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Original Article

Maslinic Acid as a Candidate Small-Molecule CDK4 Inhibitor for Lung Cancer

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ABSTRACT

Background: Lung cancer is a significant cause of cancer-related death, emphasizing the need for current targeted therapies. CDK4, an imperative regulator of cell-cycle progression and the G1/S transition**,** is a promising anticancer target. So, maslinic acid, a candidate small-molecule CDK4 inhibitor for lung cancer, was studied using integrated *in silico* and *in vitro* approaches. **Purpose:** To evaluate maslinic acid as a potential small-molecule CDK4 inhibitor and assess its anticancer activity in A549 lung carcinoma cells. **Materials and Methods:** Sequence-based domain analyses confirmed conserved protein kinase features**,** with an ATP-binding region and catalytic residues. PPI and GO enrichment analyses defined the biological role of CDK4. Molecular docking observed maslinic acid binding within the CDK4 active pocket. Cell viability assays**,** along with CDK4 mRNA and protein level analyses, were performed in A549 cells. **Results:** The target protein presented a conserved protein kinase/PK-like domain with crucial functional motifs. CDK4 was enriched in pathways related to cell-cycle regulation**,** cyclin-dependent kinase activity, and G1/S transition**.** Docking revealed stable binding of maslinic acid within the CDK4 pocket, involving van der Waals, alkyl, and Pi-alkyl interactions with residues including PHE124, LEU141, LYS142, ASP140, ILE146, SER199, VAL200, and MET197. *In vitro*, maslinic acid reduced A549 cell viability in both dose-dependent and time-dependent manners and significantly decreased CDK4 mRNA expression and protein levels. **Conclusion:** Maslinic acid shows potential as a candidate small-molecule CDK4 inhibitor for lung cancer. Its favorable CDK4 binding, association with cell-cycle pathways**,** and inhibitory effects on cell viability and CDK4 expression support further development as a potential therapeutic lead.

Keywords: A549 cells, CDK4, Domain analyses, Lung Cancer, Maslinic Acid, Molecular docking, mRNA expression, Protein Levels.

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INTRODUCTION

Lung cancer remains one of the most frequently diagnosed malignancies and a key cause of cancer-related death worldwide (Thai *et al*., 2021). Despite substantial advances in diagnosis and treatment, the clinical outcomes for many patients remain unsatisfactory due to late-stage detection, tumor heterogeneity, and the development of therapeutic resistance. These limitations have intensified the search for molecularly targeted agents that can effectively suppress key drivers of lung cancer progression (Chaudhary *et al*., 2025; Liu *et al*., 2024; Zafar *et al*., 2025).

CDK4 is a key regulator of cell-cycle progression, particularly the G1/S transition, where it partners with D-type cyclins to phosphorylate downstream targets and promote entry into DNA synthesis (Alrouji *et al*., 2025; Ding *et al*., 2020; Pellarin *et al*., 2025; Zabihi *et al*., 2023). Dysregulation of the CDK4 cyclin axis is frequently related to uncontrolled proliferation, making CDK4 an attractive therapeutic target in cancer, with lung cancer (Baker *et al*., 2022). A bioinformatics analysis revealed that the target protein has a conserved protein kinase domain, an ATP-binding site, and essential catalytic motifs, and functional annotation positioned CDK4 at the intersection of pathways involving cyclin-dependent kinase activity, cell-cycle regulation, and the mitotic G1/S transition (Cipak, 2022; Castelo-Soccio *et al*., 2020).

The use of natural compounds in the discovery of anticancer drugs has gained momentum due to their multitarget potential, biological compatibility, and structural diversity. A natural pentacyclic triterpenoid, Maslinic Acid, has become a promising bioactive molecule owing to reported pharmacological effects, including antiproliferative and pro-apoptotic activities in various cancer models (Asma *et al*., 2022; Pavithra *et al*., 2024). Nevertheless, its exact interaction with CDK4 and its potential role in lung cancer are yet to be clarified. Given that lung cancer cells are reliant on aberrant cell-cycle signaling, maslinic acid can be a strong candidate, as a small-molecule inhibitor, of CDK4 (Palmer *et al*., 2025; Ayyub *et al*., 2024; Tarique *et al*., 2017).

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This potential was supported by molecular docking, which revealed that maslinic acid can stabilize within the CDK4 pocket and bind specifically via van der Waals, alkyl, and Pi-alkyl interactions with crucial residues. This evidence indicates a structurally significant CDK4 interaction and that maslinic acid is a promising CDK4-targeting agent (Aguiar *et al*., 2025).

This hypothesis is further supported by the *in vitro* results. Maslinic acid decreased cell viability in a concentration- and time-dependent manner in A549 lung carcinoma cells, suggesting a robust cytotoxic or growth-inhibitory effect (Sulistyowaty *et al*., 2021). Moreover, therapy significantly reduced CDK4 mRNA expression and CDK4 protein levels, indicating that the anticancer effects of maslinic acid may be mediated, at least in part, by inhibition of the CDK4 signaling axis. Cooperatively, these remarks can relation molecular targeting to cellular consequences that can be measured (Goel *et al*., 2022; Qayoom *et al*., 2022).

Building on these discoveries, this paper examined maslinic acid as a potential small-molecule CDK4 inhibitor for lung cancer through combined sequence/domain analysis, functional network analysis, molecular docking, and cell-based validation. Collectively, these strategies support pursuing the development of maslinic acid as an attractive therapeutic lead in lung cancer.

MATERIALS AND METHODS

Sequence of the target protein

The 303-aa target protein was also analyzed for sequence motifs and domain architecture to identify conserved regions associated with kinase activity. First, the sequence was analyzed with ScanProsite to identify PROSITE signatures, motifs, and key functional residues (Castro *et al*., 2006; Venkataraman *et al*., 2011; Sidorczuk *et al*., 2023). This comparison determined the protein kinase region, the kinase signature, the proton acceptor motif as well as the proposed active site. The protein's structural organization was further confirmed by domain identification and domain architecture analysis using SMART (Letunic *et al*., 2021; Schultz *et al*., 1998). The protein kinase/PK-like domain was verified by SMART analysis and the functional sites, such as the ATP-binding site, magnesium-binding residue, and phosphotyrosine-related site were identified (Letunic *et al*., 2009). Combining the ScanProsite and SMART results enabled the establishment of conserved kinase-related features, such as domain organization, residue positions, and predicted catalytic sites, for functional annotation of the target protein (Cipak, 2022; Watson *et al*., 2020).

CDK4 Protein-Protein Interaction Network and Gene Ontology

The interaction between CDK4 and other proteins was studied using Protein-Protein Interaction (PPI) network and Gene Ontology (GO) biological process enrichment (Zhu *et al*., 201). The STRING database (STRING: functional protein association networks) was utilized to find key interacting partners involved in cell-cycle regulation using CDK4 as the query protein (Baker *et al*., 2022; Szklarczyk *et al*., 2025). This gene/protein set was then analyzed using GO enrichment analysis to identify significantly overrepresented biological processes. Enriched terms were plotted in a bubble plot according to signal strength, False Discovery Rate (FDR) and the number of genes. The visualization and interpretation were performed in ShinyGO, which identified key processes associated with CDK4, including cell-cycle regulation, cyclin-dependent protein kinase activity, and the G1/S transition (Rosati *et al*., 2024).

Preparation of ligands

The ligand structures were drawn using ChemDraw Ultra 8.0. Each ligand structure was then converted into SMILES format and translated using the online SMILES Translator available at the CACTUS server. The generated ligand file was saved in PDB format and subsequently converted into PDBQT format using AutoDock Tools (ADT) version 1.5.6 from The Scripps Research Institute. During this conversion, the root was detected, torsions were selected, and the number of torsions was defined to prepare the ligand for docking analysis.

Selection and preparation of the target protein

The crystal structures of CDK4 were selected as drug targets. Their three-dimensional structures, with PDB, were retrieved from the RCSB Protein Data Bank. All water molecules were removed from the protein structures, and hydrogen atoms were added in the final preparation step. The prepared PDB files were then converted into PDBQT format using AutoDock Tools (ADT) version 1.5.6 from The Scripps Research Institute for subsequent molecular docking analysis (Morris *et al*., 1998).

Molecular docking

Molecular docking simulations of the ligand molecules with CDK4 were conducted with the AutoDock 4.2 docking suite using the Lamarckian genetic algorithm (Morris *et al*., 1998; Malik *et al*., 2019; Sait *et al*., 2019; Alsamghan *et al*., 2020). The ligand (maslinic acid) was set to explore and flexible to rotate the most probable binding poses, while the receptor was kept rigid. The grid maps representing the center of the active-site pocket of the ligand were generated using Autogrid. In the present docking study, conformations were generated, and the best docked conformation was selected based on the AutoDock binding energy (Kcal/mol), for further analysis. Finally, the results were visualized using PyMOL for analysis of minimum binding energy (Kcal/mol), Ki (Inhibition constant) (μM), and hydrogen- and hydrophobic-interaction analyses of the docked inhibitor with the modeled structure.

Viability assay

A549 lung carcinoma cells were seeded into 96-well plates at a density of 5,000-10,000 cells/well and incubated at 37°C in a CO_2 incubator for 24 hr to allow cell attachment. The spent medium was then replaced with fresh culture medium containing different concentrations (0-3328 µM) of the test compound (maslinic acid CID: 73659). After 72 hr of treatment, MTT solution (5 mg/mL in PBS) was added to each well (10-20 µL per 100 µL medium), and the plates were further incubated at 37°C for 2-4 hr. Viable cells reduced MTT to formazan crystals, which were dissolved in DMSO (100-200 µL) after careful removal of the supernatant. Absorbance was recorded at 570 nm using a microplate reader. Cell viability was calculated relative to the untreated control using the formula:

$$\text{Cell viability (\%)} = (\text{Sample absorbance} / \text{Control absorbance}) \times 100$$

Dose-response curves were generated from multiple concentrations. In addition, a time-dependent viability assay was carried out at 12, 24, 36, 48, 60, 72, and 84 hr in cells treated with maslinic acid.

mRNA expression

A549 lung carcinoma cells were exposed to maslinic acid for 24 hr. After treatment, cells were collected under sterile conditions, and total RNA was isolated using an RNA extraction kit (Almanac). RNA concentration was measured using a NanoDrop spectrophotometer, while RNA integrity was verified by agarose gel electrophoresis. Subsequently, 1 µg of total RNA was used for reverse transcription to synthesize cDNA using a cDNA synthesis kit (Almanac).

Relative CDK4 mRNA expression was analyzed by quantitative real-time PCR (qPCR) using an Applied Biosystems real-time PCR instrument. The obtained Ct values were used to calculate relative gene expression by the $2^{\wedge-\Delta\Delta Ct}$ method. The primers used were: CDK4 forward, 5′-GAATCTCCAGGGAATAGGGC-3′; CDK4 reverse, 5′-CTGAAATCCTCCTGGGCTG-3′; 18S forward, 5′-GGCCCTGTAATTGGAATGAGTC-3′; and 18S reverse, 5′-CCAAGATCCAACTACGAGCTT-3′. Furthermore, CDK4 protein levels in A549 cell lysates were measured using a Human CDK4 ELISA Kit (Abcam, Cat. No. ab316258) following the manufacturer's protocol.

Statistical Analysis

All experiments were performed in triplicate. Data are presented as mean±Standard Deviation (SD). Statistical significance was determined using one-way ANOVA or Student's t-test, with a *p*-value <0.05 considered statistically significant.

RESULTS

Sequence Analysis and Domain organization

Sequence-based analysis revealed that the CDK4 protein is composed of 303 aa and contains conserved features characteristic of a protein kinase. ScanProsite identified a broad protein kinase region spanning residues 6-295 aa, indicating that most of the protein is occupied by a kinase-related functional core (Figure 1A). In addition, a protein kinase signature was detected at residues 12-35 aa, while a proton acceptor motif was recorded to residues 136-148 aa. A predicted active site was contained at residue 140, augmenting the catalytic potential of the protein (Figure 1A).

These results were further corroborated by domain organization analysis, which revealed a large PROTEIN_KINASE_DOM that spanned the entire full-length sequence, consistent with the kinase-associated profile of CDK4 (Figure 1B). Conserved kinase-related regions were mainly evident in the 12-35 aa and 136-148 aa segments, reinforcing the functional importance of these motifs (Figure 1B).

Additional confirmation was got through SMART analysis, which identified a protein kinase/PK-like domain spanning residues 3-297 aa, covering nearly the entire protein length (Figure 1C). SMART also predicted several key functional sites, including an ATP-binding site at 12-20 aa, a magnesium-binding residue via carbonyl oxygen at 145 aa, an ATP-binding residue at 158 aa, and phosphotyrosine-related site at 165 aa (Figure 1C). Together, these results indicate that CDK4 has the conserved structural and functional domain of a kinase protein and likely functions as an enzymatically active kinase.

CDK4 Interaction Network and Functional Enrichment Analysis

The protein-protein interaction (PPI) network bare that CDK4 is centrally linked with numerous important regulators of cell-cycle progression, with *CCND1, CCND2, CCNE1, CDKN1B, CDKN2B, CDKN2C, CDK6, RB1, HSP90AB1,* and *CDC37*, representing a tightly coordinated interaction landscape related to cell-cycle control (Figure 2A). The dense connectivity pattern suggests that CDK4 functions as a core regulatory node within the cyclin-dependent signaling network, chiefly through its association with cyclins, *CDK* inhibitors, and retinoblastoma-related proteins (Figure 2A and Table 1).

The biological effect of this interaction network was likewise authenticated through functional development study. The enriched Gene Ontology (GO) terms of biological processes were largely associated with regulation of cyclin-dependent protein kinase activity, regulation of cyclin-dependent protein serine/threonine kinase activity, regulation of protein serine/threonine kinase activity and regulation of cell cycle (Figure 2B). Additional meaningfully enriched terms were G1/S transition of mitotic

cell cycle, regulation of G1/S transition, regulation of mitotic cell cycle phase transition, +ve regulation of cyclin-dependent protein kinase activity (Figure 2B).

In sum, these results suggest that CDK4 plays a vital functional role in the cell-cycle progression and G1/S phase-change molecular network, supporting its use as a biologically-relevant therapeutic target (Figures 2A, 2B).

Docking of Maslinic Acid with CDK4

Molecular docking investigation demonstrated that compound (maslinic acid) binds positively contained via the CDK4 active

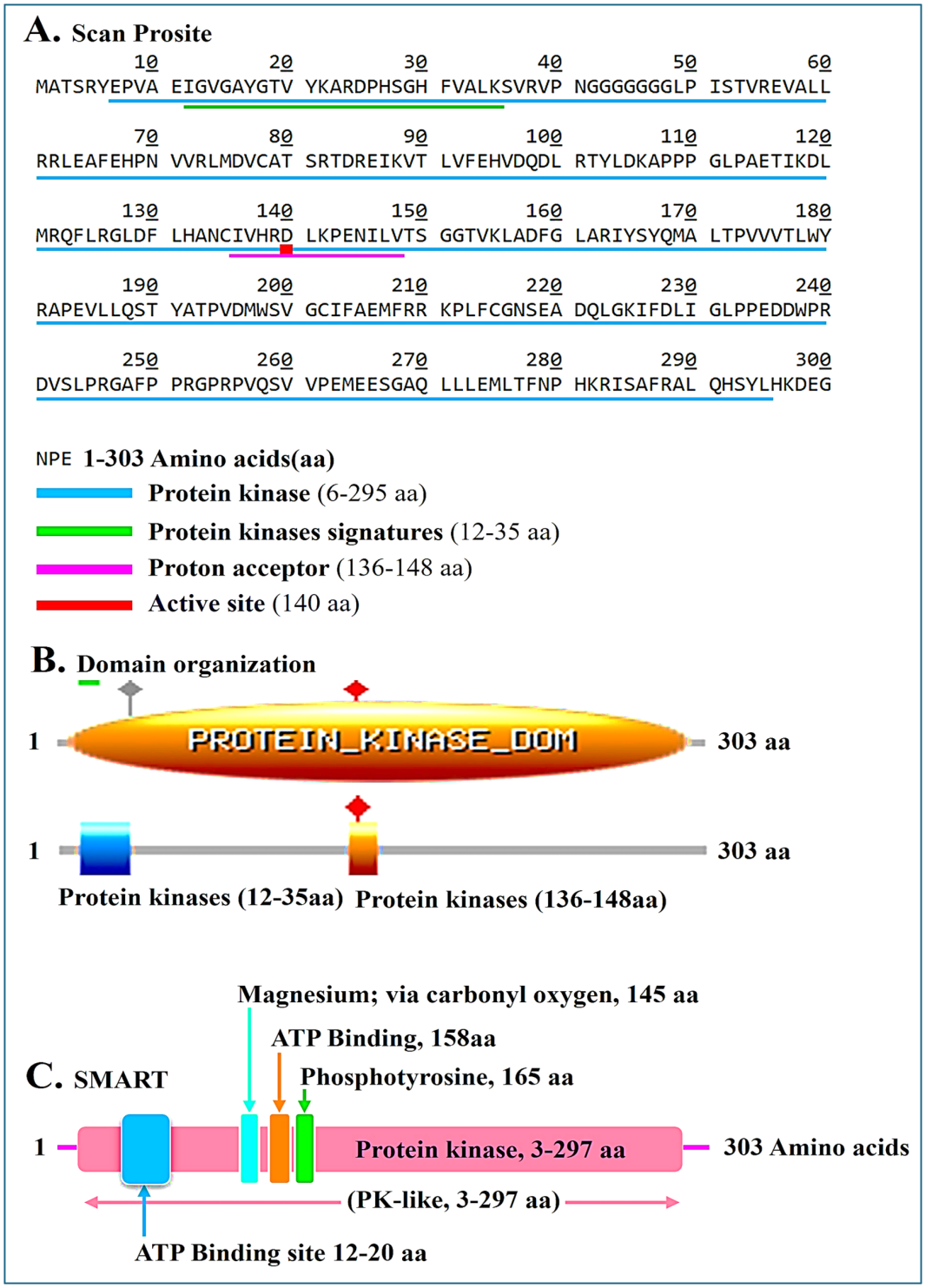

Figure 1: (A) ScanProsite tool identified many domains. (B) Analysis showed that the main PROTEIN_KINASE_DOM (C) SMART tool identified a protein kinase/PK-like domain with ATP-binding and a phosphotyrosine-related site, among other features.

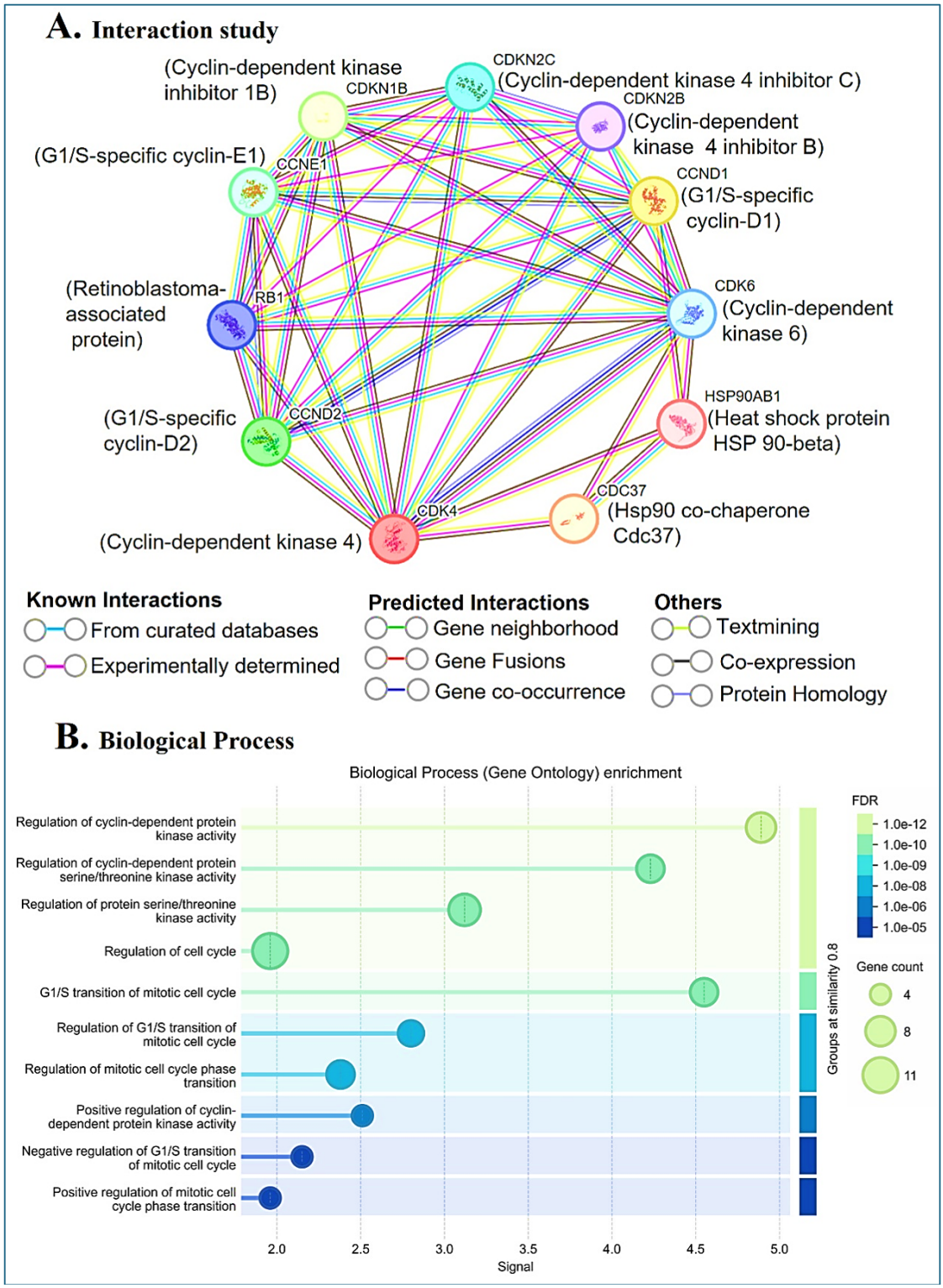

Figure 2: (A) Interaction network of CDK4 with important cell cycle regulators. (B) Gene Ontology (GO) biological process enrichment showing significant association with cell cycle regulation, G1/S transition, and cyclin-dependent protein kinase activity.

site and is stabilized via multiple interactions non-covalent interactions. The 2D interaction map showed that the ligand formed extensive van der Waals interactions with residues including HIS132, LEU128, ASP196, HIS138, ILE146, SER199, TYR180, LYS142, ASP140, and ASN145, while alkyl/Pi-alkyl interactions were observed with PHE124, LEU141, MET197, and VAL200 (Figure 3A). Such patterns of interactions suggest that maslinic acid has high hydrophobic accommodation in the binding cavity (Figure 3A).

Table 1: Predicted Functional Partners.

Interacting Proteins	Functional Partners
CDC37	Cdc37 recruits' kinases to Hsp90, stabilizes them, and inhibits the ATPase activity of HSP90AA1.
CCND1	Cyclin D1 partners with CDK4 to phosphorylate RB1, release E2F, and promote G1/S progression while integrating mitogenic and antimitogenic signals.
CDKN1B	p27^Kip1 (CDKN1B) enforces G1 arrest by inhibiting Cyclin E/A-CDK2, and modulates Cyclin D-CDK4 assembly/ activity depending on phosphorylation and stoichiometry.
CCND2	Cyclin D2 partners with CDK4 to phosphorylate RB1, release E2F, and promote G1/S progression while integrating mitogenic and antimitogenic signals.
CCNE1	G1/S-specific cyclin-E1; Essential for the control of the cell cycle at the G1/S (start) transition.
CDKN2C	CDK4 inhibitor C (CDKN2C/p18) binds CDK6 strongly (and CDK4 weakly), suppressing cell growth and proliferation in an RB-dependent manner. Member of the CDKN2 family.
CDK6	CDK6 complexes with D-type cyclins to phosphorylate RB1 (and NPM1), promoting G1/S progression and regulating proliferation vs differentiation.
RB1	RB1 binds E2F1 to repress cell-cycle genes; phosphorylation releases E2F, and RB1 supports heterochromatin via histone methylation.
CDKN2B	CDKN2B (p15) is a potent CDK4/CDK6 inhibitor implicated in TGF-β-mediated cell-cycle arrest; member of the CDKN2 family.
HSP90AB1	HSP90β is an ATP-dependent molecular chaperone that stabilizes client proteins; its ATPase cycle is tuned by co-chaperones.

The 3D docking pose also confirmed the good positioning of maslinic acid within the CDK4 binding pocket, reside within the active cavity in a stable orientation that could support ligand-protein recognition (Figure 3B). The surface representation showed that the compound fitted well in the pocket environment, which suggests that it will be able to effectively engage the target (Figure 3B).

The direct-interaction of the critical residues, including PHE124, ASP140, LEU141, LYS142, SER199, VAL200, and MET197 in binding the ligand (a full close-up view of the docked complex, Figure 3C) was highlighted. Together, these findings suggest that maslinic acid establishes a stable interaction network within the catalytic region of CDK4, supporting its potential as a promising small-molecule CDK4 inhibitor (Figures 3A-3C).

Concentration-Dependent Cytotoxic Effect of Maslinic Acid on A549 Cells

Maslinic Acid reduced the viability of A549 lung carcinoma cells in a clear concentration-dependent manner (Figure 4A). At the lowest concentration tested, 6.5 µM, cell viability remained high at ~78%, close to the untreated control (0 µM; ~78%). A slight decline was observed at 13.0 µM (~71%), followed by a more perceptible reduction at 26.0 µM (~57%) and 52.0 µM (~50%). At the intermediate concentrations, viability continued to decrease to about 41% at 104.0 µM and 39% at 208.0 µM. At 416.0 µM and 832.0 µM, cell viability remained low at approximately 36%. The higher concentrations produced the strongest inhibitory effect, with viability declining further to about 33% at 1664.0 µM and reaching its lowest level of approximately 30% at 3328.0 µM (Figure 4A). Overall, these findings show that maslinic acid exerts a marked dose-dependent antiproliferative/cytotoxic effect on A549 cells, with minimal effect at lower concentrations and substantial suppression of cell viability at higher concentrations (Figure 4A).

Time-Dependent Effect of Maslinic Acid on A549 Cell Viability

At a fixed concentration of maslinic acid (105 µM), the viability of A549 lung carcinoma cells decreased in a clear time-dependent manner (Figure 4B). At the earliest time point (12 hr), cell viability was the highest, at approximately 78%. Viability decreased significantly after 24 hr to some 49% then to almost 40 at 36 hr with protracted exposure, but thereafter, cell viability progressively decreased, but at a slower rate. At 48 hr, viability was approximately 37%, while at 60 hr and 72 hr it remained around 35%. The lowest viability was observed at the latest time point (84 hr), where it reached approximately 33% (Figure 4B).

Overall, these findings indicate that maslinic acid at 105 µM exerts a pronounced time-dependent cytotoxic/antiproliferative effect on A549 cells. The highest viability was observed at the shortest exposure time (12 hr; ~78%), whereas the lowest viability was observed after the longest exposure (84 hr; ~33%), confirming that prolonged treatment enhances the compound's inhibitory effect (Figure 4B).

Maslinic Acid Downregulates CDK4 mRNA and Protein Levels in A549 Cells

Treatment of A549 lung carcinoma cells with maslinic acid resulted in a marked concentration-dependent reduction in both CDK4 mRNA expression and CDK4 protein levels (Figures 4C, 4D).

For CDK4 mRNA expression, the untreated control showed the maximum relative expression, at approximately 5.1-fold. Upon treatment with 42.301 µM maslinic acid, CDK4 expression let fall sharply to about 1.3-fold. A further decrease was observed at 89.19 µM, where expression declined to nearly 0.8-fold. The lowest mRNA expression was observed at the highest concentration, 181.07 µM, at approximately 0.35-fold (Figure 4C). These findings indicate strong transcriptional suppression of CDK4 as Maslinic Acid concentration increases.

The same tendency was observed in CDK4 protein levels in A549 cell extracts. The control group showed the highest concentration of CDK4, at about 4000 pg/mL. After 42.301 ug of Maslinic Acid was administered, the level of CDK4 dropped to approximately 3000 pg/mL. The protein concentration was further decreased to approximately 1750 pg/mL at 89.19 µM, with the lowest protein level observed at 181.07 µM, which was very close to 1500 pg/mL (Figure 4D).

In general, these results indicate that the action of maslinic acid, in inhibiting CDK4 at both the mRNA and protein levels, is clearly dose-dependent. The highest expression and protein levels were noted in the untreated control, with the lowest levels being at the highest concentration of the test (181.07 µM), which confirmed the inhibitory activity of maslinic acid on the CDK4 axis (Figures 4C, 4D).

DISCUSSION

The present study suggests that maslinic acid may be a promising small-molecule CDK4 inhibitor in lung cancer. This is biologically significant, as disregard for the CDK4-RB-E2F axis results in uncontrolled cell proliferation and G1/S cell-cycle progression. Other recent investigations also note the growing interest in CDK4/6-targeted therapy for NSCLC and other solid tumors (Dong *et al*., 2017; Trabelsi *et al*., 2024; Zhong *et al*., 2021).

The target protein retained the main characteristics of a functional kinase, such as a protein kinase domain, kinase signature, predicted active site, and ATP-binding domain, as revealed by sequence and domain analyses (Christian *et al*., 2026; Tabana *et al*., 2023; Wang *et al*., 2021). These findings are mechanistically relevant because kinase inhibition depends on binding to the conserved ATP-binding pocket, which is central to Cdk-Rb-E2F-mediated

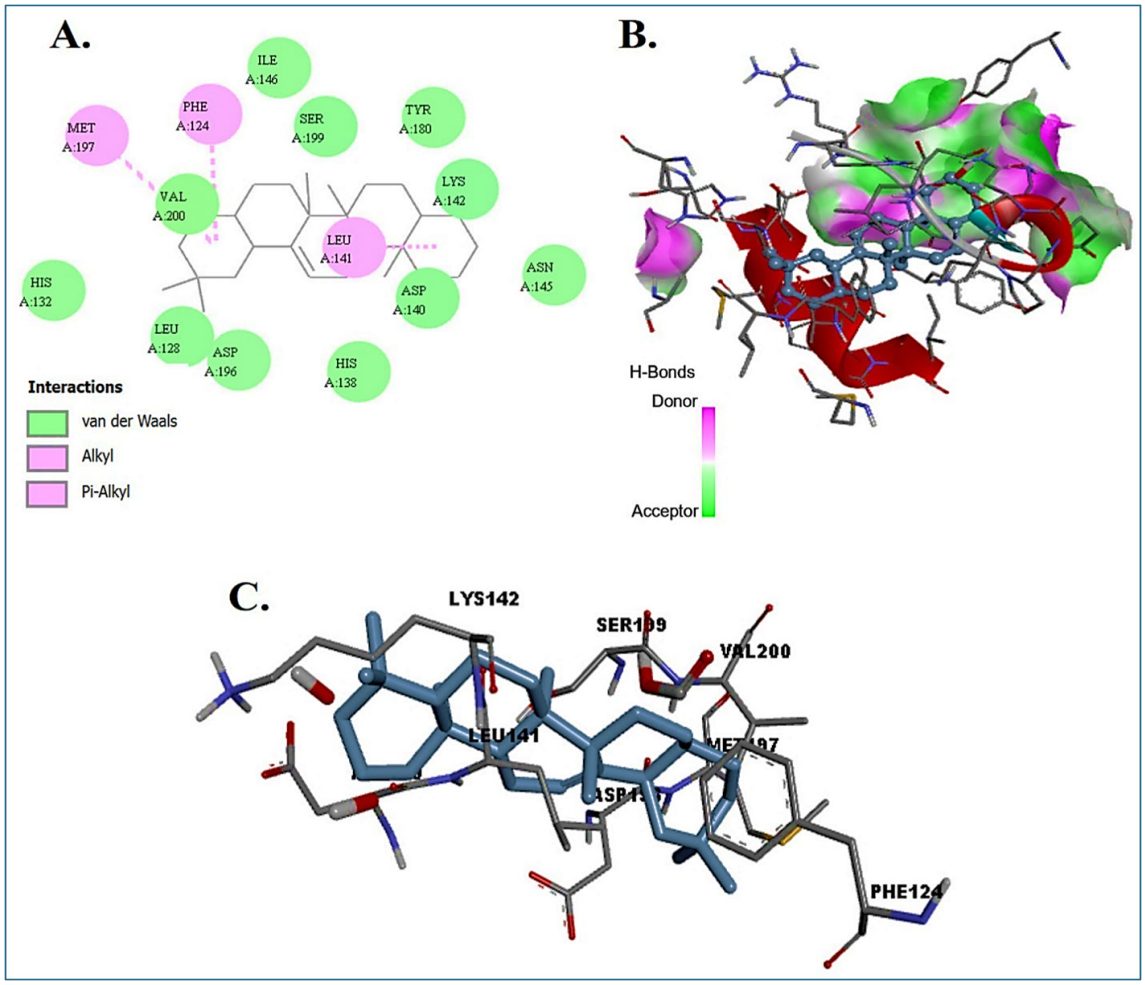

Figure 3: (A) Molecular docking of Maslinic acid with CDK4. Two-dimensional interaction map showing hydrophobic and van der Waals contacts. (B) Three-dimensional binding pose of maslinic acid within the CDK4 active pocket. (C) Close-up view highlighting key interacting residues in the binding site.

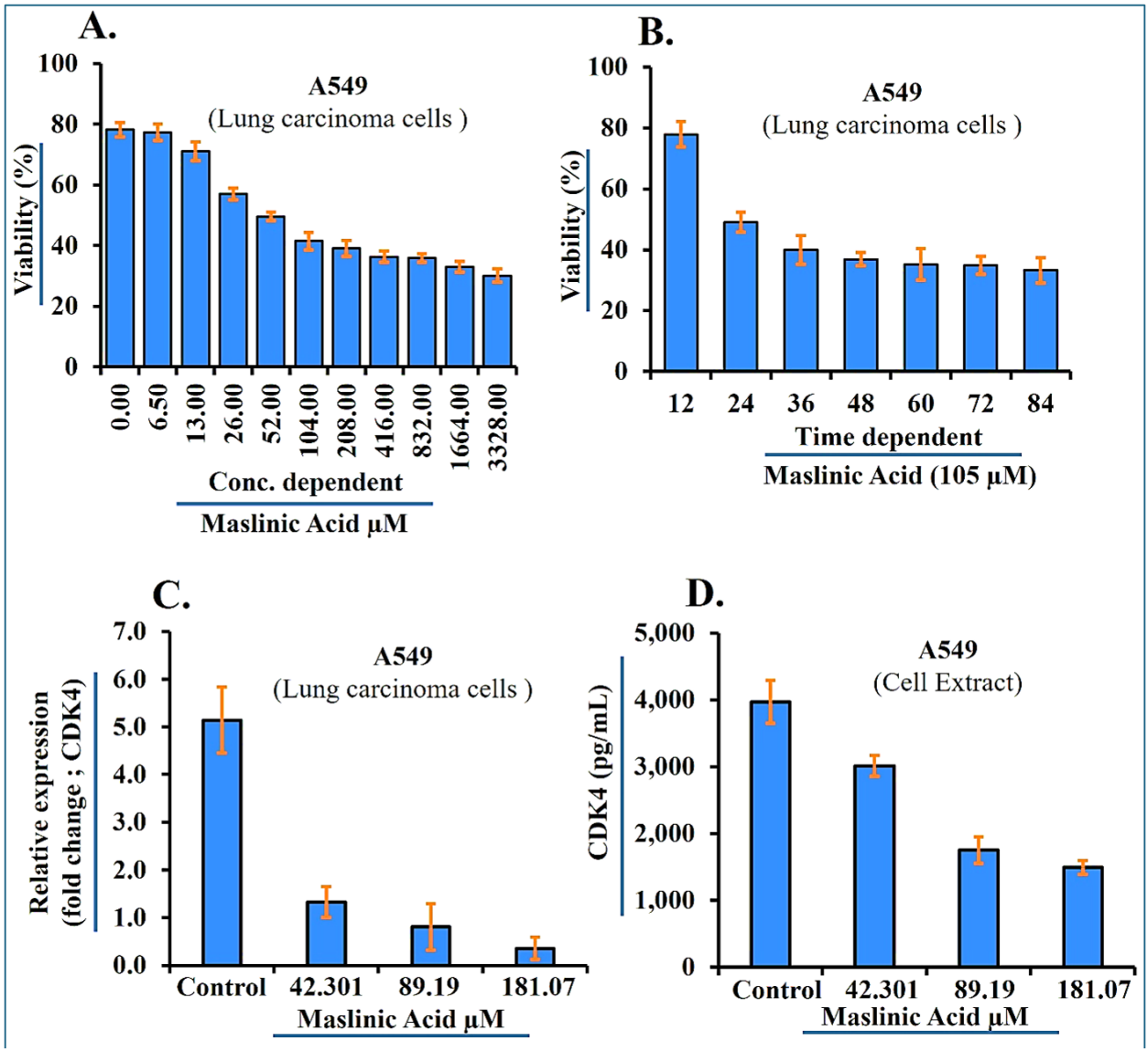

Figure 4: (A) Concentration dependent cell viability of Maslinic Acid treated A549 lung cancer cells (B) Time dependent cell viability of Maslinic Acid treated A549 lung cancer cells (C) Effect of half concentration of Maslinic Acid on the mRNA expression of CDK4 in A549 lung cancer cells (D) Result of half concentration of Maslinic Acid on the activity level of CDK4 in A549 lung cancer cells.

G1/S regulation and CDK4-driven proliferative signaling (Ahsan *et al*., 2023; Li *et al*., 2024; Narayanan *et al*., 2023).

The results of protein-protein interaction and Gene Ontology enrichment analyses further support the biological relevance of CDK4 in the current model. The close association of CDK4 with *CCND1, CCND2, CCNE1, CDKN1B, CDKN2B, CDKN2C, CDK6, RB1, HSP90AB1,* and *CDC37* places it within a tightly coordinated regulatory network governing cell-cycle progression. Similarly, the enrichment of pathways involving cyclin-dependent kinase activity, cell-cycle regulation, and the G1/S transition is consistent with CDK4's known role as a hub in tumor proliferation (Rao *et al*., 2014; Szklarczyk *et al*., 2025; Zhang *et al*., 2024). Recently, there has been evidence in the literature that CDK4 has extended oncogenic activities beyond its classical cell-cycle regulation, and is relevant to cancer progression and response to treatment.

A strength of this study is the agreement between the docking and biological results. Maslinic Acid was observed to exhibit stable binding in the CDK4 pocket in van der Waals, alkyl, and Pi-alkyl interactions with critical residues. Whereas docking does not indicate direct inhibition on its own, the interaction pattern is indicative of a functionally relevant CDK4 interaction. This fact is supported by recent data indicating that selective inhibition of CDK4 may improve antitumor activity without causing the same level of toxicity as previous CDK4/6 inhibitors (Agu *et al*., 2023; Sharmila *et al*., 2025; Torres *et al*., 2019).

The *in vitro* results also confirm this interpretation. Maslinic Acid decreased cell viability in A549 lung carcinoma cells in a concentration-dependent and time-dependent fashion, suggesting a long-term antiproliferative effect. It agrees with the reports about Maslinic Acid that is a natural pleiotropic

triterpenoid and has a wide anticancer effect (Abo *et al*., 2025; Chen *et al*., 2024). Maslinic Acid treatment significantly decreased both CDK4 mRNA and CDK4 protein levels, indicating that its antiproliferative effect may be linked to inhibition of the CDK4 axis rather than to nonspecific cytotoxicity. The study involves structural prediction, network analysis, ligand target-interaction, and cellular target suppression to support Maslinic Acid as a potential CDK4-targeting lead against lung cancer (He *et al*., 2025; Ziegler *et al*., 2024). These are initial results, with the study demonstrating *in silico* binding and *in vitro* anticancer activity, but not yet demonstrating direct CDK4 inhibition, binding affinity, or selectivity against other kinases.

CONCLUSION

The present study reveals that maslinic acid has promising potential as a small-molecule CDK4-targeting agent in lung cancer. Sequence and domain studies confirmed that the target protein has conserved structural elements of a functional protein kinase, including a kinase domain, an ATP-binding region, and key catalytic motifs. The functional enrichment and protein interaction analyses further confirmed the key role of CDK4 in cell-cycle regulation, particularly in cyclin-dependent kinase activity and at the G1/S transition. Molecular docking revealed that maslinic acid is retained in the CDK4 active site, with favorable van der Waals, alkyl, and Pi-alkyl interactions with key amino acid residues, indicating a possible ligand-target interaction. These computational observations were complemented by *in vitro* experiments on A549 lung carcinoma cells, which showed that maslinic acid decreased cell viability in a concentration- and time-dependent manner. Notably, treatment also significantly reduced CDK4 mRNA expression and CDK4 protein levels, suggesting inhibition of the CDK4 axis. Taken together, these findings indicate that maslinic acid is a potentially useful natural product to develop as a CDK4-targeted therapeutic lead for lung cancer. Although further biochemical, mechanistic, and *in vivo* validation assays are yet to be conducted, the present findings provide a solid initial basis for its future investigation in specific lung cancer therapies.

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ABBREVIATIONS

CDK4: Cyclin-dependent kinase 4; **PPI:** Protein-Protein Interaction; **GO:** Gene Ontology; **ATP:** Adenosine triphosphate; **FDR:** False discovery rate; **SMILES:** Simplified Molecular Input Line Entry System; **PDB:** Protein Data Bank; **ADT:** AutoDock Tools; **RCSB:** Research Collaboratory for Structural Bioinformatics; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **PBS:** Phosphate-Buffered Saline; **DMSO:** Dimethyl sulfoxide; **RNA:** Ribonucleic acid; **qPCR:** Quantitative Real-Time Polymerase Chain Reaction; **cDNA:** Complementary DNA; **ELISA:** Enzyme-linked immunosorbent assay; **NSCLC:** Non-Small Cell Lung Cancer; **RB:** Retinoblastoma.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

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AUTHOR CONTRIBUTIONS

Rashed Mohammed Alghamdi uniquely contributed to the conception and design of the study, data collection, analysis and interpretation of the results, and the writing and revision of the manuscript. The author has read and approved the final version of the manuscript.

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