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IN SILICO SCREENING OF SECONDARY METABOLITES DERIVED FROM MARINE FUNGI FOR ANTICANCER STUDY

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ABSTRACT:

Marine derived fungi have shown in recent years to produce a plethora of new bioactive secondary metabolites with biological activities mainly focused in the areas of antibiotic and anticancer properties. There lies immense scope of exploring marine fungus to obtain antitumor leads and drugs. In this study, we have taken seven secondary metabolites obtained from marine derived fungi that are experimentally proven to be cytotoxic against various human/cancer cell lines. Using Pharmmapper - a web server for drug target identification, we found the potential anti tumor targets of these compounds and determined their cancer relevance. The compounds taken for study are found to be druggable by Lipinski test and have a safe ADMET profile as indicated by the ToxPredict analysis.

Key words: Pharmmapper, Marine derived fungi, ADMET, Lipinski rule, Virtual screening & ChemSpider

[I] INTRADUCTION

Oceans have borne most of the biological activities on our planet and represent a resource of huge dimension for natural product chemists [1]. Marine floras, such as bacteria, actinobacteria, cyanobacteria, fungi, microalgae, seaweeds, mangroves, and other halophytes are extremely important oceanic resources, constituting over 90% of the oceanic biomass. They are taxonomically diverse, largely productive, biologically active, and chemically unique offering a great scope for discovery of new anticancer drugs [2]. Most eminently, marine derived fungi have shown in recent years to produce a plethora of new bioactive secondary metabolites with biological activities mainly focused in the areas of antibiotic and anticancer properties. Fungi from marine environment have proved to be prolific producers of structurally and chemically diverse compounds some of which feature new carbon frameworks hitherto unprecedented in nature [3]. Marine-derived fungi, are fertile producers of new structurally interesting compounds, and are recognized as an important source of structurally novel and bioactive secondary metabolites for drug discovery [4]. In this study, we have taken seven secondary metabolites obtained from marine derived fungi that are experimentally proven to

be cytotoxic against various human/cancer cell lines. Some researchers have resorted to computing methods to find the probable target proteins for active compounds, natural products or old drugs. This searching of drug targets is called "Insilico Target Fishing" or "Inverse Virtual Screening/Reverse Docking". There are a number of drug target databases and reverse docking servers developed for the purpose of performing target fishing [5]. These tools generate a tractable set of target proteins for experimental validation. We have first tested 30 compounds against Lipinski rule and toxicity analysis to check their druggability. Out of them 7 compounds obeyed Lipinski rule and were found to be druggable [6]. These compounds were submitted to PharmMapper – a web server that uses pharmacophore mapping approach for potential drug target identification and top 3 % to 5 % of proteins reported by PharmMapper were collected as potential targets and their cancer relevance was found relying on published literature.

[II] MATERIALS AND METHOD:

2.1. Ligand Preparation and virtual screening:

30 cytotoxic metabolites isolated from various marine derived fungi having IC_{50} values in micro to nanomolar range were retrieved from literature and their chemical structures were collected from ChemSpider [7]. These compounds were downloaded in MDL Mol file format and converted to a 3D Structure-Data File (sdf) format using 3D cleaning method available in ChemAxon suite. These compounds were screened using Lipinski Filter to distinguish drug like and non drug like compounds. Lipinski's filter predicts high probability of success and failure due to drug likeness for molecules, complying with two or more of the following rules:

Molecular mass less than 500 Dalton.
High lipophilicity(expressed as logP less than 5).
Less than 5 hydrogen bond donors.
Less than 10 hydrogen bond acceptors.
Molar refractivity should be between 40-130.

7 compounds passed through this filter and only these were taken for further analysis. Next, the compounds were submitted to ToxPredict [8] application of OpenTox which accepts chemical structures and automatically generates a toxicity report based on various pre calibrated toxicity models. The compounds successfully screened by Lipinski filters shown in table 1. and structure Fig: 1 to 7

Compound Name	Organism	Chemistry
Gymnastatin G	*Gymnascella dankaliensis*	Steroid
Epoxyphomalin A	*Phoma spp*	Prenylated polyketide
Conidiogenone C	*Penicillium spp.*	Diterpene
Ustusorane E	*Aspergillus ustus*	Sesquiterpenoid
Peribysin H	*Periconia byssoides*	Sesquiterpenoid
Chaetomugilin I	*Chaetomium globosum*	Azaphilone
Aspergiolide B	*Aspergillus flavus*	Anthraquinone derivative

Table 1: Compounds screened using Lipinski Filters

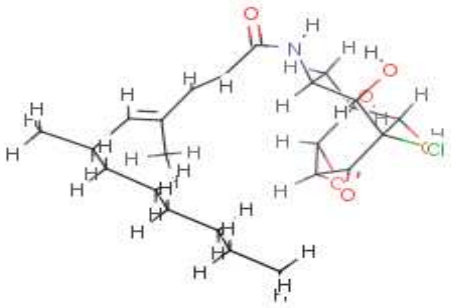

Fig1: Gymnastatin G (CID 10481523)

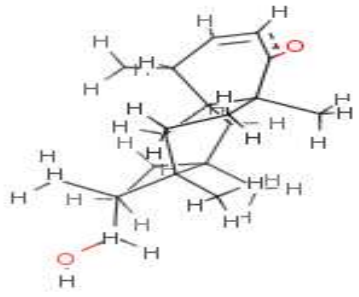

Fig2: Conidiogenone C (CID 27024829)

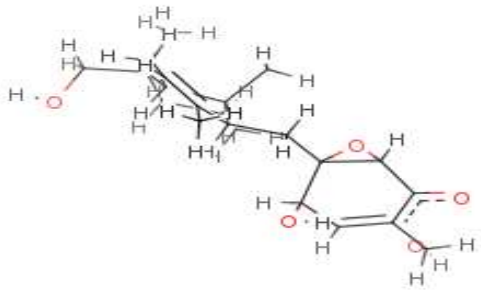

Fig3: Epoxyphomalin A (CID 26390061)

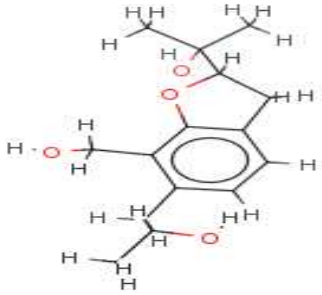

Fig4: Ustusorane E (CID 24629208)

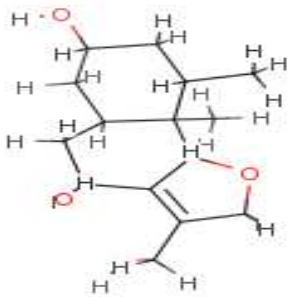

Fig5: Peribysin H (CID 9805133)

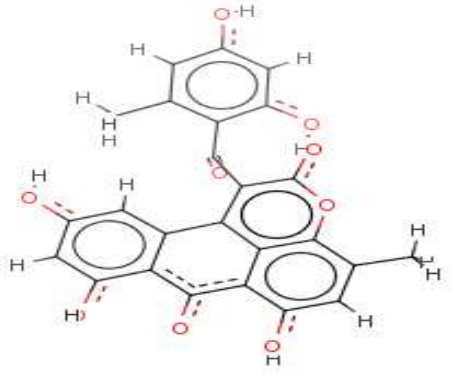

Fig6: Aspergiolide B (CID 24637894)

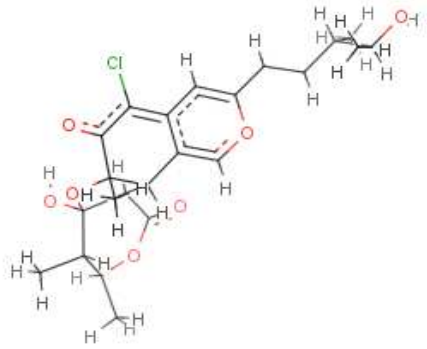

Fig7: Chetomugilin I (CID 26607402)

2.2. Drug target identification:

Target identification is the most crucial step in a modern drug discovery campaign [9]. *Insilico* drug target identification, which includes many distinct algorithms for finding disease genes and proteins, is the first step in the drug discovery pipeline. Input to PharmMapper was provided as 3d SDF file and only human protein target set was used for target screening. The output of PharmMapper run was in the form of a ranked list of hit target pharmacophore models that were sorted by fit score in descending order. The targets ranking in top 3% to 5% of the PharmMapper list are considered further for finding their cancer relevance based on published literature. From target fishing it was found that five of the seven compounds namely Epoxyphamalin A, Conidiogenone C, Ustusorane E, Peribysin H and Aspergiolide B have Heat shock protein- Hsp 90^{α} as one of their potential targets.

[III] RESULTS

3.1. Ligand Preparation and Virtual Screening:

The 30 compounds taken for study were screened using Lipinski drug filters and only 7 compounds successfully passed the screening with Lipinski Filters and their results are shown in table 2:

	GG	EA	CC	UE	PH	CI	AB
Molecular weight	**432**	**376**	**316**	**266**	**268.0**	**427.0**	**460.0**
Hydrogen bond donor	**4**	**3**	**1**	**3**	**3**	**2**	**5**
Hydrogen bond acceptor	**6**	**5**	**2**	**4**	**4**	**7**	**9**
LogP	**0.474**	**1.1**	**4.3**	**0.3**	**0.8**	**0.009**	**3.2**
Molar refractivity	**103**	**100.8**	**91.8**	**71.9**	**70.5**	**108.4**	**120.8**

GG: Gymnastatin G, EA: Epoxyphomalin A, CC: Conidiogenone C UE: Ustusorane E, PH: Peribysin H, CI: Chetomugilin I and AB: Aspergiolide B

Table 2: Screening of cytotoxic compounds of marine derived fungi using Lipinski Drug Filters

3.2. Drug target identification:

The compounds obtained after toxicity screening were taken as candidates molecules for target identification using PharmMapper web server. PharmMapper listed top 300 targets based on the fit score for each of the 7 compounds. Proteins ranked in top 3% to 5% of the output PharmMapper list were taken as potential anti tumor targets of the compounds. The some of the important target class and potential target are outlined in the table 3.

Compound Name	Predicted Target	Target class	Cancer relevance
Gymnastatin G	Thyroid hormone receptor beta[1Q4X]	Nuclear receptor	RCCC, Thyroid Pappilary Cancer
Epoxyphomalin A	Heat shock protein HSP90- alpha [1OSF]	Chaperone protein	Breast Cancer HIF-1α-positive tumors
Conidiogenone C	Heat shock protein HSP90- alpha [1OSF]	Chaperone protein	Breast Cancer HIF-1α-positive tumors
Ustusorane E	Tyrosine protein phosphatase non receptor type 1[1Q1M]	Protein Tyrosine Phosphatse	Breast Cancer
Peribysin H	Insulin like growth factor 1 receptor	Transmembrane receptor	Paediatric cancer Lung Cancer
Chaetomugilin I	Vitamin D3 Receptor [1DB1]	Nuclear Receptor	Ovarian Cancer Breast Cancer Colorectal Cancer
Aspergiolide B	Glutathione requiring Prostaglandin D synthase [1V40]	Enzyme	Intestinal tumors

Table3: Some of the important target class and potential target for cancer

[IV] DISCUSSION

In recent decades, much effort has been directed toward using natural products as a source of novel anticancer drugs. Recent reviews of drug discovery literature have shown that more than two thirds of the anticancer drugs approved between the 1940s and 2006 are either natural products or developed based on the knowledge gained from natural products [9 & 10]. In recent years, marine microorganisms have attracted great attention in the pharmaceutical community as they produce a wide variety of metabolites that are structurally unique and pharmacologically active [11 & 12]. Fungal secondary metabolites play a major role within the large group of natural products originating from marine microorganisms as they are a source of plentiful structurally unique and biologically active secondary metabolites [13]. In this study we have utilized bioinformatics analysis to reveal the molecular targets of marine fungal secondary metabolites.

[V] CONCLUSION:

Cancer is the second leading cause of death worldwide. In 2008, around 12.4 million new cancer cases had been diagnosed worldwide and 7.6 million people died of cancer that year. This accounted for 12.5% of all deaths. Most cancer treatments rely heavily on chemotherapy; however, chemotherapy has limitations. Chemotherapeutic drugs lack selectivity meaning they can also kill normal cells. They might cause multidrug resistance (MDR) as well. Therefore, the development of novel chemotherapeutic agents would play a key role in the treatment of refractory or relapsing cancer patients. Nowadays, the chemical, biological and ecological diversity of the marine ecosystem has contributed immensely potent antitumor compounds. In this study we try to screen the secondary metabolites of marine fungi are potential leads to evolve novel anti cancer drugs and they are found by PharmMapper screening to

target many of the experimentally validated targets relating to various cancers.

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