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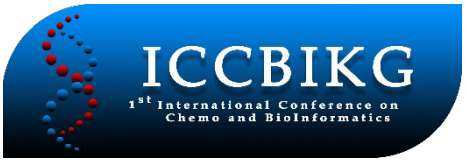
BOOK OF PROCEEDINGS



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3D-Quantitative Structure-Activity Relationship and design of novel Rho-associated protein kinases-1 (ROCK1) inhibitors

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Abstract: Rho-associated coiled-coil kinases (ROCKs) are involved in essential cellular functions such as adhesion, contraction, motility, proliferation, and cell survival/apoptosis. Four ROCK inhibitors have already been approved by the FDA and are used to treat glaucoma (ripasudil and netarsudil), cerebral vasospasm (fasudil), and graft-versus-host disease (belumosudil). Recent studies have focused on exploring the role of ROCK kinase inhibitors in cancer treatment and the development of new ROCK inhibitors. The main objective of this study was to identify critical structural features relevant to the inhibition of ROCK1 using a ligand-based 3D-QSAR (3D quantitative structure-activity relationship) method. The 3D-QSAR model for ROCK1 was created and validated using internal and external validation parameters (R^2, Q^2, R^2_{pred}, r_m^2, r'^2_m, $\overline{r_m^2}$ and Δr^2_m). The main structural features that correlate with the inhibition of ROCK1 were identified (e.g., heterocycle with hydrogen donor group like nitrogen atom) and further structural modifications of the ROCK1 inhibitors that contribute to increased activity were proposed (removal of the amino group of the oxadiazole, modification of the substituents of the phenyl ring).
Keywords: ROCK, cancer, 3D-QSAR, design

1. Introduction

Rho-associated protein kinases (ROCKs) belong to the serine-threonine protein kinases family which play an important role in the regulation of actin- and myosin-mediated processes such as cell motility, smooth muscle contraction, adhesion, and phagocytosis [1]. Physiologically, there are two isoforms of ROCK kinases, ROCK1 and ROCK2 [2]. Activation of ROCK leads to phosphorylation of myosin light chains, myosin phosphatase target subunit 1, LIM kinases, adducin and ERM proteins [3]. Besides cell motility, ROCK affects proliferation, differentiation, apoptosis, and oncogenic transmission of the cells. Disruption of ROCK kinase activity can lead to

various diseases [1-3]. For this reason, ROCK inhibitors are promising targets in the therapy of cancer, hypertension, cardiovascular and neurodegenerative diseases, smooth muscle contraction disorders, glaucoma or erectile dysfunction. To date, four ROCK inhibitors were approved by the FDA (fasudil, ripasudil, netarsudil and belumosudil) for the treatment of glaucoma, cerebral vasospasm and graft-versus-host disease [4]. The main objectives of this study were to 1) develop a 3D quantitative structure-activity relationship (3D-QSAR) model with good predictive power; 2) determine the key structural features that positively correlate with ROCK1 inhibition; 3) define the structural changes that lead to improvement in ROCK1 inhibitor activity (design of new ROCK1 inhibitors).

2. Materials and methods

The 3D-QSAR study included 48 ROCK1 inhibitors obtained from the CHEMBL database (https://www.ebi.ac.uk/chembl/) [5,6]. All compounds were divided into two clusters based on their structural similarity: pyridine derivatives (pKi: 5.563-8.167) and oxadiazole derivatives (pKi: 5.786-7.070). The Marvin Sketch program (Marvin Sketch v 16.10.24, 2016, www.chemaxon.com/) was used to determine the dominant forms for all tested compounds at a physiological pH of 7.4, while they were optimized with the semi-empirical PM3 method (Parameterized Model revision 3) using the Gaussian 098W software included in Chem3D Ultra7 (Chem3D Ultra v 7.0, 7.0, 2001, http://www.cambridgesoft.com/) [7].

The data set of 48 compounds was divided into a training set (34 ligands) and a test set (14 ligands) using Principal Component Analysis (PCA), taking into account that the entire range of pKi values is homogeneously distributed in both the training and test sets. Pentacle software 1.0.7 was used for the calculation of the alignment-independent three-dimensional molecular descriptors (GRIND) and the formation of the 3D-QSAR model [8,9]. The internal and external validation parameters were used to confirm the predictive power of the created 3D-QSAR model [10,11,12].

3. Results and discussion

The created 3D-QSAR model was used to identify structural features crucial for ROCK1 inhibition. The parameters of internal and external validation showed that the formed 3D-QSAR model was reliable and could be used to predict the activity of novel design compounds. The validation parameters for the training set of compounds, Q^2=0.760 and R^2=0.920, indicate good statistical reliability and internal predictability of the model [10,11]. In order to analyze the external predictability of the model, a test set of 14 compounds was used. The calculated values of R^2_{pred} (0.746), r_m^2, r'^2_m, $\overline{r_m^2}$ greater than 0.5, and Δr^2_m less than 0.2 indicated the possibility of applying the created 3D-QSAR model for activity prediction of newly designed molecules. Moreover, the close values of Q^2 and R^2_{pred}, as well as RMSEE (0.227) and RMSEP (0.296), confirmed the good robustness and predictive power of the created model [11,12]. The main variables that positively or negatively affected the activity of the compounds are presented by Partial Least Square (PLS) coefficient plot (**Figure 1**).

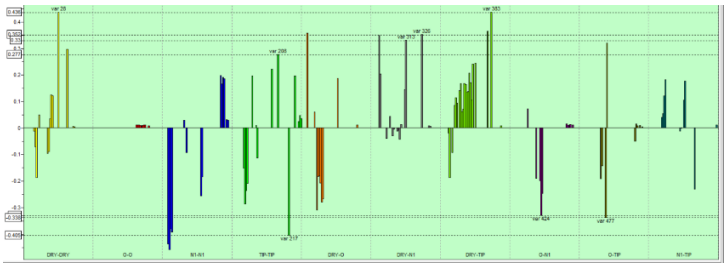

Figure 1. PLS coefficient plot for the 3D-QSAR (ROCK1) model. The most significant variables are labelled.

CHEMBL3581126 (pKi=8.100) is one of the most active compounds belonging to the first cluster. It has all favourable variables that positively correlated with ROCK1 inhibition. The favourable var28 (DRY-DRY: 11.20-11.60 Å) is described between the hydrophobic regions around the pyridine and the 1,3-benzodioxole. The optimal distance between these two groups (pyridine and 1,3-benzodioxole) is also underlined by the favourable var208 (TIP-TIP: 14.80-15.20 Å). The importance of the hydrogen accepting group of pyridine (nitrogen atom) for ROCK1 inhibition is highlighted by the favourable var313 (DRY -N1: 11.20-11.60 Å) and var326 (DRY -N1: 16.40-16.80 Å). Favourable var208, var313 and var28 are also present in the least active molecule of the first cluster, **CHEMBL3581136** (pKi=5.600), but in contrast to CHEMBL3581126 they are weakly expressed. On the other hand, the unfavourable var424 (O-N1: 10.00 - 10.40 Å) showed the negative influence of the distance between the hydrogen accepting group of pyridine (nitrogen atom) and the hydrogen donating group (nitrogen atom of the amide) on ROCK1 inhibition. It is described for both the most active and the least active molecule of the first cluster. However, besides the unfavourable var424, the unfavourable var217 (TIP-TIP: 18.40-18.80 Å) is also observed in the least active molecule (CHEMBL3581126), highlighting the negative correlation of the distance between the steric spots around pyridine and the methoxy group of the phenyl ring on ROCK1 inhibition. This variable is found in all compounds that have a methoxy group as a substituent. The lower activity of the most active compound of the second cluster -**CHEMBL1080071** (pKi=7.070) can be explained by the absence of the favourable var28 probably due to an insufficient distance between two hydrophobic regions of the molecule. Meanwhile, the favouarble var313 was found, highlighting the importance of the nitrogen atom of the oxadiazole for the activity of ROCK1 inhibitors. Moreover, the unfavourable variable 424 (O-N1: 10.00-10.40 Å) correlates negatively with the ROCK1 inhibition and is observed between the hydrogen donating group (amino group of benzoimidazole) and hydrogen accepting group (nitrogen atom of 1,3,5 – oxadiazole) of CHEMBL1080071. In one of the least active compounds of the second cluster, **CHEMBL1079208** (pKi=5.720), the favourable variables are not described, while the presence of unfavourable var477 (O-TIP:8.40-8.80 Å) emphasizes the negative influence of the distance between hydrogen donor group (amino group at position C-3 of oxadiazole) and steric spot around the benzimidazole on ROCK1 inhibition.

4. Conclusion

Taking into account all the previous results, we can assume that:

1) the presence of a hydrogen accepting group of the heterocycle (nitrogen atom of pyridine or oxadiazole) is crucial for ROCK1 inhibition due to the positive impact of var313 and var326;
2) replacement of the methoxy group with other groups (e.g., electron-withdrawing groups) may correlate positively with ROCK1 inhibition due to the elimination of the influence of unfavourable var217;
3) the removal of the amino group of the oxadiazole may positively contribute to the activity of ROCK1 inhibitors by reducing the negative influence of var477
4) the optimal distance between two hydrophobic parts of molecules can be crucial for ROCK1 inhibition due to the influence of var28. Therefore, the length of the linker should be taken into account when designing new ROCK1 inhibitors.

In accordance with the previous conclusions, some novel ROCK1 inhibitors were. Designed. Their activities were predicted using created 3D-QSAR models and some of them showed good ROCK1 inhibitory activity, MBT1 (derivative of CHEMBL3581147, which has chloro group instead of methoxy group, predicted pKi=7.520) and MBT2 (derivative of CHEMBL1080071, whose amino group of oxadiazole was removed, predicted pKi=7.340). These compounds will be further synthesized and evaluated by enzyme and cancer cell assays.

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References

[1] Julian L, Olson M F (2014) Rho-associated coiled-coil containing kinases (ROCK): structure, regulation, and functions, Small GTPases. 5(2), e29846.
[2] Amano M, Nakayama M, Kaibuchi K (2010) Rho-kinase/ROCK: A key regulator of the cytoskeleton and cell polarity. Cytoskeleton, 67(9), 545–554.
[3] Hahmann C, Schroeter T (2009). Rho-kinase inhibitors as therapeutics: from pan inhibition to isoform selectivity. Cellular and Molecular Life Sciences, 67(2), 171–177
[4] Barcelo, J., Samain, R., & Sanz-Moreno, V. (2023). Preclinical to clinical utility of ROCK inhibitors in cancer. *Trends in Cancer*
[5] Bandarage U, Hare B, Parsons J, Pham L, Marhefka C, Bemis G, … Green J (2009) 4-(Benzimidazol-2-yl)-1,2,5-oxadiazol-3-ylamine derivatives: Potent and selective p70S6 kinase inhibitors. Bioorganic & Medicinal Chemistry Letters, 19(17), 5191–5194
[6] Green J, Cao J, Bandarage U K, Gao H, Court J, Marhefka C, … Rodems S (2015) Design, Synthesis, and Structure–Activity Relationships of Pyridine-Based Rho Kinase (ROCK) Inhibitors. Journal of Medicinal Chemistry, 58(12), 5028–5037
[7] Frisch, M. J. (1998). Gaussian 98 (RevisionA.7). Pittsburgh, PA: Gaussian, Inc.
[8] Pastor M, Cruciani G, McLay I, Pickett S, Clementi S (2000) Grid-INdependent Descriptors (GRIND): A novel class of alignment-independent three-dimensional molecular descriptors. Journal of Medicinal Chemistry, 43(17), 3233–3243.

[9] Pentacle,Version1.0.6,MolecularDiscoveryLtd.,Perugia,Italy;2009
[10] Ojha P K, Roy K (2011) Comparative QSARs for antimalarial endochins: Importance of descriptor-thinning and noise reduction prior to feature selection. Chemometrics and Intelligent Laborary Systems., 109(2), 146–161
[11] Tropsha, A. (2010). Best practices for QSAR model development, validation, and exploitation. Molecular Informatics, 29(6-7), 476–488
[12] Roy P P, Roy K (2008) On some aspects of variable selection for partial least squares regression models. QSAR & Combinatorial Science, 27(3), 302–313.