	
	Sulfated Polysaccharide -carrageenans	Thailandolide B
Mass	607.5223	484.5366
logP	-2.1780	3.4173
H-bond acceptors	20	8
H-bond donors	3	2
Rotatable bonds	12	2
PSA	308.2100	119.3600
RO5 violations	2	0
RO3 violations	4	4
Refractivity	103.3117	126.3153
Atoms	60	67
Rings	2	5
Heavy atoms	37	35
Hydrogen atoms	23	32
Heteroatoms	23	8
N/O atoms	20	8
Chiral centers	10	7
R/S chiral centers	6	0

absorption, tissue distribution, bioavailability, receptor interaction, metabolism, cellular uptake, and toxicity [32]. The formulation of Lipinski's rule of five is based on the observation that orally active drugs are small and have optimal solubility in aqueous and non-polar [33].

Lipinski's rule of five states that value of ALOGP of ≤5, a molecular weight of ≤500 daltons, a number of hydrogen bonding acceptor sites (HBA) of ≤10, a number of hydrogen bonding donor sites (HBD) of ≤5 are ideal for a lead to behave as drug candidate [34-35]. Perhaps the drug likeness rule will not stratified in natural product because it is combination of bulkier functional groups connected to the parent compounds.

As per rule of veber drug likeness PSA (PSA<=140)[36] plays an important role, PSA is a commonly used medicinal chemistry metric for the optimization of a drug's ability to permeate cells. Molecules with a polar surface area of greater than 140 angstroms squared tend to be poor at permeating cell membrane. For molecules to penetrate the blood–brain barrier (and thus act on receptors in the central nervous system), a PSA less than 60 angstroms squared is usually needed[37]. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood-brain barrier penetration. More over Sulfated Polysaccharide–carrageenans contains six R/S chiral centers compared with Thailandolide B, chiral centers plays a major significance in biological activity also, the activity of drugs containing stereo centers can similarly vary between enantiomers. In addition, rotatable bonds less than ten is more prominent for compounds to obey veber drug likeness rule.

Pharmacokinetics of the compounds

Pharamacokinetics study is also known as PK studies of drug, It attempts to discover the fate of a drug from the moment that it is administered up to the point at which it is eliminated from the body[38].

Table 3: ADMET Predicted Profile of the compounds

Models	ADMET Predicted Profile	
	carrageenans	Thailandolide B
(BBB) Penetration	0.718	0.5487
Human Intestinal abs	0.969	0.8965
CYP450 2C9 Inhibitor	0.8051	0.9044
AMES Toxicity	0.6329	0.5197
Rat Acute Toxicity	2.5559	3.3941
Fish Toxicity	1.8792	0.0049
Tetrahymena Pyriformis Toxicity	0.0448	1.5957
Biodegradation	0.6676	1
Acute Oral Toxicity	0.5382	0.4633
Aqueous solubility	-2.4168	-4.3342
Caco-2 Permeability	-0.2301	0.4902
Carcinogens	0.6413	0.9214

Pharmacokinetics describes how the body affects a specific drug after administration through the mechanisms of absorption and distribution, as well as the chemical changes of the substance in the body and the effects and routes of excretion of the metabolites of the drug. From the above table 3, the observed values of each parameter express the kinetic profile of drug. Blood-Brain Barrier (BBB) Penetration of Thailandolide B is more than Sulfated Polysaccharide –carrageenans . Absorption of Thailandolide B is 0.8965 which shows it can be better absorption than Sulfated Polysaccharide –carrageenans with value of 0.969.Metabolism of enzymes in liver is more important for all the drug, the enzyme CYP450 family is required for biotransformation Thailandolide B and Sulfated Polysaccharide – carrageenans shows non inhibitor activity. According to environmental factors, both compounds are not readily biodegradable. LogS (Aqueous solubility) of the both compounds are more soluble, LogPapp of Sulfated Polysaccharide –carrageenans is -0.2301 cm/s whereas for Thailandolide B is 0.4902 cm/s.

Receptor ligand interaction

Receptor-ligand interactions are fundamental to various biological processes such as gene transcription, signal transduction, enzymatic re-actions and physiological regulation. As many proteins regulate key biological functions via interactions with small molecules, these receptor proteins are often major targets for therapeutic agents. In this study, envelope protein from HIV virus, one of the most common sexually transmitted disease which is docked with the naturally isolated compounds from algae such as Sulfated Polysaccharide–carrageenans and Thailandolide B. The various energy parameters were calculated during docking are shown in the table 4 and table 5 amino acid binding and its distance also the interaction of protein and ligand is shown in figure 3 and figure 4.

Table 4: Various energy parameters of docking

Parameters	Sulfated Polysaccharide – carrageenans	Thailandolide B
Inhibition Constant, Ki at 298.15 K	248.32 mM	54.64 uM
Estimated Free Energy of Binding	-0.83 kcal/mol	-5.82 kcal/mol
Final Intermolecular Energy	-4.29 kcal/mol	-6.36 kcal/mol
vdW + Hbond + desolv Energy	-4.67 kcal/mol	-6.42 kcal/mol
Electrostatic Energy	+0.38 kcal/mol	+0.06 kcal/mol
Final Total Internal Energy	+0.17 kcal/mol	+0.00 kcal/mol
Torsional Free Energy	+3.29 kcal/mol	+0.55 kcal/mol
Unbound System's Energy	+0.00 kcal/mol	+0.00 kcal/mol

Figure 3: Sulfated Polysaccharide –Carrageenan's binding with active site of Hiv-1 Gp120

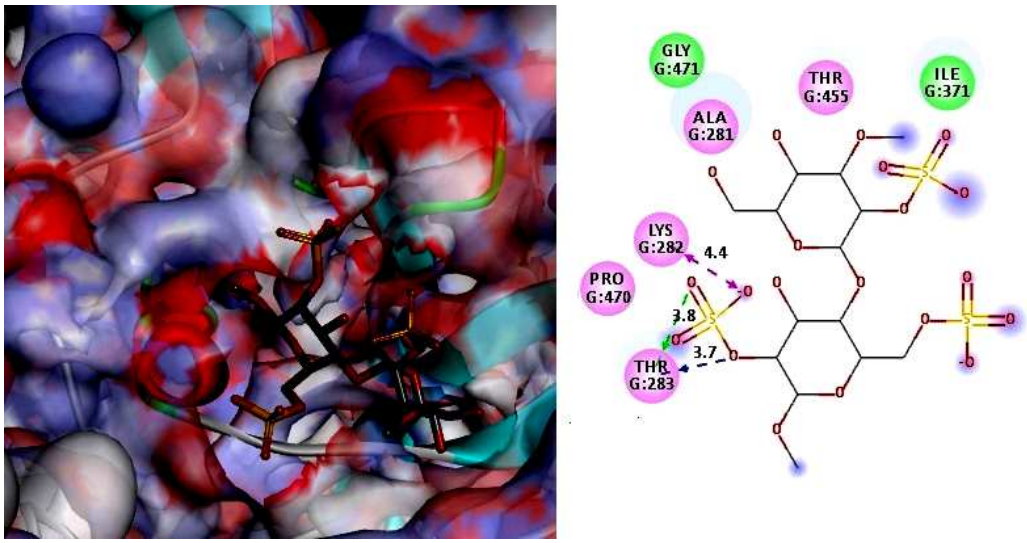


Figure 4: Thailandolide B binding with active site of Hiv-1 Gp120

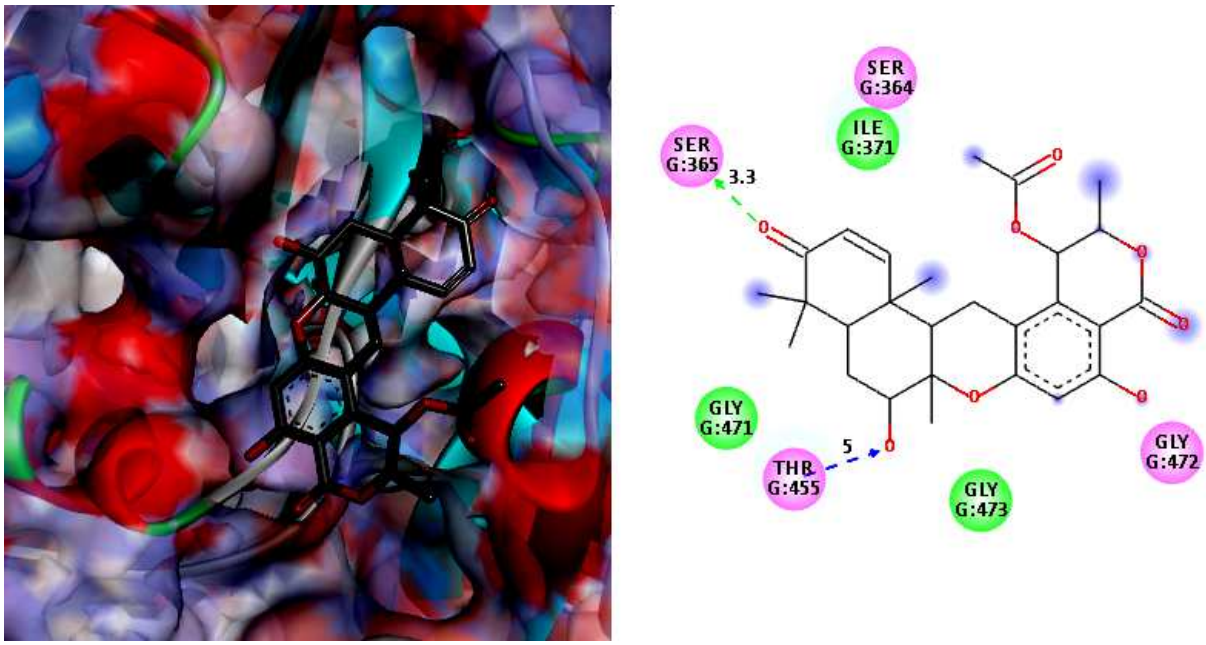


Table 5: The below mentioned table is the binding energy of ligand to the active site/binding site of the HIV-1 Gp120

LIGAND and RECEPTOR	Amnioacid Binding	Distance in Å	Binding energy kcal/mol
Sulfated Polysaccharide –carrageenans with HIV-1 Gp120	Lys282	4.4	-0.83
	Thr 283	3.7	
		3.8	
Thailandolide B with HIV-1 Gp120	Ser 365	3.3	-5.82
	Thr 455	5	

DISCUSSION

The importance of isolating active secondary metabolites is a key factor research in medical field in all over the entire world. Also producing the drug without side effects are more important parametric factor in drug discovery

process .The active metabolites from various natural sources of microbes, plants, animal and marine were used a potent chemical drugs alternative. Many pharmaceutical companies were investing the millions of dollar in the natural drug production in large quantity with fewer prices to reach the people. Algal sulfated polysaccharides are a source of numerous biological activities that may find therapeutic advantage. Hiebert.,2002[39] and Irhimeh et al., 2009[40] reports that algal polysaccharides is their potentially low bioavailability given their often high molecular weights and that algal sulfated polysaccharides will display some, albeit low, degree of oral bioavailability.

Blunt et al., 2005[41] investigated on algae and reported that more than Over 15,000 novel compounds have been chemically determined. Focusing on bioproducts, recent trends in drug research from natural sources suggest that algae are a promising group to furnish novel biochemically active substances..Another study of virus by Hidari et al., 2008[42] shows the microorganism *Cladosiphon okamuranus* composed of glucuronic acid and sulfated fucose units

Potently inhibited infection of BHK-21 cells with dengue virus type 2 (DENV-2). Witvrouw in 1997[43] reported that sulfated polysaccharides from seaweeds to inhibit the replication of enveloped viruses including herpes simplex virus (HSV), human immunodeficiency virus (HIV), and human cytomegalovirus, dengue virus and respiratory syncytial virus. The research of Witvrouw in 1997 provides the key for Insilco studies to carry out the both the compounds Sulfated Polysaccharide–carrageenans and Thailandolide B.

Kubinyi, 1998; Muller, 1995; Van Drie & Lajiness, 1998; Walters et al., 1998[44-47] stated that the process of finding novel leads for a new target is the most important and undoubtedly one of the most crucial steps in a drug development program. Today two complementary strategies are followed by experimental high-throughput screening to discover possible leads from large compound libraries, and computational methods exploiting structural information of the protein-binding site aiming at the construction of a ligand de novo or their discovery by virtual screening of large databases. The physiochemical properties and PK studies of the compound shows non carcinogen ,non inhibitor of cytochrome p450 family of enzymes and Toxicity is another important parameter that needs to be considered in this studies, commonly the values of Rat Acute Toxicity LD50 of Sulfated Polysaccharide – carrageenans is 2.5559 mol/kg and Thailandolide B is 3.3941 mol/kg. Fish Toxicity pLC50 for Sulfated Polysaccharide –carrageenans is 1.8792 mg/L and Thailandolide B is 0.0049 mg/L. Similarly for Tetrahymena Pyriformis Toxicity pIGC50 for Sulfated Polysaccharide –carrageenans is 0.0448 ug/L and Thailandolide B is 1.5957 ug/L respectively. On other hand receptor ligand interaction of the compound shows more favorable affinity for Threonine residues in envelope protein. hence Thailandolide B with lowest binding energy and least Ki value of 54.64 uM compared with Sulfated Polysaccharide –carrageenans with Ki value of 248.32 mM. Comparison of these compounds need to carried out in both in-vitro and in-vivo system to improve its bioefficay mode of theoretical prediction, thus in future the isolates form algae can be used for HIV treatment for targeting envelope protein.

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