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Research article

A molecular dynamics study of AZD4573 and Roscovitine as two potent inhibitors of cyclin dependent *kinase 9*

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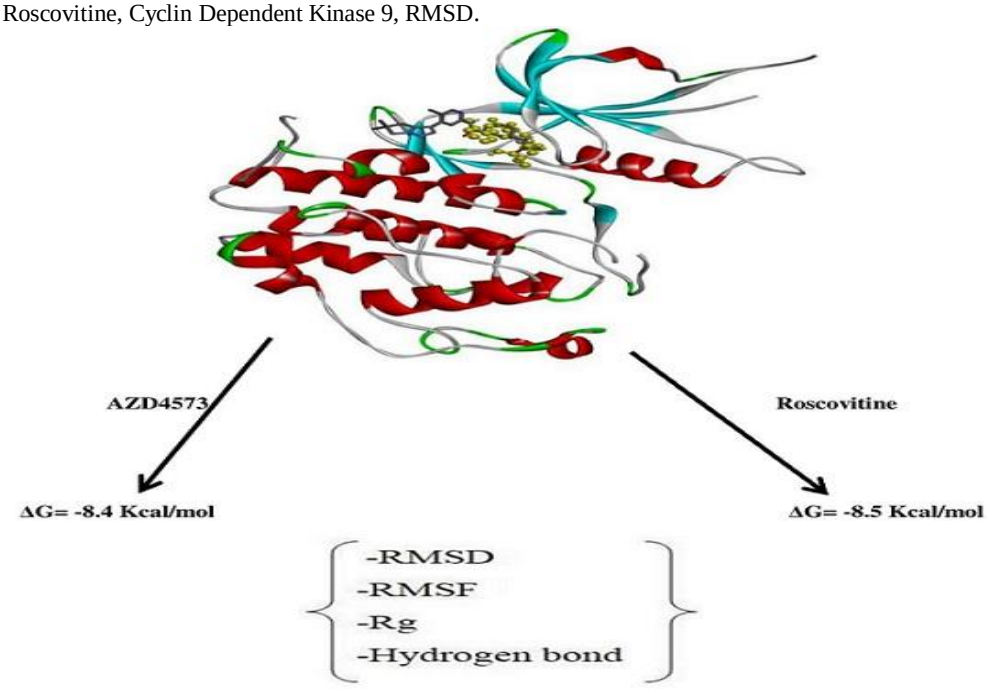
ABSTRACT

The cyclin dependent kinase 9(CDK9) was confirmed in several malignancies, notably those brought on by the dysregulation of the transcriptional elongation and mRNA maturation processes, have made CDK9 an appealing therapeutic target. The creation of selective CDK9 inhibitors continues to be extremely difficult due to the great homology of these CDKs in the catalytic domain. In this work we present two potential inhibitors, AZD4573 and Roscovitine, to detect potential binding between these compounds and the protein. These two compounds are in different experimental phases. AZD4573 were able to establish hydrogen bond with Asn154 and Asp167 with several hydrophobic bonds in the complex formed. Using the assistance of molecular dynamics (MD) simulations, the mechanism and dynamic stability of the interaction between the investigated chemicals and CDK9 were identified. It is strongly advised to consider these drug candidates as potential treatments, and we propose conducting in vivo trials to experimentally validate our findings.

Keywords: AZD4573, Roscovitine, Cyclin Dependent Kinase 9, RMSD.



INTRODUCTION

Cyclin 9-dependent kinase (CDK9) is associated to positive transcription elongation factor b (P-TEFb). This gene produces a protein that belongs to the CDK (cyclin-dependent kinase) family. Important cell cycle regulators, members of the CDK family are genetically very similar to the gene products of *Saccharomyces Cerevisiae*. The kinase was discovered to be a part of the multiprotein complex TAK/P-TEFb, which serves as an elongation factor for transcription that is guided by RNA polymerase II and phosphorylates the C-terminal domain of the RNA polymerase II large subunit of CDK9, one of a large number of CDKs that regulate cell-cycle progression and gene transcription, has been researched as a therapeutic target in Acute myeloid leukemia(AML) While being initially believed to regulate the cell cycle, CDK9 also controls the maturation of messenger RNA (mRNA), gene transcription, and other physiological functions[1,2]. The CDK9 pathway is dysregulated in AML and other hematologic malignancies, as well as in solid tumors, making it a promising target for cancer therapies[3]. The discovery of AZD4573 was by Astrazeneca, CDK9 inhibitor AZD4573 was created for transitory target engagement in humans and is acceptable for intravenous delivery. With the use of AZD4573 transient CDK9 inhibition, it is possible to destroy tumor cells by indirectly downregulating important cell survival proteins like Mcl-1[4]. (R)-Roscovitine, CY-202, and Seliciclib are some other names for Roscovitine, which was discovered at a lab in the French town of Roscoff. Roscovitine is a member of the purine family, which also includes the vitally important biological compounds ATP, cyclic AMP, NAD, FAD, adenine, and guanine. It works by interacting with the amino acids that line up the ATP-binding pocket of the CDK catalytic domain, where it competes with ATP for binding at the ATP-binding site of CDK[5]. In clinical research, Roscovitine is frequently used at doses of 200, 400, and 800 mg for various diseases, Cushing disease in a phase clinical trial 2 (NCT02160730)[6], cystic fibrosis against chronic infection by Pseudomonas aeruginosa (NCT02649751) and advanced solid tumors (NCT00999401). With an acceptable tolerance profile for the 200 and 400 mg dosages overall. However, its effectiveness and safety must still be tested on humans before being approved for use. The current work aims to use MD to investigate and compare the results of two potent molecules on CDK9. In this study we have investigated the potential of two drugs AZD4573 and Roscovitine against Cyclin Dependent Kinase 9 with the use of molecular dynamic simulation.

MATERIAL AND METHODS

Protein and ligand preparation

In the context of the study of protein-ligand interactions, the target protein cyclin dependent kinase 9(CDK9) with cyclin T and 2-amino-4-heteroaryl-pyrimidine inhibitor has been extracted from protein data bank (PDB code: 4BCG) The choice of 4BCG is due to the strong inhibition of pyrimidine derivatives, especially 12u, with high selectivity, showing that this active site is important for the inhibition of this protein[7] . A preparation step to remove the ligand and the water molecules, missing hydrogen atoms was added. The active site was determined based on the ligand binding site (pyrimidine derivatives) which is highly selective (80 times) for CDK9. The SDF files of the two ligands Roscovitine (CID:6603989) and AZD4573 (CID:124155204) were retrieved from PubChem database (http://pubchem.ncbi). The energy minimization was carried out by Chem3D using Molecular Mechanical Force Field (MMFF).

Molecular docking

The binding energy between protein and ligand was found by molecular docking using AutoDock Vina (https://vina.scripps.edu) was selected as the molecular docking tool. MGLTools 1.5.6 was used to add hydrogens and give Kollman charge for the protein .which use a computational approach which seeks to predict binding energy[2,3]. A grid box with size of (X=66 Å, Y=72 Å, Z=74 Å) and a center of (X=58.303 Å, Y= -15.694 Å, Z=-7.889 Å) was set to cover the binding site in the in the cyclin dependent kinase 9. The exhaustiveness which refers to repetitions of quantifying a protein binding site was set to 8. The ligand and the protein were converted to the PDBQT format to use it for the docking in AutoDock Vina. The best complex interaction has been analyzed by Discovery Studio 2021 client.

Molecular dynamics simulation

The molecular dynamics simulation is a very powerful tool to simulate motions and changes in the structure of the ligand and the protein and their stability. It was performed by WEBGRO Macromolecular simulation[10] which use GROMACS (Groningen Machine for chemical simulation)[11]. Structure minimization was carried out by using GROMOS96 43a1 where hydrogen bond was added by default. The ligand topology file were generated by PRODRG (https://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg)[12]. CDK9-ligand has been solvated in a cubic box with original and refined simple point charge (SPC) water model. The CDK9-ligand complexes were solvated for the two ligands as follow: for water molecules CDK9-Roscovitine (17 323 molecules), CDK9-AZD4573 (17 313 molecules). Fifty six and sixteen Na+ and Cl- respectively were added for the neutralization of the two complexes. Energy minimization parameters were set using steepest descent minimization algorithms with 5000 steps to remove any steric clash. The equilibration step was performed at 300K and 1.0 bar with (constant volume) and (constant pressure) with approximate number of frame per simulation 1000. After the simulation, outputs with data on the trajectory are generated as root mean square deviation

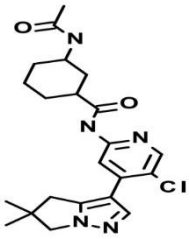
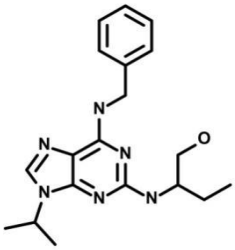
(RMSD), root mean square fluctuation (RMSF), the radius of gyration (Rg), the solvent accessible surface area (SASA) and hydrogen bonds (HBs).

RESULTS AND DISCUSSION

CDK9-ligand complexes

For CDK9, we found that the two compounds share some common hydrophobic interaction with residues Ala46, Ile25, Leu56 and Val33 (Table 1). The complex CDK9-AZD4573 has no hydrogen bonding. However, CDK9-Roscvitine has two hydrogen bonds Asp167 and Asn154 as described in other studies [1,6].

Table 1: The two active compounds and their interactions

Name	Chemical structure	Binding energy score(Kcal/mol)	pKi (µM)	Hydrogen bond	Hydrophobic
AZD4573		-8.4	0.72	Asn154, Asp167	Ala46,Ile25, Leu156,Val33, Phe103,Ala166,Val79,Lys48
Roscovitine		-8.5	0.60	-	Ala46,Ile25, Leu156,Val33

From the two molecules Roscovitine showed slightly high affinity with cyclin dependent kinase 9 as shown in the binding pocket (Figure 1a, 1b).

Roscovitine displayed two hydrogen bond the first one between carbonyl group of Asp167 residue and hydroxyl-butan-1-ol group of purine moiety (2.67Å). The second one between the carbonyl of Asn154 and hydroxyl group of purine (1.98 Å). Additionally benzyl amino group are engaged in hydrophobic interaction with Ala166, Val79, Lys48 and Phe103 in π-Alkyl and π-π interactions, also the molecule is stabilized by carbon-hydrogen bond with Cys105 (Figure 1a).

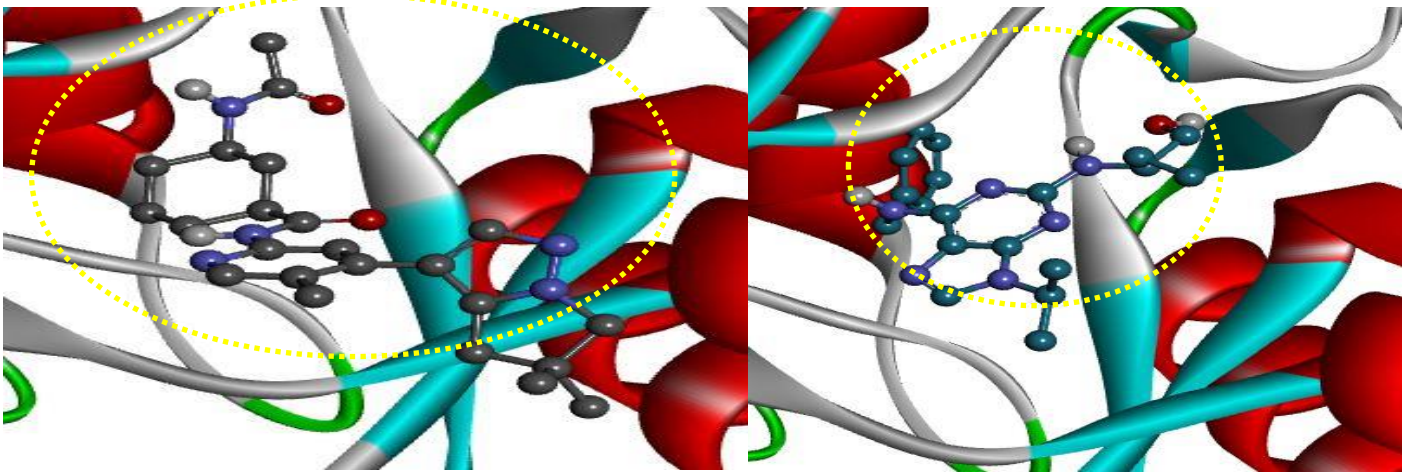
For AZD4573 the complex is stabilized by different hydrophobic π-Alky and π-π interactions. It is abundantly obvious that the stabilization of the ligands at the binding interface is greatly influenced by hydrophobic interactions. Since the two binding energies are close (Figure 1b).

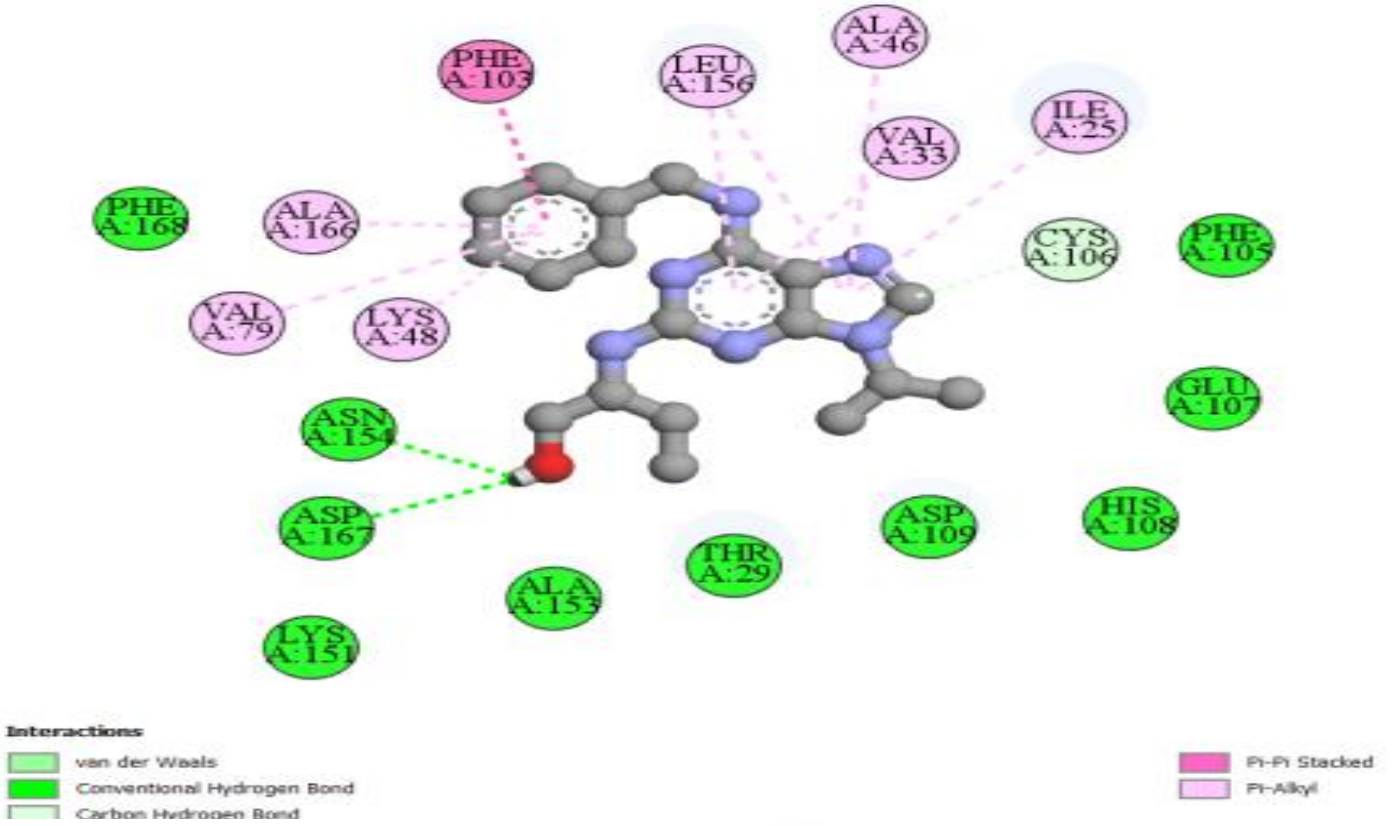
Molecular dynamic simulation of protein-ligand

Root mean square deviation

The RMSD is a measure of the difference between the backbones from its initial and final positions. The changing in conformation can be assessed by the deviation produced during the simulation[14], The smaller the deviation more stable will be the complex[15]. The free protein showed an overage of RMSD of 0.38 nm with values between 0.32 and 0.45 nm (Figure2). For the other two complexes the dynamic simulation showed higher RMSD CDK9-AZD4573 (0.54 nm) and CDK9-Roscovitine (0.50 nm), values indicating less stability of the complexes compared to the protein alone.

Figure 1: The binding site of the two selected candidates in the active site CDK9, (a) AZD4573, (b) Roscovitine




PHE A:103
LEU A:156
ALA A:46
ILE A:25
VAL A:33
PHE A:168
ALA A:166
CYS A:106
PHE A:105
VAL A:79
LYS A:48
GLU A:107
ASN A:154
ASP A:167
ASP A:109
HIS A:108
THR A:29
ALA A:153
LYS A:151
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Pi Stacked
Pi-Alkyl

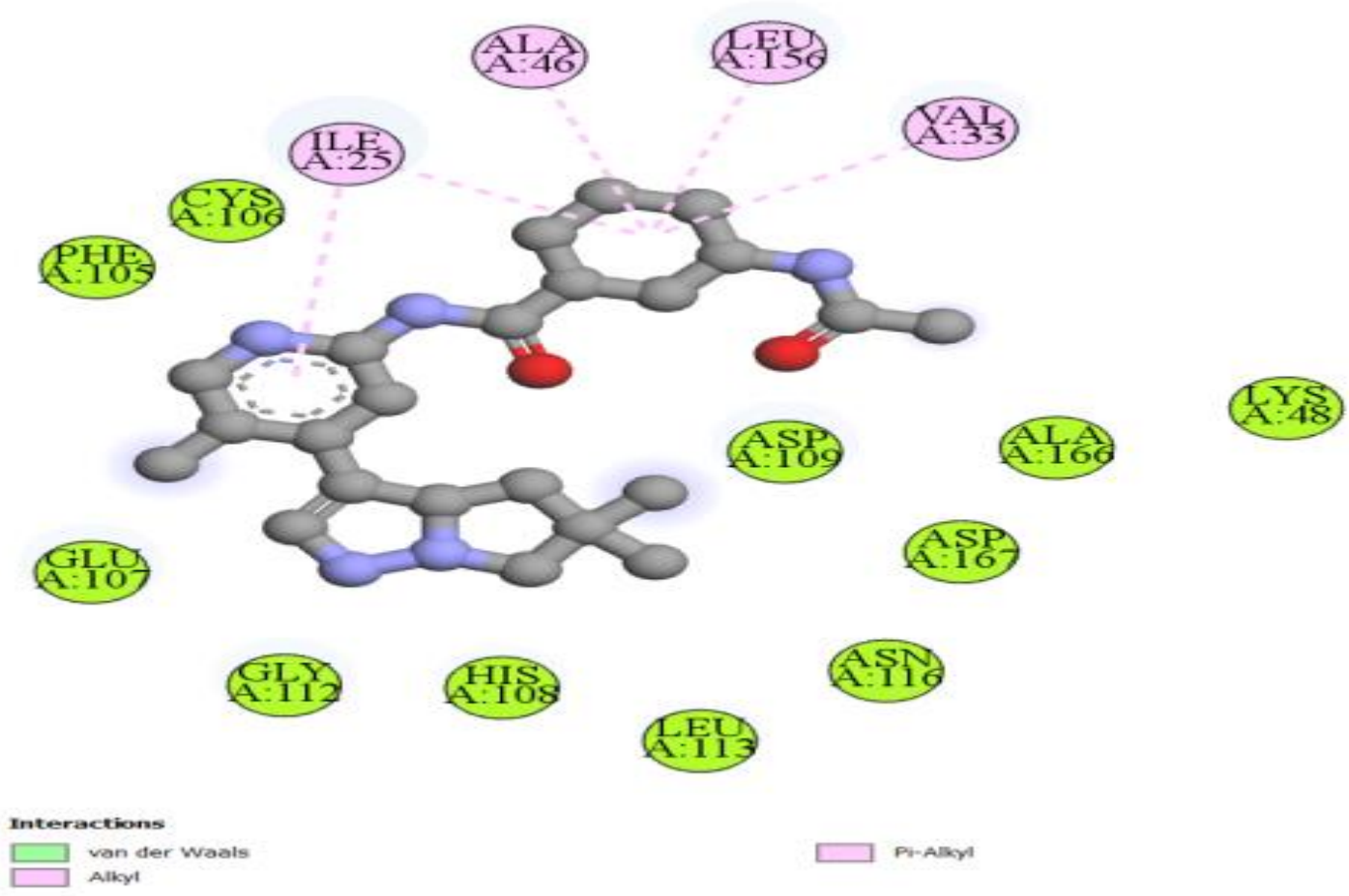
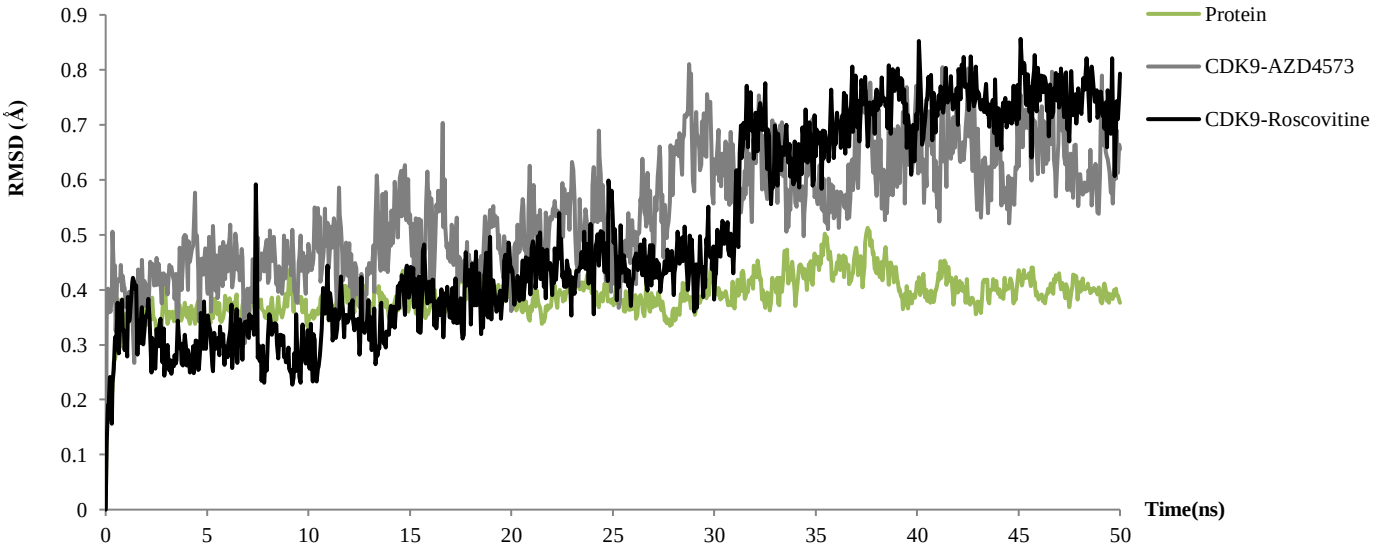

ALA A:46
LEU A:156
VAL A:33
ILE A:25
CYS A:106
PHE A:105
LYS A:48
ASP A:109
ALA A:166
ASP A:167
GLU A:107
ASN A:116
GLY A:112
HIS A:108
LEU A:113
Interactions
van der Waals
Alkyl
Pi-Alkyl

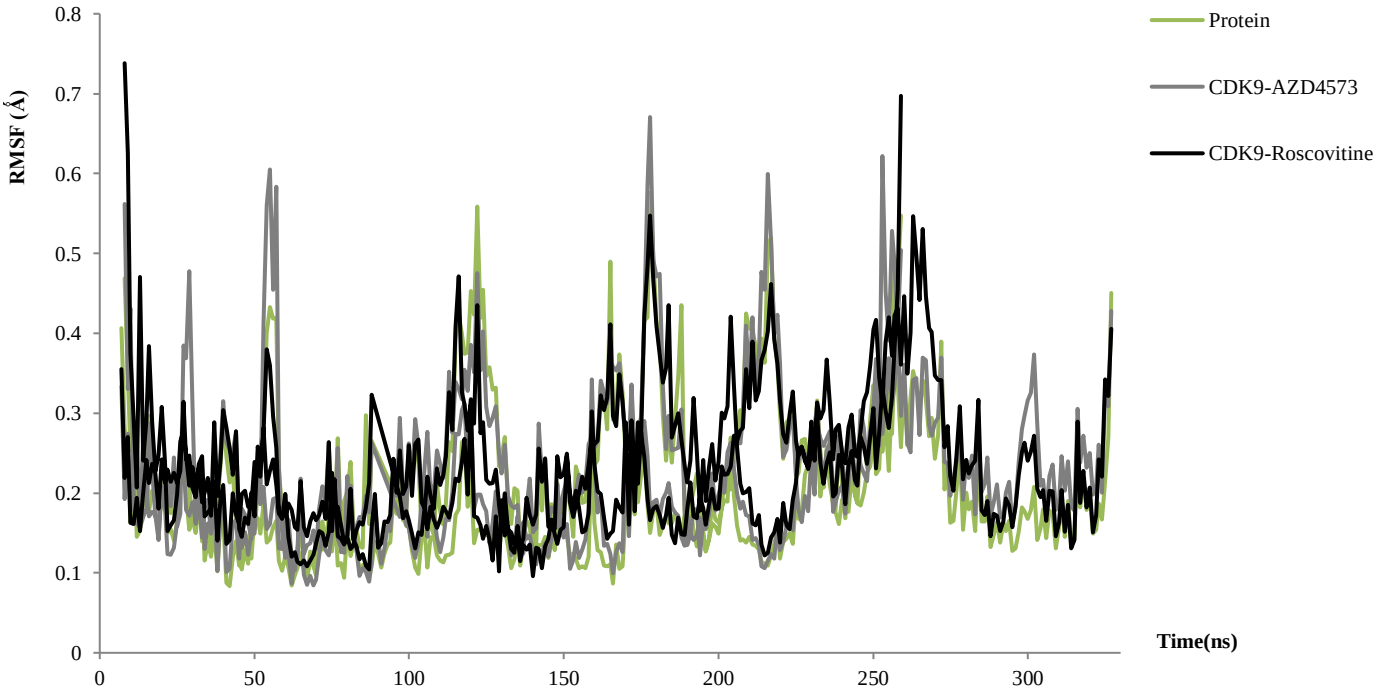
Figure 2: Molecular dynamics simulation. RMSD of the backbone for 50 ns at 300



Root mean square fluctuation

RMSF helps us to understand the internal behavior of amino acids by their flexibility in the presence and absence of the ligand. If the ligand binds well, the active amino acid groups in the binding pocket will reduce their mobility resulting in a more stable complex. Active residue Asn154, Asp167 exhibited RMSF values 0.11 nm and 0.13 nm for the free protein, however CDK9-AZD4573 and CDK9-Roscovitine showed 0.13 nm, 0.12 nm, 0.20 nm and 0.19 nm respectively. From these values it can be deduced that the protein [13]. alone is more stable with AZD4573 values close to that of the protein [13]

Figure 3: Molecular dynamics simulation. RMSF of the backbone for 50 ns at 300 K



Radius of gyration

In order to understand how inhibitors affect protein compactness, radius of gyration (Rg) asses the secondary structures of the protein. If the protein is folded consistently Rg will keep at a very constant value, from the two compounds AZD4573 exhibits good stability toward CDK9. The mean of the Rg value is 2.70, 2.70 and 2.76 nm respectively for CDK9, CDK9-AZD4573 and CDK9-Roscovitine (Figure4).

Solvent accessible surface area (SASA)

The exposition of the protein surface was analyzed by SASA, if the protein enters a stable complex the display area will be reduced so there will be less access to solvent. For AZD4573 forms a complex with less exposure to solvent during the first 20 nanoseconds, after the 20 ns Roscovitine shows less exposure on its surface. The average was 258.82, 262.48 and 254.62 nm respectively for CDK9, CDK9-AZD4573 and Roscovitine (Figure5).

Figure 4: Molecular dynamics simulation. Rg of the backbone for 50 ns at 300 K

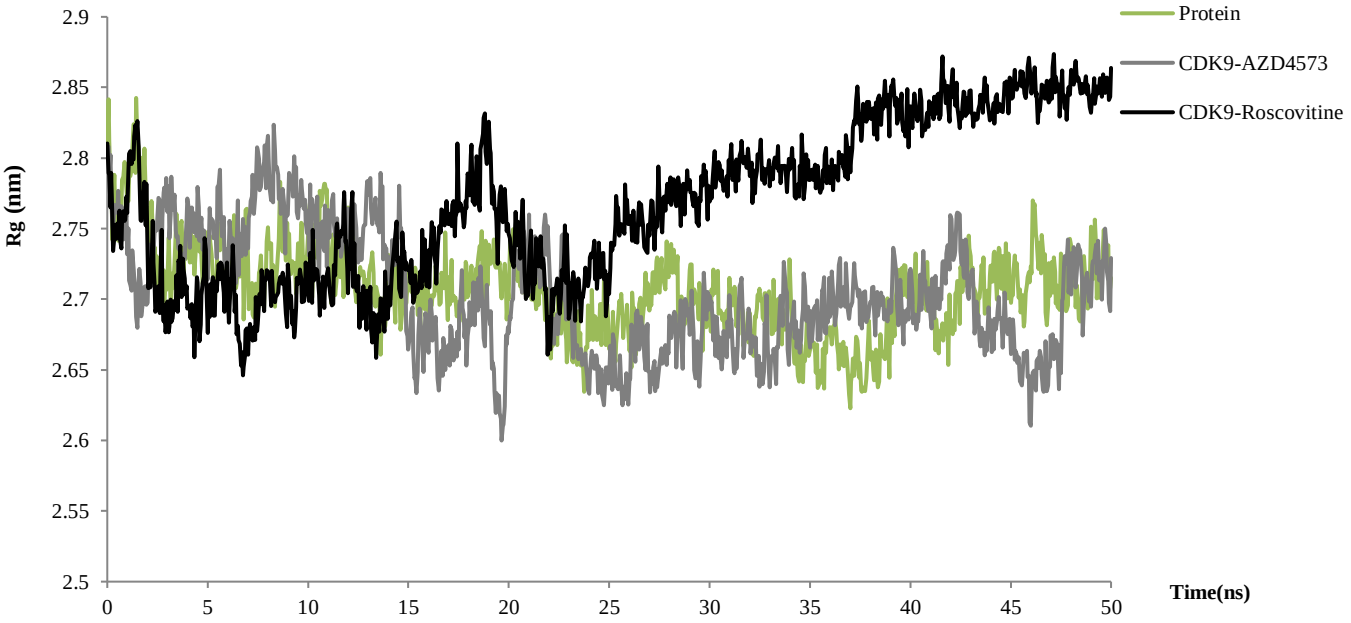
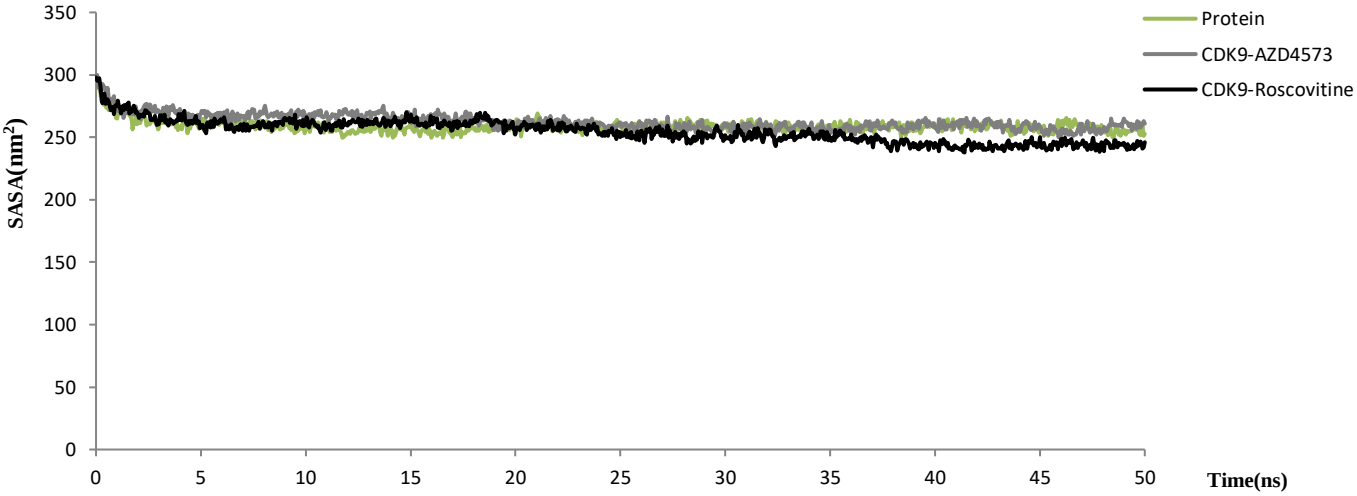


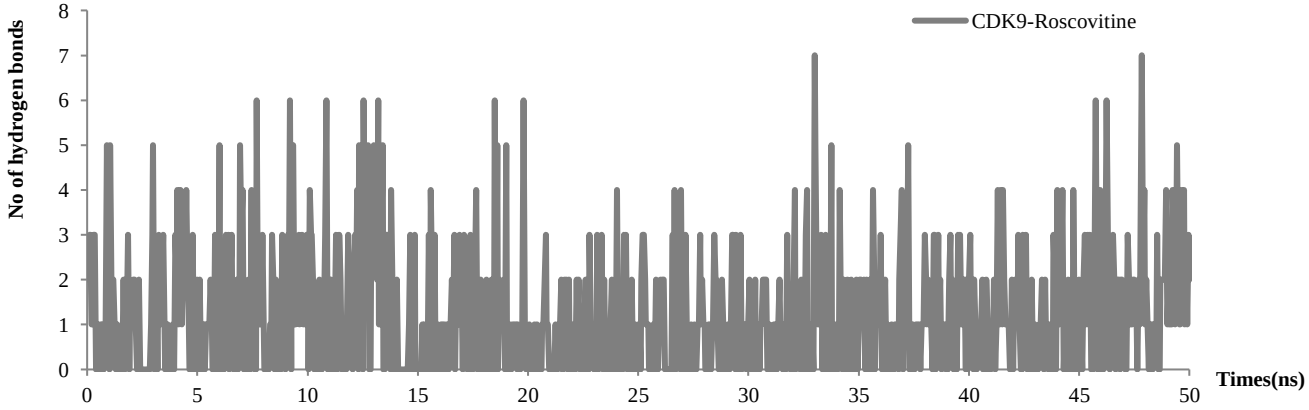
Figure 5: Molecular dynamics simulation. SASA of the backbone for 50 ns at 300 K

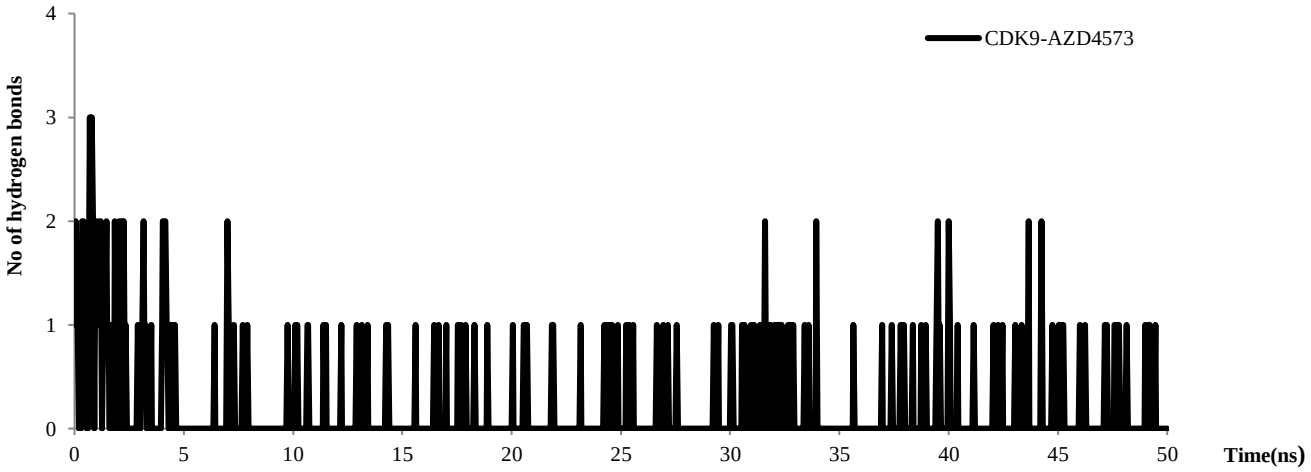


Hydrogen bonds analysis (Hbs)

The best inhibitor is one that has several hydrogen bonds with the CDK9, which means that it is a more stable complex, performed during the simulation. The highest number of hydrogen bonds(7) was found between Roscovitine and CDK9, however, AZD4573 has shown a maximum number of hydrogen bond(3) (Figure 6a,b). Furthermore, the amount of hydrogen bonds in the rigid docking and molecular dynamics was different which may be because the conformational during the molecular dynamic simulation.

Figure 6: Number of hydrogen bonds of the complex of CDK9 with Roscovitine and AZD4573





In this study we attempted to examine the interactions between AZD4573 and Roscovitine towards CDK9, Roscovitine demonstrated strong CDK9 binding. The protein alone has a superior stability profile than in the presence of the two ligands, as seen in the graphs of RMSD, RMSF, Rg, SASA, and hydrogen bonds, which were performed using molecular dynamics under the identical pressure and temperature settings for the protein alone and the two complexes. As far as hydrogen bonding is concerned, Roscovitine showed a larger number of interactions according to molecular dynamics results.

The protein binding of Roscovitine was more stable than that of AZD4573 in our study, which allowed the molecule to function properly as it had a lower deviation from the RMSD. Flexibility values (RMSF) for AZD4573 were lower, with key residues in the active site demonstrating stabilization with little to no movement. AZD4573 showed a degree of compactness comparable to proteins in their native state. The ligands showed the highest binding interaction in 150-170 regions of CDK9, where Asn154, Asp167, Ala46, Ile25, Leu156, Val33, Phe103, Ala166, Val79 and Lys48 where most dominant. In the present study, we uncovered the molecular interactions of two drug candidates. AZD4573 is a potent inhibitor of CDK9[13] inducing fast apoptosis across different hematological cancer model patient-derived xenograft(PDX) [4].

AZD4543 is a good candidate for the development of a treatment against CDK9, with an unknown pharmacodynamic and pharmacokinetic profile that should be taken into consideration in clinical trials. Molinspiration[16] was deployed to predict the molecular properties of the molecule. AZD4573 meets Lipinski's criteria of five. With a molecular weight of 429 g/mol, LogP of 3.29, number of acceptor and donor hydrogen's two and seven respectively.

Molecular dynamics has shown that AZD4573 may be an excellent candidate for further pharmacokinetic and pharmacodynamic studies while trying to better understand the interaction between CDK9-AZD4573 at the molecular level. Further in vivo and in vitro studies are recommended to evaluate the efficacy of the ligand experimentally.

CONCLUSION

The two selected compounds have been documented as having apoptotic activity and acting on the cell cycle in leukemia. In silico study by docking and molecular dynamics showed a significant action against cyclin dependent kinase 9 by AZD4573 and Roscovitine. It is assumed that our study constitutes the basis for further studies to evaluate the anti-leukemic activity of these two compounds as potential therapeutic agents, acting on a key enzyme in the regulation of cell multiplication. This study demonstrates that the combination of computational approach and medicinal chemistry can greatly contribute to the improvement of new drugs.

ACKNOWLEDGEMENTS

The authors declare that they have no conflicts of interest concerning this article.

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