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Computational Strategies in Anticancer Drug Discovery: Virtual Screening of Protein Kinase Inhibitors

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Abstract Protein kinase CK2 is a crucial target for drug discovery due to its involvement in various cellular processes and diseases, including cancer. In this study, molecular docking and molecular dynamics (MD) simulations were employed to explore the binding interactions and stability of selected ligands with *Zea mays* CK2alpha (PDB ID: 4RLK). A library of 5000 compounds was screened using AutoDock Vina, followed by a refined docking analysis of the top 50 compounds with AutoDock. The molecular dynamics simulations, performed for 100 ns using the OPLS-2005 force field in Desmond, provided insights into the conformational stability of the protein-ligand complexes. Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and hydrogen bonding analyses revealed the structural stability and dynamic behavior of the ligands within the active site. The results highlight key interactions, including hydrogen bonding, π-stacking, and hydrophobic interactions, contributing to ligand binding and stability. These findings provide valuable insights into potential CK2alpha inhibitors and their role in drug design.

Keywords Protein kinase CK2, *Zea mays* CK2alpha, Molecular docking, Molecular dynamics simulations, Binding interactions

Introduction

Cancer remains one of the most formidable global health challenges, responsible for millions of deaths each year. Despite significant advancements in treatment modalities, including surgery, radiation therapy, and chemotherapy, the search for effective and targeted anticancer drugs continues to be a major focus in medical research [1-3]. Among various molecular targets, protein kinases play a crucial role in cancer pathogenesis, making them a prime focus for drug discovery efforts [4-7]. Aberrant kinase activity is implicated in uncontrolled cell proliferation, survival, metastasis, and resistance to therapy [8-9]. Consequently, protein kinase inhibitors (PKIs) have emerged as a promising class of anticancer agents [10-11]. However, the development of these inhibitors through traditional drug discovery methods is often time-consuming, costly, and labor-intensive. Computational strategies, particularly virtual screening, have revolutionized the drug discovery paradigm by expediting the identification of potential PKIs with high efficacy and selectivity [12-13].

Protein kinases constitute a large family of enzymes that regulate various cellular processes by transferring phosphate groups to target proteins. This phosphorylation modulates protein function, impacting signaling pathways involved in cell cycle regulation, apoptosis, differentiation, and metabolism. Dysregulated kinase activity due to mutations or overexpression contributes to oncogenesis and cancer progression. Several kinases, including tyrosine kinases (TKs), serine/threonine kinases (STKs), and dual-specificity kinases, have been identified as critical mediators in malignancies [14-16].

Over the past two decades, numerous kinase inhibitors have been developed, with many gaining FDA approval for clinical use. Notable examples include imatinib (BCR-ABL inhibitor for chronic myeloid leukemia), erlotinib (EGFR inhibitor for lung cancer), and sorafenib (multi-kinase inhibitor for hepatocellular carcinoma). Despite these successes, kinase-targeted therapies face several challenges, including drug resistance, off-target effects, and limited efficacy in heterogeneous tumors. Therefore, novel PKIs with improved selectivity and potency are urgently needed. Computational strategies, particularly virtual screening, offer a promising approach to accelerate the identification and optimization of new kinase inhibitors [17-19].

Virtual screening has emerged as a pivotal strategy in the discovery of protein kinase inhibitors, offering an efficient and cost-effective alternative to traditional drug discovery methods. By leveraging computational techniques such as molecular docking, MD simulations, QSAR modeling, and pharmacophore modeling, researchers can accelerate the identification and optimization of novel anticancer agents [20-21]. While challenges remain, continuous advancements in AI-driven drug discovery and integrative computational-experimental approaches will pave the way for more effective and selective kinase inhibitors. As research in this field progresses, virtual screening will continue to play a vital role in developing targeted therapies for cancer treatment, ultimately improving patient outcomes and addressing the global burden of cancer.

Experimental Work

Hardware and Software details

The entire computational investigation was performed on Windows 10 (64-bit) operating systems with 8 GB RAM and 2.11GHz Intel® Core™ i5-10210U processor except for molecular dynamics simulations. Molecular dynamics simulations were performed on Ubuntu 14.04.5 version in the Linux environment with 4 GB RAM by Desmond. Binary distribution of PyRx python prescription 0.8 the Scripps research institute for Windows available free from https://pyrx.sourceforge.io/downloads. Maestro visulizer and Discovery studio academic versions were used for visuliztion of Interactions.

Virtual Screening of the ChemDiv Database

Procurement of Virtual Compounds

The chemical structures of 5000 ligands were downloaded from Chemdiv databse in SDF format [22].

Molecular Docking Study

The crystal structure of Zea mays CK2alpha in complex with the ATP-competitive inhibitor 4-[(E)-(fluoren-9-ylidenehydrazinylidene)-methyl] benzoate (PDB ID: 4RLK) was selected as the protein model for this study, with a resolution of 1.24 Å [23-24]. The chemical structures of the ligands were obtained from the ChemDiv database in SDF format, followed by geometry optimization and energy minimization using the MM2 force field. The crystal structure of Z. mays CK2alpha (PDB ID: 4RLK) was retrieved from the Protein Data Bank (https://www.rcsb.org/). Prior to docking, all water molecules and existing ligands were removed from the protein file, and the refined structure was imported into AutoDock for molecular docking studies. Molecular docking was carried out using AutoDock 4.2, following standard docking protocols with default parameters and an empirical free energy function.

Polar hydrogens were added to the protein, while all water molecules were excluded. The ligands were treated as flexible molecules, allowing rotation of all rotatable bonds to achieve the optimal binding conformation within the active site of CK2alpha. For validation, the native ligand, chlorobiocin, was redocked into the binding site. The grid box was centered at x = 21.3159, y = 7.8669, z = 20.1202, with dimensions of 25 × 25 × 25 and an exhaustiveness of 8. Grid maps were generated using Autogrid4, and ligand conformations were explored using the Lamarckian genetic algorithm combined with an adaptive local search method in AutoDock. Docking simulations were performed with 200 runs, and conformations were clustered based on an RMSD tolerance of 2.0 Å. The best-ranked conformation, determined by binding free energy, was selected for further analysis. Visualization of molecular interactions was conducted using Maestro Visualizer and Discovery Studio. After an initial docking of 5000 compounds using AutoDock Vina, the top 50 compounds were selected for further docking refinement in AutoDock. The grid box was repositioned at x = 21.879, y = 7.606, z = 19.725, with dimensions of 40 × 40 × 40 and a default spacing of 0.375 [25-27].

Molecular Dynamics Simulation

Molecular dynamics (MD) simulations were conducted using Desmond 2021-04 on an Acer workstation running Ubuntu 22.04. The ligands 128, 129, 1192, and 1226 complexes with 4RLK were simulated using the OPLS-2005 force field for topology generation. The system preparation involved solvation in an orthorhombic simulation box using the simple point-charge (SPC) explicit water model. To maintain physiological conditions, the system was neutralized with Na^+/Cl^- counter ions and a 0.15 M salt concentration. The receptor-ligand complex was designated with the OPLS-2005 force field, and the SPC explicit solvent model was applied. Prior to simulation, Desmond minimization was performed for 20 ps, followed by system relaxation using default protocols. The MD simulations were carried out for 100 ns under controlled conditions, maintaining a temperature of 300 K and pressure of 1.0325 bar [28-30

Results and Discussion

Molecular Docking

The molecular docking results reveal significant interactions between the tested compounds and the protein kinase active site. Compounds 128, 129, 1192, and 1226 exhibited strong binding affinities, with binding energy values ranging from -10.37 to -10.67 kcal/mol (Autodock) and docking scores from -12.4 to -12.6 (Autodock Vina). These values suggest high stability of the ligand-protein complexes (Table 1).

The hydrophobic interactions primarily involved residues such as ARG47, HIS160, GLY46, VAL95, ASP175, TRP176, PHE113, LYS68, and MET163. Compound 1192 displayed additional hydrogen bonding with ARG43, VAL116, and ASP120, which may contribute to its slightly improved binding stability compared to the others (Figure 1). The presence of key interactions with highly conserved residues within the kinase domain suggests that these compounds could serve as potent inhibitors.

Table 1: Molecular docking results of top docked complex

Compound Code	Binding Affinity (Autodock Vina)	Binding Energy (Autodock)
128	-12.6	-10.55
129	-12.4	-10.67
1192	-12.5	-10.37
1226	-12.4	-10.49

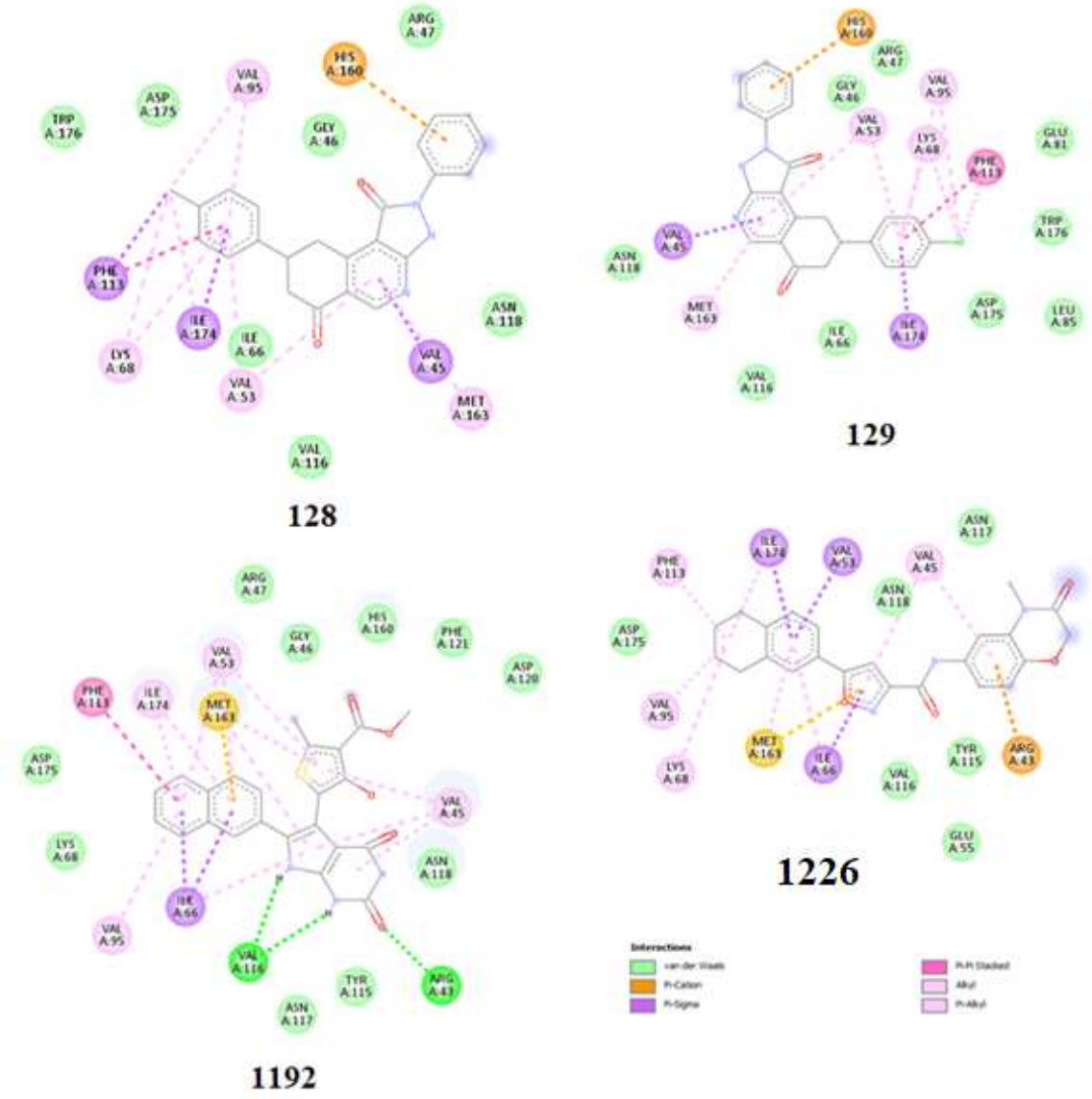

Figure 1: Binding interaction of top 4 docked complexes

Molecular Dynamics Simulations

Molecular dynamics (MD) simulations provided crucial insights into the stability and dynamic behavior of the 4RLK protein-ligand complexes. RMSD analysis revealed that while all protein-ligand systems experienced fluctuations, they largely maintained structural integrity, indicating stable interactions.

128 Complex: The protein exhibited moderate fluctuations (1.2–2.5 Å), with a slight increase

around 40 ns, suggesting structural adjustments. The ligand remained stable initially but showed increased flexibility after 40 ns, stabilizing within 1.8–2.5 Å.

129 Complex: Significant fluctuations (2.0–2.8 Å) were observed as the system equilibrated. After 80 ns, the RMSD stabilized, but ligand movement suggested possible partial dissociation.

1192 Complex: The protein exhibited higher fluctuations (2.8–3.4 Å), indicating moderate conformational changes. The ligand remained within the binding site but with higher RMSD values (4–6 Å), suggesting considerable movement.

1226 Complex: The protein RMSD stabilized between 1.8–2.4 Å after initial equilibration, indicating structural stability. However, the ligand showed greater flexibility (2.4–4.8 Å), exploring a larger conformational space within the binding pocket (Figure 2).

Overall, these findings suggest that while all ligands maintained interactions with the binding site, their stability varied, with some exhibiting greater mobility.

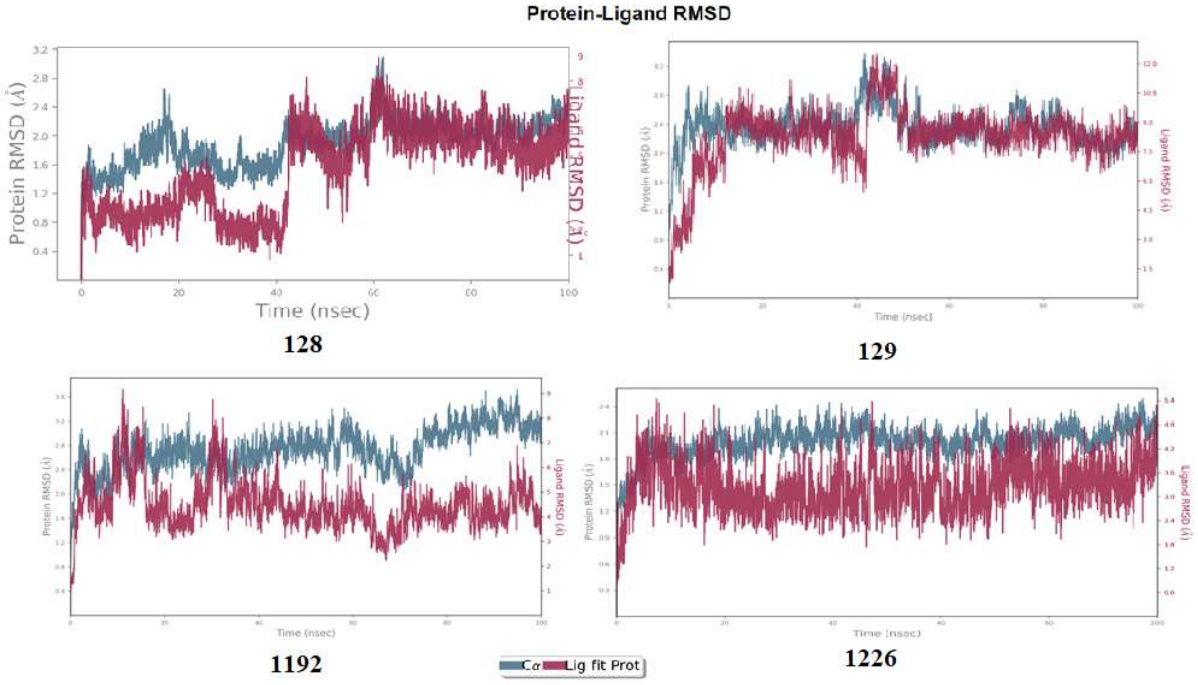

Figure 2: RMSD of protein (4RLK) and ligands (128, 129, 1192 and 1226)

The Root Mean Square Fluctuation (RMSF) analysis provides insights into the flexibility of the protein-ligand complexes, where higher RMSF values indicate more flexible regions and lower values suggest greater structural stability.

128 Complex: The RMSF plots of the protein (side chains) and ligand exhibited minor fluctuations, ranging from 0.76 to 2.5 Å, indicating localized flexibility.

129 Complex: The RMSF values fluctuated between 0.8 and 2.0 Å, suggesting relatively stable binding with moderate flexibility.

1192 Complex: The RMSF analysis showed fluctuations between 0.8 and 2.4 Å, reflecting a balanced structural adaptation.

1226 Complex: Fluctuations ranged from 0.7 to 2.4 Å, demonstrating minor variations while maintaining overall stability (Figure 3 and 4).

These results highlight the structural stability of the complexes, with variations in flexibility depending on the ligand binding dynamics.

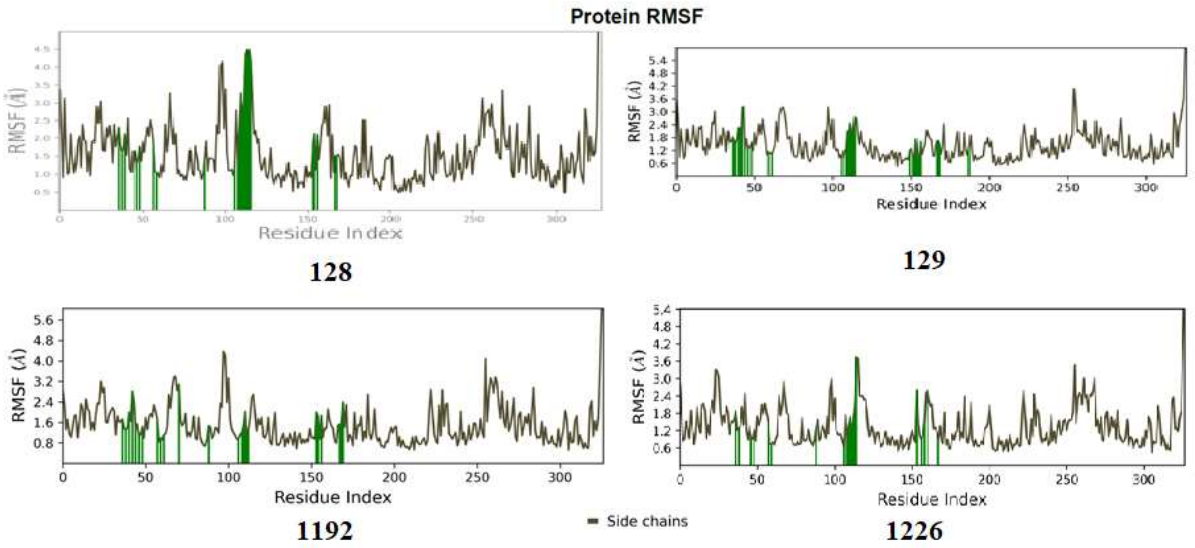

Figure 3: RMSF of side chain (4RLK) with ligands (128, 129, 1192 and 1226)

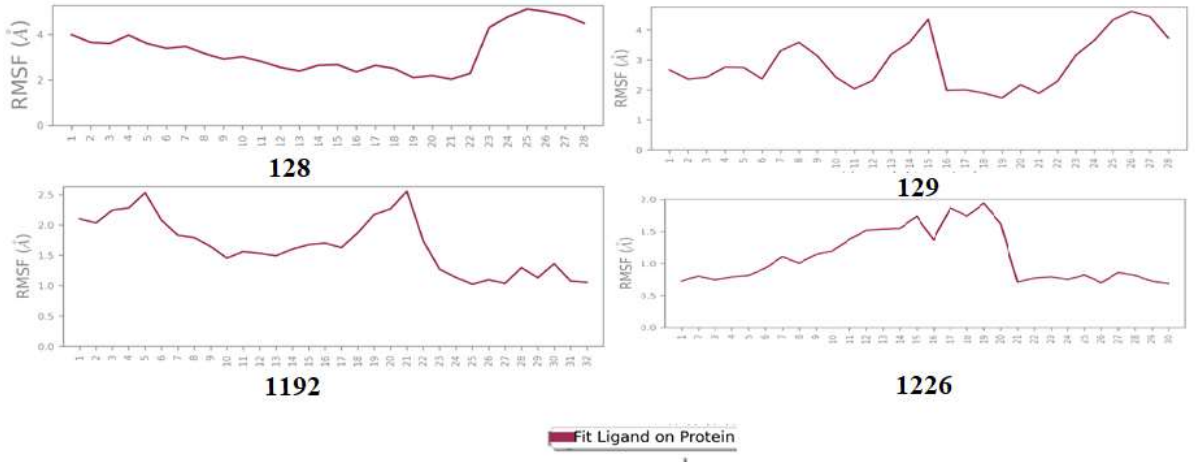

Figure 4: RMSF of ligands (128, 129, 1192 and 1226)

Protein-Ligand Interaction Analysis

128 Complex: Throughout the 100 ns trajectory, key interactions stabilizing the ligand within the binding site were identified. Pi-cation interactions (red dashed lines) were observed between the ligand and Arg43 and His160, playing a crucial role in ligand stabilization. Additionally, hydrogen bonds with Asn118 and Asp120 significantly contributed to ligand binding. Hydrophobic interactions involving Ile66, Val45, Phe113, and Met163 (labeled in green) further enhanced ligand stability within the binding pocket.

129 Complex: In this complex, His160 formed a pi-pi stacking interaction, which contributed to ligand stabilization. Ser51 and Asn161 established hydrogen bonds, enhancing ligand specificity and binding affinity. Hydrophobic residues such as Ile174 and Val53 facilitated ligand binding via van der Waals forces, stabilizing the nonpolar regions of the ligand within the binding site.

1192 Complex: Hydrophobic interactions played a critical role in ligand stabilization, particularly with Ile174, Phe113, Ile66, and Val116, forming a hydrophobic pocket that accommodated the ligand's nonpolar regions. Arg43 and His160, along with nearby water

molecules, contributed to hydrogen bonding, helping to maintain specific ligand orientations. Additionally, pi-stacking interactions provided further stability and selectivity for ligands containing aromatic rings.

1226 Complex: Key residues contributing to ligand stabilization included Val116, Met163, Val53, Phe113, Ile66, and Ile174, forming a hydrophobic pocket that accommodated the ligand's nonpolar regions. Notably, Tyr115 formed a hydrogen bond with the ligand's nitrogen (HN), with a high frequency of 95%, indicating a strong and persistent interaction. Additionally, Arg43 contributed to complex stability and specificity, particularly for ligands with aromatic rings (Figure 5).

These interactions highlight the diverse binding mechanisms across different complexes, emphasizing the role of hydrophobic contacts, hydrogen bonds, and pi-stacking interactions in ligand stabilization and specificity.

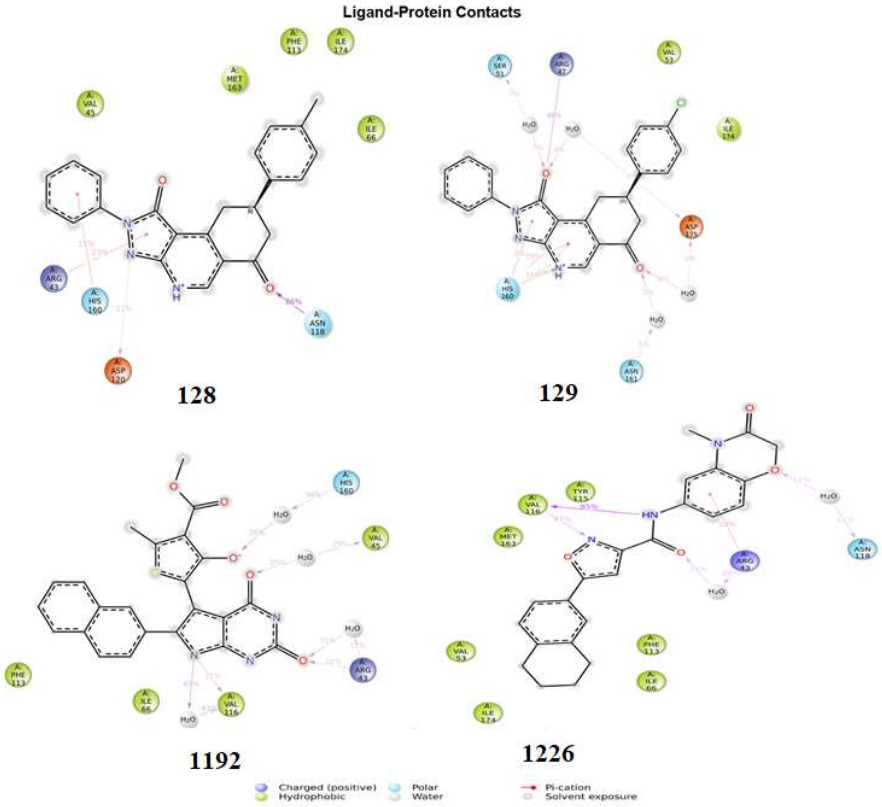

Figure 5: Binding interactions of ligands with pdb 4RLK

Summary and Conclusion

This study explored the binding interactions and stability of various ligand-protein complexes using molecular docking and molecular dynamics (MD) simulations. The computational analysis focused on four ligand-protein complexes to evaluate their structural stability, binding affinity, and key molecular interactions within the binding pocket.

Molecular docking results revealed strong binding affinities across all complexes, with significant contributions from hydrophobic interactions, hydrogen bonding, and pi-stacking interactions. The MD simulations, conducted over a 100 ns trajectory, provided deeper insights into the dynamic behavior of these complexes. Root Mean Square Deviation (RMSD) and Root

Mean Square Fluctuation (RMSF) analyses indicated that while all complexes achieved equilibrium, some exhibited greater flexibility in ligand positioning within the binding site. The 128 and 129 complexes demonstrated relatively stable ligand binding, with moderate fluctuations in RMSD and strong interactions with key residues such as His160, Arg43, and Asn118. The 1192 complex showed higher ligand movement, with hydrophobic pockets contributing significantly to stabilization. The presence of Arg43 and His160 in hydrogen bonding further enhanced ligand retention. The 1226 complex exhibited a well-defined binding pocket, where Tyr115 formed a highly persistent hydrogen bond (95%), ensuring strong ligand stabilization.

Overall, these results support the hypothesis that computational screening can effectively identify promising PKIs. Further validation through *in vitro* assays is required to confirm their biological activity and selectivity.

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Conflict of Interest: None.

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