INTERACTION PROFILE AND SAFETY ASSESSMENT OF DEGRADATION PRODUCTS OF SGLT-2 INHIBITOR DRUGS USING *IN SILICO* METHODS

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Introduction

Type II Diabetes mellitus is a complex metabolic disease whose main characteristic is hyperglycemia. If untreated, this hyperglycemic state can result in progressively worsening tissue damage, leading to a consequent reduction in patient's quality of life and life expectancy. Several classes of drugs have been studied in an attempt to reduce the morbidity and mortality attributed to the disease. One of the most recent and promising are the gliflozins, which are inhibitors of the sodium-glucose co-transporter type II (SGLT2 inhibitors)[1]. By blocking renal SGLT2, these drugs promote increased glucose excretion through urine, contributing to a reduction in blood glucose levels, with few reported side effects. However, they are subject to environmental factors such as temperature variations and/or light exposure, which can lead to the degradation of their original structures and the formation of secondary compounds known as degradation products (DPs). DPs may or may not interact with the target of the original substance, or even with other biological targets, leading to uncertain or undesirable effects. Regulatory agencies currently require the analysis of DPs in drugs marketed[2]. However, obtaining these products has become a limiting factor for *in vitro* and *in vivo* testing. Therefore, *in silico* methods are recommended as they do not depend on the quantity of analyte[3]. Thus, the objective of this work is to use artificial intelligence tools to identify potential molecular targets of SGLT2 inhibitor DPs, mapping their interaction modes and assessing the toxicological risk of each DP.

Material and Methods

Initially, a literature search was conducted using the terms "Degradation Products," "Empagliflozin," "Ertugliflozin," "Dapagliflozin," and "Canagliflozin," along with the Boolean operators "AND" and "OR" in the *Scopus* and *PubMed* databases. Fifty-four degradation products were identified of the drugs of interest. The structures were drawn in 2D and 3D using the *Maestro 14.1* program and then subjected to *in silico* toxicological analysis with the help of *ADMET Predictor 12* and *QSAR Toolbox 4.7*, enabling the classification of impurities according to ICH-M7 through two distinct predictive models. Following this step, other potential biological targets of the DPs were identified using the Target Fishing technique, using the *Swiss Target Prediction* plataform, which performs a mathematical correlation between the target ligand and known ligands in a database to suggest possible molecular targets. This provides the necessary support for conducting Molecular Docking, using the *GOLD 2024.2* program, in order to attempt to elucidate the mode of interaction between the drug candidates and their possible biological targets.

Results and Discussion

The toxicity evaluation demonstrated that all canagliflozin degradation products (DPs) have structural alerts for non-genotoxic carcinogenicity, classifying them as grade 5 according to ICH-M7, excluding the risk of mutagenicity. However, two dapagliflozin DPs, one ertugliflozin DP, and three empagliflozin DPs were classified as grade 3 risk due to the presence of an aldehyde, an aliphatic halogen, and a monohaloalkene, respectively, suggesting a mutagenic risk alert. Additionally, ten

empagliflozin DPs were predicted to be hepatotoxic, and two ertugliflozin DPs were predicted to be cardiotoxic, reinforcing the need for monitoring the chronic use of these drugs.

The search for other possible molecular targets through Target Fishing indicated a strong mathematical correlation with the adenosine A2A, A3 receptors, and the enzyme Adenosine Kinase. Redocking on these targets, as well as on SGLT2, was performed to validate the molecular docking algorithm, obtaining RMSD (root mean square deviation) values below 0.8 Å, consistent with the literature. Currently, the project is in the stage of evaluating the interactions through molecular docking to elucidate which DPs may be interacting with the mentioned targets.

Conclusion

The constant monitoring of drug impurities has become a crucial factor in ensuring the safety of medications, especially those for chronic use, such as gliflozins. This study has demonstrated that several DPs may pose a risk to human health and require attention, as toxic effects such as mutagenicity, cardiotoxicity, and hepatotoxicity, even though absent in the original molecule, may be present in its DPs. With the results of molecular docking, we hope to elucidate the possible interaction of these DPs with targets other than the primary one, and thus explain the predicted potential risks and the secondary effects observed clinically and reported in the literature.

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Bibliographic References

[1] Lim, S., Sohn, M.: *Initial combination of metformin, sitagliptin, and empagliflozin in drug-naïve patients with type 2 diabetes: Safety and metabolic effects*. Diabetes, Obesity and Metabolism. 2022. 24: pp 757-762.

[2] ANVISA - Agência Nacional de Vigilância Sanitária. RDC 53, de 04 de dezembro de 2015. *Estabelece parâmetros para a notificação, identificação e qualificação de produtos de degradação em medicamentos com substâncias ativas sintéticas e semissintéticas, classificados como novos, genéricos e similares, e dá outras providências*. Diário Oficial da União, nº 234, de 8 de dezembro de 2015.

[3] ICH - *International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Guideline M7(R2) on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk*. 2023.