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IN-SILICO STUDIES ON CYANOBACTERIAL METABOLITES AGAINST LUNG CANCER EGFR PROTEIN

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ABSTRACT

Cyanobacteria are one of the most promising groups of organisms for isolation of novel and biochemically active natural products. The strain of cyanobacterial metabolites were extracted from effluent derived *Oscillatoria terebriformis* and cultivated in the laboratory condition at Algal physiology and biotechnology, Department of Plant Biology and Plant Biotechnology through improvised CFTRI medium. The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work we have taken the Human epidermal growth factor receptor (EGFR) and identified the drugs that were used against Lung Cancer. When the receptor (1MOX) was docked with compounds such as phytol and the energy value obtained to study on cyanobacterial metabolites against LUNG CANCER EGFR and to identify potential drugs without clinical trial.

1. INTRODUCTION

Lung cancer, the most common cause of cancer-related death in men and women, is responsible for 1.3 million deaths worldwide annually, as of 2004 (WHO, 2006). The most common (including coughing up blood), and weight loss (Minna *et al* 2008). An estimated 219,440 new cases of lung cancer are expected in 2009, accounting for about 15% of cancer diagnoses. The incidence rate is declining significantly in men, from a high of 102.1cases per 100,000 in 1984 to 73.2 in 2005. In Women, the rate is approaching a plateau after along period of increase. Lung cancer is classified clinically as small cell (14%) or non-small cell (85%) (Travis *et al*., 1995) for the purposes of treatment. The most common cause of lung cancer is long-term exposure to tobacco smoke. Lung cancer is a highly aggressive malignancy presenting as metastatic disease with extremely poor prognosis.

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed (Alberto Ambesi-Impiombato and Diego di Bernardo, 2006). Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds (Daniel lednicer, 2008). One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor (Alberto Ambesi-Impiombato and Diego di Bernardo, 2006.). Docking is the process by which two molecules fit together in 3D space. Hence a study was planned to evaluate the interaction of the selected ligand with a target protein of lung cancer. Therefore, in the present study, an attempt was made to isolate metabolites from *Oscillatoria terebriformis*, a thermophilic cyanobacterium and to analyze them against the Lung cancer by using molecular docking.

2. MATERIALS AND METHODS

2.1 Cyanobacterial Culture

Oscillatoria terebriformis, a thermophilic cyanobacterium were obtained from the culture collection of Algal physiology and biotechnology, Chennai (isolated from effluent). Biomass was obtained by growing algal cultures in 20L of water and 0.25g /L of NPK fertilizer was added with a facility to pump the culture with aeration pump. The algae was grown for 20 days and harvested and then the biomass was harvested for the extraction of intracellular metabolites.

2.2 Extraction of Intracellular Metabolites: The Cyanobacterial biomass was homogenized with 100% methanol, filtered through Whatman No.1 filter paper and the filtrate was then dried under vacuum at 40^{o}C. This extract was subjected for GC-MS analysis.

2.3 Gas chromatography-Mass spectroscopy: GC-MS-QP 2010 [SHIMADZU] was used for the analysis of the intracellular compounds present in the cyanobacterial extract by using HP-5 capillary column. 1 µl of the extract was injected in the injection port with the temperature of 240°C and helium as the carrier gas. Compounds were identified by matching with known compounds in library of the instrument.

2.4 Retrieval of Protein Structure. The 3-D crystal structure of the targeted Lung cancer Epidermal Growth Factor Receptor Protein **(**ID: 1 MOX) was retrieved from the protein data bank (PDB) (www.rcsb.org/pdb). Structural and active site studies of the protein were done by using CASTP (Computed Atlas of Surface Topography of Proteins) and pymol molecular visualization software.

2.5 Compounds Screened: Five compounds namely Phytol, 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester, METHYL (7E)-7-HEXADECENOATE, 9,12-OCTADECADIENOIC ACID (Z,Z)-, HEPTADECANOIC ACID, METHYL ESTER isolated from *Oscillatoria terebriformis* were screened against the protein EGFR. The pubchem database was used for retrieving the structure of the ligand molecules.

The selected chemical structures were generated from the software (open babel). The molecular docking was performed using autodock a widely distributed public domain of molecular docking software. The inhibitor and target protein were geometrically optimized and docked using PyRx.

2.6 Docking Methods: Virtual-screening is an emerging approach and is extensively used to reduce cost, and time in drug discovery. PyRx is virtual screening software for Computational Drug Discovery (CDD), which can be used to screen libraries of compounds against potential drug targets. It uses a large body of already established open source software such as Auto Dock 4 and AutoDock Vina. These two are used as docking software.

2.7 Active Site Prediction. Active site of the target protein was predicted by using "Active site prediction tool" from SCFBio Server (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp) which requires a. pdb file as an input and this tool explains the total number of active sites along with information on their amino acid sequence, cavity points, and the average volume of the cavity.

2.8 Ligand Binding Sites Prediction: After docking the docked structure was saved as ".pdb" file and further explored to predict the binding sites using "Ligand Explorer" software. The predicted binding sites, based on the binding energy, and amino acids make up the binding cavity. Here ligand binding site represents the site where the ligands most efficiently bind with the protein, among all the active sites.

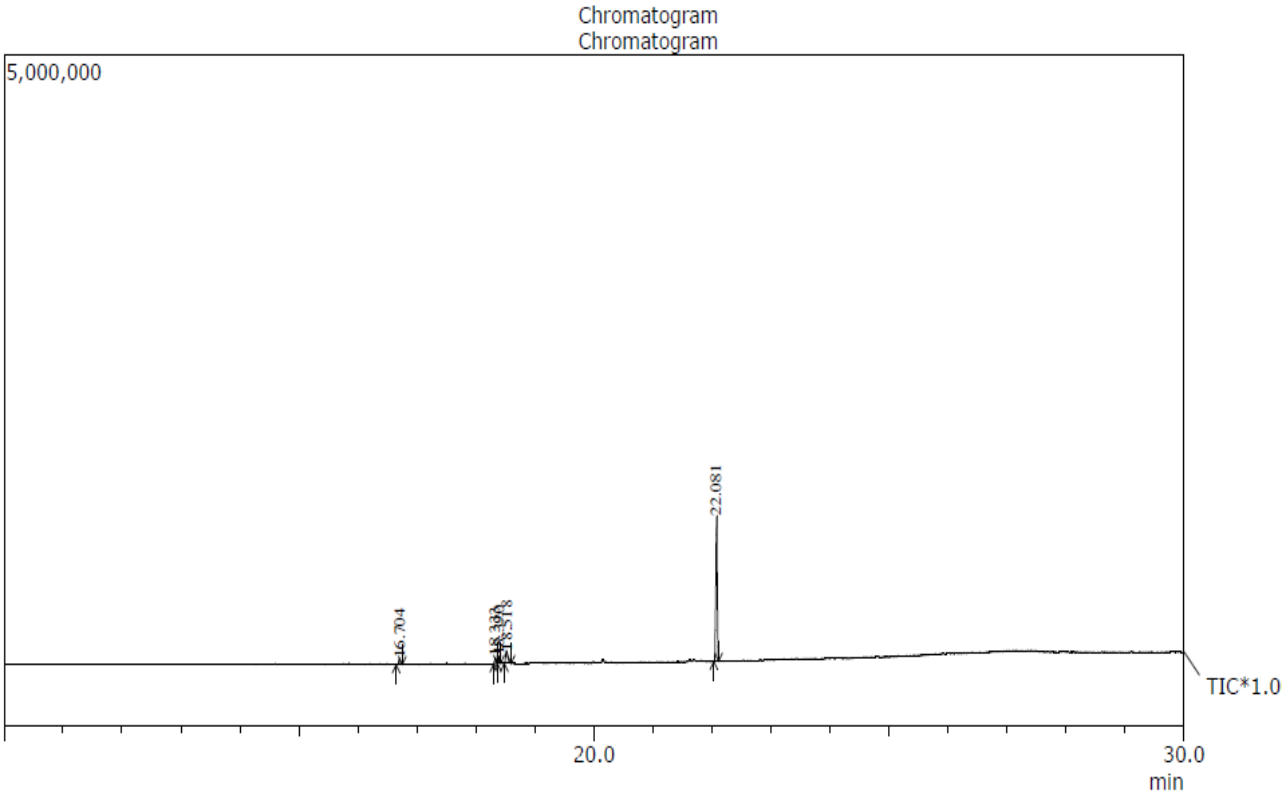
2.9 Drug Likeliness Prediction: Ligand property was predicted by using "Lipinski Drug Filters" (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp). Lipinski rule of five helps in distinguishing drug-like and non-drug-like properties and predicts high probability of success or failure due to drug likeliness for molecules. The Lipsinki filter helps in early preclinical assessment and thereby avoiding costly late-stage preclinical and clinical failures.

3. RESULTS

Cyanobacterial metabolites were extracted from *Oscillatoria terebriformis* and subjected to GC-MS analysis, as shown in Figure:1. Cyanobacterial metabolites were extracted from effluent derived *Oscillatoria terebriformis* subjected to GC-MS analysis, as shown in Figure 1. It revealed the presence of 5 metabolites with retention time ranging from 9.25 to 35.10. The maximum peak was shown by 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (79.42$^{\%}$) followed by Phytol (11.37$^{\%}$), Methyl (7E)-7-hexadecenoate (3.14%) and 9, 12-Octadecadienoic acid (2.95$^{\%}$). All the 5 isolated compounds were screened in docking analysis against the LUNG cancer protein (EGFR). Of which, only two compounds namely pthalic acid and Phytol displayed good docking scores (Table 2).

FIGURE 1

GC-MS ANALYSIS OF OSCILLATORIA TEREBRIFORMIS EXTRACT.



Peak Report TIC

PEAK#	R.TIME	AREA	AREA%	NAME
1	16.704	77448	3.12	HEPTADECANOIC ACID, METHYL ESTER
2	18.333	73068	2.95	9,12-OCTADECADIENOIC ACID (Z,Z)-
3	18.390	78011	3.14	METHYL (7E)-7-HEXADECENOATE
4	18.518	282033	11.37	Phytol
5	22.081	1970169	79.42	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
		2480729	100.00	

TABLE 1

RESULTS OF LIPINSKI DRUG FILTERS OF OSCILLATORIA TEREBRIFORMIS COMPOUNDS

Compound Name	LogP	Molecular Weight(g/mol)	Hydrogen Donor/acceptor	Molar Refractivity
Heptadecanoic Acid, Methyl Ester	8.5	284.4772	0,2	87.21
9,12-Octadecadienoic Acid (Z,Z)	4.848	280.00	1.1	86.856
Methyl (7E)-7-Hexadecenoate	5.420	268.00	0,2	82.234
Phytol	4.3658	296.00	1,1	95.561
1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester.	2.725	278.0	1.3	76.853

TABLE 2

DOCKING RESULTS OF OSCILLATORIA TEREBRIFORMIS COMPOUNDS AGAINST LUNG CANCER PROTEIN (1MOX).

S.No	Compound With Pubchem ID	Binding Site	Chemical Formula	Binding Energy (Kcal/mol).
1	Heptadecanoic Acid, Methyl Ester(CID 15609)	PHE230,ARG231	$C_{18}H_{36}O_2$	- 4.1
2	9,12-Octadecadienoic Acid (Z,Z) (CID 5460332)	SER222,ASP223	$C_{18}H_{32}O_2$	-4.2
3	Methyl (7E)-7-Hexadecenoate (CID 5364431)	LEU225,VAL226	$C_{17}H_{32}O_2$	- 4.3
4	Phytol (CID 5280435)	Glu 319,LYS 321	$C_{20}H_{40}O$	-6.4
5	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester**(**CAS Registry Number**:** 4376-20-9)	ASN 33	$C_{16}H_{22}O_4$	-5.9
6	Gemcitabine (potent drug)(CID 60750)	ARG 231, CYS 224	$C_9H_{11}F_2N_3O_4$	-5.8

FIGURE 2

AMINO ACIDS IN THE BINDING POCKET (THR 360, ASN331, SER 324, ASP355, LEU321, VAL 350) RCSB LIGAND EXPLORER

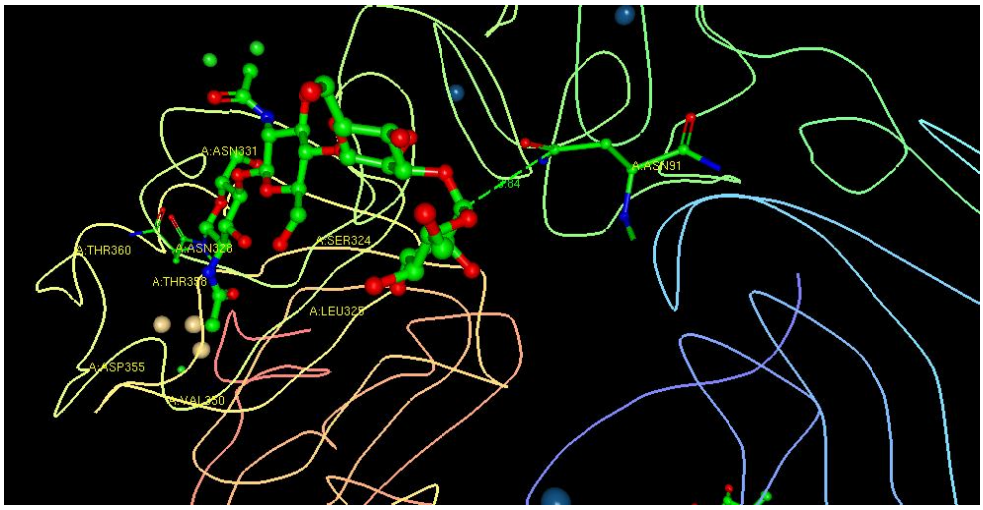
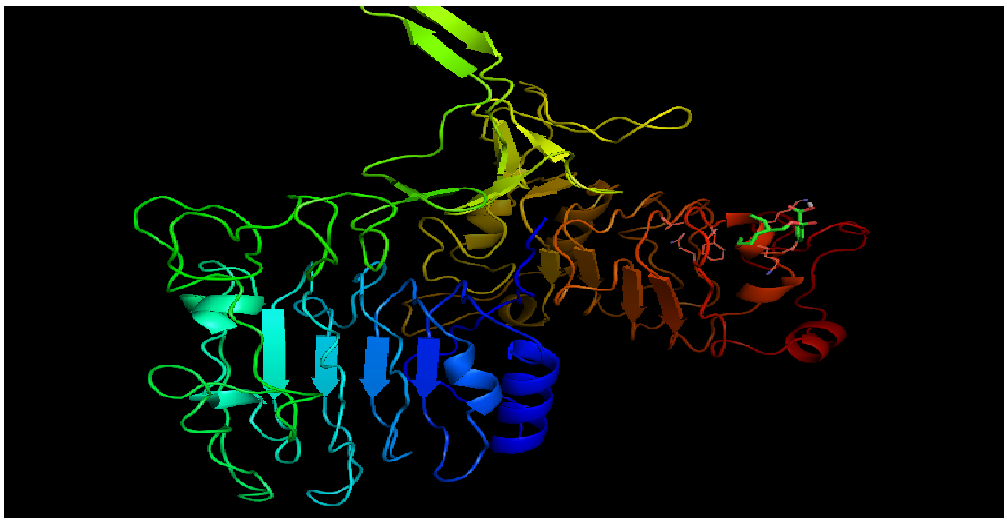


FIGURE 3

PROTEIN -LIGAND INTERACTION OF PHYTOL AND EGFR (PYMOL SOFTWARE



The biology of epidermal growth factor receptor (EGFR) suggests its potential as a target for anti-cancer therapy. Biologic therapy targeted at aberrant pathways that are unique to, or dysregulated in, tumor cells is expected to be more selective and less toxic than conventional cytotoxic therapy. Activation of EGFR by epidermal growth factor (EGF) and other ligands (amphiregulin) which bind to its extracellular domain is the first step in a series of complex signaling pathways which take the message to proliferate from the cell membrane to the genetic material deep within the cell nucleus (Bacon *et al.,* 1992).

Totally 40 active sites were predicted in the target protein by the "Active site prediction tool". PyRx is virtual screening software was used to dock fatty acid compounds against the lung cancer protein (Human Epidermal Growth Factor Receptor, PDB ID: 1 MOX**)**. The docking interaction of the protein and ligand, and the predicted ligand binding site residues are shown in Figures 2 and 3 respectively. The lowest docking score was shown by Phytol followed by 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester having the value of – 6.4 and - 5.9 kcal/mol respectively.

Commercial drug, Gemcitabine (Mini *et al.,* 2006), exhibited the docking score of – 5.8 kcal/mol as against kcal/mol for – 6.4 derived Phytol (Table 2). Higher the minus value of docking score, better would be the binding activity of the chemical ligand with the protein. Thus the docking result showed that the cyanobacterial metabolites had the high specificity and efficiency towards the target protein. However, further in vitro and in vivo experiments are needed to demonstrate the effectiveness of the cyanobacterial metabolites for inhibition of lung cancer.

4. DISCUSSION

Cyanobacteria are a prolific source of nearly 800 diverse bioactive secondary metabolites, originating mainly from nonribosomal peptide synthetase (NRPS) or mixed polyketide synthase (PKS)–NRPS biosynthesis (Welker *et al*., 2006, Tan et al 2007).Their role as antiviral, anti-tumour, antibacterial, anti-HIV and a food additive have been well established. *Oscillatoria* spp. can produce fatty acids, tetraamine, spermine and piperazine derivatives which show antimicrobial activity (Mundt *et al.,* 2003). Epidermal growth factor (EGF) is a small mitogenic protein that is thought to be involved in mechanisms such as normal cell growth, oncogenesis, and wound healing. EGF is a small 53 amino acid residue long protein that contains three disulfide bridges (Rosario García Campelo *et al.,* 2014). Recently, various pharmacological effects of Phytol have been reported. In particular, α-tocopherol, which is structurally related to phytol, has been shown to inhibit smooth muscle cell proliferation(Boscoboinik, *et al.,* 1991) Moreover, phytol can contribute to the prevention of cancer by augmenting immunological responses against tumor cells in early stages of carcinogenesis. Thus, vitamin E and related compounds have generally been regarded as inhibitors of cancer (Tomita *et al*., 1983). In addition, phytol has shown have demonstrated an anti-inflammatory activity effect (Shimizu *et al*., 1994). It was observed using Pymol that the Glycine/Lysine and tyrosine protein kinases present in the drug was the site of binding to the receptor (1MOX) and methyl group present in the probable functional groups, which resulted in a decrease in the energy values. If the drug action and pathogenesis of different types of neoplasm are clearly known, it is easier to design of newer

drugs which selectively target the tumour with no or reduced side effects. However, the exact biology of Lung cancer remains largely unclear and this offers scope for research to develop novel compounds to target the malignant cells.

The structures of lung cancer protein have been documented in recent years. Based on the literature it has been shown clearly that the secondary metabolites have been used to target the Human epidermal growth factor receptor.

CONCLUSION

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work we have taken the Human epidermal growth factor receptor (EGFR) and identified the drugs that were used against Lung Cancer. When the receptor (1MOX) was docked with compounds such as phytol and the energy value obtained. In future research work can be used further in clinical trials to test it effectiveness and for social benefit thus reducing the time and cost in drug discovery process.

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