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Research Article

SCREENING OF ALK TARGETED ANTI-LUNG CANCER INHIBITORS: AN *IN SILICO* EXPLORATION FROM NATURAL DOMAIN

Nand, M[1]., Maiti, P[2]., Chandra, S[2] and Pande, V[1*]

[1]Department of Biotechnology, Kumaun University, Bhimtal Campus Bhimtal, Uttarakhand, India
[2]Department of Botany, Kumaun University, S.S.J Campus, Almora, Uttarakhand, India

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ABSTRACT

The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that is unusually found in a various type of sarcomas. Almost 3-7% of lung tumor is contributed by ALK fusions library of therefore, is a good target for anticancer drug development. In this pursuit, we virtually screened four hundred chemically diverse bioactive natural products to get the best hits having an ALK binding affinity. Initially, all molecules were screened for their anti-cancer efficacy and drug-likeness. The docking scores and protein-ligand interactions of the obtained hits were emulated with the clinically exploited selective ALK antagonists. The results revealed seven potential hits against ALK. Additionally, all the hits were evaluated for their ADMET properties, cell-line cytotoxicity and median lethal dose (LD_{50}). The results signify that these compounds could serve as potential leads in the drug discovery process for the treatment of ALK targeted lung cancer.

INTRODUCTION

The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that is unusually found in a various type of sarcomas. Missense mutations within ALK are found in neuroblastomas, on other hand ALK fusions are also found in anaplastic large cell lymphoma, colorectal, ovarian, and non-small cell lung cancer (Gascoyne *et al.*, 2003). All ALK fusions contain the complete ALK tyrosine kinase domain. The N-terminal fusion promotes dimerization, which interned activate downstream signaling pathways of ALK. Results intern activation of cellular pathways including cell growth and cell proliferation. Almost 3–7% of lung tumor are contributed by ALK fusions (Dai *et al.*, 2018). ALK fusions are more frequently found in light smokers (<10 years) and/or never smokers (Solomon *et al.*, 2010 and Takeuchi *et al.*, 2008). Multiple different ALK rearrangements have been described in NSCLC. The majority of these ALK fusion variants are comprised of portions of the echinoderm microtubule-associated protein like 4 (EML4) gene with the ALK gene. At least nine different EML4-ALK fusion variants have been identified in NSCLC. In addition, non-EML4 fusion partners have also been identified, including KIF5B-ALK, TFG-ALK, and EML4-ALK fusions is associated with EGFR tyrosine kinase inhibitor (TKI). Rearrangement or change in a gene named ALK which are responsible for NSCLC. This change of ALK is mostly seen in non-smokers and woman who are suffering from adenocarcinoma subtype of NSCLC. The ALK gene rearrangements form an abnormal ALK protein that causes the cells to grow and spread. Drugs that target ALK include; Crizotinib (Xalkori®), Ceritinib (Zykadia™), Alectinib (Alecensa) (DiBonaventura *et al.,* 2017). These drugs inhibit the abnormal ALK product and minimize tumors in patients. Therefore, is a good target for anticancer drug development. Natural products play a vital role in anti-cancer drug development. Irinotecan, Vincristine, Etoposide, and Paclitaxel are classic examples of plant-derived compounds; Mitomycin C, Actinomycin D, Bleomycin, Doxorubicin and l-Asparaginase are examples of reported drugs coming from microbial sources and Cytarabine is the first drug originating from a marine source. Current days, new generations of Taxanes, Anthracyclines, Vinca alkaloids, Camptothecin, along with the novel class of Epothilone have been developed. Some of these are in clinical use, others in clinical trials. Other compounds obtained from marine sources (both plants and animals) like Trabectedin-ET-743, Bryostatin-1, Neovastat have also entered into clinical trials (Nobili *et al., 2009*).

Corresponding author:* **Pande, V
Department of Biotechnology, Kumaun University, Bhimtal Campus Bhimtal, Uttarakhand, India

Even though considerable research work have been done on developing drugs against lung cancer but the increasing occurrence magnitude lung cancer reflects the necessity of conducting more research on this disease. Realizing the significance of the enormous wealth of the natural compounds and the vital necessity of discovering new anti-lung cancer drug, the present investigation aims to explore the natural compounds with anti-lung cancer property by computational techniques. The outcomes of the present work have identified several novel natural anti-lung cancer compounds with future avenues for drug development against lung cancer.

The methods that were used to screen the compounds were data mining, library construction, CDRUG, molecular docking, FAF Drug3, ADMET prediction, cell-line cytotoxicity prediction and finally a calculation of median lethal dose (LD50).

MATERIALS AND METHODS

Data mining and construction of compounds library

Generally, data mining (sometimes called data or knowledge discovery) is the process of analyzing data from different angles and summarizing it into useful information. Data mining software is one of a number of analytical tools for analyzing data. The process of data mining involves in the steps like analyze data from many different dimensions, categorize and summarize then get useful information. By comparing several literatures useful information about the sources that were reported for anti-cancer compounds were investigated (Witten *et al.,* 2011).

A library was constructed by collecting four hundred compounds from selected seven diverse resources including Plants, Fungus, Lichens, Fruits, Bacteria, Cyanobacteria, Vegetables, and Nutritional and Functional Food. The selected sources are well reported for their anti-cancer properties and traditionally known for their various pharmacological activities against several diseases.

All structures are retrieved from three databases ChemSpider (http://www.chemspider.com, Accessed on 16th September 2016) (Gaulton *et al.,* 2011), PubChem (pubchem.ncbi.nlm.nih.gov, Accessed on 16th September 2015) and Zinc database used to create the library in PDB format. All the molecules in the library were annotated by molecular weight, number of rotatable bonds, logP, number of H-bond donors, number of H-bond acceptors which are important parameters for designing potent drugs and converted into different molecular formats like .mol2, .sdf, smile etc. by using Avogadro and Open Babel software (Hanwell *et al.*, 2012 and O'Boyle *et al.*, 2011 and Liew *et al.,* 2012).

Screening of compounds based on anticancer efficacy

All molecules of in-house library were again screened for their anti-cancer efficacy using CDRUG online server. Required smiles formats of all molecules ware merged into a single .sdf molecule by using Open Babel and then converted into smiles format. CDRUG server calculates molecular descriptors (relative frequency-weighted fingerprint) to implement query compounds. The similarity between the query and active compounds were measured in terms of hybrid score. At final step, a confidence level (p-value) was calculated.

Molecular docking

Docking studies were carried out using AutoDock in PyRx (Trott and Olson, 2010). PyRx is a graphical user interface for AutoDock 4.2 and Vina for performing virtual screening. Docking in AutoDock is based on Lamarckian Genetic Algorithm (LGA). In LGA the orientation/conformation of the ligand with respect to the EGFR receptor was represented through a chromosome. The constructed chromosome consists of a number of variables for ligand translation/rotation that was the same for all ligands and a number of variables for ligand flexibility that is specific to each ligand. The phenotype of a ligand was represented by its 3D coordinates after applying all the transformations in the genotype. The fitness of the ligands were calculated from its phenotype using any of the standard docking scoring functions. AutoDock uses a modification on the AMBER'95 force field with terms empirically determined by linear regression analysis from a set of protein-ligand complexes with known binding constants. Free energy (Gibbs, ΔG) is denoted by a master equation, broken down into six terms to model dispersion/repulsion, hydrogen bonding, electrostatic interactions, deviation from covalent geometry, internal ligand torsional constraints and desolvation effects:

$$\Delta G = \Delta G_{vdw} + G_{hbond} + G_{elec} + G_{conform} + G_{tor} + G_{sol}$$

Evaluation of Pharmacological properties of Hit compounds

Several *in silico* tools were employed to detect the pharmacological properties of the hit compounds.

FAF Drug3 server was used for evaluation of druglike properties, admetSAR server was used for detection of absorption, distribution, metabolism, excretion, and toxicity of hit compounds, CLC- Pred web services (www.pharmaexpert.ru, Accessed on 2nd Feb 2016) was applied for cell-line cytotoxicity prediction, GUSAR online server was exploited for calculation of median lethal dose (LD50), evaluation of common pharmacophores was done through PharmaGist web server.

RESULTS

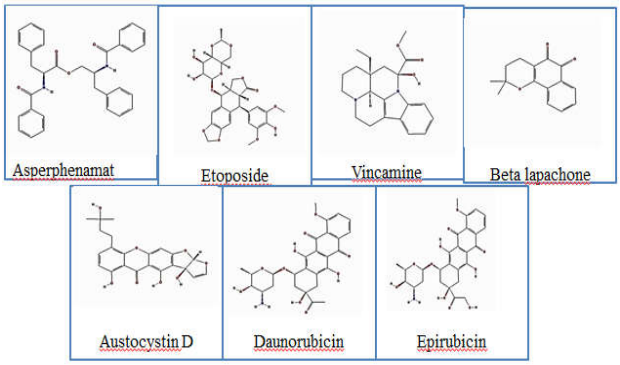

Figure 1 2D structure of screened molecules

A library was constructed by collecting four hundred compounds from diverse natural resources including Plants, Fungus, Lichens, Fruits, Bacteria, Cyanobacteria, Vegetables etc. Initially the library was screened for their anticancer efficacy and binding property with ALK protein and as a result, seven compounds were screened namely Asperphenamat, Etoposide, Vincamine, Beta lapachone, Austocystin D, Daunorubicin and Epirubicin (Fig.1). Anti-cancer efficacy of

the molecules were evaluated by CDRUG server where weighted method (RFW_FP) was used to find out the molecular fingerprints of the screened molecules by applying machine learning method and after that, a hybrid score was calculated to compute the compound similarity with the active or inactive compounds previously present in the server. The results of the screened molecules (Table-1) clearly indicates their high anticancer efficacy.

The screened molecules from present study that showed good binding properties against ALK were Etoposide, Vincamine, Epirubicin, Daunorubicin, Beta lapachone, Asperphenamat and Austocystin D with binding energy -10.5 kcal/mol, -10.0 kcal/mol, -10.1 kcal/mol, -10.9 kcal/mol, -9.8 kcal/mol, -9.8 kcal/mol and -9.5 kcal/mol respectively whereas the binding energy of reference (Entrectinib) ligand was -10.5 kcal/mol.

Table 1 CDRUG results of hit molecules

Rank	Query	Match_nscID	Match_ pubchemSID	Mean_logGI50	Match_ RFW_TC	Match_ kernel_ minmax	Cdrug_ score	p_value
1	Austocystin D	700893	527189	-7.057	1	1	1	0.00E+00
2	Beta lapachone	629749	494711	-6.233	1	1	1	0.00E+00
3	Vincamine	141540	427063	-5.167	1	1	1	0.00E+00
4	Epirubicin	123127	301181	-7.015	0.998	1	0.998	2.30E-05
5	Daunorubicin	82151	301154	-7.133	0.998	1	0.998	2.30E-05
6	Etoposide	322920	457665	-5.096	0.673	0.6	0.404	0.0439
7	Asperphenamat	708472	529840	-6.095	0.724	0.326	0.236	0.1602

Molecular docking method was applied to check the binding potential of the compounds in the binding pocket of ALK protein (PBD ID- 5FTO) using Entrectinib as reference ligand. The binding pocket consists of GLU (1197, A), MET (1199, A), MET (1199, A).

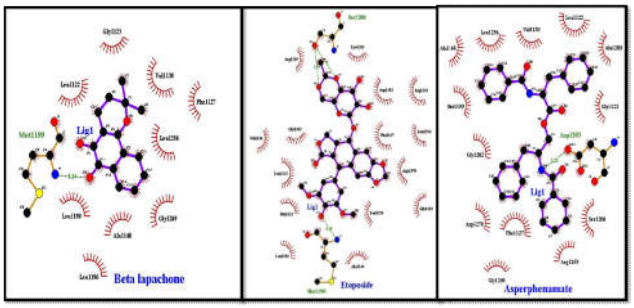

Figure 2 Lig plot analysis of screened ligands against ALK protein

Table 2 CLC- Pred (Cell-Line Cytotoxicity Predictor) of hit molecules

S. No	Compounds Name	Cell line	Cell line full Name
1	Asperphenamat	NCI-H1299	Non-small cell lung carcinoma
		NCI-H69	Small cell lung carcinoma
		NCI-H128	Small cell lung cancer
		NCI-H838	Non-small cell lung cancer. 3 stage
		A549	Lung carcinoma
		NCI-H647	Adenosquamous lung carcinoma
2	Etoposide	NCI-H322M	Non-small cell lung carcinoma
		NCI-H187	Small cell lung carcinoma
		NCI-H226	Non-small cell lung carcinoma
		HOP-92	Non-small cell lung carcinoma
		PC-6	Small cell lung carcinoma
3	Vincamine	A549	Lung carcinoma
		NCI-H838	Non-small cell lung cancer. 3stage
4	Beta lapachone	SW1573	Lung carcinoma
		HOP-18	Non-small cell lung carcinoma
5	Austocystin D	NCI-H187	Small cell lung carcinoma
		SHP77	Small cell lung carcinoma
		UCLA P-3	Lung carcinoma cell line
6	Daunorubicin	NCI-H838	Non-small cell lung cancer. 3 Stage
		DMS-114	Lung carcinoma
		NCI-H187	Small cell lung carcinoma
		SK-MES-1	Squamous cell lung carcinoma
7	Epirubicin	NCI-H838	Non-small cell lung cancer. 3 Stage
		DMS-114	Lung carcinoma
		UCLA P-3	Lung carcinoma cell line
		NCI-H187	Small cell lung carcinoma

Their hydrogen bonding with the protein were analyzed by their Lig Plot interaction (Fig.2). Further drug-like properties of the molecules were evaluated in FAF Drug3 server. The server used several rules for assessing oral bioavailability of the compounds. The rules were the Veber rule (Veber *et al.*, 2002) (≤10 rotatable bonds and tPSA ≤ 140 Å2 (or ≤12 HBA+HBD), the Egan rule (Egan *et al.*, 2000) (−1 ≤ log P ≤ 5.8 and tPSA ≤ 130 Å2) and the Bayer Traffic Lights (Lobell *et al.*, 2006) (involving tPSA, log P, MW, rotatable bonds and solubility. A compound that able to passes these filters can enter the open drug discovery program offered by the company. All of the screened molecules showed accepted results in the evaluation results. Absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of drug candidates play crucial roles in drug discovery and environmental hazard assessment. All the screened molecules showed an acceptable range of ADMET profile in the results of the admetSAR server. The Cytotoxic capability of the screened compounds was evaluated using CLC- Pred web services. The results showed the cytotoxic effect of the screened molecules on several lung cancer cell lines with highly active probability (Table 2).

GUSAR online server was exploited for calculation of median lethal dose (LD50).The 'LD50' value of compounds gives information on degrees of toxicity. LD50 stands for Lethal Dose 50%, means the dose at which half (1/2) of the number of test animals die. If a chemical showed low LD50 value it means it is highly toxic because a low dose of the chemical will kill an animal. The LD 50 values and the environmental toxicity prediction values of the all investigated compounds were in class 3 and 5 and in acceptability domain. Evaluation of pharmacophores was done through PharmaGist web server. Pharmacophore, which is the spatial arrangement of features essential for a molecule to interact with a specific target receptor, is important for achieving this goal. In the present study, PharmaGist server was used to predict the pharmacophores of the screened compounds (Table3).

Table 3 Pharmacophores of the screened ligands

S. No	Name of Molecule	Atoms	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives
1	Asperphenamat	68	24	18	3	3	6	12	0	0
2	Etoposide	67	23	18	3	4	5	11	0	0
3	Vincamine	50	19	15	3	4	4	8	0	0
4	Beta lapachone	32	8	8	2	3	0	3	0	0
5	Austocystin D	52	15	14	2	7	1	4	0	1
6	Daunorubicin	74	24	21	3	5	3	13	0	0
7	Epirubicin	68	12	12	4	2	2	4	0	0

CONCLUSION

Anaplastic lymphoma kinase (ALK) rearrangements identified in a number of non–small cell lung cancer (NSCLC) patients for whom ALK inhibitors are highly effective. Although several first and second generation inhibitors are presently available in the market but the challenge of acquired resistance also exists with them with various side effects. Keeping all these in view seven compounds from natural domain were screened with inhibitory potential against ALK. The seven screened compounds were Asperphenamat, Etoposide, Vincamine, Beta lapachone, Austocystin D, Daunorubicin and Epirubicin. All the above screened ligands showed efficient binding potential with ALK and anticancer efficacy in the result`s. All of them were also validated for several other vital pharmacological parameters and their potential as drug candidate were demonstrated in every result. Therefore the newly identified ALK inhibitors are expected to bring a lead in the treatment landscape of ALK-positive lung cancer.\\

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