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Identifying Isoononin and Candidissiol as Rho-associated protein kinase 1 (ROCK1) inhibitors: a combined virtual screening and MD simulation approach

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ABSTRACT

Rho-associated protein kinase 1 (ROCK1) is a member of the AGC family which plays crucial roles in inflammatory diseases and cancer progression. Elevated expression of ROCK1 has been reported in multiple cancer types, and thus it has emerged as a potential drug target for cancer therapeutics. In this study, we performed a structure-based virtual screening of the natural compounds taken from the IMPPAT database to find some potential molecules as inhibitors of ROCK1. For the first step, we selected the compounds based on the Lipinski rule of five, and then we filtered them based on their ADMET properties and PAINS value. After this, other parameters like binding affinities, docking score, biological properties and selectivity were calculated to find appropriate hits against ROCK1. Finally, we identified two natural compounds, Isoononin and Candidissiol, with appreciable binding affinity and selectivity towards ROCK1. Furthermore, all-atom molecular dynamics simulations were carried out on ROCK1 with the elucidated compounds, which suggested stability throughout the simulated trajectories of 100 ns. Taken together, Isoononin and Candidissiol could be considered as potential inhibitors of ROCK1 for developing anticancer therapeutics.

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Rho-associated protein kinase 1; cancer therapy; natural compounds; drug discovery; virtual screening; molecular dynamic simulation

1. Introduction

Rho-associated protein kinase 1 (ROCK1) is a serine/threonine-protein kinase that belongs to the AGC-kinase family, and is involved in the cell cycle and apoptosis (Riento & Ridley, 2003; Schofield & Bernard, 2013). ROCK1 signalling plays a vital role in cancer development and progression (Whatcott et al., 2017). ROCK1 is a multifunctional protein associated with the modulation of cell apoptosis, migration, and invasion of multiple cancer types (Whatcott et al., 2011; 2017). ROCK1 inhibition in cancer cells has been shown to decrease the migration and invasion capacity in various cancers, including pancreatic cancer (Whatcott et al., 2017). The ROCK1 is a 1354 amino acid long protein comprising a kinase domain (residues 76–338) and an AGC-kinase C-terminal (residues 341–409) (Julian & Olson, 2014). The ATP-binding and active sites residues of ROCK1 are Lys105 and Asp198, respectively. These sites play a crucial role in the functional activity of ROCK1. Structural information of ROCK1 can be exploited in developing the potentialinhibitors of ROCK1 for therapeutic applications. ROCK1 plays a major role in carcinogenesis and responsible for resistance to chemotherapy (Rath & Olson, 2012). Altered expression of ROCK1 has been detected in many malignancies such as breast, osteosarcoma, and pancreatic cancer (Kaneko et al., 2002; Provenzano et al., 2008). ROCK1 has been shown to play a crucial role in cancer progression, making it one of the prime targets to develop novel anticancer therapeutics.

In this context, phytochemicals can be a resource to discover novel ROCK1 inhibitors using a structure-based drug design approach (Anjum et al., 2021a, 2021b, 2022). It is an important domain in drug discovery because of its specificity and effectiveness (Naqvi et al., 2018). In structure-based drug design, virtual screening is one of the efficient techniques for finding potential novel compounds (Mohammad et al., 2020). It is used to identify potential compounds taken from chemical libraries that might bind to the protein structure with an appreciable affinity (Jairajpuri et al., 2020). A computer-based screening employing molecular docking-based approaches will make it easier to avoid undesirable compounds to check that would cause extra time and money. We further have some other refinement and filtering techniques that help us to get the potential drug-like compounds (Naqvi et al., 2018). Several filters are employed to screen large libraries of compounds, such as molecular docking, Lipinski filter,

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ADMET, etc., providing insight into the lead discovering process. The new advancements in structural biology technology and computational settings make it easier to discover novel drug-like compounds. Natural compounds are extensively exploited in drug discovery because they contain a wide range of chemical structures, binding specificity, and affinity (Dahiya et al., 2019; Naz et al., 2018).

In this study, we considered ROCK1 as a therapeutic target for identifying its potential inhibitors from a curated library of phytochemicals using a structure-based drug discovery approach. We have taken ~9,000 natural compounds from the Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) database (Mohanraj et al., 2018). This database provides plant-based natural compounds for the virtual screening process. The three-dimensional structure of ROCK1 was taken from the PDB database. After that, we screened 6093 compounds by applying the Lipinski rule of five filters. Based on the binding affinities and scoring function, the top 20 hits were selected, and then we ran SwissADME to filter compounds with no PAINS patterns. We ran ADMET on the pkCSM server and checked the drug likeliness, followed by PASS analysis. Finally, the top 2 compounds were selected based on their specific interactions with the critical residues of ROCK1 and evaluated in all-atom molecular dynamics (MD) simulations and principal component analysis (PCA).

2. Material and methods

2.1. Computational tools and servers

We used the InstaDock (Mohammad et al., 2021), Discovery Studio Visualizer (Studio, 2008) and GROMACS (Van Der Spoel et al., 2005) tools for docking-based virtual screening, interaction analysis, and MD simulations. The structure of the ROCK1 receptor was taken from RCSB PDB with ID: 5WNF. We remodelled the receptor structure by taking the kinase domain from residues Y76-S401, adding hydrogen to polar atoms and assigning the appropriate atom types for the virtual screening process. A set of ~9000 natural phytoconstituents was taken from the plant-based library IMPPAT database in processed form. The physicochemical properties, PAINS patterns (Baell, 2016) and ADMET properties were calculated through SwissADME (Daina et al., 2017) and pkCSM (Pires et al., 2015) web servers.

2.2. Filtration of compounds

The plant-based compounds taken from the IMPPAT database were filtered based on their respective physicochemical, binding affinity and ADMET properties. The first step of filtering the compound is based on the Lipinski rule of five (Lipinski, 2004), where we removed those compounds having any RO5 violation. The compounds were then subjected to molecular docking with ROCK1 to identify high-affinity molecules. Then, the Pan-assay interference compound filter (PAINS) was applied to avoid any compounds with a PAINS pattern (Baell, 2016). Further, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) screening was conducted to elucidate safe compounds with drug-like properties. Only compounds with well-characterized ADMET properties and noncarcinogenic patterns were considered for the subsequent PASS analysis and interaction studies.

2.3. Molecular docking-based virtual screening

The compounds filtered from RO5 analysis were subjected to the molecular docking-based virtual screening process. We performed the molecular docking of the compounds to find the potential molecules with a high binding affinity value. For docking, we used InstaDock with blind search settings of a grid of 66 Å, 77 Å, and 75 Å centralized at −19.377, 0.724, and 46.211 for X, Y, and Z coordinates, respectively. 1 Å is the value in which grid spacing was set in the default parameters. We used PyMOL (DeLano, 2002) and Discovery Studio Visualizer to find the ideal interacting binding partners for the ROCK1 binding pocket (Xiong et al., 2018).

2.4. ADMET properties of compounds

The major criteria for identifying good lead molecules for a target protein are their efficacy and safety (Naqvi et al., 2018). ADMET property tells us about the efficacy of the compound, and the pkCSM estimates these properties. In the pkCSM web server, the SMILES string was provided for each compound as input. Moreover, for neglecting Pan-assay interference compounds, we used the PAINS filter because these compounds have a high potential to bind to multiple targets.

2.5. PASS analysis: biological activity predictions

PASS (Prediction of Activity Spectra for Substances) is a bioinformatics webserver available through the Way2Drug platform (http://way2drug.com/passonline/). It helps us identify biologically activated substances from the pool of compounds based on their chemical formula (Lagunin et al., 2000). The PASS server provides a prediction score for biological properties in Pa to Pi ratio, where Pa = Probability to be active and Pi = Probability to be inactive. The high value of Pa for any biological property tells us that the chance of possessing this property in the compound is high.

2.6. MD simulations

MD simulations were performed on an HP Z840 machine at 300 K at the molecular mechanics level using the GROMACS package. We used the GROMOS 54A8 force-field (Oostenbrink et al., 2004) to simulate the ROCK1 in apo and bound states with Isoononin and Candidissiol. The ITP and GRO parameters for Isoononin and Candidissiol were generated from the PRODRG web server (Schüttelkopf & Van Aalten, 2004). The systems were centrally located in cubic boxes of 10 Å distance from the edges. The SPC216 solvent model was used in the aqueous environment to provide

Table 1. Selected hits and their binding affinities toward ROCK1.

S. No.	Compound ID	Binding Affinity
1.	CID_146680	−10.9
2.	CID_633072	−10.8
3.	CASID_53777-78-9	−10.8
4.	CID_102267534	−10.7
5.	CHEBIID_16778	−10.7
6.	CID_442731	−10.7
7.	CID_5319890	−10.6
8.	CID_11537197	−10.6
9.	CID_73657079	−10.6
10.	CID_159888	−10.5
11.	CID_443716	−10.5
12.	CID_441979	−10.5
13.	CID_179640	−10.4
14.	CID_3083586	−10.4
15.	CID_85976174	−10.4
16.	CID_5320772	−10.3
17.	CID_11988260	−10.3
18.	CID_5318619	−10.3
19.	CID_101651627	−10.3
20.	CASID_145706-88-3	−10.1

water molecules (Mark & Nilsson, 2001). We have neutralized the systems by adding an appropriate amount of counterions to make the system stable. All systems were optimized by minimizing them using the steepest descent algorithm. Then we performed the equilibration with some control over the position of NVT and NPT ensembles. Finally, in the end, we performed the md run for 100 ns for individual systems, and then the outcomes were analyzed using the GROMACS packages.

2.7. Principal component and free energy landscape analysis

To understand the motions of ROCK1 protein before and after ligand binding, we performed a PCA tool using the simulated trajectories (Lever et al., 2017). This tool is preferably used to understand folding dynamics in the protein. It is a covariance matrix-based method used to minimize or lower the multidimensional variables (Maisuradze et al., 2009). For calculating the covariance matrix, this formula was used,

$$C_{ij} = < (x_i - < x_i >) (x_j - < x_j >) >$$

where, x_i/x_j = ith/jth atom coordinates, < - > = Ensemble Coverage.

The MD trajectory of ROCK1 with Isoononin and Candidissiol was also used for the free energy landscapes (FELs) generation by diagonalizing the eigenvalues for the covariance matrix (Altis et al., 2008). It helps us to understand the changes in the conformation of ROCK1 with Isoononin and Candidissiol. Generally, the FEL talks about the stability of the protein and its complexes in the form of funnel diagrams and contour maps. The plots of ROCK1 with Isoononin and Candidissiol were formed with the help of this formula,

$$\Delta G(X) = - K_B T \ln P(x)$$

where, K_B = Boltzmann constant, T = Simulation temperature, P(x) = Principal Component's probability distribution.

3. Results and discussion

3.1. RO5 filtration of compounds

To get the potential molecule(s) with drug-like properties, we filtered out the compounds of the IMPPAT database based on the Lipinski rule of five, where we identified 6093 compounds. These compounds followed the RO5 and did not contain any violation of drug-likeness.

3.2. Molecular docking-based virtual screening

The parent library IMPPAT from which we have taken was trimmed down to 6093 compounds after filtering them based on RO5. For molecular docking-based screening of these compounds, we used InstaDock, a standalone application that makes molecular docking a one-step process. Based on the calculated docking score of ROCK1, we identified 20 top hits. After this analysis, it was found that the selected compounds exhibit appreciable affinity for ROCK1, ranging from −10.1 to −10.9 kcal/mol (Table 1).

3.3. ADMET properties of compounds

To assert drug-like candidates from the selected hits, the ADMET properties of all the compounds were calculated through the pkCSM webserver. This web server tells us about different ADMET properties based on GI absorption, BBB permeability, CaCO-2 permeability, water-solubility, AMES toxicity, etc. We used the ADMET property of the top 20 compounds, where we identified six compounds with well ADMET properties without any toxic pattern. The six compounds we identified are shown in Table 2.

3.4. PASS analysis: biological activity predictions

PASS server is a web server that predicts around 4000 types of biological activities for a compound (Lagunin et al., 2000). After analyzing the six compounds we filtered from the ADMET analysis, we identified two compounds, Isoononin and Candidissiol, in the PASS filter with desirable properties. The predicted properties of Isoononin and Candidissiol are shown in (Table 3). Both compounds show anti-cancerous, antineoplastic, anticarcinogenic and kinase inhibition properties with Pa ranging from 0.711–0.951 when Pa > Pi.

3.5. Interaction analysis

Interaction analysis was conducted based on the conformations of the compounds' binding to the ROCK1. We then selected the conformers that fit well into the binding pocket of ROCK1. Pose selection was performed by exploring each docked conformation of the selected compounds. Reference inhibitor-based pose selection shows a well superposing of docked conformers of Isoononin and Candidissiol with the crystal structure of ROCK1. The results showed that Isoononin and Candidissiol interact at the ATP-binding site of ROCK1, i.e. Lys105, along with several other important

Table 2. ADMET properties of the selected compounds.

S. No.	Molecule	Absorption *GI Absorption*	Distribution *BBB permeability*	Metabolism *CYP2D6 inhibitor*	Excretion *Renal OCT2 Substrate*	Toxicity *AMES toxicity*
1.	CID_102267534	High	Yes	No	No	No
2.	CASID_145706-88-3	High	Yes	No	No	No
3.	CID_5318619	High	No	No	No	No
4.	CID_5319890	High	Yes	No	No	No
5.	CID_5320772	High	No	No	No	No
6.	CID_9798203	High	No	No	No	No

Table 3. Selected compounds and their biological properties predicted through PASS webserver.

S. No.	Compound ID	Common name	Pa	Pi	Biological activity
1.	CASID_145706-88-3	Candidissiol	0.951	0.003	TP53 expression enhancer
			0.926	0.002	Anticarcogenic
			0.839	0.005	Antiinflammatory
			0.812	0.005	Kinase inhibitor
			0.807	0.011	Antineoplastic
2.	CID_5318619	Isoononin	0.914	0.002	Anticarcinogenic
			0.862	0.003	Histidine Kinase Inhibitor
			0.773	0.015	Antineoplastic
			0.719	0.018	HIF1A expression inhibitor
			0.711	0.004	Antioxidant

residues. These residues are critical for the functional activity of ROCK1. The depiction of the binding of Isoononin and Candidissiol is shown in Figure 1. Interaction analysis revealed that Isoononin and Candidissiol have many common interactions with the ROCK1 binding pocket (Figure 1B). They are superimposed on each other during their interactions with the ROCK1 active sites. Both compounds block the binding site of ROCK1 and bind virtuously to the deep cavity. As a result of binding, Isoononin and Candidissiol may hinder ROCK1's ATP accessibility, thus inhibiting its functionality.

Detailed interaction analyses are necessary to explore the non-covalent interactions and their types (Ali et al., 2019; Amir et al., 2020). For the detailed interactions of Isoononin and Candidissiol towards ROCK1, the selected interaction modes were examined further in 2 D plots. For Isoononin and Candidissiol, the generated 2 D plots of all possible interactions are shown in Figure 2. Isoononin and Candidissiol are seen to exhibit the same binding pattern. From the 2 D plots, it can be seen that Isoononin interacts with the ATP binding site 'Lys105' and share the common interactions as Candidissiol (Figure 2A–B). Both compounds interact with Lys105, where they reside within the ATP-binding pocket of ROCK1 and share common interactions with a known ROCK1 inhibitor 1-(4-amino-1,2,5-oxadiazol-3-yl)-5-methyl-N-({3-[(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-c]pyridin-2-yl)carbamoyl]phenyl}methyl)-1H-1,2,3-triazole-4-carboxamide, co-crystallized with PDB ID: 5WNF.

3.6. MD simulations

MD simulations of docked complexes could further refine docking models defining the protein-ligands flexibility (Naqvi et al., 2018). It gives us detailed knowledge of proteins' structure and dynamic behaviour that experimental approaches cannot provide (Amir et al., 2020; Fatima et al., 2020; Jairajpuri et al., 2020). To analyze and find the stability of the docking complexes, we run the MD simulations with the elucidated compounds Isoononin and Candidissiol in aqueous surroundings. For both the compounds, the selected poses were prepared and used in MD simulation for 100 ns. The time evaluation of different structural and dynamic parameters was recorded and analyzed to explore how ROCK1 behaves before and after the Isoononin and Candidissiol binding.

3.6.1. Structural changes and compactness

When any small molecule or a ligand binds to the protein's active site, it can induce large changes to the protein structure conformation (Guterres & Im, 2020; Shamsi et al., 2021). We examine the root-mean-square deviation (RMSD) to study these conformational changes in ROCK1 structure after the binding of elucidated compounds. RMSD helps measure the backbone fluctuations of ROCK1 before and after ligands binding (Mohammad et al., 2020). Figure 3A plot shows the time evolution of the system RMSD values. The minimal fluctuation shown in the plot signifies system stability. The RMSD plot shows that the ligand-bound states have minor fluctuations compared to ROCK1 in a free state. According to the RMSD calculated from all three plots, the simulation is in equilibrium up to 100 ns and the conformational changes over time are small. A minor RMSD fluctuation of ~0.1 nm has appeared after ligands binding (Figure 3A, upper panel). In the RMSD, the ROCK1-Isoononin complex exhibits more stability than the ROCK1-Candidissiol complex, equilibrated throughout the simulation. The RMSD distribution plot of the probability density function (PDF) shows the RMSD values mainly distributed around 0.2–0.4 nm with visible stability (Figure 3A, lower panel). In conclusion of, the RMSD results, we identified that the ligand-bound systems are stable throughout the simulation time.

To further measure the residual vibration in ROCK1, the root-mean-square fluctuation (RMSF) was plotted and studied. It helps us check the flexibility of the individual residues of all three systems (Khan et al., 2021). The plot shows that all the systems have almost the same pattern of RMSFs with increased values (Figure 3B). In this plot, the residual fluctuations are stable and minimally increased upon

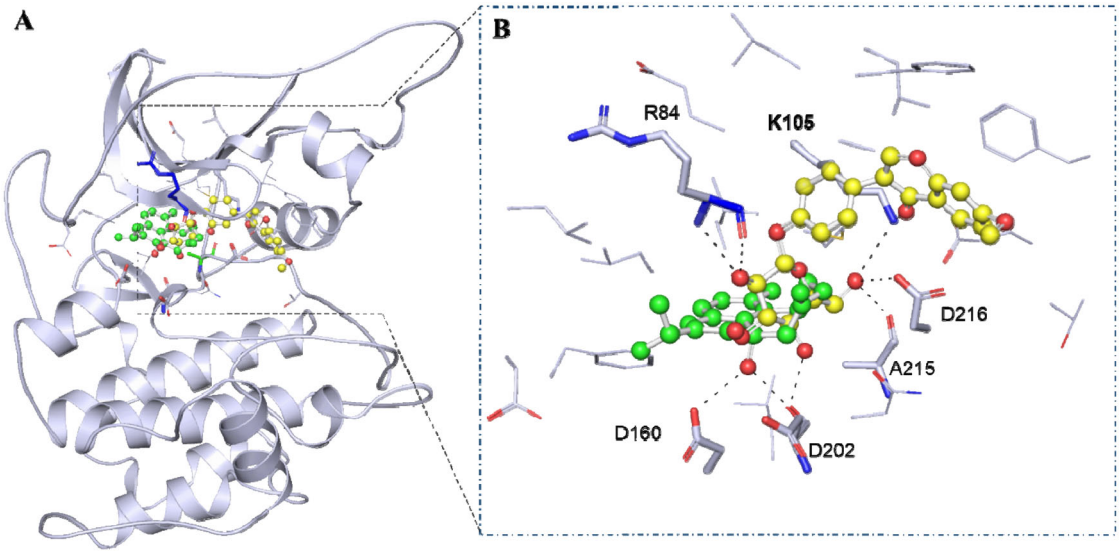

Figure 1. Structural representation of ROCK1 in complexed with Isoononin and Candidissiol. (A) Cartoon representation of ROCK1 with the selected compounds. (B) The magnified view of ROCK1 with Isoononin (green) and Candidissiol (yellow).

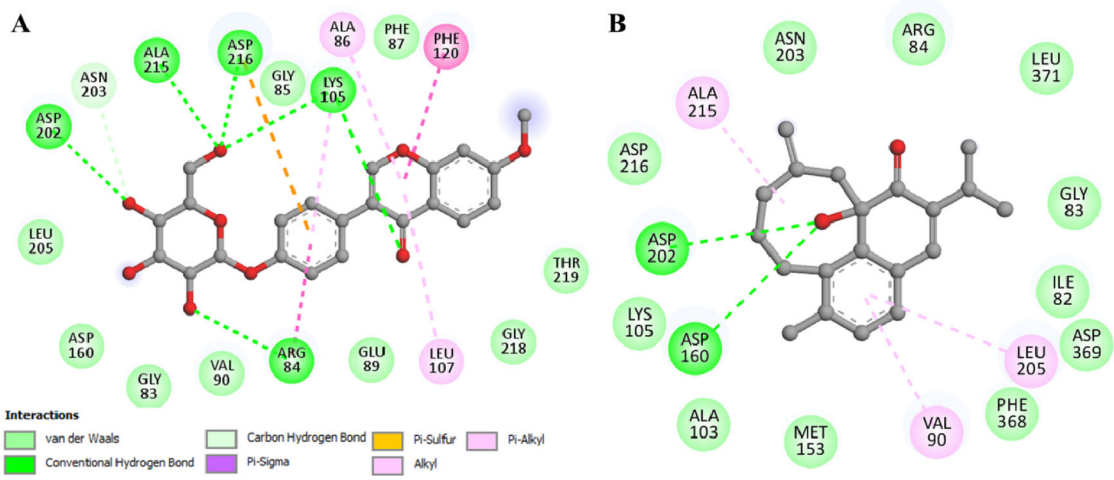

Figure 2. 2D structural representation of ROCK1 binding pocket residues and their interactions with (A) Isoononin and (B) Candidissiol.

Isoononin and Candidissiol binding, which indicates a stable protein-ligand complex. According to the RMSF analysis, the residues that interact with Isoononin and Candidissiol appeared nearly stable during the simulation and showed only minor fluctuations.

The radius of gyration (*Rg*) is the root-mean-square (RMS) distance of the atoms and then the center of mass, which is fully dependent on the tertiary structure of the protein (Lobanov et al., 2008). This parameter tells us about the structure's compactness. Time evaluation of *Rg* was conducted to determine whether ROCK1 is compact in apo and ligand-bound states or not. In MD simulation, for *Rg* values, we check both the complexes of ROCK1 with Isoononin and Candidissiol to know the structure compactness. The stable values in the trajectory of ROCK1 with Isoononin and Candidissiol range from 1.9 to 2.0 nm (Figure 4A, upper panel). According to the comparative results, ROCK1 is consistently stable in terms of structural dynamics and folding in the presence of both Isoononin and Candidissiol. Additionally, the PDF plot shows equal distribution of *Rg* values after binding Isoononin and Candidissiol to ROCK1 (Figure 4A, lower panel).

The solvent-accessible surface area (SASA) is the area of the protein that is accessible to its adjacent solvent (Richmond, 1984). It has been regarded as a key aspect in the study of protein folding and stability (Mohammad et al., 2020). The time evaluation plot of ROCK1 SASA with Isoononin and Candidissiol shows no major changes but stability during the simulation (Figure 4B). Like *Rg*, SASA also shows a similar trend in the values throughput without varying the structure's folding/unfolding and compactness. In the PDF, ROCK1 distribution and its complex with Candidissiol follow a similar pattern compared to the ROCK1-Isoononin complex (Figure 4B, lower panel).

3.6.2. Dynamics of hydrogen bonds

For stabilizing the protein structure conformation, intramolecular hydrogen bonding is important (Hubbard & Haider, 2010). The analysis helps to analyze the compactness and conformational changes of the protein (Dahiya et al., 2019). We calculated the time evaluation of intramolecular hydrogen bonding in ROCK1 before and after ligands binding in the MD simulation. This analysis explains the varying nature of intramolecular hydrogen bonding formation in ROCK1

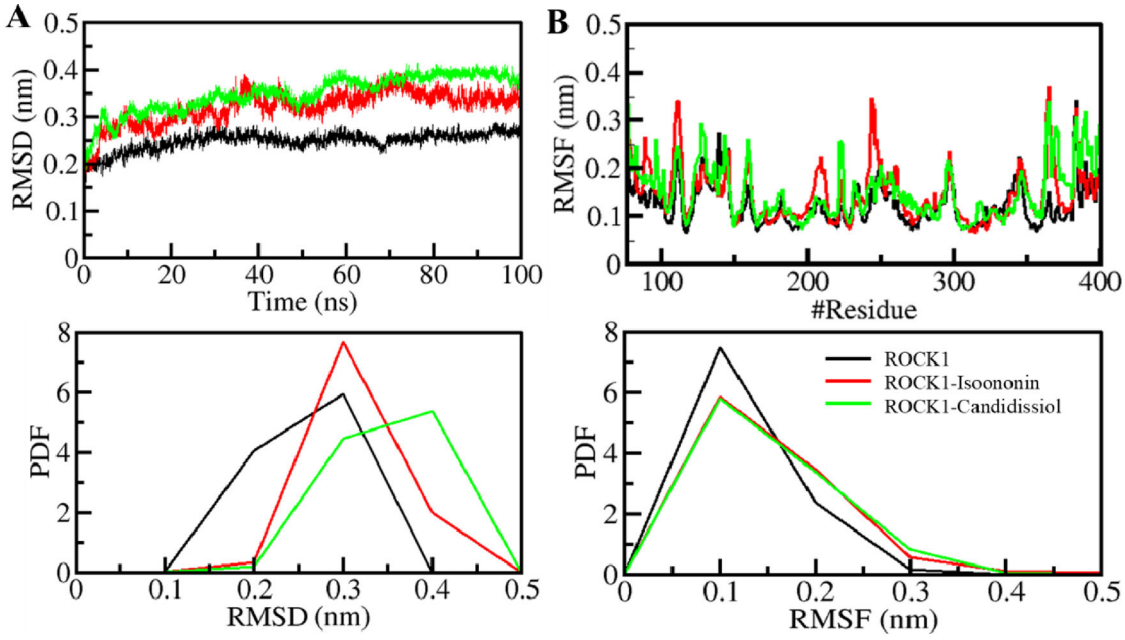

Figure 3. Structural dynamics of ROCK1 upon Isoononin and Candidissiol binding. (A) RMSD plot of ROCK1 in complexed with Isoononin and Candidissiol. (B) RMSF plot of ROCK1 and its complex with Isoononin and Candidissiol. Lower panels show the probability distribution function as PDF.

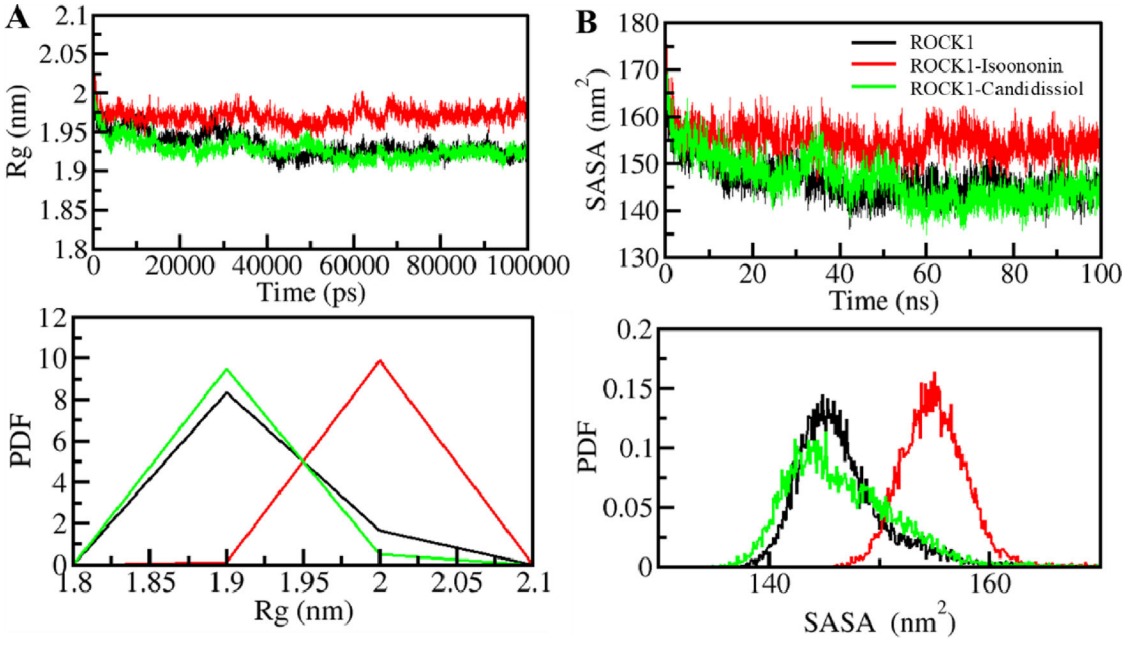

Figure 4. Structural compactness of ROCK1 upon Isoononin and Candidissiol binding. (A) The radius of the gyration plot as a function of time. (B) SASA plot of ROCK1 as a function of time before and after Isoononin and Candidissiol binding. Lower panels show the probability distribution function values as PDF.

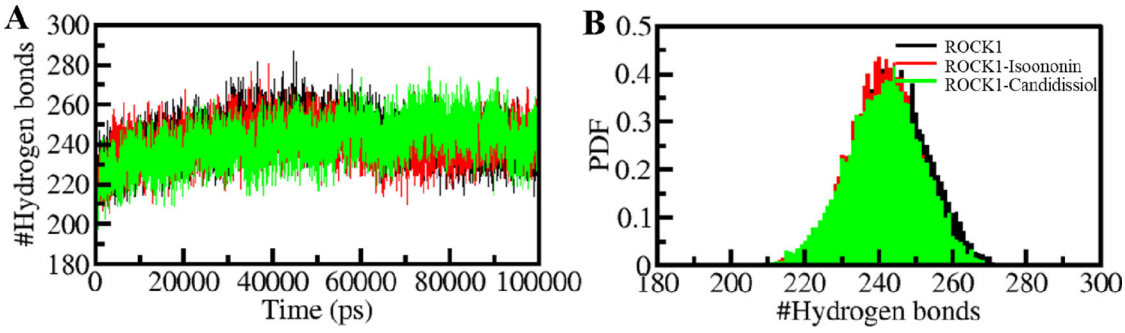

Figure 5. Intramolecular hydrogen bonding in ROCK1. (A) The time evolution of hydrogen bonds formed within 0.35 nm intra-ROCK1. (B) The PDF plot of the distribution.

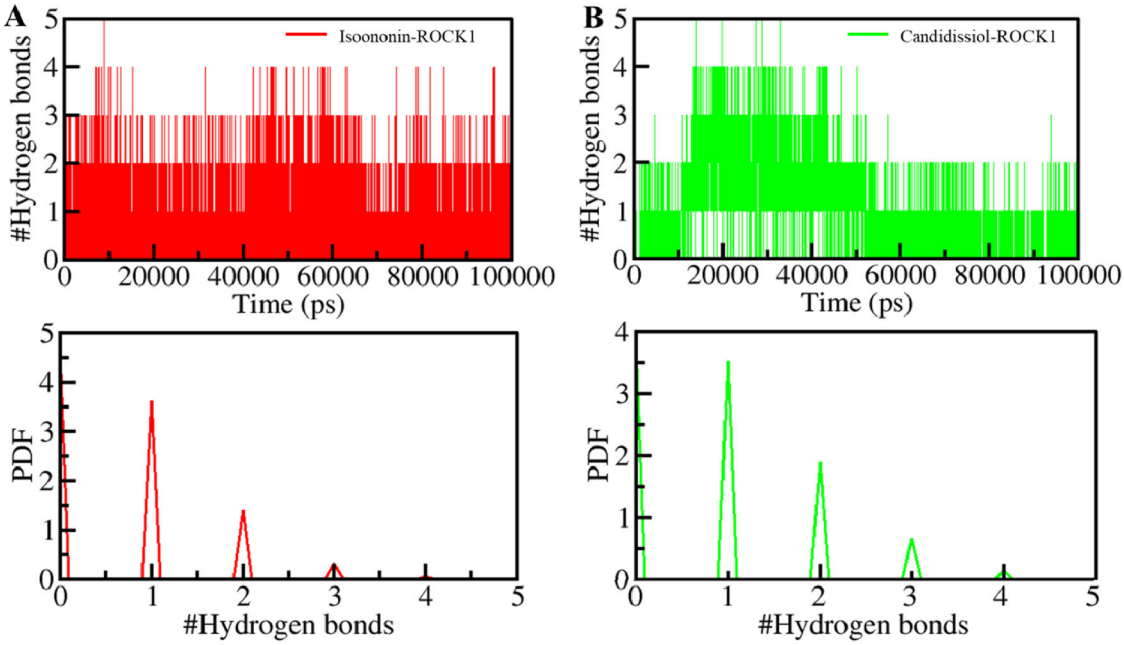

Figure 6. Time evolution of intermolecular hydrogen bonds formed within 0.35 nm between ROCK1 and (A) Isoononin and (B) Candidissiol. The lower panels show the PDF of the hydrogen bonds distribution.

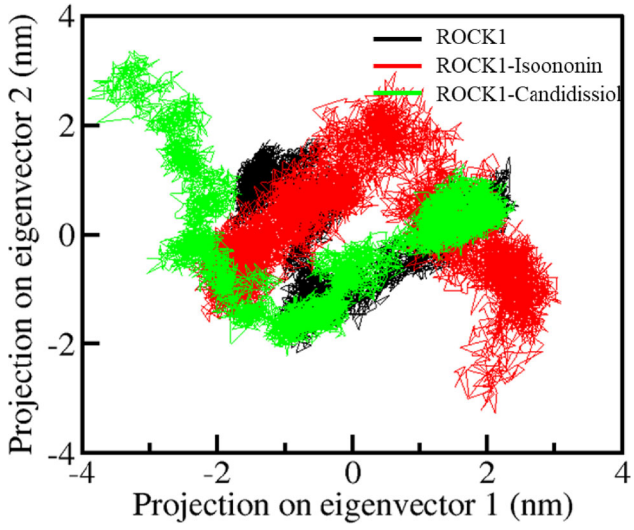

Figure 7. Conformational sampling in principal component analysis. 2 D projection of trajectories showing the conformational sampling of ROCK1, ROCK1-Candidissiol, and ROCK1-Isoononin.

with Isoononin and Candidissiol, and this variation is shown in Figure 5. This plot tells that the intramolecular hydrogen bonds formation in ROCK1 is continuous even after the binding of Isoononin and Candidissiol (Figure 5A). A good amount of constancy was shown during the simulation by all three systems in the PDF plot of the distribution of hydrogen bonding (Figure 5B).

Furthermore, the study of intermolecular hydrogen bonds was performed to determine the constancy of interactions of ROCK1 with Isoononin and Candidissiol. The average hydrogen bonds formed in the Isoononin-ROCK1 and Candidissiol-ROCK1 complex is 2 for each, which goes up to 5 bonds at a time (Figure 6, upper panels). The hydrogen bond consistency is more stable and prominent in the case of the Isoononin-ROCK1 complex than the Candidissiol-ROCK1 complex. The PDF shows a good constancy for intramolecular hydrogen bonds in both the systems, with the higher PDF values value at 1 hydrogen bond (Figure 6, lower panels). It is suggested that Isoononin and Candidissiol did not move from their initial docking position on ROCK1 due to the stable intermolecular hydrogen bonding that stabilizes the complex structures.

3.7. Principal component analysis and free energy landscapes

Principal component analysis (PCA) is a useful tool for providing information about the conformational sampling of protein structure (Maisuradze et al., 2009). So, we used this tool for the conformational sampling of ROCK1, ROCK1-Isoononin and ROCK1-Candidissiol. The PCA of the three complexes is shown in Figure 7. The plot shows that the ROCK1-Isoononin and ROCK1-Candidissiol complexes occupied most of the common essential subspace as ROCK1 in the free state. The ROCK1 free and ROCK1-Isoononin are shown in the same subspace in the EV plots. The sampling of both systems demonstrates the stability of the complexes in the simulation.

Free energy landscapes (FELs) demonstrate the folding and unfolding journey of a protein reaching its native state and achieving global minima (Altis et al., 2008). The application of FEL is to check whether the protein and protein-ligand complexes are stable or not during MD simulations. We have extracted the ROCK1, ROCK1-Isoononin and ROCK1-Candidissiol complexes' energy minima and conformational landscape using two PCs. Figure 8 shows the FELs of ROCK1, ROCK1-Isoononin and ROCK1-Candidissiol complexes. The plot tells that the Isoononin and Candidiossiol binding with ROCK1 affect the size and position of the phases confined with 1–2 global minima. The deeper blue signifies the lower

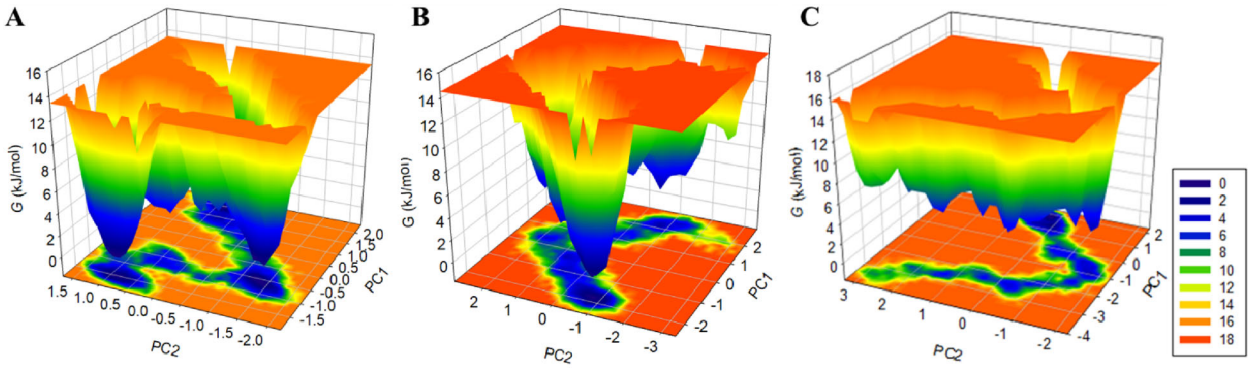

Figure 8. The free energy landscapes (FELs) of (A) free ROCK1, (B) ROCK1-Isoononin, and (C) ROCK1-Candidissiol.

energy towards global minima (Figure 8A-C). These plots demonstrate that ROCK1 is present in large single global minima expand to 2–3 basins, whereas ROCK1-Isoononin and ROCK1-Candidissiol are confined in smaller global minima with 2–3 basins.

4. Conclusions

ROCK1 is involved in numerous malignancies and is considered as a potential therapeutic target for developing effective drugs to obstruct ROCK1-mediated cancers. This study highlights the role of ROCK1as an important drug target because it functions as a positive regulator of cancer progression, growth, and various inflammatory diseases. In this study, an state-of-the-art computational approach was followed to discover potential inhibitors of ROCK1. Initially, the physicochemical properties and other drug-like properties were used to sort a library of natural products. Then, a structure-based virtual screening approach was conducted using the molecular docking approach to find high-affinity binding molecules against ROCK1. Afterward, the compounds were analyzed for ADMET, specific interactions, and PASS analysis to find safe and effective inhibitors of ROCK1. Overall, based on the binding affinity, specific interactions, and predicted biological properties, we have identified two natural products (Isoononin and Candidissiol) that have been evaluated as potent binding partners of ROCK1. We elucidated the stable binding of Isoononin and Candidissiol with ROCK1 through all-atom MD simulation, PCA, and FEL studies. Our approach proves to be useful in drug development using natural products as potent anticancer compounds of ROCK1 and as a therapeutic model for metabolic reprogramming. Overall, the presented results can be continue to be used as a guideline for the identified natural products to act as promising inhibitors of ROCK1. Present study guides to develop potent and selective small molecule inhibitors for ROCK1 to target cancer progression.

Disclosure statement

No potential conflict of interest was reported by the authors.

Informed consent statement

Not applicable.

Institutional review board statement

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Data availability statement

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