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# VIRTUAL SCREENING STRATEGY FOR IDENTIFYING NEW SMALL-MOLECULE ANTAGONISTS OF INTEGRIN αIIBβ3

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***Aim.*** To develop an optimal strategy for identifying new small-molecule antagonists of integrin αIIbβ3 using virtual screening.

***Methods.*** Molecular modeling.

***Results.*** The 7TMZ-based model demonstrated high classification accuracy (receiver operating characteristic area under the curve (ROC AUC): 84.285) and pose reproducibility (root mean square deviation (RMSD): 0.326 Å). The 3T3M-based model demonstrated high pose reproducibility (RMSD of 0.218 Å for RUC2 and 0.254 Å for RUC1).

***Conclusions.*** Two virtual screening models were developed to identify integrin αIIbβ3 antagonists that do not induce receptor unfolding. Preliminary evaluation suggests their strong potential in selecting active compounds.

***Keywords:*** virtual screening, small-molecule antagonists, integrin αIIbβ3, glycoprotein IIb/IIIa.

Integrin αIIbβ3 antagonists inhibit platelet-fibrinogen binding, preventing blood clotting. They are used to reduce thrombosis related to certain medical conditions and procedures [1].

Clinically used αIIbβ3 antagonists include the αIIbβ3-specific antibody abciximab, the cyclic heptapeptide eptifibatide, which contains a KGD (Lys-Gly-Asp) sequence, and the peptidomimetic tirofiban, which mimics the RGD (Arg-Gly-Asp) sequence [2]. Despite their anticoagulant effect, these drugs are associated with the development of thrombocytopenia [3]. A conformational change may cause this side effect, the unfolding of integrin αIIbβ3 on the platelet surface upon interaction with antagonists. It is assumed that the unfolding of integrin makes the immunogenic site on the surface of integrin accessible, which leads to an immune response, which in turn leads to the development of thrombocytopenia [4]. Such unfolding may also lead to a high level of active platelets after a decrease in the antagonist concentration in the blood plasma, which may be the reason why a number of other RGD peptidomimetics failed to receive regulatory approval [5, 6].

However, some small-molecule inhibitors are able to exert an antagonistic effect without inducing αIIbβ3 integrin unfolding. Among them are both stabilizing RGD peptidomimetics and conformationally neutral compounds of another type of binding — the RUC family. Lin, Fu-Yang, et al. proposed that stabilization of the closed integrin conformation is mediated by a specific water molecule in the fibrinogen binding site. Displacement of this water leads to Ser123-$Mg^{2+}$ contact, causing integrin unfolding [7]. Therefore, RGD peptidomimetics that stabilize this key water molecule may not have those mentioned above adverse clinical effects. And since the dominant conformation of integrin in the body is closed, the family of conformationally neutral RUC compounds is also promising.

Thus, the search for new low-molecular antagonists of integrin αIIbβ3 that don't induce receptor unfolding can result in a significant contribution to the direction of platelet aggregation inhibition.

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For this purpose, the use of the method of virtual screening based on molecular docking is promising. Virtual screening can be divided into two parts: the search for RGD peptidomimetics that stabilize the closed conformation of integrin and the search for antagonists that have the binding type of compounds of the RUC family. Two models were created for this purpose.

*Aim.* To develop an optimal strategy for identifying new small-molecule antagonists of integrin αIIbβ3 using virtual screening.

*Methods.* To identify RGD peptidomimetics that stabilize the closed conformation, a model was created based on the crystal structure of the integrin αIIbβ3 complex with the stabilizing ligand BMS4 (PDB ID: 7TMZ). This model includes the water molecule essential for stabilization and two additional molecules that interact with the $Mg^{2+}$.

To identify antagonists that have the binding type of RUC family compounds, a model was created based on the crystal structure of the RUC2-integrin αIIbβ3 complex (PDB ID: 3T3M). This model includes two molecules that mediate the hydrogen bond of the ligand with residue D232. The construction and evaluation of the models were carried out using the ICM-Pro software (Molsoft LLC, USA). The dudes module of the Tldr's Ligand Discovery Resource service (UCSF, USA) was utilized to generate the test set of compounds [8].

*Results and Discussion.* The model for identifying RGD peptidomimetics that stabilize the closed conformation demonstrated high classification accuracy and pose reproducibility for active compounds. The model achieved an ROC AUC of 84.285 (Fig. 1). The RMSD between the pose of the ligand from the 7TMZ (redocking), and the model prediction was 0.326 Å. The average RMSD between the poses of similar stabilizing RGD peptidomimetics from the crystal structures 7UCY, 7TCT, 7UJE, 7U9F, 7U9V, 7UDH, 7UBR, and the predicted ones was 0.326 Å.

The model for identifying antagonists that have RUC family binding type demonstrated relatively high pose reproducibility, with an RMSD of 0.218 Å for RUC2 (redocking) and 0.254 Å for RUC1.

The determined classification accuracy of the RGD peptidomimetic screening model may be underestimated since the list of active compounds in the test set included all compounds from the ChEMBL database with a pChEMBL value ≥ 6, regardless of interaction type. Still, the model was optimized for the selection of integrin-stabilizing compounds.

Assessing the relevant classification accuracy of the RUC family antagonist screening model is challenging due to the limited number of documented active compounds with sufficiently high activity (pChEMBL value ≥ 6).

*Conclusions.* As the result of the development of an optimal virtual screening strategy for identifying small-molecule integrin αIIbβ3 antagonists, two virtual models were created and tested. Each model is optimized for the selection of one of two types of antagonists that do not induce integrin unfolding, which can cause adverse clinical effects. Preliminary evaluation suggests that these models have strong potential for identifying suitable active compounds.

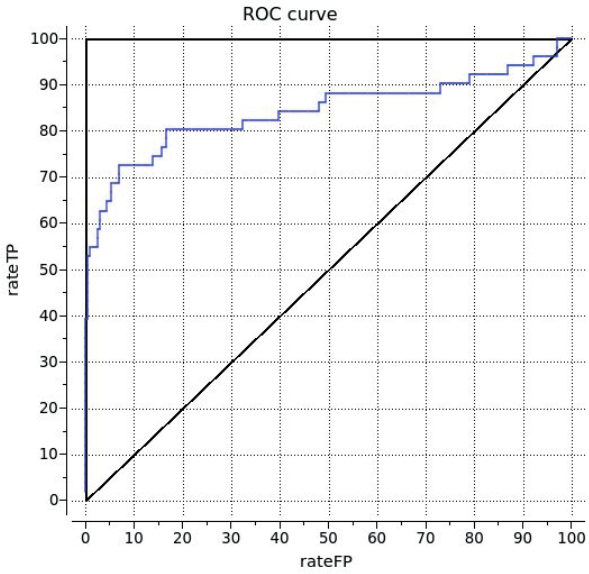


*Fig. 1.* **Receiver operating characteristic curve for the 7TMZ-based model:** rateFP — false positive rate; rateTP — actual positive rate

The determined classification accuracy of the RGD peptidomimetic screening model may be underestimated since the list of active compounds in the test set included all compounds from the ChEMBL database with a ChEMBL act value ≥ 6, regardless of interaction type. Still, the model was optimized for the selection of integrin-stabilizing compounds.

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*Author's contribution*

OC developed the virtual screening strategy, built and analyzed the virtual models, and prepared the manuscript.

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