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Research Article

IN SILICO DOCKING ANALYSIS OF BIOACTIVE COMPOUNDS FROM *CALOPHYLLUM INOPHYLLUM* L. ETHANOL LEAF EXTRACT AGAINST EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) PROTEIN

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ABSTRACT

Objective: The objective of this study was to evaluate the effective new phytocomponents from *Calophyllum inophyllum* ethanol leaf extract against breast cancer target protein of epidermal growth factor receptor (EGFR) using *in silico* docking studies.

Methods: The identification of compounds was done by gas chromatography-mass spectrometry (GC-MS) analysis. The *in silico* docking studies were carried out using Discovery Studio 4.0 software.

Results: The GC-MS analysis of ethanol leaf extract revealed the presence of eleven compounds. The docking analysis has exhibited moderate to potent inhibition with a range of dock score 3-55. 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-compound showed the dock score of 55.427.

Conclusion: The results revealed out that the compounds present in *C. inophyllum* can inhibit the EGFR protein. The plant possesses anticancer potential because of the various bioactive compounds presence which is mainly responsible for anticancer activity.

Keywords: *Calophyllum inophyllum*, Gas chromatography-mass spectrometry analysis, *In silico*, Epidermal growth factor receptor protein, Discovery Studio.

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INTRODUCTION

The epidermal growth factor (EGF) is the prototype of a large family of peptide ligands that bind to cell membrane receptors and activate a myriad of intracellular signaling pathways to control tumor cell growth, proliferation, survival, metastasis, and angiogenesis [1]. The EGF receptor (epidermal growth factor receptor [EGFR], ErbB1, or HER1) is one of a four member family of transmembrane receptors that, similar to HER2, are frequently overexpressed in cancer cells, correlating with poor prognosis [2]. EGFR therefore presents a rational target for the development of novel anticancer therapies. The best-known agents targeting EGFR is the most advanced clinical development include cetuximab, gefitinib, and erlotinib [3]. Unfortunately, currently, clinical results are disappointing due to several mutations on the EGFR kinase domain that is associated with a number of human cancer and brain tumors. Hence, there is a need for the development of novel EGFR inhibitors. It is reported that natural products derived from medicinal herbs, food sources, and marine organism are able to inhibit EGFR signaling [4].

Calophyllum inophyllum L. belongs to the family of Clusiaceae and is commonly known as "Punnai" in Tamil. It is a tree that can grow 8-20 m tall with a broad spreading crown of irregular branches, which exudes white latex when bruised. The leaves have opposite arrangements and are petiolate, thick, and shiny with numerous parallel secondary veins. Its flower arranged in axillary cymes and has a sweet, lime-like fragrance [5]. *C. inophyllum L.* ethanol leaf extract showed potent anticancer activity against MCF-7 breast cancer cell line [6]. *C. inophyllum* leaves extract showed more anti-inflammatory activity compared with *C. inophyllum* stem bark extract [7]. The (+) - calanolide A and inophyllum B isolated from *Calophyllum lanigerum* Miq. and *C inophyllum L*. showed strong activity against human immunodeficiency virus type 1 [8]. Comparative analgesic studies of leaf and stem bark of *C. inophyllum* in Swiss albino mice using acute oral toxicity assay and the results suggested that leaf extract showed more activity compared with stem bark extract [9].

In silico technique is an inexpensive technique that shortens the length of time spending in testing the efficacy drugs. Hence, the present study focused on the identification of bioactive compounds present in *C. inophyllum* ethanol leaf extract through gas chromatography-mass spectrometry (GC-MS) analysis and to screen the potential bioactive compounds as an anticancer agent by molecular docking analysis studies against EGFR protein.

MATERIALS AND METHODS

Collection of plant material

The leaves of *C. inophyllum* were collected from Ramakrishna Mission Vivekananda College Campus, Chennai, Tamil Nadu, India. The plant material was identified and authenticated by Botanical Survey of India (BSI) with ref no. BSI/SRC/5/23/2016/Tech/386.

Preparation of leaf extract

The fresh leaves were collected and washed with running tap water, chopped into small pieces and then kept in the shade dry for 30 days and then grounded using electric blender. About 50 g of powdered leaves were extracted with 300 ml of ethanol in soxhlet apparatus for 6 hrs. The extract was then concentrated at reduced pressure using rotary evaporator and stored in vials at 4°C until further analysis [10].

GC-MS analysis

The composition of the chloroform extract was established by GC-MS analysis. The analysis was performed on a JEOL GCMATE II GC-MS system in EI/CI mode equipped with a split/splitless injector (220°C), at a split ratio of 1/10, using a VF-1MS fused silica capillary column (30 m×0.25 mm i.d.; film thickness: 0.25 mm). The oven

temperature was programed from 60°C (5 minutes) to 280°C at a rate of 4°C/minutes and held at the temperature for 10 minutes. Helium was used as a carrier gas at a flow rate of 0.8 ml/minute. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. The spectrums of the components were compared with the database of known spectrum components stored in the NIST library [11].

In silico docking analysis

Generation of ligand

Structures of compounds present in *C. inophyllum* ethanol leaf extract and were attained from PUBCHEM compounds database. The same was used to predict properties of ligand such as hydrogen donors, acceptors, logP value, refractivity, pH, and molecular weight (MW). These compounds were screened for drug-likeliness according to Lipinski's rule of five [12].

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) and Lipinski's rule of five

The 2D structures were subject to ADMET analysis for solubility, intestinal absorption, hepatotoxicity, plasma protein binding ability, blood-brain barrier penetration, cytochrome P450 inhibition, and AMES mutagenicity using Discovery Studio 4.0 [13,14].

Preparation of receptor

The crystal structure of EGFR protein was retrieved from Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB ID: 1IVO) (http://www.rcsb.org/pdb/home/home.do) [15,16].

Docking analysis

Docking is virtual screening of a database of compounds (ligands) and predicting the strongest binders based on various scoring functions. Accelrys Discovery Studio 4.0 [17] was used for docking. Before docking, the ligands were prepared using the "prepare ligand" module. Receptor-ligand interactions were further optimized by molecular dynamics and clean geometry of Discovery Studio. Force field is applied in Discovery Studio and is energies and forces of each particle of the system and also defines the positional relationship between atoms that determine their energy. The docking scores, internal energy of ligands, and potential mean force (PMF) values are estimated. Root-mean-square distance (RMSD) between the docked structure and the original conformation of the inhibitor in each complex was calculated.

RESULTS

GC-MS analysis

The GC-MS results showed presence of eleven bioactive compounds in ethanol leaf extract of *C. inophyllum*. The identification of the compounds was confirmed based on the peak area, retention time (RT), and molecular formula. The active principle with their RT, molecular formula, MW, and peak area in percentage are presented in Fig. 1 and Table 1.

In silico docking analysis

Lipinski properties

The drug-likeness score of the compounds of *C. inophyllum* ethanol leaf extract was tested within the help of Lipinski's rule of five. The physicochemical properties of the compounds obtained from *C. inophyllum* ethanol leaf extracts that accept the Lipinski's rule of five, as tabulated in Table 2. Six compounds (ligands) showed drug-likeness properties and other five failed in Lipinski's rule of five.

The ligands were subjected to predict ADMET properties using the Discovery Studio client, the toxicity prediction module of the software. The predicted ADMET properties are tabulated in Table 3 and Fig 2. All the compounds exhibited non-mutagenicity as predicted by TOPKAT AMES mutagenicity. The solubility levels showed in between 1 and 3 in all compounds.

The six compounds passed in Lipinski's rule of five and ADMET property and their structures were retrieved from PUBCHEM and CHEMSPIDER database was shown in Fig. 3a-f.

The target EGFR protein structure was retrieved from PDB (PDB ID: 1IVO) with 34 active sites predicted in Discovery Studio 4.0 (Fig. 4).

Docking analysis

Docking with Discovery Studio showed the top two drug candidates namely 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1 and Benzo(b,t)-1,2,4-triazolo(4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl. The ligand internal energies and dock score of the candidates are shown in Table 4. The docking results showed that all the ligands with high internal energies, namely, 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1, and Benzo(b,t)-1,2,4-triazolo(4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl occupied the active site of EGFR protein and ligand internal energy is 2.19 and −4.173 Kcal/mol with a dock score of 55.427 and 50.325, respectively (Fig. 5a-d). The standard anticancer drug 5-fluorouracil showed dock score of 50.565 with −0.187 ligand internal energy.

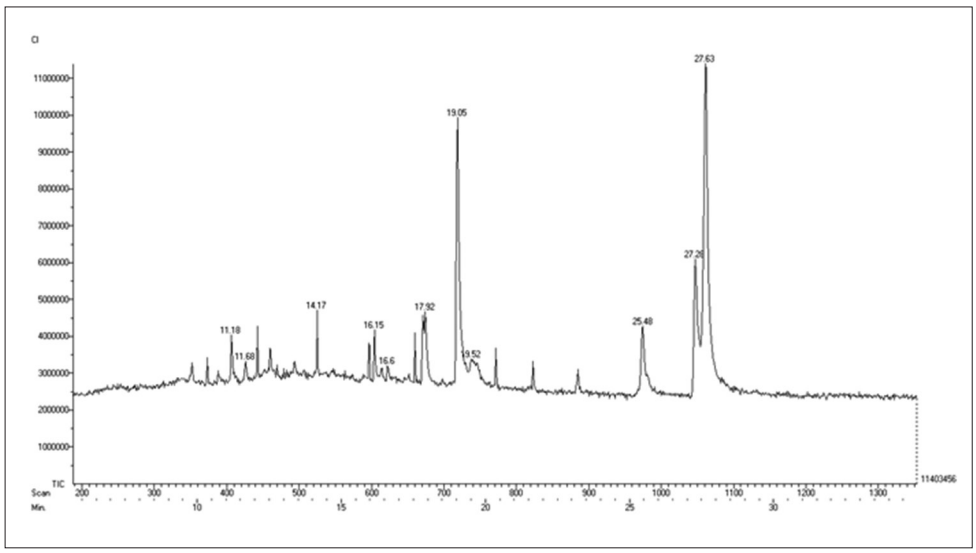

Fig. 1: Gas chromatography-mass spectrometry of ethanol leaf extract of *Calophyllum inophyllum*

Table 1: GC-MS profile of ethanol leaf extract of *Calophyllum inophyllum*

S. No.	RT	Name of the compound	PUBCHEM or CHEMSPIDER ID	Molecular weight	Molecular formulae	Peak area %
1	11.18	Caryophyllene	5322111	204.35	$C_{15}H_{24}$	5.52
2	11.68	Z, Z, Z-1,4,6,9-nonadecatetraene	5362676	260.45	$C_{19}H_{32}$	2.97
3	12.53	1,4-Methanoazalen-3-ol, decahydro-1, 5,5,8a-tetramethyl 15-(1a, 3a, 3aa, 4a, 8aa)	6432447	222.36	$C_{15}H_{26}O$	4.02
4	15.95	Z, E-2-Methyl-3, 13, Octadecadein-1-ol	5364521	280.48	$C_{19}H_{36}O$	3.95
5	16.60	E, E, Z-1,3,12-Nonadecatriene-5,14-diol	5364768	294.47	$C_{19}H_{34}O_2$	0.51
6	17.83	Hexadecanoic acid, ethyl ester	12366	284.47	$C_{18}H_{36}O_2$	13.59
7	19.10	Phytol	5280435	296.53	$C_{20}H_{40}O$	35.45
8	19.52	Dasycarpidan-1-methanol, acetate (ester)	550072	326.43	$C_{20}H_{26}N_2O_2$	1.41
9	25.48	Benz(b, t)-1,2,4-triazolo(4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl	CHEM SPIDER ID 549034	361.55	$C_{22}H_{11}N_5O$	11.48
10	27.28	2H-Benzo(cd)pyrene-2,6(1, H)-dione, 3,5,7,10-tetrahydroxy-1	5282060	376.35	$C_{22}H_{16}O_6$	15.95
11	27.63	n-Heptane, 1,1-bi (4,4-dimethylhexan-2,6-dione-1-yl)	CHEM SPIDER ID 309203	376.53	$C_{23}H_{36}O_4$	51.20

GC-MS: Gas chromatography-mass spectrometry, RT: Retention time

Table 2: Physicochemical properties of ligands accepting the Lipinski's rule of five

S. No.	Compounds name	Molecular weight <500 daltons	H-bond donor	H-bond acceptor	logP<5
1	Caryophyllene	204.35	0	0	4.75
2	Z, Z, Z-1,4,6,9-nonadecatetraene	260.48	0	0	7.31
3	1,4-Methanoazalen-3-ol, decahydro-1, 5,5,8a-tetramethyl 15-(1a, 3a, 3aa, 4a, 8aa)	222.36	1	1	3.29
4	Z, E-2-Methyl-3, 13, Octadecadein-1-ol	280.48	1	1	6.78
5	E, E, Z-1,3,12-Nonadecatriene-5,14-diol	294.47	2	2	5.55
6	Hexadecanoic acid, ethyl ester	284.47	0	2	6.96
7	Phytol	296.53	1	1	7.33
8	Dasycarpidan-1-methanol, acetate (ester)	326.43	1	4	3.44
9	Benzo (b, t)-1,2,4-triazolo (4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl	361.55	0	6	4.31
10	2H-Benzo(cd)pyrene-2,6(1, H)-dione, 3,5,7,10-tetrahydroxy-1	376.35	4	6	3.83
11	n-Heptane, 1,1-bi(4,4-dimethylhexan-2,6-dione-1-yl)	376.53	0	4	4.17

Table 3: ADMET profile for the test ligands from *Calophyllum inophyllum*

S. No.	Compound name	Solubility level	BBB level	Hepatotoxicity prediction	CYP2D6	PPB prediction	AMES mutagenicity
1	Caryophyllene	2	0	False	False	True	NM
2	Z, Z, Z-1,4,6,9-nonadecatetraene	1	4	False	True	True	NM
3	1,4-Methanoazalen-3-ol, decahydro-1, 5,5,8a-tetramethyl 15-(1a, 3a, 3aa, 4a, 8aa)	2	1	True	False	True	NM
4	Z, E-2-Methyl-3, 13, Octadecadein-1-ol	2	0	False	True	True	NM
5	E, E, Z-1,3,12-Nonadecatriene-5,14-diol	3	0	False	False	True	NM
6	Hexadecanoic acid, ethyl ester	2	0	False	False	True	NM
7	Phytol	2	4	False	False	True	NM
8	Dasycarpidan-1-methanol, acetate (ester)	2	1	True	True	True	NM
9	Benzo(b, t)-1,2,4-triazolo(4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl	1	2	True	False	True	NM
10	2H-Benzo(cd)pyrene-2,6(1, H)-dione, 3,5,7,10-tetrahydroxy-1	2	0	True	False	True	NM
11	n-Heptane, 1,1-bi (4,4-dimethylhexan-2,6-dione-1-yl)	2	0	False	False	True	NM

BBB: Blood-brain barrier, PPB: Plasma protein binding, NM: Non-mutagen, ADMET: Absorption, distribution, metabolism, excretion, and toxicity

The binding mode of the ligands within the active site of EGFR protein was analyzed using Discovery Studio 4.0. It gives 2D visualization of the drug and receptor. The binding interaction of 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1 (Fig. 5a) with the EGFR, with LYS A:455, LEU A:429, and ARG A:509 was noted and the binding interaction of Benzo(b,t)-1,2,4-triazolo(4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl with the EGFR, with THR A:249, ASN B:86, and LYS B:4 was noted. The binding interaction of 5-fluorouracil (Table and Fig. 6a-d) with the EGFR, with GLU A:133 and HIS A:159 was noted (Table 5).

DISCUSSION

Malarvizhi *et al.* 2011 reported of *C. inophyllum L.* leaf extract using alcoholic solvents by yielded seventeen compounds [18]. By comparing the earlier reports, two compounds are similar, namely, phytol and hexadecanoic acid, whereas other nine compounds were different. The difference in plant components from previous study might arise from different extraction procedure, whereas the present study of ethanol leaf extract exhibited important bioactive compounds which possess various biological activities.

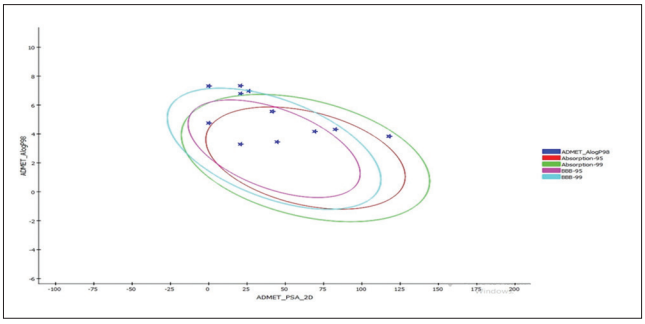

Fig. 2: Prediction of absorption, distribution, metabolism, excretion, and toxicity properties of compounds (X axis indicates the solubility of the compounds; Y axis indicates the logP values)

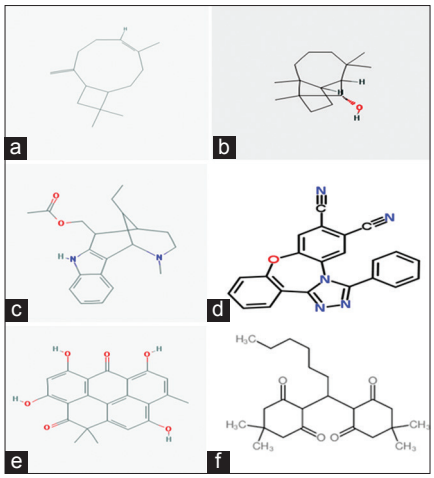

Fig. 3: (a-f) Chemical structures of ligands. (a) Caryophyllene, (b) 1,4-Methanoazalen-3-ol, decahydro-1, 5,5,8a-tetramethyl 15-(1a,3a,3aa,4a,8aa), (c) Dasycarpidan-1-methanol,acetate(ester), (d) Benzo(b,t)-1,2,4-triazolo(4,3, d)=1,4-oxazepine-6, 7-dicarbonitrile, 3-phenyl, (e) 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1, (f) n-Heptane, 1,1-bi(4,4-dimethylhexan-2,6-dione-1-yl)

Molecular docking discovers the binding geometry of two interacting molecules with known structures. It predicts the preferred orientation of receptor and ligand to each other to form a stable complex [19,20]. Currently, the use of computers to determine the binding of datasets of small molecules to known receptors is a major component of drug discovery. Rule of five evaluates certain pharmacological, biological, and ADME properties of the ligand [12,13]. The compound that passed Lipinski's rule of five to be further pursued as a potential drug because it would likely lack properties essential in its ADME. The only six compounds (e.g. 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1 and n-Heptane, 1,1-bi(4,4-dimethylhexan-2,6-dione-1-yl)) had drug-like properties. The solubility levels of the compounds were in the range 1-3, indicating good solubility. Toxicity profile of the designed derivatives was predicted using TOPKAT-like AMES mutagenicity for ligands. The results showed all the ligands were non-mutagen in AMES test [21]. The PMF values developed based on statistical analysis of the 3D structures of protein-ligand complexes, scores are calculating by summing pairwise interaction terms overall interatomic pairs of the receptor-ligand complex, a higher score indicates a stronger receptor-ligand binding affinity [22]. If the RMSD of the docked pose is less than or equal to 1.0Å from the experimentally observed conformation, the prediction is regarded to be successful. All the binding ligands the RMSD values is zero, hence the receptor-ligand interaction to be strong binding.

This study showed that *C. inophyllum* plant contains a few compounds that are capable of binding to and inhibiting the EGFR protein and

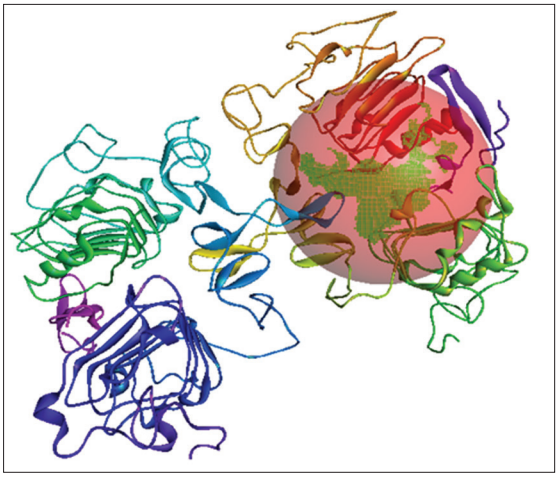

Fig. 4: Epidermal growth factor receptor Protein Data Bank ID: 1IVO with receptor cavity (total active sites: 34)

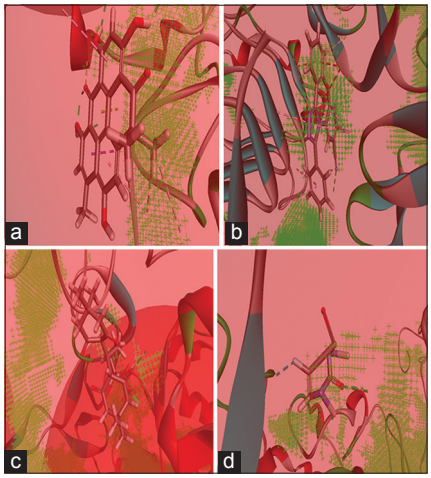

Fig. 5: Interaction of epidermal growth factor receptor (EGFR) with ligands (3D View). (a) Interaction of EGFR with 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1, (b) Interaction of EGFR with Benzo(b,t)-1,2,4-triazolo(4,3, d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl, (c) Interaction of EGFR with n-Heptane, 1,1-bi (4,4-dimethylhexan-2,6-dione-1-yl), (d) Interaction of EDFR with standard drug (5-fluorouracil)

thereby preventing cell proliferation in an uncontrolled manner. From the ADMET studies, the 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1 compound is a better drug candidate and showed the least adverse effects. Hence, 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1 could prove to be a probable anticancer drug. Mukund *et al.*, (2014) reported the GC-MS compounds, namely, Bis(2-ethyl hexylpthalate), Hexadecenoic acid methyl ester, and Phytol showed -7.9 Kcal/mol, -5.3 Kcal/mol, and -5.2 Kcal/mol binding energy against EGFR protein, respectively [23]. In the present study, the 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1 compound showed the highest dock value of 55.427, but the compounds such as n-Heptane, 1,1-bi(4,4-dimethylhexan-2,6-dione-1-yl) and Dasycarpidan-1-methanol,acetate(ester) showed lowest dock values of 37.076 and 3.165, respectively.

CONCLUSION

However, this molecular docking study is only one way of predicting the activity of the molecules involved. Therefore, *in vitro* and *in vivo* studies need to be performed on animal models to confirm the anticancerous activity of these compounds. The role of some important amino acids involved in the appropriate binding of inhibitors with the active site

Table 4: Dock score and ligand internal energy of docked ligands using Discovery Studio

S. No.	Compound name	Binding site	PMF	Ligand internal energy	Dock score	RMSD (Å)
1	Caryophyllene	-	-	-	-	-
2	1,4-Methanoazalen-3-ol, decahydro-1, 5,5,8a-tetramethyl 15-(1a, 3a, 3aa, 4a, 8aa)	-	-	-	-	-
3	Dasycarpidan-1-methanol,acetate (ester)	6	13.51	39.078	3.165	0.00
4	Benzo(b, t)-1,2,4-triazolo(4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl	5	107.06	−4.173	50.325	0.00
5	2H-Benzo(cd)pyrene-2,6(1, H)-dione, 3,5,7,10-tetrahydroxy-1	6	31.33	2.19	55.427	0.00
6	n-Heptane, 1,1-bi(4,4-dimethylhexan-2,6-dione-1-yl)	13	61.75	8.557	37.076	0.00
7	Standard drug (5-fluorouracil)	23	27.26	−0.187	50.565	0.00

PMF: Potential mean force, RMSD: Root-mean-square distance

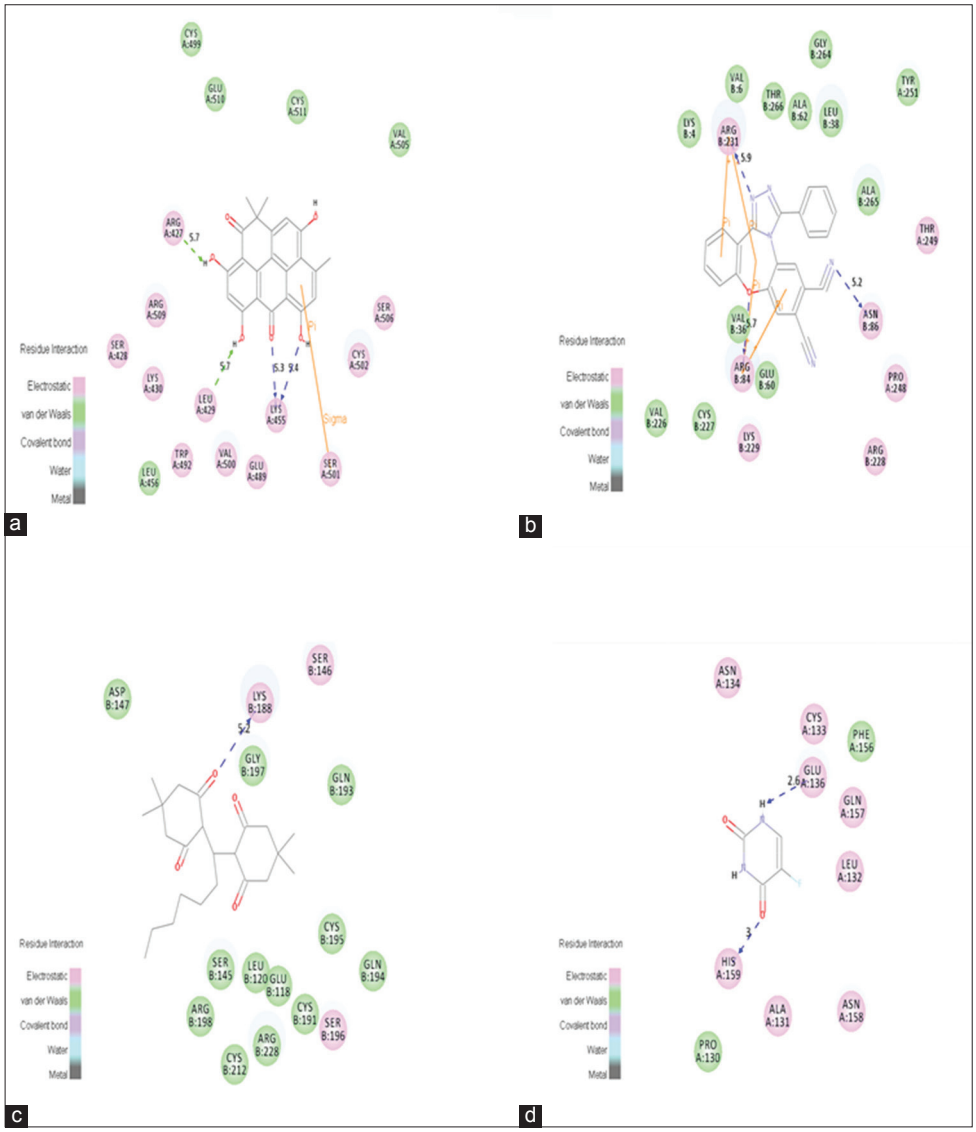

Fig. 6: Interaction of ligands with amino acid residues (a). 2H-Benzo(cd)pyrene-2,6(1,H)-dione, 3,5,7,10-tetrahydroxy-1 (b). Benzo(b,t)-1,2,4-triazolo(4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl (c). n-Heptane, 1,1-bi(4,4-dimethylhexan-2,6-dione-1-yl) (d). Standard drug (5-fluorouracil)

of EGFR protein can be helpful for designing better drugs to combat cancer.

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Table 5: Docking score with hydrogen bond interaction of detected binding site of EGFR protein crystal

S. No.	Ligand name	No. of hydrogen bonds	Amino acid residues involved in bonding	Distance of hydrogen bonds
1	2H-Benzo(cd)pyrene-2,6(1, H)-dione, 3,5,7,10-tetrahydroxy-1	10	A:LYS455:HZ2 - A:GLU489:OE2	2.08058
			A:LYS455:HZ1-5282060:O4	1.62628
			A:LYS455:HZ1-5282060:O6	1.8377
			A:SER501:HN - A:GLU489:O	2.86753
			5282060:H42 - A:ARG427:O	1.86596
			5282060:H43 - A:LEU429:O	2.4384
			5282060:H44 - A:GLU489:OE2	3.01105
			A:LYS430:HE1 - A:LYS430:O	2.63563
			A:LYS455:HE1-5282060:O4	2.87218
			A:SER501:HB1 - A:GLU489:OE2	1.85579
2	Benzo(b, t)-1,2,4-triazolo(4,3,-d)=1,4-Oxazepine-6, 7-dicarbonitrile, 3-phenyl	3	A:THR249:HG1 - C22 H11 N5 O: N15	2.89079
			B:ASN86:HD22 - C22 H11 N5 O: N15	1.9734
			B:LYS4:HE1 - C22 H11 N5 O: N3	2.53636
3	n-Heptane, 1,1-bi(4,4-dimethylhexan-2,6-dione-1-yl)	2	B:LYS188:HZ1 - C23 H36 O4:O4	2.29092
			B:LYS188:HE1 - C23 H36 O4:O4	2.61881
4	Standard drug(5-fluorouracil)	7	A:ALA131:HN - 3385:O2	2.58813
			A:GLN157:HE21 - A:GLN157:O	2.12989
			A:GLN157:HE21-3385:O2	2.77627
			A:HIS159:HD1-3385:F1	2.63395
			A:HIS159:HD1-3385:O2	1.95974
			3385:H11 - A:GLU136:OE1	0.609531
			A:HIS159:HE1-3385:F1	2.40322

EGFR: Epidermal growth factor receptor

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