DESIGN AND SYNTHESIS OF NEW COMPOUNDS WITH ANTIPLATELET ACTIVITY

Peçanha, B.R.B. [1]*; Oliveira, E.J.M.; Lima, C.H.S.[2]; Sathler, P.C. [3]; Dias, L.R.S.[1]**
[1]*Universidade Federal Fluminense/Faculdade de Farmácia, R. Mario Viana 523, Niterói, RJ, Brazil*
[2]*Universidade Federal do Rio de Janeiro/Instituto de Química, Av. Athos da Silveira Ramos 149, Rio de Janeiro, Brazil*
[3]*Universidade Federal do Rio de Janeiro/Faculdade de Farmácia, Av. Carlos Chagas Filho, 373, Rio de Janeiro, Brazil*
**brunapecanha@id.uff.br* ***lrsdias@id.uff.br*

Introduction

Computational methods, such as molecular docking and machine learning (ML), can accelerate drug discovery by predicting bioactive molecules [1]. This study focuses on thromboxane synthase (TXAS), an enzyme that plays a crucial role in the biosynthesis of thromboxane A_2 (TXA_2) in the arachidonic acid metabolic pathway. TXA_2 is a potent platelet-aggregating prostanoid involved in thrombus formation and vasoconstriction, which can lead to cardiovascular diseases [2].

In this work, new compounds were designed using computational techniques, synthesized, and evaluated for their antiplatelet activity.

Material and Methods

We generated a virtual library of new chemical structures based on pyrazolopyridine compounds with antiplatelet activity from the literature [3,4]. The generated library was subjected to a virtual screening using molecular docking simulation and ML [5].

Reagents were obtained commercially or prepared by modifying functional groups for the synthesis. TLC monitored reactions on silica gel plates using a gradient eluent and UV visualization. Structural identification of synthesized compounds was carried out using the following spectroscopic analyses: FTIR by ATR on a Shimadzu IRTracer-100; ^{1}H NMR on Varian VNMRS 500 MHz and Bruker 500 MHz spectrometers; ^{13}C NMR, DEPTQ, HSQC, and HMBC on Bruker 500 and 400 MHz spectrometers; and HRMS on a Bruker ESI-Q20 TOF in positive ion mode.

The compounds were tested for their ability to inhibit platelet aggregation in human plasma induced by arachidonic acid. Platelet aggregation was measured using a turbidimetric method and expressed as percent inhibition relative to a control. DMSO 1% was used as the solvent, and acetylsalicylic acid as a positive control [3].

Results and Discussion

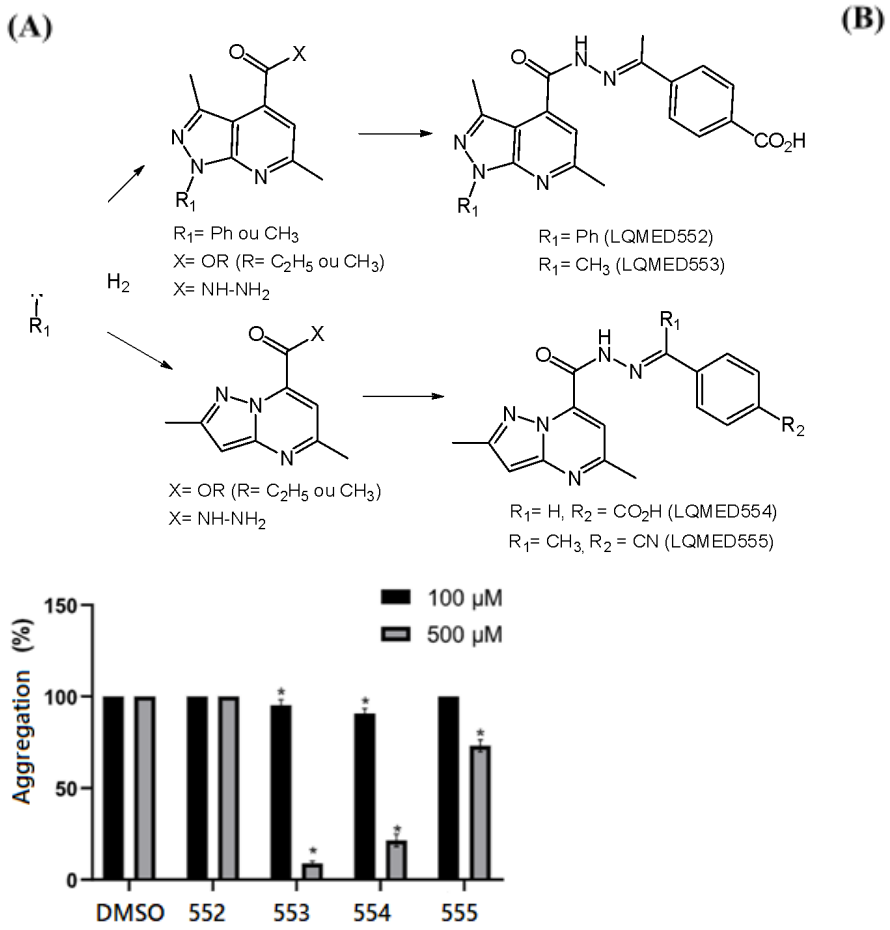
We created a virtual library of chemical structures comprising 142 proposed molecular structures related to pyrazolopyridine compounds with antiplatelet activity from the literature [3,4]. These structures were submitted to a classification model built with ML in the KNIME program to classify the structures as active or inactive. Forty-seven structures were classified as active, presenting a probability (P) of TXAS inhibition with $P \geq 0.5$ [5,6].

Four molecular structures were selected for chemical synthesis: two 1*H*-pyrazolo[3,4-*b*]pyridine and two pyrazolo[1,5-*a*]pyridine (**Fig. 1**). These were predicted to have the highest probability of activity ($P \geq 0.70$) by the classification model. The four compounds were synthesized from

5-amino-3-methyl-1*H*-pyrazoles varying R1 substituents. They were characterized by melting point and spectroscopic techniques [6].

The compounds LQMED552-555 were tested for their ability to inhibit platelet aggregation. Only those predicted with the best results for TXAS inhibition, LQMED553 (P=0.83) and LQMED554 (P=0.82), showed inhibition at a 500 µM concentration (**Fig. 1**).

Fig. 1. Synthesis (A) and *in vitro* antiplatelet activity (B) of the designed compounds: 1*H*-pyrazolo[3,4-*b*]pyridine (LQMED552 and 553) and pyrazolo[1,5-*a*]pyrimidine (LQMED554 and 555).



Conclusion

Integrating molecular docking and machine learning, virtual screening identified promising compounds for inhibiting the TXAS enzyme. This approach streamlined the discovery process for bioactive compounds by reducing the number of compounds selected for synthesis and testing.

Results suggest that the antiplatelet activity exhibited by the newly synthesized compounds is related to the predicted TXAS enzyme inhibition potential from the virtual screening and that this action is concentration-dependent.

Acknowledgments

The authors thank for the support of the sponsors FAPERJ (E-26/210.915/2021), CAPES (Finance Code 001), PQI-UFF 001/20118 for B.R.B. Peçanha, and CNPq for the scientific initiation scholarship of E. J. M. Oliveira.

Bibliographic References

[1] Maia, E.H.B. et al. Structure-Based Virtual Screening: From Classical to Artificial Intelligence. Front. Chem., 2020, 8, article 343.
[2] Mesitskaya, D. et al. Thromboxane A synthase: A new target for the treatment of cardiovascular diseases, Cardiovasc. Hematol. Agents Med. Chem., 2018, 16, (2), 81–87.
[3] Lourenço, A., et al. Synthesis and mechanistic evaluation of novel N'-benzylidenecarbohydrazide-1*H*-pyrazolo[3,4-*b*]pyridine derivatives as non-anionic antiplatelet agents, Eur. J. Med. Chem., 2017, 135, 213-229.
[4] SHI, X. Ozagrel meglumine salt and the composition, preparation method and application thereof. Shenyang Zhonghai Biotechnology Co., Ltd. (Shengyang [CN]). CN 104610153. 2015.
[5] Peçanha, B.R.B.; Flores Jr., L.A.P.; Lima, C.H.S.; Dias, L.R.S. In silico prediction of the thromboxane A synthase inhibition of antiplatelet pyrazolopyridine compounds with machine learning approach. 4th Electronic Conference for the Faculty of Pharmacy Graduate Program – UFF. November, 2022.
[6] Peçanha, B.R.B. Aplicação de técnicas de aprendizagem de máquina no planejamento de inibidores de enzimas do processo de agregação plaquetária e síntese dos compostos planejados. Doctoral thesis. UFF, 2024.