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In-Silico Validation of Niazinin-A Against Proinflammatory Mediator: Anti-Proliferative Potential

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Abstract

Background: Cancer is one of the most dreaded human diseases, that has become an ever-increasing health problem and is a prime cause of death globally. Munga, also known as Moringa oleifera Lam., is one of the most significant plants grown extensively in India. This plant, Moringa oleifera Lam, is used extensively as a dietary supplement and has valuable pharmacological properties including anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-cancer, antimicrobial, anti-oxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic action. This plant has great therapeutic properties in every area. It is a good source of milk protein, vitamin A, and vitamin C. Alkaloids, protein, quinine, saponins, flavonoids, tannin, steroids, glycosides, fixed oil, and lipids are only a few examples of the several active phytoconstituents that are present. ***Aim:*** The current work sought to elucidate the molecular basis for Niazinin A antiproliferative activity against the *VEGF-1 & AURKA*, which functions as a proinflammatory factor in proliferation. ***Method*:** A molecular docking method was employed in the current work to look for VEGFR-1 & AURKA protein inhibitors. The binding was determined by the Auto Dock software utilising a grid-based docking method. ***Results*:** The molecular docking result revealed that Niazinin A showed encouraging docking score. The docking score found to be -7& -6.11 for VEGF-1 & AURKA kcal mol^{-1} respectively. ***Conclusion:*** The interaction of ligand hits to targeted site and docking score finding it can be predicted that Niazinin A found in the plants *Moringa* exhibited good inhibitor of VEGF-1 & AURKA protein.

Keywords: Niazinin A, VEGF-1(Vascular endothelial growth factor), AURKA *(Aurora kinase A)* protein, in-silico molecular docking & Anti proliferative activity.

INTRODUCTION

The process of researching and developing novel chemicals with specific effects on humans is known as drug designing [1]. The number of microbe-caused illnesses that are multidrug resistant is growing every day, necessitating the development of a new class of antimicrobial drugs [2]. Cancer is a severe health issue and the second leading cause of mortality worldwide. The underlying reason is cell cycle dysregulation, which hinders cellular differentiation and encourages unregulated cellular proliferation [3, 4]. Therefore, it is imperative to develop novel bioactive molecules with chemical structures and modes of action that differ significantly from those of commercially available medications [5]. Discovery of medicines is a sluggish, lengthy costly and interdisciplinary technique but the new discoveries have transformed the means by which researchers create innovative therapeutic chemicals e.g. The cost of medication design is reduced by up to 50% thanks to CADD technology. The molecular docking technique [6] is used to comprehend the I drug-receptor interaction, II binding affinity, III orientation and approach of drug molecules to the target site. Precise structural modelling and accurate activity forecasting are the main objectives of docking investigations. It provides the most upbeat depiction of drug-receptor interaction and develops a new rational method to drug creation.

The *Moringa oleifera* plant is also known as the drumstick tree and the horse radish tree. Zeatin, quercetin, beta-sitosterol, kaemopferol, and caffeoylguinic acid are all present in significant and uncommon amounts in munga plants. Iron, potassium, calcium, copper, zinc, magnesium, manganese, and other essential elements are found in Moringa oleifera. Deic,

palmitic and stearic acid, saponins, glycoside, gum, and protein are the primary components of the moringa plant. Vitamins: B1, B2, B3, C, and A (8855 IU per 100g). Calcium, iron, phosphorus, and magnesium are minerals. Significant amounts of the vitamins A, B, and C, riboflavin, nicotinic acid, folic acid, pyridoxine, beta-carotene, calcium, iron, and alpha-tocopherol can be found in the leaves, flowers, and pods. The high flavonoid concentration of the Moringa genus contributes to its potent antioxidant effect. Most of the flavonoids in the genus are found in their flavanol and glycoside forms. The most common flavonoids in the genus include rutin, quercetin, rhamnetin, kaempferol, apigenin, niazinin A, and myricetin. The species' most abundant glucosinolate is 4-O-(-L-rhamnopyranosyloxy)-benzyl glucosinolate. The most prevalent phenolic acid in M. oleifera leaves is gallic acid. Additionally, elagic acid, ferulic acid, caffeic acid, o-coumaric acid, and chlorogenic acid are present in the leaves. The leaves and seeds were used to isolate -sitosterol. Antispasmodic, anti-hypertensive, anti-inflammatory, anti-fertility, anti-hyperglycemic, anti-hyperlipidemic, and hypocholesterolemic, as well as antiviral, anti-leishmanial, anti-convulsant, anti-microbial, and anticancer activities were all demonstrated by the plant [7-9].

EXPERIMENTAL WORK

Ligand Preparation:

2D Structure of ligand Niazinin-A was drawn using Chem Sketch [10], the two-dimensional structures of the prepared ligand were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structure of the prepared ligand Niazinin-A was given below:

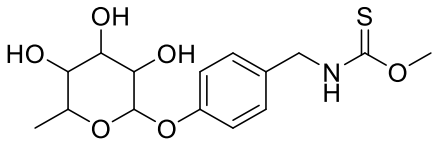


Figure 1: 2D structure of Niazinin-A

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing and grid points for both the receptors Aurora kinase-1 and VEGFR1 were given in table 1 [11-12].

Table 1: Grid parameters used in current docking analysis of Aurora kinase-1 and VEGFR1 receptors

S. No	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	Aurora Kinase-1	40	40	40	0.453	-9.284	25.387	-7.076
2	VEGFR1	40	40	40	0.392	3.911	17.995	32.857

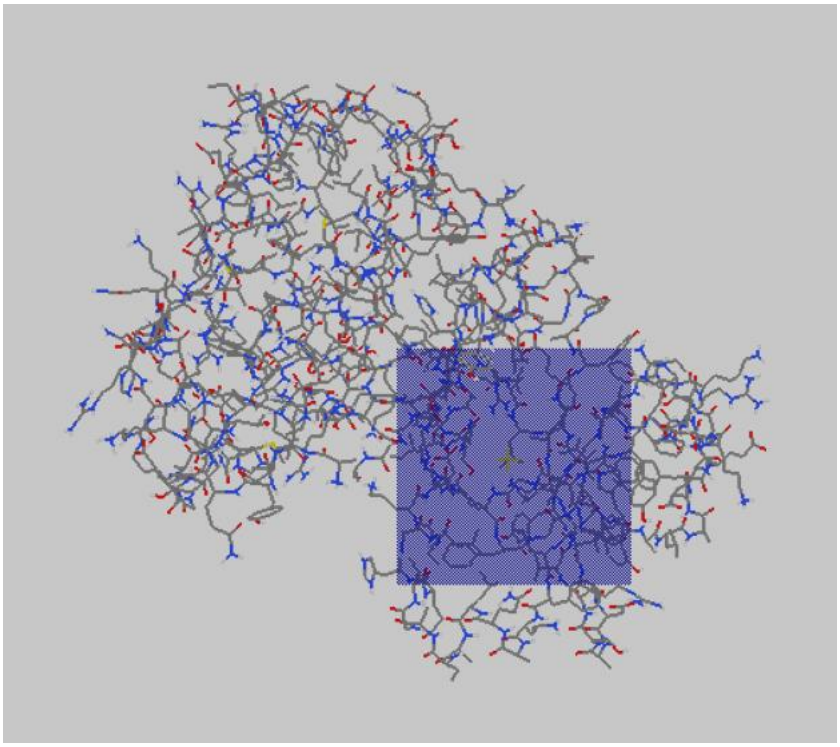


Figure 2: Grid box covering all active sites in aurora kinase-1 receptor

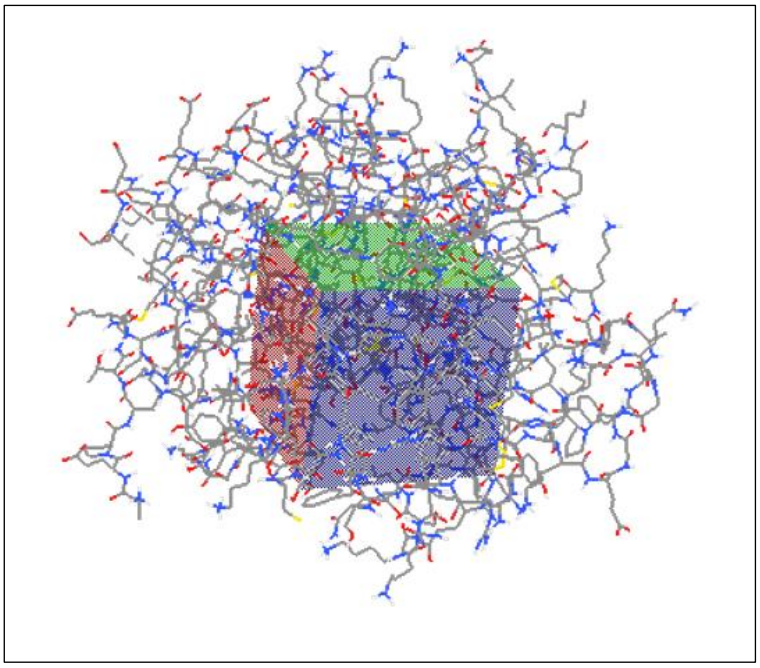


Figure 3: Grid box covering all active sites in VEGFR1 receptor

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [13-15].

Docking Study

Crystal structure

The crystal structures of the protein consisting of Aurora kinase-1 and VEGFR1 receptor were downloaded from the Protein Data Bank portal. All the primary information regarding receptor and aurora kinase-1 structures (6gra.pdb) and VEGFR1 (3hng.pdb) were registered in the Protein data bank was used [16-18]. The complex ligand for both the receptors were separated by using Chimera software.

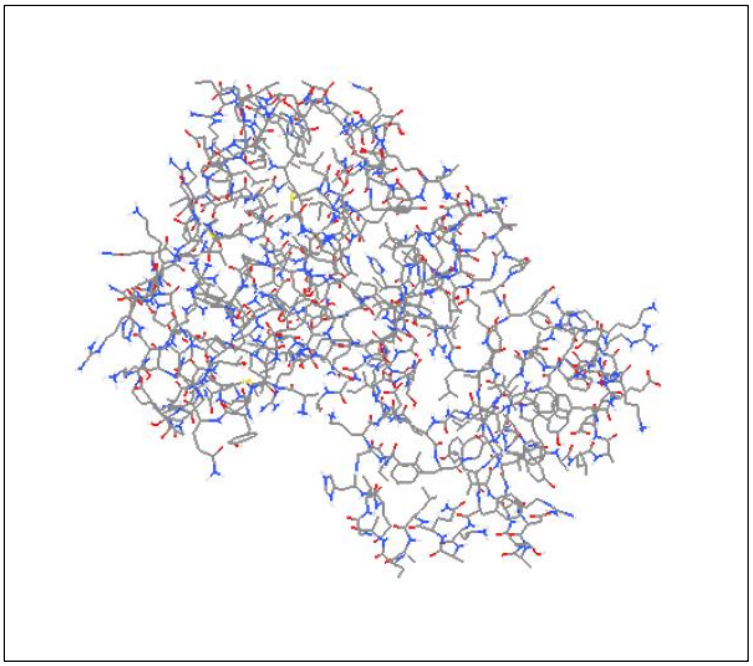


Figure 4: Crystal structure of aurora kinase-1 receptor (PDB ID-6gra)

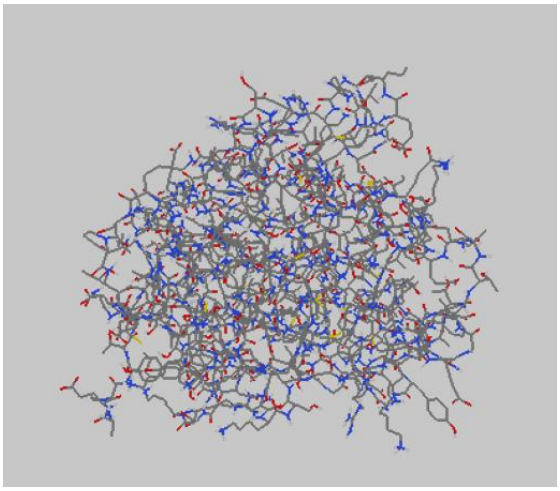


Figure 5: Crystal structure of VEGFR1 receptor (PDB ID-3hng)

Processing of Protein

Both the downloaded receptors are having only one chains, i.e. chain A, which has been selected for experimental purpose and complex ligand was removed from it. The bound ligand was separated from the macromolecular complex by using software Chimera [19].

Molecular Docking Simulation Studies

Docking of ligand niazinin-A against aurora kinase-1 and VEGFR1 receptors were performed by Autodock. All the bonds of ligand were kept flexible, while no residues in receptor were made flexible [13-15].

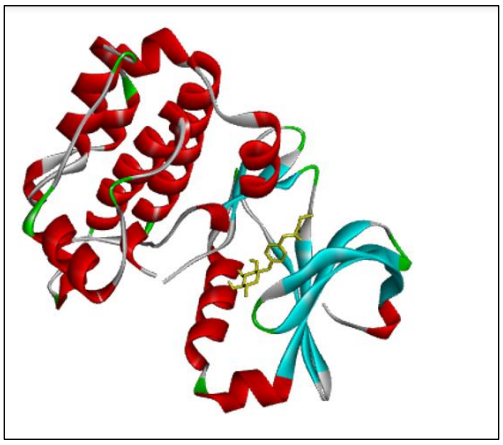


Figure 6: Binding mode of Niazinin-A within the active site of aurora kinase-1 receptor

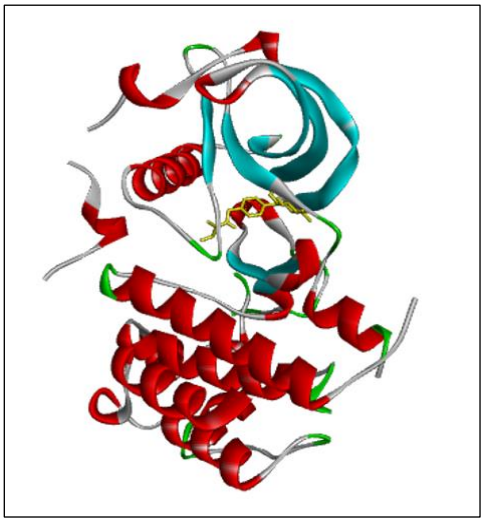


Figure 7: Binding mode of Niazinin-A within the active site of VEGFR1 receptor

Toxicity & ADME-T Studies

The ligand molecule Niazinin-A was studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [20].

RESULT AND DISCUSSION

Nature has long been a strange source of pharmaceutical components, giving us a variety of medicinal plants that produce useful phytochemicals. Niazirinin A was chosen as the lead compound for in silico molecular modelling research to evaluate the anti-proliferative ability of M. oleifera leaf bioactive against Aurora kinase-1 and VEGFR1 receptors.

The majority of cancer therapy strategies currently in use place a strong emphasis on the surgical removal of tumor masses. In addition to other chemical and physical therapies, chemotherapy and radiotherapy have considerably reduced the spread of cancer cells. These tactics are frequently combined to enhance treatment indices. It is commonly recognized that treatments like surgery, chemotherapy, and radiotherapy impede healthy cell growth as well. The severe side effects and excessive toxicity of many therapeutic procedures can contribute to a poor quality of life.

The postulated mechanism of Niazirinin A as an antiproliferative medication was clarified through in-silico molecular docking research utilising autodock. First, a target protein was chosen for the molecular modelling inquiry based on a review of the literature. Molecular docking techniques are used to identify the ideal orientation of a ligand to its molecular target with the least amount of free energy in the development of a stable complex, in accordance with a brief definition. This computational drug design strategy can be said to be more thorough, time- and money-efficient, and complete when compared to other conventional cancer therapy techniques. Another benefit of using molecular modelling in pharmaceutical research is the potential for increased production and quality.

One important measure produced by molecular docking is the binding energy between the protein and ligand. This offers details on the protein and ligand-receptor docking's binding affinity and effectiveness. The stronger the binding affinity and docking, the lower the binding energy value. In this study, the binding energies of the chosen proteins Aurora kinase-1 and VEGFR1 to Niazirinin A and amino acid residues were calculated using molecular docking analysis.

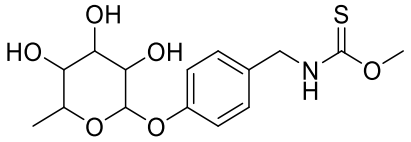
The bioactive substance Niazirinin A has the best binding affinities for Aurora kinase-1 and VEGFR1, with respective binding energies of -6.11 kcal/mol and -7 kcal/mol. According to the results of the experiment, some bioactives lessen the growth of cancerous cells, which also kills them. It promotes apoptosis at growth phase 1 (G1) and cell arrest at growth phase 2 (G2), also known as the mitotic phase (M). Table 2 contains a summary of the docking outcomes. Figure 6-7 depicts the Niazirinin A's way of binding to the VEGFR1 receptor and Aurora kinase-1's active site. Niazinin A's 2D and 3D binding interactions with Aurora kinase-1 and VEGFR1 are depicted in Fig 8–12. The binding interaction of niazinin A with Aurora kinase showed conventional hydrogen binding at Ala 273,Glu 181,Asp274 having Pi-sigma binding at Lys 162 & Glu211 along with covalent interaction at Lys 143,Val147 &Leu 210 whereas interaction of niazinin A with VEGFR-1 receptors showed conventional hydrogen binding at Cys 912 & Glu910, Pi-sigma binding at Val892 and covalent binding at Phen1041, Leu 833,Leu 1029,Val 841,Cys 1039 & Val 909.Therefore, it is of interest to design and develop new yet effective compounds against *Aurora kinase-1* and *VEGFR1* from medicinal plants. Specifically targeting VEGFR-1 signal transduction or VEGFR-1 exclusive ligands (such as PlGF or VEGF-B) may be an effective strategy to hinder the development of tumor-associated vessels. In fact, PlGF and VEGFR-1 overexpression promotes ECM invasion and resistance to anti-VEGF-A therapy in a number of tumour types. Importantly, VEGFR-1-blocking treatments are anticipated to have less side effects than those targeting VEGFR-2 since VEGFR-1 is essential for tumor-associated angiogenesis but not physiological angiogenesis. The amount of sVEGFR-1 appears to be a key factor in the development of numerous cancer types, as evidenced by studies linking the VEGF-A/sVEGFR-1 ratio found in tumour tissue, serum, or plasma samples to survival time, malignancy grade, and therapeutic response [21]. The Aurora kinases, a family of serine/threonine kinases that comprises Aurora A (AURKA), Aurora B (AURKB), and Aurora C, control the process of chromosomal segregation during mitosis (AURKC). Cell division depends on these kinases. In addition to mitosis, it is believed that aurora kinases also regulate meiosis. The removal of Aurora kinases may stop cell division and obstruct embryonic development. It is known that the Aurora kinase gene is amplified or overexpressed in a number of cancers. Inhibiting Aurora kinases has also been linked to research that suggest it may improve the effects of chemotherapy. A variety of Aurora kinase inhibitors (AKIs) have been developed over the past few decades, and they have successfully delayed the development and spread of many cancers both in vivo and in vitro, suggesting that Aurora kinases may be a potential therapeutic target.

In malignancies, AURKA also mediates the oncogenic effects of Myc (N-Myc, c-Myc, and L-Myc). In human cancers, Myc overexpression or activation is frequently observed. By interacting with the CCCTCCCCA motif in the NHE III1 region and functioning as a Myc regulator, AURKA can activate c-Myc transcription. On the other hand, c-Myc can transcriptionally up-regulate AURKA by attaching to the AURKA promoter, resulting in a positive feedback loop.

The transcription of genes linked to the cell cycle is also triggered by c-Myc activation, which encourages cell proliferation and Myc-induced lymphomagenesis [22].

The docking results were produced and shown using pymol. As shown by the test, the docking score for the selected compound is similar enough and thus reflects their maximal activity against both selected target receptor. The findings showed that the investigative molecules had higher energy values on the VEGFR1 protein, which means that Niazinin A have greater affinity and steric compatibility with VEGFR 1 protein. The pharmacokinetic profile of Niazinin A reveals that it is having good pharmacokinetic profile without presence of any major toxic effects. The pharmacokinetic and toxicity profiling results of Niazinin were shown in Figure 14.

Table 2: Results of docking of ligand Niazinin-A against aurora kinase-1 and VEGFR1 receptor

S. No	Compound Name	Structure	Binding Energy (Kcal/mole)	
			Aurora kinase-1	VEGFR1
1	Niazinin-A		-6.11	-7.0

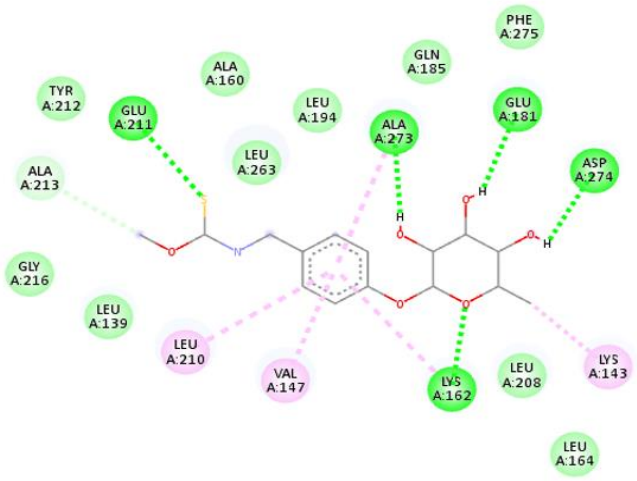


Figure 8: Two-dimensional binding mode of Niazinin-A within the active site of aurora kinase-1 receptor

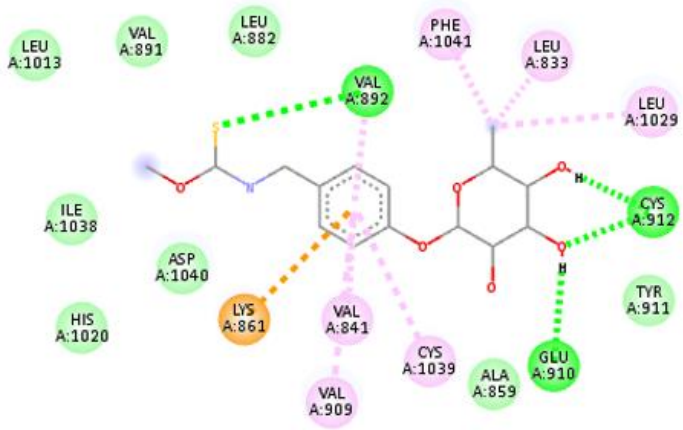


Figure 9: Two-dimensional binding mode of Niazinin-A within the active site of VEGFR1 receptor

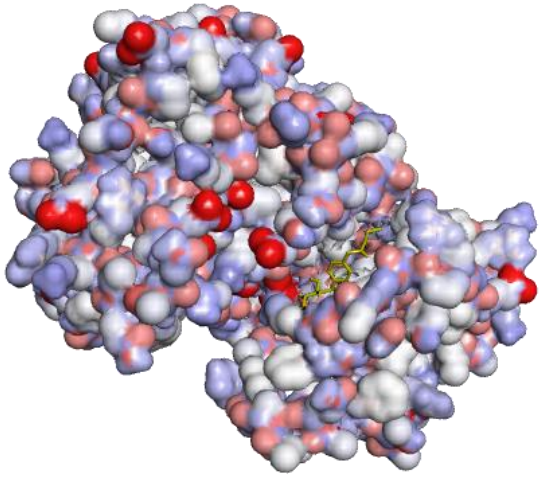


Figure 10: Three-dimensional binding conformation of Niazinin-A within the active site of aurora kinase-1 receptor

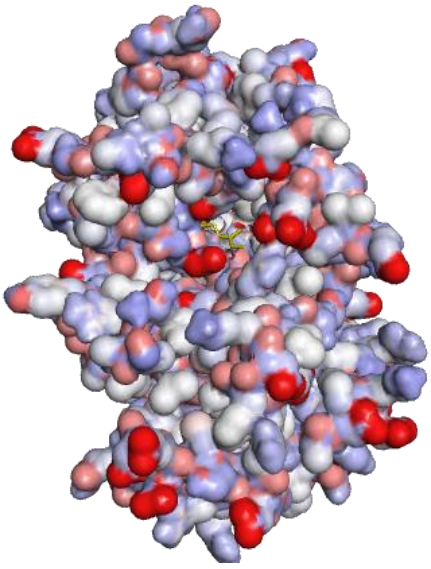


Figure 11: Three-dimensional binding conformation of Niazinin-A within the active site of VEGFR1 receptor

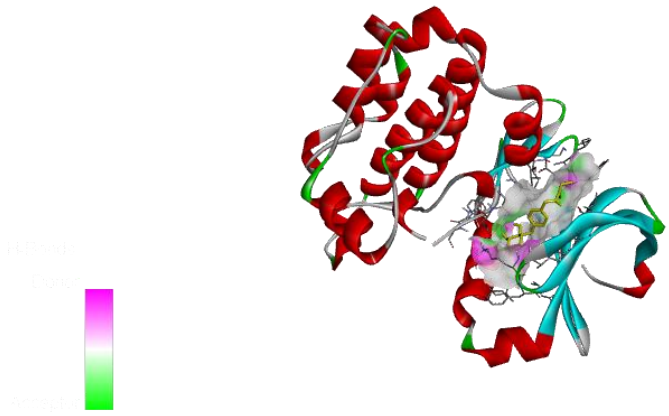


Figure 12: Three-dimensional binding mode of Niazinin-A within the active site of aurora kinase-1 receptor

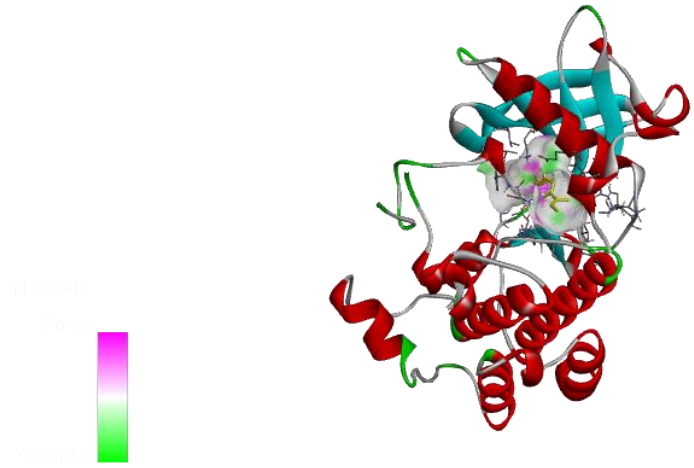


Figure 13: Three-dimensional binding mode of Niazinin-A within the active site of VEGFR1 receptor

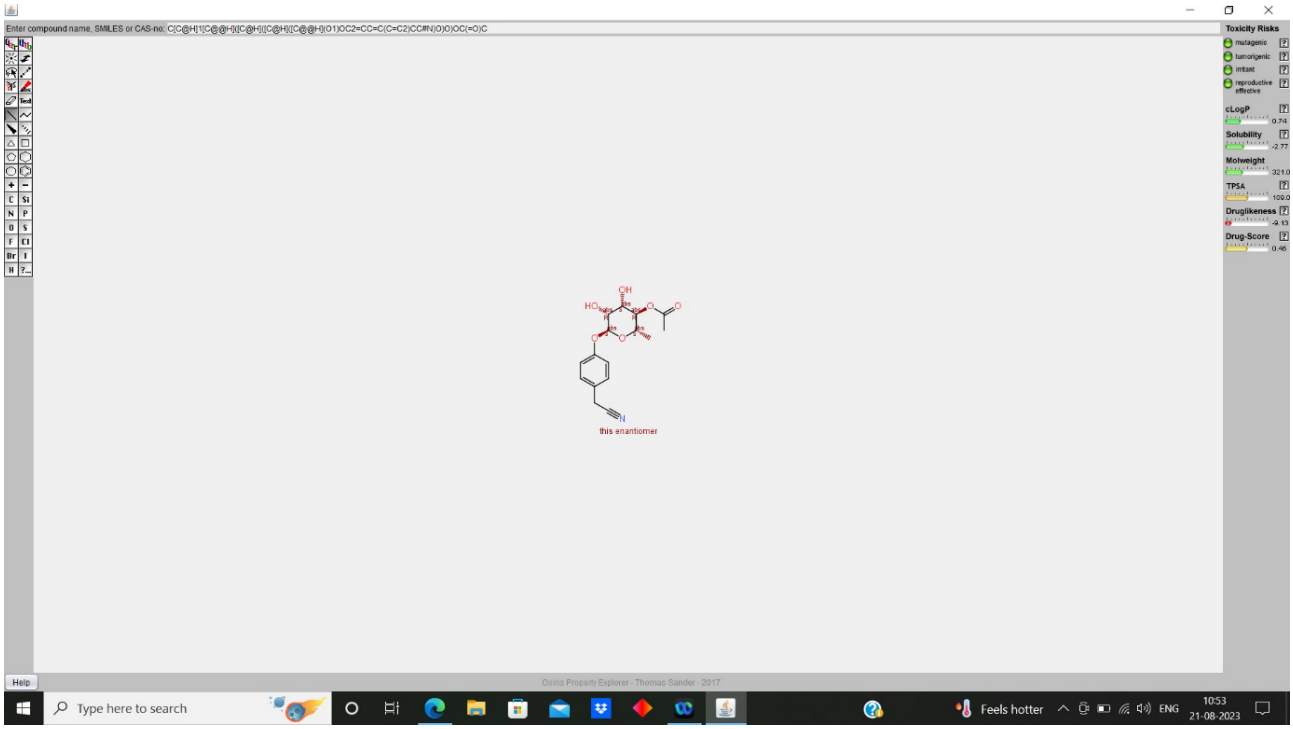


Figure 14: Pharmacokinetic and toxicity profiling of Niazirinin.

Divulgence of Investigation

The chemical interaction of Niazinin A with the amino acids in the active pockets was demonstrated by molecular docking studies with Aurora kinase-1 and the VEGFR1 receptor. Theoretically, the ligand molecule has demonstrated a positive docking score, and it is possible to predict that niazinine A is a suitable target receptor inhibitor. The main regulator of angiogenesis in cancer, oncogene expression, a number of growth factors, and hypoxia all contribute to the up-regulation of VEGF. A tumor needs blood vessels for nourishment and oxygen before it can grow larger than 1-2 mm, making angiogenesis crucial for the formation and growth of cancer. The investigation's findings indicated that Niazinin A has a high binding energy to the VEGFR1 receptor, and it is possible to infer that the chosen bioactive has an inhibitory effect on the receptor by preventing angiogenesis in cancer cells, which causes cell death.

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