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Targeting CDK2 in Lung Cancer: In-Silico Exploration of Natural Compounds Derived from *Moringa oleifera* †

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Abstract

Lung cancer, encompassing tumors that originate in the bronchi or lung parenchyma, is one of the leading causes of cancer-related mortality worldwide. Historically, it was relatively uncommon at the beginning of the 20th century; however, the sharp increase in cases over subsequent decades has been primarily attributed to the rise in smoking among both men and women. Tobacco use remains the most significant cause of lung cancer, with cigarette smokers accounting for 80–90% of cases. In this context, *Moringa Oleifera*, commonly known as the drumstick tree, has attracted attention for its potential anticancer properties. Studies have demonstrated that extracts from *M. oleifera* exhibit strong anticancer activity against various tumors, including breast cancer, by modulating cell cycle regulatory genes and promoting apoptosis. This study aims to perform an in silico screening of 191 bioactive compounds from *M. oleifera* to identify potential natural inhibitors of Cyclin-Dependent Kinase 2 (CDK2), thereby exploring novel avenues for lung cancer treatment. The obtained results revealed that the plant contains compounds with high binding affinity toward CDK2, with the best-docked compound showing a binding free energy of −8.1 kJ/mol, which also passes Lipinski's rule of five for drug-likeness.

Keywords: lung cancer; CDK2 inhibitors; *Moringa oleifera*; molecular docking; ADMET

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1. Introduction

One of the main causes of cancer-related mortality globally, lung cancer claims the lives of more than a million people each year. Non-small cell lung cancer accounts for around 80% of cases. The success rate of conventional cancer therapies, including radiation, chemotherapy, and surgery, is poor. Therefore, creating new medications is essential to halting the spread of lung cancer [1,2]. Because protein kinases play a crucial role in controlling a variety of cellular signal transduction pathways, targeting them has emerged as one of the most popular and successful cancer therapy strategies. Numerous essential physiological functions, such as transcription, metabolism, apoptosis, differentiation, cell proliferation, and survival, are regulated by these kinases. Protein kinases support both healthy cellular activity and the pathological processes linked to cancer by regulating these vital activities [3]. CDK2 is involved in many biological processes and is essential for controlling the cell cycle. In processes including DNA damage, intracellular transport, protein degradation, signal transduction, DNA and RNA metabolism, and translation,

CDK2 interacts with and phosphorylates proteins. Many human malignancies have unregulated CDK2 and its regulatory components, and there is growing evidence that inhibiting CDK2 has antitumor effects in a subset of tumours with certain hereditary characteristics. Prior CDK2 inhibitors had off-target effects and lacked specificity. A potential treatment option for CDK2-dependent malignancies is the creation of novel CDK2 inhibitors [4]. New treatment strategies must be developed in light of the rising prevalence of cancer worldwide. Because of its strong approximation skills, in-silico virtual screening of potential therapeutic compounds is gaining a lot of attention. Herbal therapy is a very effective alternative to Western medicine in the battle against cancer. Herbal medicine has evolved into a safe, non-toxic, and reasonably accessible source of cancer-curing compounds. Herbs are believed to combat the effects of ailments in the body due to their numerous characteristics. The purpose of this study is to offer a suggestion for the usage of natural substances, which are often found in the Indian subcontinent region [5]. *Moringa oleifera* is one such important medicinal plant that is used in all traditional medical systems. The drumstick tree, or *M. oleifera*, is a member of the *Moringaceae* family and a vegetable in the Brassica order [6]. In addition to being a common food in these areas, moringa is also well-known and utilised for its health advantages. It has gained the nickname "the miracle tree" among ordinary people because of its remarkable capacity to treat a wide range of illnesses, including some chronic conditions. Due to the plant's numerous uses, numerous studies were conducted to separate bioactive chemicals from different plant portions [7]. Because of their low cost, herbal plants, sometimes referred to as phytomedicine, are still widely used and trusted as an alternative in the medical sector [8]. As an anti-inflammatory, antioxidant, anticancer, antidiabetic, and cardioprotective agent, M. oleifera has a wide range of therapeutic uses. It has hepatoprotective and renoprotective properties in addition to antibacterial action. In light of this, the current study was created to find potential natural compounds from *M. oleifera* that target CDK2 by virtually screening a chemical database in order to further lung cancer treatment approaches.

2. Methodology

2.1. Protein Preparation

The full length of Cyclin-Dependent Kinase 2 (CDK2) structure downloaded from Protein Data bank (https://www.rcsb.org/). It is a high-resolution crystal structures of human cyclin-dependent kinase 2 with and without ATP: bound waters and natural ligand as guides for inhibitor design (PDB entry code: 1HCK; resolution 1. 90 Å, R-value Free: 0.272, R value work: 0.185 and R-value Observed: 0.185). This PDB id (1HCK) was chosen based on its origin and high resolution over other PDB structure IDs. The protein was prepared using the Discovery Studio 2024 (https://discover.3ds.com/discovery-studio-visualizer-download) by eliminating the cofactors, ligands, water molecules, and metal ions bound to the protein. Energy minimization was performed using SWISS PDB Viewer (https://spdbv.vital-it.ch/) to stabilize the protein structure [9].

2.2. Ligand Retrieval and Preparation

In this study, *M. oleifera* was chosen for anti- CDK2 drug development based on a literature study [2]. The PubChem Compound Identities (CIDs) of all the bioactive metabolites of the compounds found from *M. oleifera* plant were retrieved from the IMPPAT database (https://cb.imsc.res.in/imppat/). Following the removal of duplicates, compounds were selected for subsequent investigation. The IMPPAT database (https://cb.imsc.res.in/imppat/) is a repository of phytochemical data on Indian medicinal plants [10]. The database utilizes cheminformatics methodologies to evaluate its physicochemical and drug-like properties, employing various scoring methodologies. The

3D structures of the ligands were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format [11,12]. We have used Open Babel (https://sourceforge.net/projects/openbabel/) to generate a ligand library [12]. Energy minimization of the ligand compounds was performed using the global Force Field and the Conjugate Gradients algorithm of PyRx [13].

2.3. Binding Site Prediction

Amino acids involved in active pocket formation were determined using Computed Atlas for Surface Topographym of proteins (CASTp). CASTp may be a very easy and useful web-based tool for determining the topology and site pockets within the proteins. Site determination is vital to line the grid box before docking.

2.4. Virtual Screening and Molecular Docking

The molecular docking method finds more potent, selective, and efficient drug candidates [14]. Accordingly, virtual screening and blind molecular docking techniques were performed to select a limited number of ligand compounds from the ligand library with desired biological functions, capable of interacting with the binding pocket of the target protein (receptor) [13,15]. The ligand compounds were docked against the target protein CDK2 using PyRx, an open-access virtual screening tool [16]. A molecular docking program, AutoDockVina under the PyRx, was employed for this docking purpose and to estimate the binding affinities of docked complexes. With the help of the calculation of the value of energy minimization and binding energy, molecular docking predicts possible drug-target interactions [17]. In Angstrom, the X, Y, and Z coordinates of the grid box were 104.2965, 94.9090, and 82.2995, while the center of the box's core were 23.4965, 29.9481, and 40.8121, the bioactive conformations were simulated employing Autodock Vina. The docked complexes were visualized and analyzed using the BIOVIA visualizer of Discovery Studio 2024 [14].

2.5. Protein-Ligand Interaction

The protein-ligand complexes in the pdb format were displayed, edited and run via the software Discovery studio and PyMol. The protein-ligand interactions along with the hydrophobic interactions and hydrogen bonding with the complex binding residues were input for the lead inhibitor. The Discovery studio shows the amino acid of the target protein which is involved in the interaction with the ligand. The interaction profile of lead phytochemicals and the interface among heterodimers of a protein can be determined.

2.6. ADMET Analysis

The top eight ligand compounds were subjected to pharmacokinetic and drug-likeness evaluation using the SwissADME (www.swissadme.ch) web server, a widely used tool for in silico prediction of ADME (Absorption, Distribution, Metabolism, and Excretion) parameters [17]. Pharmacokinetic properties (PKs) like physicochemical properties, lipophilicity, water solubility Log S (ESOL), pharmacokinetics, drug-likeness rules (Lipinski), and medicinal chemistry (PAINS, Synthetic acces sibility) of drugs hold immense significance in pharmacological research for understanding how drugs behave within the body [18]. The Canonical SMILES of each compound were utilized as input to retrieve data on physicochemical properties (e.g., molecular weight, hydrogen bond donors and acceptors), lipophilicity (iLOGP), water solubility (Log S), pharma cokinetic parameters (e.g., gastrointestinal absorption), drug-likeness criteria (e.g., Lipinski's Rule of Five, bioavailability score), and medicinal chemistry filters (e.g., synthetic accessibility). These properties are critical for assessing the oral bioavailability and therapeutic potential of small molecules [11,17,18]. To ensure the safety profile of the selected compounds,

toxicity predictions were conducted using admetSAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2) and Protox-III (https://tox-new.charite.de/) web servers [19,20] web servers. Both servers utilize canonical SMILES as input to predict key toxicological endpoints. The admetSAR 2.0 provides insights into parameters such as AMES mutagenicity, acute oral toxicity, and hERG channel inhibition, while Protox-III evaluates potential carcinogenicity, cytotoxicity, and immunotoxicity of selected ligand compounds

3. Result & Discussion

3.1. Virtual Screening and Molecular Docking Analyses

A total of 191 chemicals from the M. oleifera plant were obtained from the IMPPAT database and virtually evaluated against the CDK2 protein. Improved therapeutic receptor interactions are associated with a higher binding free energy. All compounds have a binding free energy between −8.8 and −5 kJ/mol, with the top 6 molecules having a binding free energy between −8.8 and −8 kJ/mol. We used Pyrx software for docking analysis. The docking results of the top eight molecule are given in Table 5.

Here Niazirinin shows higher result among others is −8.1 KJ/mol.

Table 1. Site specific docking result.

Ligand	Binding Affinity
Beta-Sitostenone	−8.8
Delta7-Avenasterol	−8.7
28-Isoavenasterol acetate	−8.3
N,alpha-L-rhamnopyranosyl vincosamide	−8.3
Niazirinin	−8.1
Pterygospermin	−8

3.2. Analysis of ADME Properties

In the assessment of the potential utility of drug molecules within the body, the ADME properties of adsorption, metabolism, distribution, and excretion are considered crucial parameters. During the ADME experiments, the widely recognized Lipinski's Rule of Five was employed as a key factor in identifying the most suitable compounds. Tables 2 and 3 summarizes the drug-like characteristics of the top 6 performing ligands. After careful evaluation, the compound niazirinin was selected as the most promising candidate for further study of its molecular interactions. For analyzing ADME properties SwissADME and PKCSM virtual tools were used. For toxicity analysis protox-3 was used.

Table 2. ADME properties of most promising ligand.

Ligand	Lipinski Rule	GI Absorbtion	Water Solubility (log mol/L)	BBB Permeant	Lipophilicity Log P_{ow}
Beta-Sitostenone	Yes; 1 violation: MLOGP > 4.15	Low	1.31×10^{-7} mol/L	No	5.00
Delta7-Avenasterol	Yes; 1 violation: MLOGP > 4.15	Low	1.49×10^{-6} mol/L	No	5.12
28-Isoavenasterol acetate	Yes; 1 violation: MLOGP > 4.15	Low	9.35×10^{-8} mol/L	No	4.96
N,alpha-L-rhamnopyranosyl vincosamide	No; 3 violations	Low	5.22×10^{0} mol/L	No	2.95
Niazirinin	Yes; 0 violation	High	1.27×10^{-2} mol/L	No	2.25
Pterygospermin	Yes; 0 violation	High	5.89×10^{-6} mol/L	No	3.45

Table 3. Toxicity profiling top 8 compounds through protox-iii online server.

Compound	Cytotoxicity	Immunogenecity	Mutagenicity	Carcinogenicity
Beta-Sitostenone	Inactive	Active	Inactive	Inactive
Delta7-Avenasterol	Inactive	Active	Inactive	Inactive
28-Isoavenasterol acetate	Inactive	Active	Inactive	Active
N,alpha-L-rhamnopyranosyl vincosamide	Inactive	Active	Inactive	Active
Niazirinin	Inactive	Inactive	Inactive	Inactive
Pterygospermin	Inactive	Inactive	Inactive	Inactive

3.3. Molecular Interaction Analysis

The chosen niazirinin molecule was subjected to an ADME investigation and an examination of its molecular interactions with the receptor protein. Twelve combinations are feasible. All six are hydrogen bond, one alkyl bond and three pi-alkyl bond. In Table 8 and Figure 1, all the molecular interactions are shown.

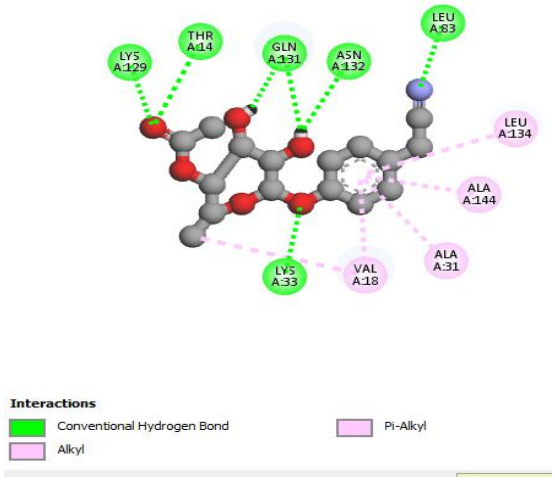

Figure 1. The 2D visualization of the molecular interaction.

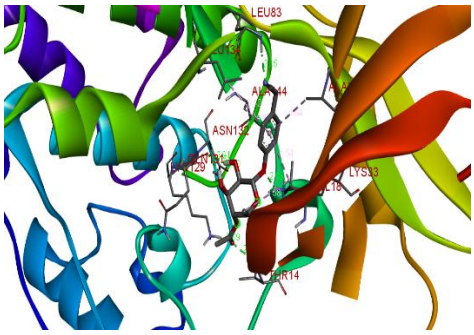

Figure 2. Closer interactions of protein ligand binding.

Table 4. Molecular Interactions Analysis Result Between niazirinin and Receptor Protein.

Name	Distance	Category	Type
THR14	2.4188	Hydrogen Bond	Conventional Hydrogen Bond
LYS33	2.02889	Hydrogen Bond	Conventional Hydrogen Bond
LEU83	2.04943	Hydrogen Bond	Conventional Hydrogen Bond
LYS129	2.08799	Hydrogen Bond	Conventional Hydrogen Bond
GLN131	2.04925	Hydrogen Bond	Conventional Hydrogen Bond
GLN131	2.01084	Hydrogen Bond	Conventional Hydrogen Bond

ASN132	2.50486	Hydrogen Bond	Conventional Hydrogen Bond
VAL18	4.57719	Hydrophobic	Alkyl
VAL18	4.5375	Hydrophobic	Pi-Alkyl
ALA31	4.84395	Hydrophobic	Pi-Alkyl
LEU134	5.4231	Hydrophobic	Pi-Alkyl
ALA144	4.4441	Hydrophobic	Pi-Alkyl

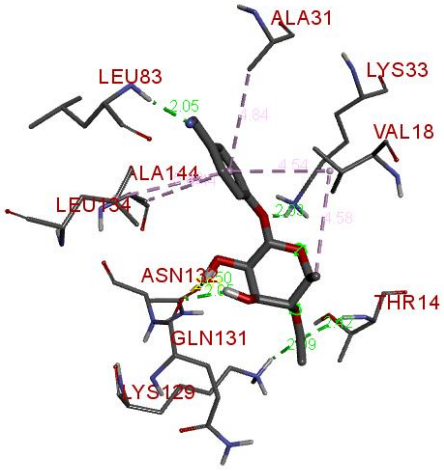

Figure 3. Molecular Interactions Analysis.

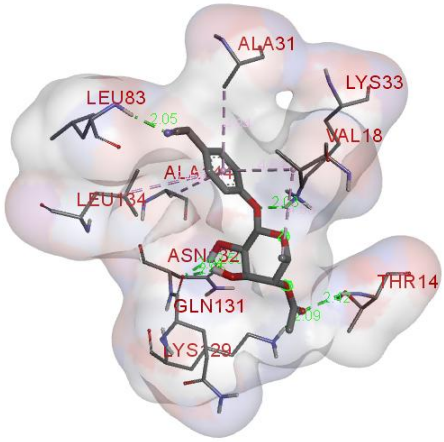

Figure 4. 3D Surface molecular interactions of the niazirinin ligand.

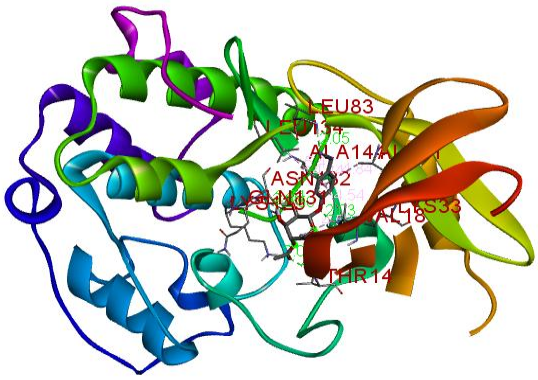

Figure 5. Protein and ligand interaction.

4. Conclusions

Niazirinin is identified as the most potent inhibitor with a binding affinity of -8.1 kJ/mol. It has high gastrointestinal absorption and favorable pharmacokinetics. It is non cyto-toxic and non-carcinogenic. It forms 6 convetional hydrogen bonds, 3 Pi-alkyl interactions and one alkyl bond with CD2. So, Niazirinin is a promising candidate for lung cancer with Strong binding affinity, favorable ADMET profile, and safety.

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Abbreviations

CDK2	Cyclin Dependent Kinase 2
GI	Gastrointestinal
DNA	Deoxyribonucleic
RNA	Ribonucleic

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