Analysis of an Insilico Interaction by a Curcumin Derivative specifically Only with the AKT1 Molecule but Not AKT2

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Abstract

Akt1 is an important macromolecule expressed excessively in many cancers. Inhibition of Akt1 by natural compounds is always advantageous for there are fewer side effects. It is well known that curcumin modifies the behavior of AKT1/AKT2/AKT3 but a natural ligand or its derivative that interacts specifically with AKT1 is one, that will be of tremendous interest in the world of cancers. For the first time, a derivative of curcumin that interacts only with AKT1 but not AKT2 is proposed by way of bioinformatics-based verification studies. Through simulation-based analysis, this research studies the interactions of curcumin derivative with AKT1 protein and compare the same with the fda approved AKT1 inhibitor, capiversatib. The curcumin derivative's energy was localized along with optimization using the PDB file 4EKL obtained from RCSB. The stability of the curcumin derivative's non-bonded interactions with the AKT1 protein was studied using Desmond MD method. The affinity of the curcumin derivative's interaction was -10.327 kcal/mol and for the fda approved molecule, Capivasertib, it was -8.339 kcal/mol. The MMGBSA value of -60.53 kcal/mol for the curcumin derivative-AKT1 complex was much better than the value of -26.65 kcal/mole for the Capivasertib-AKT1 complex. The curcumin derivative molecule did not interact with Akt2 molecule as indicated by the failure of good docking poses by the docking software. The curcumin derivative molecule was helped by hydrogen bond formations with Glu228, Ala230, Lys179, Glu278, Thr312, Tyr315 whereas for Capivasertib, the hydrogen bonds were formed at Glu234, Glu278, Glu228 and Ala230 in addition to various hydrophobic interactions for both the complexes. Molecular dynamics simulation studies confirmed the stability of the curcumin derivative molecule-Akt1 complex for the entire duration of 100 nanoseconds. Hence, the curcumin derivative molecule may target specifically the Akt1 molecule for its behavioural modification.

Keywords: AKT1 protein, Curcumin derivative, MD simulation, Interaction analysis

Introduction

AKT1 is a serine/threonine kinase also known as protein kinase B, PKB and plays an important role to influence the survival and proliferation of the cancer cells (Nitulescu et al. 2018). AKT1, AKT2, AKT3 are three isoforms with similar but distinct functions, and are collectively known as AKT. Many cancer cell types involve excessive expression of AKT1, which helps to evade apoptosis. AKT1 also has a role in angiogenesis and metastasis. Hence, it is vital to inhibit AKT signalling to block cancer progression (Fratev et al. 2021) . In cancer therapy, it is becoming imperative to target AKT1 which will be significant in treatment. In cervical cancers, it has been seen that AKT1 inhibition led to decrease in cell growth via CD73. Platelet derived growth factor (PDGF) an important mitogenic factor activates AKT1 via PI3K signalling pathway and is dysregulated in cancers. PTEN, a regulator of AKT1, plays an important role in the cell cycle regulation and, is often inactivated by mutations in cancers (Mirza et al. 2023).

The mutations in PTEN gene leads to deregulation of mTOR activity (Matsumoto et al. 2016) and high AKT1 activity in many types of cancers including breast, lung, prostate, brain among others. Many

natural compounds exhibit anticancer activity by activating PTEN activity. Likewise, Curcumin, a natural compound from Curcuma Longa is known to inhibit all the three isoforms of AKT (Yu S, Shen 2008). Several available therapeutic synthetic inhibitors are pan-AKT inhibitors and effective but their usage is limited because of side effects. It is very essential to develop molecules that can specifically target AKT1 with no/limited side effects.In this research, a derivative of curcumin is shown to exhibit specificinsilico interaction with Akt1 using the 4EKLpdb file whereas the same molecule fails to interact with AKT2 obtained through the 2JDR pdb file.

Materials and methods

Software and Modules

Schrodinger and Desmond simulation software (Ayaz, P et al. 2023) were used to obtain the necessary knowledge about the interactions at the atomic level. The same set of softwares are used to understand the stability of the complexes formed.The software modules, namely, protein preparation, Grid generation, Site map prediction, Ligprep, and glide docking (Da Silva et al. 2023) are used to study molecular mechanical interactions of the complex between curcumin derivative and Akt1. Quantum Mechanical procedural software consists of the modules that monitors simulation's real time dynamism, interactions and its quality (Bitencourt-Ferreira et al. 2019; Kuki and Nielsen, 2010). The QM software was used to study the stability of the curcumin derivative – Akt1 complex in a better way.

Protocol for preparing the Ligand and the Protein

To promote interaction with the AKT1 protein (4EKL pdb), the energy of the curcumin derivative molecule must be reduced to a local energy level that ensures its stability. The protein was preprocessed by setting on the Assign bond order using the CCD database along with hydrogens substituted (Lima et al. 2022). In the preparation method, the PDB file 4EKL was used as an entry for the source. The metal and disulfide linkages are made using zero-order. The pH level of 7.0 +/- 2.0 was generated using the Epik module.

The protein after preprocessing was assigned an optimised H-Bond using the PROPKA optimisation technique (Davies et al. 2008) and sample water orientation. Finally, the protein was minimised using the OPLS4 force field computation, RMSD coverage of heavy atom set as 0.30 A. Any water molecule beyond 5 A from the PDB ligand was removed. The curcumin derivative molecule was prepared for docking by the LigPrep technique. By enabling parameters of OPLS4 force field, the Epik algorithm was utilised to desalt the system. In total, 64 stereoisomers of the ligand molecule were created.

Analysis of the binding pocket by the Grid generation method

The receptor grid-generating procedure used the ligand position in the pdb protein 4EKL as a suitable site for binding for the curcumin derivative and capavasertib ligands. The VdW radius scaling factor is set to 1.0, with a cutoff partial charge of 0.25. The enclosing box was sized to fit the workspace ligand's centroid, and the dock ligands were set to be 12 inches long.

Set Up for Docking Ligands

Scaling factor approach was set to 0.80 for ligand docking, and partial charge cutoff for the van der Waals radii was set to 0.15. Using the extra precision technique, the flexible ligand sampling setup was used in conjunction with the imported grid (Friesner et al., 2004). For bias sampling of torsions, all predefined functional groups were used along with ring conformations and sample nitrogen inversion. Finally, Epik state penalties raised the docking score. The energy for the ring sampling window was set at 2.5 kcal/mol for the

generation of conformers, and the dielectric constant value as a function of distance was chosen at 2.0 for minimization (Malla et al2022).The Prime-MMGBSA approach was also used to investigate binding energies with Akt1 (4EKL) protein for better poses of curcumin derivative molecules and Capavasertib. The solvated model was set as VSGB and force field protocols was set as OPLS4, according to L. B. Silva et al. (2023).

Stability studies of Dynamic Simulation

The stability of best-docked pose of the Curcumin derivative and Capavasertib was analysed using the system builder protocol in Desmond software. The curcumin derivative - 4EKL system was initially solvated for molecular dynamic simulation using the SPC solvent model with orthorhombic boxes as boundary conditions, and the size of buffer box was estimated at a distance of 10.0 A.

Furthermore, the system was neutralised with sodium and chloride ions, and it's final volume was minimised based on the system's surface occupation. All the settings for trajectory recording like the energy gap, simulation length, time interval was set according to Sathya et al 2023. For the NPT ensemble class, 1000 frames were created at normal temperature and pressure after relaxing the model before the simulation.The completed simulation was loaded, and the average length of the block was set to 10.0 ps for the trajectory quality analysis. The simulation quality analysis technique is used to generate graphs using the parameters Potential energy (kcal/mol), Total energy (kcal/mol), pressure (bar), temperature (k), and volume (A3). The simulation interaction diagram module calculated the RMSD, RMSF, and Torsion values of a complex across a time scale of 100 ns.

Results and Discussion

The energy values of protein Akt1 and the ligands were adjusted to a localized minimum to ensure stability using suitable processes for docking analysis. Fig 1 shows a protein overlay before and after minimization. The amino acids Ser205, Glu278, Glu440, and Tyr 229 constituting the loop of the binding area were foundclose to the conformational modifications that were significant. Likewise, the original and energy-minimized states of the ligand molecule (Pincock &Torupka, 1969) were superimposed. Configurational modifications were observed in the cyclic ring segments whose RMSD is 0.329. The final energy of the protein was found to be -1379.516 kcal/mol, while the energy of the molecule was 21.13653 kcal/mol.

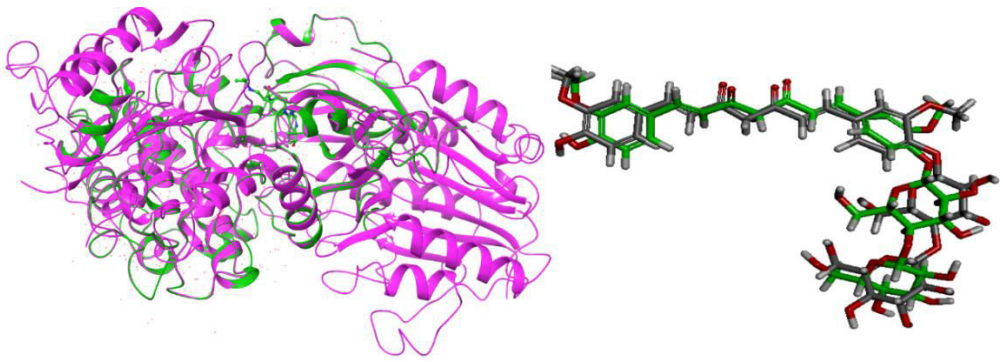

Fig 1 Superimposed images of the Protein 4EKL's secondary structure before (green) and after (pink) after minimization.Superimposed structures of Curcumin derivative before (green) and after (red) after energy minimization

The binding grid pockets(Fig 2) for theactive site with X, Y, Z coordinates set as -28.03, 5.22, and 10.89 was used to bind the Curcumin derivative and Capivasertib.

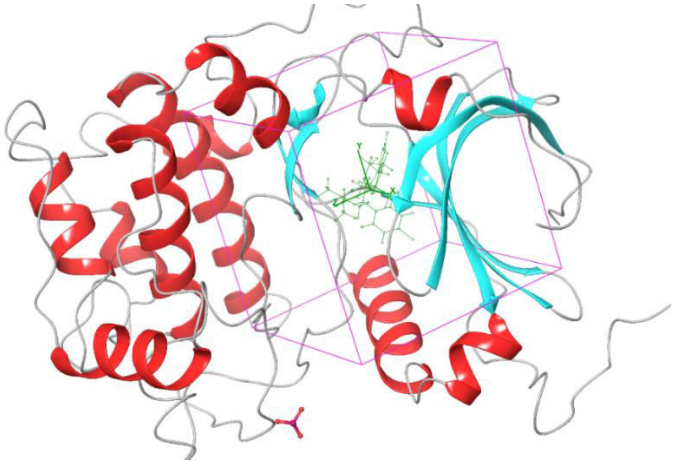

Fig 2. 4EKL protein's Active site.

Docking Analysis of Curcumin derivative and Capavasertib

Glide docking procedures revealed that Capivasertib and Curcumin derivatives formed excellent poses in the locations for binding. The curcumin derivative's binding site energy was found to be -10.327 kcal/mol, while Capivasertib had an energy of -8.339 kcal/mol(Table 1). When compared to Capivasertib, the Curcumin derivative wasaided well by water molecules, and the hydroxyl groups of the curcumin derivative helped to generate 7 H-bonds with the amino acids Ala230, Glu228, Thr312, Lys179, Tyr315, Glu278 and the -OH group of the sugars.Furthermore, 3 polar residues Thr211, Thr291, Thr312, 13 amino acids Ala177,Met227, Tyr229, Ala230, Val164, Phe438, Phe161, Leu156,Met281, Leu347, Phe236, Tyr315 (Hydrophobic), 5 negatively charged amino acids Asp292, Glu228, Glu314, Glu341, Glu278 help modify the curcumin derivative configuration for good binding(Fig 3). An Mmgbsa value of -60.53 kcal/molfor the binding of curcumin derivative with the active site confirms the strength of the binding.. For the AKT2 molecule, the pdb file 2JDR was prepared according to the protocols but the curcumin derivative molecule failed to dock with it.

Table 1.Docking energy values of Curcumin derivative and Capavasertibmolecules

Ligand Molecules	Docking Energy at Active site (kcal/mol)	Mmgbsa Energy
Curcumin derivative	-10.327	-60.53
Capivasertib	-8.339	-26.65

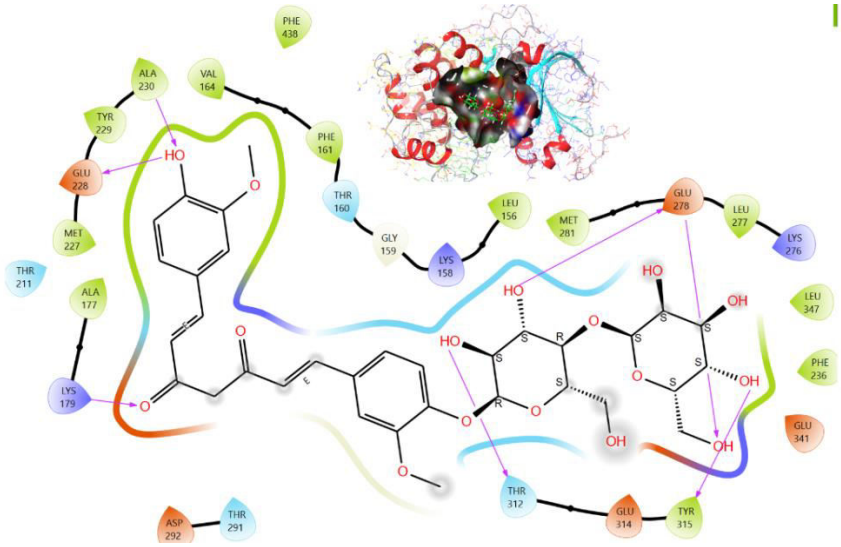

Fig 32-Dimensional representation of the interaction between amino acids and curcumin derivativein the 4EKL PDB's active site

Fig 4 depicts the interaction of the typical medication Capivasertib with the protein's active site. The image demonstrated that the FDA-approved Capivasertib formed five different types of interactions such as the formation of hydrogen bondswith Glu234, Glu278, Glu228, Ala230 and also the π-cationic contact between Lys179 and the chloro benzene ring.

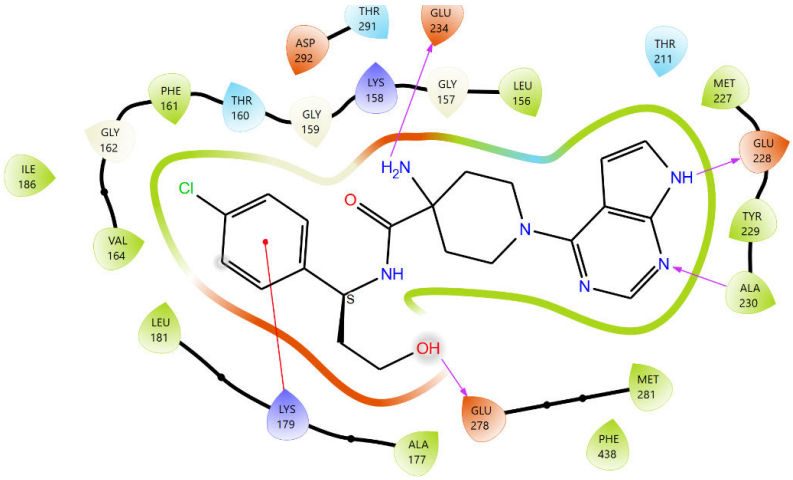

Fig4.Capivasertib's interactionat the active site of the protein

Simulation Dynamics Stability Analysis

Interaction studies alone cannot establish the Curcumin derivative's potential efficacy and bond formation. It should be dynamically tested utilising MD simulation experiments (Navabshan et al, 2021; Galvo Lopes et al, 2023). The Curcumin derivative and Capivasertib molecule complexes were dissolved in water (data for Capivasertib are not included in this preprint) and neutralised with Na+ and Cl- ions.The system builder protocol included 8373 water molecules for the 100ns simulation trials for the Curcumin derivative active site binding complex (Fig. 5).

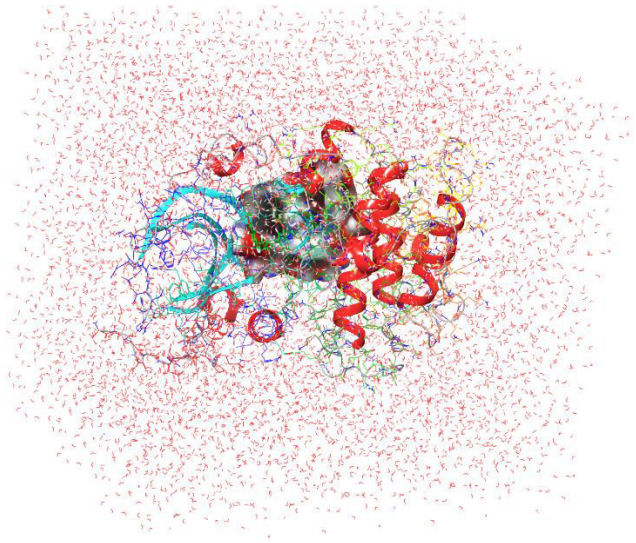

Fig5Curcumin derivative binding to the active site in a solvated model.

The quality of the molecular dynamic simulation run was investigated, and the parameter values were discovered to be within a suitable range. As demonstrated in Fig. 6, the complicated simulation quality has trustworthy values for volume (282940.527 ±339.548), pressure (1.08361 bar), temperature (298.675 ±0.665), and potential energy (-97221.942± 77.132). The overall energy values of -79484.457±86.321 kcal/mol demonstrated that the 4ekl protein-curcumin derivativecombination remained stable across the 100 ns simulation.

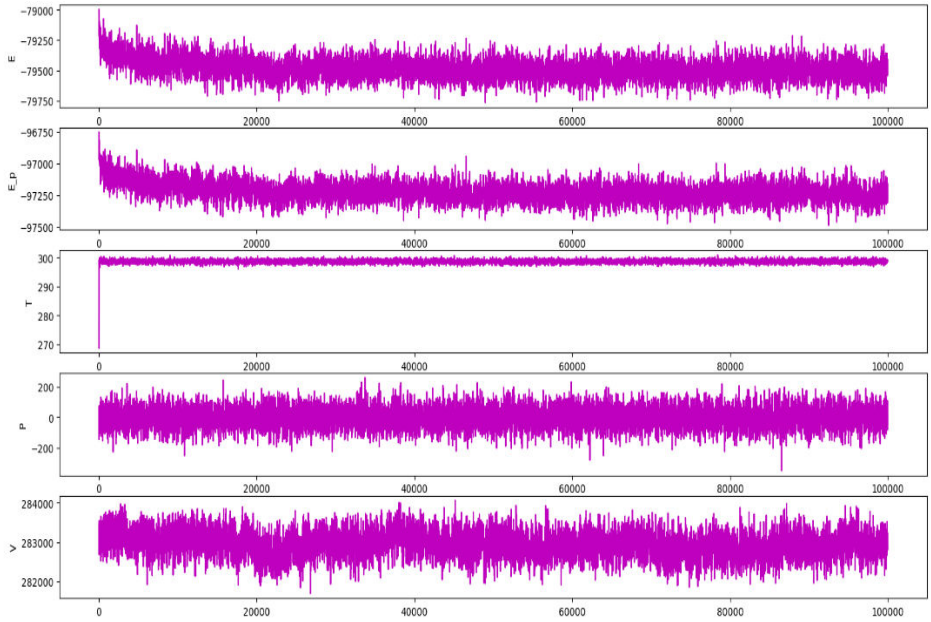

Fig6.Curcumin derivative-4EKL proteinsimulation run quality analysis report for 100 ns

Root Mean Squared Deviation(RMSD) and Fluctuation (RMSF) of the Protein-ligand Interactions in the active site

The 4ekl-curcumin derivative complex began to stabilise at 20 nanoseconds, according to the RMSD graph, with an RMSD range of 3.50 - 4.00 (Fig. 7). Between 50 and 55 ns, the RMSD values varied just marginally. Based on the RMSD after the 39th ns, the complex was laterconfirmed to be completely stabilised.

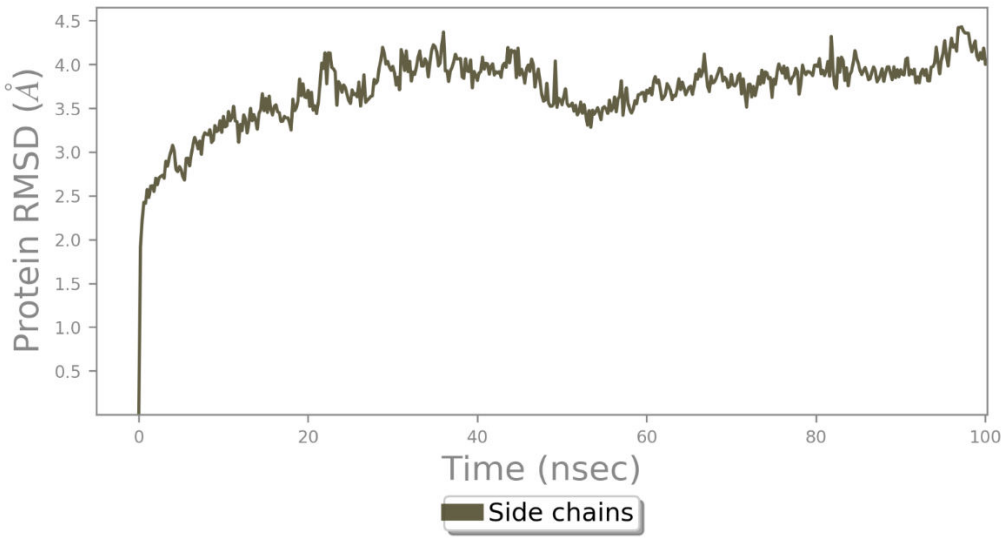

Fig7RMSD of different conformations of 4ekl-curcumin derivative at 100ns.

The interactions between curcumin derivative and 4ekl protein shown in Fig. 8, favored the stablityfor 100ns. During the simulation, the sugar moiety's four hydroxyl groups made considerable interactions with Glu A228 (94%), Leu A156 (42%), Ala A230 (91%), Lys A179 (42%), and one ketonic hydrogen bond with Phe A161 (80%). These interactions and amino acid combinations help to stabilise the complex and decrease oscillations.

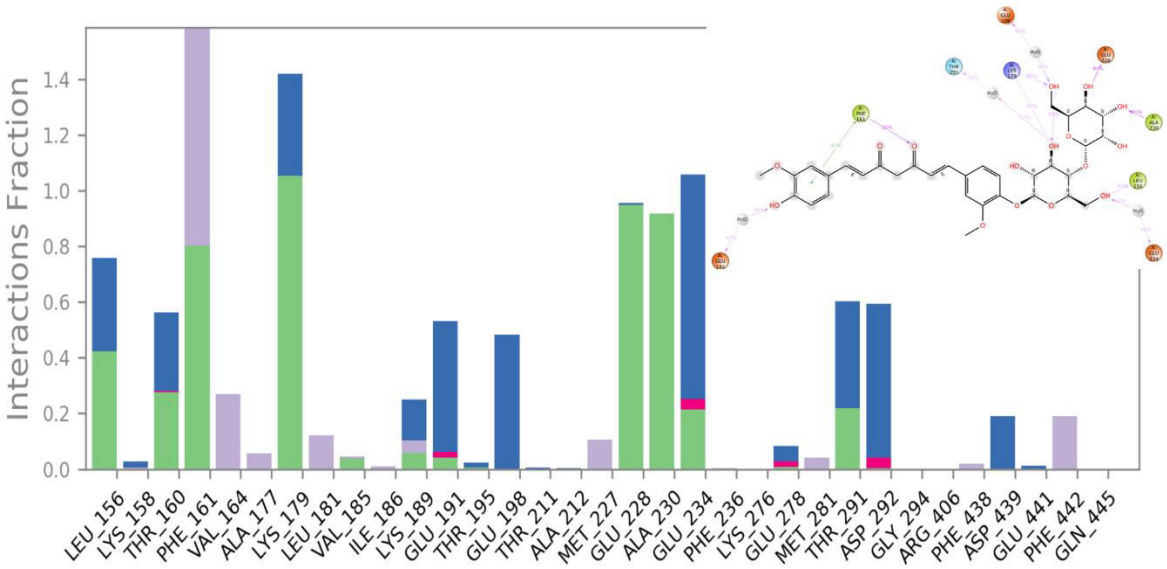

Fig8. Interaction chart of thecurcumin derivative during the 100ns simulation

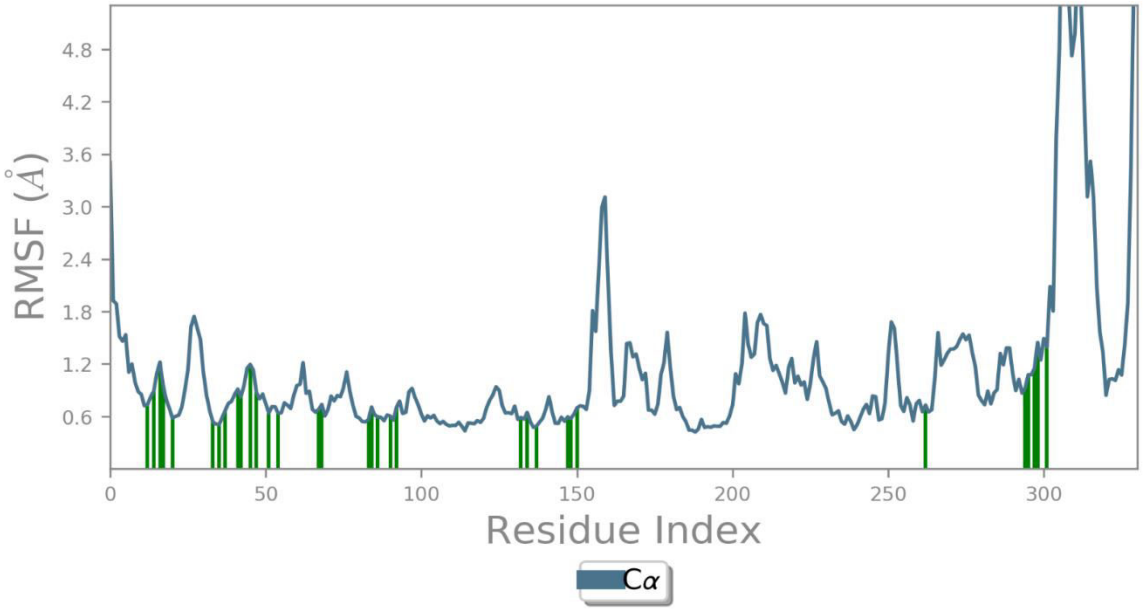

Fig 9. Curcumin derivative-4EKL protein's RMSF.

Fig9 depicts the residues establishing bonds with the protein as green bonds. The residues 15-25, 32-52, 71-73, 84-91, 140-150, 261, 289-300 bonded well and formed the protein's sturdy coils and sheets structure.

The Curcumin derivative molecule binds very strongly in the active site. The MD tests confirmed the stability of the 4ekl protein-curcumin derivative complex while binding. This research also suggests that a curcumin derivative could be a promising compound for changing the behaviour of the AKT1 protein (4ekl).

Conclusion

The results of this study's interaction analysis and stability examinations revealed that the curcumin derivative molecule is capable of specifically altering the behaviour of AKT1. The MD stability analysis encourages the formation of unbreakable non-bonded links, which keep the molecule stable within the protein.

Conflict of Interest

There is no conflict of Interest

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