



Article

Inhibition of Casein Kinase 1 δ as a Novel Therapeutic Strategy for Amyotrophic Lateral Sclerosis: A Theoretical Study

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Abstract

Amyotrophic Lateral Sclerosis is a progressive neurodegenerative disease characterized by the degeneration of motor neurons and the pathological accumulation of phosphorylated TDP-43. Casein kinase one delta (CK1 δ) has been identified as a key regulator of this aberrant phosphorylation, making it a promising therapeutic target. In this theoretical study, 26 structurally diverse compounds were evaluated against CK1 δ using molecular docking, molecular dynamics simulations, and binding free energy calculations. Among them, BZH exhibited the most stable interaction with CK1 δ (-46.53 ± 1.94 kcal/mol). An inverse correlation was observed between theoretical affinity and experimental IC₅₀ values, supporting the predictive validity of the computational approach. Pharmacokinetic analysis indicated that IMF and BIP show good oral absorption and the ability to cross the blood–brain barrier. At the same time, the toxicological profile classified all compounds in toxicity Class IV (moderate risk). Additionally, dynamic migration toward an alternative pocket was observed during simulation, highlighting the importance of considering protein flexibility in drug design. This study proposes BZH, IMF, and BIP as promising CK1 δ inhibitors for future experimental validation in the treatment of ALS.

Keywords: amyotrophic lateral sclerosis (ALS); CK1 δ ; molecular docking; molecular dynamics; MMPBSA; virtual screening; neurodegeneration



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1. Introduction

Neurodegenerative diseases (NDs) are the leading cause of physical and cognitive disability worldwide, affecting around 15% of the global population [1]. Among the NDs, Amyotrophic Lateral Sclerosis (ALS) is rare, heterogeneous, progressive, and fatal, affecting the central nervous system [2]. Its hallmark feature is the simultaneous degeneration of upper motor neurons, located in the motor cortex, and lower motor neurons, which connect the spinal cord to the muscles. This neuronal damage leads to both motor and extra-motor clinical signs such as muscle weakness, atrophy, cramps, fasciculations, spasticity,

clumsiness, and hyperreflexia. Over time, these symptoms lead to generalized paralysis that eventually results in the death of the patient, commonly due to respiratory failure [3–5]. Approximately 90% of ALS cases are sporadic, with no apparent heritability, while 10% are familial or genetic in origin [6,7]. ALS incidence peaks between the ages of 60 and 79, with a higher prevalence in males [8], and an estimated incidence rate of 1.68 per 100,000 people per year, depending on the region [9]. Despite scientific advances in this disease, the prognosis remains poor, with a median survival of 3 to 5 years after diagnosis [4]. To date, there is no definitive cure, and there are only a few therapies that slow disease progression. One such therapy is Riluzole, the only FDA-approved drug, which has been shown to extend survival in some cases by up to 3 months [10,11]. Therefore, there is an urgent need to develop innovative therapeutic approaches that improve the quality of life for these patients. ALS lacks a single etiology and is caused by the interaction of diverse molecular pathways [6,12].

One of the most critical findings in recent years has been the pathological involvement of the DNA-binding protein TAR 43 (TDP-43) [13–15], encoded by the *TARDBP* gene, which regulates RNA processes in the nucleus, such as transcriptional regulation, alternative splicing, and mRNA stabilization. However, under pathological conditions, TDP-43 undergoes cytoplasmic mislocalization, accompanied by hyperphosphorylation and other modifications that promote its accumulation and the formation of toxic cytoplasmic inclusions [16]. The cytoplasmic mislocalization of TDP-43 is a hallmark and key toxic event in ALS, although the mechanisms regulating it are not yet fully understood [17]. Among these regulators, casein kinase one delta (CK1 δ), a serine/threonine enzyme involved in multiple cellular processes, has been identified as one of the main contributors to the aberrant phosphorylation of TDP-43 [18]. In the report by Choksi [19], it was demonstrated that phosphorylation of TDP-43 by CK1 ϵ promoted oligomerization and increased in vivo toxicity. Similarly, in the study by Hicks [20], endoplasmic reticulum stress induced the cytoplasmic accumulation of phosphorylated TDP-43 in a CK1-dependent manner in motor neuron-like cells. Furthermore, Liachko [21] showed in *C. elegans* that phosphorylation of TDP-43 by kinases such as CK1 significantly increased its neurotoxicity, as evidenced by motor dysfunction and motor neuron degeneration. Finally, Martínez-González [22] reported that inhibition of CK1 δ reduced the aberrant phosphorylation of TDP-43, attenuated its toxicity, and preserved motor neurons in both cellular and murine models of ALS. Due to the critical role of CK1 δ in the pathological phosphorylation of TDP-43, its inhibition presents a promising therapeutic strategy in ALS.

However, current CK1 δ inhibitors still present experimental limitations regarding their specificity. Therefore, molecular modeling, molecular docking, and molecular dynamics simulations could provide a detailed understanding of protein behavior at the molecular level and help develop greater specificity toward potential inhibitors [23]. The objective of this study is to identify potential inhibitors targeting CK1 δ through molecular dynamics simulations and to provide theoretical insights for the development of optimized experimental studies in ALS.

2. Results

The structure of CK1 δ , obtained from the PDB database (ID: 6RCG) [24], contained multiple chains and several crystallographic water molecules. We used UCSF Chimera v1.18 software [25] to remove all extraneous molecules, including water and potential ions. Missing residues in the CK1 δ sequence were identified and reconstructed by homology modeling with the MODELLER tool [26] integrated into Chimera. The processed structure of CK1 δ , composed of 296 residues, was solvated in a cubic box of 8.3436 nm per side using the TIP3P water model. The addition of Cl[−] atoms neutralized the net charge of the

system, and additional Na^+ and Cl^- ions were incorporated to reach a physiological ionic concentration of 0.15 M. In total, we added 17,340 water molecules to the system.

Furthermore, we minimized the forces on the system using the steepest descent algorithm down to a tolerance of 1.0 kJ/mol. Next, we followed with thermal (309.65 K) and pressure (1 bar) equilibration steps, both lasting 10 ns. A 500 ns molecular dynamics simulation with one fs steps was then run (see Figure 1a). We performed Ramachandran diagram analysis to assess the structural conformation of the CK1 δ protein. Of the 261 non-glycine/proline residues analyzed, 221 residues (84.7%) were located in the most favored regions, 34 (13.0%) in additionally allowed regions, and 5 (1.9%) in generously allowed regions, representing 99.6% of the residues. We located only one residue (0.4%) in the disallowed regions. This evaluation excluded glycine and proline residues, as well as terminal residues, from the analysis (see Figure 1b).

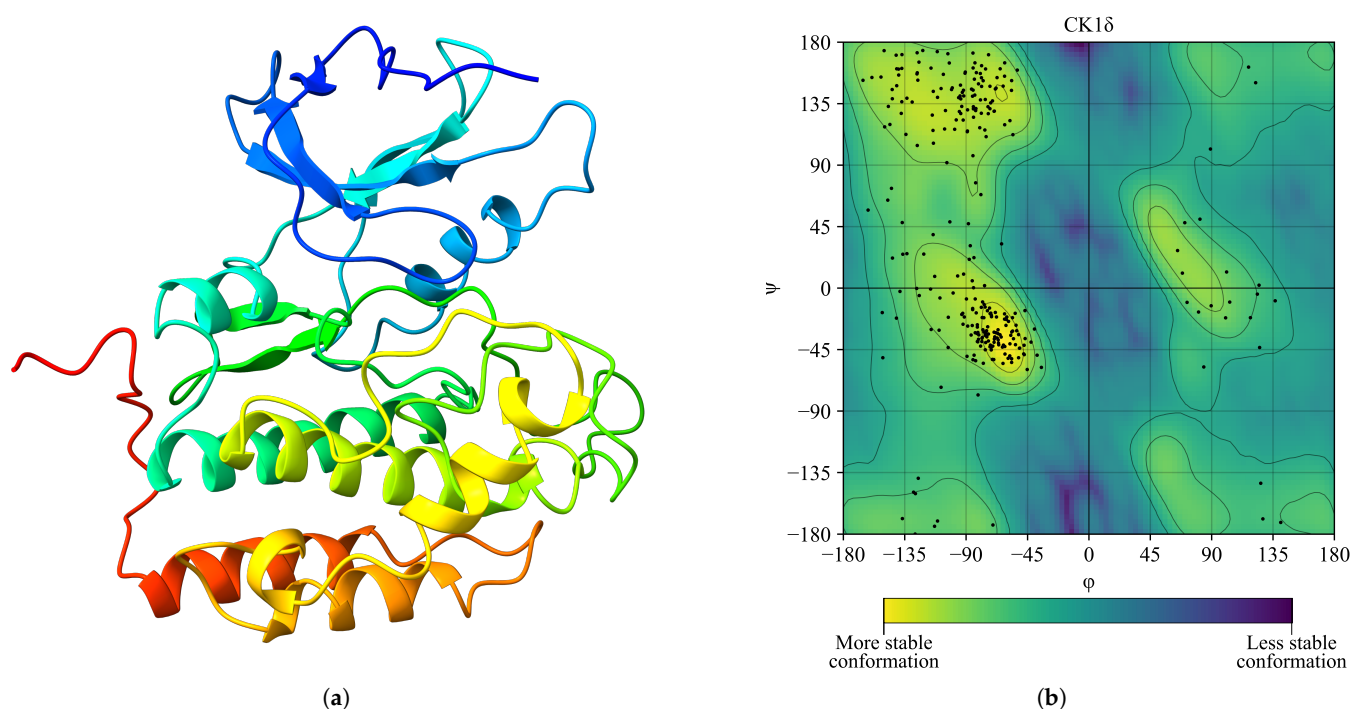


Figure 1. Stabilized structure of CK1 δ (ID: 6RCG) and residue stability analysis after 500 ns of simulation. (a) Structure of CK1 δ . (b) Ramachandran diagram, the yellow color shows a more stable conformation and purple color shows a less stable conformation.

By analyzing the root mean square deviation (RMSD) of the distances (see Figure 2a), we observe that the system reaches a state of structural equilibrium after 100 ns, with average fluctuations of around 0.3 nm until the end of the molecular dynamics simulation, indicating overall stability of the protein conformation. In Figure 2b, we present the variation in hydrogen bonds in the protein structure during the molecular dynamics simulation trajectory. We obtained an almost constant average of 230 bonds throughout the simulation, reflecting stable interactions.

In the root mean square deviation (RMSD) of the residue (see Figure 2c), we have identified peaks of higher mobility between residues near positions 144 to 150 and between 220 and 255, probably in flexible regions. Likewise, we observe concerning the radius of gyration (see Figure 2d), an initial compaction process that stabilizes after 100 ns, with constant values around 1.94 nm. Finally, the solvent accessible surface area (SASA) (see Figure 2e) shows a gradual decrease during the first 150 ns, followed by a stabilization phase at 200 nm, reflecting the clustering of the observed structure.

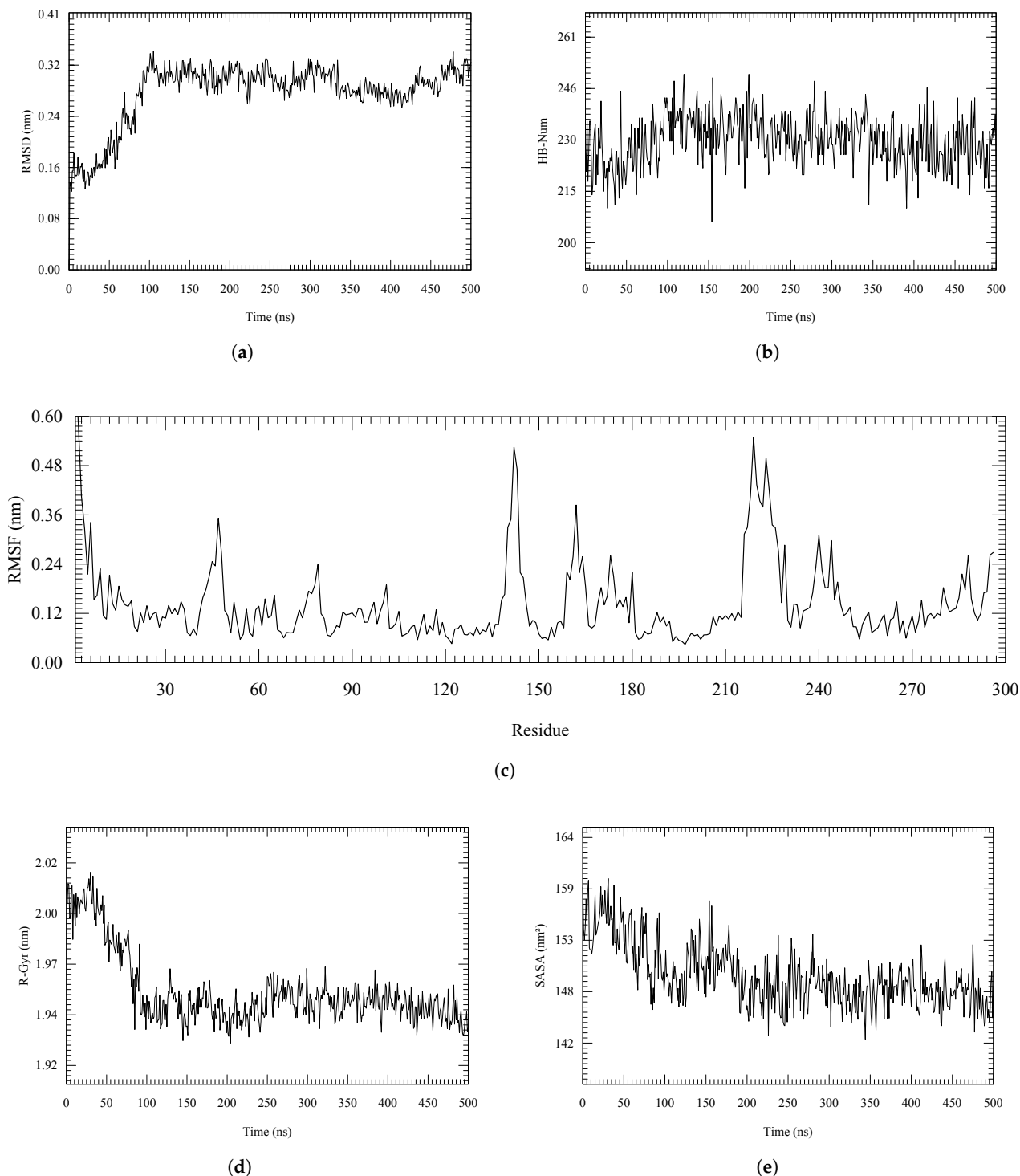


Figure 2. Structural properties of CK1 δ during a 500 ns molecular dynamics simulation. (a) Root mean square deviation (RMSD). (b) Number of hydrogen bonds. (c) Root mean square fluctuation per residue (RMSF). (d) Radius of gyration (R-Gyr). (e) Solvent-accessible surface area (SASA).

In Figure 3, we present the two-dimensional (2D) structures that we identified in databases with potential inhibitory activity against CK1 δ . We have found potential ligands from the families of amines, benzimidazoles, benzothiazoles, imidazoles, lamellarines, pyrimidines, and triazatines. Among all the structures, we identified two as hydrochlorides (AMI and PYR).

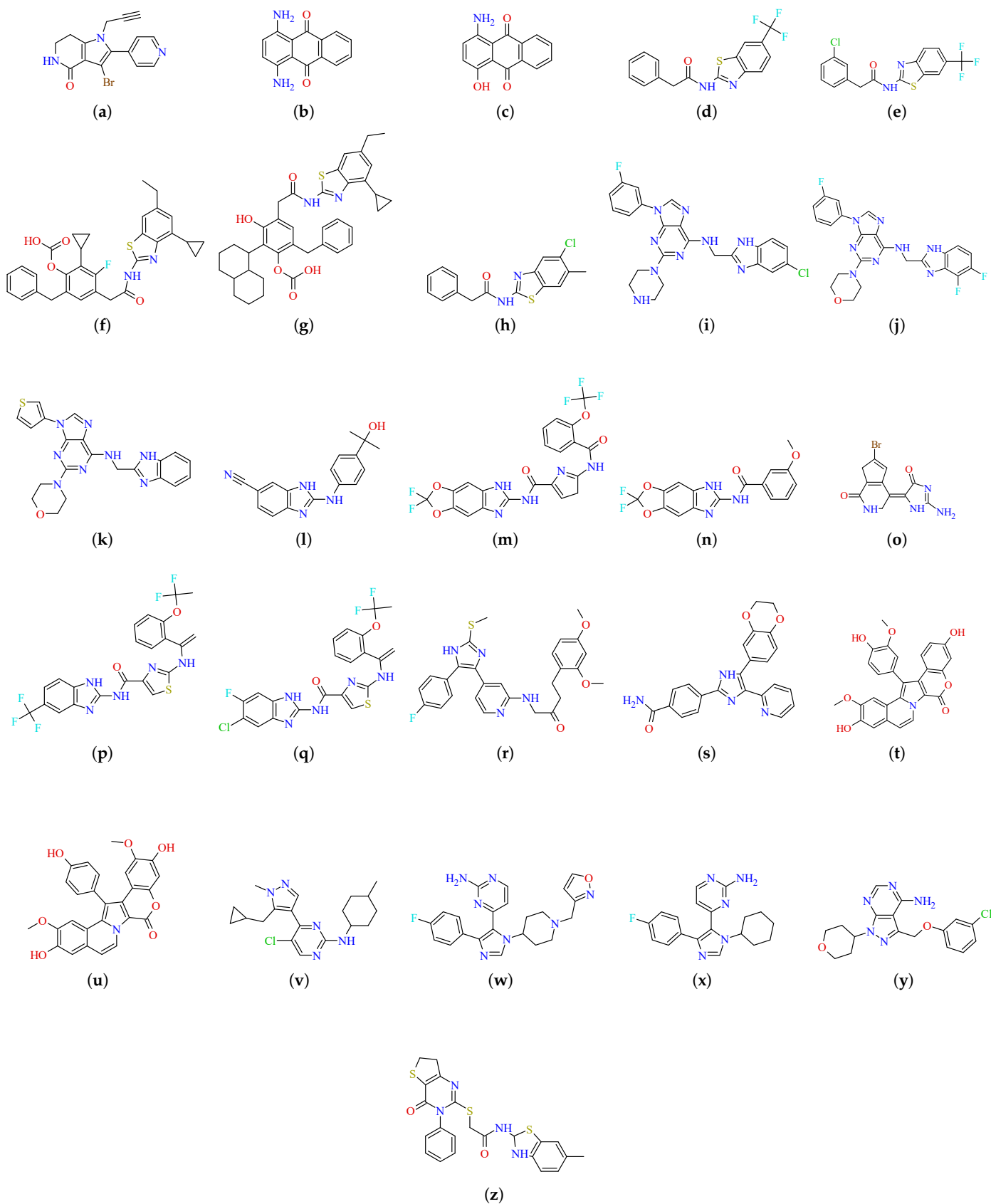


Figure 3. Two-dimensional structures of all compounds classified by chemical family: Amine, Benzimidazole, Benzothiazole, Imidazole, Lamellarin, Pyrimidine, and Triazatin. (a) AML. (b) AMQ. (c) AHQ. (d) BZF. (e) BZT. (f) BZC. (g) BZH. (h) BZM. (i) BIP. (j) BIM. (k) BIT. (l) BIC. (m) DXB. (n) DXM. (o) HYA. (p) IMT. (q) IMF. (r) IMB. (s) IMD. (t) LAM. (u) LAX. (v) PMP. (w) PYP. (x) PYI. (y) PYR. (z) TTA.

Pharmacologically relevant cavities of the CK1 δ protein were analyzed using the CavityPlus server [27] to identify potential inhibitor binding regions. Table 1 presents the results for the nine cavities with the highest binding potential. The highest scoring cavity obtained a value of 1058, while the lowest scored reached -1274 , in arbitrary pharmacological units. In general, the higher the DrugScore value, the higher the probability that a molecule can interact favorably with that region of the protein. Conversely, negative values suggest a low probability of interaction. It is important to emphasize that we based this analysis on a static protein structure; under physiological conditions, flexibility and structural motion can influence the binding affinity of inhibitors.

Table 1. Analysis of druggable pockets of the CK1 δ protein.

Protein	Pocket	DrugScore	Druggability
CK1 δ	2	1058	Strong
	4	22	Medium
	1	14	Medium
	5	-375	Weak
	3	-651	Weak
	7	-782	Weak
	8	-1200	Weak
	9	-1183	Weak
	6	-1274	Weak

Figure 4 shows the binding pockets identified in the CK1 δ protein using the CavityPlus server. Each pocket is marked with a specific color and numbered according to the data presented in Table 1. The colors are only referential; they have no meaning except to identify them.

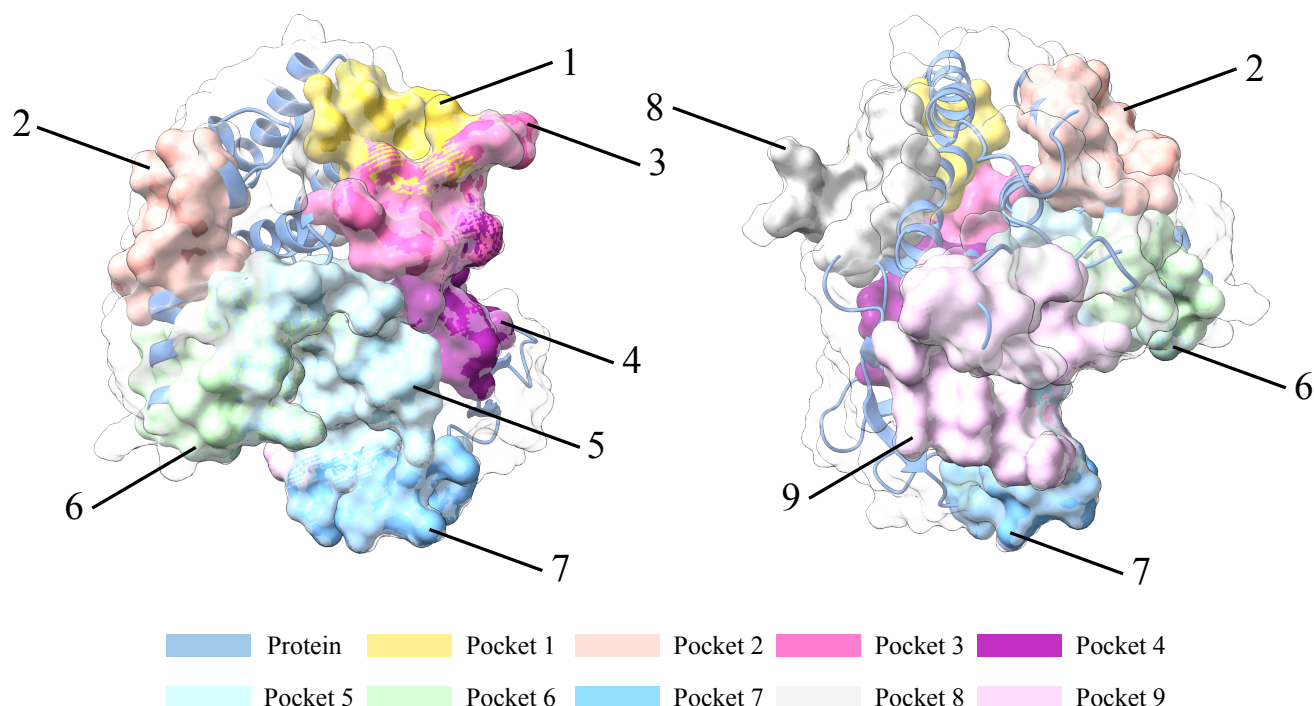


Figure 4. Binding pockets identified in the CK1 δ protein.

We used UNI-DOCK [28] to dock the inhibitors to the stabilized CK1 δ protein. For each ligand, we performed 2500 simulation events, resulting in a total of 75,000 dockings. In each case, we selected the complex with the lowest predicted affinity energy or the one with the greatest absolute energy (see Table 2).

It is important to emphasize that the energy values presented are for reference only; we employ force fields that use geometric parameters, such as those derived from Van der Waals volumes. When analyzing the binding energy results for the complexes with CK1 δ , six compounds with the highest affinities were identified: DXB with a binding energy of -8.14 kcal/mol, followed by BIM (-7.70 kcal/mol), BZH (-7.66 kcal/mol), BIP (-7.32 kcal/mol), IMT (-7.21 kcal/mol), and IMF (-7.17 kcal/mol). In contrast, the compound with the lowest affinity was AMQ, with a binding energy of -5.27 kcal/mol. The docking energies obtained in Table 2 are only referential, since they are based on probabilistic models and simplified force fields. To obtain more accurate interaction data, we performed 100 ns molecular dynamics simulations under constant physiological conditions. The last 10 ns was analyzed using the MMPBSA method, evaluating the interaction free energy and its energy components.

Table 2. Docking and MMPBSA energy components for ligand binding to CK1 δ (kcal/mol).

Ligand	Dock	MMPBSA Components					
		VdW	Elec	Pol	SASA	SAV	Total
BZH	-7.663	-52.70 ± 1.93	-4.12 ± 0.12	16.37 ± 0.05	-6.07 ± 0.09	10.30 ± 0.10	-46.53 ± 1.94
IMF	-7.171	-40.00 ± 1.36	-2.27 ± 1.28	10.76 ± 0.45	-4.51 ± 0.01	6.25 ± 0.45	-36.02 ± 1.92
IMB	-6.806	-37.25 ± 3.07	-6.30 ± 0.62	14.96 ± 0.15	-4.41 ± 0.21	10.56 ± 0.26	-32.99 ± 3.14
TTA	-6.533	-38.15 ± 1.03	-3.32 ± 1.29	12.76 ± 0.58	-4.13 ± 0.02	8.62 ± 0.58	-32.85 ± 1.75
BIP	-7.322	-35.48 ± 0.94	-5.00 ± 0.18	12.83 ± 0.24	-3.79 ± 0.01	9.04 ± 0.24	-31.44 ± 0.99
BZC	-7.076	-36.14 ± 0.79	-0.61 ± 0.08	9.80 ± 0.20	-4.23 ± 0.10	5.57 ± 0.22	-31.18 ± 0.83
IMT	-7.211	-34.69 ± 1.59	1.65 ± 1.36	6.49 ± 1.02	-3.87 ± 0.04	2.62 ± 1.02	-30.43 ± 2.33
IMD	-7.035	-33.19 ± 0.73	-9.09 ± 0.18	15.88 ± 0.08	-3.71 ± 0.06	12.17 ± 0.10	-30.11 ± 0.75
BIM	-7.704	-34.46 ± 1.43	-1.85 ± 0.32	10.61 ± 0.37	-3.93 ± 0.10	6.68 ± 0.38	-29.63 ± 1.51
PMP	-6.103	-31.87 ± 0.42	-4.68 ± 0.20	11.32 ± 0.30	-3.66 ± 0.02	7.66 ± 0.30	-28.89 ± 0.55
PYP	-7.104	-32.66 ± 0.71	0.80 ± 0.23	7.42 ± 0.06	-4.17 ± 0.03	3.26 ± 0.07	-28.61 ± 0.75
LAX	-6.517	-32.81 ± 1.64	-2.43 ± 0.64	10.24 ± 1.25	-3.51 ± 0.06	6.73 ± 1.26	-28.51 ± 2.16
LAM	-6.813	-32.58 ± 1.25	2.32 ± 0.97	5.26 ± 0.38	-3.27 ± 0.00	1.99 ± 0.38	-28.27 ± 1.63
BIT	-6.685	-32.82 ± 1.38	-3.60 ± 0.05	11.81 ± 0.37	-3.55 ± 0.04	8.26 ± 0.37	-28.16 ± 1.43
BIC	-6.090	-30.74 ± 0.91	-1.40 ± 0.33	8.79 ± 0.68	-3.85 ± 0.00	4.94 ± 0.68	-27.19 ± 1.19
PYR	-6.093	-28.74 ± 0.38	-1.37 ± 0.16	6.39 ± 0.09	-3.23 ± 0.07	3.16 ± 0.11	-26.95 ± 0.42
PYI	-6.885	-27.40 ± 0.90	-0.29 ± 0.03	6.74 ± 0.02	-3.48 ± 0.06	3.26 ± 0.06	-24.42 ± 0.90
AMI	-5.570	-27.42 ± 0.23	-4.38 ± 1.38	10.75 ± 0.63	-3.16 ± 0.15	7.58 ± 0.65	-24.22 ± 1.54
BZF	-6.044	-27.48 ± 0.59	-3.83 ± 0.88	11.05 ± 0.75	-3.60 ± 0.03	7.45 ± 0.75	-23.86 ± 1.30
BZT	-6.090	-26.35 ± 0.32	-1.20 ± 0.77	7.09 ± 0.49	-3.34 ± 0.02	3.75 ± 0.49	-23.79 ± 0.97
BZM	-6.004	-26.11 ± 0.36	-1.42 ± 0.81	7.34 ± 0.58	-3.00 ± 0.01	4.34 ± 0.58	-23.19 ± 1.06
DXB	-8.137	-26.02 ± 0.77	-11.40 ± 2.75	20.33 ± 1.93	-3.07 ± 0.04	17.25 ± 1.93	-20.16 ± 3.45
HYA	-5.648	-21.52 ± 0.43	-2.90 ± 0.37	7.31 ± 0.43	-2.52 ± 0.01	4.79 ± 0.43	-19.63 ± 0.71
AHQ	-5.276	-23.37 ± 1.28	-2.04 ± 0.58	9.52 ± 0.10	-2.69 ± 0.08	6.83 ± 0.13	-18.57 ± 1.41
DXM	-6.435	-17.90 ± 0.63	-1.61 ± 1.91	6.70 ± 1.71	-2.28 ± 0.00	4.42 ± 1.71	-15.09 ± 2.64
AMQ	-5.273	-7.85 ± 0.31	-0.52 ± 0.58	2.32 ± 0.19	-0.97 ± 0.21	1.35 ± 0.28	-7.03 ± 0.71

Dock: Docking score. **VdW:** Van der Waals energy. **Elec:** Electrostatic energy. **Pol:** Polar solvation energy. **SASA:** Solvent-accessible surface area. **SAV:** Solvation energy. **Total:** Total MMPBSA energy. All values in kcal/mol; errors are $\pm$ standard deviation.

Table 2 shows the interaction free energies between CK1 δ and the different inhibitors, presenting a binding free energy scale of BZH > IMF > IMB > TTA > BIP. The value for BZH was -46.53 ± 1.94 kcal/mol, while for BIP it was -31.44 ± 0.99 kcal/mol. When analyzing

the binding free energies by Van der Waals interactions, we observed the following order: BZH > IMF > TTA > IMB > BIP. The value for BZH was -52.70 ± 1.93 kcal/mol, while for BIP it was -35.48 ± 0.94 kcal/mol. In the case of electrostatic interactions, the order was IMB > BIP > BZH > TTA > IMF. The highest value was for IMB with -6.30 ± 0.62 kcal/mol, and the lowest for IMF with -2.27 ± 1.28 kcal/mol. Regarding the polar solvation energy, we observed the following order: BZH > IMB > TTA > BIP > IMF. The highest value was for BZH with 16.37 ± 0.05 kcal/mol, while the lowest value was for IMF with 10.76 ± 0.45 kcal/mol. When examining the solvent accessible surface area (SASA), we found the following order: BZH > IMF > IMB > TTA > BIP. BZH showed the most favorable contribution with -6.07 ± 0.09 kcal/mol, while BIP showed the least favorable with -3.79 ± 0.01 kcal/mol. Finally, regarding the contribution of solvent-accessible volume (SAV), the order was IMB > BZH > TTA > BIP > IMF. The highest value was for IMB, with 10.56 ± 0.26 kcal/mol, and the lowest for IMF, with 6.25 ± 0.45 kcal/mol. These results suggest that the compound BZH exhibits potential inhibitory activity against CK1 δ .

Among the twenty-six structurally diverse compounds analyzed through molecular docking using UNI-DOCK [28], DXB initially showed the highest predicted binding affinity (-8.14 kcal/mol), followed by BIM, BZH, and BIP. However, this ranking changed significantly when we performed molecular dynamics simulations and MMPBSA binding free energy calculations. BZH emerged as the most stable and promising inhibitor, with the most favorable free energy (-46.53 ± 1.94 kcal/mol), while DXB showed a less favorable interaction profile (-20.16 ± 3.45 kcal/mol). These findings underscore the limitations of relying solely on static docking scores, which do not account for conformational dynamics or solvent effects. The MMPBSA decomposition further highlighted BZH's strong Van der Waals interactions and favorable SASA contributions. Altogether, this reinforces the need to integrate dynamic methods, such as MMPBSA, to better reflect the real biophysical behavior of the complex. Several studies support this notion: Gohlke and Klebe [29] emphasized the need to consider system flexibility and the biological environment. At the same time, Hou [30] noted that the accuracy of MMPBSA is highly dependent on simulation parameters such as duration. Additionally, Kollman [31] demonstrated that combining molecular dynamics with explicit solvation models significantly enhances the prediction of binding free energies and complex stability.

In Figure 5, we presented the energetic decomposition of the ligand-CK1 δ binding complexes, broken down into their main components: Van der Waals, electrostatics, polar solvation, and SASA for the BZH-CK1 δ complex. It is important to emphasize that negative energies are contributive while positive energies are destabilizing. We observed that the interaction is primarily due to a substantial contribution from Van der Waals interactions, which represent the most significant component of the total binding energy.

In Figure 6, we showed the root mean square deviation (RMSD) analysis of ligand-CK1 δ complexes during 100 ns of molecular dynamics simulation. All systems reach structural stability after the first 20 ns. BZH-CK1 δ , BIP-CK1 δ , and IMF-CK1 δ complexes show more stable trajectories, whereas the IMB-CK1 δ complex exhibits greater fluctuations over time. Furthermore, the probability density function (PDF) suggests that BZH-CK1 δ , BIP-CK1 δ , and IMF-CK1 δ complexes present narrow and centered peaks, indicating structural stability. In contrast, IMB-CK1 δ complexes show broad and dispersed distributions, reflecting higher conformational flexibility.

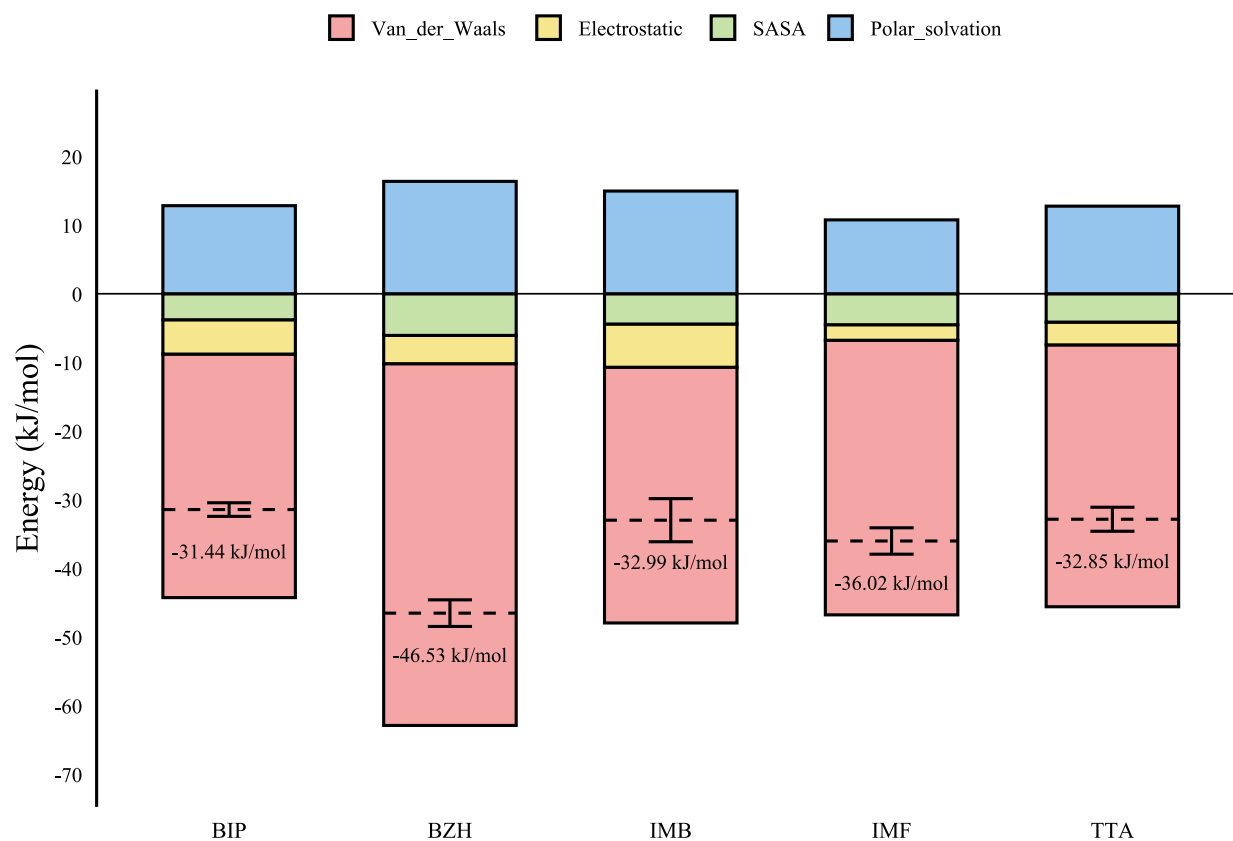


Figure 5. Energy decomposition of the binding energy of ligand-CK1 δ complexes according to MMPBSA analysis.

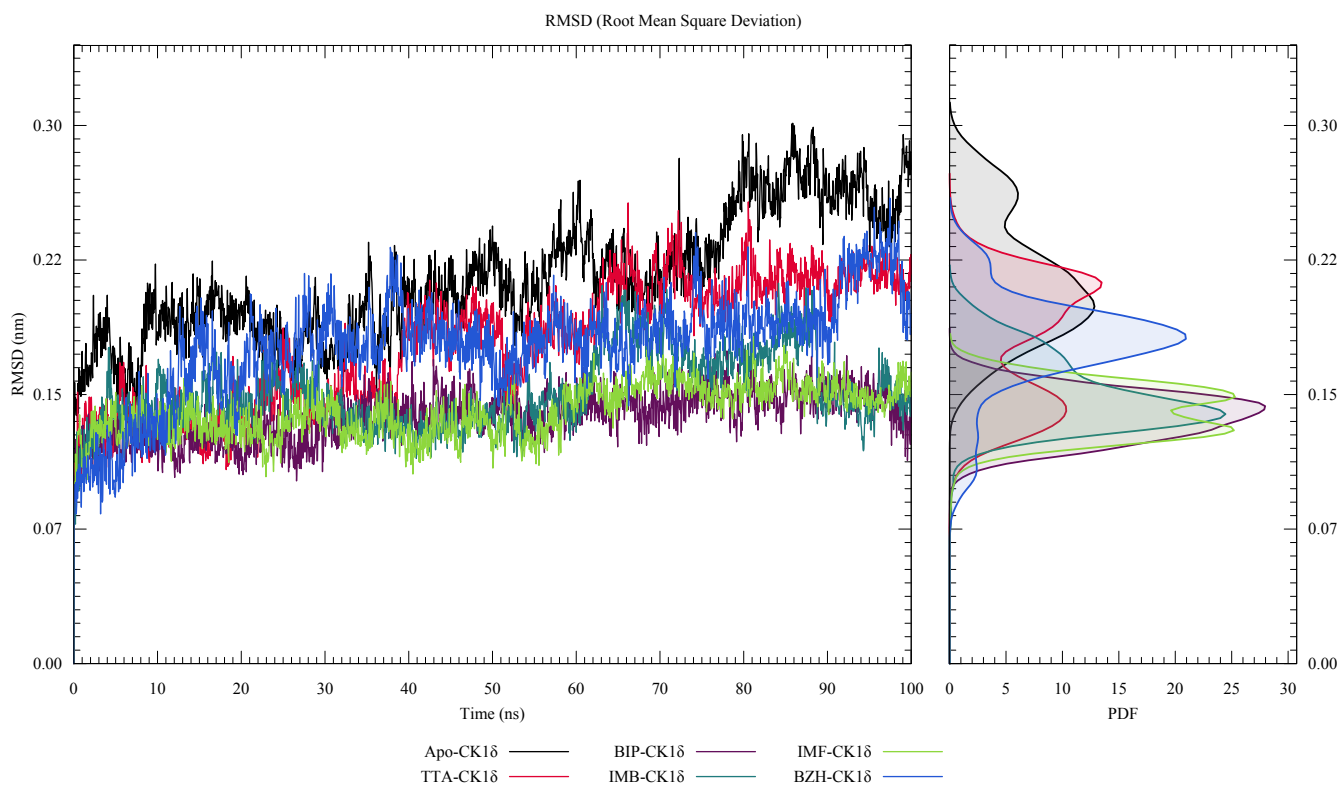


Figure 6. Root mean square deviation (RMSD) analysis of ligand-CK1 δ complexes over a 100 ns molecular dynamics simulation.

In Figure 7, RMSF analysis shows that residual fluctuations are most pronounced in specific regions, around residues 140, 160, 220, and 240, suggesting localized flexibility that does not compromise the structural integrity of the system. Furthermore, in Figure 8, the radius of gyration (Rg) analysis shows that the IMF-CK1 δ and BZH-CK1 δ complexes present the lowest and most stable values over time, indicating greater structural compaction. In contrast, the TTA-CK1 δ complex shows slightly higher values, suggesting less compaction. The PDF distribution reinforces these findings, with more concentrated peaks towards low values.

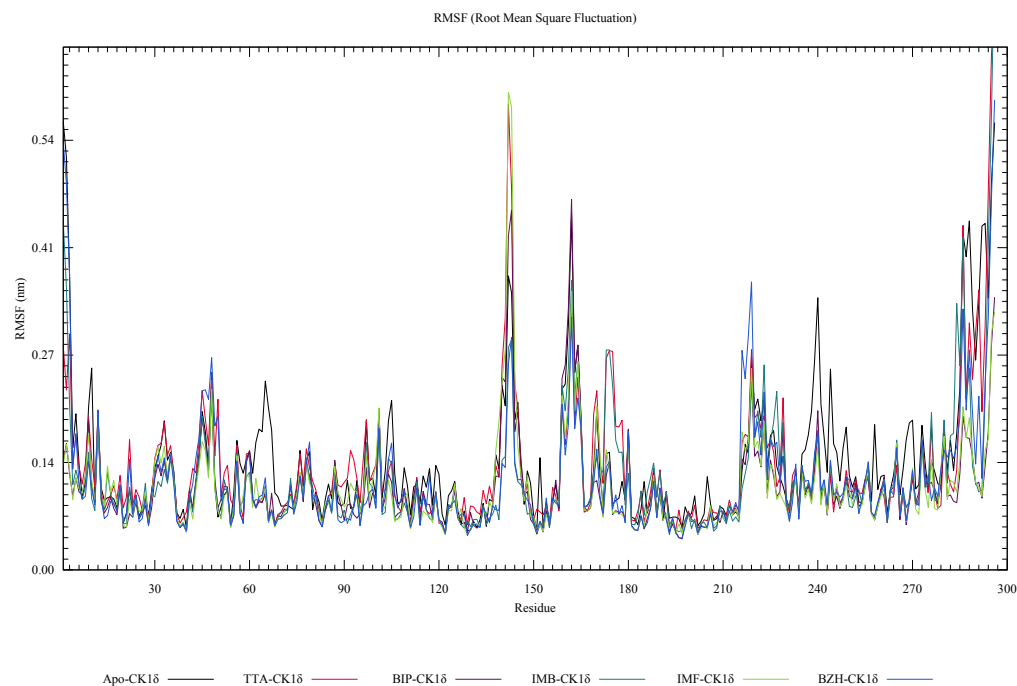


Figure 7. Residue mean square fluctuation (RMSF) analysis of ligand-CK1 δ complexes during a 100 ns molecular dynamics simulation.

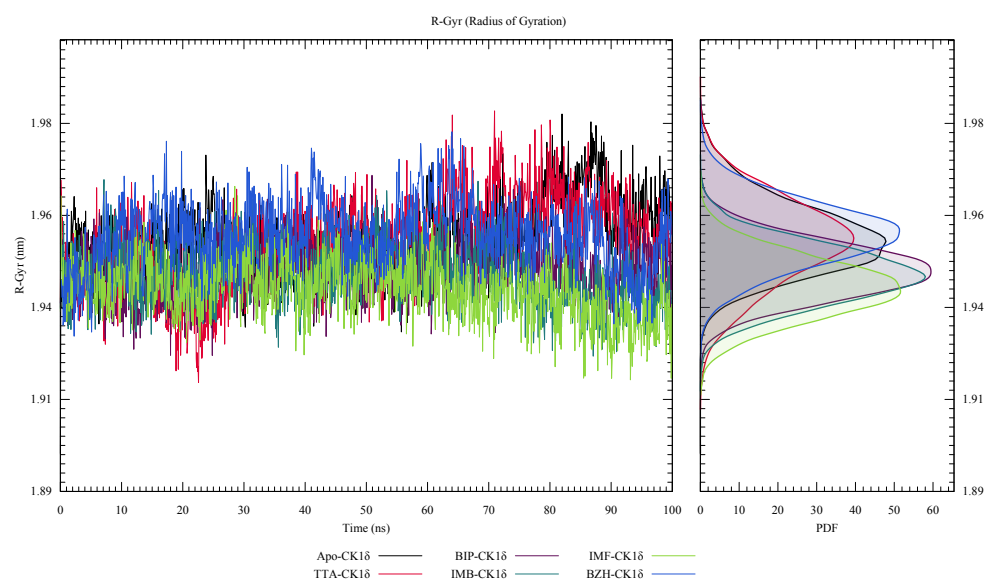


Figure 8. Radius of gyration (Rg) analysis of ligand-CK1 δ complexes during a 100 ns molecular dynamics simulation.

In Figure 9, we show that when analyzing the hydrogen bonds, they remain virtually unchanged throughout the trajectory, indicating that these interactions do not significantly influence the overall stability of the system during the simulation.

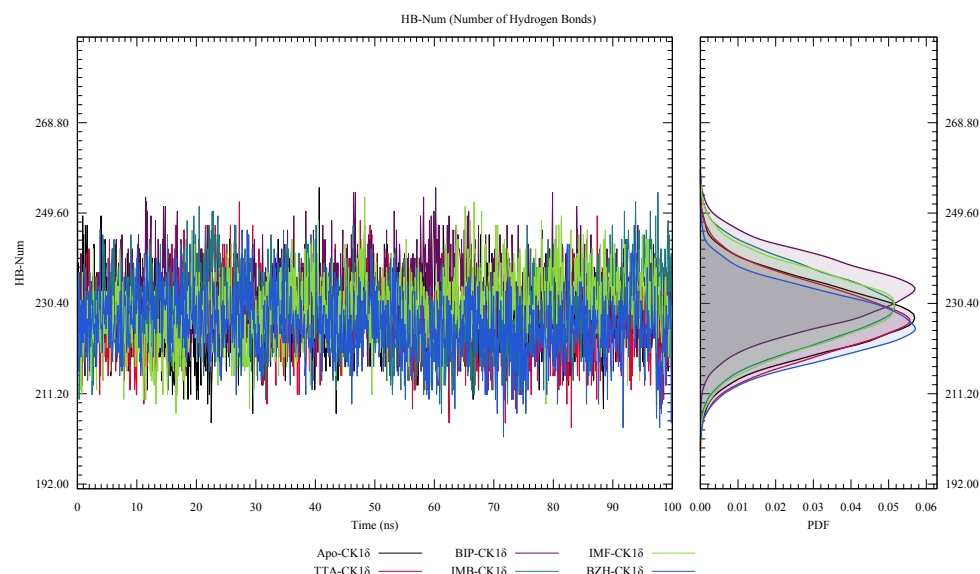


Figure 9. Number of hydrogen bonds (HBs) in ligand-CK1 δ complexes during a 100 ns molecular dynamics simulation.

Similarly, in Figure 10, we observe that the SASA values remain relatively constant in all systems, without significant variations. It suggests that we are facing a general structural stability throughout the simulation.

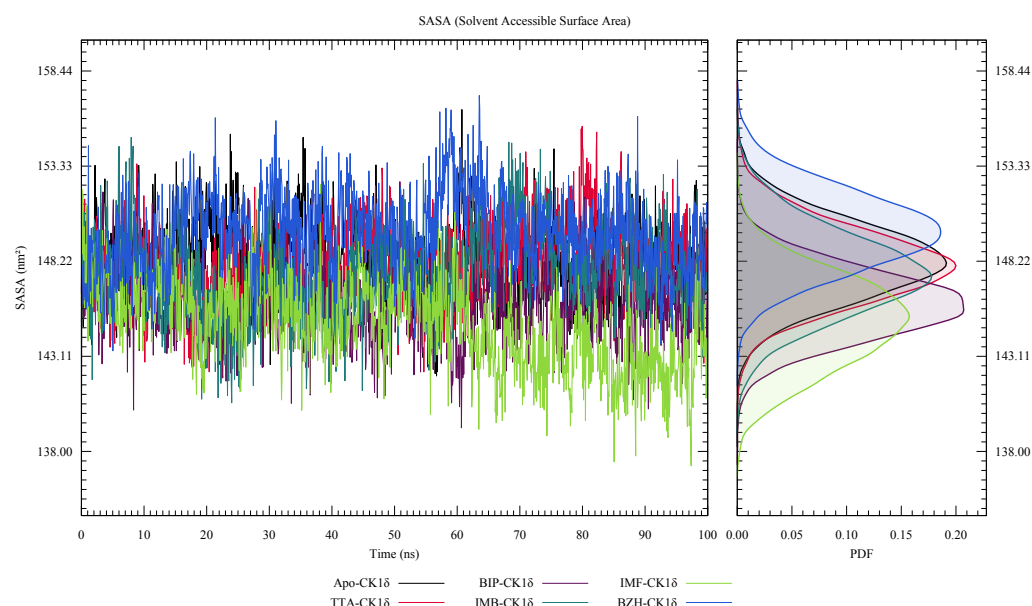


Figure 10. Solvent-accessible surface area (SASA) of ligand-CK1 δ complexes during a 100 ns molecular dynamics simulation.

In Figure 11, we observed that the BZH-CK1 δ complex maintains more negative and stable binding free energy values during the simulation, suggesting a stronger interaction with the protein. This result is consistent with the data presented in Table 2, where BZH presents the most favorable value, with -46.53 ± 1.94 kcal/mol. In contrast, the TTA-CK1 δ , IMF-CK1 δ , and IMB-CK1 δ complexes present less negative values, and BIP-CK1 δ has the

highest, with -31.44 ± 0.99 kcal/mol, indicating a comparatively lower affinity. The PDF distribution confirms this trend, with a defined peak shifted towards low values for BZH.

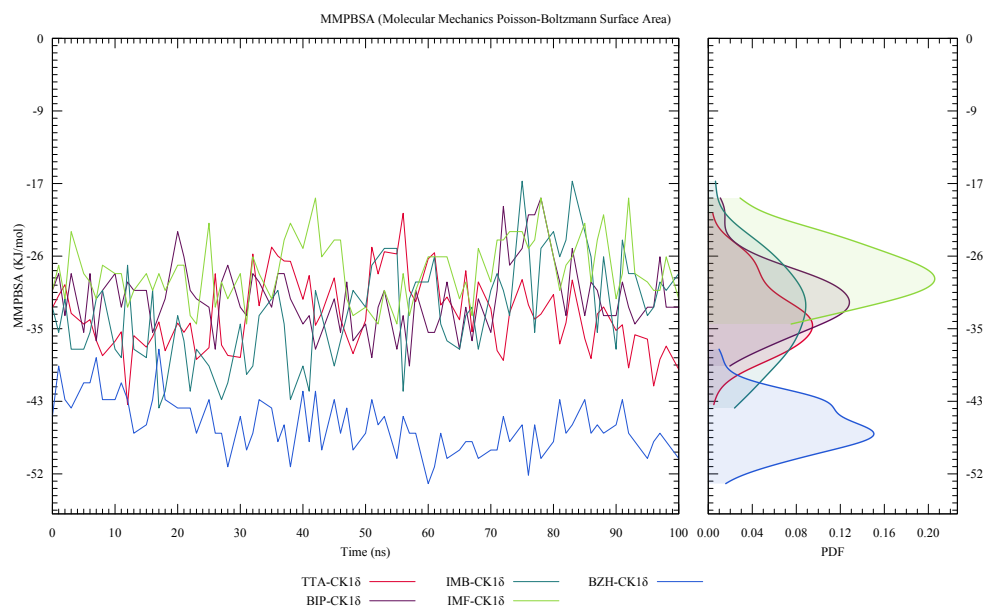


Figure 11. Binding free energy calculated by MMPBSA for ligand-CK1 δ complexes during a 100 ns molecular dynamics simulation.

In Figure 12a, we showed that pocket 2 was identified as the site with the highest druggable potential, while pocket 6 scored the lowest. However, Figure 12b shows that most ligands, following the molecular dynamics simulation, converge in the region corresponding to pocket 6. When analyzing the structural properties of CK1 δ at the end of the molecular dynamics simulation, we considered only the five complexes with the better free energy of interaction.

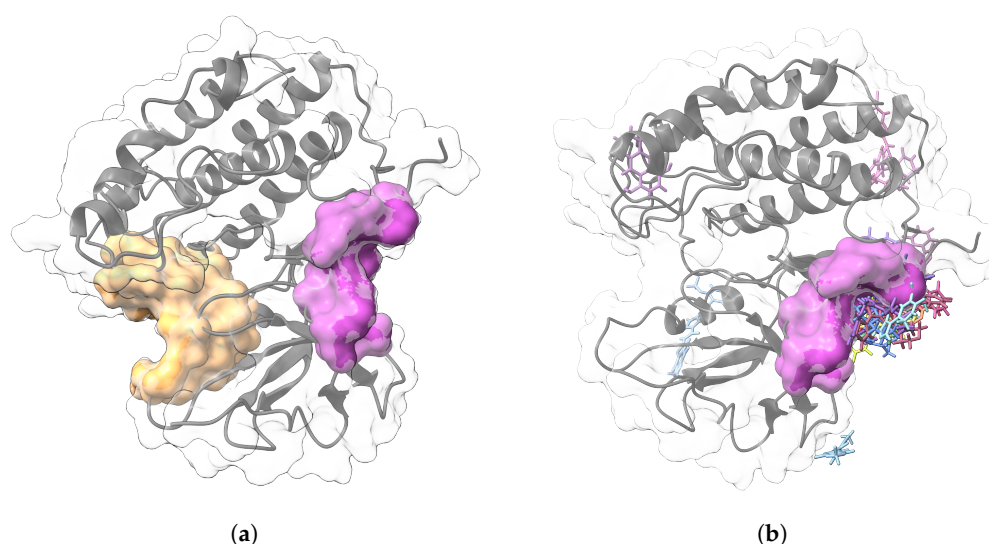


Figure 12. The best-scoring predicted protein pockets and ligand-preferred pockets in the protein after 100 ns of molecular dynamics simulation. (a) Representation of the pharmacological pockets predicted by CavityPlus, highlighting pocket 2 and pocket 6. (b) Final distribution of the ligands after 100 ns of molecular dynamics, demonstrating a common interaction zone with CK1 δ .

In Figure 13a, we showed an overview of the BZH-CK1 δ complex after 100 ns of molecular dynamics simulation, where we have found that the BZH ligand is embedded in

a surface pocket of the protein. Figure 13b shows a close-up of the binding pocket, revealing specific interactions between BZH and several key residues. The ligand orientation allows for multiple hydrophobic and polar contacts.

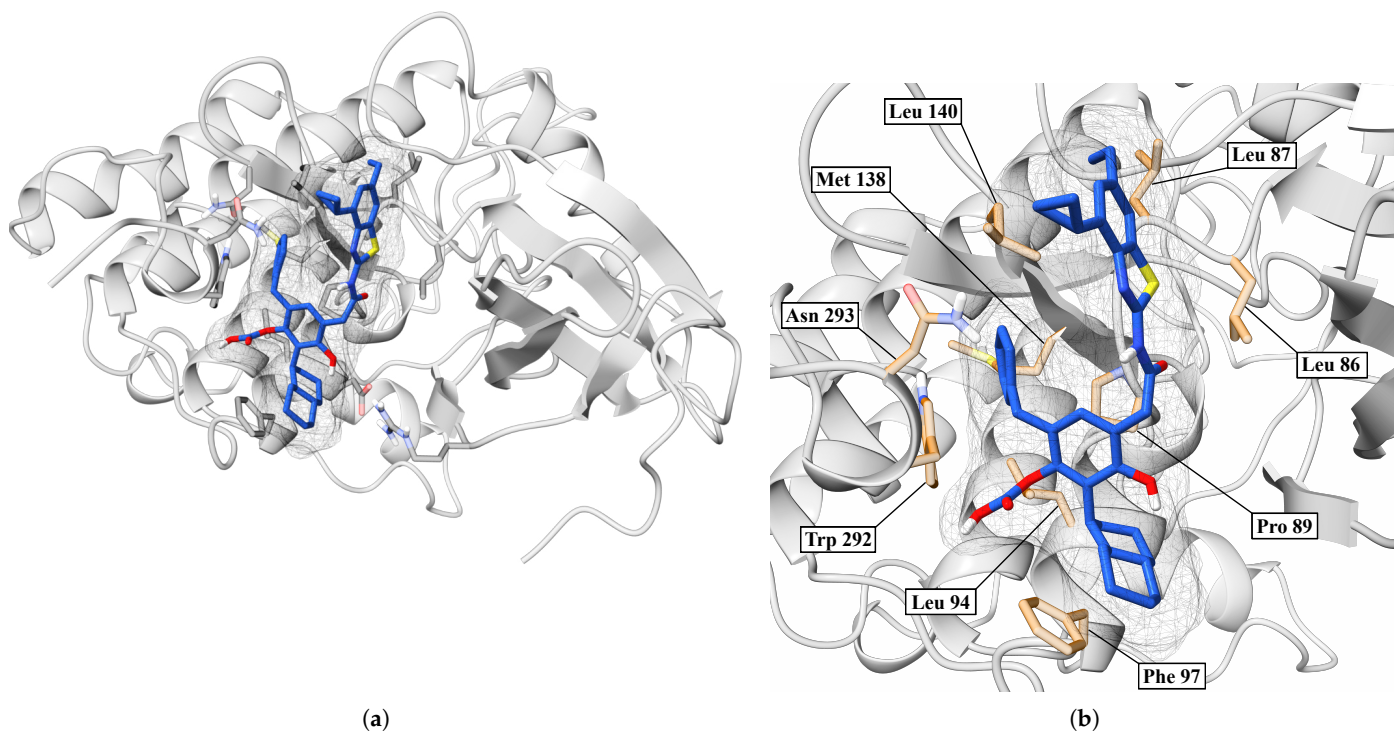


Figure 13. Interaction between CK1 δ and the BZH ligand. (a) Overview. (b) Close-up.

In Figure 14, we observed the surface electrostatic potential mapping of the BZH-CK1 δ complex after 100 ns of molecular dynamics. The binding site shows a predominantly blue and green distribution, indicating areas with positive or neutral charge, which means that these regions have positive or apolar amino acids.

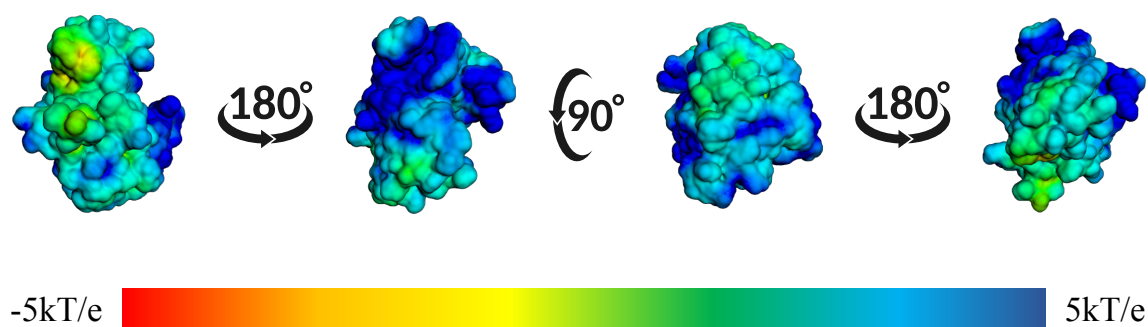


Figure 14. Mapping of the surface electrostatic potential of the BZH-CK1 δ complex calculated using APBS after 100 ns of molecular dynamics. The different orientations show the potential distribution, where the blue regions represent positive charges and the red ones the negative charges, according to the kT/e scale.

In Figure 15a, we performed a Ramachandran plot analysis to assess the structural conformation of the BZH-CK1 δ complex. Of the 261 non-glycine/proline residues analyzed, 218 residues (83.5%) were located in the most favored regions, 38 (14.6%) in additionally allowed regions, and 5 (1.9%) in generously allowed regions, representing 100% of the residues. We did not locate residues (0.0%) in the disallowed regions. This assessment excluded glycine and proline residues, as well as terminal residues, from the analysis. In Figure 15b, BZH interacts with several CK1 δ residues, including LEU86, LEU87, PRO89,

LEU94, PHE97, MET138, LEU140, TRP292, and ASN293. Although we did not identify hydrogen bonds, the average interaction distance was 3.7 Å.

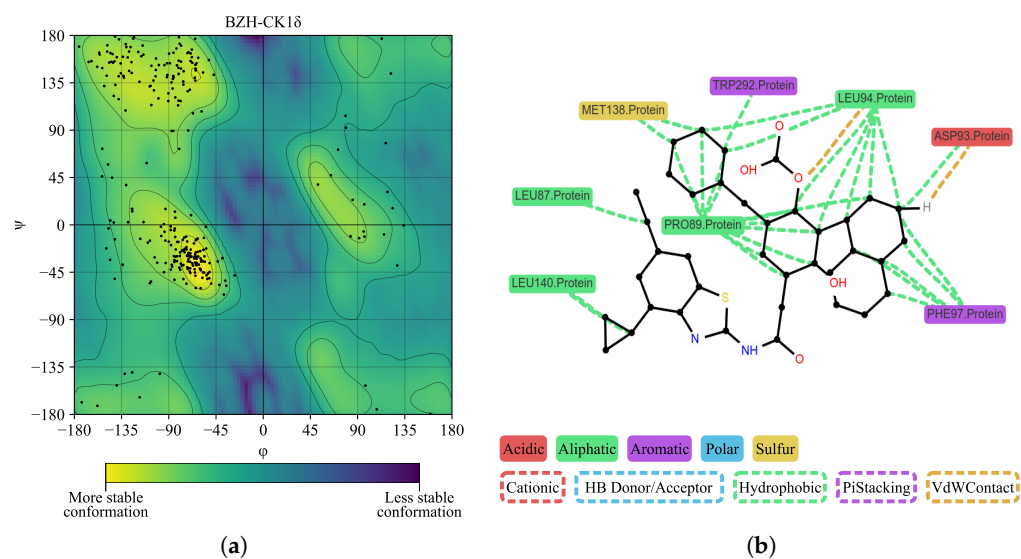


Figure 15. Ramachandran diagram and molecular interactions of the BZH-CK1δ complex after 100 ns of molecular dynamics. (a) Ramachandran diagram, the yellow color shows a more stable conformation and purple color shows a less stable conformation. (b) Molecular interactions.

In Figure 16a, we showed an overview of the IMF-CK1δ complex after 100 ns of molecular dynamics. In the case of the IMF, the ligand remains stably anchored in a cavity on the protein surface. On the other hand, Figure 16b shows a close-up of the binding site, where the IMF makes direct contact with key residues. The ligand orientation allows for multiple hydrophobic and polar contacts.

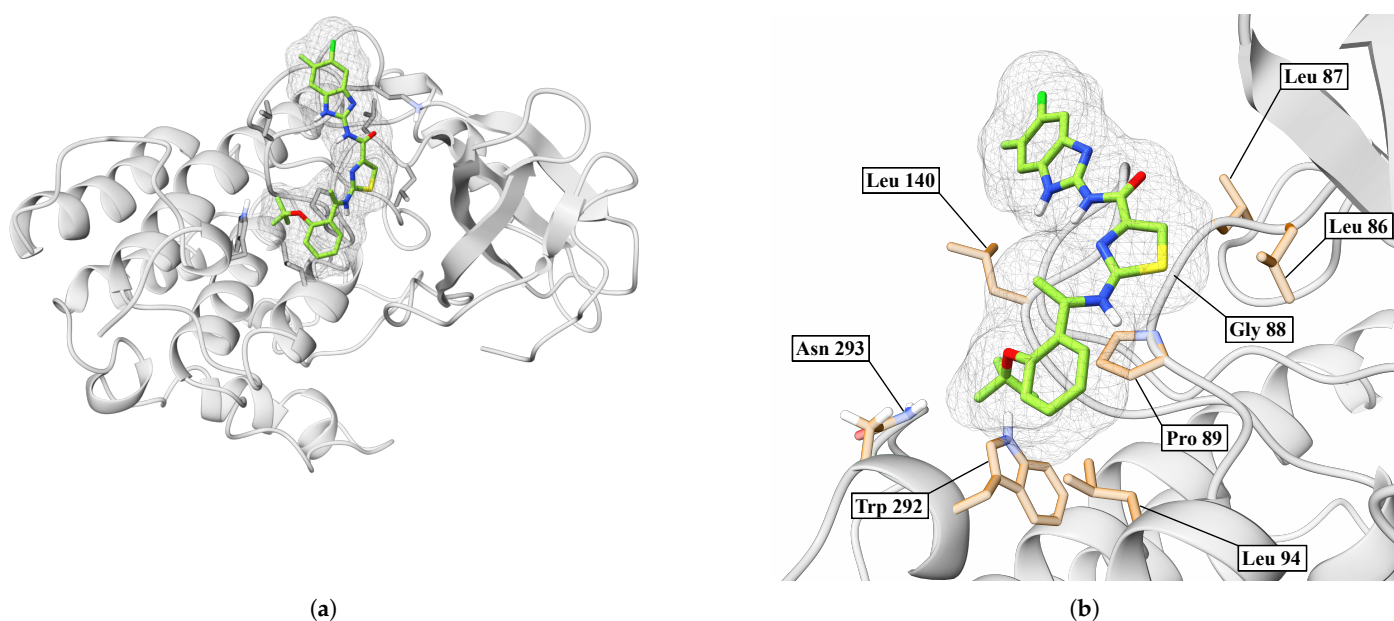


Figure 16. Interaction between CK1δ and the IMF ligand. (a) Overview. (b) Close-up.

In Figure 17, we showed the surface electrostatic potential mapping of the IMF-CK1δ complex, calculated at the end of the molecular dynamics simulation. Rotated views of the protein reveal that the IMF binding site is surrounded primarily by regions of positive charge. The predominance of blue areas indicates positive electrostatic potentials, while the green-yellow areas correspond to neutral or slightly negative charges.

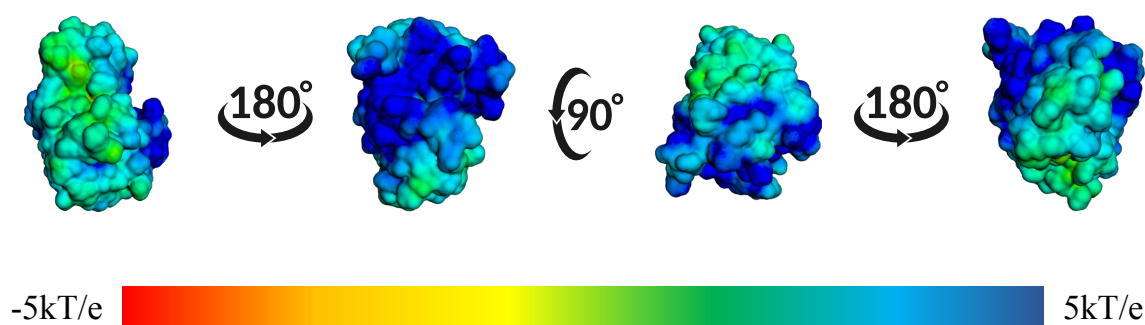


Figure 17. Mapping of the surface electrostatic potential of the IMF-CK1 δ complex calculated using APBS after 100 ns of molecular dynamics. The different orientations show the potential distribution, where blue regions represent positive charges and red regions represent negative charges, according to the kT/e scale.

In Figure 18a, we performed a Ramachandran plot analysis to assess the structural conformation of the IMF-CK1 δ complex. Of the 261 non-glycine/proline residues analyzed, 218 residues (83.5%) were located in the most favored regions, 39 (14.9%) in regions with extra permissions, and 3 (1.1%) in regions with generous permissions, representing 99.6% of the residues. We located only one residue (0.4%) in the regions with disallowed permissions. In Figure 18b, we observed interactions between BZH and several CK1 δ residues, notably LEU86, LEU87, PRO89, LEU94, PHE97, MET138, LEU140, TRP292, and ASN293. We did not detect hydrogen bonds. The average interaction distance was 3.7 Å.

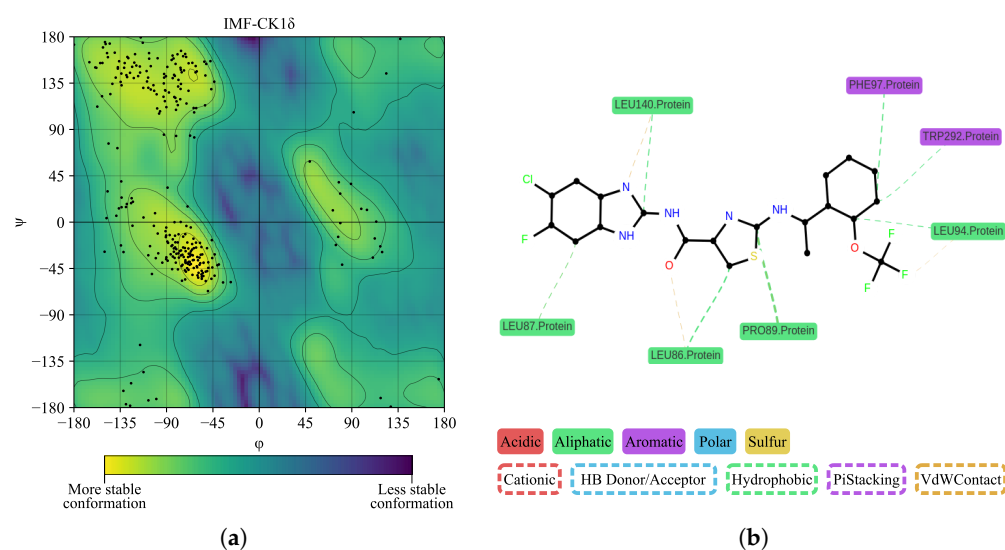


Figure 18. Ramachandran diagram and molecular interactions of the IMF-CK1 δ complex after 100 ns of molecular dynamics simulations. (a) Ramachandran diagram, the yellow color shows a more stable conformation and purple color shows a less stable conformation. (b) Molecular interactions.

In Figure 19a, we showed a general view of the IMF-CK1 δ complex after 100 ns of molecular dynamics simulation. The IMF ligand remains stably anchored in a cavity on the protein surface. Additionally, in Figure 19b, we showed a close-up of the binding site, where IMF enters direct contact with key residues.

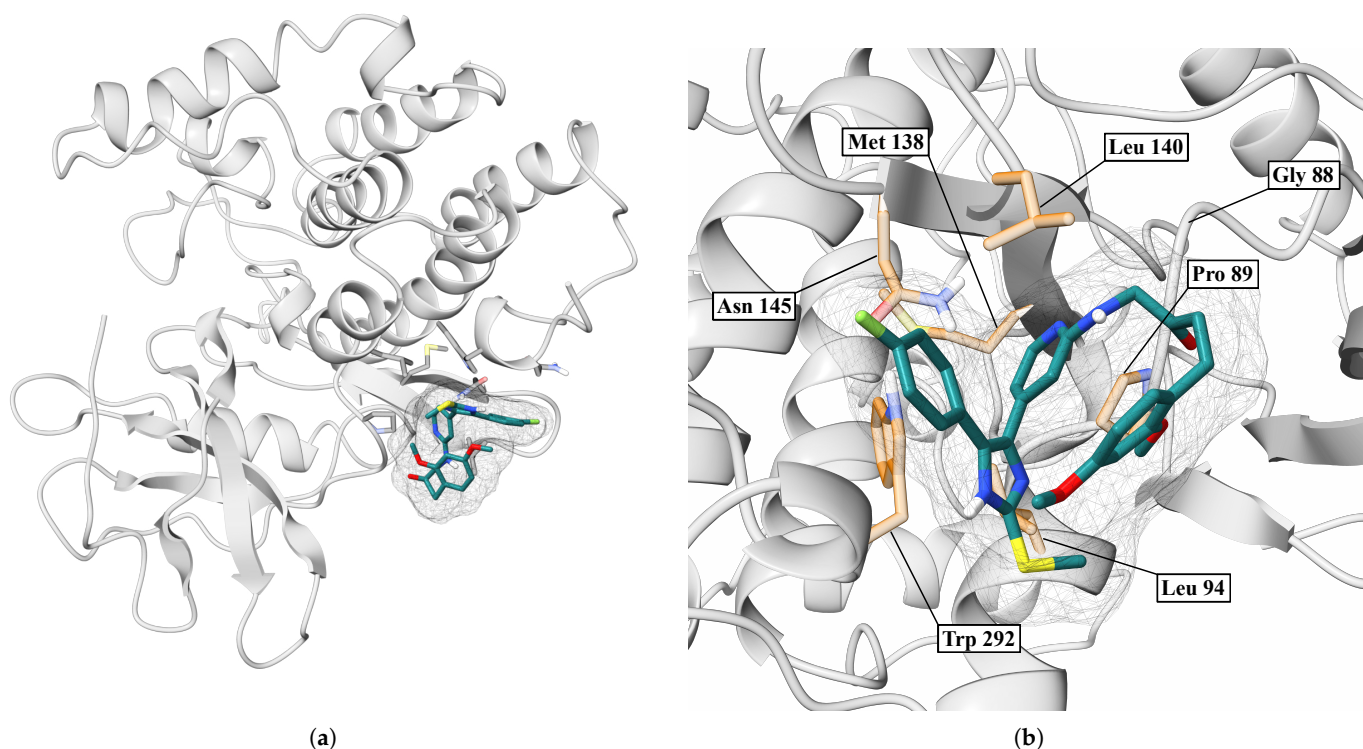


Figure 19. The IMB-CK1 δ complex after 100 ns of molecular dynamics simulation, showing the key residues involved in the interaction with IMB. (a) Overview. (b) Close-up.

In addition, in Figure 20, we showed the surface electrostatic potential mapping of the IMB-CK1 δ complex, calculated at the end of the molecular dynamics simulation. A clear predominance of blue areas is observed on the protein surface, suggesting a predominance of positive charges.

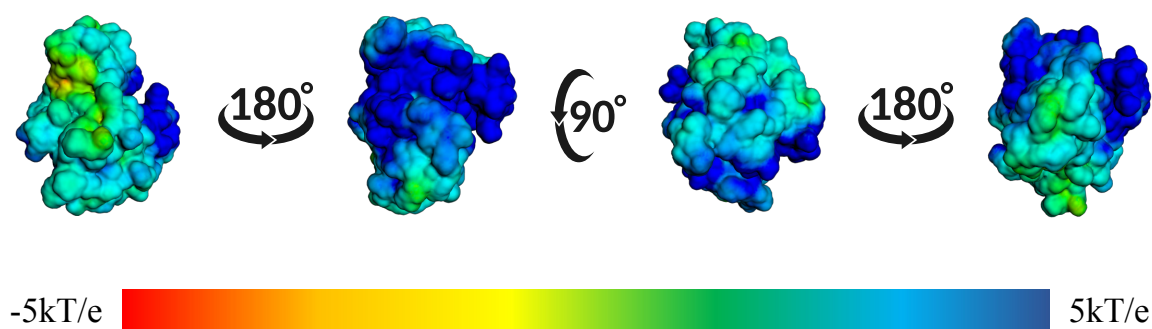


Figure 20. Mapping of the surface electrostatic potential of the IMB-CK1 δ complex calculated using APBS after 100 ns of molecular dynamics. The different orientations show the potential distribution, where blue regions represent positive charges and red regions represent negative charges, according to the kT/e scale.

In Figure 21a, we performed a Ramachandran plot analysis to evaluate the structural conformation of the IMB-CK1 δ complex. Of the 261 non-glycine/proline residues analyzed, 214 residues (82.0%) were located in the most favored regions, 40 (15.3%) in regions with extra permissions, and 5 (1.9%) in regions with generous permissions, representing 99.2% of the residues. We located only two residues (0.8%) in the disallowed regions. This evaluation excluded glycine and proline residues, as well as terminal residues, from the analysis. On the other hand, in Figure 21b, interactions between IMB and CK1 δ residues are observed,

including PRO89, LEU140, TRP292, MET138, GLY88, LEU94, and ASN145. We did not identify hydrogen bonds. The average contact distance was 3.64 Å.

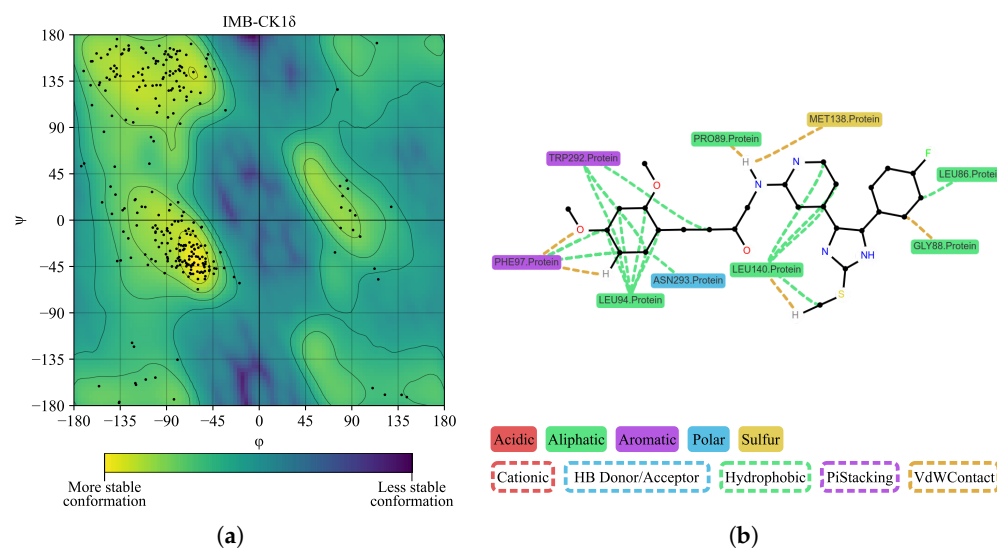


Figure 21. Ramachandran diagram and molecular interactions of the IMB-CK1 δ complex after 100 ns of molecular dynamics simulation. (a) Ramachandran diagram, the yellow color shows a more stable conformation and purple color shows a less stable conformation. (b) Molecular interactions.

Likewise, in Figure 22a, we show an overview of the TTA-CK1 δ complex after 100 ns of molecular dynamics simulation. The TTA ligand remains stably anchored in a cavity on the protein surface. Figure 22b shows a close-up of the binding site, where TTA makes direct contact with key residues.

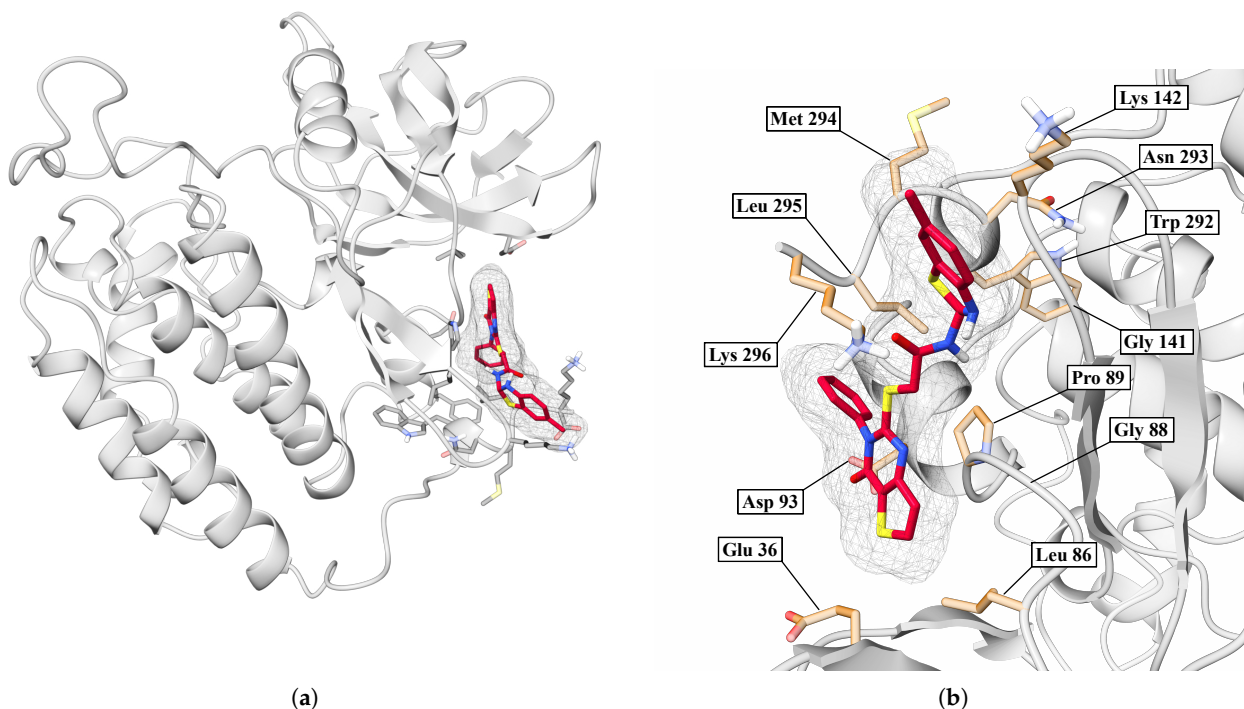


Figure 22. Interaction between CK1 δ and the ligand TTA after 100 ns of molecular dynamics simulation. (a) Overview. (b) Close-up.

On the other hand, we showed in Figure 23 the surface electrostatic potential mapping of the TTA-CK1 δ complex, calculated at the end of the molecular dynamics simulation.

We can observe a clear predominance of blue areas on the protein surface, suggesting a predominance of positive charges or positive residues.

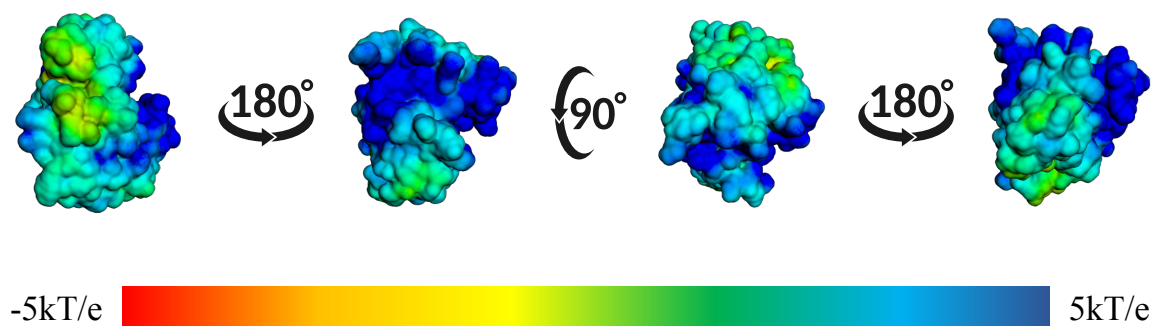


Figure 23. Mapping of the surface electrostatic potential of the TTA-CK1 δ complex calculated using APBS after 100 ns of molecular dynamics. The different orientations show the potential distribution, where blue regions represent positive charges and red regions represent negative charges, according to the kT/e scale.

We performed a Ramachandran plot analysis, as shown in Figure 24a, to assess the structural conformation of the TTA-CK1 δ complex. Of the 261 non-glycine/proline residues analyzed, 216 (82.8%) were located in the most favored regions, 40 (15.3%) in regions with additional permissions, and 5 (1.9%) in regions with generous permissions, representing 100% of the residues. We did not locate residues (0.0%) in the disallowed regions. This assessment excluded glycine and proline residues, as well as terminal residues, from the analysis. On the other hand, in Figure 24b, interactions between TTA and CK1 δ residues, including LEU295, GLY88, LYS142, ASN293, GLU36, LEU86, PRO89, ASP93, GLY141, TRP292, MET294, and LYS296, are observed. We did not detect hydrogen bonds at this interface. The average contact distance was 3.67 Å.

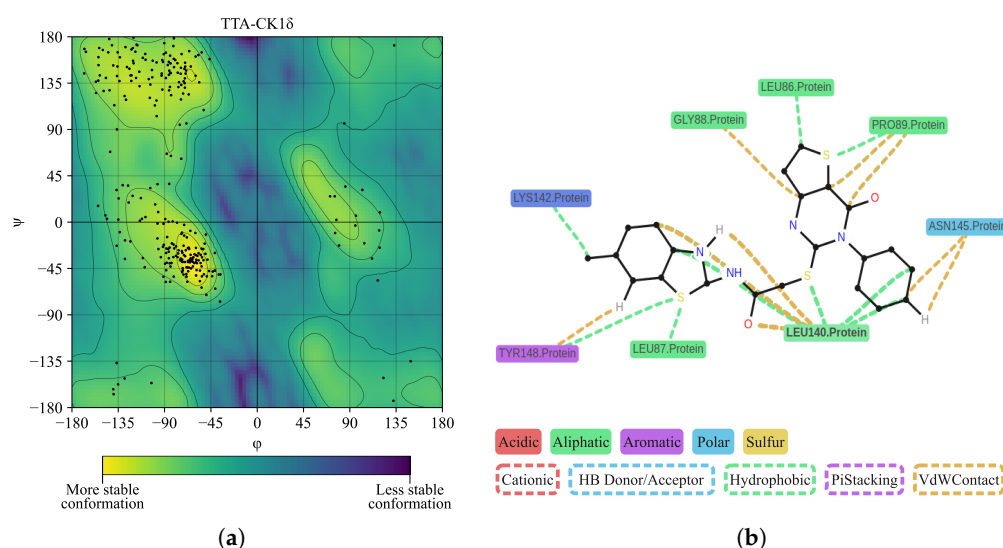


Figure 24. Ramachandran diagram and molecular interactions of the TTA-CK1 δ complex after 100 ns of molecular dynamics simulation. (a) Ramachandran diagram, the yellow color shows a more stable conformation and purple color shows a less stable conformation. (b) Molecular interactions.

In Figure 25a, we showed an overview of the BIP-CK1 δ complex after 100 ns of molecular dynamics simulation. The BIP ligand remains stably anchored in a cavity on the protein surface. Likewise, Figure 25b shows a close-up of the binding site, where BIP makes direct contact with key residues.

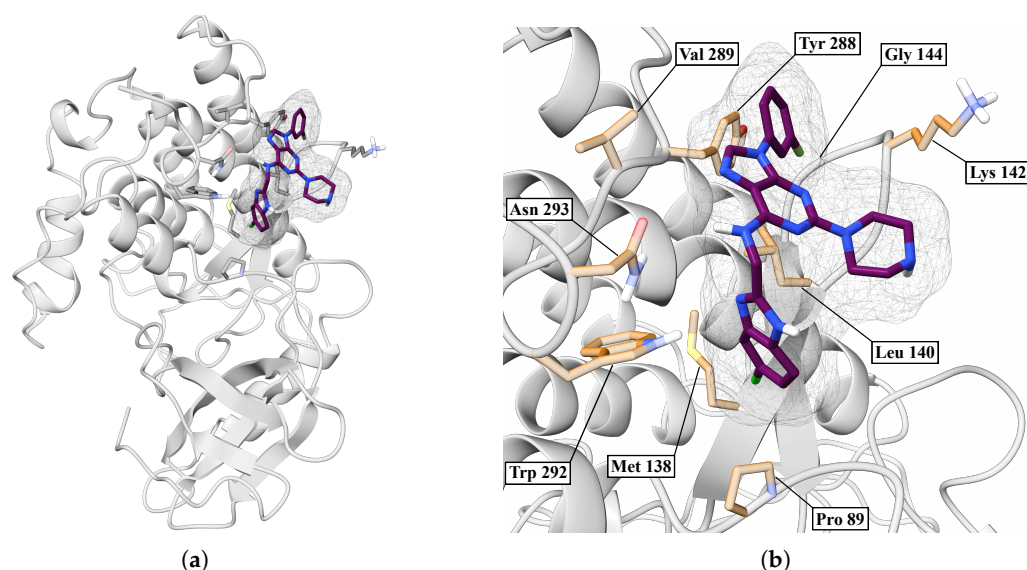


Figure 25. Interaction between CK1 δ and the ligand BIP complex after 100 ns of molecular dynamics simulation. (a) Overview. (b) Close-up.

Additionally, we showed in Figure 26 the electrostatic surface potential of the BIP-CK1 δ complex, calculated at the end of the molecular dynamics simulation. We observed the BIP ligand-binding pocket to be surrounded primarily by blue and green regions, indicating a predominantly positively charged zone with areas of neutral potential.

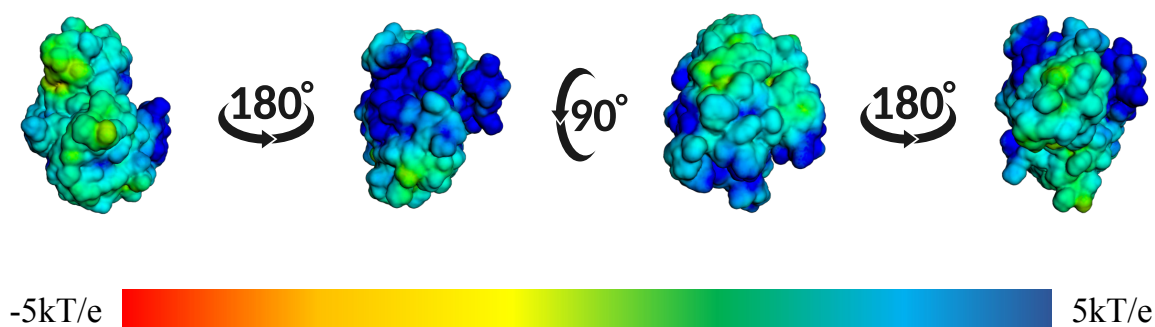


Figure 26. Mapping of the surface electrostatic potential of the BIP-CK1 δ complex calculated using APBS after 100 ns of molecular dynamics. The different orientations show the potential distribution, where blue regions represent positive charges and red regions represent negative charges, according to the kT/e scale.

In Figure 27a, we performed a Ramachandran plot analysis to assess the structural conformation of the BIP-CK1 δ complex. Of the 261 non-glycine/proline residues analyzed, 217 residues (83.1%) were located in the most favored regions, 37 (14.2%) in regions with extra permissions, and 6 (2.3%) in regions with generous permissions, representing 99.6% of the residues.

We located only one residue (0.4%) in the disallowed regions. This evaluation excluded glycine and proline residues, as well as terminal residues, from the analysis. In Figure 27b, interactions between BIP and CK1 δ residues are observed, particularly TYR288, LEU140, GLY141, GLY144, PRO89, MET138, LYS142, TRP292, and ASN293. We detected only one hydrogen bond between BIP and residue GLY144. The average interaction distance was 3.65 Å.

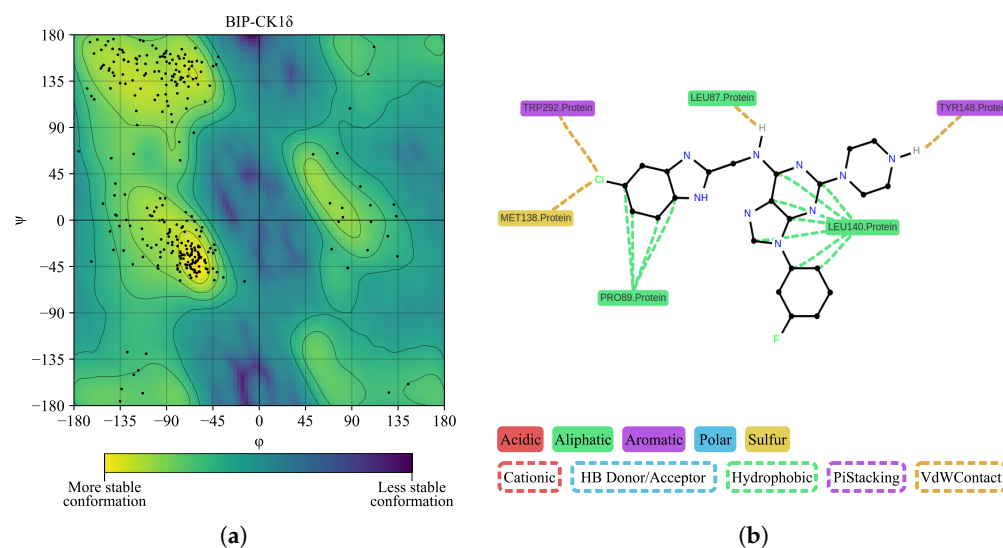


Figure 27. Ramachandran diagram and molecular interactions of the BIP-CK1 δ complex after 100 ns of molecular dynamics simulation. (a) Ramachandran diagram, the yellow color shows a more stable conformation and purple color shows a less stable conformation. (b) Molecular interactions.

Moreover, we found that BZH interacts with residues LEU86, LEU87, PRO89, LEU94, PHE97, MET138, LEU140, TRP292, and ASN293. According to Knippschild et al. [32], MET82 acts as a gatekeeper residue within the ATP-binding pocket of CK1 δ . Also, Long et al. [33] identified LEU85, MET80, and MET82 as essential for inhibitor binding. In contrast, Córdova-Bahena et al. [34] and Sunkari et al. [35] confirmed the participation of GLU85 and LEU87 as relevant interaction sites. The presence of LEU87 among our interaction residues supports these findings (Table 3).

Table 3. Summary of the interactions between BZH, IMF, IMB, TTA, and BIP with the amino acid residues within the active site of CK1 δ .

Ligand	Interacting Residues	H. Bonds	Dist. (Å)
BZH	LEU86, LEU87, PRO89, LEU94, PHE97, MET138, LEU140, TRP292, ASN293	—	3.70
IMF	LEU86, LEU87, GLY88, PRO89, LEU94, LEU140, TRP292, ASN293	—	3.64
IMB	PRO89, LEU140, TRP292, MET138, GLY88, LEU94, ASN145	—	3.64
TTA	LEU295, GLY88, LYS142, ASN293, GLU36, LEU86, PRO89, ASP93, GLY141, TRP292, MET294, LYS296	—	3.67
BIP	TYR288, LEU140, GLY141, GLY144, PRO89, MET138, LYS142, TRP292, ASN293	GLY144	3.65

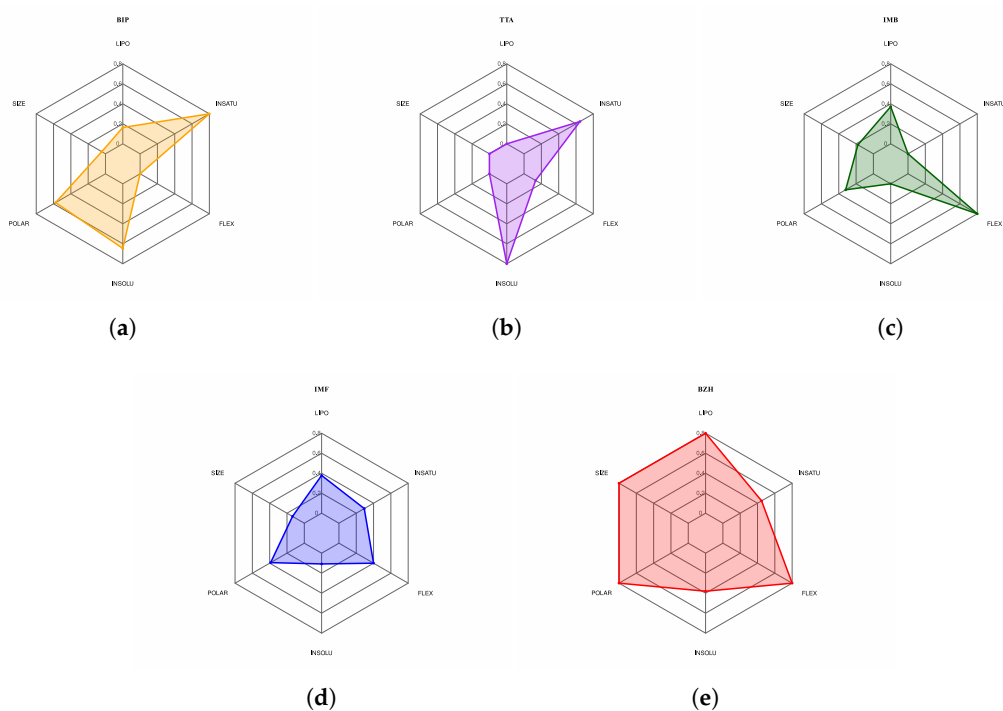
When we evaluated the pharmacokinetic properties (ADME) of BZH, IMF, IMB, TTA, and BIP using the ADMETlab 3.0 server [36]. All ligands, except IMB and BZH, complied with Lipinski's rule, while none of them complied with Veber's rule, which could affect their flexibility and oral bioavailability. Even so, all of them presented acceptable molecular weight values (MW < 650 Da), logP between 2.1 and 5.0, and TPSA < 110 Å², which favored their absorption. Regarding the blood–brain barrier (BBB), IMB and BIP showed brain permeation capacity, while BZH, IMF, and TTA did not cross it (see Table 4).

Table 4. Pharmacokinetic parameter evaluation (ADME) of CK1 δ ligands.

Ligand	MW	HBD	HBA	LogP	TPSA	RB	Lipinski	Veber	HIA	BBB
BIP	477.16	3	9	3.384	99.58	5	Accepted	—	—	+++
TTA	468.07	2	6	2.113	76.02	6	Accepted	—	—	—
IMB	506.18	2	7	5.006	89.13	11	Rejected	—	—	—
IMF	497.03	3	7	5.054	91.93	8	Accepted	—	—	++
BZH	638.28	3	7	8.339	108.75	11	Rejected	—	—	—

MW: molecular weight in Da; HBD: hydrogen bond donors; HBA: hydrogen bond acceptors; TPSA: topological polar surface area; RB: rotatable bonds; HIA: human intestinal absorption; BBB: blood–brain barrier permeability.

We conduct a radar plot analysis, which reveals contrasting physicochemical profiles among the evaluated compounds. In Figure 28a, BIP displays high unsaturation (≈ 0.8) and moderate levels of polarity and insolubility, but low lipophilicity, size, and flexibility, suggesting a small and specific molecule, albeit with potential permeability limitations. In Figure 28b, TTA stands out for its high unsaturation (≈ 0.75) and insolubility (≈ 0.8), but with low lipophilicity, polarity, size, and flexibility values, indicating an unfavorable profile for oral bioavailability. In Figure 28c, IMB displays high flexibility (≈ 0.85) and moderate lipophilicity (≈ 0.55), accompanied by medium polarity and size, which may favor permeability, although its low unsaturation and insolubility limit its specificity. In Figure 28d, IMF shows a balanced profile with intermediate values in all properties, which positions it as one of the most promising candidates. In contrast, in Figure 28e, BZH exhibits extreme values in size, lipophilicity, polarity, and flexibility (all ≥ 0.9), with low insolubility (≈ 0.4), which configures an unfavorable profile for oral absorption. Finally, in Figure 28e, it is observed that IMF and BIP are the compounds with the most balanced profiles and suitable for bioavailability, while BZH, IMB, and TTA present properties that could compromise their pharmacological development.

**Figure 28.** Oral bioavailability radar plots. The plots display six key properties: lipophilicity, size, polarity, solubility, flexibility, and saturation. (a) BIP. (b) TTA. (c) IMB. (d) IMF. (e) BZH.

We evaluated bioavailability properties using the cooked egg model. We located BIP, IMF, IMB, and TTA in the yolk; however, given their characteristics, only BIP and IMF could

cross the blood–brain barrier (BBB). In contrast, BZH, outside the egg, presents unfavorable physicochemical properties for both absorption and brain penetration. We classified BIP, IMF, and BZH as P-gp substrates, which favors their intracerebral permanence. From our analysis, we found that TTA and IMB compounds are actively excreted from the central nervous system (Figure 29).

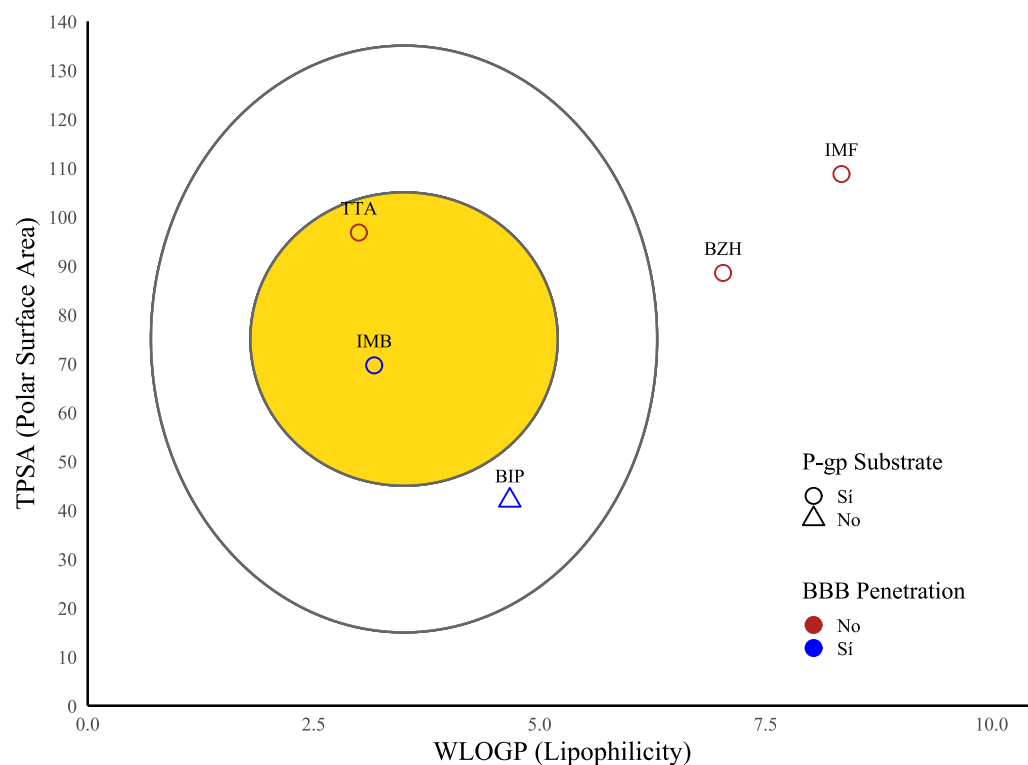


Figure 29. Boiled egg plot showing the lipophilicity (LogP) vs. the topological polar surface area (TPSA) of BIP, TTA, IMB, IMF, and BZH.

Since safety is an essential requirement for drug development, the toxicity of BIP, TTA, IMB, IMF, and BZH compounds was assessed using the ADMETlab 3.0 platform [36]. According to the standard classification, all compounds were assigned to acute oral toxicity class IV, indicating moderate toxicity (LD_{50} between 300 and 2000 mg/kg). IMB and BZH presented the highest probabilities of hepatotoxicity (0.849 and 0.831, respectively), whereas all compounds showed low carcinogenicity (<0.06) and a moderate risk of neurotoxicity, with BIP being the highest in the latter aspect (0.271). Taken together, the results suggest that BIP and IMF possess a more favorable toxicological profile, whereas IMB and BZH might require further experimental validation to confirm their safety (Table 5).

Table 5. Toxicity assessment results of BIP, TTA, IMB, IMF, and BZH analyzed using ADMETlab 3.0.

Compound	Hepatotoxicity	Carcinogenicity	Neurotoxicity	LD_{50} (Oral, Rat, mol/kg)	Toxicity Class
BIP	0.768	0.040	0.271	0.503	IV
TTA	0.715	0.052	0.251	0.550	IV
IMB	0.849	0.032	0.212	0.497	IV
IMF	0.812	0.037	0.242	0.512	IV
BZH	0.831	0.039	0.262	0.567	IV

All toxicity predictions are based on in silico models. LD_{50} values are given in mol/kg. Toxicity classes follow the globally harmonized system (GHS).

From a pharmacokinetic perspective, IMF and BIP stand out as the most promising candidates, showing good oral absorption and the ability to cross the blood–brain barrier (BBB), as predicted by ADMETlab 3.0 [37]. Both comply with Lipinski’s rule [38] and exhibit a balanced physicochemical profile in terms of lipophilicity, size, polarity, and flexibility, as shown in Figure 28. In contrast, IMB and BZH do not meet key bioavailability criteria, showing properties that could limit their efficacy, such as low brain permeability or high molecular size. In terms of toxicity, all compounds were in class IV (LD_{50} between 300 and 2000 mg/kg), indicating a moderate risk. However, the IMF and BIP showed lower probabilities of causing liver or neurological damage. Overall, IMF emerges as the most balanced candidate for future applications in the central nervous system, followed by BIP.

We compared the experimental inhibitory activity (IC_{50}) against CK1 δ of the 26 compounds evaluated, which were compiled based on the available literature data. We summarized in Table 6 the binding free energy and IC_{50} values compounds, we observed an inverse relationship between binding free energy and IC_{50} ; with more favorable energies (≤ -36 kcal/mol), such as BZH (-46.53 kcal/mol, $IC_{50} \approx 39.7$ μ M), IMF (-36.02 kcal/mol, $IC_{50} = 0.085$ μ M), and IMB (-32.99 kcal/mol, $IC_{50} = 0.004$ μ M), showed higher inhibitory potency. In contrast, molecules such as AMQ (-7.03 kcal/mol) and HYA (-19.63 kcal/mol) presented IC_{50} higher than 0.3 μ M, suggesting a lower functional affinity. It is consistent with that reported by Walton et al. [39], who identified compounds capable of inhibiting CK1 δ with very low IC_{50} concentrations.

Table 6. Binding free energies and IC_{50} values of selected compounds against CK1 δ .

Compound	Binding Energy (kcal/mol)	IC_{50} (μ M)	Reference
BZH	-46.53 ± 1.94	≈ 39.7000	[40]
IMF	-36.02 ± 1.92	0.0850	[41]
IMB	-32.99 ± 3.14	0.0040	[42]
TTA	-32.85 ± 1.75	0.9300	[43]
BIP	-31.44 ± 0.99	0.0040	[44]
BZC	-31.18 ± 0.83	≈ 34.0000	[40]
IMT	-30.43 ± 2.33	0.0029	[41,45]
IMD	-30.11 ± 0.75	0.3000	[44–46]
BIM	-29.63 ± 1.51	0.0440	[44,47]
PMP	-28.89 ± 0.55	0.0005–0.0200	[48]
PYP	-28.61 ± 0.75	0.0039	[49,50]
LAX	-28.51 ± 2.16	0.8000	[51]
LAM	-28.27 ± 1.63	0.4100	[51]
BIT	-28.16 ± 1.43	0.0440	[52]
BIC	-27.19 ± 1.19	2.3200	[53]
PYR	-26.95 ± 0.42	0.7000	[39,54]
PYI	-24.42 ± 0.90	0.0130	[39,44,55–58]
AMI	-24.22 ± 1.54	0.7000	[59]
BZF	-23.86 ± 1.30	47.0000	[60–62]
BZT	-23.79 ± 0.97	0.0230	[22,60,61,63]
BZM	-23.19 ± 1.06	0.2900	[64]
DXB	-20.16 ± 3.45	0.0200	[65]
HYA	-19.63 ± 0.71	0.0300	[66–69]
AHQ	-18.57 ± 1.41	0.6600	[70]
DXM	-15.09 ± 2.64	0.0700	[65]
AMQ	-7.03 ± 0.71	0.3300	[64]

Binding energies were computed from molecular dynamics simulation. IC_{50} values were obtained from experimental sources.

Finally, it is essential to mention that, while static analysis with CavityPlus [71] identified pocket 2 as the most druggable (DrugScore = 1058), most ligands dynamically migrated

towards pocket 6 during the simulation. We found that Bakan et al. [72] described this behavior by showing that structural flexibility allows some proteins to activate initially inactive regions. Furthermore, as reported by Córdova-Bahena et al. [34], docking can suggest an initial binding site, but only with molecular dynamics can it be confirmed whether the complex was truly stable and if that pocket was functional under dynamic conditions. These findings highlight the importance of complementing docking with molecular dynamics, since some regions only adopt a suitable shape to bind inhibitors when the protein is in motion. We presented results from the investigation that demonstrate the energetic efficiency of the CK1 δ –ligand interaction and should not necessarily align with QSAR models as presented in ADMET.

3. Computational Methods

We downloaded the initial three-dimensional conformation of Cyclin-Dependent Kinase 1 delta (CK1 δ) from the Protein Data Bank (PDB) (<http://www.rcsb.org>) [73,74], using the PDB ID: 6RCG [24]. We cleaned the molecule by removing all crystallographic water molecules. Missing fragments in the protein chain were reconstructed through homology modeling using MODELLER [26], integrated into the UCSF Chimera v1.18 interface [25]. The complete structure, free of artifacts and ready for subsequent simulations, was named “CK1 δ ”.

We selected the ligands studied through a literature review (Figure 3). Of the compounds selected for their occurrence in the literature regarding interaction with CK1 δ , only one (AMI) is of natural origin; the rest are synthetic compounds in a preclinical research stage. For those whose structures were not available in databases such as PubChem [75] or DrugBank [76], we used the DECIMER server, a tool based on artificial intelligence for chemical structure recognition, to extract SMILES notation from graphical representations [77]. We verified the SMILES strings obtained and converted them into .sdf format using RDKit [78] in Python [79]; similar to the protein, we assigned each ligand a unique ID (Table 1). We performed Ligand parameterization and generation of molecular formats required for simulation locally using MglTools [80], AmberTools [81], and LigParGen [82], the latter applying the OPLS force field [83] to assign protein–ligand interaction parameters after docking.

Virtual screening of the ligands was performed on the known active site of the CK1 δ protein using UNI-DOCK software, version 1.1 [28]. We configured the docking protocol to run 2500 iterations, generating 30 conformers per iteration for each ligand. We selected the conformers with the most favorable interaction energies for each ligand for further analysis.

We refined the atomic charges initially assigned by LigParGen [82] after docking for protein–ligand simulations. Subsequently, we calculated Hirshfeld charges using the CAM-B3LYP functional [84] in Gaussian 16 [85].

The structure of the CK1 δ protein, as well as the protein–ligand complexes, was optimized and equilibrated through molecular dynamics simulations using GROMACS software, version 2024.5 [86,87]. We centered the protein system in a cubic geometry simulation box. We subsequently solvated the box with TIP3P water molecules [71]. To ensure the electrical neutrality of the system, chlorine (Cl[−]) and sodium (Na⁺) counterions were added. We initially minimized the system to resolve potential steric conflicts and reduce interatomic forces. Subsequently, we carried out two equilibrium stages: a simulation in the NVT (constant number of particles, volume, temperature) ensemble at 309.65 K for 10 ns, followed by a simulation in the NPT (constant number of particles, pressure, temperature) ensemble at a pressure of 1 bar for 10 ns, ending with a molecular dynamics simulation (SDM) of 500 ns, we performed for the protein structure and the complexes the same procedure, varying only the SDM time to 100 ns for complexes.

Once we optimized the protein structure, we performed a preliminary analysis to identify and characterize potential ligand binding sites, focusing on pockets with a high probability of being pharmacologically relevant or interacting with ligands. This process was performed on the protein structure alone and post-interaction to verify possible changes in the pockets and interaction sites. We identified potential pockets and binding sites using CavityPlus [27].

The protein–ligand complexes obtained from the virtual screening were subjected to production molecular dynamics simulations using GROMACS software, version 2024.5 [86,87], following the optimization and equilibration steps described previously. We performed a 100 ns simulation in an isothermal-isobaric system.

We analyzed trajectories obtained from molecular dynamics simulations to assess the stability and dynamic behavior of the complexes. Metrics such as root mean square deviation (RMSD) [88], root mean square fluctuation (RMSF) [89], radius of gyration (Rg), and number of hydrogen bonds (HBs) were calculated, as well as the Solvent Accessible Surface Area (SASA) [86,86]. Additionally, we calculated the binding free energy of the complexes using the molecular mechanics Poisson–Boltzmann surface area (MMPBSA) method [90,91].

We identified specific interactions such as hydrogen bonds, π – π interactions, and hydrophobic contacts between ligands and key residues. We also assessed changes in surface electrostatic charge distribution using APBS [92] and ChimeraX visualization [37].

We predicted the pharmacokinetic properties and ADMET parameters of the candidate ligands using ADMETlab 3.0 [36]. We analyzed key descriptors such as molecular weight, logP, TPSA, HBD, HBA, and rotatable bonds to assess drug-likeness based on Lipinski's and Veber's rules. The boiled egg model was used to predict gastrointestinal absorption, blood–brain barrier permeability, and P-glycoprotein interactions. Additionally, *in silico* toxicity assessments included hepatotoxicity, neurotoxicity, carcinogenicity, and acute oral toxicity (LD₅₀).

We assessed the stereochemical quality of the structures using Ramachandran diagrams generated with Ramachandraw [93]. We calculated electrostatic potentials with APBS [92]. We performed 3D visualization and structural manipulation with ChimeraX [37] and RDKit [78]. We used Open Babel for chemical structure processing, and we plotted numerical results with Gnuplot [94].

4. Conclusions

In this study, we identified BZH as the most stable inhibitor of the CK1 δ protein, following a combined docking, molecular dynamics, and free energy analysis (MMPBSA), highlighting its interaction with key catalytic residues. We found that the compounds with the best binding typically have lower IC₅₀, confirming the usefulness of the computational approach in predicting efficacy. IMF and BIP stand out as the most promising candidates due to their good absorption and ability to reach the brain, unlike others, such as IMB, which, although potent, may not be effective in the body. Finally, we demonstrated that molecular dynamics simulation allows the identification of functional regions not visible in static analyses, such as pocket 6, highlighting the importance of complementing docking with dynamic simulations for a more realistic and accurate prediction of ligand–protein behavior.

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References

1. Van Schependom, J.; D'haeseleer, M. Advances in Neurodegenerative Diseases. *J. Clin. Med.* **2023**, *12*, 1709. [\[CrossRef\]](#)
2. Ryan, M.; Heverin, M.; McLaughlin, R.L.; Hardiman, O. Lifetime Risk and Heritability of Amyotrophic Lateral Sclerosis. *JAMA Neurol.* **2019**, *76*, 1367. [\[CrossRef\]](#)
3. Al-Chalabi, A.; van den Berg, L.H.; Veldink, J. Gene discovery in amyotrophic lateral sclerosis: Implications for clinical management. *Nat. Rev. Neurol.* **2016**, *13*, 96–104. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Xu, X.; Shen, D.; Gao, Y.; Zhou, Q.; Ni, Y.; Meng, H.; Shi, H.; Le, W.; Chen, S.; Chen, S. A perspective on therapies for amyotrophic lateral sclerosis: Can disease progression be curbed? *Transl. Neurodegener.* **2021**, *10*, 29. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Mehta, P.; Kaye, W.; Bryan, L.; Larson, T.; Copeland, T.; Wu, J.; Muravov, O.; Horton, K. Prevalence of Amyotrophic Lateral Sclerosis - United States, 2012–2013. *Morb. Mortal. Wkly. Rep. Surveill. Summ.* **2016**, *65*, 1–12. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Chen, S.; Sayana, P.; Zhang, X.; Le, W. Genetics of amyotrophic lateral sclerosis: An update. *Mol. Neurodegener.* **2013**, *8*, 28. [\[CrossRef\]](#)
7. Ravits, J.M.; La Spada, A.R. ALS motor phenotype heterogeneity, focal, and spread: Deconstructing motor neuron degeneration. *Neurology* **2009**, *73*, 805–811. [\[CrossRef\]](#)
8. Feldman, E.L.; Goutman, S.A.; Petri, S.; Mazzini, L.; Savelieff, M.G.; Shaw, P.J.; Sobue, G. Amyotrophic lateral sclerosis. *Lancet* **2022**, *400*, 1363–1380. [\[CrossRef\]](#)
9. Marin, B.; Boumédiene, F.; Logrosino, G.; Couratier, P.; Babron, M.C.; Leutenegger, A.L.; Copetti, M.; Preux, P.M.; Beghi, E. Variation in worldwide incidence of amyotrophic lateral sclerosis: A meta-analysis. *Int. J. Epidemiol.* **2016**, *46*, 57–74. [\[CrossRef\]](#)
10. Hoxhaj, P.; Hastings, N.; Kachhadia, M.P.; Gupta, R.; Sindhu, U.; Durve, S.A.; Azam, A.; Auz Vinuesa, M.J.; Bhuvan; Win, S.H.; et al. Exploring Advancements in the Treatment of Amyotrophic Lateral Sclerosis: A Comprehensive Review of Current Modalities and Future Prospects. *Cureus* **2023**, *15*, e45489. [\[CrossRef\]](#)
11. Czaplinski, A.; Yen, A.A.; Simpson, E.P.; Appel, S.H. Slower Disease Progression and Prolonged Survival in Contemporary Patients With Amyotrophic Lateral Sclerosis: Is the Natural History of Amyotrophic Lateral Sclerosis Changing? *Arch. Neurol.* **2006**, *63*, 1139. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Brotman, R.G.; Moreno-Escobar, M.C.; Joseph, J.; Munakomi, S.; Pawar, G. *Amyotrophic Lateral Sclerosis*; StatPearls: Treasure Island, FL, USA, 2025.
13. Garcia-Montojo, M.; Fathi, S.; Rastegar, C.; Simula, E.R.; Doucet-O'Hare, T.; Cheng, Y.H.H.; Abrams, R.P.M.; Pasternack, N.; Malik, N.; Bachani, M.; et al. TDP-43 proteinopathy in ALS is triggered by loss of ASRGL1 and associated with HML-2 expression. *Nat. Commun.* **2024**, *15*, 4163. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Droppelmann, C.A.; Campos-Melo, D.; Noches, V.; McLellan, C.; Szabla, R.; Lyons, T.A.; Amzil, H.; Withers, B.; Kaplanis, B.; Sonkar, K.S.; et al. Mitigation of TDP-43 toxic phenotype by an RGNEF fragment in amyotrophic lateral sclerosis models. *Brain* **2024**, *147*, 2053–2068. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Oiwa, K.; Watanabe, S.; Onodera, K.; Iguchi, Y.; Kinoshita, Y.; Komine, O.; Sobue, A.; Okada, Y.; Katsuno, M.; Yamanaka, K. Monomerization of TDP-43 is a key determinant for inducing TDP-43 pathology in amyotrophic lateral sclerosis. *Sci. Adv.* **2023**, *9*, eadf6895. [\[CrossRef\]](#)
16. Jo, M.; Lee, S.; Jeon, Y.M.; Kim, S.; Kwon, Y.; Kim, H.J. The role of TDP-43 propagation in neurodegenerative diseases: Integrating insights from clinical and experimental studies. *Exp. Mol. Med.* **2020**, *52*, 1652–1662. [\[CrossRef\]](#)
17. Wu, C.C.; Jin, L.W.; Wang, I.F.; Wei, W.Y.; Ho, P.C.; Liu, Y.C.; Tsai, K.J. HDAC1 dysregulation induces aberrant cell cycle and DNA damage in progress of TDP-43 proteinopathies. *EMBO Mol. Med.* **2020**, *12*, e10622. [\[CrossRef\]](#)

18. Ko, V.I.; Ong, K.; Cleveland, D.W.; Yu, H.; Ravits, J.M. CK1 δ/ϵ kinases regulate TDP-43 phosphorylation and are therapeutic targets for ALS-related TDP-43 hyperphosphorylation. *Neurobiol. Dis.* **2024**, *196*, 106516. [\[CrossRef\]](#)
19. Choksi, D.K.; Roy, B.; Chatterjee, S.; Yusuff, T.; Bakhoun, M.F.; Sengupta, U.; Ambegaokar, S.; Kayed, R.; Jackson, G.R. TDP-43 Phosphorylation by casein kinase I ϵ promotes oligomerization and enhances toxicity in vivo. *Hum. Mol. Genet.* **2013**, *23*, 1025–1035. [\[CrossRef\]](#)
20. Hicks, D.A.; Cross, L.L.; Williamson, R.; Rattray, M. Endoplasmic Reticulum Stress Signalling Induces Casein Kinase 1-Dependent Formation of Cytosolic TDP-43 Inclusions in Motor Neuron-Like Cells. *Neurochem. Res.* **2019**, *45*, 1354–1364. [\[CrossRef\]](#)
21. Liachko, N.F.; Guthrie, C.R.; Kraemer, B.C. Phosphorylation Promotes Neurotoxicity in aCaenorhabditis elegansModel of TDP-43 Proteinopathy. *J. Neurosci.* **2010**, *30*, 16208–16219. [\[CrossRef\]](#)
22. Martínez-González, L.; Rodríguez-Cueto, C.; Cabezudo, D.; Bartolomé, F.; Andrés-Benito, P.; Ferrer, I.; Gil, C.; Martín-Requero, Á.; Fernández-Ruiz, J.; Martínez, A.; et al. Motor neuron preservation and decrease of in vivo TDP-43 phosphorylation by protein CK-1 δ kinase inhibitor treatment. *Sci. Rep.* **2020**, *10*, 4449. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Akharume, F.; Adediji, A. Molecular dynamic (in silico) modeling of structure-function of glutelin type-B 5-like from proso millet storage protein: Effects of temperature and pressure. *J. Food Sci. Technol.* **2022**, *60*, 114–122. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Chaikuad, A.; Arrowsmith, C.; Edwards, A.; Bountra, C.; Roush, W.; Knapp, S. Crystal Structure of Casein Kinase 1 Delta (CK1 Delta) Complexed with SR3029 Inhibitor: 6rcg. 2019. Available online: <https://www.rcsb.org/structure/6RCC> (accessed on 15 October 2025).
25. Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera—A visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, *25*, 1605–1612. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Webb, B.; Sali, A. Comparative Protein Structure Modeling Using MODELLER. *Curr. Protoc. Bioinform.* **2016**, *54*, 5.6.1–5.6.37. [\[CrossRef\]](#)
27. Xu, Y.; Wang, S.; Hu, Q.; Gao, S.; Ma, X.; Zhang, W.; Shen, Y.; Chen, F.; Lai, L.; Pei, J. CavityPlus: A web server for protein cavity detection with pharmacophore modelling, allosteric site identification and covalent ligand binding ability prediction. *Nucleic Acids Res.* **2018**, *46*, W374–W379. [\[CrossRef\]](#)
28. Yu, Y.; Cai, C.; Wang, J.; Bo, Z.; Zhu, Z.; Zheng, H. Uni-Dock: GPU-Accelerated Docking Enables Ultralarge Virtual Screening. *J. Chem. Theory Comput.* **2023**, *19*, 3336–3345. [\[CrossRef\]](#)
29. Gohlke, H.; Klebe, G. Approaches to the description and prediction of the binding affinity of small-molecule ligands to macromolecular receptors. *Angew. Chem. (Int. Ed. Engl.)* **2002**, *41*, 2644–2676. [\[CrossRef\]](#)
30. Hou, T.; Wang, J.; Li, Y.; Wang, W. Assessing the Performance of the MM/PBSA and MM/GBSA Methods. 1. The Accuracy of Binding Free Energy Calculations Based on Molecular Dynamics Simulations. *J. Chem. Inf. Model.* **2010**, *51*, 69–82. [\[CrossRef\]](#)
31. Kollman, P.A.; Massova, I.; Reyes, C.; Kuhn, B.; Huo, S.; Chong, L.; Lee, M.; Lee, T.; Duan, Y.; Wang, W.; et al. Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models. *Accounts Chem. Res.* **2000**, *33*, 889–897. [\[CrossRef\]](#)
32. Knippschild, U.; Krüger, M.; Richter, J.; Xu, P.; García-Reyes, B.; Peifer, C.; Halekotte, J.; Bakulev, V.; Bischof, J. The CK1 Family: Contribution to Cellular Stress Response and Its Role in Carcinogenesis. *Front. Oncol.* **2014**, *4*, 96. [\[CrossRef\]](#)
33. Long, A.; Zhao, H.; Huang, X. Structural Basis for the Interaction between Casein Kinase 1 Delta and a Potent and Selective Inhibitor. *J. Med. Chem.* **2012**, *55*, 956–960. [\[CrossRef\]](#)
34. Córdova-Bahena, L.; Sánchez-Álvarez, A.A.; Ruiz-Moreno, A.J.; Velasco-Velázquez, M.A. Repositioning of Etravirine as a Potential CK1 ϵ Inhibitor by Virtual Screening. *Pharmaceuticals* **2021**, *15*, 8. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Sunkari, Y.K.; Meijer, L.; Flajolet, M. The protein kinase CK1: Inhibition, activation, and possible allosteric modulation. *Front. Mol. Biosci.* **2022**, *9*, 916232. [\[CrossRef\]](#)
36. Fu, L.; Shi, S.; Yi, J.; Wang, N.; He, Y.; Wu, Z.; Peng, J.; Deng, Y.; Wang, W.; Wu, C.; et al. ADMETlab 3.0: An updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. *Nucleic Acids Res.* **2024**, *52*, W422–W431. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Meng, E.C.; Goddard, T.D.; Pettersen, E.F.; Couch, G.S.; Pearson, Z.J.; Morris, J.H.; Ferrin, T.E. UCSF ChimeraX: Tools for structure building and analysis. *Protein Sci.* **2023**, *32*, e4792. [\[CrossRef\]](#)
38. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3–26. [\[CrossRef\]](#)
39. Walton, K.M.; Fisher, K.; Rubitski, D.; Marconi, M.; Meng, Q.J.; Sládek, M.; Adams, J.; Bass, M.; Chandrasekaran, R.; Butler, T.; et al. Selective Inhibition of Casein Kinase 1 ϵ Minimally Alters Circadian Clock Period. *J. Pharmacol. Exp. Ther.* **2009**, *330*, 430–439. [\[CrossRef\]](#)
40. Joshi, K.; Goyal, S.; Grover, S.; Jamal, S.; Singh, A.; Dhar, P.; Grover, A. Novel group-based QSAR and combinatorial design of CK-1 δ inhibitors as neuroprotective agents. *BMC Bioinform.* **2016**, *17*, 515. [\[CrossRef\]](#)

41. Bischof, J.; Leban, J.; Zaja, M.; Grothey, A.; Radunsky, B.; Othersen, O.; Strobl, S.; Vitt, D.; Knippschild, U. 2-Benzamido-N-(1H-benzo[d]imidazol-2-yl)thiazole-4-carboxamide derivatives as potent inhibitors of CK1 δ/ϵ . *Amino Acids* **2012**, *43*, 1577–1591. [[CrossRef](#)]
42. Halekotte, J.; Witt, L.; Ianes, C.; Krüger, M.; Bührmann, M.; Rauh, D.; Pichlo, C.; Brunstein, E.; Luxemburger, A.; Baumann, U.; et al. Optimized 4,5-Diarylimidazoles as Potent/Selective Inhibitors of Protein Kinase CK1 δ and Their Structural Relation to p38 α MAPK. *Molecules* **2017**, *22*, 522. [[CrossRef](#)]
43. García-Reyes, B.; Witt, L.; Jansen, B.; Karasu, E.; Gehring, T.; Leban, J.; Henne-Bruns, D.; Pichlo, C.; Brunstein, E.; Baumann, U.; et al. Discovery of Inhibitor of Wnt Production 2 (IWP-2) and Related Compounds As Selective ATP-Competitive Inhibitors of Casein Kinase 1 (CK1) δ/ϵ . *J. Med. Chem.* **2018**, *61*, 4087–4102. [[CrossRef](#)]
44. Bibian, M.; Rahaim, R.J.; Choi, J.Y.; Noguchi, Y.; Schürer, S.; Chen, W.; Nakanishi, S.; Licht, K.; Rosenberg, L.H.; Li, L.; et al. Development of highly selective casein kinase 1 $\delta/1\epsilon$ (CK1 δ/ϵ) inhibitors with potent antiproliferative properties. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4374–4380. [[CrossRef](#)]
45. Rena, G.; Bain, J.; Elliott, M.; Cohen, P. D4476, a cell-permeant inhibitor of CK1, suppresses the site-specific phosphorylation and nuclear exclusion of FOXO1a. *EMBO Rep.* **2004**, *5*, 60–65. [[CrossRef](#)]
46. Liu, G.; Li, H.; Zhang, W.; Yu, J.; Zhang, X.; Wu, R.; Niu, M.; Liu, X.; Yu, R. Csnk1a1 inhibition modulates the inflammatory secretome and enhances response to radiotherapy in glioma. *J. Cell. Mol. Med.* **2021**, *25*, 7395–7406. Erratum in *J. Cell. Mol. Med.* **2022**, *26*, 2754–2755. [[CrossRef](#)] [[PubMed](#)]
47. Su, Z.; Song, J.; Wang, Z.; Zhou, L.; Xia, Y.; Yu, S.; Sun, Q.; Liu, S.S.; Zhao, L.; Li, S.; et al. Tumor promoter TPA activates Wnt/ β -catenin signaling in a casein kinase 1-dependent manner. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E7522–E7531. [[CrossRef](#)] [[PubMed](#)]
48. Ball, B.J.; Stein, A.S.; Borthakur, G.; Murray, C.; Kook, K.; Chan, K.W.; Stein, E.M. Trial in Progress: A Phase I Trial of BTX-A51 in Patients with Relapsed or Refractory AML or High-Risk MDS. *Blood* **2020**, *136*, 18–19. [[CrossRef](#)]
49. Cunningham, P.S.; Ahern, S.A.; Smith, L.C.; da Silva Santos, C.S.; Wager, T.T.; Bechtold, D.A. Targeting of the circadian clock via CK1 δ/ϵ to improve glucose homeostasis in obesity. *Sci. Rep.* **2016**, *6*, 29983. [[CrossRef](#)]
50. Wager, T.T.; Chandrasekaran, R.Y.; Bradley, J.; Rubitski, D.; Berke, H.; Mente, S.; Butler, T.; Doran, A.; Chang, C.; Fisher, K.; et al. Casein Kinase 1 δ/ϵ Inhibitor PF-5006739 Attenuates Opioid Drug-Seeking Behavior. *ACS Chem. Neurosci.* **2014**, *5*, 1253–1265. [[CrossRef](#)]
51. Baunbæk, D.; Trinkler, N.; Ferandin, Y.; Lozach, O.; Ploypradith, P.; Rucirawat, S.; Ishibashi, F.; Iwao, M.; Meijer, L. Anticancer Alkaloid Lamellarins Inhibit Protein Kinases. *Mar. Drugs* **2008**, *6*, 514–527. [[CrossRef](#)]
52. Monastyrskyi, A.; Nilchan, N.; Quereda, V.; Noguchi, Y.; Ruiz, C.; Grant, W.; Cameron, M.; Duckett, D.; Roush, W. Development of dual casein kinase 1 $\delta/1\epsilon$ (CK1 δ/ϵ) inhibitors for treatment of breast cancer. *Bioorg. Med. Chem.* **2018**, *26*, 590–602. [[CrossRef](#)]
53. Hua, Z.; Huang, X.; Bregman, H.; Chakka, N.; DiMauro, E.F.; Doherty, E.M.; Goldstein, J.; Gunaydin, H.; Huang, H.; Mercede, S.; et al. 2-Phenylamino-6-cyano-1H-benzimidazole-based isoform selective casein kinase 1 gamma (CK1 γ) inhibitors. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5392–5395. [[CrossRef](#)]
54. Meng, Q.J.; Maywood, E.S.; Bechtold, D.A.; Lu, W.Q.; Li, J.; Gibbs, J.E.; Dupré, S.M.; Chesham, J.E.; Rajamohan, F.; Knafels, J.; et al. Entrainment of disrupted circadian behavior through inhibition of casein kinase 1 (CK1) enzymes. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 15240–15245. [[CrossRef](#)]
55. Adler, P.; Mayne, J.; Walker, K.; Ning, Z.; Figeys, D. Therapeutic Targeting of Casein Kinase 1 δ/ϵ in an Alzheimer's Disease Mouse Model. *J. Proteome Res.* **2019**, *18*, 3383–3393. [[CrossRef](#)] [[PubMed](#)]
56. Badura, L.; Swanson, T.; Adamowicz, W.; Adams, J.; Cianfrogna, J.; Fisher, K.; Holland, J.; Kleiman, R.; Nelson, F.; Reynolds, L.; et al. An Inhibitor of Casein Kinase 1 ϵ Induces Phase Delays in Circadian Rhythms under Free-Running and Entrained Conditions. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 730–738. [[CrossRef](#)] [[PubMed](#)]
57. Cheong, J.K.; Hung, N.T.; Wang, H.; Tan, P.; Voorhoeve, P.M.; Lee, S.H.; Virshup, D.M. IC261 induces cell cycle arrest and apoptosis of human cancer cells via CK1 δ/ϵ and Wnt/ β -catenin independent inhibition of mitotic spindle formation. *Oncogene* **2011**, *30*, 2558–2569. [[CrossRef](#)] [[PubMed](#)]
58. Sundaram, S.; Nagaraj, S.; Mahoney, H.; Portugues, A.; Li, W.; Millsaps, K.; Faulkner, J.; Yunus, A.; Burns, C.; Bloom, C.; et al. Inhibition of casein kinase 1 δ/ϵ improves cognitive-affective behavior and reduces amyloid load in the APP-PS1 mouse model of Alzheimer's disease. *Sci. Rep.* **2019**, *9*, 13743. [[CrossRef](#)]
59. Saito, A.N.; Matsuo, H.; Kuwata, K.; Ono, A.; Kinoshita, T.; Yamaguchi, J.; Nakamichi, N. Structure-function study of a novel inhibitor of the casein kinase 1 family in *Arabidopsis thaliana*. *Plant Direct* **2019**, *3*, e00172. [[CrossRef](#)]
60. Alquezar, C.; Salado, I.G.; de la Encarnación, A.; Pérez, D.I.; Moreno, F.; Gil, C.; de Munain, A.L.; Martínez, A.; Martín-Requero, Á. Targeting TDP-43 phosphorylation by Casein Kinase-1 δ inhibitors: A novel strategy for the treatment of frontotemporal dementia. *Mol. Neurodegener.* **2016**, *11*, 36. [[CrossRef](#)]

61. Morales-Garcia, J.A.; Salado, I.G.; Sanz-San Cristobal, M.; Gil, C.; Pérez-Castillo, A.; Martínez, A.; Pérez, D.I. Biological and Pharmacological Characterization of Benzothiazole-Based CK-1 δ Inhibitors in Models of Parkinson's Disease. *ACS Omega* **2017**, *2*, 5215–5220. [CrossRef]
62. Posa, D.; Martínez-González, L.; Bartolomé, F.; Nagaraj, S.; Porras, G.; Martínez, A.; Martín-Requero, Á. Recapitulation of Pathological TDP-43 Features in Immortalized Lymphocytes from Sporadic ALS Patients. *Mol. Neurobiol.* **2018**, *56*, 2424–2432. [CrossRef]
63. Martinez-Gonzalez, L.; Cuevas, E.P.; Tosat-Bitrián, C.; Nozal, V.; Gil, C.; Palomo, V.; Martín-Requero, Á.; Martinez, A. TTBK1 and CK1 inhibitors restore TDP-43 pathology and avoid disease propagation in lymphoblast from Alzheimer's disease patients. *Front. Mol. Neurosci.* **2023**, *16*, 1243277. [CrossRef]
64. Lee, J.W.; Hirota, T.; Peters, E.C.; Garcia, M.; Gonzalez, R.; Cho, C.Y.; Wu, X.; Schultz, P.G.; Kay, S.A. A Small Molecule Modulates Circadian Rhythms through Phosphorylation of the Period Protein. *Angew. Chem. Int. Ed.* **2011**, *50*, 10608–10611. [CrossRef] [PubMed]
65. Richter, J.; Bischof, J.; Zaja, M.; Kohlhof, H.; Othersen, O.; Vitt, D.; Alscher, V.; Pospiech, I.; García-Reyes, B.; Berg, S.; et al. Difluoro-dioxolo-benzimidazol-benzamides As Potent Inhibitors of CK1 δ and ϵ with Nanomolar Inhibitory Activity on Cancer Cell Proliferation. *J. Med. Chem.* **2014**, *57*, 7933–7946. [CrossRef] [PubMed]
66. Meijer, L.; Thunnissen, A.M.; White, A.; Garnier, M.; Nikolic, M.; Tsai, L.H.; Walter, J.; Cleverley, K.; Salinas, P.; Wu, Y.Z.; et al. Inhibition of cyclin-dependent kinases, GSK-3 β and CK1 by hymenialdisine, a marine sponge constituent. *Chem. Biol.* **2000**, *7*, 51–63. [CrossRef] [PubMed]
67. Plisson, F.; Prasad, P.; Xiao, X.; Piggott, A.M.; Huang, X.c.; Khalil, Z.; Capon, R.J. Callyspongisines A-D: Bromopyrrole alkaloids from an Australian marine sponge, *Callyspongia* sp. *Org. Biomol. Chem.* **2014**, *12*, 1579–1584. [CrossRef]
68. Wan, Y.; Hur, W.; Cho, C.Y.; Liu, Y.; Adrian, F.J.; Lozach, O.; Bach, S.; Mayer, T.; Fabbro, D.; Meijer, L.; et al. Synthesis and Target Identification of Hymenialdisine Analogs. *Chem. Biol.* **2004**, *11*, 247–259. [CrossRef]
69. Zhang, H.; Khalil, Z.; Conte, M.M.; Plisson, F.; Capon, R.J. A search for kinase inhibitors and antibacterial agents: Bromopyrrolo-2-aminoimidazoles from a deep-water Great Australian Bight sponge, *Axinella* sp. *Tetrahedron Lett.* **2012**, *53*, 3784–3787. [CrossRef]
70. Cozza, G.; Gianoncelli, A.; Montopoli, M.; Caparrotta, L.; Venerando, A.; Meggio, F.; Pinna, L.A.; Zagotto, G.; Moro, S. Identification of novel protein kinase CK1 delta (CK1 δ) inhibitors through structure-based virtual screening. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5672–5675. [CrossRef]
71. Aliev, A.E.; Kulke, M.; Khaneja, H.S.; Chudasama, V.; Sheppard, T.D.; Lanigan, R.M. Motional timescale predictions by molecular dynamics simulations: Case study using proline and hydroxyproline sidechain dynamics: Proline Force Field Parameters. *Proteins Struct. Funct. Bioinform.* **2013**, *82*, 195–215. [CrossRef]
72. Bakan, A.; Meireles, L.M.; Bahar, I. ProDy: Protein Dynamics Inferred from Theory and Experiments. *Bioinformatics* **2011**, *27*, 1575–1577. [CrossRef]
73. Burley, S.K.; Berman, H.M.; Bhikadiya, C.; Bi, C.; Chen, L.; Costanzo, L.D.; Christie, C.; Duarte, J.M.; Dutta, S.; Feng, Z.; et al. Protein Data Bank: The single global archive for 3D macromolecular structure data. *Nucleic Acids Res.* **2018**, *47*, D520–D528. [CrossRef]
74. Burley, S.K.; Berman, H.M.; Kleywegt, G.J.; Markley, J.L.; Nakamura, H.; Velankar, S. Protein Data Bank (PDB): The Single Global Macromolecular Structure Archive. In *Protein Crystallography*; Springer: New York, NY, USA, 2017; pp. 627–641. [CrossRef]
75. Kim, S.; Chen, J.; Cheng, T.; Gindulyte, A.; He, J.; He, S.; Li, Q.; Shoemaker, B.A.; Thiessen, P.A.; Yu, B.; et al. PubChem 2023 update. *Nucleic Acids Res.* **2022**, *51*, D1373–D1380. [CrossRef] [PubMed]
76. Wishart, D.S. DrugBank: A comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res.* **2006**, *34*, D668–D672. [CrossRef] [PubMed]
77. Rajan, K.; Zielesny, A.; Steinbeck, C. DECIMER: Towards deep learning for chemical image recognition. *J. Cheminform.* **2020**, *12*, 65. [CrossRef] [PubMed]
78. Landrum, G.; Tosco, P.; Kelley, B.; Rodriguez, R.; Cosgrove, D.; Vianello, R.; Sriniker; Gedeck, P.; Jones, G.; Kawashima, E.; et al. rdkit/rdkit: 2025_03_4 (Q1 2025) Release. 2025. Available online: <https://zenodo.org/records/15773589> (accessed on 15 October 2025).
79. Van Rossum, G.; Drake, F. *Python 3 Reference Manual: (Python Documentation Manual Part 2)*; Documentation for Python; CreateSpace Independent Publishing Platform: North Charleston, SC, USA, 2009.
80. Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30*, 2785–2791. [CrossRef]
81. Case, D.A.; Aktulga, H.M.; Belfon, K.; Cerutti, D.S.; Cisneros, G.A.; Cruzeiro, V.W.D.; Forouzesesh, N.; Giese, T.J.; Götz, A.W.; Gohlke, H.; et al. AmberTools. *J. Chem. Inf. Model.* **2023**, *63*, 6183–6191. [CrossRef]
82. Dodda, L.S.; Cabeza de Vaca, I.; Tirado-Rives, J.; Jorgensen, W.L. LigParGen web server: An automatic OPLS-AA parameter generator for organic ligands. *Nucleic Acids Res.* **2017**, *45*, W331–W336. [CrossRef]

83. Jorgensen, W.L.; Maxwell, D.S.; Tirado-Rives, J. Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids. *J. Am. Chem. Soc.* **1996**, *118*, 11225–11236. [[CrossRef](#)]
84. Yanai, T.; Tew, D.P.; Handy, N.C. A new hybrid exchange-correlation functional using the Coulomb-attenuating method (CAM-B3LYP). *Chem. Phys. Lett.* **2004**, *393*, 51–57. [[CrossRef](#)]
85. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Petersson, G.A.; Nakatsuji, H.; et al. *Gaussian~16 Revision C.01*; Gaussian Inc.: Wallingford, CT, USA, 2016.
86. Abraham, M.; Alekseenko, A.; Basov, V.; Bergh, C.; Briand, E.; Brown, A.; Doijade, M.; Fiorin, G.; Fleischmann, S.; Gorelov, S.; et al. GROMACS 2024.5 Manual. 2025. Available online: <https://manual.gromacs.org/documentation/2024.5/install-guide/index.html> (accessed on 15 October 2025).
87. Abraham, M.J.; Murtola, T.; Schulz, R.; Páll, S.; Smith, J.C.; Hess, B.; Lindahl, E. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **2015**, *1–2*, 19–25. [[CrossRef](#)]
88. Rooijers, K.; Kolmeder, C.; Juste, C.; Doré, J.; de Been, M.; Boeren, S.; Galan, P.; Beauvallet, C.; de Vos, W.M.; Schaap, P.J. An iterative workflow for mining the human intestinal metaproteome. *BMC Genom.* **2011**, *12*, 6. [[CrossRef](#)] [[PubMed](#)]
89. Sang, P.; Yang, Q.; Du, X.; Yang, N.; Yang, L.Q.; Ji, X.L.; Fu, Y.X.; Meng, Z.H.; Liu, S.Q. Effect of the Solvent Temperatures on Dynamics of Serine Protease Proteinase K. *Int. J. Mol. Sci.* **2016**, *17*, 254. [[CrossRef](#)] [[PubMed](#)]
90. Jurrus, E.; Engel, D.; Star, K.; Monson, K.; Brandi, J.; Felberg, L.E.; Brookes, D.H.; Wilson, L.; Chen, J.; Liles, K.; et al. Improvements to the APBS biomolecular solvation software suite. *Protein Sci.* **2017**, *27*, 112–128. [[CrossRef](#)]
91. Kumari, R.; Kumar, R.; Lynn, A. g_mmpbsa-A GROMACS Tool for High-Throughput MM-PBSA Calculations. *J. Chem. Inf. Model.* **2014**, *54*, 1951–1962. [[CrossRef](#)]
92. Berendsen, H.J.C.; Postma, J.P.M.; van Gunsteren, W.F.; Hermans, J. Interaction Models for Water in Relation to Protein Hydration. In *Intermolecular Forces*; Springer: Dordrecht, The Netherlands, 1981; pp. 331–342. [[CrossRef](#)]
93. Hooft, R.W.; Sander, C.; Vriend, G. Objectively judging the quality of a protein structure from a Ramachandran plot. *Bioinformatics* **1997**, *13*, 425–430. [[CrossRef](#)]
94. O’Boyle, N.M.; Banck, M.; James, C.A.; Morley, C.; Vandermeersch, T.; Hutchison, G.R. Open Babel: An open chemical toolbox. *J. Cheminform.* **2011**, *3*, 33. [[CrossRef](#)] [[PubMed](#)]

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