DOCKING-BASED SCREENING AND INTERACTION ANALYSIS OF β-LACTAMASE INHIBITORS TARGETING TEM-1, KPC AND BcII

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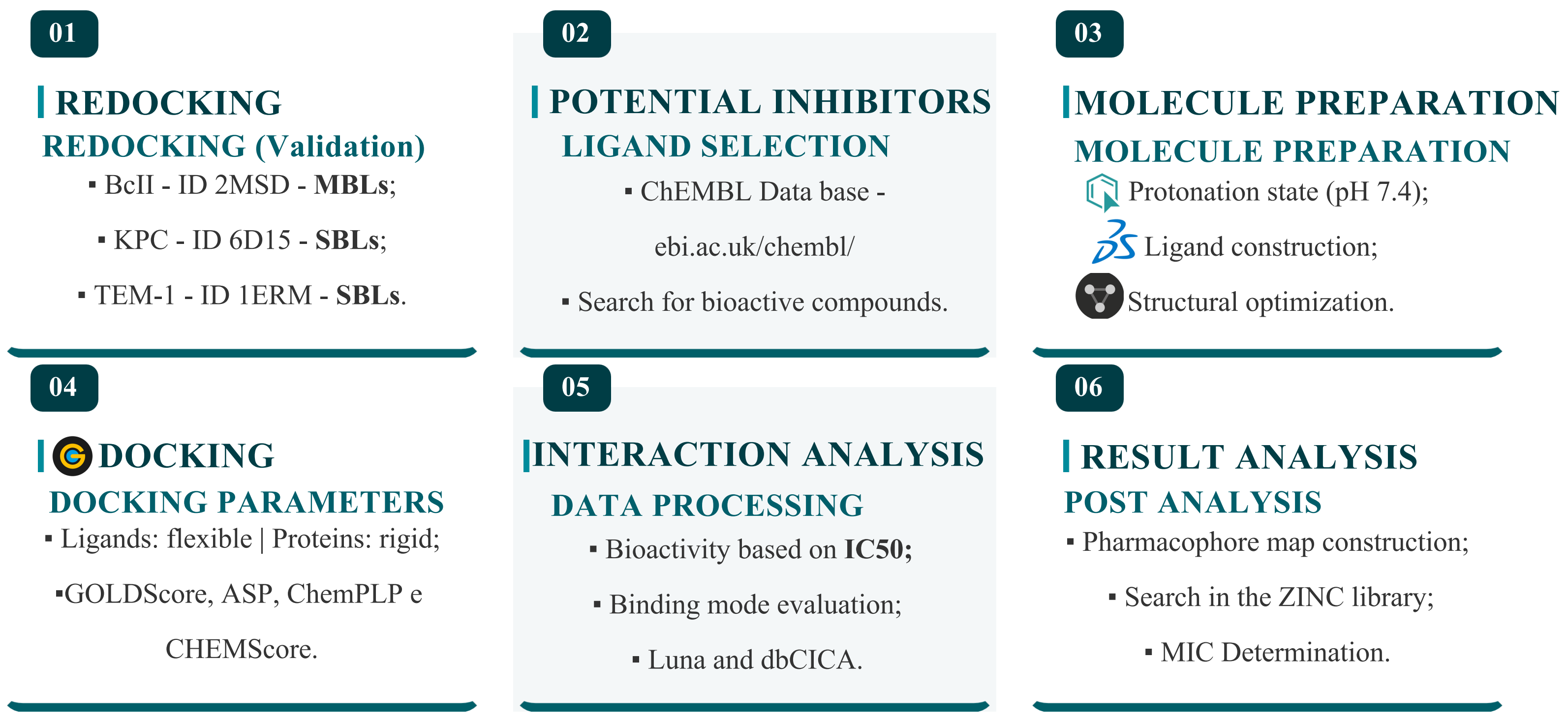
Introduction and Objectives

Antimicrobial Resistance (AMR) is a major global public health threat, with the WHO GLASS 2025 Report highlighting its continuous expansion. A primary mechanism of resistance is the production of β-lactamases, enzymes that inactivate antibiotics through ring hydrolysis. These are classified into Serine-β-lactamases (SBLs), which utilize a serine-dependent mechanism, and Metallo-β-lactamases (MBLs), which require zinc ions for catalysis. This project employs in silico screening and molecular docking to identify novel inhibitors for TEM-1, KPC, and BcII. The goal is to discover therapeutic adjuvants capable of restoring the efficacy of clinically available β-lactam antibiotics.

Materials and methods

The ChEMBL database was used to identify bioactive molecules against TEM-1 (PDB: 1ERM), KPC (PDB: 6D15), and BcII (PDB: 2M5D) enzymes. The computational workflow performed for these molecules is illustrated in Figure 1.

Figure 1. *Workflow of the applied methodology.*



01

REDOCKING

REDOCKING (Validation)

- BcII - ID 2MSD - **MBLs**;
- KPC - ID 6D15 - **SBLs**;
- TEM-1 - ID 1ERM - **SBLs**.

02

POTENTIAL INHIBITORS

LIGAND SELECTION

- ChEMBL Data base - ebi.ac.uk/chembl/
- Search for bioactive compounds.

03

MOLECULE PREPARATION

MOLECULE PREPARATION

- Protonation state (pH 7.4);
- Ligand construction;
- Structural optimization.

04

DOCKING

DOCKING PARAMETERS

- Ligands: flexible | Proteins: rigid;
- GOLDScore, ASP, ChemPLP e CHEMScore.

05

INTERACTION ANALYSIS

DATA PROCESSING

- Bioactivity based on **IC50;**
- Binding mode evaluation;
- Luna and dbCICA.

06

RESULT ANALYSIS

POST ANALYSIS

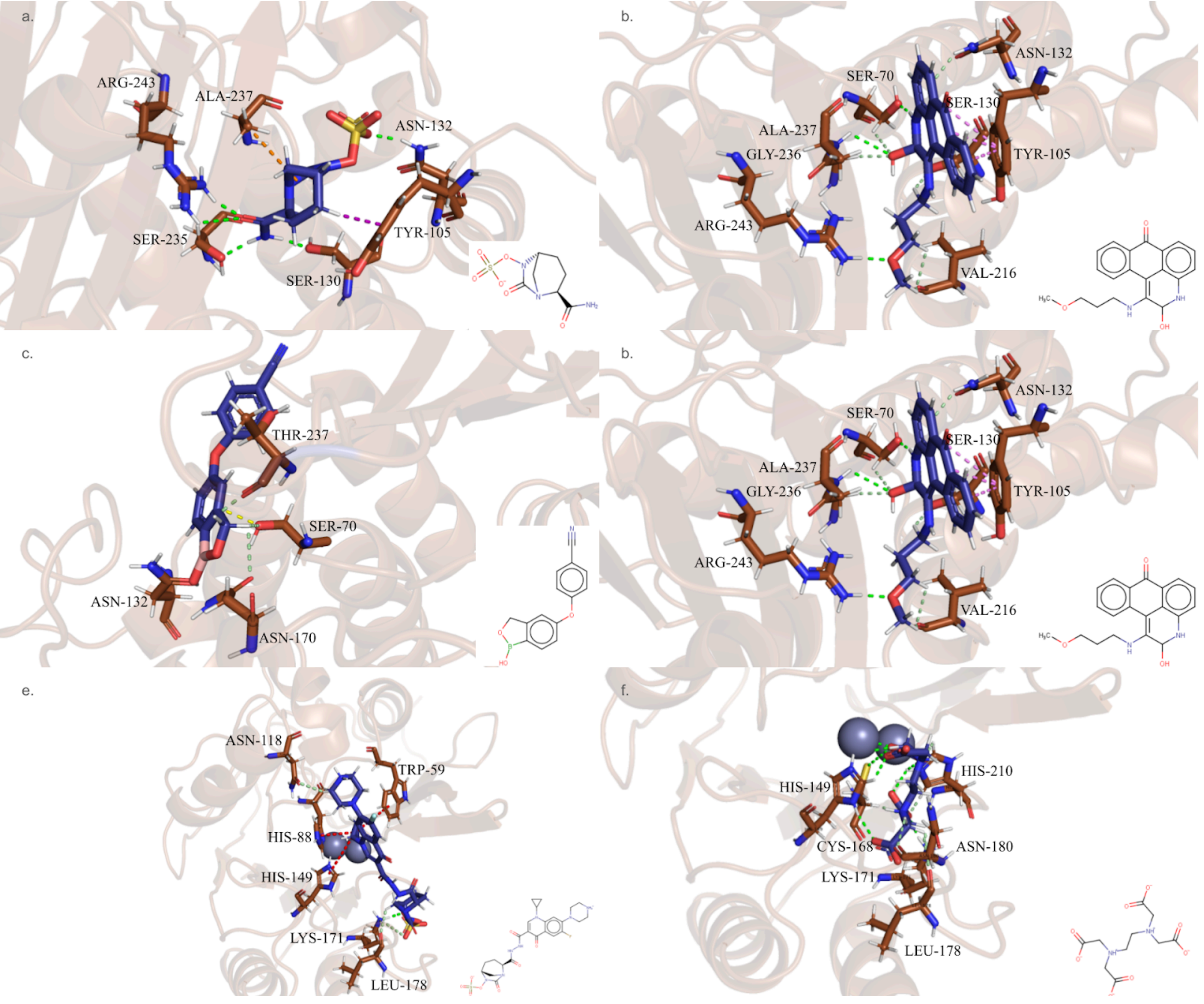
- Pharmacophore map construction;
- Search in the ZINC library;
- MIC Determination.

A total of 188 potential inhibitors of TEM-1, 88 of KPC, and 72 of BcII were analyzed.

Results

After validation of the method, molecular docking was performed using compounds from the ChEMBL database with known bioactivity (IC_{50}). Figure 3 shows the docking results for the molecules with the highest and lowest bioactivity against the enzymes TEM-1, KPC, and BcII, respectively.

Figure 2. *A–B: highest/lowest bioactivity (TEM-1); C–D: highest/lowest (KPC); E–F: highest/lowest (BcII).*



A and B represent the molecules with the highest and lowest bioactivity for the TEM-1 enzyme; C and D correspond to those with the highest and lowest bioactivity for KPC; and E and F to the molecules with the highest and lowest bioactivity against BcII.

After docking and data processing, the large-scale intermolecular interaction post-processing method (LUNA) was applied. Molecular fingerprints were generated and used for contact analysis using the dbCICA method. The results of the interaction analysis (dbCICA) are presented in Table 1.

Table 1. *r^2 values obtained from dbCICA analysis.*

Enzyme	Class	Scoring Function	r^2
TEM-1	A	GoldScore	0,61
KPC	A	GoldScore	0,9
BcII	B	ASP	0,84

Table 2 presents the critical contacts predicted by the best-performing dbCICA models for the three enzymes.

Table 2. *Contacts from the best dbCICA models*

Model	Positive contacts	Negative contacts
TEM1	A:THR235:CB (3); A:THR235:O (3); A:ALA212:CB(2); A:SER70:CB.	A:ARG218:CZ; A:GLU214:OE1; A:TYR80:CZ; Internal:LIG:C1-C15; Internal:LIG:C3-C4.
KPC	A:TRP105:CE3 (1), A:LYS73:NZ (2), A:THR237:CG2 (1), A:SER70:OG (1).	A:ARG220:NH2 (2), A:TWB301:C18 (2), A:TWB301:C17 (1), A:TWB301:P11 (1).
BcII	A:ARG248:CZ;A:ARG248:NE; A:ARG248:NH1;A:ARG248:NH2; A:ARG218:CZ; A:ARG218:NE; A:ARG218:NH1; A:ARG218:NH2; z:LIG9999:S1;z:LIG9999:S; A:PRO142:CB;A:TYR80:CE1; A:TYR80:CD1.	A:ALA212:CB; A:GLU214:CG; A:GLU214:OE2; A:SER45:CB; A:VAL191:CG2; LIG:C; LIG:C1; LIG:C2; LIG:C3; LIG:C5; LIG:C6; LIG:C8; LIG:C10; LIG:C15; LIG:N3; LIG:O .

As presented in Table 2, the model identified essential molecular interactions. For TEM-1, the highlights include Thr235, Ala212, and the critical contact with Ser70 (the nucleophile for catalytic attack). In KPC, interactions with Lys73 and Ser70 corroborate the deprotonation and enzymatic activation mechanism, while Thr235 acts in substrate anchoring. In contrast to SBLs, the model for BcII (MBL) focuses on the metal interface, highlighting important contacts with Arg248 and Arg218. During antimicrobial catalysis, these residues are responsible for stabilizing the ligand's carboxylate group. Furthermore, contacts with the ligand's sulfur atoms indicate direct coordination with Zinc ions, an essential factor for interfering with catalysis. This metal interface is the critical determinant of MBL activity, as the ligand's interaction with the metal center displaces the catalytic water molecule and stabilizes the transition state via carbonyl group polarization. Additionally, contact with Tyr80 contributes to ligand stabilization within the enzymatic cavity.

Conclusion

The results indicate a promising model for the development of broad-spectrum inhibitors against the studied enzymes. LUNA and dbCICA analyses showed high statistical reliability, with k-fold validation values of 0.90 (KPC), 0,84 (BcII) and 0.6 (TEM-1), demonstrating model robustness. The identification of critical contacts provides a basis for pharmacophore modeling and the selection of compounds from the ZINC database for in vitro evaluation, supporting the rational design of drugs with improved affinity and selectivity.

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