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Computational investigation of marine bioactive compounds against E6 oncoprotein of Human Papilloma Virus-HPV16

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ABSTRACT

Objective: Cervical cancer is the most leading cause of mortality and morbidity in women. Most of these are caused by human papilloma virus (HPV) which are sexually transmitted. Among 200 HPV types, high-risk HPV16 persists in humans and results in precancerous lesions and cervical cancer. The viral E6 oncoprotein which is necessary for malignant conversion of HPV 16 is used as the potential target for the inhibition of HPV infection. The present study aims to investigate the inhibitory activities of the seventy-four bioactive compounds from different marine organisms against the viral E6 oncoprotein of HPV16 using computational techniques. **Methods:** Virtual screening technique has been applied to identify the potent bioactive compounds against E6 oncoprotein of HPV16 using Molinspiration and is subjected to drug-likeliness assessment using the Molsoft server. Molecular docking was carried out for E6 protein (4XR8) with selected hits obtained from virtual screening method and their binding energies were determined. Further Molecular Dynamic Simulation (MDS) studies of the obtained protein-bioactive inhibitor complex were performed to analyze the stability and conformation. **Results and Conclusion:** Four potential hits were identified from virtual screening and finalized against HPV16. Molecular docking studies revealed Salicylihalamide B from *Haliclona species* has shown the better interaction with E6 oncoprotein and gives the best binding energy of -8.92 Kcal/mol. The MDS studies inferred that the complex was found to be steady after 40 ns. As an outcome, Salicylihalamide B plays a promising role against E6 protein of HPV16 and hence can act as a template for further studies on cervical cancer drug candidates.

INTRODUCTION

Human papilloma virus codes for 5.2% of all cancers worldwide. Cervical cancer is one of the significant mortality amongst women due to human papilloma virus infection. Human papilloma virus (HPVs) are small, circular double-stranded DNA viruses with a genome of approximately 8 kb (White *et al*., 2012). HPV comprise more than 200 types of infections, among which the high-risk HPV types 16 and 18 are the main cause and account for about 70% of cervical cancer. One HPV type (HPV 16) emerges as profoundly connected with malignancies at a few distinctive anatomical locations such as cervix, penis, anus, oropharynx, esophagus (Liyanage *et al*., 2013) and bladder (Husain *et al*., 2009; Li *et al*., 2011). HPV type 16 is the most oncogenic type, followed by type 18 as the next most virulent (Hoory *et al*., 2008). HPV16 belongs to the genus of Alpha-papilloma virus and a member of species 9. The genome organization of HPV16 is organized into three regions: an early region (E), a late region (L), and a non-coding long control region (LCR). The early region encodes six non- structural proteins: E1, E2, E4, E5, E6, and E7. Late region encodes two structural proteins: L1 and L2. The non-structural proteins E1, E2, E4, and E5 proteins are required for viral DNA replication, the E6 and E7 proteins cooperate to transform and immortalize cells, and the L1 and L2 proteins are needed for the production of viral particles (Ledwaba *et al*., 2004; Munger *et al*., 2004). Among the non-structural protein products, E6 protein plays a role in the induction and maintenance of cellular transformation. The HPV 16 E6 protein consists of 151 amino acids and has a molecular weight of 18 kDa with two zinc fingers and also has a high content of α-helical and β-sheet secondary structures (Tungteakkhun *et al*., 2008).

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There are different interacting partners of E6 protein, the first and most vital cellular target of E6 protein is p53. HPV E6 oncoprotein interacts with several other cellular targets such as E6-AP, p53, CBP/P300. The importance of p53-mediated apoptosis has been recognized in terms of maintaining homeostasis and preventing neoplastic transformation. E6 forms a ternary complex with p53 and E6 associated protein (E6AP) resulting in the degradation of p53 via ubiquitination pathway (Huibregtse *et al*., 1991). E6 also interacts with transcriptional co-activators CBP/P300 is directly independent of proteins known to bind the co-activators, such as p53 (Patel *et al*., 1999*)*. The E6 protein binds to three domains of CBP/P300 and affects the transcriptional activity of the co-activators. High-risk HPV E6 binds to E6-AP (E6 Associated Protein) inside its N-terminal substrate recognition domain (Huibregtse *et al*., 1993a), and formation of a stable E6-E6AP complex precedes association with p53, thereby redirecting the substrate specificity of E6AP towards p53 (Huibregtse *et al*., 1993b). Hence E6AP also plays an essential role in E6 directed degradation of p53. Compared to other cellular targets, Thomas *et al*. (1999) evidenced that p53 activities is controlled in association with E6-AP and E6* proteins and leads to the replication of the virus and undoubtedly an E6-p53 association gives fundamental importance in the pathogenesis of HPV and represents as an important therapeutic target for many important human cancers (Thomas *et al*., 1999).

Marine sponges have been positioned at the top because of the discovery of bioactive compounds with potential pharmaceutical applications. Specialists in the field of natural products chemistry propose that sponges have the potential to provide future drugs against important diseases such as a range of viral diseases, malaria, inflammations, immunosuppressive diseases and various malignant neoplasms (Alcaraz *et al*., 2006; Molinski *et al*., 2009*;* Simmons *et al*., 2005; Gordaliza *et al*., 2010). Sponges produce various natural components and metabolites by isolation and screening of bioactive substances lead to the discovery of several chemicals with antiviral properties (Sipkema *et al*., 2005). Therefore, marine sponges are considered as a prosperous source of chemical diversity and health benefits for developing drug candidates, cosmetics, nutritional supplements, and molecular probes that can be supported to enhance the healthy lifespan of humans (Perdicaris *et al*., 2013). Sponges of the genus Haliclona are noticeable for producing a variety of secondary metabolites, most commonly bioactive alkaloids. Our study focuses on the in silico identification of potential bioactive inhibitors from marine algal sources against cervical cancer which is caused by HPV16 E6 oncoprotein.

MATERIALS AND METHODS

Retrieval of E6 protein structure

The three-dimensional structure of E6 protein was retrieved from PDB and hence we considered the entry 4XR8 as a target for marine algal bioactive compounds. In contrast to all other entries of E6 protein, 4XR8 shows larger position availability in X-ray method with a resolution of 2.25 Å (http://www.uniprot.org/uniprot/P03126). The RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank) is a repository for three-dimensional structural information of large biomolecules such as proteins and nucleic acids.

Construction of ligand dataset from marine sources

Among marine invertebrates, marine sponges from phylum Porifera is the most prevailing group responsible for discovering a huge number of natural products, that have been used as a template to develop therapeutic drugs. These natural products have a wide range of therapeutic properties including antimicrobial, antioxidant, antihypertensive, anticoagulant, anticancer, anti-inflammatory, wound healing and immune modulator, and other medicinal effects (Perdicaris *et al*., 2013). The bioactive compounds from various marine organisms such as different types of algae, sponge, bacteria which can act against the viral protein E6 were collected from the literature. 74 bioactive compounds from marine organisms were collected which forms the ligand dataset. PubChem database provides information about the chemical compounds, structure and their biological activities. Structure Data File (SDF) of the bioactive compounds was retrieved from PubChem database and converted to PDB format using OpenBabelGUI (O'Boyle *et al*., 2011). Accordingly, compound structures were saved in MOL format, later it is converted to PDB using OpenBabelGUI.

Virtual Screening of bioactive compounds

74 bioactive compounds from marine sources were involved in this study (Michalak *et al*., 2015; Simmons *et al*., 2005; Jimenez *et al*., 2009*;* Hartog *et al*., 1999; Adams *et al*., 2013; Jiao *et al*., 2011; Pal *et al*., 2014; Plaza *et al*., 2010; Francavilla *et al*., 2012; Kakinuma *et al*., 2001; Pereira *et al*., 2012; Saeidnia *et al*., 2014; Asgharpour *et al*., 2015; Yu *et al*., 1981; Panayotova *et al*., 2013*;* Michiels *et al*., 2011; Karsten *et al*., 1998; Groniger *et al*., 2000; Gupta *et al*., 2011; Apt *et al*., 1995; Sajilata *et al*., 2008*;* García-Vilas *et al*., 2016; Krohn, 2007; Manam *et al*., 2005; Domozych *et al*., 2012*;* Murakami *et al*., 2001; Isbrucker *et al*., 2003; Oliveira *et al*., 2012; Ponder *et al*., 2011; Aoki *et al*., 2004). Virtual screening offers a computational prediction of binding affinity for a large set of compounds. Screening of large libraries of compounds can be performed using docking. The final outcomes are positioned to propose basic theories of how the ligands hinder the target, a key objective in lead optimization. Virtual screening technique is used to construct the libraries of the marine bioactive compounds and also filters out the relevant bioactive compounds against the viral protein E6 using two softwares such as Molinspiration and Molsoft. All the bioactive compounds were optimized using Argus lab 4.0.1 using AM1 (Austin model 1) force field (http://www.arguslab.com/arguslab.com/ArgusLab.html).

Physicochemical and drug-likeness property prediction

Molinspiration tool, (http://www.molinspiration.com/cgi-bin/properties) is used in this study to calculate the molecular properties of bioactive compounds. Lipinski's filter includes properties like molecular weight, ClogP, polar surface area, number of hydrogen bond donors and acceptors, number of atoms, violations, rotational bonds, and volume. The Druglikeness score of the bioactive compounds was predicted using Molsoft software (http://molsoft.com/mprop/). The qualitative concept drug-likeness reveals information about how a drug responds to components like bioavailability and also checks the toxicity of the compound. Theoretically, a drug-like substance has a log P range of −0.4 to 5.6, molecular weight 180 to 500 Daltons,

molar refractivity of 40-130, which is related to the volume and molecular weight of the molecule, also it has 20-70 atoms and follow other Lipinski's rules (Ghose *et al*., 1999).

Molecular docking studies

Molecular docking is the best way to estimate the interaction between two molecules (Arumugam *et al*., 2013). Thus, to refine the retrieved hits from the previous analysis, docking was performed for all the hit compounds with the target protein E6. AutoDock is an automated docking procedure for predicting the interaction of ligands with bio-macromolecular targets. The four Bioactive compounds such as ascorbic acid, frigocyclinone, salicylihalamides A and B sieved out from the virtual screening technique were docked against the viral protein E6 using AutoDock tools 4.2 (Morris *et al*., 2009). Hydrogen atoms are added to the protein structure and Kollman charges are added to the protein and saved in .pdbqt format. After detecting the root, the ligand files are saved in .pdbqt format. A Lamarckian genetic algorithm is used as a docking parameter to find globally optimized conformation. The grid box dimension was set to 60 × 60 × 60 and for the remaining parameter default settings were applied. At the end of a docking with multiple runs, a cluster analysis was performed (Sundarrajan *et al*., 2014). The bioactive compounds were analyzed based on their binding energy values.

Molecular dynamics simulation studies

The molecular dynamics simulation (MDS) calculates the stability of the protein-ligand complex. The docked protein-ligand complex obtained from Autodock program was considered as starting model for MDS. It is used to investigate the thermodynamics of biological macromolecules and their complexes. MDS of the protein-ligand complex with lowest binding energy was performed using GROMACS 4.5.5 (Groningen Machine for Chemical Simulations). The protein topology file was prepared using GROMOS96 43a1 force field (Schuler *et al*., 2001). Ligand topology file and force field parameter file were prepared using PRODRG server (Schüttelkopf *et al*., 2004). The whole system was subjected to 50 ns MDS at 300 K temperature and 1 bar pressure. The potential energy fluctuations, Root mean square deviation of alpha carbon atoms, Root mean square fluctuation and radius of gyration were monitored.

RESULTS AND DISCUSSION

Role of E6 protein in HPV16

E6 protein is a major transforming protein and also acts as a critical factor in cervical carcinogenesis of HPV16. E6 binds with E6AP ubiquitin-protein ligase and inactivates P53 via proteasome degradation pathway thereby DNA damage and chromosomal instabilities occur which leads to cell proliferation and cancer development in humans. Therefore, to prevent this we manually selected 74 marine bioactive compounds from literature sources which can compete with E6 protein.

Virtual screening of bioactive compounds from marine sources

74 biologically active compounds from different marine organisms involved in this study have been listed in Table 1. The 2D structure file is given in S. Figure 1. Virtual screening involves evaluation of a large number of small compounds based on their several molecular properties, using computational methods. We subjected our bioactive compounds to virtual screening specifically to calculate physicochemical properties and drug likeliness score using Molinspiration and Molsoft softwares. The SMILES (Simplified Molecular-Input Line-Entry System) notation of the bioactive compounds were used to calculate the molecular properties and drug likeliness score. The compounds which obey Lipinski's rule were further screened using molecular docking and simulation studies. Lipinski's rule components which are molecular weight < 500, log P not greater than 5, Hydrogen bond donors not more than 5 and Hydrogen bond acceptors not more than 10, number of atoms from 20 to 70, 10 or fewer rotatable bonds. The properties like milogP, TPSA, number of atoms, hydrogen bond donors and acceptors, molecular weight and rotational bonds of the marine bioactive compounds were predicted (Table 2). 74 bioactive compounds were first subjected to molinspiration to check the physicochemical characteristics of the compound and as a result, we filtered 24 bioactive compounds which obey the Lipinski's rule. These compounds were further subjected to drug-likeness score prediction using molsoft. Four compounds show above 0.5 drug-likeness score was selected as a good drug candidate and proceeds for further studies. In Molsoft software also the SMILES notation is used to check the drug-likeness score of the compounds. The drug-likeness score of the potential bioactive compounds is listed in Table 3. As an outcome of the virtual screening technique, we finalized four bioactive compounds such as Ascorbic acid from *Ascophyllum Nodosum*, Frigocyclinone from *Streptomyces Griseus*, Salicylihalamides A and B from *Haliclona Species* may have potential to act against the viral protein E6 and further investigated for molecular docking studies.

Molecular docking studies of E6 protein with bioactive inhibitors

Elucidation of ligand binding mechanism in the essential phase to achieve selective and potent drugs for any target. Therefore, the four bioactive compounds (Ascorbic acid, Frigocyclinone, Salicylihalamides A and B) were docked separately into the binding site of the target protein E6 using AutoDock 4.2. The Protein-ligand interaction binding energies give a better understanding about how well the drugs bind to the E6 protein molecule. Among the four bioactive compounds, Salicylihalamide B from *Haliclona Species* had shown the better interaction with E6 protein with a binding energy of −8.92 Kcal/mol. Ascorbic acid (−3.82), Frigocyclinone (−8.01), Salicylihalamide A (−8.76) have lesser binding energy values than Salicylihalamide B and their binding site residues were identified using Pymol (Table 4). The four bioactive compounds interacted with E6 protein and formed a complex which was visualized using pymol and their 2D interaction patterns were identified using Discovery studio visualizer (Figure 1). E6 protein (green) represented as a surface model and the ligands are represented as ball and stick model. The residues interacting with ascorbic acid form hydrogen bond interaction with aliphatic amino acids LEU99, LEU110 and hydrophilic amino acid ASP49. It also forms vanderwaals interaction with aliphatic amino acids

PRO112, CYS111, PRO109, ILE101, LYS115, LEU100 and carbon-hydrogen bond interaction with LEU110 (Figure 1e). Frigocyclinone forms hydrogen bond interaction with aromatic group TRP132 and aliphatic group LEU100, vanderwaals interaction with aliphatic groups LEU67, GLY107, LEU50, ILE101, SER74, GLY130, LEU99, Carbon-Hydrogen bond with SER71, strong hydrophobic pi-sigma interaction with LEU100 and ARG131 and hydrophobic pi-alkyl interaction with ARG102 and ARG131 (Figure 1f). Salicylihalamide A forms hydrogen bond interaction with hydrophilic ARG102 and hydrophobic CYS51, vanderwaals interaction with GLY130, LEU100, VAL53, PHE45, TYR32, SER74, SER71, GLN107, ARG131, ILE101, hydrophobic pi-sigma interaction with VAL62, hydrophobic alkyl interaction with LEU67 and pi-alkyl interaction with LEU50, TRP132 (Figure 1g). Salicylihalamide B forms hydrogen bond interaction with ARG102 and CYS51, vanderwaals interaction with both aliphatic and aromatic amino acid groups VAL53, PHE45, TYR32, GLN107, SER71, SER74, ARG131, TRP132, GLY130, hydrophobic Alkyl interaction with LEU67 and LEU100 and strong pi-alkyl interaction with aliphatic groups VAL62 and LEU50 (Figure 1h).

Table 1: Dataset table – Seventy-four bioactive compounds from marine sources used for the study.

Bioactive Compounds	Compound Family	Algae	Algal Family	References
Docosahexaenoic acid	PUFA*	*Schizotrychium*	*Thraustochytriacea*	Hartog *et al*. (1999)
Eicosapentaenoic acid	PUFA*	*Porphyridium cruentum*	*Porphyridiophyceae*	Asgharpour *et al*. (2015)
Beta carotene	Carotenoids	*Dunaliella salina*	*Dunaliellaceae*	Hartog *et al*. (1999)
Lutein	Carotenoids	*Muriellopsis*	*Chlorophyceae*	Hartog *et al*. (1999)
Astaxanthin	Keto carotenoid	*Hematococcus pluvialisis*	*Haematococcaceae*	Hartog *et al*. (1999)
Griffithsin	Lectin	*Griffithsia*	*Wrangeliaceae*	Adams *et al*. (2013)
Fucoidan	Sulfated polysaacharide	*Fucus vesciculosis*	*Fucaceae*	Jiao *et al*. (2011)
Lambda carrageenan	Sulfated polysaacharide	*Gigartina*	*Gigartinaceae*	Jiao *et al*. (2011)
Phloroglucinol	Polyphenol	*Ecklonia species*	*Lessoniaceae*	Michalak *et al*. (2015)
Phycoerythrobilin	Phycobiliproteins	*Callithamnion roseum*	*Callithamniaceae*	Yu *et al*. (1981)
Dictyol C	Diterpenes	*Dictyota ciliolate*	*Dictyotaceae*	Michalak *et al*. (2015)
Dictyol H	Diterpenes	*Dictyota ciliolate*	*Dictyotaceae*	Michalak *et al*. (2015)
Alpha tocopherol	vitamin E	*Ulva rigida*	*Ulvaceae*	Panayotovo *et al*. (2013)
Ascorbic acid	Vitamin C	*Ascophyllum Nodosum*	*Fucaceae*	Michiels *et al*. (2011)
Beta glucans	Polysaacharide	*Laminaria Digitata*	*Laminariceae*	Michalak *et al*. (2015)
Laminarin	Polysaacharide	*laminaria species*	*Laminariceae*	Michalak *et al*. (2015)
Palythine	Mycosporine-like amino acid	*Chaetomorpha aerea*	Cladophoraceae	Karsten *et al*. (1998)
Shinorine	Mycosporine-like amino acid	*Gelidium latifolium*	*Gelidiaceae*	Groniger *et al*. (2000)
Dolabellanes	Diterpenes	*Dictyota ciliolate*	*Dictyotacaea*	Gupta *et al*. (2011)
Fucosterol	Sterols	*Laminaria species*	*Laminariceae*	Pal *et al*. (2014)
Desmosterol	Sterols	*Palmaria species*	*Palmariaceae*	Pal *et al*. (2014)
Agar	Sulfated polysaacharide	*Gracilaria species*	*Gracilariaceae*	Pal *et al*. (2014)
Fucoxanthin	Carotenoid	*Macrocystis pyrifera*	*Laminariaceae*	Apt *et al*. (1995)
Zeaxanthin	Carotenoid	*Microcystis aeruginosa*	*Microcystaceae*	Sajilata *et al*. (2008)
Dolastatin 15	Linear peptide	*Dolabella auricularia*	*Aplysiidae*	Simmons *et al*. (2005)
E7389	Macrocyclic polyether	*Halichondria okadai*	*Halichondriidae*	Simmons *et al*. (2005)
Discodermolide	Lactone	*Discodermia dissolute*	*Theonellidae*	Simmons *et al*. (2005)
LAF-389	Ɛ-Lactam peptide derivative	*Jaspis digonoxea*	*Ancorinidae*	Simmons *et al*. (2005)
Curacin A	Thiazole lipid	*Lyngbya majuscula*	*Oscillatoriaceae*	Simmons *et al*. (2005)
DMMC	Cyclic depsipeptide	*Lyngbya majuscula*	*Oscillatoriaceae*	Simmons *et al*. (2005)
Salinosporamide A	Bicyclic λ-lactam-β lactone	*Salinospora species*	*Micromonosporaceae*	Simmons *et al*. (2005)
Laulimalide	Macrolide	*Cacospongia mycofijiensis*	*Thorectidae*	Simmons *et al*. (2005)
Eleutherobin	Diterpene glycoside	*Eleutherobia species*	*Alcyoniidae*	Simmons *et al*. (2005)
Sarcodictyin A	Diterpene	*Sarcodictyon roseum*	*Clavulariidae*	Simmons *et al*. (2005)

Peloruside A	Macrocyclic lactone	*Mycale hentscheli*	*Mycalidae*	Simmons *et al.* (2005)
Salicylihalamide A	Polyketide	*Haliclona species*	*Chalinidae*	Simmons *et al.* (2005)
Salicylihalamide B	Polyketide	*Haliclona species*	*Chalinidae*	Simmons *et al.* (2005)
Thiocoraline	Depsipeptide	*Micromonospora marina*	*Micromonasporaceae*	Simmons *et al.* (2005)
Asciidemin	Aromatic alkaloid	*Didemnum species*	*Didemnidae*	Simmons *et al.* (2005)
Lamellarin D	Pyrrole alkaloid	*Lamellaria species*	*Velutinidae*	Simmons *et al.* (2005)
Dictyodendrin	Pyrrolocarbazole derivatives	*Dictyodendrilla verongiformis*	*Dictyodendrillidae*	Simmons *et al.* (2005)
ES-285 (Spisulosine)	Alkyl amino alcohol	*Mactromeris polynyma*	*Mactridae*	Simmons *et al.* (2005)
Aeroplysinin I	Alkaloid	*Aplysina aerophoba*	*Aplysinidae*	Jimenez *et al.* (2009); Garcia-vilas *et al.* (2016)
Aureoverticillactam	Macrocyclic lactam	*Streptomyces aureoverticillatus*	*Streptomycetaceae*	Jimenez *et al.* (2009)
Bonactin	Macrotetrolide antibiotic	*Streptomyces species*	*Streptomycetaceae*	Jimenez *et al.* (2009)
Caprolactones	Lactone	*Streptomyces species*	*Streptomycetaceae*	Jimenez *et al.* (2009)
Chandrananimycin	Benzoxazines	*Actinomadura species*	*Thermomonosporaceae*	Jimenez *et al.* (2009)
Diazepinomicin	Alkaloid	*Micromonospora species*	*Micromonosporaceae*	Jimenez *et al.* (2009)
Frigocyclinone	Angucyclinone antibiotic	*Streptomyces griseus*	*Streptomycetaceae*	Jimenez *et al.* (2009)
Glaciapyrrole C	Pyrrolosesquiterpenes	*Streptomyces species*	*Streptomycetaceae*	Jimenez *et al.* (2009)
Helquinoline	Tetrahydroquinoline antibiotic	*Janibacter limosus*	*Intrasporangiaceae*	Jimenez *et al.* (2009)
Himalomycin A	Peptide antibiotic	*Streptomyces Parvulus*	*Streptomycetaceae*	Jimenez *et al.* (2009); Krohn *et al.* (2007)
Himalomycin B	Peptide antibiotic	*Streptomyces Parvulus*	*Streptomycetaceae*	Jimenez *et al.* (2009); Krohn *et al.* (2007)
Komodoquinone A	Anthracycline	*Streptomyces species*	*Streptomycetaceae*	Jimenez *et al.* (2009)
Lajollamycin	Nitro-tetraene spiro-β-lactone-λ-lactam antibiotic	*Streptomyces nodosus*	*Streptomycetaceae*	Jimenez *et al.* (2009); Manam *et al.* (2005)
Marinomycin B	Antibiotics	*Marinispora*	*Marine actinomycete*	Jimenez *et al.* (2009)
MKN-349A	Cyclic tetrapaptide	*Nocardiopsis species*	*Nocardiopsaceae*	Jimenez *et al.* (2009)
Trioxacarcin	Antibiotics	*Streptomyces species*	*Streptomycetaceae*	Jimenez *et al.* (2009)
Phytosterols	Steroids	*Dunaliella Tertiolecta*	*Dunaliellaceae*	Francavilla *et al.* (2012)
Glutathione (GSH)	Antioxidant	*Ishige okamurai*	*Phaeophyta*	Kakinuma *et al.* (2001)
Gamma-linolenic acid	PUFA	*Codium species*	*Chlorophyta*	Pereira *et al.* (2012)
Beta-sitosterol	Sterol	*Gracilaria salicornia*	*Gracilariaceae*	Saeidnia *et al.* (2014)
Xylan	Polysaacharide	*Ulvales*	*Ulvophyceae*	Domozych *et al.* (2012)
Phytol	Diterpene alcohol	*Synechocystis species*	*Merismopediaceae*	Plaza *et al.* (2010)
Palmitoleic acid	Monounsaturated Fatty acid	*Synechocystis species*	*Merismopediaceae*	Plaza *et al.* (2010)
Squalamine	Aminosteroid	*Squalus acanthias*	*Squalidae*	Simmons *et al.* (2005)
Cemadotin	Linear peptide	*Dollabella auricularia*	*Aplysiidae*	Simmons *et al.* (2005)
Soblidotin	Linear peptide	*Dollabella auricularia*	*Aplysiidae*	Simmons *et al.* (2005)
Agosterol A	Steroid	*Spongia species*	*Spongiidae*	Jimenez *et al.* (2009); Murakami *et al.* (2001)
Dictyostatin-1	Polyketide	*Spongia species*	*Spongiidae*	Jimenez *et al.* (2009); Isbrucker *et al.* (2003)
Laurenditerpenol	Diterpene	*Laurencia intricata*	*Rhodomelaceae*	Jimenez *et al.* (2009); Oliviera *et al.* (2012)
Lissoclinolide	Fatty acid	*Ascidian*	*Ascidiacea*	Jimenez *et al.* (2009)
Neoamphimedine	Alkaloid	*Xestospongia species*	*Petrosiidae*	Jimenez *et al.* (2009); Ponder *et al.* (2011)
Smenospongorine	Seskviterpene	*Dactylospongia elegans*	*Thorectidae*	Jimenez *et al.* (2009); Aoki *et al.* (2004)

*PUFA- Polyunsaturated fatty acid.

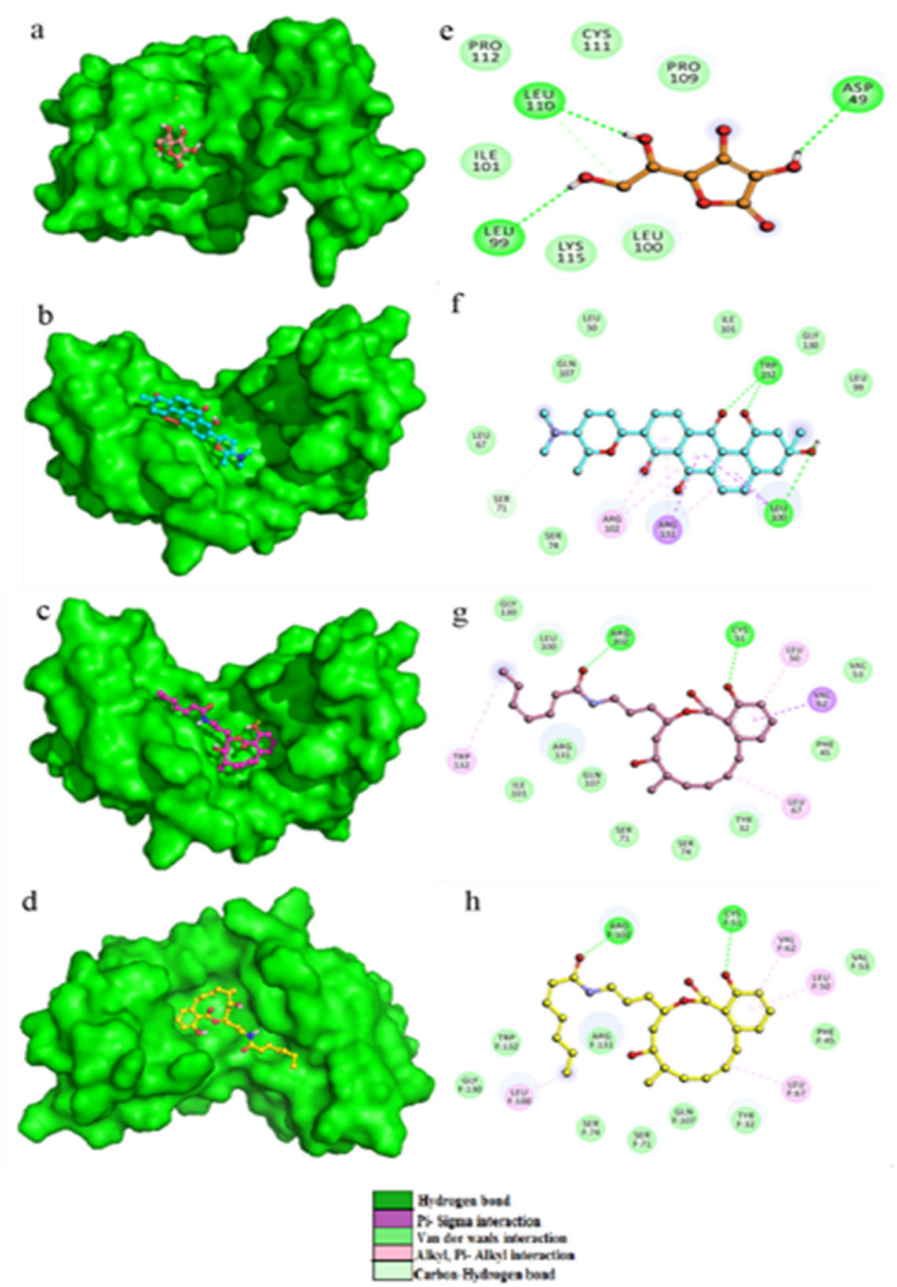

Fig. 1: a: Ascorbic acid-E6 protein complex, b: Frigocyclinone-E6 protein complex, c: Salicylihalamide A-E6 protein complex, d: Salicylihalamide B-E6 protein complex, e-h: 2D interaction pattern of four ligands with E6.

Table 2: Molecular properties of Bioactive compounds predicted using Molinspiration property calculator.

S. No	Bioactive Compounds	miLogP	TPSA	Atoms	MW	#ON	#OHNH	#Violations	#ROTB	Volume
1	Ascorbic acid	-1.4	107.22	12	176.12	6	4	0	2	139.71
2	Frigocyclinone	3.62	104.14	34	463.53	7	2	0	2	418.12
3	Salicylihalamide A	4.32	95.86	32	439.55	6	3	0	6	425.87
4	Salicylihalamide B	4.32	95.86	32	439.55	6	3	0	6	425.87

miLogP: LogP (partition coefficient); TPSA: topological polar surface area; MW: Molecular weight; #ON: number of hydrogen bond acceptors; #OHNH: number of hydrogen bond donors; #ROTB: number of rotational bonds.

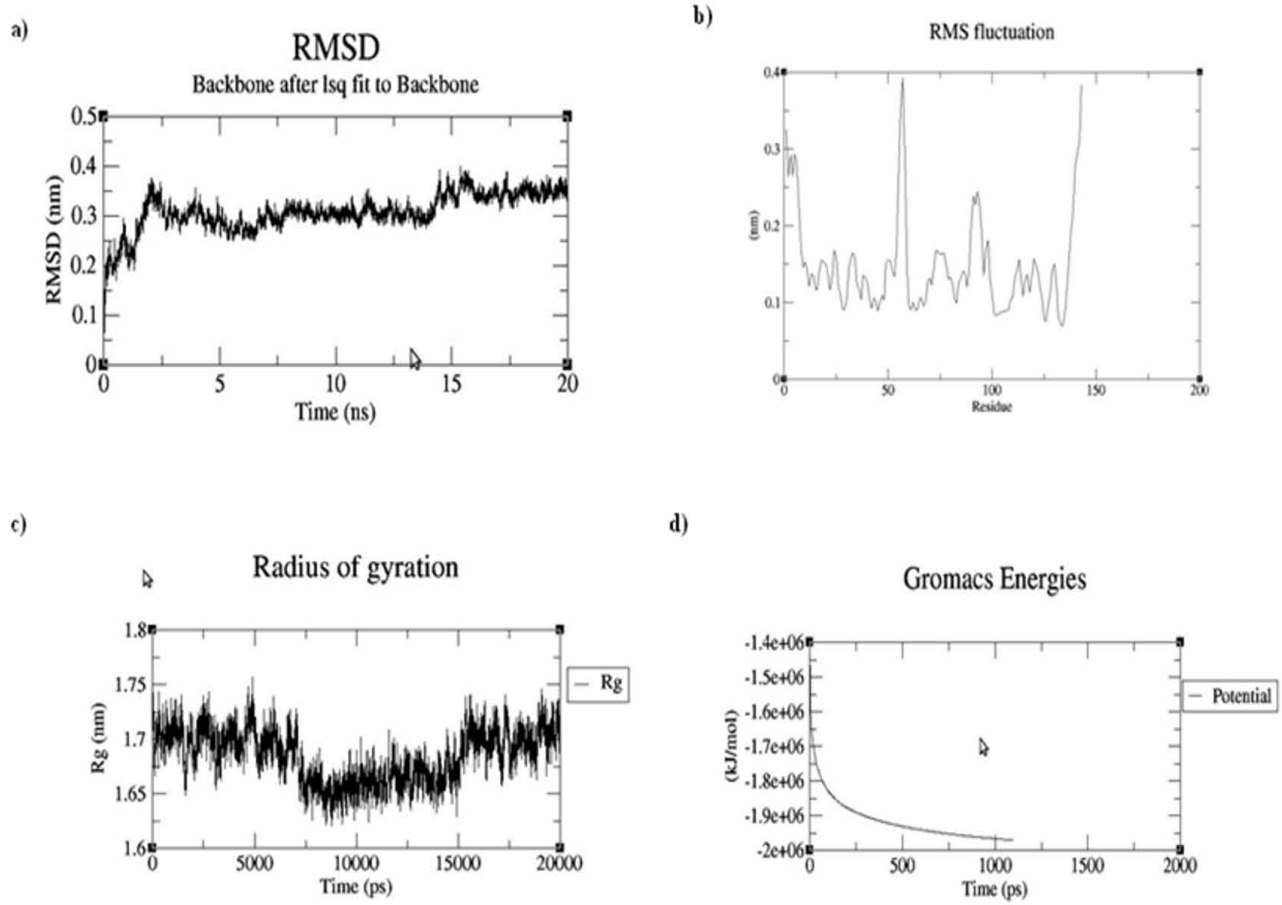

Fig. 2: a: RMSD plot of the backbone atoms of the E6 protein-Salicylihalamide B complex, b: RMSF shows the backbone of the fluctuation of atoms of E6 protein-Salicylihalamide B complex, c: Radius of gyration of the protein has an average value at 1.74 nm, d: Potential energy plot depicting the stability of the protein.

Table 3: Druglikeness prediction of bioactive compounds using Molsoft software.

S. No	Bioactive Compounds	Druglikeness score
1	Ascorbic acid	0.84
2	Frigocyclinone	0.93
3	Salicylihalamide A	1.01
4	Salicylihalamide B	1.01

Interacting residues such as LEU100, TRP132, SER74, ARG102, LEU50 were found in all the docked complexes. The presence of residues at their respective positions are important for the binding of E6 protein. A group of arginine residues forms the rim cap over the E6 helix groove and plays a remunerative role in the binding and recognition of small molecules (Rietz *et al*., 2016). The aromatic group of tryptophan is found to involve in the pi-alkyl stacking interaction. The rim arginine ARG102 forms a hydrogen bond with Salicylihalamide B. TRP132 and ARG131 found to contribute to the interaction through vanderwaal's forces and lies in the C-terminal region of the E6 protein. As an outcome, Salicylihalamide B gives the best binding interaction pattern with E6 protein and also it may act as a lead molecule to compete with E6-E6AP complex and prevents its activation. The Salicylihalamide B and E6 protein complex are then further validated for its stability using molecular dynamic simulation studies.

Molecular dynamics and simulation analysis

In order to examine the conformational stability, MDS was performed. From molecular docking studies, Salicylihalamide B – HPV16 E6 protein complex was subjected to MDS studies. GROMACS – 4.5.5 version is used to check the stability and the compactness of the complex. GROMOS96 43a1 forcefield was used for E6 protein. GROMOS87/GROMACS file with polar/Aromatic hydrogens is used as drg.gro file and the ligand topology file was used as drg.itp file. The lowest binding energy conformation was taken as initial conformation and solvated using SPC (single point charge) water molecule and neutralized by adding chlorine ions. Finally, 50 ns MD simulation was performed for the E6 Protein–Salicylihalamide B complex and the results were monitored. The entire system remained steady all through the MD simulation process. Various trajectory analysis like potential energy, RMSD, and RMSF of the backbone atoms, radius of gyration of the protein was monitored and they were analyzed to interpret the convergence, fluctuations and the stability of the

protein-ligand complexes. RMSD analysis shows the Protein-ligand complex exhibits numerous smaller peaks, and at 40 ns it started to converge and attained stability (Figure 2a). The protein structural flexibility during the simulation process was noticeable from the small drifts observed in the RMSD plot. The dynamic stability of the Protein-ligand complex was also determined based on the root mean square fluctuation which reflects the mobility of the residues around its mean position. RMSF shows major fluctuations at the residues 75, 110 and 130 and minor fluctuations in the residues 30, 60 and 80 (Figure 2b). The radius of gyration maintains the compactness of protein at 1.75 nm (Figure 2c). The potential energy plot shows the protein-ligand complex reaches its stability close to 460 ps (Figure 2d). Overview of this study is presented in Figure 3. At the end of the simulation period, the complex undergoes minor conformational changes and attains its stability.

CONCLUSION

The results obtained from the present work shows that the bioactive compounds from marine algal sources also play a vital role in interacting with Human papilloma virus (HPV 16) which causes oral and cervical cancers in humans. Our results suggest that Salicylihalamide B from *Haliclona species* (sponge) obeyed all the rules of virtual screening and can precisely bind to the active pocket of the E6 protein with better conformational stability. Hence, Salicylihalamide B can be proposed as the potent bioactive compound against target E6 of HPV16 and may be furthermore explored as an anticancer drug. Along with potential anti-tumor property, in this study, Salicylihalamide B may conceivably be considered to have the capability of anticancer property.

Table 4: Binding energy and interacting residues of selected bioactive compounds with E6 protein.

S. No.	Ligands	Binding Energy*	Binding site residues involved in interactions
1	Ascorbic acid	-3.82	LEU110, LEU99, ASP49, LYS115
2	Frigocyclinone	-8.01	TRP132, LEU100
3	Salicylihalimide A	-8.76	ARG102, CYS51
4	Salicylihalimide B	-8.92	ARG102, CYS51

*Kcal/mol.

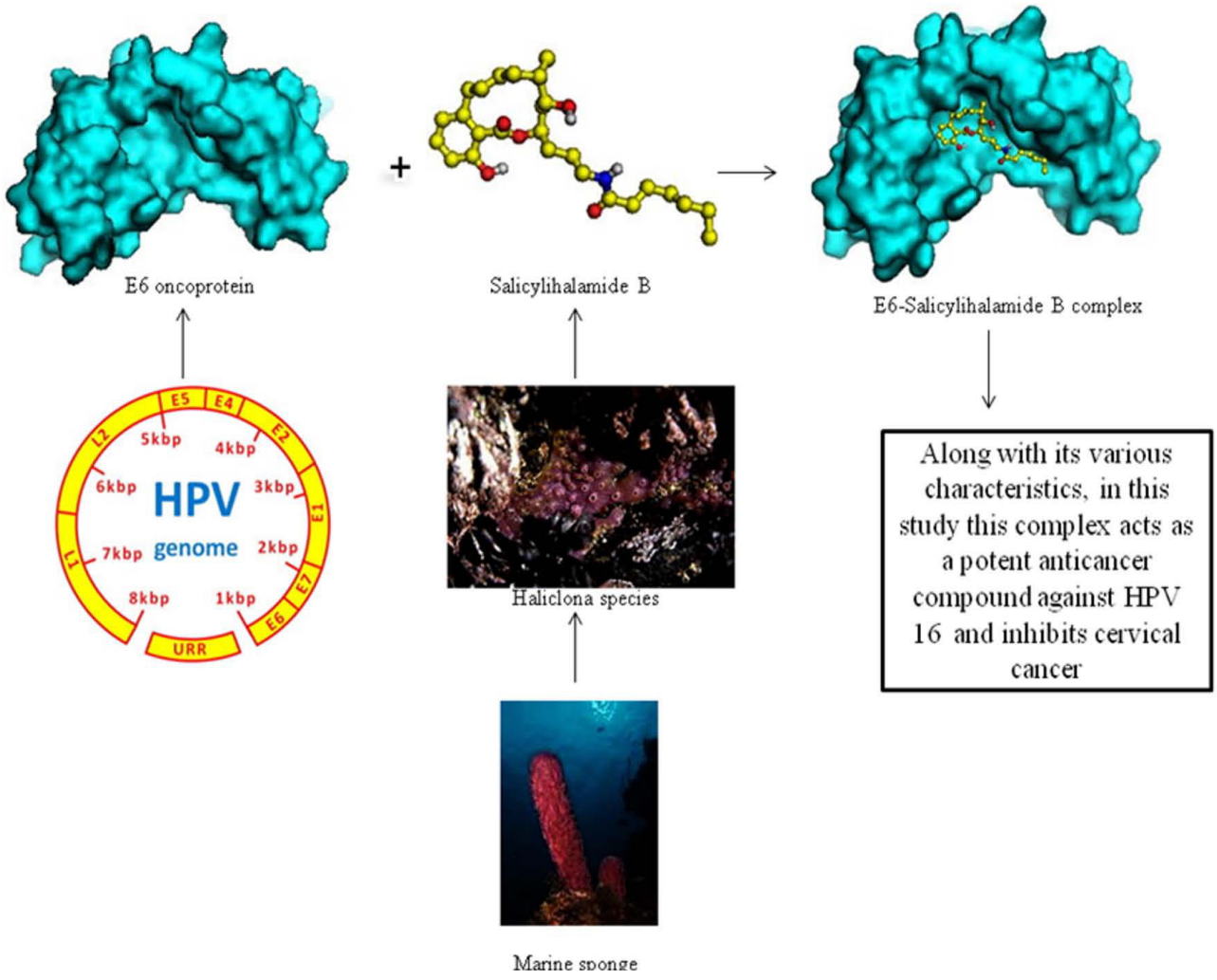

Fig. 3: Overview of this study.

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