IOCXe 2026 Conference
The 1st International Online Conference on Xenobiotics
22–23 June 2026 | Online
MDPI

In silico evaluation of emerging pharmaceutical contaminants and their interactions with human proteins: implications for environmental toxicity

Gabriel Cornetta Ferraz Ribeiro[1], Leandro Cardoso de Morais[2], Vitória Regina Lima de Oliveira[3]
[1] Graduate Program in Environmental Sciences, São Paulo State University (UNESP), Sorocaba, Brazil
[2] Department of Environmental Sciences, São Paulo State University (UNESP), Sorocaba, Brazil
[3] Department of Biotechnology, São Paulo State University (UNESP), Assis, Brazil

INTRODUCTION & AIM

INTRODUCTION

The increasing presence of pharmaceutical compounds in aquatic environments has raised concerns regarding their impacts on human health and ecosystems. These substances are considered emerging contaminants due to their persistence, high solubility, and continuous release through industrial effluents and wastewater.

Computational approaches such as molecular docking have become valuable tools for evaluating xenobiotic interactions with biological targets, allowing preliminary toxicological assessment and prediction of metabolic interactions.

Human metabolic enzymes such as Cytochrome P450 3A4 (CYP3A4) and N-acetyltransferase 2 (NAT2) play essential roles in phase I and phase II xenobiotic biotransformation and may interact with pharmaceutical pollutants present in environmental matrices.

OBJECTIVE

To investigate the interactions between selected pharmaceutical contaminants and human metabolic enzymes using molecular docking simulations and evaluate their potential toxicological implications under chronic environmental exposure.

METHOD

Protein structures of Cytochrome P450 3A4 (CYP3A4, PDB ID: 1TQN) and N-acetyltransferase 2 (NAT2, PDB ID: 2PFR) were obtained from the Protein Data Bank, while Amoxicillin and Sulfamethoxazole structures were retrieved from PubChem.

Protein preparation included removal of solvent molecules and non-essential crystallographic components. The heme group (HEM) in CYP3A4 and coenzyme A (CoA) in NAT2 were maintained due to their catalytic relevance.

Ligands were converted from SDF to PDB using OpenBabel and prepared in PDBQT format using MGLTools 1.5.7.

Docking simulations were performed using AutoDock Vina 1.2.5 (exhaustiveness = 100; grid size = 30 × 30 × 30 Å; spacing = 0.375 Å), centered on catalytically relevant regions.

Binding affinity, RMSD, and ligand orientation were analyzed. RMSD values <2.0 Å were considered reliable. For CYP3A4, ligand–heme iron distance was additionally evaluated to estimate catalytic feasibility.

RESULTS & DISCUSSION

MOLECULAR DOCKING RESULTS

System	Affinity (kcal/mol)	RMSD (Å)	Main Observation
Amoxicilin — CYP3A4	-8.77	<2.0	Strong binding affinity but weak catalytic orientation
Sulfamethoxazhole — NAT2	-7.82	<2.0	Stable interaction near acetylation region

DISCUSSION

CYP3A4 – Amoxicillin Interaction:

Amoxicillin showed favorable binding affinity toward CYP3A4 (~−8.7 kcal/mol). However, the relatively large ligand–heme iron distance (~6.5 Å according to Figure 1) suggests suboptimal orientation for catalytic oxidation, since substrate proximity to the heme iron is a critical factor for CYP-mediated metabolism.

These findings indicate that stable binding does not necessarily correspond to efficient biotransformation, suggesting possible enzyme occupancy without effective metabolism. From an environmental toxicology perspective, reduced metabolic efficiency may favor amoxicillin persistence in exposed organisms, potentially contributing to chronic exposure effects and interactions with other xenobiotics.

Fe–LIGAND DISTANCE INTERPRETATION (CYP34)

Distance (Å)	Interpretation
3 - 5	Possible catalytic metablosim
5 - 8	Weak interaction
> 8	Binding whithout catalytic relevance

NAT2 – Sulfamethoxazole Interaction:

Sulfamethoxazole showed favorable binding affinity toward NAT2 (~−7.8 kcal/mol) and RMSD values below 2 Å, indicating stable and reliable docking poses. The ligand was positioned near the catalytic region and in close proximity to coenzyme A (CoA) according to Figure 2, suggesting a structurally favorable orientation for acetylation-related interactions. Given the polymorphic nature of NAT2, these interactions may contribute to interindividual variability in metabolic response and toxicological susceptibility. From an environmental toxicology perspective, chronic exposure to sulfamethoxazole may influence human metabolic pathways and increase the risk of long-term toxicological effects.

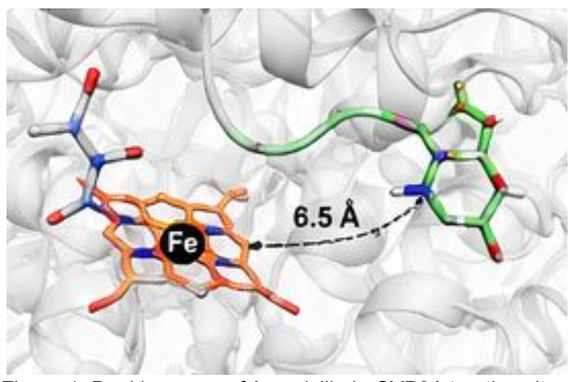

Figura 1. Docking pose of Amoxicilin in CYP3A4 active site. The distance between ligand and heme iron is -6.5Å.

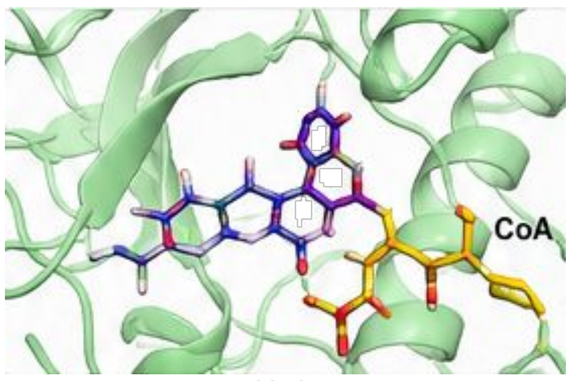

Figure 2. Docking pose of Sulfamethoxazhole in NAT2 active site near the CoA-binding (acetylation) region.

CONCLUSION

- The findings demonstrated that pharmaceutical contaminants can interact with human metabolic enzymes involved in xenobiotic biotransformation.
- **Amoxicillin** exhibited a strong binding affinity toward CYP3A4; however, its catalytic orientation relative to the heme iron center was limited, suggesting a lower likelihood of effective metabolism.
- **Sulfamethoxazole s**howed stable interactions near the NAT2 catalytic region, indicating a potential influence on enzyme-mediated metabolic processes.
- These results emphasize the importance of assessing pharmaceutical pollutants as emerging contaminants that may affect metabolic pathways under conditions of chronic environmental exposure.
- Molecular docking proved to be a valuable computational approach for preliminary toxicological assessment, enabling the prediction of potential interactions between xenobiotics and metabolic enzymes.

REFERENCES

[1] Forli, Stefano, et al. **“Computational Protein–Ligand Docking and Virtual Drug Screening with the AutoDock Suite”**. Nature Protocols, v. 11, n. 5, maio de 2016, p. 905–919. https://doi.org/10.1038/nprot.2016.051.
[2] Guengerich, F. Peter. **“Cytochrome P450 and Chemical Toxicology”**. Chemical Research in Toxicology, v. 21, n. 1, janeiro de 2008, p. 70–83. https://doi.org/10.1021/tx700079z.
[3] Hein, David W. **“Molecular Genetics and Function of NAT1 and NAT2: Role in Aromatic Amine Metabolism and Carcinogenesis”.** Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, v. 506–507, 30 setembro 2002, p. 65–77. https://doi.org/10.1016/S0027-5107(02)00153-7.