

Computational analysis of *Piper* spp.-derived natural products as anti-cancer potential through targeting aurora kinase A

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Abstract. Aurora kinase A (AURKA) overexpression correlates with increased tumor grade, resistance to chemotherapy, and poor breast cancer prognosis. Targeting AURKA is a promising way to suppress breast cancer development. Several species of the genus *Piper* have been famously used as anti-cancer agents due to their bioactive compounds. The bioactive compounds were carefully selected based on their toxicity using computational methods via the SwissADME and ProTox 3.0 web servers. The target protein prediction and network analysis toward cancer cells were then performed using the SwissTargetPrediction webserver, STRING database version 12.0, and the Cancer Hallmarks webserver. Analysis of bioactive compounds against target protein in breast cancer was performed using UALCAN, GEPIA and TIMER 2.0. The validation structure was analyzed with SAVESv6.1 webserver and ProSA-web. Molecular docking was performed using PyRx 0.8. The molecular dynamics simulation was performed using YASARA. The result showed that epi- γ -eudesmol, guaiol acetate, γ -cadinene, and safrrole were selected as drug candidates. The analysis showed that guaiol acetate had the lowest acute toxicity, making it the most favorable candidate. Based on intersection analysis, AURKA was identified as most likely therapeutic target for breast cancer therapy. In addition, AURKA was known to have higher upregulation in cancer cells which leads to poor prognosis. Regarding the immune system, AURKA significantly promotes pro-tumor immune cell infiltration and suppresses anti-tumor immune cells. Structural validation analysis showed acceptable stereochemical quality. The molecular docking results showed that guaiol acetate and AT9283 bind to the same catalytic site around the highly conserved Asp-Phe-Gly motif and the ATP-binding pocket. The molecular dynamics analysis showed that AURKA-guaiol acetate complex exhibited lower structural stability and mobility than AURKA-AT9283 complex. In addition, AURKA-guaiol acetate complex exhibited less compact structure and greater fluctuations. In conclusion, guaiol acetate shows potential as a cancer drug candidate as it has lower toxicity and evident targeted cancer activity. Guaiol acetate may mimic AT9283 as an AURKA-targeted inhibitor, although it has slightly lower potency.

Keywords: AURKA; breast cancer; dynamics simulation; natural products; *Piper* spp.

1. Introduction

The development of novel and targeted therapeutic strategies is essential to overcome the limitations of conventional cancer treatment, such as drug resistance and systemic side effects. Molecular oncology has identified key regulators of cell division as attractive therapeutic targets, notably the serine/threonine kinase, Aurora kinase A (AURKA), a critical oncogenic driver in breast cancer [1]. At the molecular level, AURKA functions as a high-fidelity catalyst for ATP-dependent phosphorylation, governing centrosome maturation and

spindle assembly. While its role in maintaining chromosomal stability is vital, its overexpression triggers a cascade of aneuploidy and genomic instability [2-4].

From a drug discovery standpoint, the therapeutic potential of AURKA inhibition is well-validated [5], yet the development of "Type I" or "Type II" inhibitors is plagued by a significant selectivity hurdle. The ATP-binding catalytic domains of the Aurora family (AURKA, B, and C) exhibit high sequence homology and structural conservation within the family. Most existing small-molecule inhibitors rely on a core

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scaffold that targets the highly conserved hinge region, leading to off-target cross-reactivity with AURKB [6, 7]. This lack of specific binding underscores the need for novel chemical scaffolds that can exploit subtle topographic differences in the AURKA binding pocket such as the gatekeeper residue or the solvent-exposed regions to achieve higher degree of isoform selectivity.

A significant proportion of anticancer drugs is currently derived from natural products [8], as well as offer a diverse chemical structure and are widely used in cancer therapy due to their availability, broad biological activity, and generally lower toxicity compared to many synthetic drugs. Natural products often possess high stereochemical complexity, a greater proportion of sp^3 -hybridized carbons, and unique oxygen or nitrogen-rich frameworks that provide precise spatial orientation for hydrogen-bonding networks, which perfect to interact with biological macromolecules, making them ideal for non-canonical kinase inhibitors candidate [9]. For example, some natural compounds can enhance immune-related chemical signaling within tumors, thereby strengthening antitumor immune responses [10, 11]. In addition, they can induce intracellular signaling pathways that cause apoptosis in tumor cells. Beyond

their direct cytotoxic effects, natural products have also been shown to improve chemotherapy sensitivity, making cancer medication more effective [12].

The *Piper* genus represents a rich, yet under-explored, repository of bioactive secondary metabolites. In addition to antioxidant and anti-inflammatory activities, *Piper* species exhibit significant anticancer potential, making them promising candidates for drug discovery [13-15]. For instance, piperine, a major compound from *Piper* species, has been reported to inhibit cell cycle progression, suppress angiogenesis, and induce cancer cell death [16]. Another bioactive compound, β -caryophyllene oxide, has been shown to downregulate MMP-2, p-p38, and p-ERK in human fibrosarcoma cells [17]. Given the therapeutic significance of these bioactive compounds, this study identified and evaluated the major bioactive compounds present in *Piper* species using an integrative *in silico* approach. The primary objective was to explore potential anti-cancer candidates, particularly those that could inhibit the AURKA protein, a key regulator in breast cancer progression. The systematic and integrative methodology used in this study is illustrated in the flowchart (Figure 1).

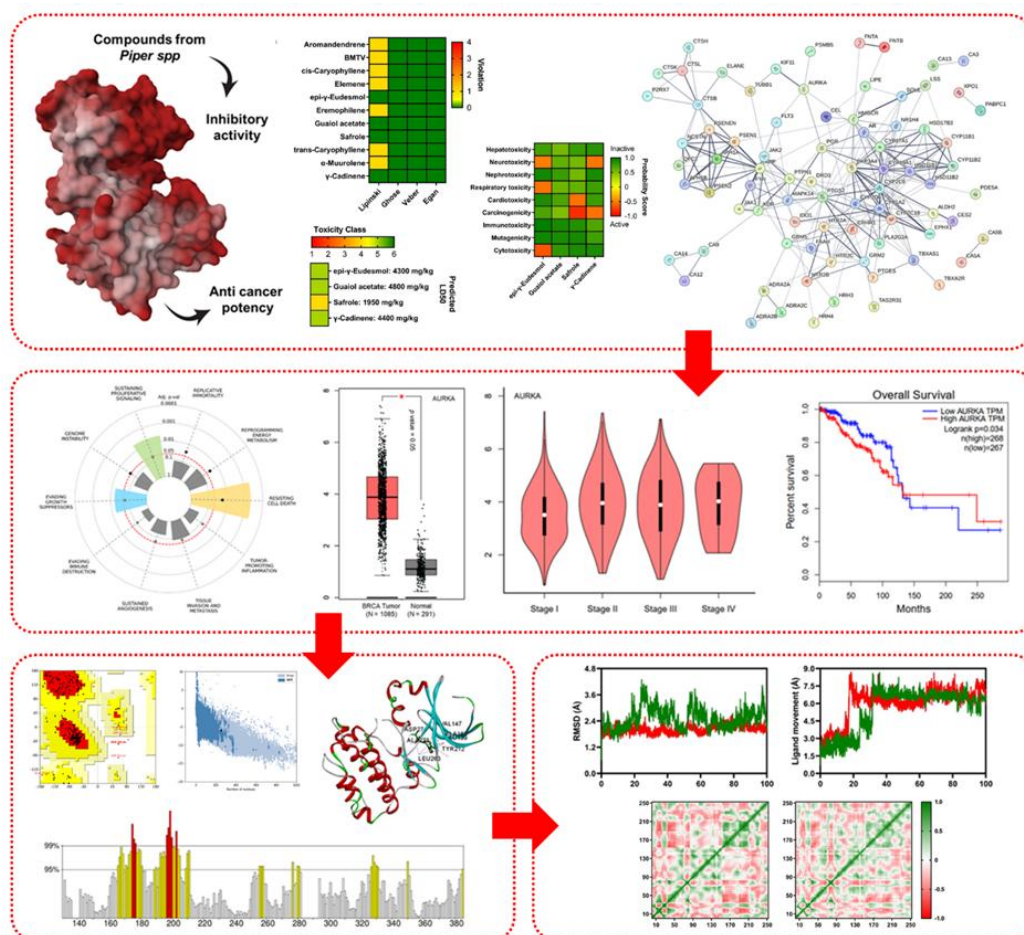


Figure 1. A flowchart illustrating the approach to the present study, which encompasses virtual screening, target protein selection with enrichment analysis, molecular docking, and molecular dynamics simulation.

2. Computational details

2.1. Drug-likeness screening and toxicity prediction

Gas chromatography–mass spectrometry (GC-MS) analyses of several *Piper* spp., including *Piper mutabile*,

Piper leptostachyum, *Piper laosanum*, *Piper boehmeriifolium*, *Piper carnibracteum*, *Piper albispicum*, and *Piper longum* have revealed the presence of common volatile phytochemicals. A drug-likeness screening was carried out to assess the drug-like

properties of the major bioactive compounds in those species [18]. The selection was analyzed using the SwissADME web server (<http://www.swissadme.ch/>) [19]. Subsequently, compounds that met the drug-likeness criteria underwent toxicity prediction using the ProTox 3.0 webserver (<https://tox.charite.de/protox3/>) to assess toxicity classification, predicted LD₅₀, and the probability of organ-specific toxicity induction [20]. The evaluated toxicity parameters included hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity.

2.2. Target protein prediction and network analysis of selected bioactive compounds

After assessing drug-likeness screening and toxicity prediction, compounds that fulfilled the criteria were further analyzed for target protein prediction. Target protein prediction was conducted using the SwissTargetPrediction webserver (<http://www.swisstargetprediction.ch/>) [21]. Next, a network analysis was performed using the STRING database version 12.0 (<https://string-db.org/>) to examine the connections between the predicted target proteins [22]. To further understand the role of these proteins in cancer progression, a cancer hallmark analysis was conducted using the Cancer Hallmarks webserver (<https://cancerhallmarks.com/>) [23].

2.3. Identification and functional analysis of specific target proteins in breast cancer

An intersection using Venn diagram (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was used to identify the specific target proteins of active compounds found in *Piper* spp. This approach compared the predicted target proteins of *Piper* spp. compounds with the top 100 upregulated genes in TNBC, HER2+, and luminal breast cancer subtypes. The upregulated gene data were obtained from the public database UALCAN (<https://ualcan.path.uab.edu/>) [24]. Once the specific targets were identified, enrichment analysis was conducted by comparing tumor and normal samples. Additionally, prognosis analysis was performed using GEPIA (<http://gepia.cancer-pku.cn/>) [25]. Finally, a correlation analysis assessed the relationship between gene expression and immune infiltration. This analysis used TIMER 2.0 (<http://timer.cistrome.org/>) [26].

2.4. Protein structure validation and molecular docking

Structural validation was performed to confirm the quality and reliability of the target protein structure used in this study. The target protein structure was sourced from the RCSB PDB database (<https://www.rcsb.org/>) [27], and validated using the SAVESv6.1 webserver (<https://saves.mbi.ucla.edu/>) and ProSA-web (<https://prosa.services.came.sbg.ac.at/prosa.php>) [28].

Before molecular docking, the structures of 11 dominant bioactive compounds from *Piper* spp. were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) [29]. These compounds included aromandendrene (CID: 91354), bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl- (CID: 557142), cis-caryophyllene (CID:

5281522), elemene (CID: 6918391), epi- γ -eudesmol (CID: 6428429), eremophilene (CID: 12309744), guaiol acetate (CID: 240122), saffrole (CID: 5144), trans-caryophyllene (CID: 5281515), α -muurolene (CID: 12306047), and γ -cadinene (CID: 92313). The reference drug used as a control was AT9283 (CID: 135398495), a known AURKA inhibitor [5]. Molecular docking was performed using AURKA (PDB ID: 8JF4), which had previously undergone structural validation [30]. Docking simulations were executed using PyRx 0.8 software (<https://pyrx.sourceforge.io/>) [31], and the docking results were visualized using BIOVIA Discovery Studio Visualizer (<https://www.3ds.com>).

2.5. Molecular dynamics simulation for protein-ligand complex

Molecular dynamics (MD) simulation was performed to assess the stability and flexibility of the protein-ligand complex following molecular docking. The simulation was conducted under physiologically relevant conditions, with parameters set to pH 7.4, 0.9% NaCl concentration, 310 K, 0.997 g/cm³ water density, and 1 atm pressure. Several parameters including root mean square deviation (RMSD) backbone, RMSD ligand movement, RMSD ligand conformation, root-mean-square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), hydrogen bonding (H-bond) interactions, binding energy, and dynamic cross-correlation matrix (DCCM) were carried out during MD simulation. The MD simulation was conducted using YASARA software (<https://www.yasara.org/>) [32].

3. Results and discussion

3.1. Drug-likeness screening and toxicity prediction of bioactive compounds in *Piper* spp.

A study was conducted to evaluate the chemical compounds present in various *Piper* species, including *Piper mutabile*, *Piper leptostachyum*, *Piper laosanum*, *Piper boehmeriifolium*, *Piper carnibracteum*, *Piper albispicum*, and *Piper longum*. These compounds were found in varying levels, high, moderate, low, or absent, and only the most abundant compounds were selected in this study, as their high natural availability makes them easier to obtain [18]. This selection also aimed to facilitate extraction and maximize their therapeutic applications.

Only eleven of the 37 identified compounds were consistently found in several *Piper* spp. species, namely aromandendrene, BMTV, cis-caryophyllene, elemene, epi- γ -eudesmol, eremophilene, guaiol acetate, saffrole, trans-caryophyllene, α -muurolene, and γ -cadinene (Figure 2A). Subsequently, a drug-likeness screening was performed using several parameters, including Lipinski, Ghose, Veber, and Egan rules. The results indicated that four of the eleven compounds possessed the requisite physicochemical characteristics to be considered as drug-like including epi- γ -eudesmol, guaiol acetate, γ -cadinene, and saffrole (Figure 2B). The selected compounds were then further analyzed through toxicity prediction to assess their safety profiles. The parameters used in this prediction included toxicity

classification, LD₅₀ values, and the potential of the compounds to induce toxicity in specific organs (Figures 2C and 2D). Based on the results, we highlighted that safrole has the lowest LD₅₀, indicating higher acute toxicity. However, the other compounds, such as epi- γ -eudesmol, guaial acetate, and γ -cadinene, fall within the same toxicity classification and have high LD₅₀ values, suggesting lower acute toxicity. Among these compounds, guaial acetate was identified as having the

lowest overall toxicological risk, with no significant flags for organ-specific toxicities like hepatotoxicity or neurotoxicity. Overall, drug-likeness screening and toxicity prediction are crucial steps in drug discovery. These processes help select compounds with the highest potential as drug candidates. Moreover, toxicity prediction provides an early assessment of the compounds' toxic effects on organs, thereby accelerating drug discovery and reducing research costs and time.

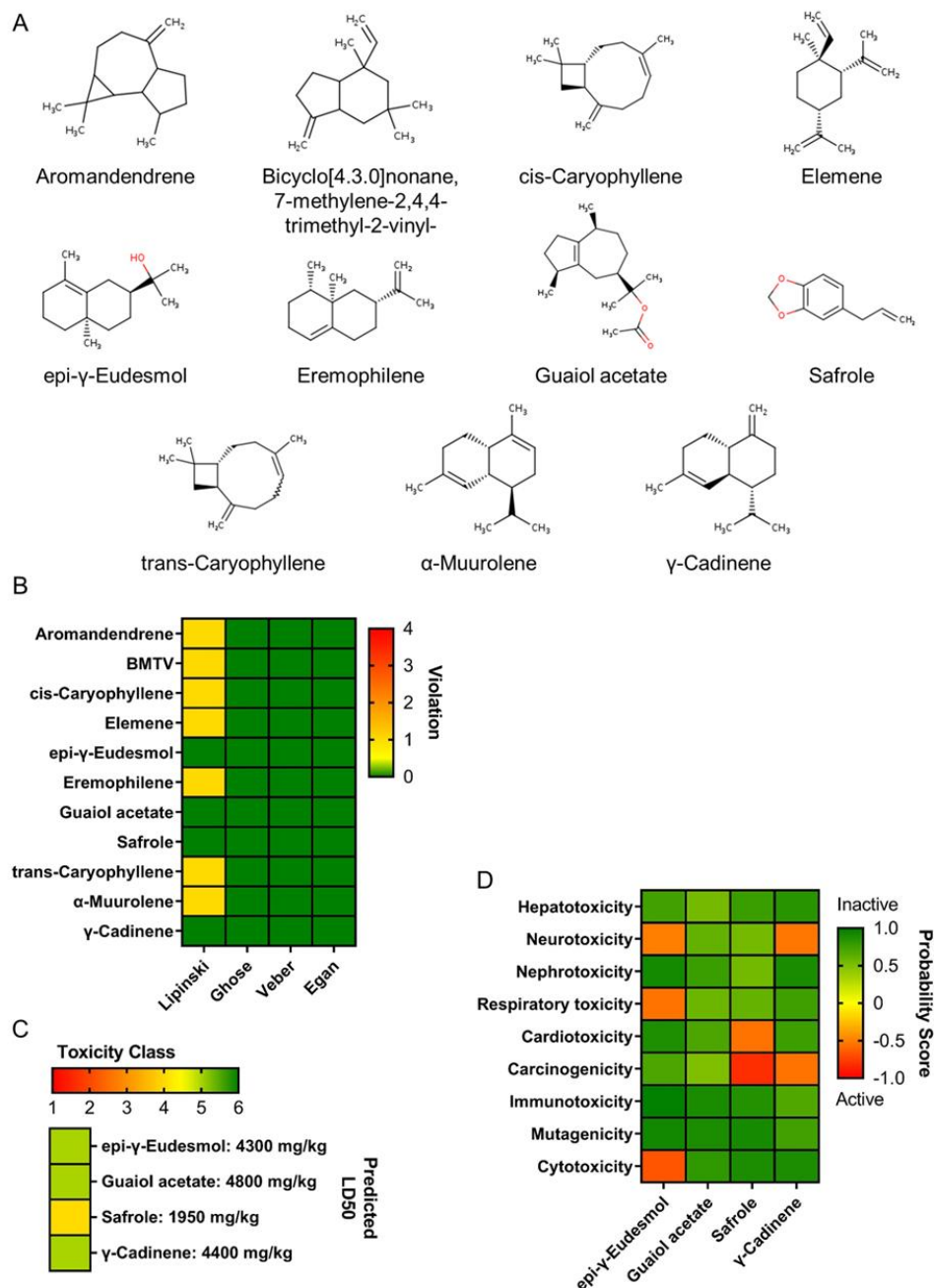


Figure 2. Virtual screening of major bioactive compounds from *Piper* spp. A). 2D structures of the major bioactive compounds. B). Drug-likeness screening. C). Toxicity classification and LD₅₀ prediction. D). Prediction of compounds with potential organ-specific toxicity.

3.2. Identification of AURKA as a potential therapeutic target in breast cancer

Subsequently, target protein prediction for guaial acetate was performed, identifying approximately 80 potential target proteins. A network analysis was then conducted to assess the interactions among these

proteins (Figure 3A). The results revealed that some proteins exhibited significant interactions, while others showed minimal interaction, as indicated by the number of connecting lines between them.

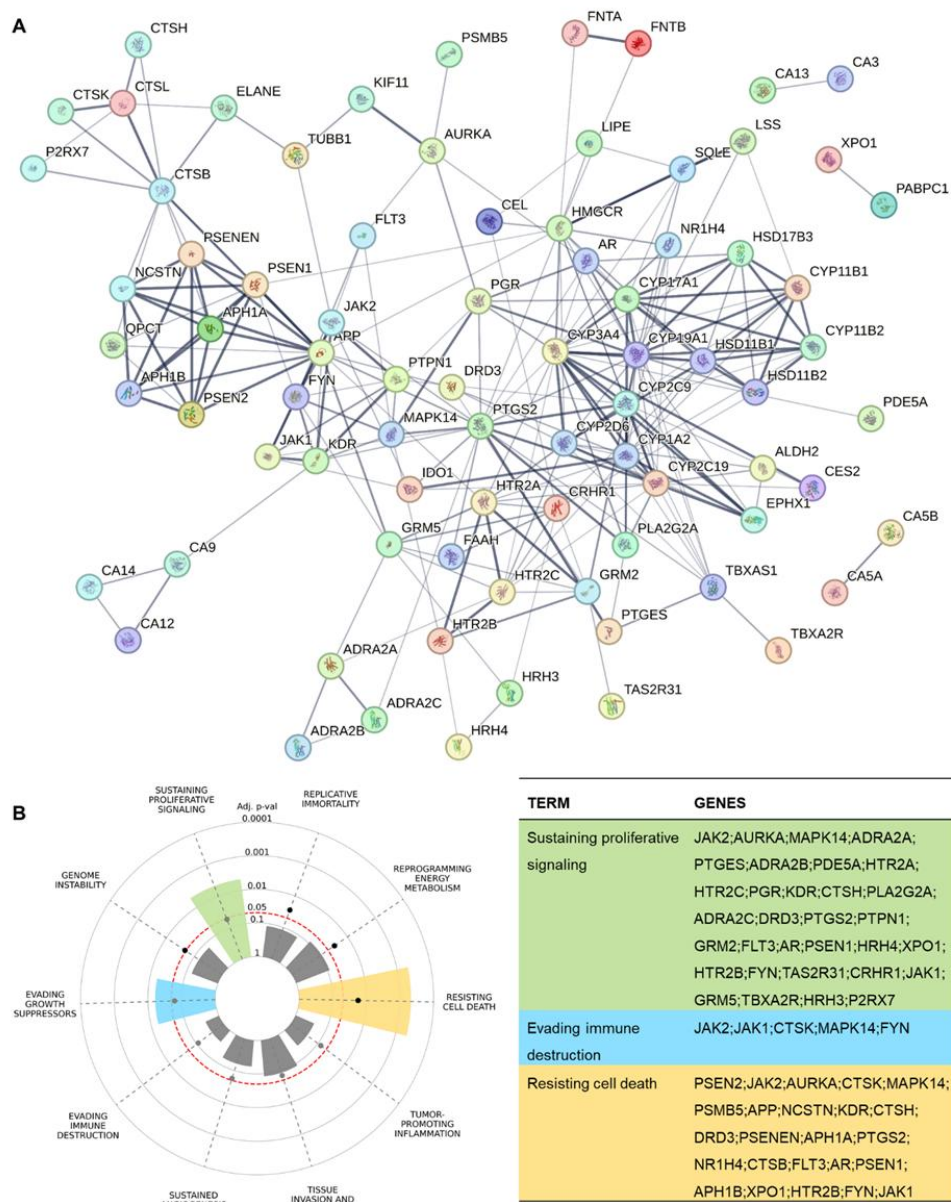


Figure 3. Target protein prediction and its role in cancer progression. A). Protein-protein interactions among predicted target proteins. B). Prediction of cancer hallmarks.

Furthermore, a cancer hallmark analysis was conducted to gain deeper insights into these proteins' functional roles and identify those involved in cancer progression (Figure 3B). This identification process facilitates the optimization of biomarker predictions, particularly those playing crucial roles in specific phases of cancer development and progression. Consequently, cancer treatment strategies can be improved by targeting these key biomarkers.

To further refine target protein prediction for breast cancer, we identified the intersection between guaiol acetate target proteins and the top 100 upregulated genes across breast cancer subtypes, including triple-negative breast cancer (TNBC), HER2-positive, and luminal breast cancer (Figure 4A). From this intersection analysis, AURKA emerged as the only potential therapeutic target for breast cancer treatment (Figure 4B). Subsequent enrichment analysis comparing gene expression in tumor versus normal samples from breast

cancer patients revealed that AURKA is significantly upregulated in tumor samples (Figure 4C). Further analysis of AURKA expression across breast cancer stages indicated that its expression remains relatively consistent across stages, suggesting that AURKA could be a viable therapeutic target regardless of stage (Figure 4D). Finally, Kaplan-Meier analysis of overall survival showed that the high AURKA expression group had a steeper decline in survival than the low AURKA expression group, indicating that high AURKA expression is associated with poor prognosis (log-rank p-value = 0.034). However, AURKA's role remains unclear in disease-free survival analysis, as no significant difference was observed between the two groups (Figure 5E). Overall, targeting AURKA could be a promising therapeutic strategy for breast cancer patients due to its role in cancer progression and its association with poor prognosis.

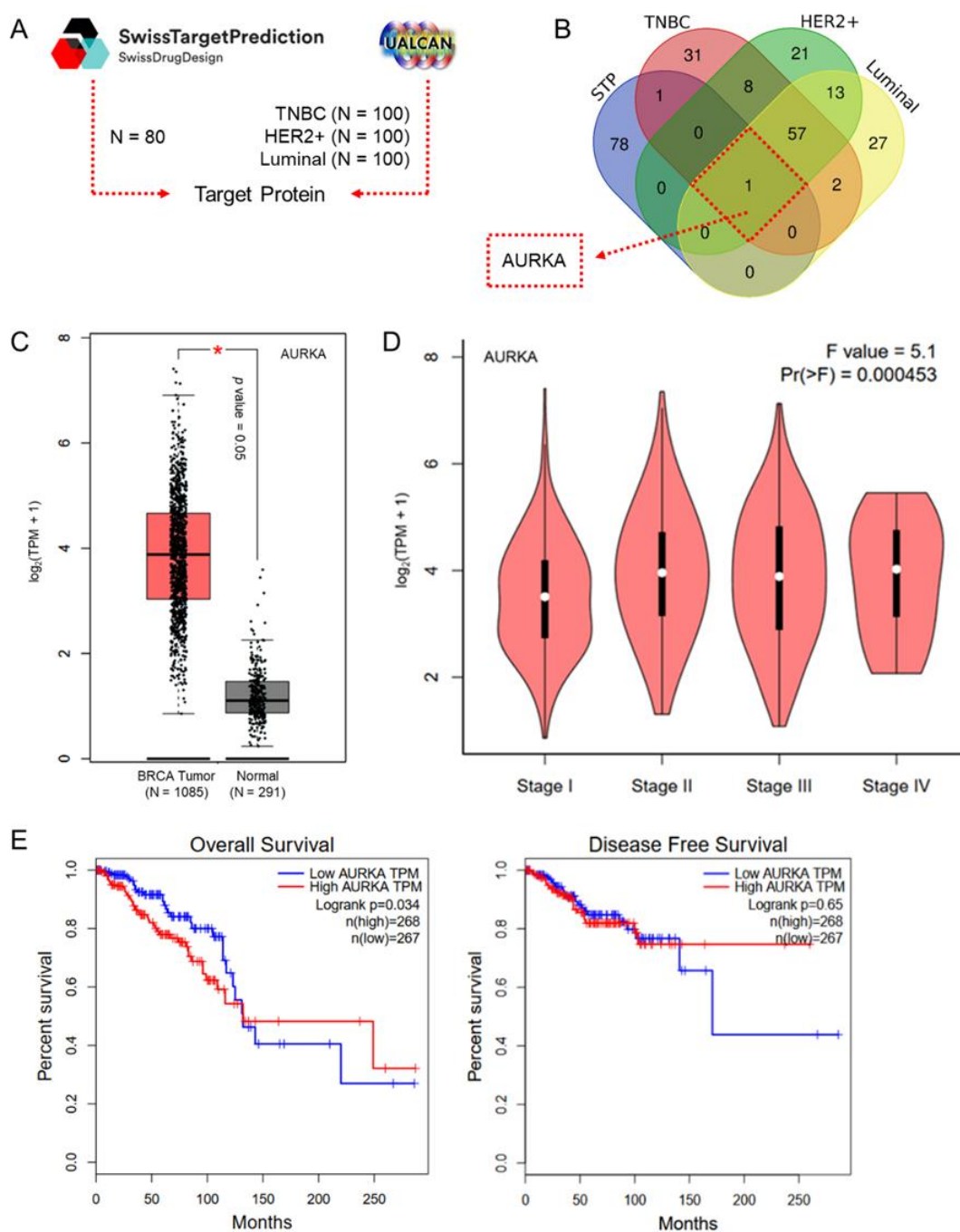


Figure 4. Target protein selection and enrichment analysis. A). Intersection of target proteins for guaiol acetate with the top 100 upregulated genes from various breast cancer subtypes, including TNBC, HER2+, and luminal breast cancer, based on public data.

B). AURKA identified as a potential target protein for breast cancer treatment using *Piper* spp. bioactive compounds. C). Comparative analysis of AURKA gene expression between tumor and normal breast tissue based on public datasets. D). Gene expression levels of AURKA across different stages of breast cancer. E). Overall survival and disease-free survival analyses.

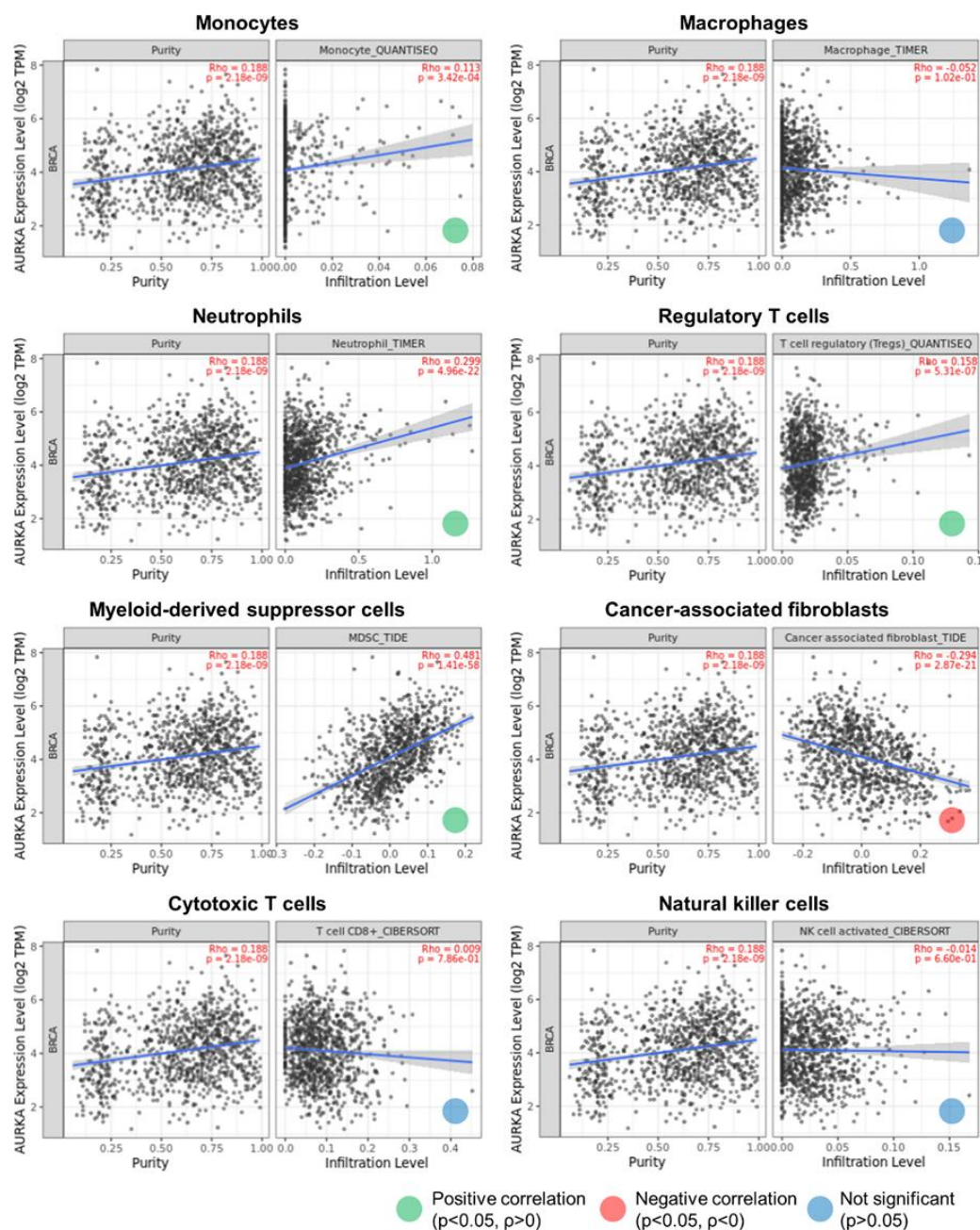


Figure 5. Correlation analysis of AURKA gene expression in breast cancer patients with tumor microenvironment component infiltration, based on public data.

On the other hand, the correlation analysis between AURKA expression and the infiltration levels of various immune and stromal cell types in breast cancer revealed a positive correlation with monocytes, neutrophils, regulatory T cells, and myeloid-derived suppressor cells (Figure 5). This suggests that AURKA expression in breast cancer may contribute to tumor progression by promoting the infiltration of pro-tumor immune cells. However, specific immune cell components, such as macrophages, cytotoxic T cells, and natural killer cells, showed no significant correlation with AURKA expression. Cancer-associated fibroblasts, known as stromal cells, also negatively correlated with AURKA expression. The lack of correlation with macrophages, cytotoxic T cells, and natural killer cells suggests that AURKA expression may not directly impact the anti-tumor immune response. Together, these findings

indicate that targeting AURKA could be a potential therapeutic strategy for breast cancer by reducing the recruitment and activation of immunosuppressive immune cells within the tumor microenvironment, thereby preventing cancer progression and improving immunotherapy efficacy.

3.3. Structural validation analysis and computational analysis of AURKA-ligand interaction

Structural validation was performed to ensure the reliability and quality of the protein structure used in molecular docking and dynamics simulations, including Ramachandran plot analysis, structural quality and error assessment, and Z-score and energy profile analysis. According to the Ramachandran plot analysis, approximately 85.1% of residues were found in most favored regions, 12.2% in additionally allowed regions,

and a small fraction (1.4%) in generously allowed and disallowed regions (Figure 6A). These findings confirm that the protein model exhibits good stereochemical quality. Furthermore, the ERRAT plot analysis supports this, showing an overall quality factor of 80.252 and only minor structural inconsistencies, as indicated by red and yellow peaks (Figure 6B). Additionally, the Z-score value of the protein model was within the expected range for a well-folded protein, reaching -6, further

supporting its validity. Notably, the energy profile analysis indicated that the protein model is structurally stable, as reflected by the dominant negative energy values of residues, suggesting better stability (Figure 6C). These results confirm that the AURKA protein model is high-quality, reliable, and structurally accurate, making it suitable for molecular docking and dynamics simulation studies.

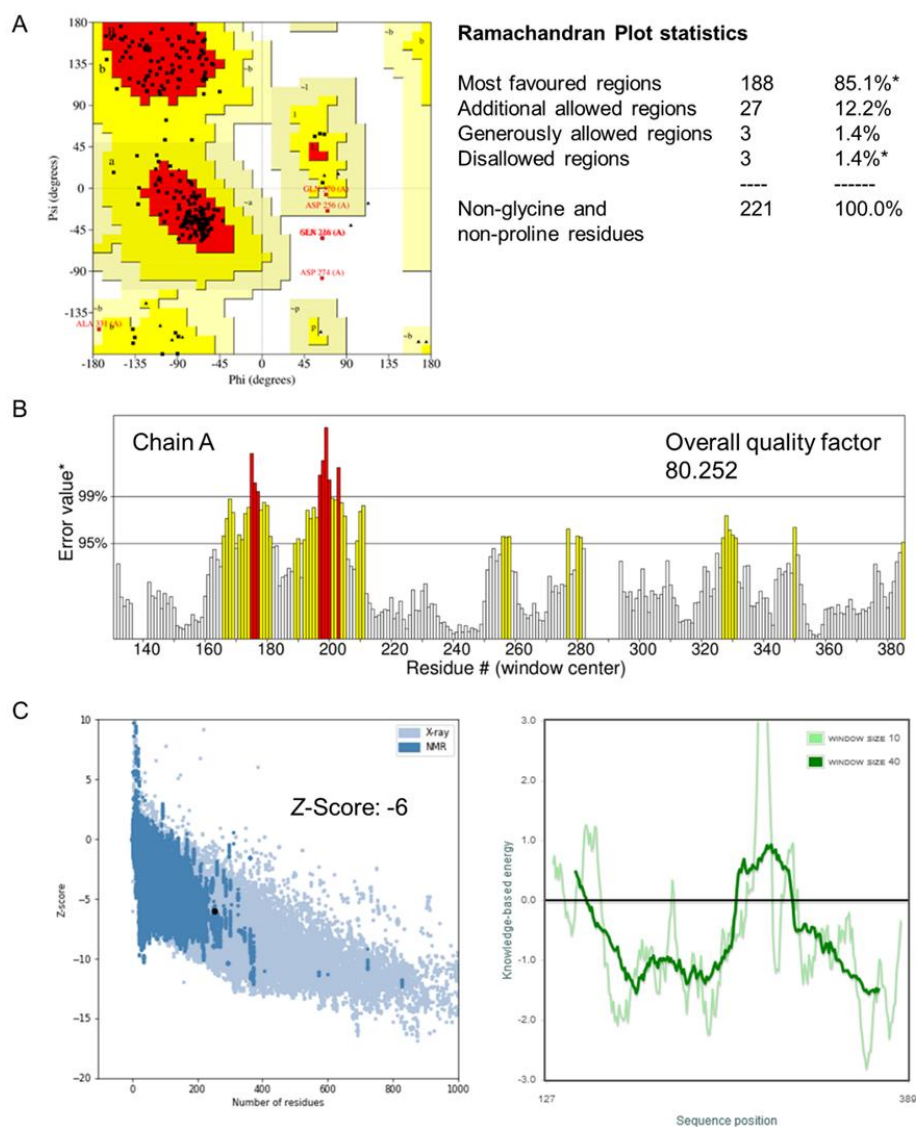


Figure 6. AURKA protein structure validation. A). Ramachandran plot assessment using SAVES. B). ERRAT plot evaluation through SAVES. C). Protein structure analysis using ProSA-web.

Molecular docking and dynamics simulation approaches were used to evaluate the binding location, stability, flexibility, and potential effectiveness of guaiaol acetate and AT9283 targeting AURKA over a 100 ns simulation period. Molecular docking results showed that both compounds occupied the same binding site around the highly conserved Asp-Phe-Gly motif and the

ATP-binding pocket, which resides between the N- and C lobes, indicated by shared interaction with Asp274, Leu263, Val147, and Lys162 [33]. Further supported by the molecular dynamics results, it demonstrated conformational changes at 0, 50, and 100 ns (Figure 7 and 8).

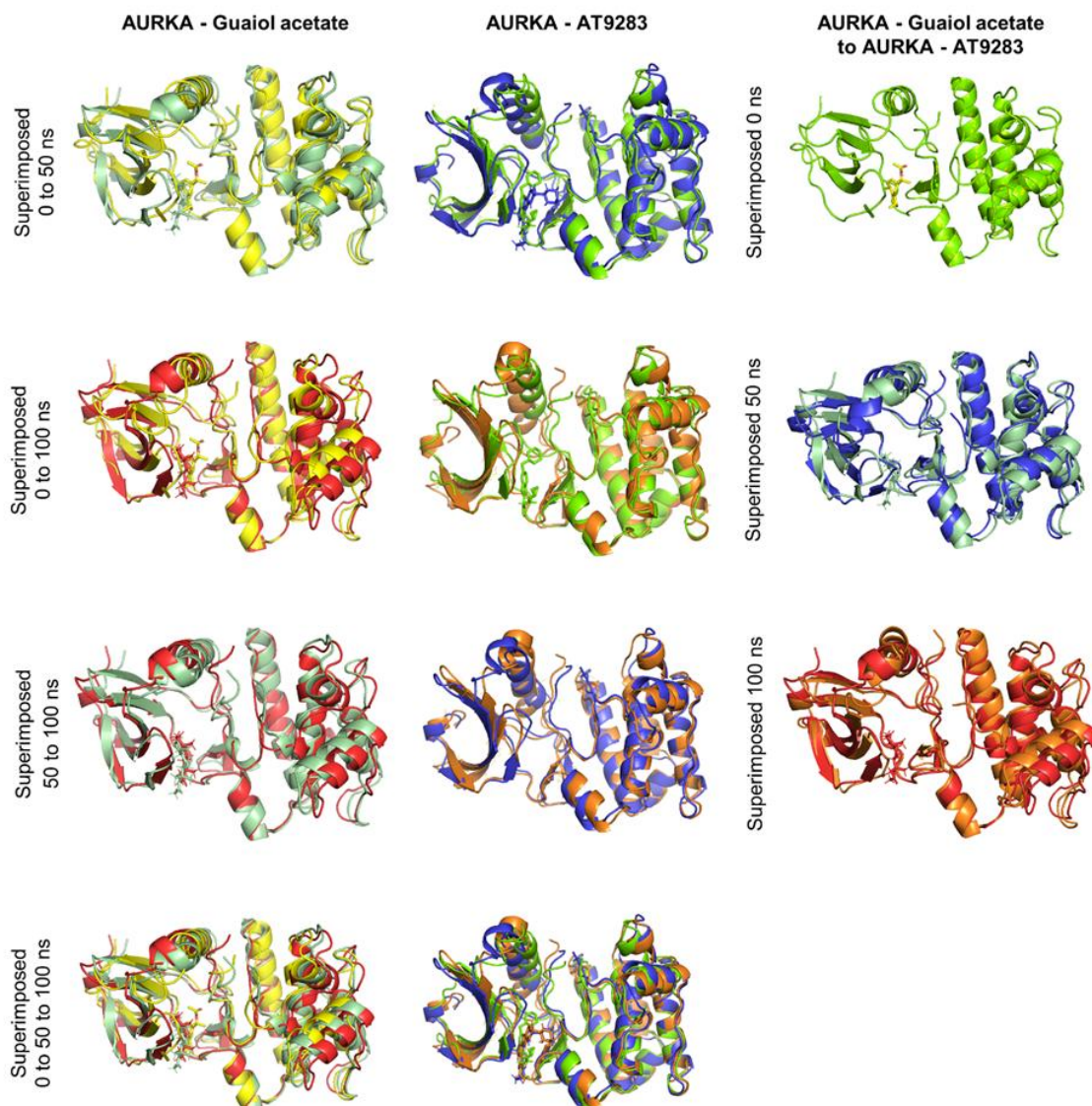


Figure 7. Protein-ligand complex movements and structural changes during molecular dynamics simulation.

During the simulation, it is clear that both ligands were positioned within the same binding pocket at the initial conformation. The AURKA-guaiol acetate complex exhibited one hydrogen bond and multiple alkyl interactions, which might contribute to ligand stabilization within the binding pocket. In contrast, the AURKA-AT9283 complex displayed multiple hydrogen bonds, Pi-stacking, and alkyl interactions, suggesting a stronger initial binding stability. During the intermediate stage, a noticeable conformational shift was observed in the AURKA-guaiol acetate complex, where the hydrogen bond was lost, indicating increased ligand mobility. Meanwhile, the AURKA-AT9283

complex remained stable, maintaining hydrogen bonds and other interactions, suggesting a consistent binding affinity. The protein-guaiol acetate complex appeared more stable at the final stage than at the intermediate stage. However, unlike AT9283, no hydrogen bonds were observed, indicating a weaker overall binding affinity. Interestingly, AT9283 maintained stable interactions throughout the simulation, demonstrating strong, persistent binding to the target protein. These findings suggest that while guaiol acetate shows some stability, its binding affinity is weaker than that of AT9283, which maintains robust, consistent interactions with AURKA throughout the simulation.

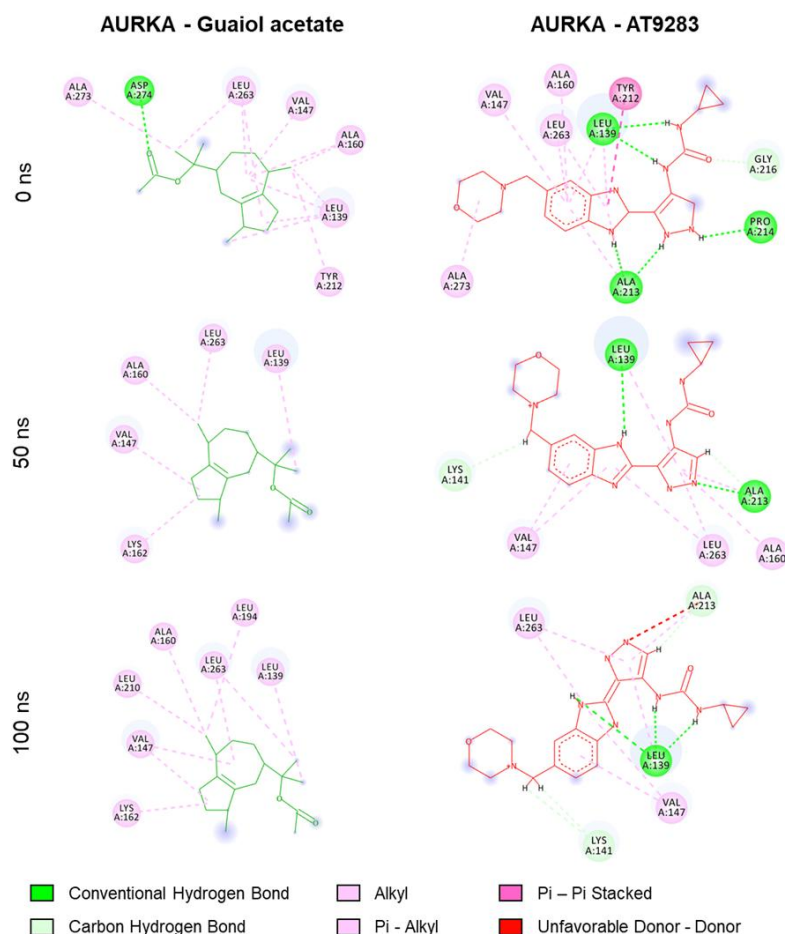


Figure 8. Ligand movements and conformational changes during molecular dynamics simulation, identified through alterations in chemical interactions.

Several molecular dynamics simulation parameters were analyzed to evaluate the structural stability, ligand mobility, flexibility, structural compactness, and binding affinity. The root-mean-square deviation (RMSD) analysis revealed that the complex with AT9283 (red) rapidly reaches equilibrium and maintains a stable RMSD of approximately 1.8 Å. In contrast, the complex with guaol acetate (green) shows significantly higher deviation, fluctuating around 2.4 Å, indicating greater structural instability in the protein when this ligand is present (Figure 9A). Both compounds demonstrate substantial ligand movement, exceeding 7.5 Å from their initial positions, within the binding pocket. This movement is observed around 20-30 ns. While the AT9283 complex (red) reaches equilibrium more rapidly, guaol acetate (green) exhibits a dramatic shift around the 30 ns mark before achieving equilibrium (Figure 9B). AT9283 underwent frequent conformational changes, indicating lower rigidity and stability than guaol acetate, which maintained a more stable conformation throughout the simulation (Figure 9C). Root mean square fluctuation (RMSF) analysis revealed similar profiles for both ligands. However, key residues in the guaol acetate complex showed increased flexibility, suggesting a weaker stabilizing effect on the overall protein structure compared to AT9283 (Figure 9D).

The radius of gyration (Rg) analysis revealed that the AURKA-AT9283 complex had a lower Rg, indicating a

more compact and stable structure. In contrast, the AURKA-guaol acetate complex had higher Rg values, suggesting a less compact structure and greater fluctuations, consistent with a less stable overall structure (Figure 9E). Additionally, solvent-accessible surface area (SASA) analysis showed that AT9283 consistently had lower SASA values, indicating that the protein adopts a more compact, tightly folded structure upon binding its potent inhibitor. Conversely, guaol acetate exhibited elevated SASA values, indicating a greater tendency for the protein complex to adopt a more unfolded conformation, thereby increasing its interaction with the surrounding solvent. These data corroborate the radius of gyration data perfectly (Figure 9F). Hydrogen bonding plays a crucial role in ligand stability within the active site. Both AT9283 and guaol acetate exhibited similar hydrogen bonding patterns (Figure 9G). However, binding energy calculations indicated that AT9283 had a significantly lower binding energy, signifying a highly stable complex than guaol acetate, which demonstrated weaker binding affinity (Figure 9H). The dynamic cross-correlation matrix (DCCM) analysis revealed that AT9283 promoted stronger correlated motions in AURKA, as indicated by more green regions. In contrast, guaol acetate showed more red regions, indicating stronger anti-correlated motions and greater structural disruption upon binding to AURKA (Figure 10). This suggests that AT9283 stabilizes AURKA more effectively than guaol acetate.

Overall, AT9283, a known AURKA inhibitor, demonstrated superior stability, lower ligand movement, strong hydrogen bonding, and higher binding affinity. Meanwhile, guaiol acetate exhibited higher flexibility,

increased solvent exposure, and weaker binding, indicating that structural modifications may be necessary to enhance its inhibitory potential against AURKA for breast cancer treatment.

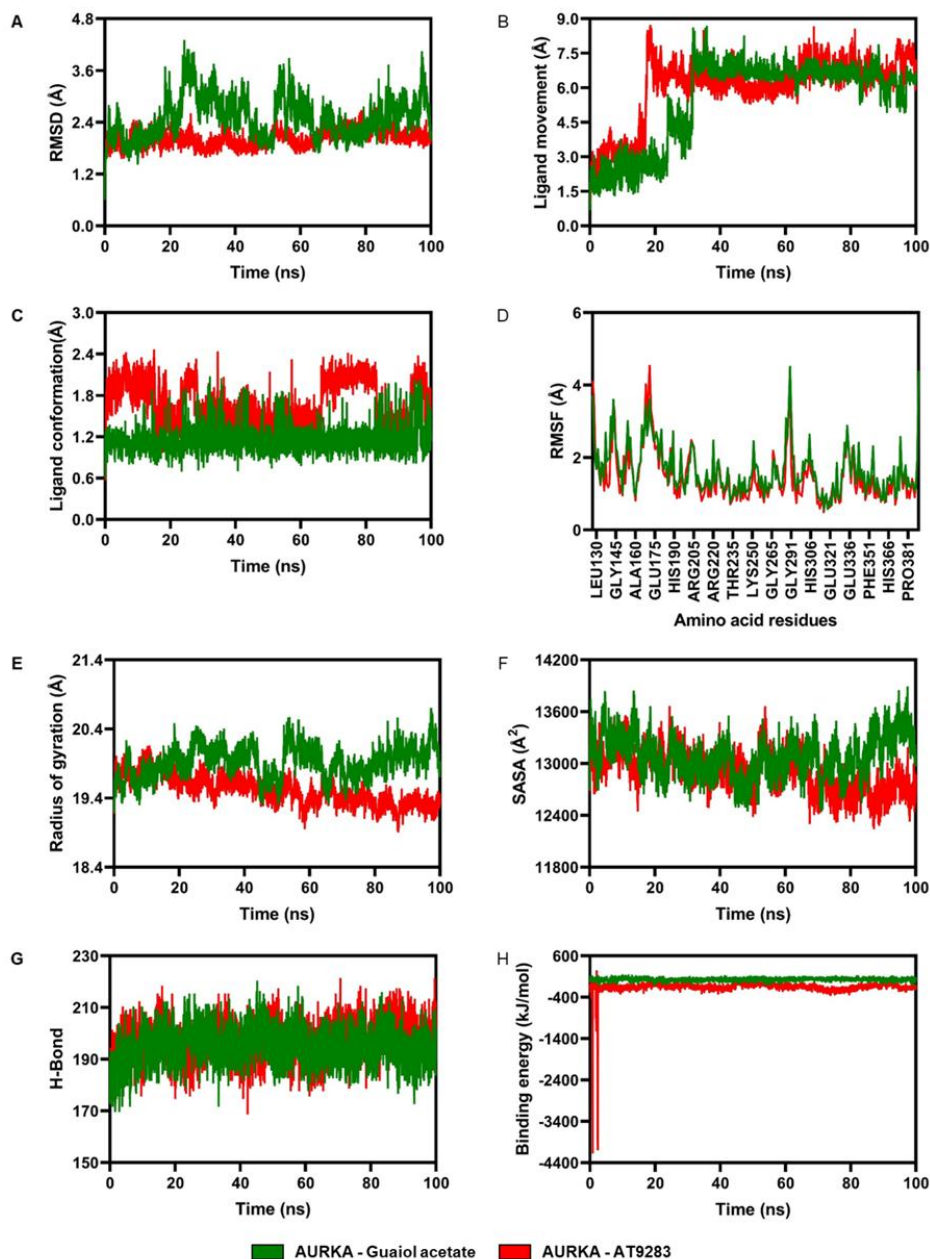


Figure 9. Molecular dynamics simulations of guaiol acetate and AT9283 against the target protein, AURKA. A). Backbone RMSD plot. B). Ligand movement RMSD plot. C). Ligand conformation RMSD plot. D). RMSF plot. E). Radius of gyration plot. F). SASA plot. G). Hydrogen bond interaction plot. H). Binding energy plot.

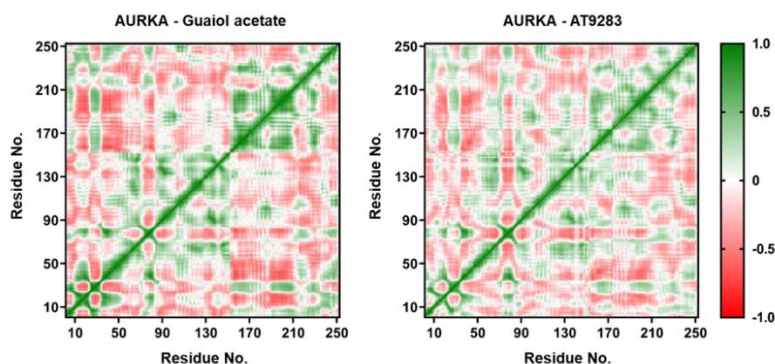


Figure 10. Dynamics cross-correlation matrix analysis of AURKA-ligand complex.

AURKA is a member of the highly conserved family of serine/threonine kinase family, which also includes Aurora Kinase B (AURKB) and Aurora Kinase C (AURKC). These family members share a high degree of sequence homology, particularly within their C-terminal catalytic domains, where AURKA and AURKB exhibit 71% identity. This structural similarity is not merely a taxonomic observation but also a foundational concept with profound implications for drug development. It presents a significant pharmacological challenge in designing inhibitors with high specificity, a factor that has dictated the trajectory and clinical outcomes of many drug candidates in this class. Fundamentally, the mechanistic link between AURKA and cancer is multifaceted. Aberrant AURKA activity is a direct driver of oncogenic transformation, promoting genomic instability by inducing centrosome amplification and aneuploidy, hallmarks of cancer. Beyond its impact on chromosome segregation, hyperactivated AURKA phosphorylates a host of pathological substrates, thereby engaging and amplifying critical oncogenic signaling pathways, including those driven by Myc, NF κ B, and Wnt/ β -catenin [4, 34]. AURKA's involvement in tumorigenesis is frequently reinforced through in positive feedback loops with its targets. It phosphorylates proteins such as PHLDA1 and ALDH1A1, which modulate its levels, establishing a reciprocal loop that maintains its upregulation and hyperactivation in cancer cells. This self-sustaining cycle creates a notable vulnerability. Targeting a substrate within this loop may disrupt the entire system and reverse AURKA hyperactivation, presenting an alternative therapeutic strategy to direct kinase inhibition [35, 36].

The full-length human AURKA protein is a 403-amino acid polypeptide organized into three distinct functional domains. At its N-terminus (approximately residues 1-125) lies a structurally disordered region of variable sequence primarily responsible for mediating protein-protein interactions and dictating the subcellular localization of the kinase, for example, by targeting it to the centrosome. The central and largest portion of the protein is the highly conserved catalytic kinase domain (approximately residues 125-391), which harbors the active site and is responsible for phosphotransfer activity. Finally, a short C-terminal tail contains a conserved "Destruction Box" (D-box) motif. This sequence acts as a degron, marking AURKA for recognition by the anaphase-promoting complex/cyclosome (APC/C) and subsequent proteasome-mediated degradation, a process that ensures its timely removal at the end of mitosis [37, 38]. The catalytic functions of AURKA are executed within a deep cleft situated at the interface of the N- and C-lobes. This active site, also referred to as the ATP-binding pocket, is a predominantly hydrophobic cavity shaped to accommodate the ATP molecule in a precisely oriented manner, facilitating the transfer of its terminal phosphate group to a serine or threonine residue on a target substrate protein [39, 40].

The simulation of the AURKA-guaiol acetate complex demonstrates a certain degree of instability. The initial docking pose at 0 ns shows a favorable

hydrogen bond with the catalytically crucial residue Asp274. This residue is the first amino acid of the highly conserved DFG (Asp-Phe-Gly) motif, which acts as a conformational switch for the active 'DFG-in' and inactive 'DFG-out' states of the kinase. In the active 'DFG-in' conformation, the side chain of Asp274 points into the active site, where it is correctly positioned to coordinate the magnesium ion (Mg²⁺) required for ATP binding and the subsequent phosphotransfer reaction [39, 41]. Several hydrophobic contacts with residues that line the ATP-binding pocket support this initial polar interaction, including Leu139, Val147, and Leu263 [41]. In the 50 ns snapshot, the initial hydrogen bond with Asp274 is lost and does not reform. Without this specific polar anchor, the ligand becomes highly mobile within the active site, as demonstrated by the high movement values in the ligand movement graph. This indicates that the complex now relies on a different and weaker set of non-specific alkyl interactions until the end of the simulation. Although guaiol acetate remains tucked into the ATP-binding site, its failure to regain contact with the DFG motif and its reliance solely on weaker bonds limit its potential as an ATP-competitive inhibitor. Critically, these weaker bonds made the complex adopt a less compact conformation with higher Rg and SASA values, and rendered AURKA intrinsically unstable, with higher random and uncoordinated motions rather than the organized dynamics observed in the DCCM. Thus, it may exhibit a lower effect on ATP binding, leading to the arrest of AURKA-mediated phosphorylation and the induction of apoptosis in cancer cells. This suggests a reduced inhibitory effect on ATP binding, consequently hindering the arrest of AURKA-mediated phosphorylation and the induction of apoptosis in cancer cells [5, 6].

Meanwhile, the simulation of the AURKA-AT9283 complex revealed a distinctive pattern. Instead of directly interacting with and inhibiting the DFG motif, the AT9283 establishes a network of interactions, including multiple hydrogen bonds with hinge region residues such as Ala213 and Leu139, which normally form crucial hydrogen bonds that anchor the adenine base of ATP within the active site [38], as well as extensive hydrophobic contacts with key residues like Val147 and Leu263, which particularly control the ATP access to the deeper part of the binding pocket [39]. Although it still experiences a similar degree of ligand movement as the guaiol acetate, those stronger hydrogen bonds secure its place in the ATP-binding site, affecting AURKA's internal dynamics. It is suggested that the AT9283 complex exhibits highly structured, well-defined patterns of both correlated and anti-correlated motions. This indicates that the stable binding of the inhibitor locks the kinase into a specific conformational state in which its structural domains move as coordinated, rigid units. The prominent anti-correlated motions are characteristic of the functional hinge-bending between the N- and C-lobes, suggesting that AT9283 restricts random fluctuations and organizes the protein's dynamics. This induced state of ordered, coordinated motion is critical for the inhibitor stabilizing its target in a functionally inhibited conformation. These

stable inhibitions of AURKA might repress AURKA expression in cancer cells.

4. Conclusions

The bioactive compound guaiol acetate, derived from the Piper genus, is strongly considered a potential drug candidate for targeting AURKA. Evidenced suggest that AURKA promotes cancer development and is associated with pro-tumor immune cells. Guaiol acetate evidently lowers acute toxicity and mimics AT9283 by occupying catalytic sites in a conserved area. AURKA-Guaiol acetate complex exhibited lower structural stability, mobility, and compactness than AURKA-AT9283 complex.

Declaration of interest

We declare there is no conflict of interest in this study.

Acknowledgement

The authors thank Universitas Negeri Malang for its support of this study.

References

- [1]. K. Polyak, Heterogeneity in breast cancer, *The Journal of Clinical Investigation* 121 (2011) 3786–3788. Doi: 10.1172/JCI60534
- [2]. Q. Zhou, C. Tao, J. Yuan, F. Pan, R. Wang, Knowledge mapping of AURKA in oncology: An advanced bibliometric analysis (1998–2023), *Heliyon* 10 (2024) e31945. Doi: 10.1016/j.heliyon.2024.e31945
- [3]. K. Nikhil, K. Shah, The significant others of aurora kinase A in cancer: combination is the key, *Biomarker Research* 12 (2024) 109. Doi: 10.1186/s40364-024-00651-4
- [4]. M. Chen, H. Zhu, J. Li, D. Luo, J. Zhang, W. Liu, J. Wang, Research progress on the relationship between AURKA and tumorigenesis: the neglected nuclear function of AURKA, *Annals of Medicine* 56 (2024) 2282184. Doi: 10.1080/07853890.2023.2282184
- [5]. R. Du, C. Huang, K. Liu, X. Li, Z. Dong, Targeting AURKA in cancer: molecular mechanisms and opportunities for cancer therapy, *Molecular Cancer* 20 (2021) 15. Doi: 10.1186/s12943-020-01305-3
- [6]. S. Chung, E. Sperier, K. Graciano, X. Hu, H. Guo, J. Xu, Aurora kinases, emerging critical targets for cancer treatment and related new therapeutic strategies, *Bioorganic Chemistry* 163 (2025) 108633. Doi: 10.1016/j.bioorg.2025.108633
- [7]. O. Gautschi, P.C. Mack, A.M. Davies, P.N. Lara, D.R. Gandara, Aurora kinase inhibitors: A new class of targeted drugs in cancer, *Clinical Lung Cancer* 8 (2006) 93–98. Doi: 10.3816/CLC.2006.n.036
- [8]. G.M. Cragg, J.M. Pezzuto, Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents, *Medical Principles and Practice* 25 (2016) 41–59. Doi: 10.1159/000443404
- [9]. C.F. Stratton, D.J. Newman, D.S. Tan, Cheminformatic comparison of approved drugs from natural product versus synthetic origins, *Bioorganic & Medicinal Chemistry Letters* 25 (2015) 4802–4807. Doi: 10.1016/j.bmcl.2015.07.014
- [10]. G. Nuzzo, G. Senese, C. Gallo, F. Albiani, L. Romano, G. d'Ippolito, E. Manzo, A. Fontana, Antitumor potential of immunomodulatory natural products, *Marine Drugs* 20 (2022) 386. Doi: 10.3390/md20060386
- [11]. J.L. Huo, W.J. Fu, Z.H. Liu, N. Lu, X.Q. Jia, Z.S. Liu, Research advance of natural products in tumor immunotherapy, *Frontiers in Immunology* 13 (2022) 972345. Doi: 10.3389/fimmu.2022.972345
- [12]. A. Naeem, P. Hu, M. Yang, J. Zhang, Y. Liu, W. Zhu, Q. Zheng, Natural products as anticancer agents: Current status and future perspectives, *Molecules* 27 (2022) 8367. Doi: 10.3390/molecules27238367
- [13]. B. Salehi, Z.A. Zakaria, R. Gyawali, S.A. Ibrahim, J. Rajkovic, Z.K. Shinwari, T. Khan, J. Sharifi-Rad, A. Ozleyen, E. Turkdonmez, M. Valussi, T.B. Tumer, L. Monzote Fidalgo, M. Martorell, W.N. Setzer, Piper species: A comprehensive review on their phytochemistry, biological activities and applications, *Molecules* 24 (2019) 1364. Doi: 10.3390/molecules24071364
- [14]. C.K. Woon, F.B. Ahmad, N.H. Zamakshshari, Chemical constituents and biological activities of Piper as anticancer agents: A review, *Chemistry & Biodiversity* 20 (2023) e202300166. Doi: 10.1002/cbdv.202300166
- [15]. J. Wang, K. Nikhil, K. Viccaro, L. Chang, J. White, K. Shah, Phosphorylation-dependent regulation of ALDH1A1 by Aurora kinase A: Insights on their synergistic relationship in pancreatic cancer, *BMC Biology* 15 (2017) 10. Doi: 10.1186/s12915-016-0335-5
- [16]. V.R. Umapathy, A. Dhanavel, R. Kesavan, P.M. Natarajan, S.B., P.V., Anticancer potential of the principal constituent of Piper nigrum, piperine: A comprehensive review, *Cureus* 16 (2024) e54425. Doi: 10.7759/cureus.54425
- [17]. H.W. Jo, M.M. Kim, β -Caryophyllene oxide inhibits metastasis by downregulating MMP-2, p-p38 and p-ERK in human fibrosarcoma cells, *Journal of Food Biochemistry* 46 (2022) e14468. Doi: 10.1111/jfbc.14468
- [18]. Q.H. Nguyen, T.T. Nguyen, V. Truong, C.T.T. Do, H.T. Tran, S.K. Nguyen, P.H. Nguyen, T.H. Nguyen, N.D. Le, D.T. Nguyen, T.P.T. Nguyen, V.P. Nguyen, T.A. Nguyen, O. Koshovyi, A. Raal, New insights into the chemical composition of Piper spp. collected in the northern region of Vietnam, *Biochemical Systematics and Ecology* 112 (2023) 104770. Doi: 10.1016/j.bse.2023.104770
- [19]. A. Daina, O. Michielin, V. Zoete, SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, *Scientific Reports* 7 (2017) 42717. Doi: 10.1038/srep42717

- [20]. P. Banerjee, E. Kemmler, M. Dunkel, R. Preissner, ProTox 3.0: A webserver for the prediction of toxicity of chemicals, *Nucleic Acids Research* 52 (2024) W513–W520. Doi: 10.1093/nar/gkae303
- [21]. A. Daina, O. Michielin, V. Zoete, SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules, *Nucleic Acids Research* 47 (2019) W357–W364. Doi: 10.1093/nar/gkz382
- [22]. D. Szklarczyk, R. Kirsch, M. Koutrouli, K. Nastou, F. Mehryary, R. Hachilif, A.L. Gable, T. Fang, N.T. Doncheva, S. Pyysalo, P. Bork, L.J. Jensen, C. von Mering, The STRING database in 2023: Protein-protein association networks and functional enrichment analyses for any sequenced genome of interest, *Nucleic Acids Research* 51 (2023) D638–D646. Doi: 10.1093/nar/gkac1000
- [23]. O. Menyhart, W.J. Kothalawala, B. Györfy, A gene set enrichment analysis for the cancer hallmarks, *Journal of Pharmaceutical Analysis* 15 (2024) 101065. Doi: 10.1016/j.jpha.2024.101065
- [24]. D.S. Chandrashekar, S.K. Karthikeyan, P.K. Korla, H. Patel, A.R. Shovon, M. Athar, G.J. Netto, Z.S. Qin, S. Kumar, U. Manne, C.J. Creighton, S. Varambally, UALCAN: An update to the integrated cancer data analysis platform, *Neoplasia* 25 (2022) 18–27. Doi: 10.1016/j.neo.2022.01.001
- [25]. Z. Tang, C. Li, B. Kang, G. Gao, C. Li, Z. Zhang, GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses, *Nucleic Acids Research* 45 (2017) W98–W102. Doi: 10.1093/nar/gkx247
- [26]. T. Li, J. Fan, B. Wang, N. Traugh, Q. Chen, J.S. Liu, B. Li, X.S. Liu, TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells, *Cancer Research* 77 (2017) e108–e110. Doi: 10.1158/0008-5472.CAN-17-0307
- [27]. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank, *Nucleic Acids Research* 28 (2000) 235–242. Doi: 10.1093/nar/28.1.235
- [28]. M. Wiederstein, M.J. Sippl, ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins, *Nucleic Acids Research* 35 (2007) W407–W410. Doi: 10.1093/nar/gkm290
- [29]. S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B.A. Shoemaker, P.A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang, E.E. Bolton, PubChem 2023 update, *Nucleic Acids Research* 51 (2023) D1373–D1380. Doi: 10.1093/nar/gkac956
- [30]. G. Tang, W. Wang, C. Zhu, H. Huang, P. Chen, X. Wang, M. Xu, J. Sun, C.J. Zhang, Q. Xiao, L. Gao, Z.M. Zhang, S.Q. Yao, Global reactivity profiling of the catalytic lysine in human kinome for covalent inhibitor development, *Angewandte Chemie International Edition* 63 (2024) e202316394. Doi: 10.1002/anie.202316394
- [31]. S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx, *Methods in Molecular Biology* 1263 (2015) 243–250. Doi: 10.1007/978-1-4939-2269-7_19
- [32]. K. Ozvoldik, T. Stockner, E. Krieger, YASARA Model-Interactive molecular modeling from two dimensions to virtual realities, *Journal of Chemical Information and Modeling* 63 (2023) 6177–6182. Doi: 10.1021/acs.jcim.3c01136
- [33]. W. Pitsawong, V. Buosi, R. Otten, R.V. Agafonov, A. Zorba, N. Kern, S. Kutter, G. Kern, R.A. Pádua, X. Meniche, D. Kern, Dynamics of human protein kinase Aurora A linked to drug selectivity, *eLife* 7 (2018) e36656. Doi: 10.7554/eLife.36656
- [34]. A. Goyal, S. Gupta, Y. Gupta, N. Tandon, Proposed guidelines for screening of hyperglycemia in patients hospitalized with COVID-19 in low resource settings, *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* 14 (2020) 753–756. Doi: 10.1016/j.dsx.2020.05.039
- [35]. E.O. Johnson, K.-H. Chang, Y. de Pablo, S. Ghosh, R. Mehta, S. Badve, K. Shah, PHLDA1 is a crucial negative regulator and effector of Aurora A kinase in breast cancer, *Journal of Cell Science* 124 (2011) 2711–2722. Doi: 10.1242/jcs.084970
- [36]. Y.H. Wang, S.L. Morris-Natschke, J. Yang, H.M. Niu, C.L. Long, K.H. Lee, Anticancer principles from medicinal piper (hú jiāo) plants, *Journal of Traditional and Complementary Medicine* 4 (2014) 8–16. Doi: 10.4103/2225-4110.124811
- [37]. N. Varshney, R.K. Pandey, A. Mishra, S. Kumar, H.C. Jha, Aurora kinase A: Integrating insights into cancer, inflammation, and infectious diseases, *Gut Microbes Reports* 1 (2024) 2419069. Doi: 10.1080/29933935.2024.2419069
- [38]. E. Willems, M. Dedobbeleer, M. Digregorio, A. Lombard, P.N. Lumapat, B. Rogister, The functional diversity of Aurora kinases: A comprehensive review, *Cell Division* 13 (2018) 7. Doi: 10.1186/s13008-018-0040-6
- [39]. C. Arter, L. Trask, S. Ward, S. Yeoh, R. Bayliss, Structural features of the protein kinase domain and targeted binding by small-molecule inhibitors, *The Journal of Biological Chemistry* 298 (2022) 102247. Doi: 10.1016/j.jbc.2022.102247
- [40]. G.M.T. Cheetham, R.M.A. Knegt, J.T. Coll, S.B. Renwick, L. Swenson, P. Weber, J.A. Lippke, D.A. Austen, Crystal structure of Aurora-2, an oncogenic serine/threonine kinase, *Journal of Biological Chemistry* 277 (2002) 42419–42422. Doi: 10.1074/jbc.C200426200
- [41]. E.F. Ruff, J.M. Muretta, A.R. Thompson, E.W. Lake, S. Cyphers, S.K. Albanese, S.M. Hanson, J.M. Behr, D.D. Thomas, J.D. Chodera, N.M. Levinson, A dynamic mechanism for allosteric activation of Aurora kinase A by activation loop phosphorylation, *eLife* 7 (2018) e32766. Doi: 10.7554/eLife.32766

Received: 28.11.2025

Received in revised form: 09.03.2026

Accepted: 13.03.2026