28-29 September 2023,
Kragujevac, Serbia

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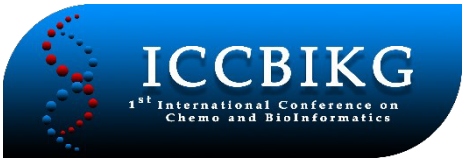
2nd International Conference on Chemo and Bioinformatics

ICCBIKG_2023

BOOK OF PROCEEDINGS



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Virtual Docking, design and *in silico* ADMET profiling of novel Rho-associated protein kinases-1 (ROCK1) inhibitors

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Abstract: Overexpression of Rho-associated protein kinases has been associated with various diseases, including tumors. None of the approved ROCK inhibitors are used for cancer treatment. However, some of them have been shown to have anti-tumor potential. The main objective of this study was to develop novel ROCK1 inhibitors using the structure-based method, molecular docking, and prediction of pharmacokinetic properties using the ADMET predictor. The key interactions that strongly correlate with the activity of ROCK1 inhibitors are hydrogen bonds between amino acid residues Met156, Glu154 and the hinge region of the inhibitors, indicating possible structural changes in the hinge region of studied compounds. On the other hand, the lack of interactions between 1,3-benzoxadiol moiety and the enzyme presents a promising approach for further structural modifications in order to design more effective ROCK1 inhibitors.

All the important interactions between the developed ROCK1 inhibitors and the binding site of the enzyme were established. They also showed acceptable pharmacokinetic properties and could be further used for synthesis and evaluation by various biological assays.

Keywords: molecular docking, ADME, design, ROCK1

1. Introduction

Rho-associated protein kinases (ROCKs) are serine-threonine kinases that have been strongly associated with the development and progression of various tumors by regulating the function of several downstream target proteins [1]. Overexpression of ROCKs leads to phosphorylation of myosin light chain, myosin phosphatase target subunit 1, LIM -kinases, ERM proteins, etc [1]. Altogether, it leads to tumorigenesis and increases the motility of cancer cells, which probably promotes the metastasis of various tumors [1]. Four ROCK inhibitors are already in clinical use (fasudil, ripasudil, netarsudil and belumosudil), but none of them is used for cancer treatment. However, some of them, such as fasudil, have shown great antitumor potential in several *in vitro* and *in vivo* studies [2]. The recent research has focused on the development of novel potent ROCK inhibitors with multitarget activity profile. The main objective of this

study was to design novel ROCK1 inhibitors using CADD (computer-aided drug design). Specifically, the structure-based method, molecular docking, and pharmacokinetic properties predicted by ADMET predictor were used to design novel ROCK1 inhibitors with stronger activity and improved pharmacokinetic properties.

2. Materials and methods

The molecular docking study was performed using GOLD 2022 software [3]. The crystal structure of ROCK1 (PDB: 4YVC) was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/). The ROCK1 inhibitors with their pIC_{50} values were obtained from the ChEMBL database (https://www.ebi.ac.uk/chembl/). The dominant microspecies of all compounds at physiological pH were selected using the program Marvin Sketch Sketch 6.1.0 (Chem Axon 2013) [4] and geometrically optimized using the semi-empirical PM3 method [5]. Discovery Studio 2020 was used to visualize the key interactions between the enzyme and ROCK1 inhibitors [6]. Ligands were ranked based on their affinity for ROCK1 using the ChemPLP score function. The virtual docking procedure was validated for further prediction of novel design inhibitors by calculating the Root Mean Square Deviation (RMSD) values, which should be below 2Å. The RMSD value was calculated by comparing the poses of heteroatoms of the co-crystallized ligand with the re-docked ligand. The binding site was defined by default according to the position of the co-crystalized ligand and all atoms within 8.0 Å of the co-crystallized ligand were selected. The Physicochemical and pharmacokinetic properties of all studied compounds were calculated by ADMET Predictor 8.5.0. [7] and were compared to already known ROCK1 inhibitor – fasudil.

3. Results and discussion

According to their chemical structure, all studied compounds can be divided into two clusters: cluster I containing pyridine derivatives and cluster II which contains oxadiazole derivatives.

Figure 1 shows the key interactions between the ROCK1 and the most active compound (CHEMBL3581126, pKi=8.150), as well as the least active compound (CHEMBL3581136, pKi= 5.600) from the cluster I, while the main interactions between the ROCK1 and the most active (CHEMBL1080071, pKi=7.070) and the least active compound (CHEMBL1079208, pKi=5.720) of cluster II are shown in **Figure 2**.

Previous studies have shown the importance of hydrogen bonding between ROCK1 inhibitors and Met156 and Glu154 of the enzyme for optimal binding to the active site of ROCK1 [8]. In the most active compounds of the I cluster, including CHEMBL3581126 (**Figure 1**), hydrogen bond is established only between the nitrogen atom of the pyridine and Met156, while it is not observed in the least active molecule, CHEMBL3581136 (**Figure 1**), suggesting its importance for ROCK1 inhibition. Considering the cluster II, a hydrogen bond between the amino group (benzimidazole part of the molecule) and Glu154 is formed only in the most active molecule (CHEMBL1080071), while it is not described in the least active compounds, including CHEMBL1079208. In addition, the oxadiazole derivatives probably did not anchor adequately in the binding site of ROCK1

because the distance between the two heterocycles was not appropriate, as also shown by the 3D-QSAR study. Based on the results of previous studies, modification of this part of the molecule should be considered to increase the activity of ROCK1 inhibitors- e.g., replacement of the pyridine with other heterocycles such as pyrazole in order to establish hydrogen bonds with Met156 and Glu154.

Numerous Van der Waals interactions have been observed for both clusters between pyridine, thiazole, benzimidazole, oxadiazole and various amino-acid residues (Leu205, Val92, Met153, Ala215, Ala103), and some of them are already described in the literature as significant for anchoring in the active site of ROCK1 [10]. 1,3-benzodioxole did not form an important interaction with the binding site of the enzyme. Therefore, this part presents an opportunity for further structural modifications.

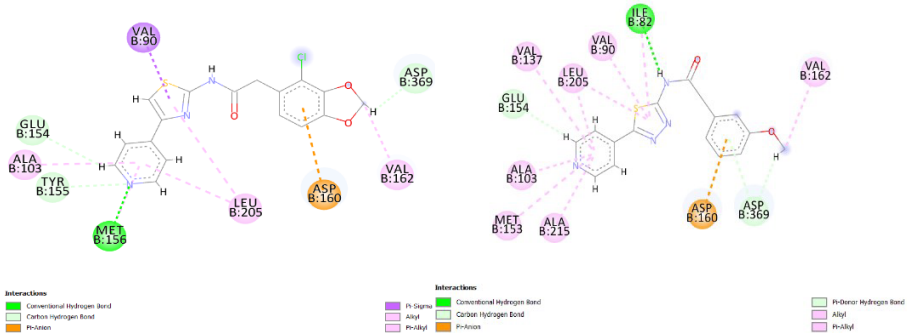

Figure 1. 2D diagram of the most important CHEMBL3581126 – ROCK1 interactions (left) and the most significant CHEMBL3581136 – ROCK1 interactions (right).

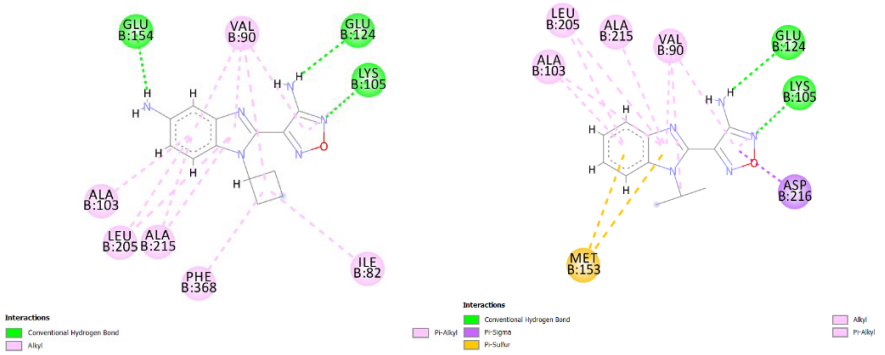

Figure 2. 2D diagram of the most important CHEMBL1080071 – ROCK1 interactions (left) and the most significant CHEMBL1079208– ROCK1 interactions (right).

The ChemPLP score values are mostly consistent with the activity of ROCK1 inhibitors in cluster I (e.g., CHEMBL3581126 – 65.35; CHEMBL3581136 – 60.09 – the lowest score), while there are some exceptions in cluster II.

Based on the results of the molecular docking study and the RMSD value obtained after redocking (0.293 Å) we can conclude that the docking procedure is valid and can be further used for the design of new ROCK1 inhibitors. The key interactions of the most active designed inhibitors according to the ChemPLP Score Values (**MB1** – ChemPLP Score Value 70.38; **MB2** - ChemPLP Score Value 69.23) are shown in **Fig.3**.

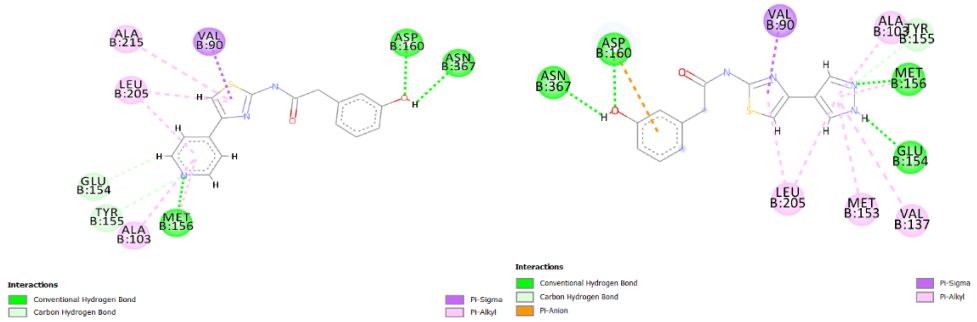

Figure 3. 2D diagram of the most important MB1 – ROCK1 interactions (left) and the most significant MB2 – ROCK1 interactions (right).

In terms of pharmacokinetic properties, designed compound MB1 (89%) is likely to cross the blood-brain barrier (BBB), which is comparable to fasudil (99%) and the most active compound from the dataset (CHEMBL3581126 - 94%), while MB2 has a low potential to cross the BBB (59%). Unlike Fasudil, CHEMBL3581126 and MB2, whose TOX risk is 1, MB1 has no TOX risk (TOX risk=0). Therefore, we can assume that MB1 will have a better safety profile.

4. Conclusion

A molecular docking study was performed, and based on the results of this study, a structural modification of the most active ROCK1 inhibitor (CHEMBL3581126) was proposed. A series of new ROCK1 inhibitors were developed, and MB1 and MB2 showed the best ChemPLP score values and pharmacokinetic properties. These compounds will be used for further synthesis and biological evaluation.

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