IN SILICO INVESTIGATION OF POTENTIAL BIOLOGICAL TARGETS OF TRIAZINE-BASED SULFONAMIDES RELATED TO NEPHROTOXICITY

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ABSTRACT
Sulfonamides are antibiotics that inhibit folic acid production in bacteria, thereby preventing their growth. They are commonly used in the treatment of infections and may be repurposed for other applications, such as herbicides. The inhibition of acetolactate synthase, an essential enzyme for the biosynthesis of amino acids in plants, represents an important target for weed control. This study aimed to investigate potential biological targets related to the nephrotoxicity of sulfonamide-based herbicides through *in silico* approaches. The methodology involved bioactivity screening of the compounds using the PharmMapper and ProTox 3.0 servers. The results suggested possible renal risks and identified seven biological targets that may potentially interact with the sulfonamides investigated in this study: Cathepsin B, Phospholipase A2, Carbonic Anhydrase 2, Aliphatic Amidase, Androgen Receptor, GDP-mannose 6-dehydrogenase, and Prothrombin. The identification of these biological targets may contribute to understanding how such sulfonamides interact in the human body, supporting the development of more selective, safer, and sustainable pesticides.

Keywords: Acetolactate synthase; toxicity; herbicides.

INTRODUCTION

Sulfonamides are bacteriostatic antibiotics that compete with para-aminobenzoic acid (PABA), which is essential for folic acid production in bacteria (Connor, 1998; Henry, 1943; Tacic et al., 2017). Discovered in 1935, they are widely used in the treatment of respiratory, urinary, gastrointestinal, and skin infections (Azevedo-Barbosa et al., 2020). The chemical structure of sulfonamides, which includes a sulfonamide group and an amino group, allows for modifications leading to diverse applications, including the development of herbicides (Ovung & Bhattacharyya, 2021).

Herbicides that inhibit acetolactate synthase (ALS) are effective against weeds and exhibit low toxicity levels in humans. However, excessive use can result in resistance and adverse effects. Through theoretical chemistry analyses, Aguiar and collaborators (2024) suggested that sulfonamides may interact with acetolactate

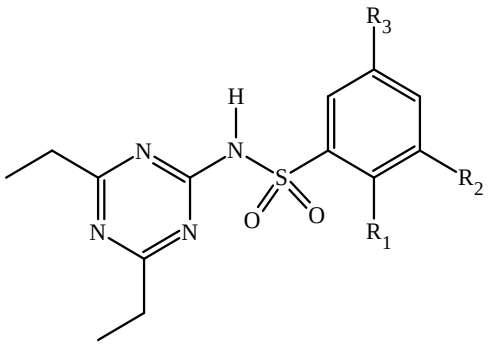
synthase (ALS), an enzyme responsible for the biosynthesis of the branched-chain amino acids leucine, isoleucine, and valine in weeds (Duggleby & Siew, 2000). It has been verified that the pharmacophoric groups of sulfonamides coincide with those of sulfonylureas, and the intermolecular interaction patterns within the active site of the biological target are similar (Aguiar et al., 2023, 2024). These findings suggest that sulfonamides present potential herbicidal activity through this mechanism of action.

Nevertheless, the interaction of these sulfonamides with the human organism may lead to adverse effects, such as renal impairment, since the renal pathway is the primary route for drug and xenobiotic excretion. This study aimed to assess whether herbicidal sulfonamides may cause nephrotoxicity in individuals exposed to these compounds during their use in agricultural weed control.

METHODOLOGY

The developed sulfonamides contain a triazine ring in their structure (Figure 1). With the aid of the ProTox 3.0 server – Prediction of Toxicity of Chemicals (ProTox-3.0 - Prediction of TOXicity of chemicals (charite.de)) (Banerjee et al., 2024), the toxicity of the 10 sulfonamides was evaluated in relation to potential cancer-related issues. Subsequently, with the support of the PharmMapper server (PharmMapper (lilab-ecust.cn)) (Liu et al., 2010), a search was performed for potential biological targets. This server employs compounds from its database to carry out an integrated correspondence of pharmacophoric groups through statistical methods in order to identify the biological targets.

Figura 1. Estrutura Geral das sulfonamidas com potencial herbicida.



Fonte. Aguiar et al. (2024).

RESULTS AND DISCUSSION

The *in silico* analyses suggest that this group of sulfonamides may cause potential nephrotoxicity issues, with seven biological targets identified as capable of interacting with the compounds. Table 1 shows the identified targets, their sites of action, main effects, and type of involvement in nephrotoxicity:

Tabela 1. Proteínas identificadas e seus efeitos principais relacionadas a nefrotoxicidade de sulfonamidas com potencial herbicida.

Proteína	Local de Ação	Efeito Principal	Envolvimento
Catepsina B	Lisossomos das células renais	Indução de apoptose em células renais quando ativada em excesso	Direto
Fosfolipase A2	Membranas celulares e tecido renal	Catalisa liberação de ácidos graxos e lisofosfolipídios, causando inflamação renal	Direto
Anidrase Carbônica 2	Células renais, principalmente nos túbulos	Regulação do equilíbrio ácido-base, desequilíbrio pode causar prejuízos renais	Direto
Amídase Alifática	Células renais e outros tecidos	Hidrólise de amidas alifáticas, podendo gerar intermediários tóxicos	Indireto
Receptor de Andrógenos	Células renais e tecidos alvo dos andrógenos	Regulação hormonal, alterações podem afetar a função renal	Indireto
GDP-manose 6-desidrogenase	Células renais, envolvida na matriz extracelular	Biossíntese de glicosaminoglicanos, sua inibição pode levar a fibrose renal	Indireto
Protrombina	Sangue e tecido renal	Participa da coagulação sanguínea, disfunção pode afetar a microcirculação renal	Indireto

Fonte. Autoria Própria.

Among these proteins, cathepsin B, phospholipase A2, and carbonic anhydrase II showed a direct involvement in nephrotoxicity. Cathepsin B, a lysosomal protease, can induce apoptosis in renal cells when excessively released or activated, leading to structural and functional damage in the kidneys (Schramm et al., 2024). Phospholipase A2 is an enzyme that plays a role in inflammatory processes, releasing fatty acids and lysophospholipids, which can trigger inflammation of renal tissue, resulting in lesions and impaired kidney function (Zhang et al., 2023). Carbonic anhydrase II, essential for the regulation of the acid–base balance in the body, can cause imbalances in the kidneys, affecting renal homeostasis and leading to functional impairment (García-Llorca et al., 2024).

Among the proteins indirectly involved in nephrotoxicity are aliphatic amidase, the androgen receptor, GDP-mannose 6-dehydrogenase, and prothrombin. Aliphatic amidase plays a role in the hydrolysis of aliphatic amides. Although its main function is not directly related to the kidneys, the hydrolysis of these amides may generate toxic intermediates that, in excess, can indirectly damage renal tissue. The androgen receptor is involved in hormonal regulation. Alterations in this receptor may indirectly

affect kidney function, since androgenic hormones influence the physiological balance of the kidneys (Barnett & Cummings, 2018).

GDP-mannose 6-dehydrogenase plays a key role in maintaining the extracellular matrix of renal cells. Its inhibition can lead to the accumulation of extracellular matrix components, promoting renal fibrosis, which indirectly impairs kidney function (Gatson & Singh, 2007). Finally, prothrombin can indirectly affect kidney function by impairing renal microcirculation. Alterations in coagulation may compromise blood flow in renal capillaries, resulting in ischemia and impaired nephron function (Kwiatkowska et al., 2021).

These findings highlight the complexity of the interactions between sulfonamides and biological targets in the kidneys. Evidence suggests that, in addition to the direct effects observed on primary proteins, interference with secondary enzymatic and regulatory functions may also contribute to nephrotoxicity. This study paves the way for further research into the mechanisms underlying the interaction of these sulfonamides with the biological targets predicted through in silico analyses.

CONCLUSION

Using *in silico* tools, it was possible to predict potential kidney-related toxicity problems associated with direct or indirect exposure to the sulfonamides described in this study. In addition, potential biological targets related to nephrotoxicity were identified during the analyses. This investigation contributes to the identification of possible risks and to a better understanding of how these substances may affect renal health. Further *in vitro* and *in vivo* studies are essential for the development of safer and more effective herbicides, minimizing adverse impacts on both the organism and the environment.

REFERENCES

Aguiar, A. S. N., Costa, R. F., Borges, L. L., Dias, L. D., Camargo, A. J., & Napolitano, H. B. (2023). Molecular basis of two pyrimidine-sulfonylurea herbicides: from supramolecular arrangement to acetolactate synthase inhibition. *Journal of Molecular Modeling*, *29*(8), 241. https://doi.org/10.1007/s00894-023-05629-x

Aguiar, A. S. N., Costa, R. F., Borges, L. L., Dias, L. D., & Napolitano, H. B. (2024). Structural-based analysis of sulfonamide derivatives: from solid states to acetolactate

synthase enzyme interactions. *Rendiconti Lincei. Scienze Fisiche e Naturali*, *35*(1), 109–129. https://doi.org/10.1007/s12210-024-01228-x

Azevedo-Barbosa, H., Dias, D. F., Franco, L. L., Hawkes, J. A., & Carvalho, D. T. (2020). From Antibacterial to Antitumour Agents: A Brief Review on The Chemical and Medicinal Aspects of Sulfonamides. *Mini-Reviews in Medicinal Chemistry*, *20*(19), 2052–2066. https://doi.org/10.2174/1389557520666200905125738

Banerjee, P., Kemmler, E., Dunkel, M., & Preissner, R. (2024). ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research*, *52*(W1), W513–W520. https://doi.org/10.1093/nar/gkae303

Connor, E. E. (1998). Sulfonamide antibiotics. *Primary Care Update for OB/GYNS*, *5*(1), 32–35. https://doi.org/10.1016/S1068-607X(97)00121-2

Duggleby, R. G., & Siew, S. (2000). Acetohydroxyacid Synthase. *Article in Journal of Biochemistry and Molecular Biology*, *33*(1), 1–36. https://doi.org/https://www.researchgate.net/publication/298812558

García-Llorca, A., Carta, F., Supuran, C. T., & Eysteinsson, T. (2024). Carbonic anhydrase, its inhibitors and vascular function. *Frontiers in Molecular Biosciences*, *11*. https://doi.org/10.3389/fmolb.2024.1338528

Henry, R. J. (1943). THE MODE OF ACTION OF SULFONAMIDES. *Bacteriological Reviews*, *7*(4), 175–262. https://doi.org/10.1128/br.7.4.175-262.1943

Liu, X., Ouyang, S., Yu, B., Liu, Y., Huang, K., Gong, J., Zheng, S., Li, Z., Li, H., & Jiang, H. (2010). PharmMapper server: a web server for potential drug target identification using pharmacophore mapping approach. *Nucleic Acids Research*, *38*(suppl_2), W609–W614. https://doi.org/10.1093/nar/gkq300

Ovung, A., & Bhattacharyya, J. (2021). Sulfonamide drugs: structure, antibacterial property, toxicity, and biophysical interactions. *Biophysical Reviews*, *13*(2), 259–272. https://doi.org/10.1007/s12551-021-00795-9

Schramm, W. C., Bala, N., Arekar, T., Malik, Z., Chacko, K. M., Lewis, R. L., Denslow, N. D., Scindia, Y., & Alli, A. A. (2024). Enrichment of Bioactive Lipids in Urinary Extracellular Vesicles and Evidence of Apoptosis in Kidneys of Hypertensive Diabetic Cathepsin B Knockout Mice after Streptozotocin Treatment. *Biomedicines*, *12*(5), 1038. https://doi.org/10.3390/biomedicines12051038

Tacic, A., Nikolic, V., Nikolic, L., & Savic, I. (2017). Antimicrobial sulfonamide drugs. *Advanced Technologies*, *6*(1), 58–71. https://doi.org/10.5937/savteh1701058T

Zhang, J., Fan, Z., Wang, P., & Zhang, A.-H. (2023). Phospholipase A2 Receptor Antibodies and Clinical Prognosis in Patients with Idiopathic Membranous Nephropathy: An Updated Systematic Review and Meta-Analysis. *Kidney and Blood Pressure Research*, *48*(1), 102–113. https://doi.org/10.1159/000529415