



IN SILICO EVIDENCE OF THE EFFICACY OF PHYTOCOMPONENTS AS COMPETITIVE INHIBITORS AGAINST A TOBACCO SMOKE CARCINOGEN IN NON-SMALL CELL LUNG CANCER

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ABSTRACT

Cigarette smoke is a major risk factor in the development of Non-small cell lung cancer (NSCLC). Nicotine- derived nitrosamine ketone (NNK) is an important carcinogen present in cigarette smoke that induces tumor metastasis by activation of a protein kinase cascade, including Focal adhesion kinase (FAK). The present investigation was aimed at employing molecular docking studies to establish binding interactions and comparison of phytochemicals from different herbs and selected Rosmarinic acid derivatives against the target protein FAK with the purpose of blocking the binding of Nicotine-derived nitrosamine ketone (NNK). Molegro virtual docker (MMV) and Hex (version 8.0.0) software was used as docking tool for investigating the interaction of the protein active site residues with the phytochemicals. Molegro molecular viewer (MMV) and Biovia Discovery studio 2017 R2 visualizer were used to identify the amino acid interactions between phytochemicals and protein and also to calculate binding scores. The molecular interaction of NNK with the target protein was also done to obtain the binding energy of this carcinogen with the protein. Rosmarinic acid derivatives manifested the highest docking score with the target protein FAK. In comparison, NNK showed weaker binding score with FAK. In addition the derivatives showed common interacting amino acids. Hence, docking studies indicated that phytochemicals and certain derivatives could effectively bind to the same active site of FAK and block its interaction with NNK. Thus, in nicotine exposed NSCLC patients, Rosmarinic acid derivatives may work as effective competitive binders.

Keywords: Non-small cell lung cancer (NSCLC). Nicotine-derived nitrosamine ketone (NNK), Focal adhesion kinase (FAK), molecular docking, Rosmarinic acid

Lung cancer is the leading cause of cancer related death in men and women worldwide (Manjunath, 2018). Mendes *et al.* (2016) have reported that Non-small cell lung cancer accounts for 85% of all lung cancer cases and is the commonest type of lung cancer diagnosed. There are diverse environmental and genetic factors involved in the pathogenesis of lung cancer but tobacco smoking is still the main risk factor for lung cancer and around 81% of lung cancer deaths are tobacco smoke related. The risk increases with quantity and duration of smoking in patients with lung cancer. As observed by Malhotra *et al.* (2016) inhalation of smoke from tobacco in its many forms including cigarettes, cigars, water pipes, cigarillos, Diospyros wrapped bidis, etc., greatly increase the risk of non-small cell lung cancer.

Cigarette smoke contains 8000 compounds including around 70 carcinogens; some of them are tobacco specific carcinogens such as nitrosamines, polycyclic aromatic hydrocarbons (PAHs) and aromatic amines (Hecht and Szabo, 2014). The chemical conversion or biotransformation converts nicotine present in the smoke into a carcinogenic product Nitrosamine 4- (methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) during curing or smoking (Xue *et al.*, 2014). NNK can induce the activation of the Focal adhesion kinase (FAK)

signaling pathway which consequently contributes to metastasis in non-small cell lung cancer (Shen *et al.*, 2012).

The treatment of non-small cell lung cancer has improved and currently various treatment options are available but non selectivity of medicines results in the loss of healthy cells at the time of treatment. Hence there has been no breakthrough in the research and no treatment with long term efficacy is available. There is therefore, an urgent need to develop a novel strategy that selectively destroys tumor cells without damaging normal cells (Chen *et al.*, 2017).

Focal adhesion kinase (FAK), a non-receptor tyrosine kinase that localizes at focal adhesion sites and detected as over-expressed in many solid tumors including lung cancer. The localization of focal adhesion kinase is a crucial step for tumor survival and metastasis, which clearly suggests that its expression leads triggers multiple cellular functions essential for tumor survival signaling (Golubovskaya *et al.*, 2013).

Researchers (Umadevi *et al.*, 2013; Greenwell and Rahman, 2015) have demonstrated that plant phytochemicals have properties that can control cancer by inhibiting cell proliferation, halting metastasis or even by improving inflammatory symptoms in patients.

The main tenet of the present study was to identify the binding interaction of NNK with focal adhesion kinase using a computational approach and evaluate the possibility of blocking the active site of FAK with natural phytocomponents as competitive binders/inhibitors.

Materials and methods

PROTEIN AND LIGAND STRUCTURES:

In the present study, phytocomponents and various rosmarinic acid derivatives present in culinary herbs having potential therapeutic role against the target protein FAK. The crystal structure of FAK (PDB ID: 4NY0) was obtained from RCSB (Research Collaboratory for Structural Bioinformatics) protein data bank (Figure 1). The ligands were obtained from PubChem (<http://pubchem.ncbi.nlm.nih.gov/>) in structure data format (SDF) (Figure 2). The ligands and protein were prepared by removing all the heteroatoms (i.e., nonreceptor atoms such as water, ions, etc.).

Molecular docking studies:

Molegro virtual docker (MVD) v 5.0 and Hex (version 8.0.0) software were used for molecular docking along with Molegro molecular viewer (MMV) and Biovia discovery studio 2017 R2 visualizer for calculating docking score and identifying amino acid interactions were used.

Molecular docking was employed to establish binding of interaction between FAK and selected phytocomponents from different culinary herbs. The derivatives of the phytocomponent that resulted in highest binding were also docked against the target protein FAK and identified that phytocomponent and derivative are effective in binding with the protein and block the binding site for carcinogen NNK.

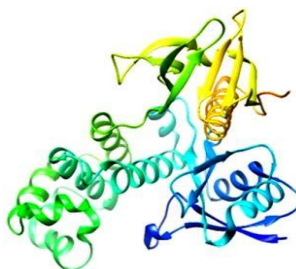


Figure 1: 3D structure of Focal Adhesion Kinase (PDB ID: 4NY0)

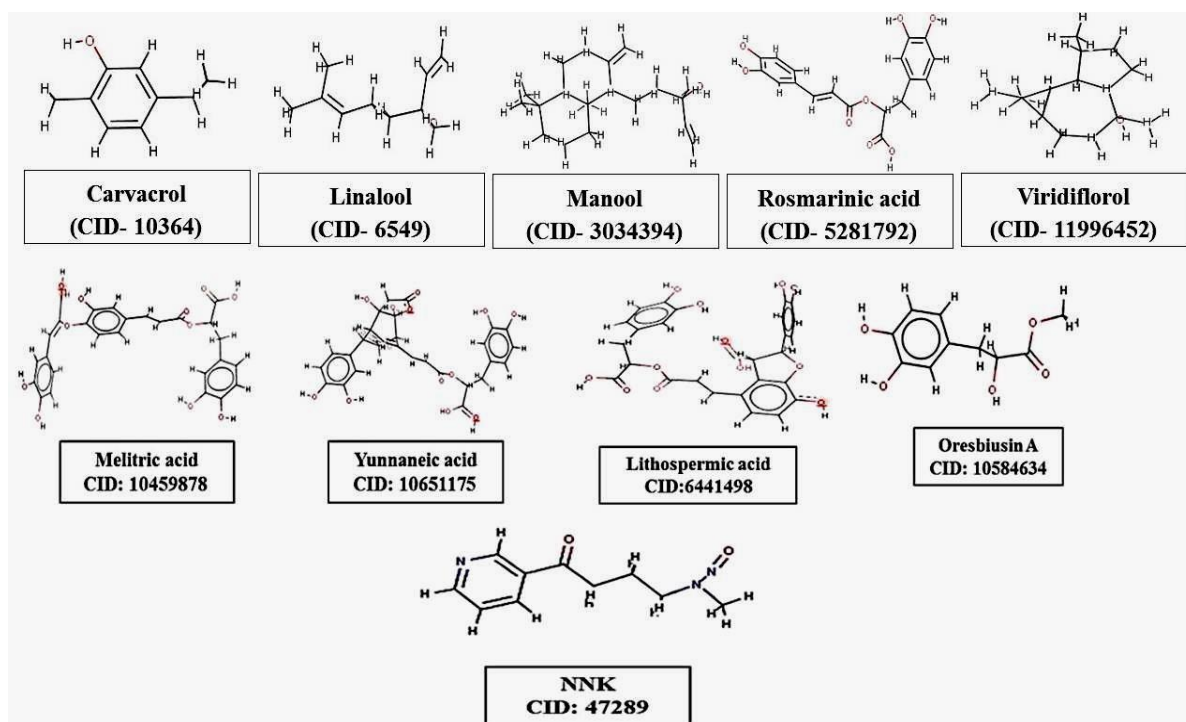


Figure 2: 3D structures of ligands

RESULTS

Protein-ligand docking was performed using Molegro virtual docker (MVD) and the binding scores were calculated by Molegro molecular viewer (MMV). The results obtained indicated that rosmarinic acid binds with lowest docking score (i.e. highest binding energy) of -130.551 Kcal/mol with FAK (Table 1). The interaction site-specific key amino acids were Arg 125, Arg 127, Leu 129, Tyr 251, Glu 256 and Tyr 347. Figure 3 shows the binding pose of rosmarinic acid with focal adhesion kinase.

Table 1: Interaction profile of FAK with phytochemicals

Protein + Ligands	Docking Score (Kcal/mol)	H Bond	Steric Interactions
FAK + Rosmarinic acid	-130.551	Arg 125, Arg 127, Leu 129, Tyr 251, Glu 256, Tyr 347	Trp 97, Arg 125, Arg 127, Leu 129, Tyr 251, Phe 253, Lys 255, Glu 256, Ile 274, Tyr 347
FAK + Manool	-92.662	Arg 127	Arg 127, Tyr 251, Glu 256
FAK + Viridiflorol	-86.732	Tyr 42, Lys 121	Phe 40, Tyr 42, Lys 121
FAK + Linalool	-80.975	Arg 252	Tyr 251, Arg 252, Tyr 347
FAK + Carvacrol	-69.791	Ile 126, Gln 150	Ile 126, Arg 127, Gln 150, Lys 255

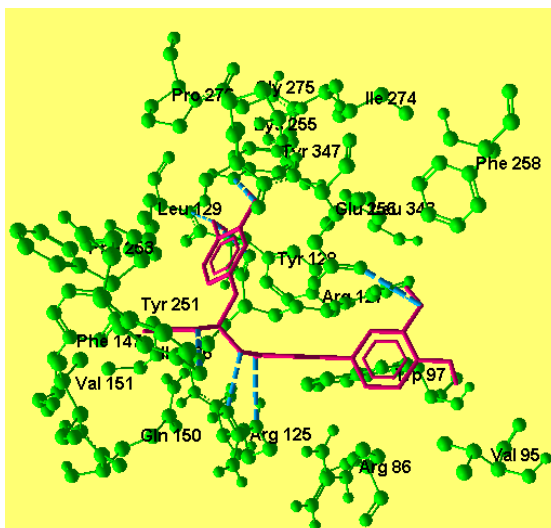


Figure 3: Binding pose of FAK with Rosmarinic acid

Further, the rosmarinic acid derivatives were docked with the target protein to identify the effectiveness of their binding to FAK. The results revealed that the derivatives show effective binding with FAK (Table 2). The target protein was then interacted with NNK to identify the binding sites for NNK on focal adhesion kinase. The binding site involves three amino acid interactions on FAK (Figure 4). Derivatives Lithospermic acid and Oresbiusin A also shows these amino acid interactions at the binding site that are similar to NNK (Figure 5). Hex software was used for this study and Biovia discovery studio for identification of amino acid interactions.

Table 2: Interaction profile of FAK with Rosmarinic acid derivatives

Protein-Ligand	Docking (Kcal/mol)	Score	Steric Interactions
FAK-Lithospermic acid	-315.98		Gly 132, Ser 248, Arg 252, Phe 253, Asp 254, Glu 277
FAK-Melitrin acid	-345.06		Pro 49, Thr 50, Trp 52, Gln 149, Tyr 180, Lys 204, Arg 205
FAK-Yunnaneic acid F	-337.89		Val 95, Trp 97, Arg 125, Arg 127, Asp 154, Glu 256, Asn 339
FAK- Oresbiusin A	-195.46		Gly 132, Phe 133, Leu 134, Ser 248, Pro 276, Glu 277

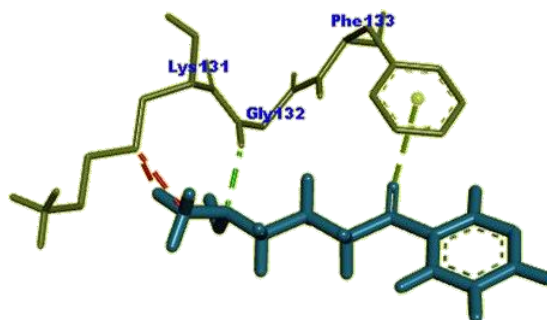
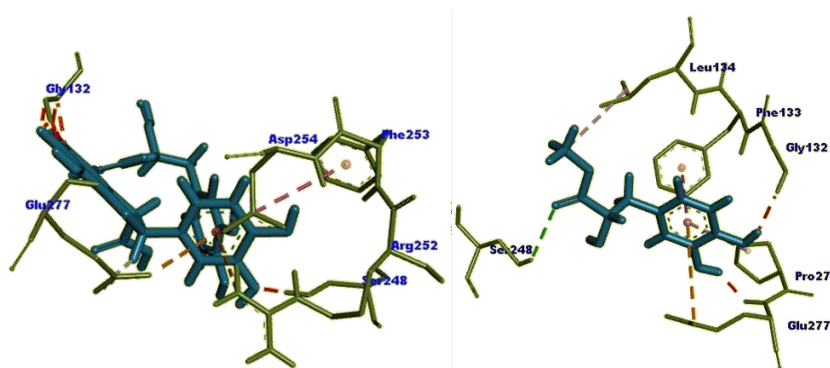


Figure 4: Binding pose of FAK with NNK



(a) (b)

Figure 5: Binding pose of (a) Lithospermic acid with FAK and (b) Oresbiusin A with FAK

DISCUSSION

Tobacco smoking is a critical health threat in our country and a major health concern worldwide; it causes millions of death annually. O'Keffe *et al.* (2018) have reported a mortality rate of 70 % in men and 55 % in women who are diagnosed with lung cancer and are smokers. Nitrosamine 4- (methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is one of the main carcinogens that plays a critical role in tumor development. Metabolically activated NNK can induce mutations in oncogenes and tumor suppressor genes by forming DNA adducts. The formation of adducts and induction of mutations are well recognized to be prime initiators of tumor growth (Czyzykowski *et al.*, 2016).

Studies by Shen *et al.* (2012) have revealed that NNK can enhance the activation of FAK signaling pathway which can contribute to cell proliferation and metastasis in non-small cell lung cancer. The *in-silico* observations of the present study have divulged the molecular interaction and binding, providing evidence to show that phytochemicals from certain culinary herbs can effectively bind with FAK. Rosmarinic acid is one of the phytochemicals that yielded a docking score to indicate highest binding energy with the target protein suggesting its efficiency in binding with strong affinity to the active site of FAK.

Further, the data revealed that in addition to rosmarinic acid, its derivative also binds to FAK with a strong binding interaction. The results also demonstrate that Lithospermic acid and Oresbiusin A (two rosmarinic acid derivatives) have higher binding affinity than NNK for the active site of the enzyme FAK. Thus these derivatives can competitively block the binding of NNK to the target protein active site. In further validation of this finding, it was observed that the rosmarinic acid derivatives and the NNK carcinogen share binding proclivity to similar amino acid sequence at their binding sites, which is responsible for the binding interaction with FAK. Gly 132 and Phe 133 are the key amino acids involved in the bonding at the active site of FAK. The binding interaction of rosmarinic acid derivatives was higher as compared to scores obtained for NNK, which was detected to be -191.09 Kcal/mol. This suggested that the phytochemicals of rosmarinic acid and its derivatives manifested greater binding affinity than NNK for the active site of FAK and could possibly competitively displace the carcinogen from its binding and subsequent activation of Focal adhesion kinase (FAK).

CONCLUSION

The molecular docking study indicated the strong binding affinity of the phytochemicals of rosmarinic acid and its derivatives to the active site of the enzyme focal adhesion kinase. These naturally occurring molecules could therefore possibly block the binding of NNK to FAK by behaving as competitive inhibitors. The present *in-silico* study therefore provides evidence of the efficacy of rosmarinic acid and its derivatives in inhibiting the activation of FAK by the tobacco associated carcinogenic product (NNK) and consequently curb its tumour inducing metastatic activity.

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