# Discovery of inhibitors for focal adhesion kinase (FAK): Molecular modeling study for combating cancer

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*Abstract* - **Focal adhesion kinase (FAK) is a tyrosine kinase that controls the signals leading to invasion and metastasis and is involved in many aspects of the metastatic process including adhesion, migration, and invasion. It is known that FAK is activated from integrin and growth factor receptors by auto-phosphorylation followed by subsequent activation of other functional phosphorylation sites to advance the signals to downstream pathways, such as AKT. Based on these facts, FAK is thought to play a critical role in malignant behavior including proliferation, survival, and invasion. FAK is over expressed in many tumors, including those derived from the head and neck, colon, breast, prostate, liver, and thyroid. Inhibition of FAK signaling by over expression of dominant-negative fragments of FAK reduces invasion of glioblastomas and ovarian cancer cells. FAK therefore represents an important target for the development of anti-neoplastic and anti-metastatic drugs. We have attempted with the help of virtual screening and molecular docking approach using Lamarckian Genetic Algorithm to see the binding mode of extracts and compounds from the Rhizomes of Veratrum dahuricum. The study conducted involved virtual screening of nearly 115 molecules on basis of structure similarity of active molecules from roots of Veratrum dahuricum. Molecular docking using Lamarckian Genetic Algorithm was carried out for these ligands and the result gave binding energies which were in the range of -12.28 kcal/mol to -1.14 kcal/mol. The top 4 best docked protein were visualized using UCSF chimera which resulted in finding intricate atomic scale properties between ligand and active site of FAK, PBD ID- 2JKO. The top molecules then were run for in-silico ADMET and druglikeness properties showed promising results. Further in-vitro and in-vivo study is required on these molecules as the binding mode provided hints for the future design of new FAK inhibitors with higher potency and specificity.**

*Index Terms*—**Veratrum dahuricum, cyclopamine, germine, antitumor activity.**

## I. INTRODUCTION

Focal adhesion kinase was discovered in 1990's in two different studies. The first study discovered that FAK protein is non-receptor tyrosine kinase protein with molecular weight of 120 KDa. FAK is located at the focal adhesion sites and is activated through phosphorylation by integrin, growth factor receptors, cytokines receptors or G-protein coupled receptor. The second study shows that FAK is one of the potential substrates for v-Src oncogene. FAK is considered as integrin-dependent tyrosine phosphorylated protein and located at focal adhesion sites. FAK was named based on it's cellular localization in the Focal adhesion site [1]. The function of the focal adhesion kinase is classified into kinase dependent and kinase independent. The kinase independent activity of FAK is induces through integrin signaling pathway. The kinase dependent activity of FAK plays an important role in the regulation of cell adhesion and migration in both normal and cancer cells. On the other hand, the kinase-independent activity is involved in protein-protein interaction, protein scaffold properties and nuclear localization of FAK. The localization of FAK into the nucleus is essential for inducing cellular proliferation and survival through interacting and degrading p53 [2]. The regulation of cellular migration through integrin signaling pathway and FAK is responsible for pathogenesis of cancer and other diseases. Accordingly, FAK is considered as potential drug target for combating cancer and cardiovascular diseases. The *homo sapiens FAK* gene sequence is highly similar with other organisms such as *Mus musculus*, *Gallus gallus* and *Xenopus* [3, 4]. In human genome, FAK gene is located in chromosome 8 and in mouse FAK gene is located in chromosome 15. The FAK structure composed of C-terminal domain that contains the FAT (focal adhesion targeting) sequences. In addition, FAK composed of central catalytic domain and FERM N-terminal domain, which share structure similarity with several proteins such as talin and tyrosine kinase JAK family [5, 6].

.The metastatic cancer cells have the ability to obtain invasive phenotype for invading distant organs. This phenotype is acquired through the disruption of the physiological interaction of the cancer cells with the surrounding ECM. In the normal conditions, the cell-matrix interaction is essential for maintaining cellular proliferation, polarity survival and differentiation through several receptors. Those receptors are able to transduce the signals from the extracellular matrix into the cell, which results in the initiation of several signaling pathways. On the other hand, disruption of signaling pathways induced by the ECM results in development of abnormal physiological conditions such as cancer [7].

Focal adhesion kinase is a non-receptor tyrosine kinase that is activated through the interaction with several receptors such as integrin and growth hormones receptors. Several studies have reported that cellular migration is induced by integrin signaling through FAK. In addition, the over expression or aberrant activation of FAK has been associated with metastatic and invasive

prospective of several human cancers. Chromsome 8 encompass *PTK2*, which encode for focal adhesion kinase protein (FAK) and is correlated with the susceptibility to the development of ovarian cancer [5, 6].
Accordingly, Cancer genome atlas exhibited that high expression level of FAK in several human cancers such as late stages of breast cancer (approximately 26%) and ovarian cancer is associated with the bad prognosis. Moreover, tissue array study of several human cancers showed that increasing the activity of FAK through TYR 397 phosphorylation is associated with increasing the progression of the disease. Unlike the other oncogenes such as PI3K, limited missense mutations has been discovered in PTK2 gene. Increasing of FAK activity results in the amplification of PTK2 gene [4, 8 & 9].

## II. MATERIALS AND METHODS

FAK plays a critical role in the biological processes of cancer cells, so FAK has been proposed as a potential target in cancer therapy and small molecule inhibitors for use as potential cancer therapies. Accordingly, this study aims to elucidate the structural properties and binding mode of derivatives of Germine, an extract and compound from the Rhizomes of *Veratrum dahuricum* showing antitumor activity. Molecular modeling approach with a focus of Structure based drug designing will be applied to a set of 115 derivatives of Germine, which might selectively inhibit the kinase [10].

### a) PROTEIN SELECTION

Two focal adhesion kinase proteins from different organisms (*Homo sapiens* and *Gallus gallus*) were selected and downloaded from the protein databank [15] with PDB. They were selected based on their resolution value. FAK protein was retrieved from Uniprot database [16] for homology modeling. This step is essential for the evaluating the selection of FAK protein with PDB ID "2JKO" for performing molecular modeling and docking studies.

### b) VIRTUAL SCREENING AND IN-SILICO GENERATION OF LIGANDS

115 drevivatives of Germine were generated and obtained from the PubChem database on the basis of structure similarity, Isomers and substructure search. All the selected molecules were drawn using ChemSketch software and converted to docking formate .pdb extension using Open babel.

### c) PROTEIN OPTIMIZATION AND ENERGY MINIMIZTION

The Hetam atoms (ligand) and water molecules found in the in the PDB file with PDB entry "2JKO" were removed using Discovery studio v4.5 [17]. This process is essential to prepare the suitable conditions for the molecular docking. Additionally, the energy minimization removes the bad contact in the protein structure using SPDBV Swiss-PDB viewer software v4.1 [18].

### d) MOLECULAR DOCKING

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. All the molecular docking procedures were performed using AutoDock4 v1.5.6 [19] using genetic algorithm.

### e) SELECTION OF POTENT INHIBITORS ON THE BASIS OF LEAST BINDING ENERGY AND LIPINSKI'S RULE OF 5

Top 3 molecules with least binding energies and their corresponding coordinates were selected for performing Lipinski's rule of 5 analysis. Notably, This step was performed using UCSF chimera software v1.11 [20] to illustrate the 3D of complex protein-ligand structures. In addition, the analysis of docked protein binding pocket from another prospective view was performed using Discovery studio software v4.5 [21].

### f) PREDICTION OF PHARMACOKINESTICS PROPERTIES OF SELECTED MOLECULES

The molecule which have shown H-bond with the active site residues or any other residues of the binding pocket were selected to predict their Molecular properties and drug likeness score, toxicity and bioactivity. All these properties were calculated using online servers such as organic chemistry server [22] and molsoft server [23].

## III. RESULTS AND DISCUSSION

Top 4 molecule will be selected based on their least bing energy score . The genetic algorithm were used during the preparation of docking files, which perform 10 different runs for each molecule to obtain the least binding for each drug. Drugs that formed H-bond with any amino acid residues of the protein-binding pocket were selected to predict their Drug likeness score, molecular properties and ADMET studies.

| Molecule Number | Ile 428 | Met 499 | Cys 502 | Thr 503 | Gly 505 | Leu 553 | Leu 567 | Other |
|---|---|---|---|---|---|---|---|---|
| **77** | - | - | - | - | - | - | - | ARG426 |
| **98** | - | - | - | - | - | - | - | - |
| **115** | - | - | H-Bond | - | - | - | - | - |
| **71** | - | - | H-Bond | - | - | - | - | - |

Table shows the data analysis of docking results of top 4 selected molecules. Molecules that formed H-bond with any of binding pocket amino acid residues were selected for prediction of molecular properties, Druglikness score, toxicity and bioactivity.

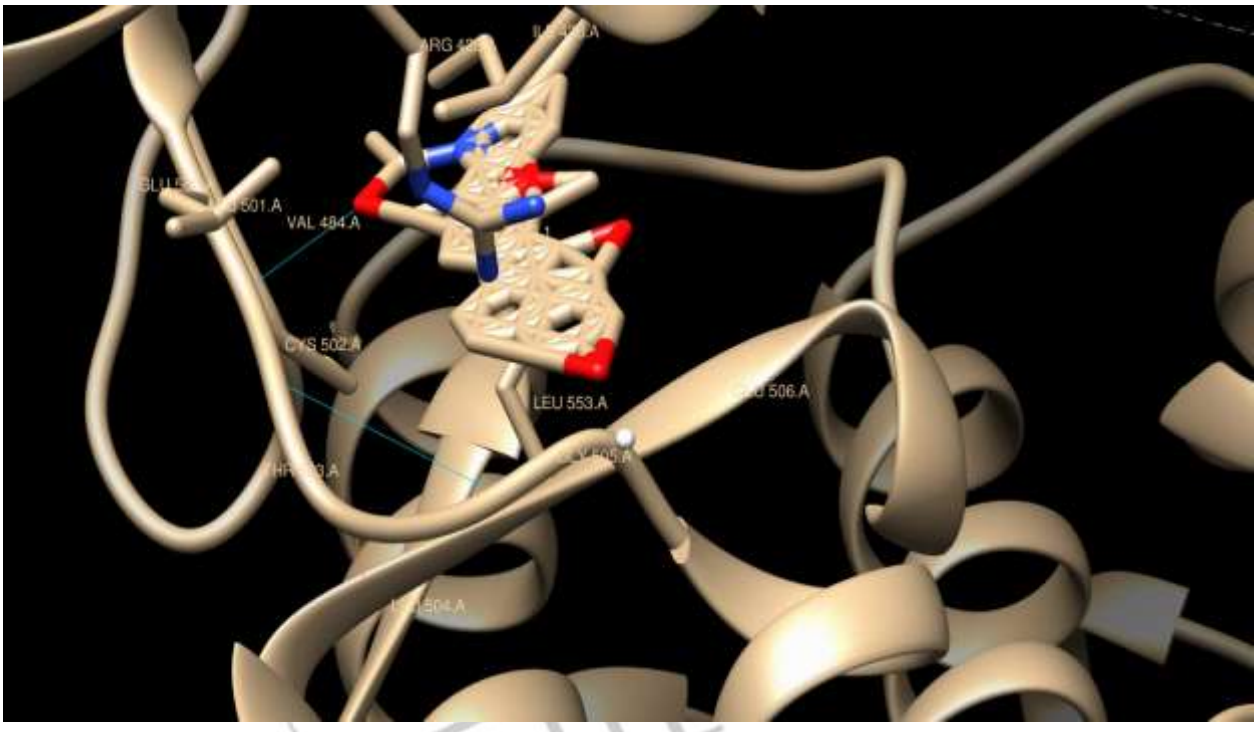


Figure 1: Shows the docking result and complex protein-ligand 3D structure

The previous figure illustrate the formation of H-bond between molecule 115 with chemical formula "$C_{29}H_{41}NO_4$ " with binding pocket amino acid residue CYS 502.

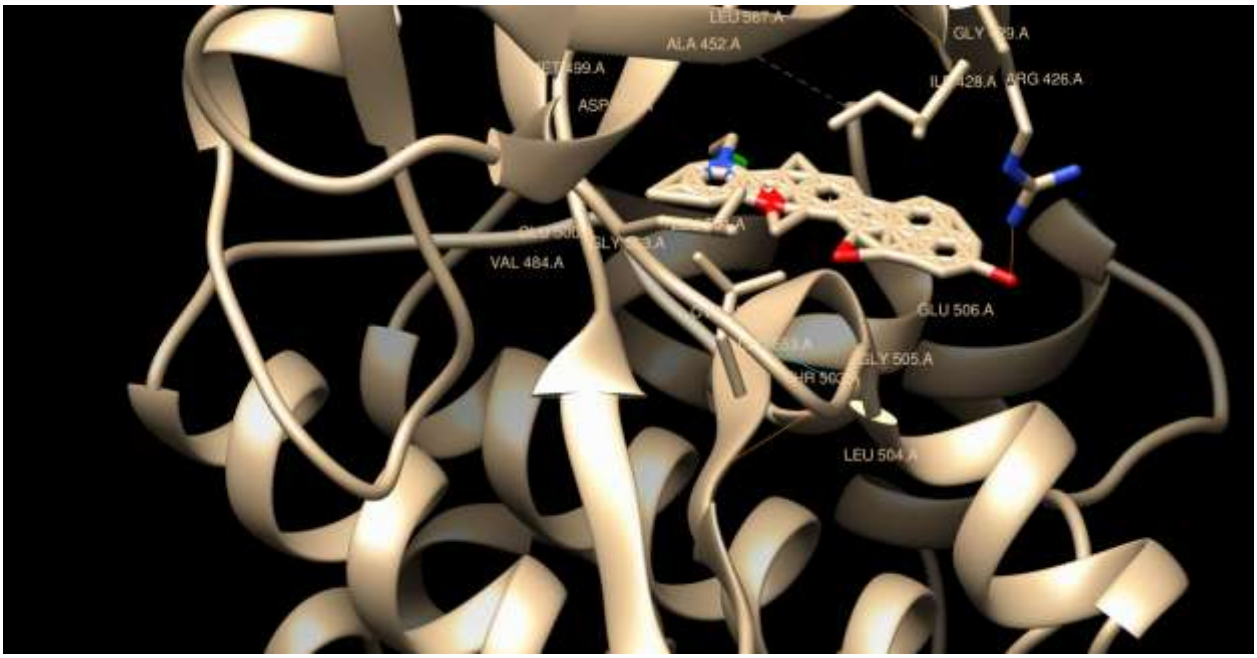


Figure 2: shows the docking result and complex protein-drug 3D structure

Molecule number 77 with chemical formula "$C_{29}H_{40}ClNO_3$ " with binding pocket residue ARG 426.

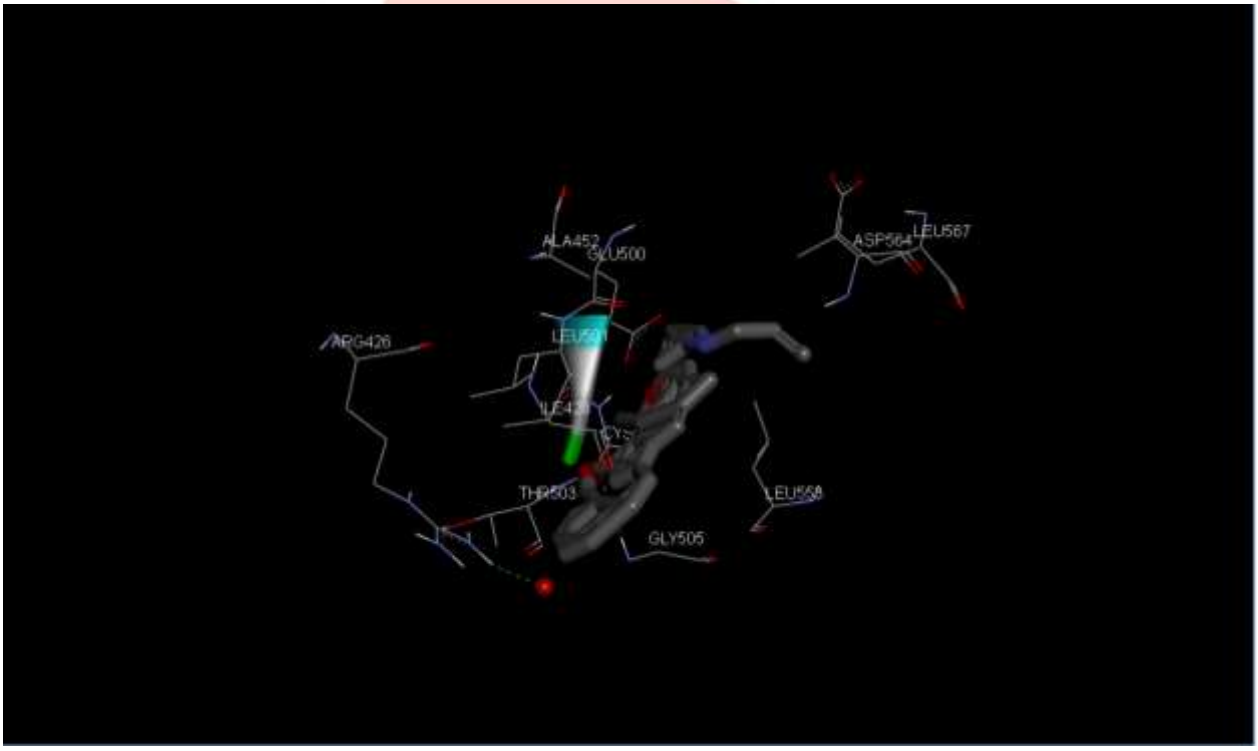


Figure 3: shows the docking result and 3D structure of binding pocket in complex with molecule 77

The previous figure illustrates the analysis of docked binding pocket from different prospective view. The green line between the ligand and the binding pocket amino acid residue indicate for H-bond.

**a) PREDICTION OF PHARMACOKINETICS PROPERTIES**

Molecules that formed H-bond with any amino acid residues of the binding pocket were selected to predict their pharmacokinetic properties such as molecular properties and Drug-likeness score, toxicity and bioavailability. The

following figures illustrate the calculated properties of the selected molecules based on their ability to form H-bond with binding pocket residues

**Molecule number: 115**
**Molecular formula:** $C_{29}H_{41}NO_4$
**Molecular weight:** 467.30
**Number of HBA:** 4
**Number of HBD:** 1
**MolLogP :** 4.73
**MolLogS :** -4.93 (in Log(moles/L)) 5.46 (in mg/L)
**MolPSA :** 53.31 $A^2$
**MolVol :** 560.14 $A^3$
**Number of stereo centers:** 10
**Drug-likeness model score:** 0.43

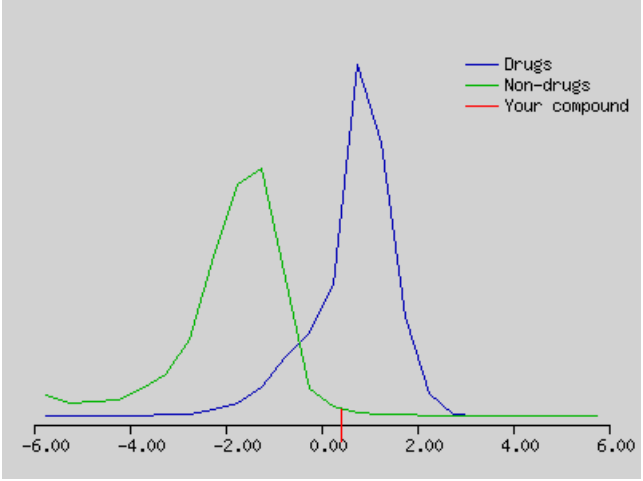


Figure 4: shows the drug likeness score of particular molecule. The X-axis illustrates the molecular properties of particular and the Y-axis illustrates the % of dataset.

**b)Toxicity and bioactivity**

The following figure illustrates the calculated toxicity and bioactivity features of the selected molecule. The green color indicates for no risk associated such as mutagenic, irritant or reproductive effects, which could be used in further studies.

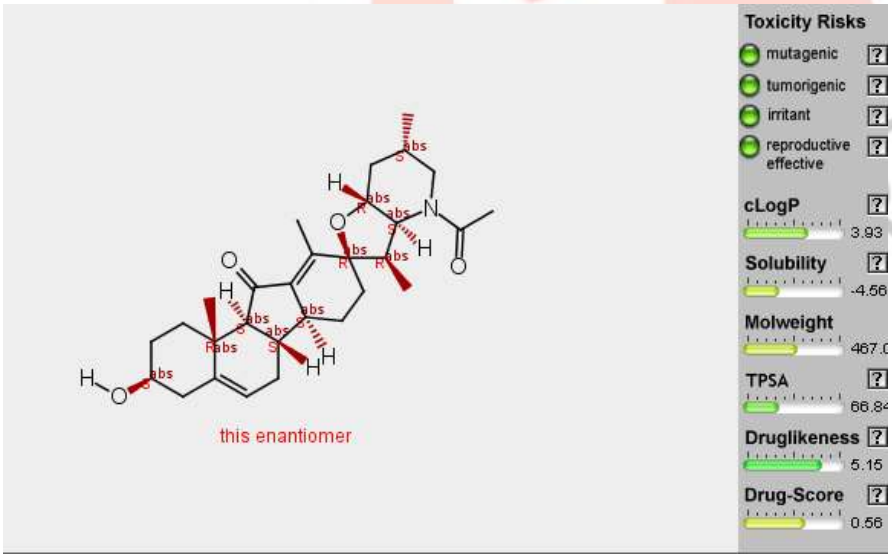


Figure 54: shows the calculated toxicity and bioactivity features of molecule 115 "$C_{29}H_{41}NO_4$".

The calculated Molecular properties and toxicity risk showing promising results, which could be used for further studies.

## IV. CONCLUSION

The top molecules were run for in-silico ADMET and drug likeness properties showed promising results. Further in-vitro and in-vivo study is required on these molecules as the binding mode provided hints for the future design of new FAK inhibitors with higher potency and specificity.

## V. ACKNOWLEDGEMENT

The author would like to thank Madhav Institute of Technology and Science, for its support for the project.

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