

## Research Article

# Structure Based Virtual Screening of Selected Phytochemicals against C1156y Mutant Anaplastic Lymphoma Kinase (ALK) using Molecular Docking and Admet Prediction

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### Abstract

Lung cancer is one of the fatal malignancies in both men and women worldwide. Anaplastic Lymphoma Kinase (ALK) rearrangements triggered fusion of echinoderm microtubule-associated protein-like 4 (EML4) and ALK increasing the tyrosine kinase activity leading to cell proliferation and tumorigenesis causing Non-Small Cell Lung Cancer (NSCLC). Crizotinib emerged as a first generation ALK inhibitor; however it developed resistance within a year of initiation of treatment in few patients deteriorating therapeutic outcomes. Various secondary mutations in ALK are responsible for crizotinib resistance in patients. C1156Y is a point mutation seen in patients resistant to crizotinib. Although second and third generation ALK inhibitors are currently available, there is still a need to develop new ALK inhibitors as the existing compounds are developing resistance. In this study we screened 940 phytochemicals against C1156Y mutant ALK to identify binding affinities. The results showed that three steroidal alkaloids Conessine, Solanocapsine and Muldamine exhibited binding affinity (-9.0 kcal/mol) and is predicted to show activity in CNS for the prevention and management CNS metastasis. We conclude that these 3 steroidal alkaloids are worth for further *in vitro* and *in vivo* studies to emerge as potent C1156Y mutant ALK inhibitor.

**Keywords:** C1156Y mutant ALK; Phytochemicals; Molecular docking; Structure based screening

### Introduction

Lung cancer is one of the deadliest malignancies accounting for 1.5 million deaths worldwide. Lung cancer can be broadly divided based on histology into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC accounts for almost 80%-85% of the lung cancer cases [1]. Anaplastic Lymphoma Kinase (ALK) rearrange-

ments are seen in 5% of NSCLC patients, equivalent to over 60,000 patients diagnosed annually worldwide [2]. Apart from NSCLC, ALK is also found in anaplastic large cell lymphoma, neuroblastoma and inflammatory myofibroblastic tumour [3]. ALK gene is located on chromosome 2 (2p23). It encodes for ALK receptor tyrosine kinase which belongs to insulin receptor super family [4]. In NSCLC, ALK rearrangements lead to fusion of echinoderm microtubule-associated protein-like 4 (EML4) and ALK further lead to the activation of kinase activity [5]. So, ALK inhibitors emerged as best therapeutic agents against ALK rearranged NSCLC. Crizotinib belongs to the first generation ALK inhibitors with outstanding efficacy and safety. However resistance to crizotinib developed within one or two years of treatment initiation [6].

Point mutations in ALK lead to crizotinib drug resistance. Many secondary mutations like C1156Y, L1196M etc. are identified within the kinase domain of EML4-ALK. C1156 residue of ALK is located at the non-active site and thus has no direct interaction with crizotinib; however, C1156Y displaces crizotinib and affects the conformations of loop 1122-1131,  $\beta$ -sheet 1145-1152, and  $\alpha$ -helix 1157-1174 [7]. Although second and third generation ALK inhibitors are present, there is still an immense need of ALK inhibitors due to different mutations in ALK. So, in this study we have focused on phytochemicals as they have reported various pharmacological activities.

## Method and Methodology

### Preparation of protein

The X-Ray Diffraction structure of C1156Y Mutant ALK (PDB ID:2YJS) with resolution 1.9Å was retrieved from Protein Data Bank (PDB). The hetero atoms present in the crude PDB file were removed. Kollman charges and polar hydrogen atoms are added to satisfy their appropriate charges in Autodock Vina and the file is converted to PDBQT format [8].

### Identification of binding pockets

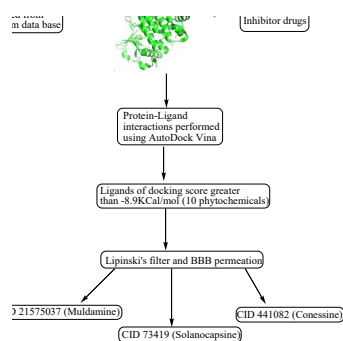
Active pockets in 2YJS receptor molecule were predicted using Computed Atlas of Surface Topography of Proteins (CASTp) program[9] (<http://sts.bioe.uic.edu/castp/>). One binding pocket was predicted with solvent accessible surface area and volume of 633.8 and 1019.2 respectively. These catalytic sites are further used for identifying ligands with best affinity to the receptor.

### Preparation of ligands

The phytochemicals (940) along with Standard ALK inhibitors viz. Ceritinib, Alectinib, Brigatinib, Crizotinib and Lorlatinib were considered for molecular docking study. 3D structures of ligands were retrieved from Pub Chem compound database at NCBI (<https://pubchem.ncbi.nlm.nih.gov/>). Ligands downloaded in SDF format are converted to PDB format using PyMOL (<https://pymol.org/2/>). In Auto Dock Vina, gastgeir charges and hydrogens were added and converted to PDBQT format.

### Molecular docking

Following protein and ligand preparation, molecular docking analysis was performed using Autodock Vina to assess their binding affinities. To define the binding site a grid box was set at  $44 \times 48 \times 16$  Å along the X, Y and Z axes respectively at grid resolution of 0.4 Å. The various conformations for ligand in docking procedure were generated. The dock score of the best poses docked into the receptor for all the ligands was considered. The docking scores of standard ALK inhibitors were compared with phytochemicals to identify the phytochemicals with best affinity to mutated ALK receptor (Figure 1).



**Figure 1:** Flow chart of structure based screening of phytochemicals against mutant ALK.

### ADME and druglikeness

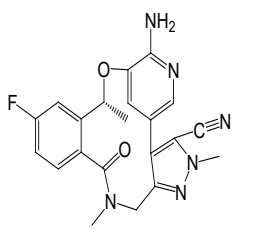
Subsequent to molecular docking, phytochemicals with higher affinity to receptor than standard ALK inhibitors were further studied for prediction of Absorption, Distribution, Metabolism, Excretion (ADME) parameters and drug likeliness in SwissADME web tool [10](<http://www.swissadme.ch/>). Predicted parameters like Molecular weight, Numer of H-bond acceptors, H-bond donors, LogPo/w (Lipophilicity), LogS (solubility), GI absorption, Blood brain barrier(BBB) permeation were recorded. Lipinski's filter was applied for examining drug likeliness.

### Results and Discussion

Initially docking was carried for standard ALK inhibitors and best binding affinities were exhibited by Alectinib (-8.9 kcal/mol) and Lorlatinib (-8.9 kcal/mol) as shown in Table 1 and low affinity was exhibited by crizotinib clearly explaining that C1156Y mutation significantly affects its binding to ALK

**Table 1:** Structures and Binding affinities of standard ALK inhibitors.

Standard Alk Inhibitors	Binding affinity (Kcal/mol)	Structures
1. Ceritinib	-7.9	
2. Alectinib	-8.9	
3. Brigatinib	-8.5	
4. Crizotinib	-7.8	

5. Lorlatinib	-8.9	
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The 940 phytochemicals were also docked and 10 phytochemicals exhibited dock score greater than (-8.9 kcal/

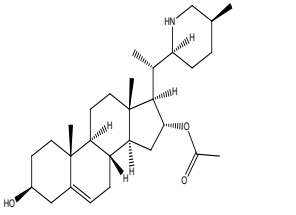
mol). These were screened for further investigation as they exhibited greater binding affinities than Alectinib and Lorlatinib Table 2. CID 11316166 (Cavicularin) exhibited the highest affinity of -9.9 Kcal/mol followed by CID 5315779 (Cepharanoloine) and CID 5492110 (Ochnaflavone) of binding affinity -9.8 kcal/mol each. Alectinib and Lorlatinib showed interaction with ASP 1270, MET 1199, LYS 1150, ARG 1253, ASP 1203. Whereas the shortlisted phytochemicals interacted with ASP 1270, MET 1199, ASP 1203, ARG 1275, GLY 1269, GLY 1123 showing that they are binding to catalytic residues.

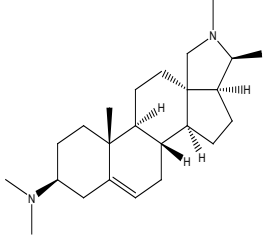
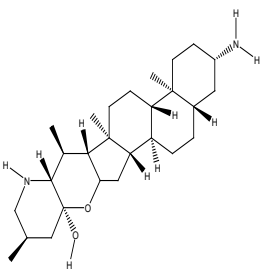
**Table 2:** Binding affinities, Pharmacokinetic parameters and druglikeness of shortlisted phytochemicals.

Phytochemicals	Binding Affinity (kcal/mol)	Molecular weight (g/mol)	Number of H-bond acceptors	Number of H-bond donors	Log Po/w	Log S	GI absorption	BBB permeant	Lipinski filter
CID 11316166	-9.9	422.4	4	3	5.06	-7.98	High	No	Yes; 1 violation
CID 5315779	-9.8	592.6	8	1	5	-7.76	High	No	Yes; 1 violation
CID 5492110	-9.8	538.4	10	5	4.01	-7.14	Low	No	Yes; 1 violation
CID 10206	-9.4	606.71	8	0	5.36	-7.98	High	No	Yes; 1 violation
CID 76972186	-9.4	598.6	8	2	4.51	-6.09	High	No	Yes; 1 violation
CID 15922818	-9.3	611.53	14	9	-0.07	-3.7	Low	No	No; 3 violations
CID 442105	-9.2	518.7	3	3	3.75	-6.88	High	Yes	No; 2 violations
CID 73419	-9	430.67	4	3	4	-5.65	High	Yes	Yes; 1 violation
CID 441082	-9	356.59	2	0	4.36	-5.04	High	Yes	Yes; 1 violation
CID 21575037	-9	518.7	3	3	3.75	-6.88	High	Yes	Yes; 1 violation

CNS involvement is seen in advanced ALK rearranged NS-CLC. The major therapeutic issue in ALK rearranged NS-CLC patients is control and prevention of CNS metastasis [2]. Crizotinib failed to show promising outcomes in controlling CNS metastasis. However the second generation ALK inhibitors v.i.z. Alectinib and brigatinib were active for CNS metastasis [11]. So, it is desired that the phytochemical of choice shows its activity in CNS as well by BBB permeation. Among the shortlisted phytochemicals only 3 were predicted to cross BBB and showed only 1 violation in lipinski's rule of five revealing favorable drug-like properties Table 3.

**Table 3:** Structure and Binding affinities of Steroidal alkaloids.

Phytochemicals	Binding affinity (Kcal/mol)	Structures
CID 21575037	-9	

CID 441082	-9	
CID 73419	-9	

These three phytochemicals are steroidal alkaloids with a perhydro-1, 2-cyclopentanophenanthrene nucleus. Steroidal alkaloids are the natural nitrogen-containing compounds that are present in many edible or medicinal plants. Steroidal alkaloids are studied extensively and showed to have anti-microbial, anti-nociceptive, anti-inflammation and anticancer properties [12].

Conessine is a steroid alkaloid derived from many plants of family Apocynaceae. Conessine is well known as a Histamine antagonist selective to H3 subtype with high BBB penetration and is also found to have long CNS clearance time [13]. It's high affinity to C1156Y mutant ALK and high BBB permeation reveals conessine can be studied further and may emerge as a novel phytochemical for ALK rearranged NSCLC patients with C1156Y mutation. Solanocapsine is derived from *Solanum pseudocapsicum*. It is studied previously for its acetylcholine esterase inhibition activity [14]. Muldamine, a phytosterol alkaloid was extracted from the roots and rhizomes of *Veratrum californicum* [15]. Muldamine is unexplored for its biological activity. So, there is a need to highlight these steroidal alkaloids for emerging as anti-oncogenic drugs against ALK rearranged NSCLC.

## Conclusion

In the present study we screened various phytochemicals against C1156Y mutant ALK. Highest affinity was shown by CID 11316166. However considering BBB permeation three steroidal alkaloids CID 73419, CID 441082, CID 21575037 showed affinity to mutant ALK. We conclude that further *in vitro* and *in vivo* studies should be performed to assess the therapeutic potential of these three steroidal alkaloids against mutant ALK rearranged NSCLC.

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## Conflicts of Interest

We hereby declare that the authors have no conflicts of interest

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