Citric Acid as a Precursor for Novel Synthetic Pathways to Bioactive Molecules

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Citric acid (CA) has emerged as a highly versatile, renewable, and economically viable bioproduct that extends far beyond its traditional industrial applications, offering significant potential as a building block in synthetic organic chemistry. The thermal condensation of CA with various primary amine precursors has been primarily used for the development of carbon dots (CDs) [1]. However, an important yet insufficiently explored aspect of these processes is the potential of the simultaneously generated small-molecule fluorophores. The literature indicates that these small molecules possess structures remarkably similar to the core scaffolds of numerous bioactive compounds. Despite the well-documented nature of CA-amine condensation, there are only limited studies focusing on the potential of specific reaction products, their modification, and pharmacological evaluation.

The primary objective of this research is to evaluate the feasibility of using simple reactions between CA and primary amines to obtain novel compounds with potential biological activity. The resulting derivatives share common structural elements, specifically 2-pyridone and pyrrolo[3,4-c]pyridine scaffolds. These are highly privileged motifs in medicinal chemistry, extensively reported in the literature as promising agents exhibiting anti-inflammatory properties [2–5]. However, the conventional synthetic approaches for these scaffolds are often multi-step processes that require the use of various catalysts and organic solvents. Consequently, implementing a straightforward synthetic pathway starting from citric acid offers a highly attractive and more sustainable alternative.

During the course of the work, various synthetic and modification approaches were explored. The research involved typical two-component condensation reactions between CA and selected amines, as well as three-component approaches. Furthermore, the mechanisms underlying these three-component reactions were investigated experimentally. Additionally, to increase the structural diversity of the proposed ligands, the compounds obtained via the initial two-component reactions were subjected to targeted structural modifications. These approaches included secondary condensations, implementation of Mannich reactions, the oxidation of sulfur atoms to sulfoxides and sulfones, as well as aminolysis involving the opening of the pyrrole ring in pyrrolo[3,4-c]pyridine compounds. To guide the synthetic efforts and estimate the therapeutic potential of the proposed structures, the designed compounds were subjected to standard *in silico* screening. Specifically, molecular docking was employed to model the binding affinity and interactions of the proposed derivatives with cyclooxygenase enzymes (COX-1 and COX-2), which are common targets for anti-inflammatory agents. Molecular docking studies were performed using known ligands as references, followed by visualization of interactions within the binding pocket. Beyond predicting biological activity, the physicochemical profiles of these virtual structures were thoroughly investigated. Parameters related to drug-likeness (e.g., Lipinski's Rule of Five [6]) and predicted bioavailability were assessed *in silico* using established online predictive platforms.

As part of the conducted analyses, synthetic pathways were developed to obtain the target structures. Molecular docking revealed that most of the theoretically designed compounds exhibit a higher binding affinity for cyclooxygenase-1 (COX-1) than for cyclooxygenase-2 (COX-2). The vast majority of these compounds possess parameters indicating favorable bioavailability and comply with Lipinski's Rule of Five.

In summary, this study demonstrates that a straightforward and sustainable synthetic pathway starting from citric acid can successfully yield novel, bioavailable compounds with promising anti-inflammatory potential.

REFERENCES

[1] W. Kasprzyk, T. Świergosz, P. P. Romańczyk, J. Feldmann and J. K. Stolarczyk, (2022), Nanoscale, 14368–14384.
[2] Wójcicka, A.; Redzicka, A. (2021) An Overview of the Biological Activity of Pyrrolo[3,4-c]pyridine Derivatives. *Pharmaceuticals*, 14, 354. https://doi.org/10.3390/ph14040354
[3] Mostafa M.K. Amer, Maged A. Aziz, Wesam S. Shehab, Magda H. Abdellattif, Samar M. Mouneir, (2021) "Recent advances in chemistry and pharmacological aspects of 2-pyridone scaffolds", *Journal of Saudi Chemical Society*, 25(6), https://doi.org/10.1016/j.jscs.2021.101259.
[4] Davana S. Gonçalves, Samara M. de S. Melo, Andrey P. Jacomini, Michael J. V. da Silva, Karlos E. Pianoski, Franciele Q. Ames, Rafael P. Aguiar, Alisson Felipe Oliveira, Hélito Volpato, Danielle L. Bidóia, Celso V. Nakamura, Ciomar A. Bersani-Amado, Davi F. Back, Sidnei Moura, Fávero R. Paula, Fernanda A. Rosa, (2020) "Synthesis of novel 3,5,6-trisubstituted 2-pyridone derivatives and evaluation for their anti-inflammatory activity", *Bioorganic & Medicinal Chemistry*, 28(12), https://doi.org/10.1016/j.bmc.2020.115549
[5] Krzyżak, E., Szkatuła, D., Wiatrak, B., Gębarowski, T., & Marciniak, A. (2020). Synthesis, Cyclooxygenases Inhibition Activities and Interactions with BSA of *N*-substituted 1*H*-pyrrolo[3,4-c]pyridine-1,3(*2H*)-diones Derivatives. *Molecules*, *25*(12), 2934. https://doi.org/10.3390/molecules25122934
[6] Lipinski CA (2004) "Lead- and drug-like compounds: the rule-of-five revolution". *Drug Discovery Today: Technologies*. 1 (4): 337–341. doi:10.1016/j.ddtec.2004.11.007