RATIONAL DESIGN OF POTENTIAL KPC-2 CARBAPENEMASE INHIBITORS THROUGH A BIOCHEMOINFORMATICS PIPELINE

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Introduction

Antimicrobial resistance has emerged as one of the greatest global public health challenges, compromising antibiotic efficacy and significantly limiting therapeutic options for infections caused by multidrug-resistant bacteria. Among the priority pathogens, carbapenem-resistant Klebsiella pneumoniae represents a critical threat, with resistance being primarily associated with the production of the KPC-2 carbapenemase, a class A β-lactamase capable of hydrolyzing β-lactam antibiotics and markedly reducing the effectiveness of current antimicrobial therapies (Batista & Ribeiro, 2020; Antimicrobial Resistance Collaborators, 2022; WHO, 2024; Belay et al., 2024; Cardoso et al., 2026).

In this context, rational drug design, combined with biochemoinformatics and computational chemistry, has become an efficient strategy for accelerating the discovery of novel enzyme inhibitors while reducing development costs, screening time, and the number of compounds requiring experimental validation (Piccirillo & Amaral, 2018; Wu et al., 2025). The integration of in silico approaches—including molecular similarity searching, pharmacokinetic (ADMET) prediction, toxicity assessment, and molecular docking—enables the rational prioritization of compounds with enhanced affinity toward therapeutic targets, thereby improving the efficiency of the early stages of drug discovery (Goodsell et al., 2020; Cardoso et al., 2026).

In the present study, relebactam, a clinically approved class A and class C β-lactamase inhibitor administered in combination with imipenem/cilastatin, was employed as the reference compound for the identification of structurally related molecules. The retrieved compounds were subsequently subjected to a hierarchical virtual screening pipeline comprising molecular similarity analysis, ADMET prediction, in silico toxicity assessment, and molecular docking against the KPC-2 carbapenemase.

Accordingly, this study aimed to develop and implement a biochemoinformatics pipeline for the rational design and prioritization of potential KPC-2 carbapenemase inhibitors,

contributing to the identification of bioactive candidates for the development of novel enzyme inhibitors through computational drug discovery strategies.

Materials and Methods

The identification of potential KPC-2 carbapenemase inhibitors was carried out using a hierarchical biochemoinformatics pipeline, integrating molecular similarity searching, pharmacokinetic (ADMET) prediction, in silico toxicity assessment (TOPKAT), and molecular docking to rationally prioritize bioactive compounds. An overview of the computational workflow employed in this study is presented in Figure 1.

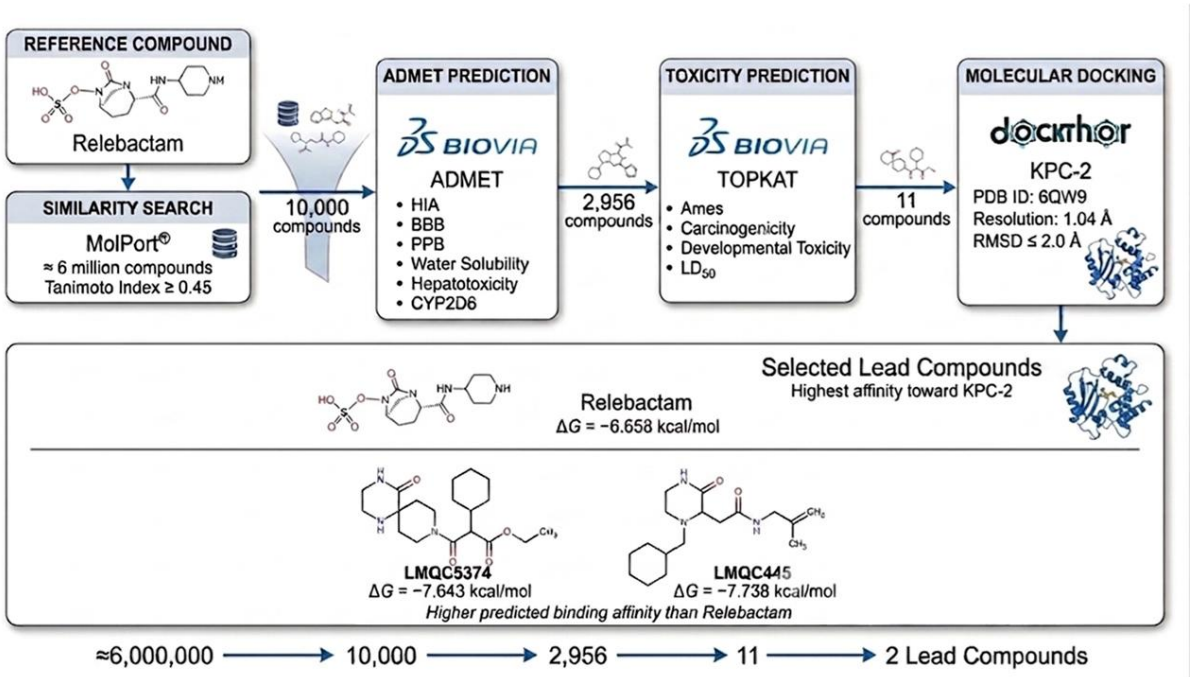

Figure 1. Hierarchical biochemoinformatics pipeline employed for the rational design of potential KPC-2 carbapenemase inhibitors. Relebactam was used as the reference compound for molecular similarity-based virtual screening, followed by pharmacokinetic (ADMET), toxicity (TOPKAT), and molecular docking filters. This hierarchical strategy reduced an initial library of approximately six million compounds to two lead candidates (LMQC5374 and LMQC445), both exhibiting more favorable predicted binding affinities toward KPC-2 than relebactam.

Relebactam was selected as the reference compound for structural similarity searching against the MolPort® database (approximately six million commercially available compounds). Similar compounds were retrieved using a Tanimoto similarity coefficient ≥ 0.45, yielding an initial dataset of 10,000 molecules.

The selected compounds were subsequently evaluated through in silico pharmacokinetic (ADMET) and toxicity (TOPKAT) predictions using Discovery Studio® v16.0 (Dassault Systèmes BIOVIA, 2016). Compounds exhibiting the most favorable pharmacokinetic and toxicological profiles were prioritized for the subsequent molecular docking analyses (Dassault Systèmes BIOVIA, 2016; Papp-Wallace et al., 2019; Roberts et al., 2022).

The approved compounds were then subjected to molecular docking using the DockThor web server (https://dockthor.lncc.br/v2/) and the crystallographic structure of the KPC-2 carbapenemase (PDB ID: 6QW9, resolution 1.04 Å) (Tooke et al., 2019). The docking protocol was validated by re-docking the co-crystallized ligand (MK7), adopting a root-mean-square deviation (RMSD) ≤ 2.0 Å as the validation criterion.

The final prioritization of candidate molecules was based on their predicted binding free energy (ΔG) together with the interaction profile established with the catalytic residues located within the active site of the KPC-2 enzyme.

Results and Discussion

Molecular Similarity Screening

The molecular similarity search performed against the MolPort® database using relebactam as the reference compound and a Tanimoto similarity coefficient ≥ 0.45 yielded an initial dataset of 10,000 structurally related molecules. This similarity threshold provided a chemically diverse yet structurally representative library, preserving the key molecular features of the reference inhibitor while ensuring sufficient structural variability for subsequent virtual screening analyses.

The selected dataset constituted a robust starting point for the hierarchical screening workflow, enabling the efficient identification of compounds with the potential to retain the molecular recognition properties required for KPC-2 inhibition.

In Silico Pharmacokinetic Prediction (ADMET)

The 10,000 selected compounds were subjected to *in silico* ADMET prediction, considering key pharmacokinetic parameters including human intestinal absorption (HIA), aqueous solubility, blood–brain barrier (BBB) permeability, hepatotoxicity, CYP2D6 inhibition, and plasma protein binding (PPB).

Considering the intended oral route of administration, only compounds located within the 95% and 99% confidence ellipses generated by Discovery Studio®, which indicate a higher probability of favorable oral bioavailability, were retained for the subsequent screening stages (Figure 2). This filtering step resulted in the selection of 2,956 compounds (29.6%), while 7,044 molecules (70.4%) were excluded from further analyses.

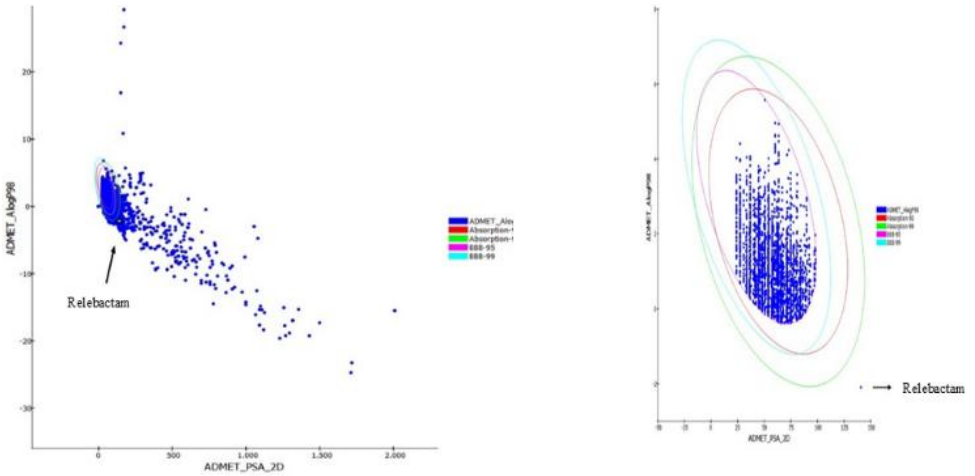

Figure 2. ADMET screening of compounds retrieved from the MolPort® database. (A) Distribution of the 10,000 molecules selected through molecular similarity searching (Tanimoto index ≥ 0.45). (B) Distribution of the 2,956 compounds prioritized after ADMET filtering, highlighting molecules located within the confidence ellipses that indicate a higher probability of favorable oral bioavailability.

Overall, the prioritized compounds exhibited a favorable pharmacokinetic profile, characterized by good predicted intestinal absorption, good to excellent aqueous solubility, low blood–brain barrier permeability, absence of predicted hepatotoxicity, and no CYP2D6 inhibition (Table 1). Collectively, these results indicate that the selected molecules possess physicochemical and pharmacokinetic characteristics compatible with the subsequent stages of the rational drug discovery pipeline.

Table 1. Predicted pharmacokinetic properties of relebactam and the lead compounds selected after ADMET screening.

Compound	Water Solubility	Human Intestinal Absorption	Blood–Brain Barrier	Hepatotoxicity	CYP2D6 Inhibition	Plasma Protein Binding
Relebactam (Reference compound)	4	2	4	true	false	false
LMQC - 445	3	0	3	false	false	false
LMQC - 5374	3	0	3	false	false	false

Water solubility: 3 = good; 4 = excellent. Human intestinal absorption: 0 = good; 2 = moderate. Blood–brain barrier permeability: 3 = low penetration; 4 = undefined. Hepatotoxicity: False = non-hepatotoxic; True = predicted hepatotoxic. CYP2D6 inhibition: False = non-inhibitor. Plasma protein binding: False = low binding; True = high binding.

Results and Discussion

In Silico Toxicity Prediction

The 2,956 compounds that successfully passed the ADMET screening were subsequently evaluated for their toxicological profiles using the TOPKAT module implemented in Discovery Studio®.

The prioritized compounds exhibited an overall favorable toxicity profile (Table 2), being consistently predicted as non-carcinogenic according to both the FDA and National Toxicology Program (NTP) models, as well as non-mutagenic in the Ames test.

Table 2. Predicted toxicological properties of relebactam and the lead compounds selected after TOPKAT.

Estrutura	FDA e NTP predictions				Ames	DTP	TD 50 mg/kg Rato	LD 50 g/kg
	Male mouse	Female mouse	Male rat	Female rat				
Relebactam (pivô)	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-mutagenic	Toxic	1,79215	3,83657
LMQC - 5374	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-mutagenic	Non-toxic	24,5666	5,44411
LMQC - 445	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-mutagenic	Non-toxic	36,3274	9,60084

DTP: DTP, Developmental Toxicity Potential; TD_{50}, Tumorigenic Dose; LD_{50}, Median Lethal Dose; FDA, Food and Drug Administration; NTP, National Toxicology Program.

Regarding the Developmental Toxicity Potential (DTP), all prioritized compounds were predicted to be non-toxic, in contrast to the reference compound, relebactam, which was classified as presenting developmental toxicity. Furthermore, the predicted oral LD_{50} values indicated low acute oral toxicity for the selected candidates.

The application of these toxicological filters dramatically reduced the dataset from 2,956 to only 11 compounds, demonstrating the effectiveness of the hierarchical screening strategy in eliminating compounds with undesirable safety profiles while preserving chemically promising candidates for molecular docking analyses.

Molecular Docking

The molecular docking protocol was initially validated through re-docking of the co-crystallized ligand (MK7), yielding an RMSD below 2.0 Å, thereby confirming the reliability and reproducibility of the docking methodology adopted in this study (Ramírez et al., 2018).

The 11 compounds that successfully passed the toxicological screening were subsequently docked into the active site of the KPC-2 carbapenemase. Among them, the four compounds exhibiting the highest predicted binding affinities were prioritized for detailed analysis.

Relebactam displayed a predicted binding free energy of −6.658 kcal/mol, whereas LMQC5374 (−7.643 kcal/mol) and LMQC445 (−7.378 kcal/mol) exhibited significantly more favorable binding affinities (Table 3). These findings indicate that both compounds interact more strongly with the catalytic pocket of KPC-2 than the clinically approved reference inhibitor.

Table 3. Predicted binding free energies (ΔG) and principal interactions established between the lead compounds and the KPC-2 carbapenemase (PDB ID: 6QW9).

Compound	ΔG(kcal/mol)	Hydrogen-Bond Interactions	Hydrophobic Interactions
Relebactam (MK7)	-6.658	THR237, SER130, THR235	-
LMQC 5374	-7.643	GLU276, THR237,SER70	TRP105
LMQC 445	-7.378	SER70, SER130	TRP105

Beyond the improved binding affinities, both lead compounds preserved key interactions with catalytically relevant residues within the KPC-2 active site. LMQC5374 established hydrogen-bond interactions with Glu276, Thr237, and Ser70, together with

hydrophobic interactions involving Trp105. Similarly, LMQC445 formed hydrogen bonds with Ser70 and Ser130, while also maintaining hydrophobic interactions with Trp105 (Figure 3).

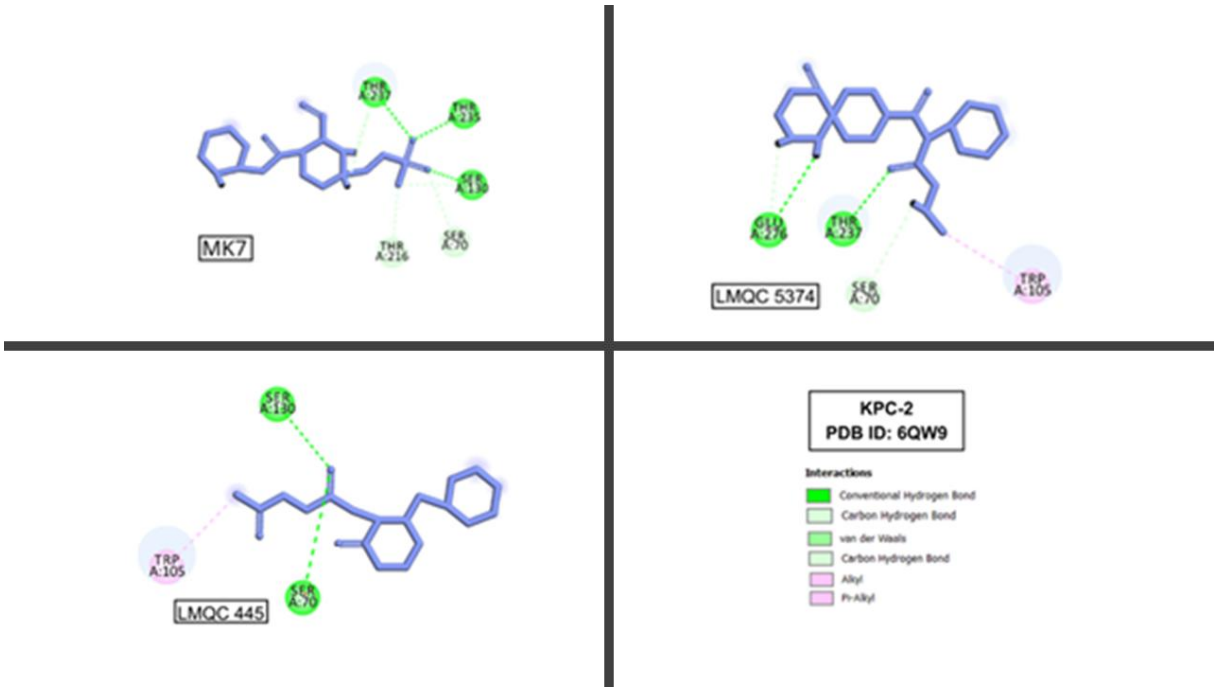

FigurE 3. Interações dos compostos líderes (LMQC5374 e LMQC445) com os resíduos do sítio ativo da carbapenemase KPC-2 (PDB ID: 6QW9).

The conservation of these critical interactions, particularly with residues known to participate in substrate recognition and catalytic activity, suggests that both lead compounds possess a favorable molecular recognition pattern within the KPC-2 active site and therefore represent promising candidates for further optimization and experimental validation.

Conclusions

The hierarchical biochemoinformatics pipeline developed in this study proved to be a robust strategy for the rational design of potential KPC-2 carbapenemase inhibitors, enabling the reduction of an initial library of approximately six million compounds to two lead candidates with favorable pharmacokinetic and toxicological profiles and higher binding affinity than relebactam. These findings reinforce the potential of computational chemistry as a valuable tool for the rational discovery of novel antimicrobials and provide a solid foundation for the experimental validation and structural optimization of the selected compounds..

Acknowledgments

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