IN SILICO PREDICTION OF THE THROMBOXANE A SYNTHASE INHIBITION OF ANTIPLATELET PYRAZOLOPYRIDINE COMPOUNDS WITH MACHINE LEARNING APPROACH

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Introduction

Thromboxane A synthase (TXAS) is an enzyme of arachidonic acid (AA) pathway responsible for synthesizing TXA_2, a potent platelet aggregating that plays a vital role in thrombotic events [1]. Virtual screening (VS) using molecular docking is a helpful tool for identifying putative inhibitors for several targets through scoring functions to predict the ligand-protein binding affinity [2]. In this context, machine learning algorithms enable to obtain classification models to improve the ranking of potential compounds by molecular docking [2]. This work aimed to identify putative inhibitors of TXAS (TXASI) in pyrazolopyridine derivatives that showed antiplatelet activity *in vitro* [6-10]. In this study we used a machine learning classification model based on molecular docking and physicochemical properties of ligands.

Methods

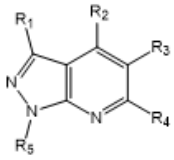
ChemBL database (www.ebi.ac.uk/chembl) was used to search TXASI and to create an inhibitors dataset to train the model. After removing duplicated structures, inorganic salts, and undefined stereoisomerism, we considered two sets of 144 compounds each: active set ($pIC_{50} \geq 7.3$) and inactive set ($pIC_{50} \leq 7.0$). We used the following programs: Open Babel [3] to generate the 3D structures (pH=7.8); GOLD [4] to perform the molecular docking; DataWarrior [5] for physicochemical properties; and KNIME (www.knime.com) to develop the classification models. The datasets were normalized, filtered by linear correlation, and partitioned in the training and test sets (70:30). The tenfold internal cross-validation was carried out with Extreme Gradient Boosting (XGBoost). The best metric model was used in the VS of a dataset of antiplatelet pyrazolopyridine compounds [6-10].

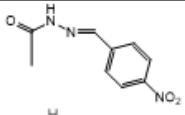
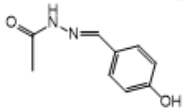
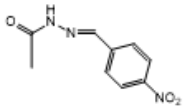
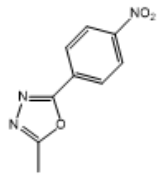
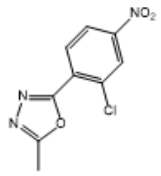
Results / Discussion

The ChemPLP classification model with stratified random partition mode showed the best metric of receiver operating characteristic curve (AUC-ROC=0.82), Matthew's correlation coefficient (MCC=0.49), and F1 (0.74), with a recall of 74% of actives, 75% of inactives and accuracy of 74%. The prediction for all compounds in the test and dataset was considered reliable. Our results predicted that 6 of 187 pyrazolopyridine derivatives from the dataset would be active as TXASI (Table 1). In this group, 116 compounds without the carbohydrazide moiety in R_2 [6] were previously tested *in vitro* for inhibition of collagen-induced platelet aggregation and only one compound was predicted as TXASI (3a). Compounds with carbohydrazide [7-9] and 1,3,4-oxadiazole moiety [10] in R_2 were previously tested *in vitro* for inhibition of AA-induced platelet aggregation. We predicted as active compounds three of the set of 63 carbohydrazide derivatives (1a-c) and two of the 8 compounds with the 1,3,4-oxadiazole group (2a-b) in R_2. The predicted active compounds showed hydrogen bond interactions in the active site of TXAS, observed in molecular docking. The amino acid residues Asn103, Ser113, Gln334, Ala341 and Thr411 interacts with H-bonding donors (HBD) or acceptors (HBA) at the R_2 and R_3 with NH_2 and OH (3a), carbohydrazide (1a-b) and oxadiazole (2a-b). In R_2, a 4-substituted phenyl ring in compounds 1a-c and 2a-b with HBD or HBA atoms from NO_2 and OH

also seems relevant to activity. Besides, the exchange of a methyl group in 1a for a phenyl in 1c at R_4 position of the pyrazolopyridine ring influenced the loss of interaction with Asn103.

Table 1. Pyrazolopyridines derivatives predicted as TXAS inhibitors by a machine learning classification model.



N°	R_1	R_2	R_3	R_4	R_5	P(active) TXAS	Inhibition of AA-induced aggregation (%)
1a	CH_3		H	CH_3	Ph	0,74	95,1
1b	CH_3		H	CH_3	Ph	0,74	96,8
1c	CH_3		H	Ph	Ph	0,60	92,2
2a	CH_3		H	Ph	Ph	0,69	97,0
2b	CH_3		H	Ph	Ph	0,60	NT
3a	H	NH_2	CH_2OH	Ph-4-OCH_3	Ph-4-OCH_3	0,63	NT

1a-c [7-9]; 2a-b [10]; 3a [6]; NT= not tested

Conclusion

We use machine learning classification models in the VS of antiplatelet pyrazolopyridine derivatives in the TXAS target. The predicted TXASI showed H-bond interactions in R_2 and R_3 positions with amino acids residues of the enzyme's active site. The position R_4 of the pyrazolopyridine ring also seems to be important for these interactions. These results can be useful for the design of new antiplatelet pyrazolopyridine derivatives.

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