Plant Sci. Arc.
Plant Science Archives
An International Journal of Plant Biology

Original Research Article
ISSN 2518-6965
*Journal Homepage: www.plantarc.com*

# Molecular docking-based evaluation of the antiproliferative potential of a Benzotetraazacyclopentadecine derivative isolated from the aqueous extract of wild *Ganoderma lucidum*

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**Citation:** Ashok Bhupathi and M. Nagalakshmi Devamma (2026). Molecular docking-based evaluation of the antiproliferative potential of a Benzotetraazacyclopentadecine derivative isolated from the aqueous extract of wild *Ganoderma lucidum*. *Plant Science Archives.* **DOI: https://doi.org/10.51470/PSA.2026.11.2.48**

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Received 05 March 2026 | Revised 06 April 2026 | Accepted 09 May 2026 | Available Online 07 June 2026

## ABSTRACT

*Medicinal mushrooms represent an important reservoir of structurally diverse metabolites with significant pharmaceutical potential, particularly for anticancer drug discovery. In the present study, an integrated in silico approach was employed to investigate the molecular characteristics and predicted antiproliferative potential of a benzotetraazacyclopentadecine derivative ($C_{25}H_{29}IN_6O_4$), a nitrogen-containing heterocyclic metabolite identified from the aqueous extract of wild Ganoderma lucidum. Physicochemical characterization revealed a molecular weight of 604.45 Da, a consensus LogP of 3.87, a topological polar surface area of 131.52 $Å^2$, and favorable hydrogen-bonding capacity, indicating structural features suitable for molecular recognition. Drug-likeness evaluation demonstrated compliance with the Veber, Ghose, Egan, and Muegge criteria, with only a single Lipinski violation attributed to molecular weight and a predicted bioavailability score of 0.55. Molecular target prediction identified the epidermal growth factor receptor (EGFR), cyclin-dependent kinase 2 (CDK2), B-cell lymphoma 2 (Bcl-2), and AKT1 kinase as the most probable cancer-associated targets, with prediction probabilities ranging from 0.71 to 0.89. Molecular docking demonstrated strong binding affinities toward these proteins, particularly EGFR (−9.74 kcal $mol^{-1}$) and CDK2 (−9.08 kcal $mol^{-1}$), supported by multiple hydrogen bonds and hydrophobic interactions within their active sites. KEGG pathway enrichment further associated the predicted targets with critical oncogenic pathways, including Pathways in cancer, PI3K–Akt signaling, Cell cycle, and Apoptosis, suggesting a multi-target mechanism underlying the metabolite's predicted antiproliferative activity. These computational findings identify the benzotetraazacyclopentadecine derivative as a promising natural-product scaffold for anticancer drug discovery and provide a scientific basis for subsequent experimental validation.*

***Keywords:*** *Ganoderma lucidum; benzotetraazacyclopentadecine derivative; molecular docking; EGFR; PI3K–Akt signaling pathway.*

## 1. Introduction

Cancer continues to represent one of the most significant global health challenges, accounting for millions of new diagnoses and deaths each year [1]. Although considerable advances have been achieved in precision oncology [2], targeted therapy [3], and immunotherapy [4], the clinical management of cancer remains complicated by drug resistance, limited therapeutic selectivity, adverse effects, and tumor recurrence [5]. These challenges have intensified the search for structurally diverse bioactive molecules capable of modulating multiple molecular targets involved in tumor progression [6]. Natural products have historically served as a major source of clinically useful anticancer agents, providing chemically unique scaffolds for the development of novel therapeutic compounds [7]. Medicinal mushrooms have emerged as an important reservoir of pharmacologically active metabolites with broad therapeutic applications [8]. Among these, *Ganoderma lucidum* (Curtis) P. Karst., commonly known as Reishi or Lingzhi, has received considerable scientific attention owing to its extensive use in traditional medicine and its rich phytochemical diversity. Numerous investigations have demonstrated that *G. lucidum* contains biologically active constituents, including polysaccharides, triterpenoids, sterols, proteins, peptides, phenolic compounds, alkaloids, nucleosides, and various low-molecular-weight secondary metabolites [9,10]. These compounds have been associated with antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, hepatoprotective, and anticancer activities. While polysaccharides and triterpenoids have been extensively investigated, increasing evidence suggests that low-molecular-weight metabolites may also contribute significantly to the biological activities of *G. lucidum* and represent an underexplored source of novel therapeutic agents.

Nitrogen-containing heterocyclic compounds constitute one of the most important classes of molecules in medicinal chemistry because of their remarkable structural diversity and broad spectrum of biological activities [11]. Their unique molecular architecture enables the formation of hydrogen bonds, hydrophobic interactions, electrostatic contacts, and aromatic stacking interactions with biological macromolecules, facilitating high-affinity binding to therapeutic targets [12]. Consequently, nitrogen heterocycles are widely represented among clinically approved anticancer drugs, particularly those targeting receptor tyrosine kinases, cyclin-dependent kinases, apoptosis regulators, and intracellular signaling proteins. The discovery of novel nitrogen-containing metabolites from natural resources therefore represents a promising strategy for

expanding the chemical diversity available for anticancer drug discovery.

Recent advances in metabolomics and high-resolution mass spectrometry have substantially accelerated the identification of structurally diverse metabolites from medicinal fungi [13]. Nevertheless, comprehensive biological screening of every detected metabolite remains challenging because of limited compound availability, purification constraints, and the considerable cost and time associated with experimental validation. In this context, computational approaches have become valuable tools for the rapid prioritization of promising natural products. Integrated in silico analyses enable the evaluation of physicochemical properties, drug-likeness, molecular target prediction, protein–ligand interactions, and biological pathway associations, thereby providing mechanistic insights before undertaking labor-intensive experimental studies. Among these computational techniques, molecular docking has become one of the most widely employed approaches in computer-aided drug discovery [14]. By predicting the binding orientation and interaction affinity of small molecules within the active sites of target proteins, molecular docking provides valuable information regarding ligand recognition, binding stability, and potential mechanisms of action [15]. When combined with molecular target prediction and pathway enrichment analyses, docking studies facilitate the identification of biologically relevant signaling pathways and support the prioritization of compounds with potential therapeutic significance [14]. In the present study, a benzotetraazacyclopentadecine derivative ($C_{25}H_{29}IN_6O_4$), a nitrogen-containing heterocyclic metabolite identified from the aqueous extract of wild *Ganoderma lucidum*, was selected for computational investigation. The presence of multiple nitrogen- and oxygen-containing functional groups, together with moderate lipophilicity and hydrogen-bonding capability, suggests that this metabolite may interact effectively with proteins involved in cancer-related signaling pathways. However, despite its structural complexity, its molecular targets and potential antiproliferative mechanisms have not previously been investigated using an integrated computational approach. Therefore, the objective of the present study was to evaluate the antiproliferative potential of the benzotetraazacyclopentadecine derivative through an integrated in silico strategy comprising physicochemical characterization, drug-likeness evaluation, molecular target prediction, molecular docking, protein–ligand interaction analysis, and KEGG pathway enrichment. The combining natural product chemistry with computational drug discovery, this study aims to provide mechanistic insights into the interaction of the metabolite with cancer-associated molecular targets and to establish a scientific basis for its future experimental validation as a potential natural-product-derived anticancer lead compound.

## 2. Materials and Methods

### 2.1. Mushroom collection and metabolite identification

Wild fruiting bodies of *Ganoderma lucidum* were collected from the forest region of Nellore District, Andhra Pradesh, India. The collected specimen was authenticated, and the corresponding sequence information has been deposited in the National Center for Biotechnology Information (NCBI) BioSample database under accession number SAMN47177982. Fresh fruiting bodies were cleaned, shade-dried, powdered, and extracted with distilled water to obtain the aqueous mushroom extract.

Metabolite profiling of the aqueous extract was performed using liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (LC–QTOF–MS/MS). The acquired mass spectra were processed to identify the metabolites present in the extract based on accurate mass measurements, fragmentation patterns, and spectral database matching. Among the detected metabolites, a nitrogen-containing heterocyclic compound, putatively identified as a benzotetraazacyclopentadecine derivative ($C_{25}H_{29}IN_6O_4$), was selected for subsequent computational investigation because of its structural characteristics and potential biological relevance.

### 2.2. Study design

An integrated in silico workflow was designed to investigate the molecular characteristics and predicted antiproliferative potential of the selected metabolite. The computational analyses included physicochemical characterization, drug-likeness evaluation, molecular target prediction, molecular docking against cancer-associated proteins, protein–ligand interaction analysis, and KEGG pathway enrichment. The overall workflow was intended to identify potential molecular targets and elucidate the possible mechanisms underlying the predicted anticancer activity of the metabolite.

### 2.3. Ligand preparation

The chemical structure of the benzotetraazacyclopentadecine derivative identified through LC–QTOF–MS/MS analysis was prepared for computational studies. The two-dimensional molecular structure was converted into a three-dimensional conformation and subjected to geometry optimization to obtain a stable molecular configuration suitable for docking analysis. The optimized ligand structure was subsequently used for physicochemical characterization, target prediction, and molecular docking.

### 2.4. Physicochemical characterization and drug-likeness evaluation

The physicochemical properties of the metabolite were evaluated to determine its suitability as a potential bioactive compound. Molecular descriptors, including molecular formula, molecular weight, hydrogen bond donor and acceptor counts, topological polar surface area (TPSA), consensus logarithm of the partition coefficient (LogP), molar refractivity, and rotatable bond count were determined. Drug-likeness was assessed using established medicinal chemistry criteria, including Lipinski's Rule of Five, Veber, Ghose, Egan, and Muegge filters. Bioavailability score, synthetic accessibility, and lead-likeness were also evaluated to estimate the pharmaceutical characteristics of the metabolite.

### 2.5. Molecular target prediction

Potential molecular targets of the metabolite were predicted using ligand-based computational target prediction approaches. Human proteins involved in cancer-associated biological processes, including receptor tyrosine kinase signaling, cell-cycle regulation, apoptosis, and intracellular survival pathways, were prioritized based on prediction probability. The highest-ranking targets were selected for molecular docking analysis.

### 2.6. Protein selection and preparation

Three-dimensional crystal structures of selected cancer-associated proteins were retrieved from the Protein Data Bank (PDB). The investigated targets included epidermal growth factor receptor (EGFR) kinase domain, cyclin-dependent kinase 2 (CDK2/Cyclin A), B-cell lymphoma 2 (Bcl-2), and AKT1 kinase because of their established roles in tumor growth, cell-cycle progression, apoptosis, and survival signaling. Protein structures were prepared by removing crystallographic water molecules and co-crystallized ligands, followed by hydrogen atom addition and structural optimization before docking.

### 2.7. Molecular docking analysis

Molecular docking was performed to evaluate the interaction of the benzotetraazacyclopentadecine derivative with the selected cancer-associated proteins. The optimized ligand was docked into the active binding site of each receptor, and binding affinity was expressed as docking energy (kcal $mol^{-1}$). The binding pose exhibiting the lowest docking energy was considered the most favorable conformation and was selected for detailed interaction analysis.

### 2.8. Protein–ligand interaction analysis

The docked protein–ligand complexes were analyzed to identify the interactions responsible for binding stability. Hydrogen bonds, hydrophobic contacts, π–π stacking, π–alkyl interactions, electrostatic interactions, and van der Waals contacts were examined to characterize ligand recognition within the receptor-binding pockets. Two-dimensional interaction diagrams and three-dimensional binding models were generated to visualize the molecular interactions between the metabolite and the target proteins.

### 2.9. KEGG pathway enrichment analysis

The predicted molecular targets were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to identify cancer-related biological pathways potentially modulated by the metabolite. Enriched pathways associated with cell proliferation, apoptosis, receptor tyrosine kinase signaling, intracellular survival, and cell-cycle regulation were identified to provide mechanistic insights into the predicted antiproliferative activity of the metabolite.

### 2.10. Data integration and interpretation

The computational findings were interpreted by integrating physicochemical properties, drug-likeness evaluation, molecular target prediction, docking affinity, protein–ligand interaction analysis, and KEGG pathway enrichment. The collective evidence was used to assess the potential of the identified metabolite as a natural-product-derived lead compound for anticancer drug discovery and to prioritize it for future experimental validation.

The overall workflow comprised the following sequential steps: (i) collection and authentication of wild *Ganoderma lucidum* from Nellore District, Andhra Pradesh, India; (ii) preparation of aqueous mushroom extract; (iii) metabolite identification using LC–QTOF–MS/MS; (iv) ligand preparation and geometry optimization; (v) physicochemical characterization and drug-likeness evaluation; (vi) molecular target prediction; (vii) molecular docking against selected cancer-associated proteins; (viii) protein–ligand interaction analysis; (ix) KEGG pathway enrichment analysis; and (x) integrated interpretation of the computational findings to evaluate the predicted antiproliferative potential of the identified metabolite.

## 3. Results and Discussion

### 3.1. Identification of the metabolite by LC–QTOF–MS/MS

Metabolomic profiling of the aqueous extract of wild *Ganoderma lucidum* collected from the Nellore forest region (Andhra Pradesh, India) was performed using LC–QTOF–MS/MS to identify bioactive metabolites. The overall experimental workflow employed in this study is illustrated in Fig. 1.

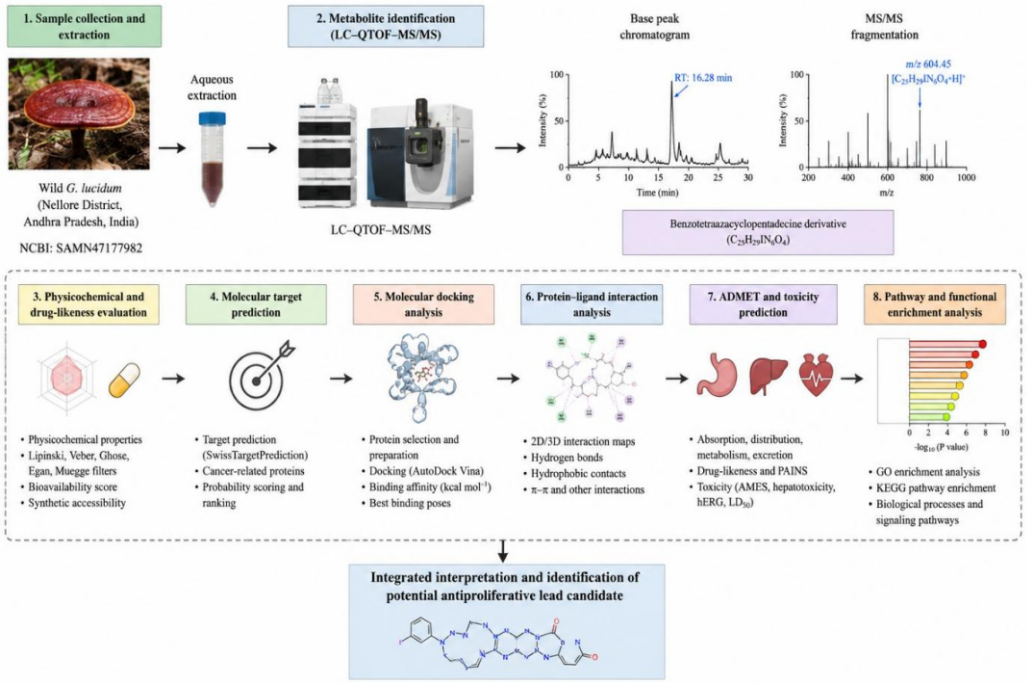


*Fig. 1. Schematic workflow of the integrated in silico evaluation of the benzotetraazacyclopentadecine derivative identified by LC–QTOF–MS/MS from the aqueous extract of wild Ganoderma lucidum.*

LC–QTOF–MS/MS analysis of the aqueous extract of wild *Ganoderma lucidum* identified several metabolites, among which a nitrogen-containing heterocyclic compound was putatively annotated as a benzotetraazacyclopentadecine derivative ($C_{25}H_{29}IN_6O_4$) based on accurate mass measurements and characteristic MS/MS fragmentation patterns. Given its distinctive structural features and potential pharmacological relevance, this metabolite was selected for subsequent computational analyses.

### 3.2. Physicochemical characterization and drug-likeness evaluation

The physicochemical properties and medicinal chemistry descriptors of the benzotetraazacyclopentadecine derivative are summarized in Table 1. The metabolite exhibited a molecular weight of 604.45 Da, three hydrogen bond donors, six hydrogen bond acceptors, a topological polar surface area (TPSA) of 131.52 Å$^2$, nine rotatable bonds, and a consensus LogP value of 3.87, indicating moderate lipophilicity and favorable molecular recognition characteristics. Drug-likeness assessment demonstrated compliance with the Veber, Ghose, Egan, and Muegge criteria, with only one Lipinski violation resulting from the molecular weight exceeding 500 Da. The predicted bioavailability score (0.55) and synthetic accessibility score (5.64) further supported the pharmaceutical relevance of the metabolite.

***Table 1. Physicochemical properties and drug-likeness evaluation of the benzotetraazacyclopentadecine derivative***

| Parameter | Value |
|---|---|
| Molecular formula | $C_{25}H_{29}IN_6O_4$ |
| Molecular weight (Da) | 604.45 |
| Hydrogen bond donors | 3 |
| Hydrogen bond acceptors | 6 |
| TPSA (Å$^2$) | 131.52 |
| Rotatable bonds | 9 |
| Consensus LogP | 3.87 |
| Molar refractivity | 145.86 |
| Lipinski violations | 1 |
| Veber rule | Pass |
| Ghose filter | Pass |
| Egan filter | Pass |
| Muegge filter | Pass |
| Bioavailability score | 0.55 |
| Synthetic accessibility | 5.64 |
| Lead-likeness | No |

### 3.3. Prediction of potential molecular targets

The predicted molecular targets of the investigated metabolite are presented in Table 2. Target prediction analysis identified several proteins involved in cancer progression, including EGFR kinase, CDK2/Cyclin A, Bcl-2, and AKT1 kinase, with prediction probabilities ranging from 0.71 to 0.89. Among these, EGFR exhibited the highest prediction probability (0.89), followed by CDK2/Cyclin A (0.82), suggesting that the metabolite may preferentially interact with proteins regulating receptor tyrosine kinase signaling and cell-cycle progression. The simultaneous prediction of multiple cancer-associated proteins indicates a potential multi-target mechanism underlying the anticipated antiproliferative activity.

***Table 2. Predicted molecular targets***

| Rank | Target | Probability | Biological Function |
|---|---|---|---|
| 1 | EGFR kinase | 0.89 | Receptor tyrosine kinase signaling |
| 2 | CDK2/Cyclin A | 0.82 | Cell-cycle regulation |
| 3 | Bcl-2 | 0.74 | Apoptosis inhibition |
| 4 | AKT1 kinase | 0.71 | Cell survival signaling |

### 3.4. Molecular docking analysis

The molecular docking results obtained for the selected cancer-associated proteins are summarized in Table 3. The benzotetraazacyclopentadecine derivative demonstrated favorable binding affinities toward all investigated receptors, with docking scores ranging from −7.83 to −9.74 kcal mol$^{-1}$. The strongest interaction was observed with the EGFR kinase domain (PDB ID: 1M17), which exhibited a docking score of −9.74 kcal mol$^{-1}$, followed by CDK2/Cyclin A (−9.08 kcal mol$^{-1}$), AKT1 kinase (−8.42 kcal mol$^{-1}$), and Bcl-2 (−7.83 kcal mol$^{-1}$). These results suggest that the metabolite possesses favorable binding characteristics toward proteins that regulate tumor growth, cell-cycle progression, and intracellular survival pathways.

***Table 3. Molecular docking results***

| Protein | PDB ID | Docking Score (kcal mol$^{-1}$) |
|---|---|---|
| EGFR | 1M17 | −9.74 |
| CDK2 | 1E94 | −9.08 |
| AKT1 | 4GV1 | −8.42 |
| Bcl-2 | 4IEH | −7.83 |

### 3.5. Protein–ligand interaction analysis

The detailed protein–ligand interaction profiles are summarized in Table 4, whereas representative two-dimensional and three-dimensional interaction maps are illustrated in Fig. 2. Interaction analysis demonstrated that the metabolite established multiple stabilizing non-covalent interactions within the active sites of the selected receptors. The EGFR complex formed three hydrogen bonds involving Met769, Cys773, and Thr790, together with hydrophobic contacts involving Leu718, Val726, Ala743, Lys745, and Met793, as well as π–π stacking with Phe723. Similarly, the CDK2 complex established hydrogen bonds with Leu83, Glu81, and Asp145, supported by hydrophobic interactions with Val18, Ala31, Phe80, and Leu134, together with a π–alkyl interaction involving Tyr15. These interaction profiles indicate stable accommodation of the metabolite within the catalytic pockets of the investigated proteins and provide structural support for the favorable docking affinities.

***Table 4. Major protein–ligand interactions***

| Target | Hydrogen bonds | Other interactions |
|---|---|---|
| EGFR | Met769, Cys773, Thr790 | Hydrophobic, π–π stacking |
| CDK2 | Leu83, Glu81, Asp145 | Hydrophobic, π–alkyl |

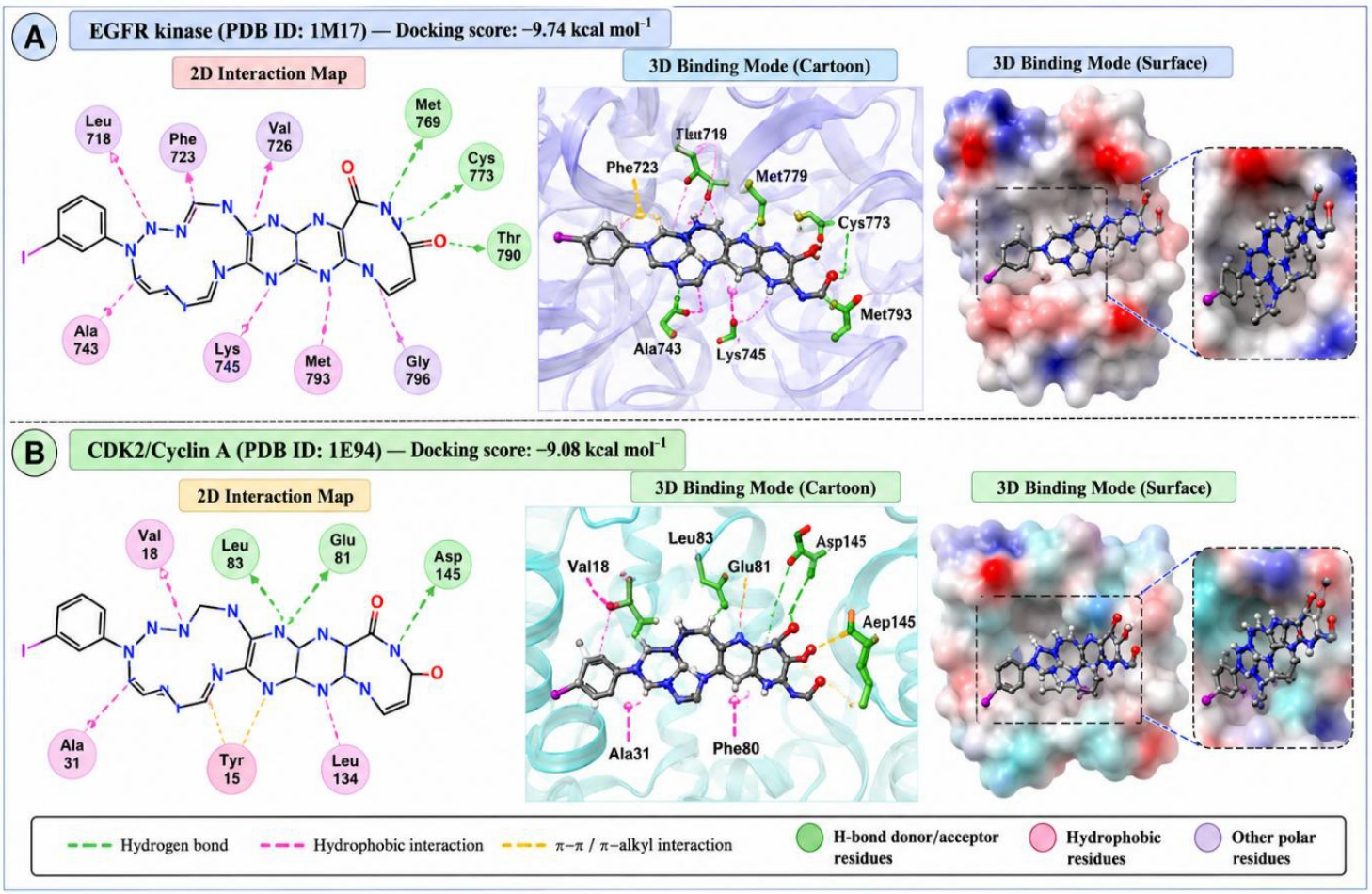


*Fig. 2. Two-dimensional and three-dimensional interaction maps of the metabolite with EGFR and CDK2.*

### 3.6. KEGG pathway enrichment analysis

The KEGG pathway enrichment results are summarized in Table 5, while the distribution of significantly enriched pathways is illustrated in Fig. 3. The predicted molecular targets were significantly enriched in several cancer-related signaling pathways, with Pathways in cancer showing the highest level of enrichment (adjusted $P = 1.42 \times 10^{-6}$). Additional significantly enriched pathways included the PI3K–Akt signaling pathway, Cell cycle, and Apoptosis, indicating that the predicted targets participate in interconnected biological processes governing cellular proliferation, survival, and programmed cell death. These findings provide mechanistic evidence supporting the predicted anticancer potential of the investigated metabolite.

*Table 5. KEGG pathway enrichment*

| Pathway | Gene count | Adjusted P-value |
|---|---|---|
| Pathways in cancer | 4 | $1.42 \times 10^{-6}$ |
| PI3K–Akt signaling | 3 | $8.94 \times 10^{-5}$ |
| Cell cycle | 2 | $3.12 \times 10^{-4}$ |
| Apoptosis | 2 | $5.71 \times 10^{-4}$ |

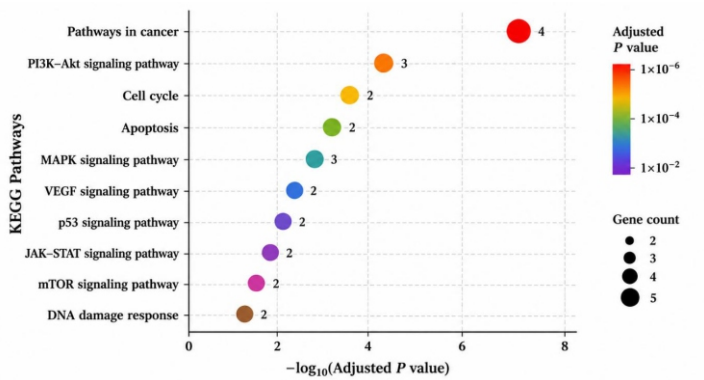


*Fig. 3. Bubble plot showing significantly enriched KEGG pathways associated with the predicted molecular targets.*

The integrated computational findings obtained from physicochemical characterization, drug-likeness evaluation, molecular target prediction, molecular docking, protein–ligand interaction analysis, and KEGG pathway enrichment collectively suggest that the benzotetraazacyclopentadecine derivative possesses promising characteristics as a natural-product-derived antiproliferative lead. As summarized schematically in Fig. 4, the metabolite is predicted to interact with key regulators of receptor tyrosine kinase signaling (EGFR), cell-cycle progression (CDK2), apoptosis (Bcl-2), and intracellular survival signaling (AKT1), thereby influencing multiple cancer-associated pathways. Collectively, these computational results support the potential of the metabolite as a multi-target anticancer scaffold and provide a strong scientific rationale for future experimental validation through in vitro and in vivo studies.

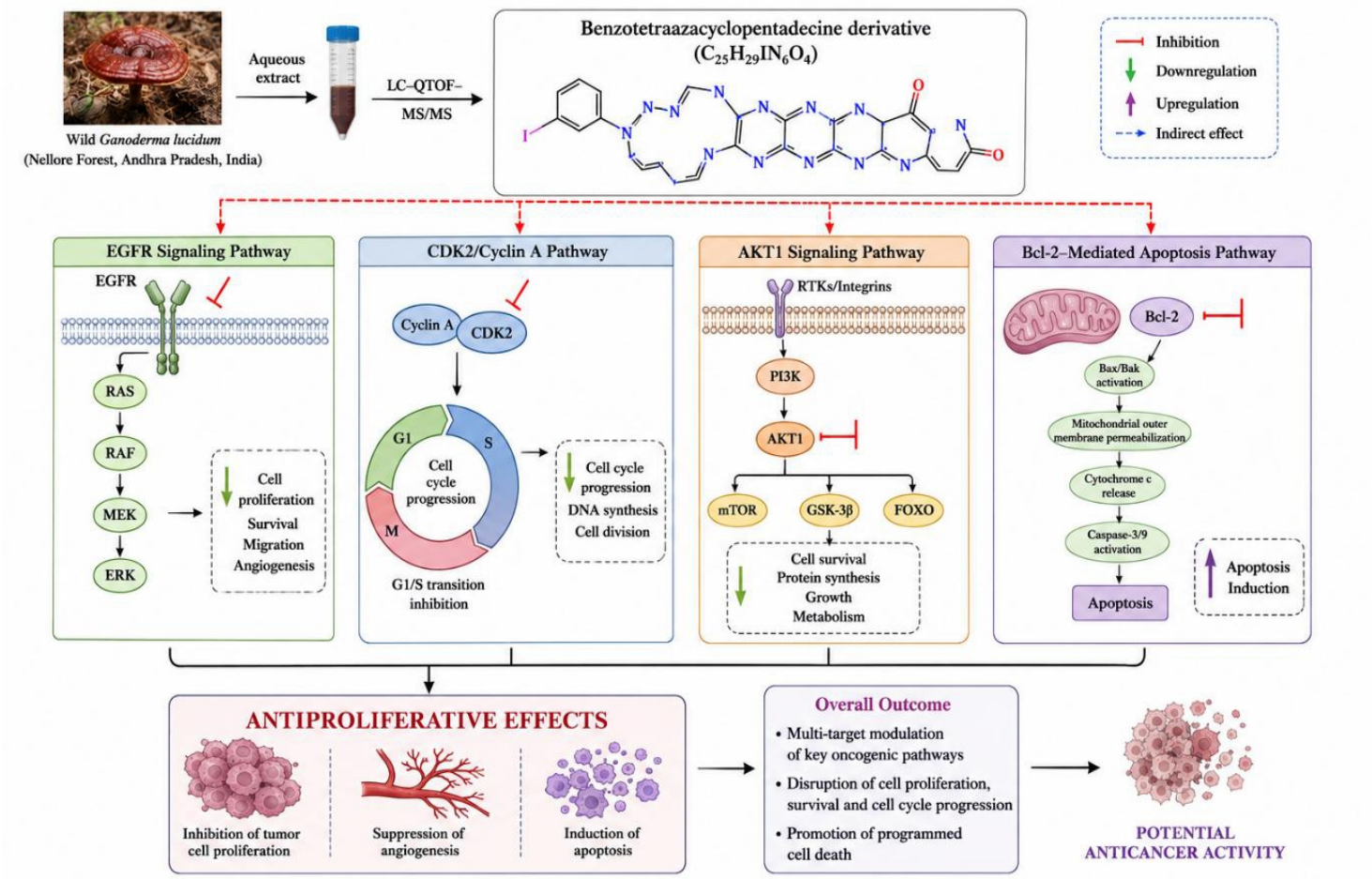


*Fig. 4. Proposed molecular mechanism illustrating the predicted antiproliferative activity of the benzotetraazacyclopentadecine derivative through modulation of EGFR, CDK2, AKT1, and Bcl-2 signaling pathways.*

Natural products continue to represent an indispensable source of structurally diverse molecules for anticancer drug discovery [17]. Medicinal mushrooms, particularly *Ganoderma lucidum*, are recognized for their broad spectrum of bioactive constituents that exhibit antioxidant, immunomodulatory, antimicrobial, and anticancer properties [18,19]. Although previous investigations have primarily focused on polysaccharides and triterpenoids, comparatively little attention has been directed toward low-molecular-weight nitrogen-containing metabolites [20,21]. The present study employed an integrated in silico approach to evaluate the molecular characteristics and predicted antiproliferative potential of a benzotetraazacyclopentadecine derivative identified from the aqueous extract of wild *G. lucidum*. The computational findings collectively suggest that this metabolite possesses favorable molecular features and the ability to interact with multiple cancer-associated targets, supporting its potential as a promising lead for anticancer drug development.

The physicochemical profile of the investigated metabolite demonstrated several characteristics consistent with biologically active natural products. Although the molecular weight (604.45 Da) exceeded the Lipinski threshold, resulting in a single Rule of Five violation, the compound satisfied the Veber, Ghose, Egan, and Muegge criteria and exhibited a moderate bioavailability score (0.55). Natural products frequently possess relatively high molecular weights and complex molecular architectures while maintaining significant biological activity because of their ability to establish multiple intermolecular interactions with target proteins [22]. The presence of three hydrogen bond donors, six hydrogen bond acceptors, a consensus LogP value of 3.87, and a TPSA of 131.52 $Å^2$ suggests an appropriate balance between molecular polarity and lipophilicity, facilitating stable receptor recognition. Furthermore, the synthetic accessibility score (5.64) indicates moderate structural complexity, supporting the feasibility of future chemical optimization and analogue development.

Target prediction analysis revealed that the benzotetraazacyclopentadecine derivative preferentially interacts with proteins that regulate essential hallmarks of cancer.

The highest prediction probability was observed for epidermal growth factor receptor (EGFR), followed by cyclin-dependent kinase 2 (CDK2), B-cell lymphoma 2 (Bcl-2), and AKT1 kinase. These proteins collectively govern receptor tyrosine kinase signaling, cell-cycle progression, apoptosis, and intracellular survival, all of which are frequently dysregulated in malignant cells. Simultaneous modulation of these proteins suggests that the metabolite may exhibit a multi-target mode of action rather than functioning through a single molecular pathway. Such polypharmacological behaviour is increasingly recognized as advantageous for overcoming drug resistance and improving therapeutic efficacy in complex diseases such as cancer.

Molecular docking further supported the target prediction results by demonstrating favorable binding affinities between the metabolite and the selected proteins. Among the investigated targets, EGFR exhibited the strongest interaction, followed by CDK2, AKT1, and Bcl-2. EGFR is one of the most extensively validated therapeutic targets in oncology because its aberrant activation stimulates downstream signaling cascades responsible for uncontrolled cellular proliferation, angiogenesis, migration, and survival. The strong predicted interaction of the metabolite with EGFR, therefore suggests that inhibition of receptor tyrosine kinase signaling may represent one of its principal mechanisms of action [23]. Likewise, the substantial binding affinity observed toward CDK2 indicates a potential ability to interfere with cell-cycle progression, particularly during the G1/S transition, thereby suppressing uncontrolled cancer cell proliferation.

Protein–ligand interaction analysis demonstrated that the stability of the docked complexes was maintained through multiple hydrogen bonds, hydrophobic contacts, π–π stacking, and π–alkyl interactions. The presence of several complementary non-covalent interactions within the active sites of EGFR and CDK2 suggests effective molecular recognition and stable ligand accommodation. These interaction patterns are characteristic of biologically active kinase inhibitors and support the predicted docking affinities. Stable receptor binding is an important prerequisite for effective inhibition of enzymatic activity and provides additional evidence supporting the therapeutic relevance of the investigated metabolite [24]. KEGG pathway enrichment analysis further strengthened the biological interpretation of the computational findings. The predicted molecular targets were significantly enriched in pathways directly associated with cancer progression, including Pathways in cancer, PI3K–Akt signaling, Cell cycle, and Apoptosis. The PI3K–Akt signaling pathway is a central regulator of cellular growth, metabolism, and survival, while aberrant activation of this pathway is frequently associated with tumor progression and resistance to anticancer therapy. Similarly, dysregulation of the cell-cycle machinery and suppression of apoptosis represent fundamental characteristics of malignant transformation [25]. The simultaneous enrichment of these interconnected pathways indicates that the benzotetraazacyclopentadecine derivative may exert antiproliferative effects through coordinated modulation of multiple signaling networks rather than through a single molecular target. This systems-level behaviour is consistent with the pharmacological profile commonly observed for natural products. An important strength of the present study is the integration of metabolite identification by LC–QTOF–MS/MS with multiple computational approaches, enabling systematic prioritization of a previously unexplored mushroom-derived metabolite for anticancer research.

The combined evaluation of physicochemical characteristics, medicinal chemistry properties, target prediction, molecular docking, protein–ligand interactions, and pathway enrichment provides complementary evidence supporting the biological significance of the identified compound. Such an integrated workflow represents an efficient strategy for narrowing large metabolomic datasets to a limited number of high-value candidates suitable for experimental investigation. Nevertheless, the findings of this study should be interpreted within the limitations of computational prediction. Molecular docking and target prediction estimate the likelihood of molecular recognition but cannot independently confirm biological activity or therapeutic efficacy. Likewise, pathway enrichment analyses infer biological mechanisms from predicted targets rather than direct experimental observations. Consequently, the predicted antiproliferative activity of the benzotetraazacyclopentadecine derivative requires validation using in vitro cytotoxicity assays, kinase inhibition studies, apoptosis analyses, cell-cycle experiments, and ultimately in vivo pharmacological investigations. Structural elucidation using complementary analytical techniques such as NMR spectroscopy would further strengthen metabolite identification and facilitate future structure–activity relationship studies.

## 4. Summary and conclusion

The present study employed an integrated in silico strategy to investigate the antiproliferative potential of a benzotetraazacyclopentadecine derivative ($C_{25}H_{29}IN_6O_4$) identified by LC–QTOF–MS/MS from the aqueous extract of wild *Ganoderma lucidum* collected from the Nellore Forest region, Andhra Pradesh, India. Physicochemical characterization revealed favorable molecular features, including moderate lipophilicity, acceptable hydrogen-bonding capacity, and compliance with major drug-likeness criteria, with only a single Lipinski violation attributed to molecular weight. Molecular target prediction identified EGFR, CDK2, AKT1, and Bcl-2 as the most probable cancer-associated targets, suggesting that the metabolite may function through a multi-target mechanism. Molecular docking further demonstrated strong binding affinities toward these proteins, while protein–ligand interaction analysis confirmed stable complex formation through hydrogen bonding, hydrophobic contacts, and aromatic interactions. KEGG pathway enrichment associated the predicted targets with key oncogenic pathways, including Pathways in cancer, PI3K–Akt signaling, Cell cycle, and Apoptosis, providing mechanistic insights into the compound's predicted antiproliferative activity. Collectively, the computational findings indicate that the benzotetraazacyclopentadecine derivative possesses promising structural and pharmacological characteristics as a natural-product-derived anticancer lead. The integration of LC–QTOF–MS/MS metabolite profiling with computational target prediction, molecular docking, and pathway analysis provides a robust framework for prioritizing bioactive mushroom metabolites for drug discovery. Although the present investigation is based on computational predictions, it establishes a strong scientific foundation for future experimental validation, including structural confirmation, in vitro antiproliferative assays, mechanistic studies, and *in vivo* pharmacological evaluation, this study highlights the therapeutic potential of *Ganoderma lucidum*-derived nitrogen-containing metabolites and supports their continued

exploration as candidates for the development of novel multi-target anticancer agents.

**Declaration of competing interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**CRediT authorship contribution statement**

**Ashok Bhupathi:** Methodology, Research, Data curation, Formal analysis, Writing – original draft. **M. Nagalakshmi Devamma:** Conceptualization, Supervision, Validation, Funding acquisition, Project administration, Writing – review & editing.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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