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Molecular Docking of Phytocompounds from Plants of the Lamiaceae Family Targeting the Oncogene Protein [PDB ID: 5p21]

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Abstract

Traditionally, plants such as *Mentha spicata*, *Plectranthus amboinicus*, and *Ocimum sanctum* from Lamiaceae are used for anticancer and other medicinal purposes. The present study deals with the analysis of the binding affinity mechanism of 10 selected compounds (apigenin, benzaldehyde, eugenol, geraniol, limonene, luteolin, vanillin, niacin, linoleic acid and butyric acid) of a few plants belonging to Lamiaceae family against the cancer targets oncogene protein (PDB ID-5P21) using Autodock 4.0 software. Based on the result, most of the selected herbal lead compounds were effective on the target oncogene protein. Predominantly, luteolin showed maximum interaction with oncogene protein (binding score -7.83) followed by apigenin (-7.65), eugenol (-6.36), niacin (-6.26) and vanillin (-6.07). This result will be helpful to select anticancer drugs from the Lamiaceae family effectively at a low cost. Further, the selected significant compounds will be tested in *in vitro* and *in vivo* studies.

Keywords: *Molecular docking; Mentha spicata; Plectranthus amboinicus; Ocimum sanctum*

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Introduction

Cancer is still a growing health problem worldwide. It is characterized by the irregular proliferation of cells, from which it progresses from normal to cancerous cells [1]. However, cancer to some degree is a preventable disease, as cancer risk can be reduced by avoidance of cancer-causing biological, chemical, and physical agents, in addition to the habitual consumption of cancer-protective foods. Medicinal plants occupy an important position for being the paramount sources of drug discovery in the modern era. Plants have been indispensable in treating diverse forms of diseases including cancer. According to World Health Organization (WHO) 80% of the people living in the rural areas depend on medicinal plants as primary health care system [2]. These practices are closely based on the knowledge of traditional use of medicinal plants. Natural herbal products are formulated with a combination of constituents from plants. Medicinal properties and health benefits of plants are attributed to their bioactive components, phytochemicals. A variety of phytochemicals are known to possess antitumor (antiproliferative, cancer-preventive, and apoptosis-inducing), antimicrobial (microbial growth-inhibiting, antibacterial, and antifungal), anti-inflammatory, analgesic, anesthetic, antioxidant (lipid peroxidation-inhibiting and radical scavenging), neuroprotective, and antiplatelet (platelet aggregation-inhibiting and antithrombotic) activity. Different types of effective drugs are being used to

enhance anticancer activities. Proper understanding of the complex synergistic interaction of various constituents of anticancer herbs would help in formulating the design to attack the cancerous cells without harming the normal cells of the body [3]. There are numerable scientific studies that have focused on the pharmacological activity of bio-active components from plants, increasing interest from scientific community as cancer suppressant.

Epidemiological studies suggest that the daily intake of certain phytochemicals can reduce the incidence of several types of cancers [4]. Thus, chemoprevention by dietary phytochemicals emerges as one of the most promising approaches for reducing the risk of cancer development. On the other hand, phytochemicals act in synergy with chemotherapeutic drugs to overcome cancer cell drug resistance, and further application of specific phytochemicals may also allow the use of lower concentrations of drugs in cancer treatment with increased efficacy. Bioinformatics plays a key role in creating useful information from raw biological data. Drug discovery is a complex and costly effort, where few drugs that reach the clinical testing phase make it to market. High throughput screening (HTS) is the primary method used by the pharmaceutical industry to identify initial lead compounds. Unfortunately, HTS has a high failure rate and is not efficient to identify viable drug leads. These shortcomings have encouraged the development of alternative methods to drive the drug discovery process. Drug discovery and development are time and resources consuming processes. So, the alternative method is the computational approach, and it consumes less time to screen the ligands on the basis of biological structures [5]. The drug and receptor binding can be studied by molecular docking approaches [6]. Molecular docking mainly focuses on free energy minimization during the binding of ligand and protein, with necessary conformation changes and it is also used for rational drug design, such as ligand-based and structured-based approaches, as well as systems biology modeling [7].

The Lamiaceae (Labiatae) is one of the most diverse and widespread plant families in terms of ethnomedicine and its medicinal value is based on the concentration of the volatile oil [8]. This family consists of more than 4000 species in 200 genera. Many species within this family are medicinal plants that are used for cancer therapy as well as food in raw and cooked forms. For example, *Mentha spicata*, *Plectranthus amboinicus*, and *Ocimum sanctum* from Lamiaceae are used for the treatment of cancer. However, there is limited scientific validation of their activities. So the present study was carried out to evaluate the binding ability of their compounds with oncogene protein [5P21] using docking analysis.

Material and Methods

A. Preparation of Protein Structure

Bioinformatics is seen as an emerging area with the potential to significantly progress how drugs are found and brought to clinical trials. Protein was downloaded from Protein Data Bank (PDB). The PDB ID of the target protein is 5P21. All water molecules were detached and in the final stage hydrogen atoms were added to a receptor molecule.

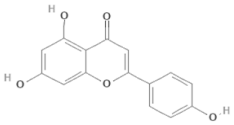
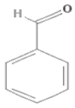
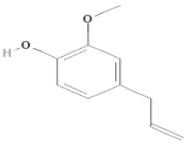
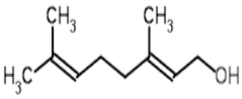
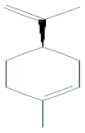
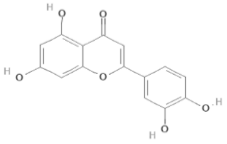
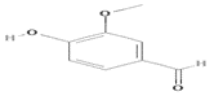
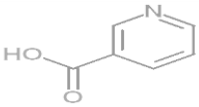
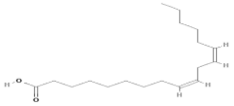
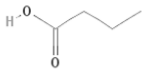
B. Preparation of Ligand Structure

Totally, 10 compounds of plants belonging to Lamiaceae were selected as ligands considering their biological activities (apigenin, benzaldehyde, fugenol, geraniol, limonene, luteolin, vanillin, niacin, linoleic acid and butyric acid). The structures of these anti-cancer compounds were drawn by using "ChemDraw". The structure of ligands is shown in Table 1. The docking analysis of anti-cancer drugs and oncogene protein (PDB I.D-5P21) were conceded by Auto-dock software. Docking permits virtually screening a database of compounds and predicting the most solid binders based on their scoring functions. It explores ways in which two molecules, such as drugs and an oncogene protein receptor fit together and dock each other well. The molecules binding to a receptor hinder its function and thus act as a drug [9]. Anticancer drugs and oncogene protein receptors were identified via docking and their relative strengths were evaluated using molecular dynamics and their binding affinities using free energy simulations. Anti-cancer drugs were docked with the oncogene protein receptor using parameters by default in Auto-dock software.

C. AutoDock

It is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoGrid calculates the energy of the noncovalent interactions between the protein and probe atoms that are located in the different grid points of a lattice that defines the area of interest. As a result of these calculations the output file of the protein-ligand complex with flexible residues and the ligand located within the binding pocket was obtained. Each structure was scored and ranked by the program by the calculated interaction energy.

Table 1: Structure of ligands from 3 different plants (*Mentha spicata*, *Plectranthus amboinicus*, and *Ocimum sanctum*)

S. No	Compound name	Chemical structure	Chemistry
1.	Apigenin		Flavone
2.	Benzaldehyde		Aldehyde
3.	Eugenol		Phenylpropanoids
4.	Geraniol		Terpenoid
5.	Limonene		Terpene
6	Luteolin		Flavone
7.	Vanillin		Phenolic aldehyde
8.	Niacin		Vitamin
9.	Linoleic acid		Fatty acids
10.	Butyric acid		Carboxylic acid

D. Protein-Ligand Interaction Using Auto-Dock 4.0

Auto-dock is an electronic structure program that is based on quantum mechanics. It foretells the potential energies, molecular structures, geometry optimization of structure, vibration frequencies of coordinates of atoms, bond length, bond angle and reactions pathway [10-11]. The oncogene protein receptor was docked against the obtained ligand using Auto-dock 4.0 to find the reasonable binding geometries and discover the protein-ligand connections. Docking of the protein-ligand complex was mainly targeted only on to the predicted active site i.e Gly13. Docking simulations were performed by selecting "Auto-Dock" as the docking engine.

E. Selection of lead compounds from Lamiacea Family

The reported chemical constituents of *Lamiaceae* were selected as lead compounds they are, apigenin, benzaldehyde, eugenol, geraniol, limonene, luteolin, vanillin, niacin, linoleic acid and butyric acid. Their structure was drawn using ACD/Chemsketch 2012 version 14.01 and it was saved as MDL molfiles for docking studies.

F. X-ray structure of anticancer target protein

The X-ray three-dimensional structure of the protein target was retrieved from the protein data bank (www.rcsb.org). The target is the oncogene protein (PDB ID: 5P21).

G. Docking tools

Molecular docking was performed using Autodock 4.0 software. This software is used for the estimation of energy during the interaction and to identify the best flexible ligand pose with minimum energy. The scoring function is based on the intramolecular interaction of ligands and protein during docking.

I. Analysis of docked protein-ligand complex structure

The best orientation for the ligand-protein complex was selected based on the H-bond energy score, rerank scoring (linear combination of E-inter (steric, Van der Waals, H-bonding and electrostatics), and Moldock scoring.

J. Oncogene

When certain normal genes have developed a mutation in most of the oncogenes are known as proto-oncogenes. Proto-oncogenes are the genes that mainly control what type of cell it is and how often it divides [12]. When a proto-oncogene creates mutation (changes) into an oncogene, it turns out to be a "bad" gene that can permanently change or be activated when it is not invented to be. This leads to the uncontrolled growth of the cell, which causes cancer. A few cancer syndromes are developed through inherited mutations of proto-oncogenes that cause the oncogene to be turned on (Activated). For example, multiple endocrine neoplasia type 2 (MEN2) is developed by an inherited mutation in the gene known as RET. People affected by this syndrome which develop an unusual thyroid cancer called medullary cancer of the thyroid. Mostly cancer-causing mutations concerning oncogenes are acquired, not inherited. They mainly stimulate the oncogenes through chromosomal rearrangements, gene repetition, or mutation. Tumour suppressor genes have normal genes that reduce cell division, repair DNA mistakes, or tell cells when to die (This process is called apoptosis or programmed cell death). Improper function of tumour suppressor genes reveals the cells can produce out-of-control, which leads to cancer [13].

Results and Discussion

The protein-ligand interaction plays a significant role in structural-based drug design [6, 14]. In the present work, cancer target oncogene protein (5P21) was docked with 10 compounds of Lamiaceae family. During the docking process, best minimum negative binding score was generated for each ligand.

A. Molecular Docking

Molecular docking studies were performed with auto-dock tool and for this docking of oncogene protein (PDB I.D-5P21) was docked with 10 different anticancer lead compounds and calculated the binding energy of these complex structures. When all the compounds show the binding energy with the oncogene protein. All complex shows negative binding energy but we have selected only minimum negative binding energy because it shows excellent binding to the receptor. The binding energy score of 10 leads compounds were 7.65, -5.13, -6.36, -5.25, -4.96, -7.83, -6.07, -6.26, -5.48, -5.18 respectively for Apigenin, Benzaldehyde, Eugenol, Geraniol, Limonene, Luteolin, Vanillin, Niacin, Linoleic acid and Butyric acid (Table 2 and “Figures 1-5”). From this analysis, we have selected the top 5 highest negative binding energy scoring (Table 3) compounds and visualized and analysed the result using UCSF CHIMERA. The phytocompounds, Luteolin was found to bind at the binding site of oncogene protein with the highest negative binding energy -7.83 Kj/Mol followed by Apigenin (-7.65), Eugenol (-6.36), Niacin (-6.26) and Vanillin (-6.07) (Table 2). Thus, it is inferred that all the compounds above the plants have potent anticancer activity.

In-silico molecular docking is one of the most powerful techniques to discover novel ligands for receptors of known structure and thus play a key role in structure-based drug design [15]. Molecular docking continues to holds great promise in the field of computer-based drug design which screens small molecules by orienting and scoring them in the binding site of protein. The genus Mentha have also been shown to have many biological activities and especially antitumoural activity. Many studies have demonstrated the effect of Mentha species inhibiting the cell proliferation of numerous tumour cells by acting on mitochondrial dysfunction, apoptosis induction, and autophagy processes [16]. The GC-MS analysis of *Mentha spicata* contains benzaldehyde and D–Limonene [17]. Ethanolic extract of *Plectranthus amboinicus* shows significant anticancer activity against MCF-7 cancer cell line [18]. The GC-MS analysis of *Plectranthus amboinicus* contains many volatiles and nonvolatile compounds it includes apigenin, eugenol, geraniol, limonene and luteolin [19].

Phytochemical compounds of *O. sanctum* such as eugenol, linoleic acid and β-sitosterol prevent skin, liver, oral and lung cancers by increasing the antioxidant activity, inducing apoptosis, altering the gene expression and inhibiting metastasis [20]. Sridevi *et al* (2016) reported that *O. sanctum* extract shows anticancer activity by decreasing cell proliferation, increasing intracellular ROS, alternation in mitochondrial membrane potential and apoptosis in NC1- H460 cell line [21]. Apigenin was reported to suppress various human cancers *in vitro* and *in vivo* through multiple biological effects, such as triggering cell apoptosis and autophagy, inducing cell cycle arrest, suppressing cell migration and invasion, and stimulating an immune response [22]. Eugenol shows inhibitory activity against breast cancer cell lines [23]. Geraniol exhibits a significant anticancer effect against colo-205 cancer cell line by inducing apoptosis in colo-205 cells. The apoptosis was also associated with the upregulation of Bax and the downregulation of Bcl-2 expressions, indicative of mitochondrial apoptosis. Moreover, geraniol could trigger DNA damage and G2/M cell cycle arrest in colo-205 cells [24]. Limonene shows antiproliferative and antitumour activity against lung cancer cell line A549 [25]. Luteolin can inhibit the in vivo growth of gastric tumors, this mechanism may correlate with downregulated expression of VEGF-A and MMP-9 [26]. Vanillin suppressed metastasis in breast cancer cell lines [27]. Treatment with niacin activates the PI3K/Akt cascade in the A431 human epithelial carcinoma cell line [28]. Linoleic acid is a major compound in almond oil that may have anticancer and anti-proliferative effects on colon cancer cells through molecular signaling pathways and, thus could be a novel therapeutic agent [29]. Novel butyric acid prodrugs provide a promising treatment strategy for malignant gliomas as single agents and in combination with radiation therapy [30]. The above studies are supported in our present work. Thus it is concluded that the compounds under study may be used as lead for the preparation of a drug for the treatment of cancer.

Table 2: Docking score of anticancer ligands with oncogene protein.

S.NO	Receptor	Ligand	Run	Docking score
1	Oncogene protein	Apigenin	1	-7.65
2	Oncogene protein	Benzaldehyde	1	-5.13
3	Oncogene protein	Eugenol	3	-6.36
4	Oncogene protein	Geraniol	7	-5.25
5	Oncogene protein	Limonene	6	-4.96
6	Oncogene protein	Luteolin	1	-7.83
7	Oncogene protein	Vanillin	4	-6.07
8	Oncogene protein	Niacin	10	-6.26
9	Oncogene protein	Linoleic acid	1	-5.48
10	Oncogene protein	Butyric acid	5	-5.18

Table 3: Binding score of anticancer ligands with oncogene protein.

S.NO	Molecule	Run	Binding score
1	Luteolin	1	-7.83
2	Apigenin	1	-7.65
3	Eugenol	3	-6.36
4	Niacin	10	-6.26
5	Vanilin	4	-6.07

Conclusion

This study established that the selected compounds have a notable interaction with the target oncogene protein. Among the selected ten compounds, luteolin had wide, strong affinity with the target oncogene protein. Followed by apigenin, eugenol, niacin, and vanillin had strong affinity with oncogene protein. Hence, it is recommended that the significant bioactive compound should be investigated further as an anticancer drug through clinical trials.

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Figure 1: Interaction of luteolin with oncogene protein.

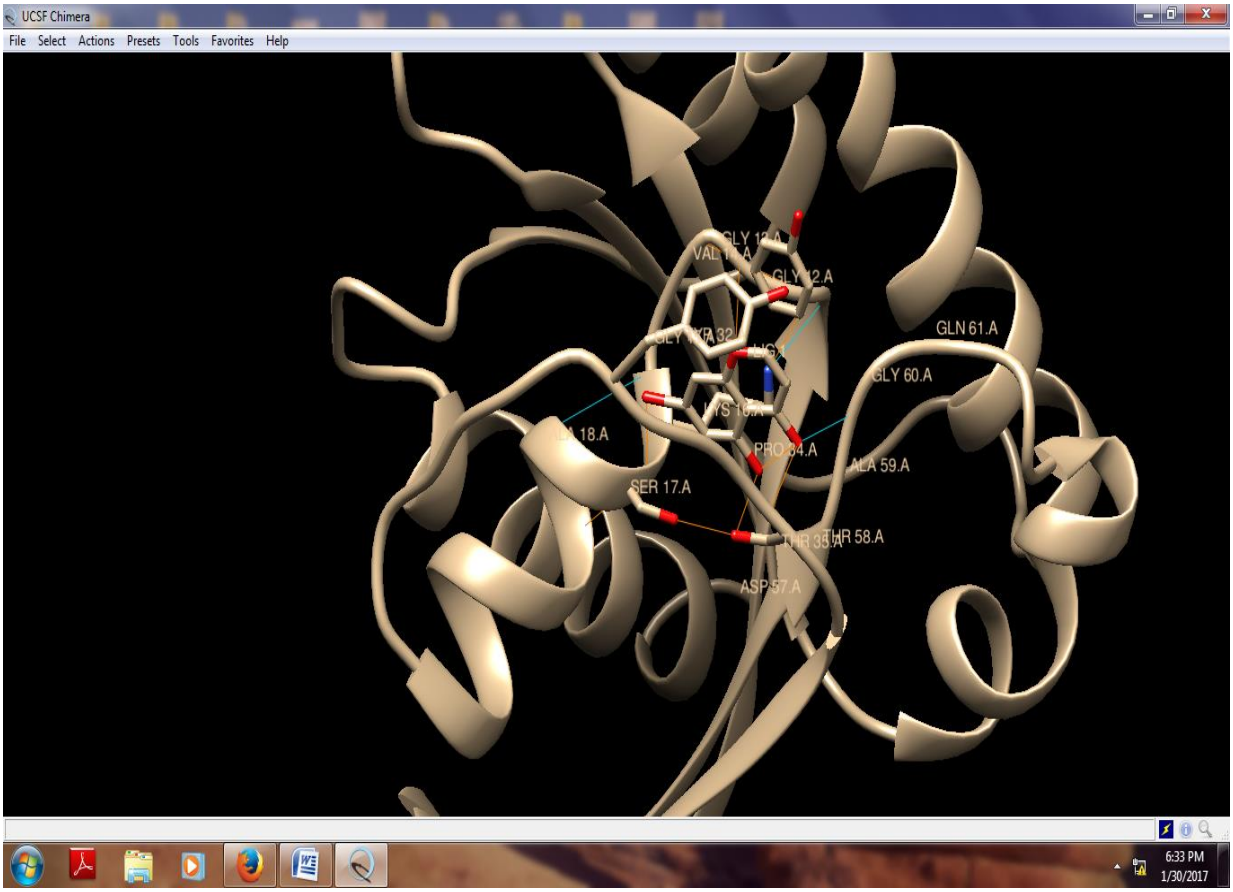

Figure 2: Interaction of apigenin with oncogene protein.

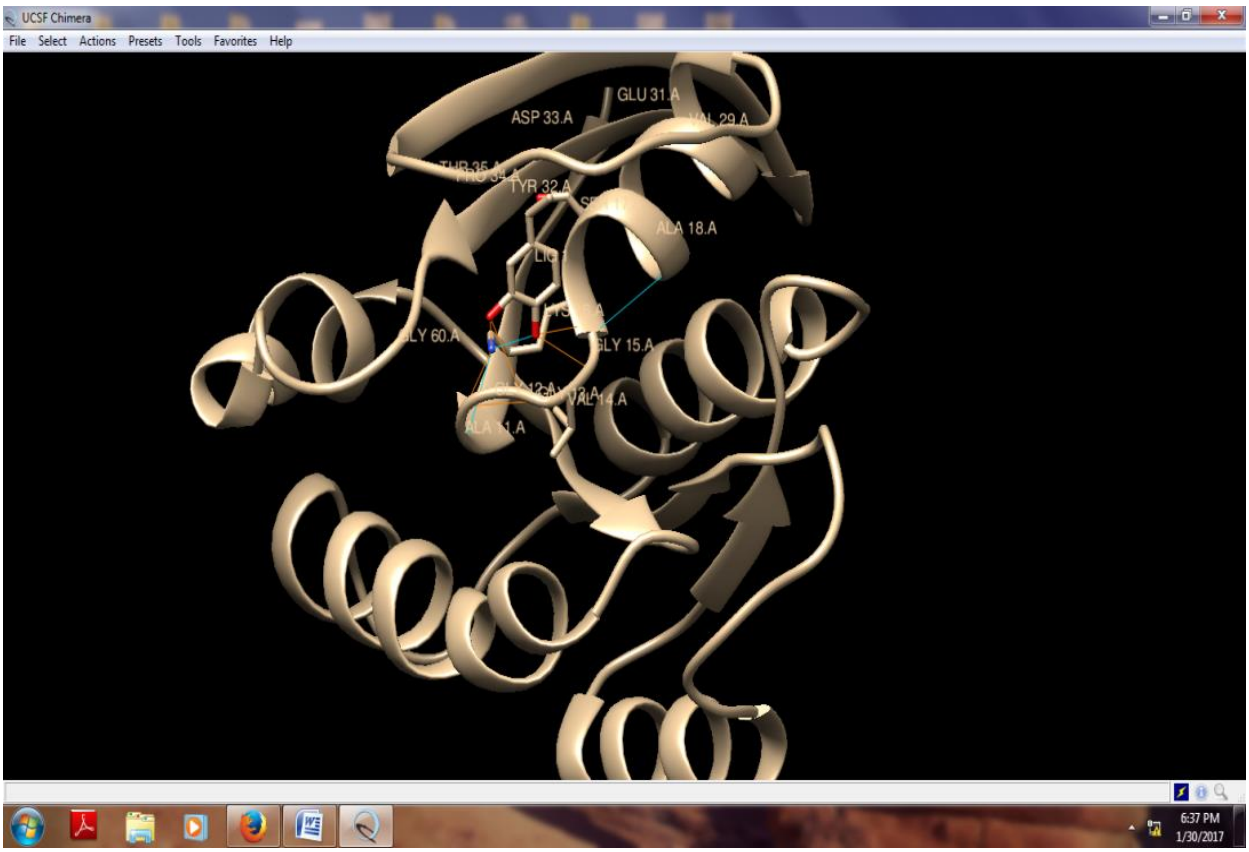

Figure 3: Interaction of eugenol with oncogene protein.

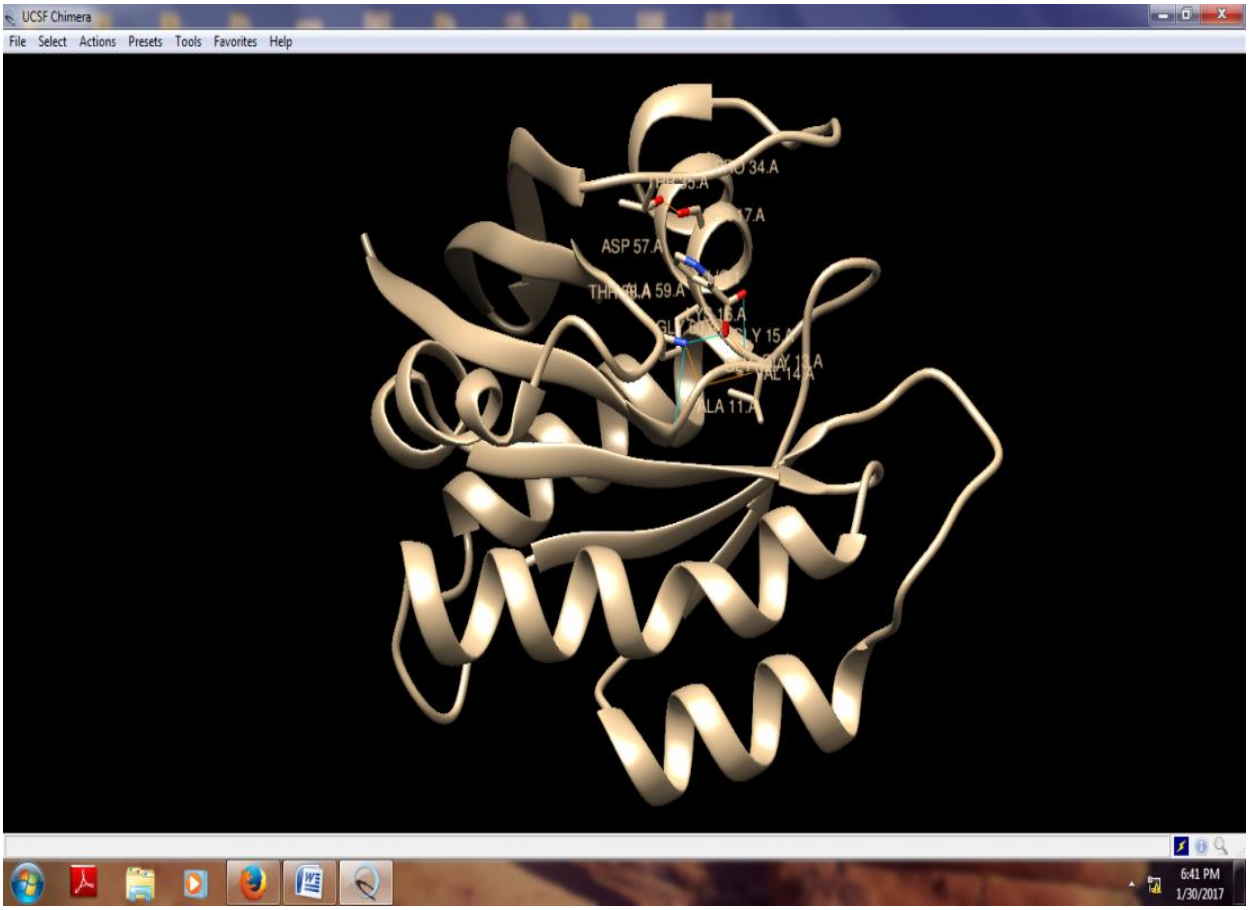

Figure 4: Interaction of niacin with oncogene protein.

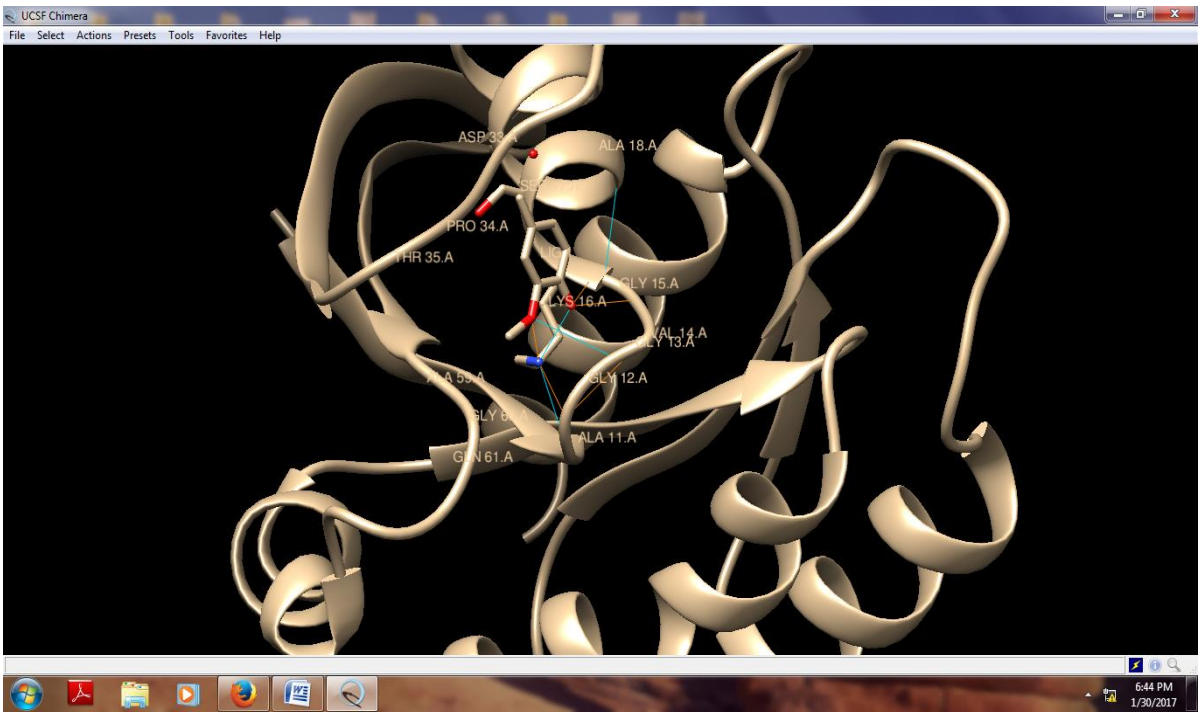

Figure 5: Interaction of vanillin with oncogene protein

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