Research Article

# The Potency of Marine Natural Compounds – An *In Silico* Approach

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**Accepted on:** 28-07-2016; **Finalized on:** 30-09-2016.

**ABSTRACT**

Marine natural compounds, as therapeutic agent, are the focus of this era. Few FDA approved drugs are already available in the market. This study focuses on identifying the activity of compounds using flexible docking isolated from the marine sponges - *Diplastrella* sp, genus *Lendenfeldia, Ancorina* sp. and *Coscinaderma* sp., and to identify their critical chemical features, with reliable geometric constraints that can be used as an effective drug. The compounds under study have been known to have anti-HIV activity and is further analysed for other antiviral activities by computational approach. These compounds fulfill the Lipinski's rule of five and have showed optimum ADMET properties that are desirable as a drug. Density functional theory (DFT) studies were conducted for these compounds. Molecular docking studies were carried out against four viral proteins (PDB ID 3H5S- Hepatitis C virus, 4TVH-HIV, 3MS6-Hepatitis B virus & 3B7E-H1N1). All these were carried out using Discovery Studio version 4.0. The compounds Diplyne C Sulfate and Coscinamide A showed more than five hydrogen bond interaction with the Hepatitis B & C virus and HIV proteins with reliable geometric constraints. These drugs have been previously identified as anti-HIV using in-vitro studies and further the results obtained through the computational approach can be used to validate the activity of these compounds against Hepatitis B & C virus which causes chronic liver disease.

**Keywords:** Marine sponges; anti-HIV; Lipinski's rule; ADMET; DFT; Molecular docking.

## INTRODUCTION

Oceans cover more than 70% of the earth's surface and represent an enormous resource for potential bioactive compounds. These have been utilized in various industrial and human health related applications in past few decades[1]. The marine sources include; bacteria, fungus, sponges, algae, and other microorganisms. Various clinical trials have been conducted using novel chemotherapeutic agents and have revealed novel mechanisms of action. Although there has been tremendous progress in medicine, newly developing defense mechanisms of bacteria, fungi and viruses, infectious diseases are still a major threat to public health.

Marine sponges have been found to be the richest sources of pharmacologically-active chemicals due to their efficient defense mechanisms against external invaders[2]. These metabolites are classified chemically as alkaloids, terpenoids, phenazines, polyketides, glycosides, phenols, amino acid analogues, nucleosides, porphyrins, aliphatic cyclic peroxides and sterols, fatty acid products and peptides.

Numerous different products have been discovered in sponges and their accompanying microorganisms each year and are being utilized in industry and in the field of medicine[3,4].

As infective microorganisms evolve and develop newer resistance mechanisms, the marine sponge provides novel leads against them, which includes cancer, viral, bacterial, fungal, protozoal, and so on. Many marine natural products have efficaciously advanced to the late stages of clinical trials[5]. Antiviral and anti-HIV activities have been observed from samples of Diplastrella sp., *Myrmekioderma styx*, *Zyzzya fuliginosa*, *Ancorina* sp., *Coscinaderma* sp. etc. In a recent review, over 132 marine natural products obtained during the period 2002 to 2011 exhibited anti-HIV activity[6].

The human immunodeficiency virus (HIV), a challenging disease, has become a serious menace to global public health in the last decades because of the rapid development of viral resistance[7]. Furthermore, the drugs in use have limited benefits due to their adverse side effects. The discovery of new novel anti-HIV agents that have unique mechanism of action and that are affordable with low toxicity to the host remains priority in the current times.

Hepatitis C and Hepatitis B causes chronic liver disease found in both children and adults. Humans are the only known natural hosts of HCV. It is still a major cause of concern in public health systems worldwide. It causes both acute and chronic hepatitis. Even though host immune mechanisms detects and targets HCV, it is able to establish and maintain a life-long persistent infection. HCV has evolved multiple strategies to survive the host immune system. Persistent infection by these viruses leads to cirrhosis and hepatocellular carcinoma. At present there is neither a selective antiviral therapy nor a preventive vaccine for HCV. HCV also has the ability of direct cell-to-cell transmission. Recent advances in understanding of HCV protein structures, genome and its lifecycle have exposed numerous target sites for potential pharmacological intervention[8,9]. The diagnosis involves detection of anti-HCV antibodies in serum. The difference

between acute and chronic infections is not clearly legible. Most patients are non-responsive to the current therapy (i.e. combination of pegylated interferon (IFN)-α and ribavirin.) given[10].

Hepatitis B virus causes liver injury by the immune response that is generated against the virus-infected liver cells.

A series of different stages are observed in the life cycle of each patient's infection, with variation in the level of viral load at each successive stage. Viral mutations affect replication and may enhance liver injury[11].

Among the various genotypes of HBV, A & D have been well documented in different parts of India. Many HBV genotypes emerge because the reverse transcriptase lacks a proofreading function[12,13].

## MATERIALS AND METHODS

### Marine Bioactive Compounds

The bioactive compounds chosen were identified through literature survey and their structures and chemical properties were obtained from Pubchem Compound database which is available in Table 1. Seven out of ten compounds satisfied the Lipinski's rule of 5. The compounds are 1-Aminocyclopropanecarboxylic acid, LMPR0104240002 or (-)-2,7-Dolabelladiene-6beta, 10alpha, 18-triol, Isobatzelline C, Diplyne C Sulfate, Dehydrofurodendin, Cyanthiwigin B, Ancorinolate C, Ptilomycalin A, Homophymine A, Batzelladine L.

### Proteins

Antiviral proteins were selected and the structures were attained from Protein Data Bank (PDB). The proteins are; Crystal structure of Hepatitis B X-Interacting Protein (**3MS6**, chains A &B), HIV Protease (PR) dimer in closed form with TL-3 in active site and fragment AK-2097 in the outside/top of flap (**4TVH**, chains A&B), Hepatitis C virus polymerase NS5B (**3H5S**, Chain A) and Neuraminidase of A/Brevig Mission/1/1918 H1N1 strain (**3B7E**, Chains A & B) shown in Figure 1. The Proteins were prepared using default settings of 'prepare protein' protocol of Discovery Studio 4.0 version. The unwanted water molecules and the ligands attached to the proteins were removed and the amino acid (target) residues suggested by the software were selected for docking. The active site residues of the Hepatitis C, B and HIV proteins as reported by the software are; TRP6, LYS7, THR12, ASP25, GLY27, ALA28, ASP29, ASP30, PRO44 LYS45, MET46, ILE47, GLY48, GLY49, ILE50, PHE53, LYS55, VAL56, ARG57, CYS67, HIS69, PRO81, VAL82, ILE84, THR91, GLN92, GLY94 (**4TVH**); THR12, ASP25, THR36, GLU68 A:SER69, ASP70, LEU55 A:ASN71, HIS79, ASP80 (**3MS6**); PHE193, PRO197, ARG200, SER288, ASN291, ASN316, GLY317, ASP318, CYS366, ASN411, MET414, TYR415, GLN446, ILE447, TYR448, GLY449, SER556 (**3H5S**).

### Molecular Docking

The forcefield of the Chemistry at Harvard Macromolecular mechanics (CHARMm) in the'prepare ligands' protocol was applied to 3D molecules mentioned above. A flexible and comprehensive empirical energy function is used by CHARMm which is a summation of many individual energy terms. The hydrogen bonds were added and their partial charges estimated and assigned. The proteins were optimized using 'prepare protein' protocol. These processes were carried out using Discovery Studio V4.0.

Absorption, Distribution, Metabolism, and Excretion – Toxicity (ADMET) were analysed and Coscinamide A, LMPR0104240002 or (-)-2,7-Dolabelladiene-6beta, 10alpha, 18-triol, Isobatzelline C, Diplyne C Sulfate, Dehydrofurodendin, Cyanthiwigin B, Ancorinolate C, Ptilomycalin A, showed good ADMET properties and were selected for docking studies (Figure 2 and Table 2).

Density functional theory (DFT) studies also were carried out for these compounds shown in Tables 3 & 4. Flexible docking protocol was used for the docking of the seven desirable bioactive compounds that showed good ADMET properties into the 'A Chains' of the three antiviral proteins of Hepatitis B, HIV, Hepatitis C and Influenza (H1N1) viruses were selected respectively. The docking results are followed by energy refinement and dockscore analysis of the best poses.

## RESULTS AND DISCUSSION

Seven chemical compounds derived from the marine sponges (*Diplastrella* sp, genus *Lendenfeldia, Ancorina* sp. and *Coscinaderma* sp.) were docked into four antiviral proteins (3H5S, 4TVH, 3MS6 and 3B7E). The docked compounds were selected based on their ADMET properties, good interaction, binding energy and dock score.

### ADMET & DFT Studies

Those compounds that are seen within the four ellipses can be termed as eligible since it satisfies the ADMET features which are Absorption, Aqueous Solubility, Blood Brain Barrier, CYP2D6 Binding, and Hepatotoxicity.

The HOMO-LUMO energy gap of a molecule plays a vital role in determining its bioactive properties. The HOMO energy describes the capacity of electron donor, whereas LUMO energy describes the capacity of electron acceptor, and the gap distinguishes the chemical stability.

The HOMO to LUMO transition indirectly explains their interacting ability with the target molecules. The two compounds that have shown the maximum number of interactions with the viral proteins have the least total energy values (Diplyne C Sulfate total energy is -3957.79035662 and for Coscinamide A it is -3646.58371462).

### Molecular Docking

Out of the seven compounds docked only two to three compounds showed best interaction with the active site residues each of the three antiviral proteins. The results

are described in Table 5 and Figure 3. The active site residues with which the ligands docked are as follows; PRO63, VAL64, VAL65, GLN77, ALA49, LYS78 of **3MS6**, GLY557, ASP559, CYS451, GLN446, GLN449, ARG200 of **3H5S** and GLN2, ILE3, THR96, ASN98, LEU97, ASN98 of **4TVH**. No interaction was observed between the H1N1 protein 3B7E and the selected ligands. The interaction has also taken place with the residues that have not been selected for the proteins in study (not indicated as in PDB). Clinical trials are required to understand the activity of the docked compounds with the viral proteins since new residues have interacted with the selected ligands.

**Table 1:** Structure and Chemical properties of Marine Derived Compounds

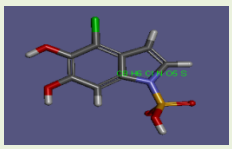
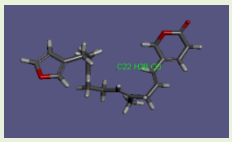
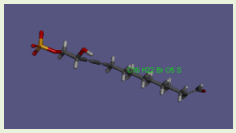
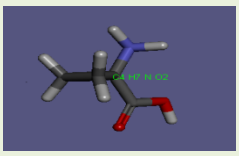
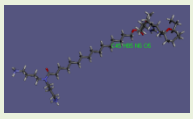
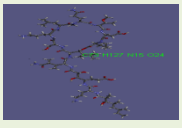
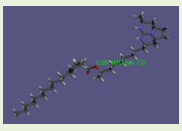
| S. No. | Compound Name | Compound Structure/Molecular Formula | Molecular Weight | Hydrogen Bond Donor/Acceptor |
|---|---|---|---|---|
| 1 | Ancorinolate C |  | 286.644709 g/mol | 3/5 |
| 2 | Cyanthiwigin B |  | 300.43512 g/mol | 0/2 |
| 3 | Dehydrofurodendin |  | 340.45592 g/mol | 0/3 |
| 4 | Diplyne C Sulfate |  | 407.31982 g/mol | 2/5 |
| 5 | Isobatzelline C |  | 235.6696 g/mol | 1/3 |
| 6 | LMPR0104240002 |  | 322.48216 g/mol | 3/3 |
| 7 | Coscinamide A |  | 101.10388 g/mol | 2/3 |
| 8 | Ptilomycalin A |  | 823.63072 g/mol | 4/8 |
| 9 | Homophymine A |  | 1598.87558 g/mol | 19/26 |
| 10 | Batzelladine_L |  | 652.99622 g/mol | 2/4 |

**Table 2:** ADMET Descriptors

| S. No. | Compound Name | Solubility[a] | BBB[b] | CYP2D6[c] | Hepatotoxic[d] | ADMET_AlogP98[e] |
|---|---|---|---|---|---|---|
| 1 | Coscinamide A | -5.485 | -0.213 | -2.20678 | 3.27481 | 3.778 |
| 2 | Ancorinolate C | -2.587 | -1.19 | -7.35768 | 0.275732 | 1.891 |
| 3 | Cyanthiwigin B | -6.033 | 0.649 | -0.32865 | -7.35222 | 4.368 |
| 4 | Dehydrofurodendin | -5.495 | 0.958 | -3.27533 | -10.504 | 5.582 |
| 5 | Diplyne C Sulfate | -2.857 | -0.27 | -5.87523 | -8.50869 | 3.804 |
| 6 | Isobatzelline C | -1.246 | -1.345 | -7.53675 | 0.101275 | -0.388 |
| 7 | LMPR0104240002 | -3.043 | -0.207 | -5.85273 | -6.43391 | 3.026 |
| 8 | Batzelladine L | -5.271 | - | -3.3462 | -9.01502 | 8.034 |
| 9 | Homophymine A | -6.343 | | -15.721 | -8.54869 | -3.574 |
| 10 | Ptilomycalin A | -2.86 | | -6.63059 | -24.0838 | 7.377 |

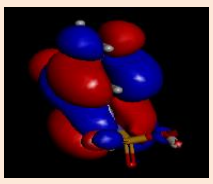
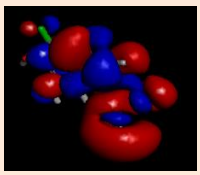
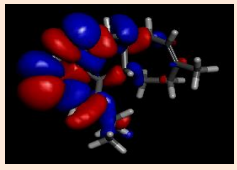
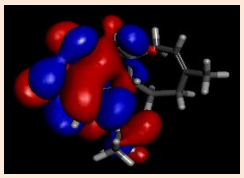
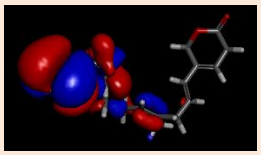
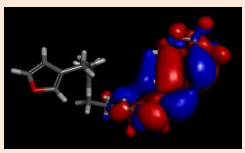
a: Predicts the solubility, b: blood brain barrier penetration, c: Predicts cytochrome P4502D6enzyme inhibition, d: Predicts the occurrence of dose-dependent human hepatoxicity.

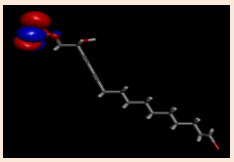
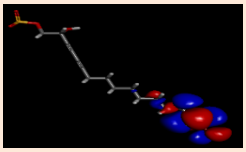
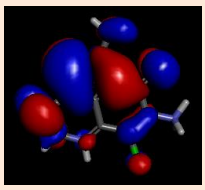
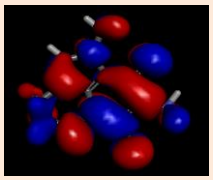
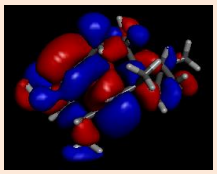
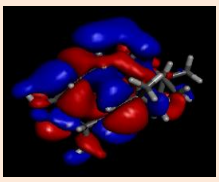
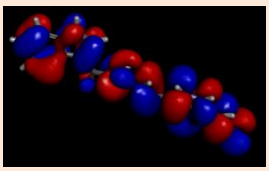
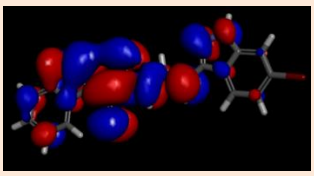
**Table 3:** Density Functional Theory Studies

| S. No. | Compound Name | Total Energy (Kcal/mol) | Binding Energy (Kcal/mol) | HOMO Energy (Kcal/mol)[a] | LUMO Energy (Kcal/mol)[b] | Dipole Mag |
|---|---|---|---|---|---|---|
| 1 | Ancorinolate C | -1749.94241284 | -3.74634743 | -0.28900033 | -0.28263095 | 17.83086028 |
| 2 | Cyanthiwigin B | -921.10356454 | -9.48861889 | -0.17627138 | -0.07197175 | 3.14926246 |
| 3 | Dehydrofurodendin | -1071.20424872 | -10.14405679 | -0.19332283 | -0.06190552 | 2.10316318 |
| 4 | **Diplyne C Sulfate** | **-3957.79035662** | **-7.88103950** | **-0.03688071** | **0.00315111** | **20.04091485** |
| 5 | Isobatzelline C | -1118.26630980 | -4.83145994 | -0.48347242 | -0.44286123 | 3.88120809 |
| 6 | LMPR0104240002 | -999.30466733 | -10.30731632 | -0.17575251 | -0.01453988 | 0.48165963 |
| 7 | **Coscinamide A** | **-3646.58371462** | **-8.53970515** | **-0.17573967** | **-0.11448433** | **1.68569490** |

**a**: Highest Occupied Molecular Orbital (HOMO), **b**: Lowest Unoccuppied Molecular Orbital (LUMO)

**Table 4:** HOMO and LUMO Energy Visualization

| S. No. | Compound Name | HOMO Energy | LUMO Energy |
|---|---|---|---|
| 1 | Ancorinolate C |  |  |
| 2 | Cyanthiwigin B |  |  |
| 3 | Dehydrofurodendin |  |  |

| | | | |
|---|---|---|---|
| 4 | Diplyne C Sulfate |  |  |
| 5 | Isobatzelline C |  |  |
| 6 | LMPR0104240002 |  |  |
| 7 | Coscinamide A |  |  |

▲ Electron acceptors are mentioned in red and the electron donors are in blue.

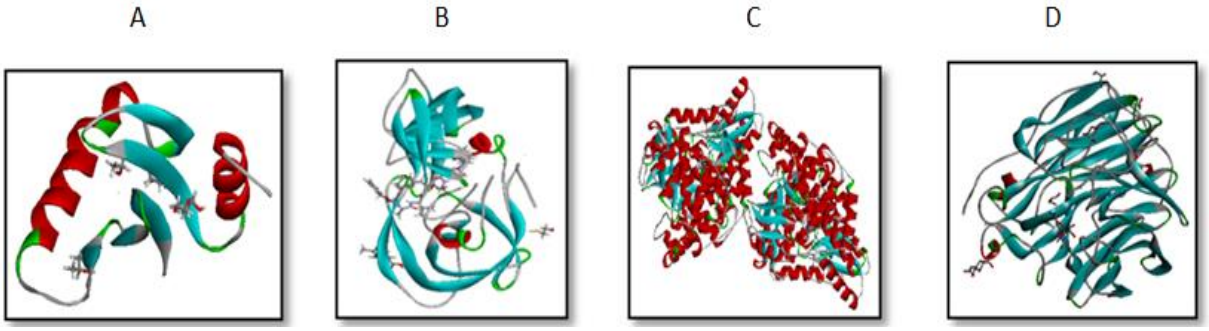



**Figure 1:** Protein Structure Obtained from PDB (Protein Data Bank). A. 3MS6, B. 4TVH, C. 3H5S and D. 3B7E

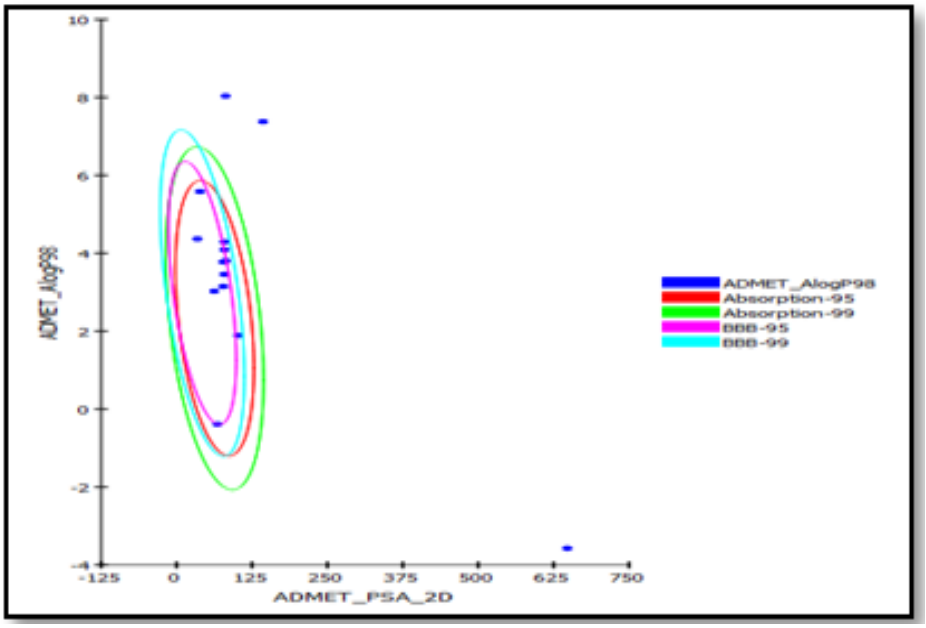
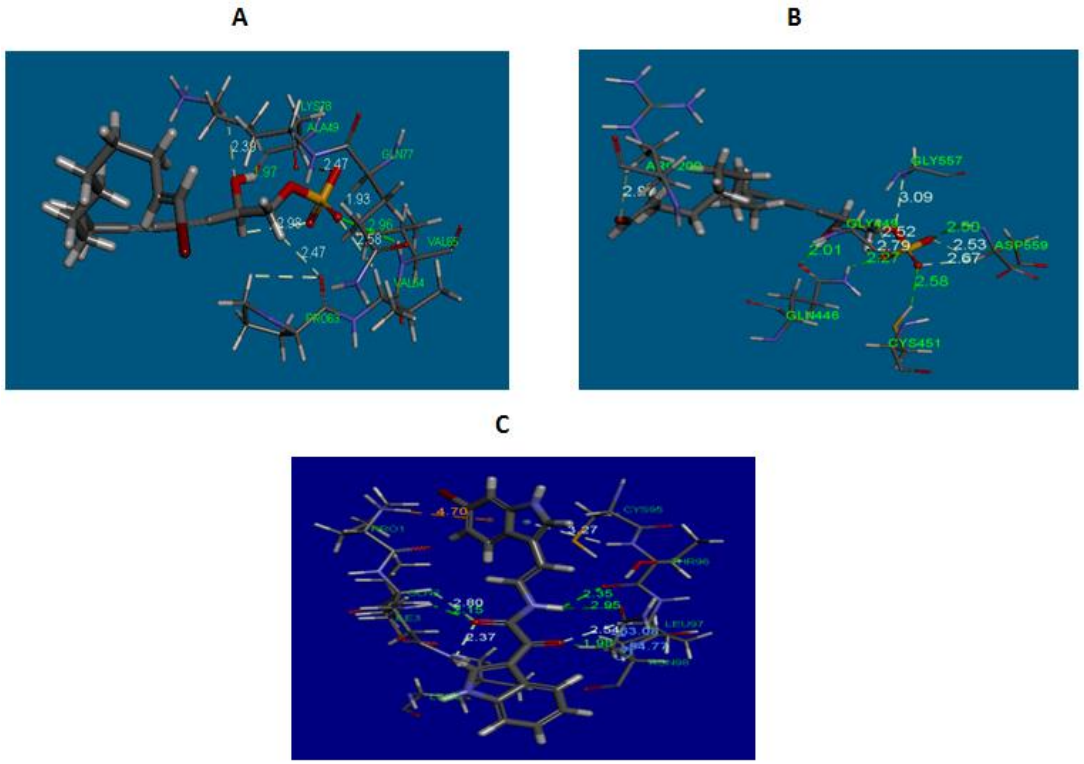



**Figure 2:** ADMET Properties




×Green dotted lines indicates hydrogen bonding between the amino acid residues and the compound. The decimal values indicate the distance of Hydrogen bond.

**Figure 3:** Flexible Docking Results of the Best Poses with Maximum Interactions with the Three Proteins. A. 3MS6 with Diplyne C Sulfate, B. 3H5S with Diplyne C Sulfate and C. 4TVH with Coscinamide A.

**Table 5:** Flexible Docking Results of the Best Poses

| S. No. | Compound Name | Protein | CDocker Energy | LibDock Score | Binding Energy | Hydrogen Bond Interaction* | Distance |
|---|---|---|---|---|---|---|---|
| 1 | Diplyne C Sulfate | 3MS6 | 7.027 | 67.774 | -48.4078 | O-H…O[ALA49]<br>[LYS78] C-H…O<br>[ALA49] C-H…O<br>[VAL65]N-H…O<br>[VAL64] C-H…O<br>C-H…O [PRO63]<br>[GLN77] C-H…O | 1.97<br>2.39<br>2.47<br>2.96<br>2.58<br>2.47<br>1.93 |
| 2 | Diplyne C Sulfate | 3H5S | 13.333 | 76.84 | -58.3785 | [ARG200] C-H…O<br>O-H…O [GLN446]<br>C-H…O [GLN446]<br>C-H…O [GLY449]<br>[GLY557] C-H…O<br>[GLN446] N-H…O<br>[ASP559] C-H…O<br>[ASP559] C-H…O<br>[ASP559] N-H…O<br>[CYS451] S-H…O | 2.87<br>2.01<br>2.52<br>2.79<br>3.09<br>2.27<br>2.67<br>2.53<br>2.50<br>2.58 |
| 3 | Coscinamide A | 4TVH | 25.593 | 70.411 | -64.9802 | [GLN2]C-H…O<br>[ILE3] N-H…O<br>N-H…O [THR96]<br>N-H…O [ASN98]<br>[LEU97] C-H…O<br>[ASN98] N-H…O | 2.80<br>2.15<br>2.35<br>2.95<br>2.54<br>1.98 |

× Hydrogen bond interaction between the compound and the active site residue of the viral protein

The search for new classes of antiviral drugs has become vital due to the long-term toxicity to the host and the acquired resistance to the currently available drugs.

There is an increase in the discovery of natural products with antiviral activity from the last decade. In this work the antiviral potential of some of the compounds from marine sponges were evaluated *in silico* and favorable results have been obtained.

Good interaction indicates effective inhibition of the viral proteins that were selected. Apart from the compounds interactions described in Table 5 the others (Ancorinolate C, Cyanthiwigin B, Dehydrofurodendin, Isobatzelline C, LMPR0104240002) also interacted well with the active site residues of the proteins signifying their potential as antiviral drugs.

These compounds have already proven to be effective against HIV proteins with cell based assays.

Hepatitis B virus (HBV) is one of the major global public health problems, followed by HIV and Hepatitis C virus (HCV). The lack of vaccines for HIV and HCV thus needs effective management therapies; this work is thus a step towards finding new drugs for cure.

## CONCLUSION

Viruses like HIV, HCV, H1N1, HBV etc. have become resistant to the currently available drugs and also continue to acquire newer resistance mechanisms towards the newly discovered drugs. Hence, the necessity to find new advanced drugs and drug targets to combat this issue.

The availability of marine compounds are enormous and lots more are to be discovered and isolated. The results obtained from this study helps in visualizing and understanding the inhibitory effects of the compounds. New residues have been identified for ligand interaction.

This method is also useful in accurately predicting the effects of a compound on different proteins of interest before implementing them in the drug synthesis process.

Here we have concluded that these compounds have good inhibitory effects against viral proteins and needs further clinical studies to validate their antiviral activity.

**Acknowledgement:** Authors are thankful to Department of Biotechnology (DBT), New Delhi, India.

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**Source of Support:** Nil, **Conflict of Interest:** None.