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Development of novel *Staphylococcus aureus* ß-Lactamase Inhibitor through Mb-Isoster and Glide Docking Software

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Abstract: Staphylococcus aureus *is an opportunistic bacterium that causes diseases in immunocompromised individuals and is among the pathogens responsible for hospital-acquired infections. Antibiotic-resistant strains like MRSA and VRSA have become a growing concern over the years. One of their mechanisms of antibiotic resistance is the production of inactivating enzymes called class A β-lactamases. In this study, we aimed to find a new drug capable of binding to the* S. aureus *β-lactamase enzyme and inhibit its activity, making multidrug-resistant* S. aureus *susceptible to β-lactam antibiotics again. We targeted the three-dimensional structure of* S. aureus *PC-1 β-lactamase, solved by X-ray crystallography at 2.0 Å resolution. We performed molecular docking using Schrödinger Suite's Glide software to screen a library of 228 potential inhibitors, derived from clavulanic acid by bioisosteric replacement through MB-Isoster software. We discovered 5 new compounds that bind more strongly to* S. aureus *β-lactamase than clavulanic acid, a known inhibitor. We also evaluated their pharmacokinetic properties using Schrödinger Suite's QikProp software and found one ligand with a satisfactory Percent Human Oral Absorption of 82.976%. Our study demonstrates the potential of bioisosteric replacement as a strategy for drug discovery. Future work will test the efficacy and safety of our inhibitors in vivo.*

Keywords: *Staphylococcus aureus*; β-lactamase; Molecular Docking; Bioisosterism.

Desenvolvimento de novo inibidor de ß-Lactamase de *Staphylococcus aureus* através do Software Mb-Isoster e Glide Docking

Resumo: Staphylococcus aureus *é uma bactéria oportunista que causa doenças em indivíduos imunocomprometidos e está entre os patógenos responsáveis por infecções hospitalares. Cepas resistentes a antibióticos como a MRSA e a VRSA tornaram-se uma preocupação crescente ao longo dos anos. Um dos seus mecanismos de resistência a antibióticos é a produção de enzimas inativantes chamadas β-lactamases de classe A. Neste estudo, visamos encontrar um novo fármaco capaz de se ligar à enzima β-lactamase da* S. aureus *e inibir sua atividade, tornando a* S. aureus *multirresistente suscetível aos antibióticos β-lactâmicos novamente. Escolhemos como alvo a estrutura tridimensional da β-lactamase da* S. aureus *PC-1, resolvida por cristalografia de raios X à resolução de 2,0 Å. Realizamos o docking molecular usando o software Glide do Schrödinger Suite para fazer a triagem de uma biblioteca de 228 potenciais inibidores, derivados do ácido clavulânico por substituição bioisostérica através do software MB-Isoster. Descobrimos 5 novos compostos que se ligam mais fortemente à β-lactamase da* S. aureus *do que o ácido clavulânico, um inibidor conhecido. Também avaliamos suas propriedades farmacocinéticas usando o software QikProp do Schrödinger Suite, e encontramos um ligante com um Percent Human Oral Absorption satisfatório de 82,976%. Nosso estudo demonstra o potencial da substituição bioisostérica como uma estratégia para a descoberta de fármacos. Trabalhos futuros testarão a eficácia e a segurança dos nossos inibidores in vivo.*

Palavras-chave: *Staphylococcus aureus*; β-lactamase; Docking Molecular; Bioisosterismo.

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Introduction

In 2001, the World Health Organization (WHO) published a report entitled *"WHO Global Strategy for Containment of Antimicrobial Resistance"*, which recognized antimicrobial resistance as one of the main public health challenges of the 21st century (WHO, 2001).

WHO also published a document entitled *"Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics"*. It contains a list of pathogen strains resistant to antibiotics and was published to encourage the scientific community to prioritize research of new antibiotics targeting such strains. Among the twelve families of pathogenic agents included, the *Staphylococcus aureus* strains are mentioned as of high priority, namely, *S. aureus* resistant to methicillin (MRSA), to vancomycin (VRSA) and with intermediate sensitivity (GISA) (WHO, 2017).

Staphylococcus aureus is a gram-positive bacterium responsible for a wide range of human diseases including septicemia, endocarditis, pneumonia, and joint infections. It has a cell wall which is essential for its growth, pathogenicity, and virulence. One of the main components of its cell wall is a carbohydrate called peptidoglycan (FEIL et al., 2003; GLACHI et al., 2018).

The peptidoglycan is a rigid exoskeletal-like structure that surrounds the cell's plasma membrane. Its function is to provide resistance to turgor, helping preserve the integrity of the cell membrane by preventing osmotic lysis. For this reason, the enzymes involved in peptidoglycan biosynthesis are potential targets, because their inactivation can disrupt the cell wall synthesis and integrity, rendering the bacterium susceptible to osmotic lysis (GLACHI et al., 2018). Among such enzymes are the penicillin-binding proteins.

Penicillin-binding proteins (PBPs) catalyze key reactions in the final step of peptidoglycan biosynthesis. These are the targets of antibiotics from the β-lactam family such as cephalosporin and penicillin, the first antibiotic used to combat *S. aureus* infections (ENRIGHT et al, 2002; ITO et al, 2014).

Penicillin exhibited favorable efficacy against *S. aureus* until the 1960s, when the emergence of resistant strains became apparent. As a result, in 1959, a semi-synthetic penicillin known as methicillin was introduced to treat infections caused by such strains. However, in 1961, there were already reports of *S. aureus* strains that had acquired resistance to methicillin (MRSA) in the United Kingdom (ENRIGHT et al., 2002).

MRSA has strong resistance to β-lactam antibiotics due to several mechanisms. These mechanisms include decreasing the transport of β-lactams to the periplasmic space by reducing porins, changing the targets of β-lactams in PBPs, using efflux pumps to exclude β-lactams, and producing β-lactamases that inactivate these antibiotics. Therefore, one potential strategy to overcome MRSA's resistance to β-lactams is to target these β-lactamases (CRISÓSTOMO et al., 2001; KANEHISA; GOTO, 2000).

The aim of this research was to design novel inhibitors of the β-lactamase enzyme of S. aureus, which is responsible for its resistance to β-lactam antibiotics. We used a computational approach to identify potential inhibitors based on the structure of clavulanic acid, a known β-lactamase inhibitor. We performed bioisosteric modifications on clavulanic acid to generate a virtual library of candidates, and then evaluated their binding affinity and pharmacokinetic properties using molecular docking and absorption, distribution, metabolism, and excretion (ADME) prediction tools. The details of the employed methods are described in the following sections.

Materials and methods

This research employed a couple of bioinformatics tools, many of which are a part of the *Schrödinger Suite*, developed by Schrödinger, LLC. Schrödinger Suite is a set of software tools used in drug discovery and materials science. They are united under a single user interface called *Maestro* (SCHRÖDINGER, 2020e).

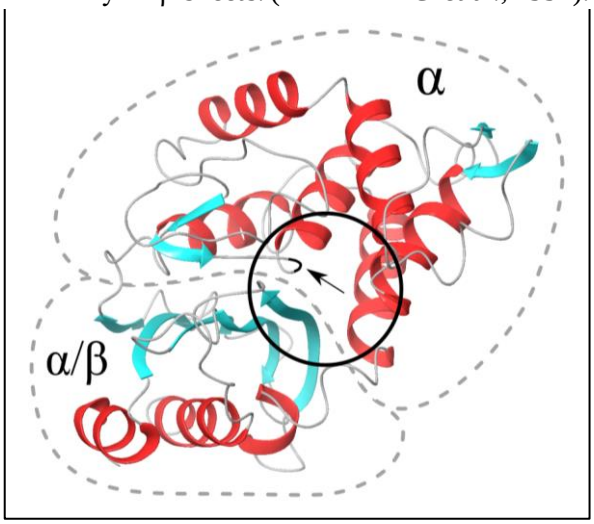
In addition to the Schrödinger Suite, MB-Isoster was used in ligand-receptor pose analysis and bioisosteric generation of drug candidates (ELIAS, 2018).

β-lactamase from *Staphylococcus aureus* PC-1

β-lactamases are hydrolytic enzymes that catalyze the opening of the β-lactam ring of β-lactams, such as penicillins, cephalosporins, and carbapenems (GALLENI et al., 1995). *S. aureus* PC-1 β-lactamase was chosen for this study. It is encoded by the blaZ gene, which can be found in the UniProt Knowledgebase (UniProtKB) through the primary accession number (or "Entry") P00807 (UNIPROT, 2016).

This class A β-lactamase consists of a single chain of amino acids. Its three-dimensional structure consists of two domains: the α domain and the α/β domain (Figure 1). The α domain is mainly composed of α-helices, as the β-sheets are short. In contrast, the α/β domain has a few α-helices and long β-sheets. The active site of the enzyme is located at a depression in the interface region between the two domains and includes the catalytic Ser70 (HERZBERG et al., 1997).

Figure 1. β-lactamase from *S. aureus* PC-1. The image shows the α domain (α) and the α/β domain (α/β) of the enzyme. The black circle indicates the location of the active site, and the arrow points to the catalytic serine 70 residue, in black. Red: α-helices. Cyan: β-sheets. (HERZBERG et al., 1997).



Source: From the authors.

The three-dimensional structure of β-lactamase from *S. aureus* PC-1 used for docking was downloaded from the Protein Data Bank. Its ID within the database is 3BLM, and it was solved by X-ray crystallography at 2.0 Å resolution (https://www.rcsb.org/structure/3BLM) (HERZBERG, 1991).

Receptor preparation

The 3BLM PDB file was first loaded to Maestro. With tools provided by Maestro's user interface, all water molecules and ions were deleted, leaving only β-lactamase's chain to be prepared. Because the enzyme does not have more than one chain, deleting irrelevant chains was unnecessary. Also, the enzyme had no metals bonded to its amino acid residues, so no correction of dummy atom types was necessary.

Schrödinger Suite's Protein Preparation Wizard software was used to prepare β-lactamase for molecular docking. The software's panel presents a couple of options that must be adjusted before running. A detailed description of the setup is provided below (SCHRÖDINGER, 2020c, 2020f).

Under the section *Preprocess the Workspace structure*, there are a couple of tasks that can be enabled by the user. *Assign bond orders* was enabled to allow for the orders of covalent bonds between atoms to be fixed. *Add hydrogens* was enabled so that the protonation states of atoms could be corrected. *Create disulfide bonds* was enabled to allow the software to create disulfide bonds between sulfurs of cysteine residues that are close together. Finally, *cap termini* was enabled to add ACE (N-acetyl) and NMA (N-methyl amide) groups

to uncapped N and C termini. Once the panel was all set, the enzyme was preprocessed (SCHRÖDINGER, 2015d).

The next step was to optimize the hydrogen bond network, which can be achieved through the reorientation of hydroxyl and thiol groups, water molecules, amide groups of glutamine (Gln) and asparagine (Asn), and the imidazole ring of histidine (His). It can also be achieved through the prediction of protonation states of histidine, glutamic acid and aspartic acid; and tautomeric states of histidine. These optimizations are necessary because the orientation of these groups cannot be determined from the crystallized structure (SCHRÖDINGER, 2015d).

We performed the optimization using the *H-bond assignment* tool of the Protein Preparation Wizard. Protein pKa predictions were made through PROPKA software (pH 7.0) (OLSSON et al., 2011).

Receptor grid generation

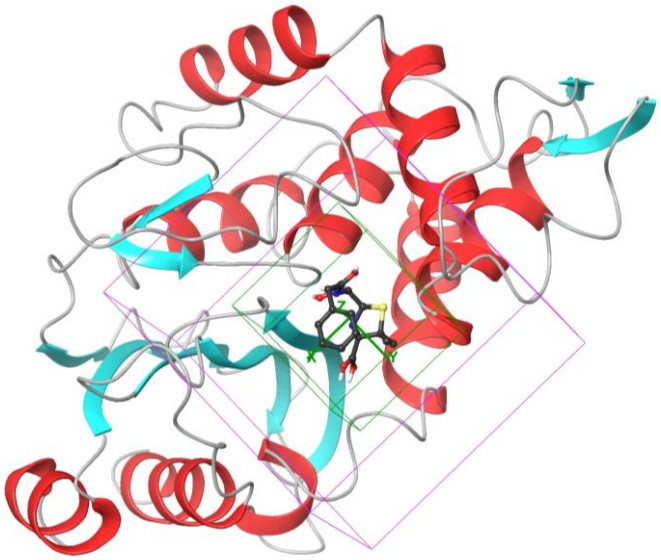
As stated earlier, the active site of β-lactamase is located in the interface region between the α and α/β domains, close to the Ser70 catalytic residue. This active site catalyzes the degradation of β-lactams whether they are antibiotics or suicide inhibitors such as clavulanic acid (HERZEBERG, 1997).

For this reason, the crystallized structure of *S. aureus* β-lactamase linked to a degraded β-lactam was searched in PDB in order to generate the grid around this ligand. The chosen structure was a mutant *S. aureus* PC-1 β-lactamase complexed with a degraded benzylpenicillin in the active site, solved by X-ray diffraction method at 1.76 Å resolution. Its PDB ID is 1GHP (https://www.rcsb.org/structure/1GHP).

According to Chen and Herzberg (2001), "The overall fold of the mutant protein is the same as that of the native protein, with rmsd between α-carbon atom positions of 0.2 Å. The two replaced residues have not altered the active site architecture significantly." For this reason, the grid built around the degraded benzylpenicillin can be used to perform ligand docking to non-mutant β-lactamase.

The grid was built around the degraded benzylpenicillin through Schrödinger Suite's Receptor Grid Generation module set up to its default settings.

Figure 2: Non-mutant β-lactamase (3BLM) and generated grid around the degraded benzylpenicillin.



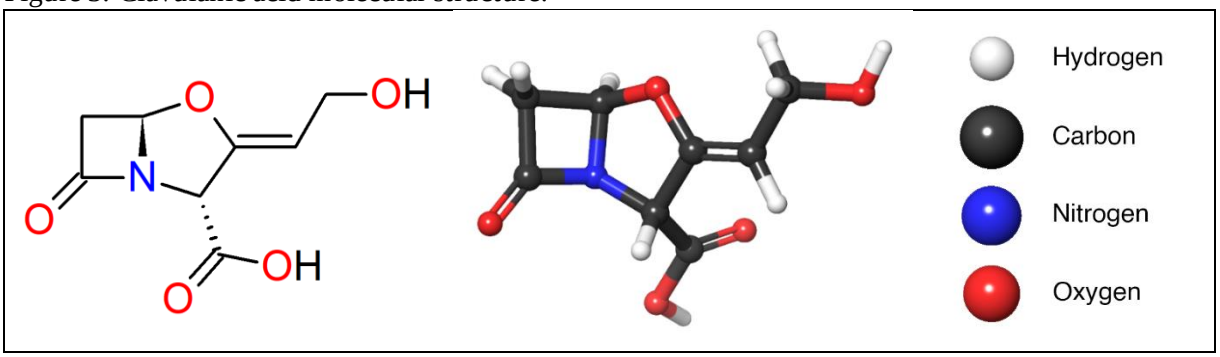
Source: From the authors.

Clavulanic acid

Clavulanic acid, or clavulanate, is a naturally occurring β-lactam. It was isolated for the first time from the bacterium *Streptomyces clavuligerus* in 1976 and exhibited the ability to inactivate β-lactamases in both gram-positive and gram-negative bacteria. Administration of amoxicillin plus clavulanate in humans shows a great synergistic effect, increasing the effectiveness in fighting off bacteria, and it is a drug combination currently used for therapeutic purposes. An important feature of this synergy is that, when administered alone, clavulanate presents low antimicrobial activity. However, when combined with amoxicillin, it significantly reduces its MIC (Minimum Inhibitory Concentration). This suggests it protects amoxicillin from being hydrolyzed by β-lactamase (BROWN et al., 1976; CHEN; HERZBERG, 1992; DRAWZ; BONOMO, 2010).

Just like β-lactam antibiotics, clavulanic acid has a β-lactam ring (Figure 3). It works as a "suicide" inhibitor, undergoing hydrolysis in place of the antibiotic as well as secondary chemical reactions at the active site of the enzyme, which causes it to be irreversibly inactivated (DRAWZ; BONOMO, 2010).

Figure 3: Clavulanic acid molecular structure.



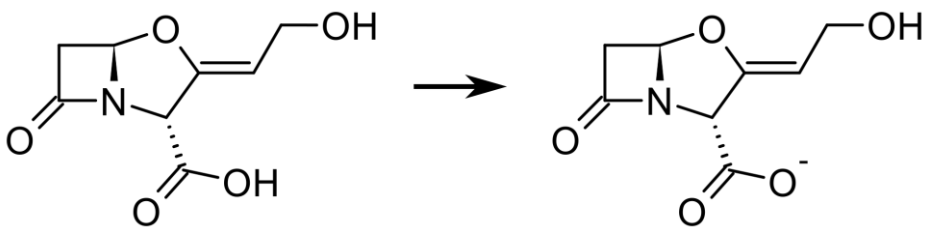
Source: From the authors.

Before docking the ligand to the receptor, the ligand molecule needs to be prepared to ensure that its three-dimensional representation is faithful to what would occur in a protein-ligand interaction. This means adjusting its bond lengths, angles, and torsions (SCHRÖDINGER, 2015a, 2015b).

Also, missing hydrogens must be added, charged groups corrected to match what occurs in aqueous medium with physiological pH values, and structures generated to represent various absolute configurations of chiral centers and possible tautomers (SCHRÖDINGER, 2015a, 2015b).

The first step was to download the three-dimensional structure of clavulanic acid from DrugBank in SDF format (WISHART et al., 2006). We then prepared the molecule using Schrödinger Suite's LigPrep module. It was set up to generate the possible ionization states at the pH range of 7.0 ± 2.0, which is the physiological pH range. It was also set up to generate tautomers and to keep absolute configurations of chiral centers unchanged since clavulanic acid is a specific enantiomer (SCHRÖDINGER, 2020b). The result of the process was clavulanate (the ionized form of clavulanic acid) (Figure 4).

Figure 4. Transformation of clavulanic acid into clavulanate performed by LigPrep.



Source: From the authors.

Clavulanate Glide docking

We performed the docking of clavulanic acid to *S. aureus* β-lactamase using Schrödinger Suite's Glide software. It was configured to perform XP docking (extra-precision mode of Glide), generate a file including the ligand and receptor poses, generate up to 30 poses per binder, create a report file containing each pose's *Extra-Precision Glide Score* (XP GScore), not use input partial charges, sample hydrogen inversions, sample ring conformations, add Epik state penalties to docking score, and perform post-docking minimization. No constraints were added to the docking process (FRIESNER et al., 2004; FRIESNER et al., 2006; HALGREN et al., 2004; SCHRÖDINGER, 2020a).

The grid created around the degraded benzylpenicillin was the one used for docking.

Once Glide docking was completed, a file was created containing clavulanate's lowest XP GScore pose at the active site of the prepared β-lactamase. This file was then exported in SDF format for later use in MB-Isoster.

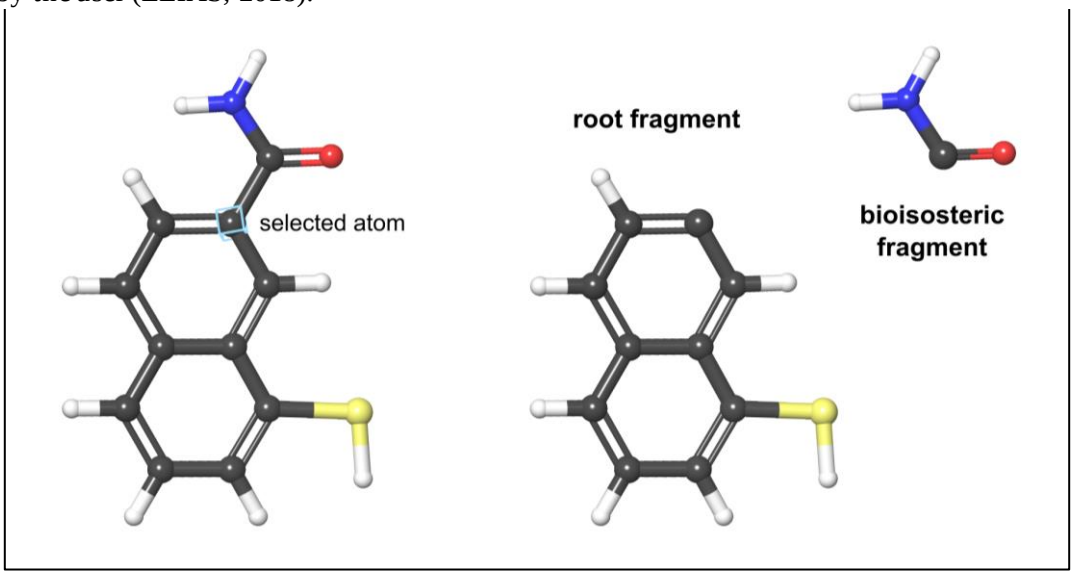
Bioisosteres generation

Bioisosterism is a useful technique to search for new bioactive compounds. It involves replacing biologically active chemical groups of a molecule with other groups of similar physical-chemical properties, such as pKa, log P, molecular volume, and electronic distribution, among others (BARREIRO; FRAGA, 2008).

Developed by Thiago Castilho Elias, MB-Isoster is a software designed to assist researchers with the application of bioisosterism in the search for new bioactive compounds. For this work, two tools must be highlighted: *Compute Energy* and *Make Bioisosteres* (ELIAS, 2018).

By inserting an SDF file of a ligand-receptor complex, the *Compute Energy* tool calculates the Van der Waals Free Energy (VdWE) and the Electrostatic Free Energy (QE) involved in the interactions of each ligand atom with the receptor atoms. It then displays the Total Free Energy (TE) of each ligand atom from highest to lowest. With this information at hand, the researcher may choose which functional groups to change in order to improve the ligand-receptor interaction (ELIAS, 2018).

Figure 5. Example of division of a molecule into *root fragment* and *bioisosteric fragment*. Cyan box: atom selected by the user (ELIAS, 2018).



Source: From the authors.

Once these chemical groups are chosen, the researcher may use the *Make Bioisosteres* tool. It builds bioisosteres by changing the chemical group starting from the selected atom. Essentially, it works by dividing the molecule into two fragments: a larger one called *root fragment*, and a smaller one called *bioisosteric fragment*, which includes the selected atom along with the chemical group branching off of it. The program then searches its virtual library for bioisosteres of the bioisosteric fragment and creates several molecules by attaching these bioisosteres to the root fragment (ELIAS, 2018).

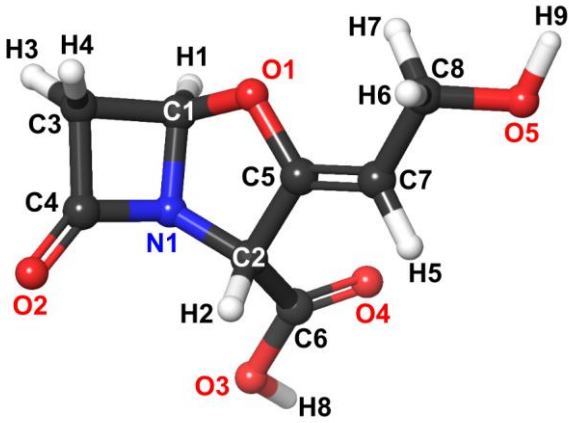
To make the bioisosteric ligands from clavulanate, the SDF file of clavulanate's best pose docked to β-lactamase was loaded to MB-Isoster, and through the Compute Energy tool, a list of the TEs of each clavulanate atom with the enzyme was generated. The results are displayed in Table 1.

Table 1: TE of each clavulanate atom with the β-lactamase residues. Oxygen 3 has no assigned VdWE nor TE because it has a non-zero formal charge.

Clavulanate's atom	VdWE (kcal/mol)	QE (kcal/mol)	TE (kcal/mol)
O1	-0.553	309.013	308.459
O2	0.149	258.788	258.937
O3	-	0.000	-
O5	8.892	415.946	424.839
N1	-0.663	383.973	383.310
O4	-0.236	265.609	265.373
H2	0.922	0.000	0.922
H1	0.061	0.000	0.061
H7	-0.179	0.000	-0.179
H3	-0.224	0.000	-0.224
H6	-0.246	0.000	-0.246
H5	-0.314	0.000	-0.314
H4	-0.324	0.000	-0.324
C7	-0.770	0.000	-0.770
C3	-1.058	0.000	-1.058
C5	-0.989	-153.984	-154.972
C2	-0.686	-155.212	-155.898
C8	-0.497	-163.560	-164.057
H8	-0.192	-243.010	-243.203
C1	-0.124	-263.342	-263.465
C6	-0.437	-267.296	-267.733
C4	-1.067	-384.778	-385.846

Source: from the Authors.

Figure 6: Numbering of clavulanate atoms according to MB-Isoster.



Source: From the authors.

As seen in Table 1, all oxygens showed unfavorable interaction values. Therefore, all were chosen as targets for bioisosteric substitutions, except Oxygen 1, because it distinguishes clavulanic acid from β-lactam antibiotics, which possess a sulfur atom in its place. Replacing Oxygen 1 with sulfur could generate a compound with antibacterial activity, and this is not the aim of the study.

The software didn't return VdWE and TE values for Oxygen 3 because it was ionized in the docked molecule. Despite this, it makes up a carboxyl group along with Carbon 6 and Oxygen 4, which had one of the highest total energy values. For this reason, O3, O4 and C6 were selected for bioisosteric changes.

The nitrogen atom was not chosen because of its importance to β-lactams reaction with the catalytic residue of β-lactamase, Ser70 (SACHA et al., 2008).

Once the bioisosteres were generated, a numbering system was created to assign a number to each ligand. Table 2 explains the system.

Table 2: Bioisosteres numbering system demonstration. Numbers other than 00 indicate an altered atom/functional group. In the example below, the number 37 indicates a replacement beginning at O2 by the functional group it represents: an imina.

Atom selected for replacement	C6	O2	O3	O4	O5
Atom/functional group number	00	37	00	00	00

Source: From the authors.

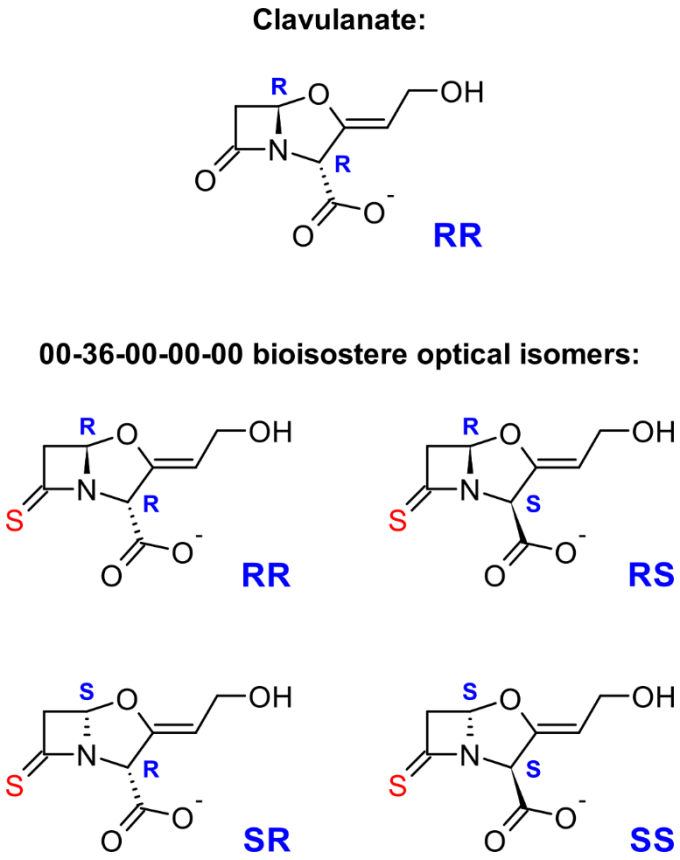
Bioisosteric ligands preparation

Once loaded to Maestro, some bioisosteres had bromine or chlorine atoms replaced by dummy atoms. These atoms were manually corrected through the user interface.

We prepared the bioisosteres using LigPrep. It was set up to generate the possible ionization states in the pH range 7.0 ± 2.0 (physiological pH range), generate tautomers, and generate all possible isomers based on inversions of the chiral centers. The process generated a total of 206 structures from the initial 35 bioisosteres (SCHRÖDINGER, 2020b).

Because clavulanic acid has 2 chiral centers that were preserved during the bioisosteres generation, all bioisosteres had 2 chiral centers. So, by going through LigPrep's ligand preparation, 4 optical isomers were generated for each bioisostere: RR, RS, SR and SS.

Figure 7: Bioisostere optical isomers. Notice that the same chiral centers are present in clavulanate and in the bioisosteres. There are 4 possible optical isomers for each bioisostere, exemplified here by the 00-36-00-00-00 bioisosteres.



Source: From the authors.

Glide docking of non-hybrid bioisosteres

The same Glide settings and grid were used to dock the bioisosteres to β-lactamase. The bioisosteres were grouped by atom target of bioisosteric substitution, and docking was performed for each group individually, with the module set up to return the 15 best poses of each group.

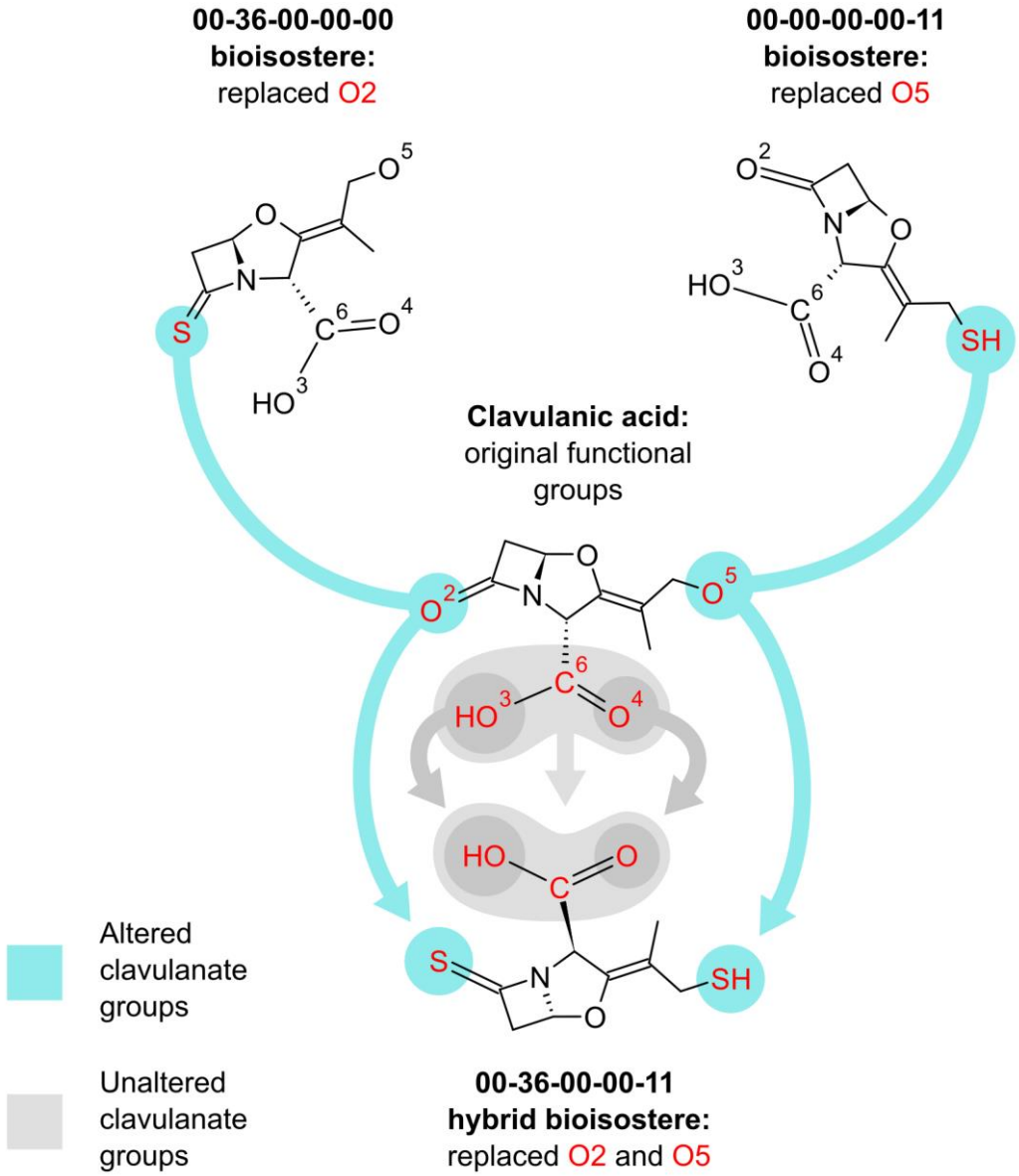
Creation and preparation of hybrid ligands

Hybridization of the bioisosteric structures was performed in order to investigate whether the combination of two or more bioisosteric functional groups could generate molecules with even better XP GScores. We started by selecting the bioisosteres with the lowest XP GScores from each group (Table 3). The hybridization process was conducted manually using Maestro's build tools.

The purpose of creating hybrid structures was to provide two options for each previously selected functional groups: either the clavulanic acid structure or the structure of a bioisostere from Table 3. Figure 8 shows an illustrative example.

Figure 8: Hybridization example. To create a hybrid bioisostere, O2 was replaced by the same substituent as 00-36-00-00-00, and O5 was replaced by the same substituent as 00-00-00-00-11. The other three groups of

clavulanate were kept unchanged in the new molecule.



Source: From the authors.

Hybridization yielded 10 hybrid bioisosteres which were numbered according to the system described previously. We prepared them using LigPrep setup to generate ionization states in the 7.0 ± 2.0 pH range. *Generate tautomers* was disabled since they had already been built manually, and *Retain specified chiralities* was selected to preserve the configuration of chiral centers. **Glide docking of hybrid bioisosteres**

We performed docking of the hybrid bioisosteres to β-lactamase using the same settings as for the non-hybrid bioisosteres docking. The module was set up to return the top 15 poses.

Obtained results were compared to those of clavulanate and non-hybrid bioisosteres, and the 5 compounds with the best XP GScores were selected for computation of pharmacokinetic descriptors.

Computation of pharmacokinetic descriptors

Having a good affinity for the receptor is not the only requirement for a drug to elicit its therapeutic effect. It also needs to be properly absorbed and distributed throughout the body, and it should not be metabolized and excreted before having enough time to generate the desired effect (GOLAN et al., 2014).

For this reason, the research included a last step to determine whether lead compounds had suitable ADME properties. QikProp was used to calculate the pharmacokinetic descriptors of the 5 best compounds. The descriptors are *Percent Human Oral Absorption* and *#stars* (SCHRÖDINGER, 2020d).

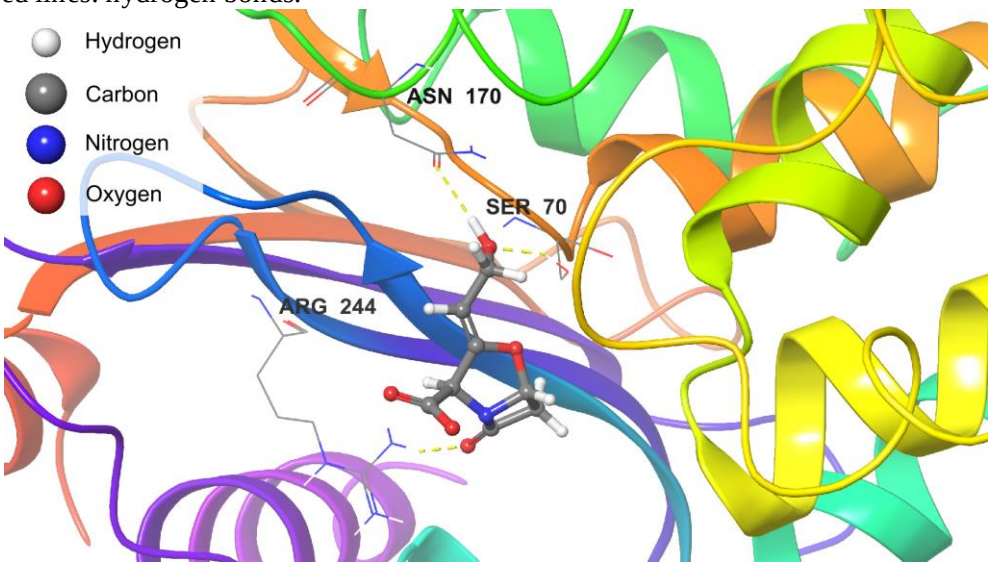
Percent Human Oral Absorption represents the predicted human oral absorption on a 0 to 100% scale, while *#stars* indicates the number of descriptors or properties that fall outside the 95% range of similar values for known drugs (SCHRÖDINGER, 2015c).

Results and Discussion

Clavulanate glide docking

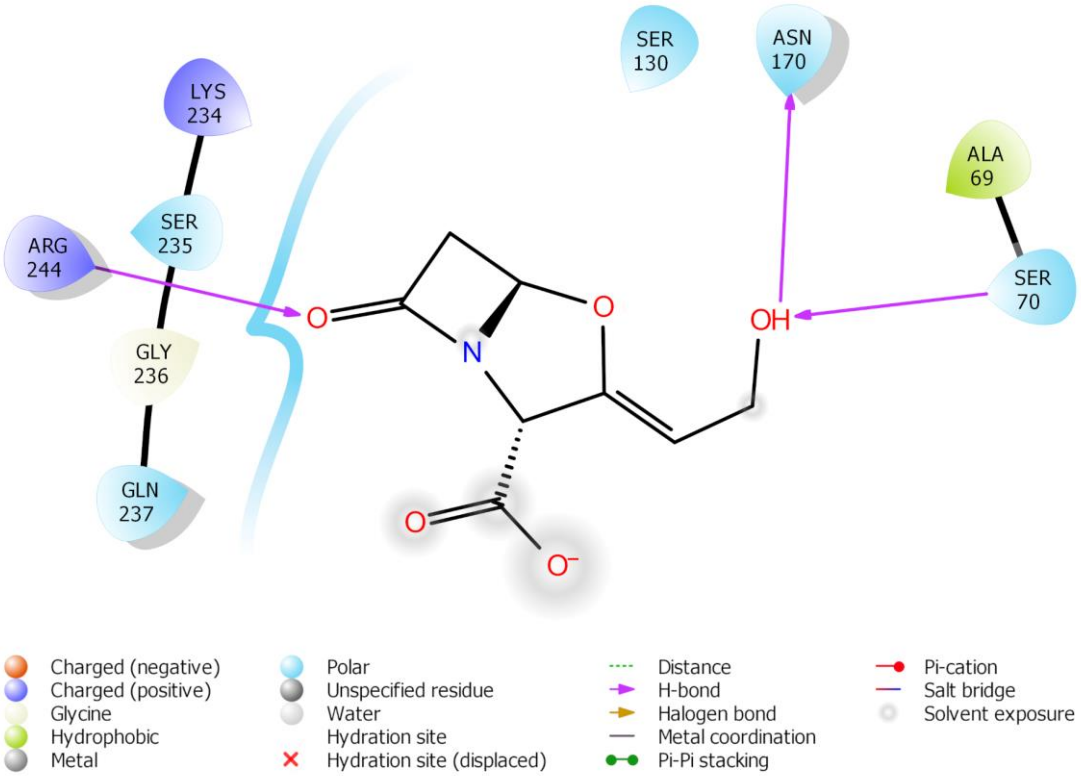
Docking of clavulanate to β-lactamase returned 9 poses, with the best one receiving an XP GScore of -4.651 kcal/mol. Figures 9 and 10 show a 3D representation of the best pose and the ligand-receptor interactions.

Figure 9: The best pose of clavulanate docked at the active site of β-lactamase. All steric contacts are optimal. Yellow dashed lines: hydrogen bonds.



Source: From the authors.

Figure 10: Diagram of ligand-receptor interactions of clavulanate with β-lactamase.



Source: From the authors.

Bioisosteres Glide docking

Glide docking XP of non-hybrid bioisosteres returned the 15 best poses for each bioisosteric group. XP GScores of the best ligand poses from each bioisosteric group are shown in Table 3. More complete data is presented in Graph 1.

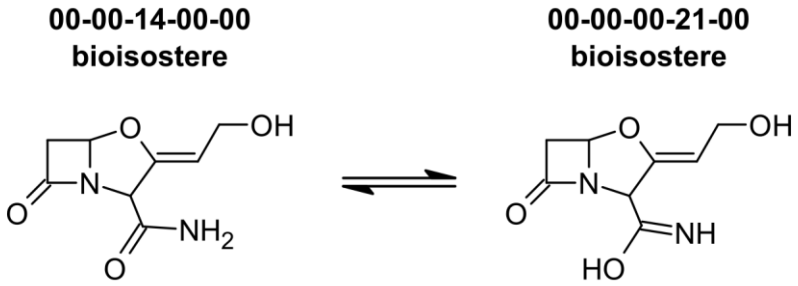
Table 3: Molecules with the lowest XP GScore from each bioisosteric group.

Bioisosteric group	Ligand number	XP GScore
C6	26-00-00-00-00 RS	-7.494
O2	00-36-00-00-00 RR	-5.662
O3	00-00-14-00-00 SS	-5.530
O4	00-00-00-21-00 SS	-5.530
O5	00-00-00-00-11 SR	-7.734

Source: From the authors.

Notice that ligands 00-00-14-00-00 SS and 00-00-00-21-00 SS show the same XP GScore. This happened because bioisosteres 00-00-14-00-00 and 00-00-00-21-00 are tautomers of each other, which caused the sets of structures generated by processing them through LigPrep to be the same. In essence, ligands 00-00-14-00-00 SS and 00-00-00-21-00 SS are identical in structure and docking pose.

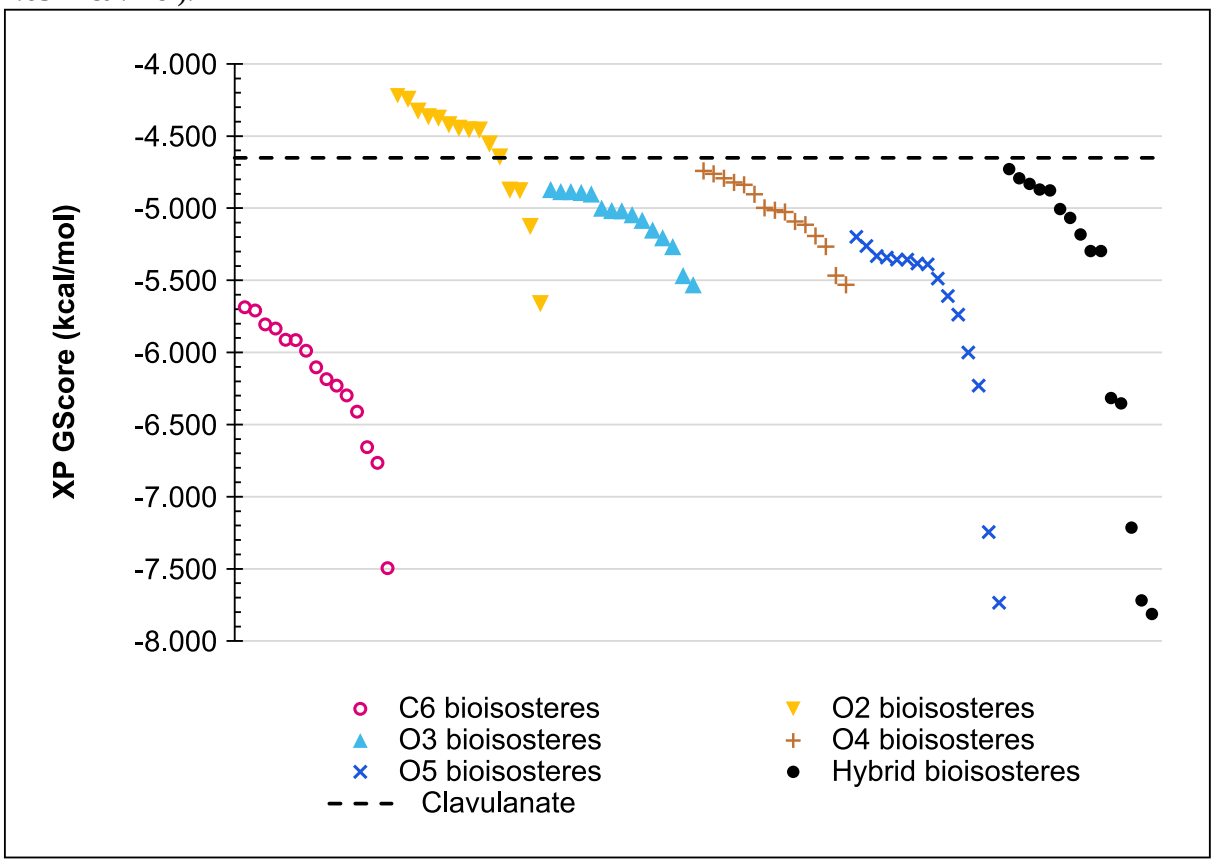
Figure 11: Bioisosteres 00-00-14-00-00 and 00-00-00-21-00 are tautomers of each other.



Source: From the authors.

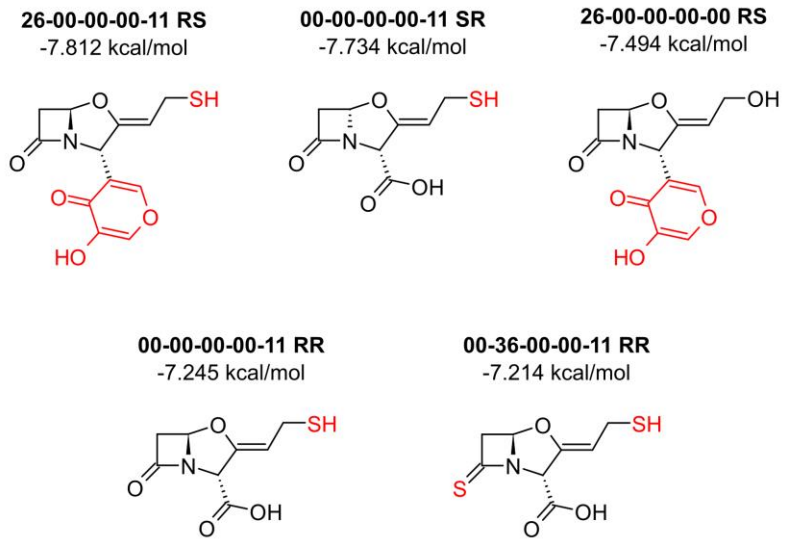
Glide docking XP of hybrid bioisosteres returned 15 poses. The 5 best compounds returned the 6 best results, which can be seen as the markers below -7.000 kcal/mol in Graph 1. Figure 12 shows the names, chiralities, XP GScores and structures of these 5 ligands best poses.

Graph 1: Bioisosteres XP GScores. The black dashed line indicates clavulanate's best pose XP GScore (-4.651 kcal/mol).



Source: From the authors.

Figure 12: Top 5 ligands in non-ionized form with their respective lowest XP GScores.



Source: From the authors.

Computation of pharmacokinetic descriptors

Table 4: *Percentage Human Oral Absorption* and *#stars* of the leading molecular docking compounds.

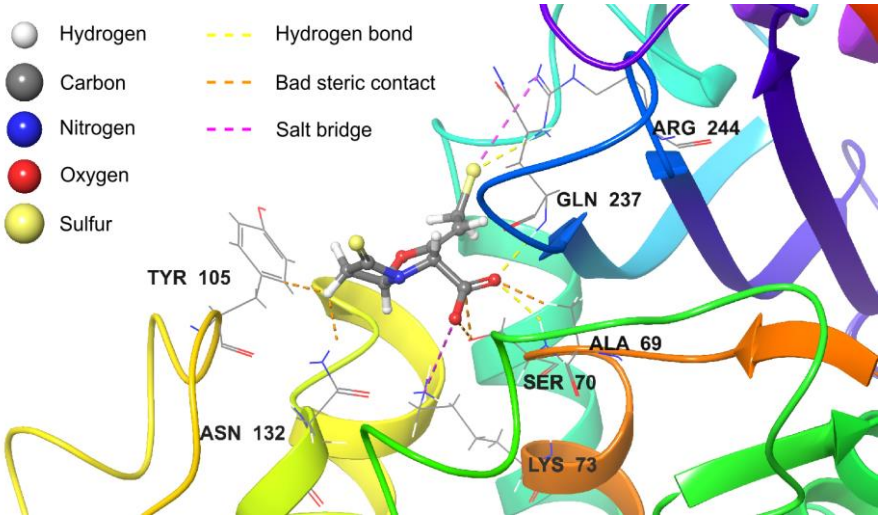
Compound	Percentage of Human Oral Absorption (%)	*#stars*
00-36-00-00-11 RR	82.976	1
26-00-00-00-11 RS	70.044	0
00-00-00-00-11 SR	63.461	0
00-00-00-00-11 RR	57.223	0
26-00-00-00-00 RS	52.805	0

Source: From the authors.

Percentage Human Oral Absorption and *#stars* values are shown in Table 4. By analyzing the data, we see that 00-36-00-00-11 RR is the only compound with a high *Percentage Human Oral Absorption* (>80%). In regards to the *#stars* descriptor, the recommended values fall within the range of 0 to 5.

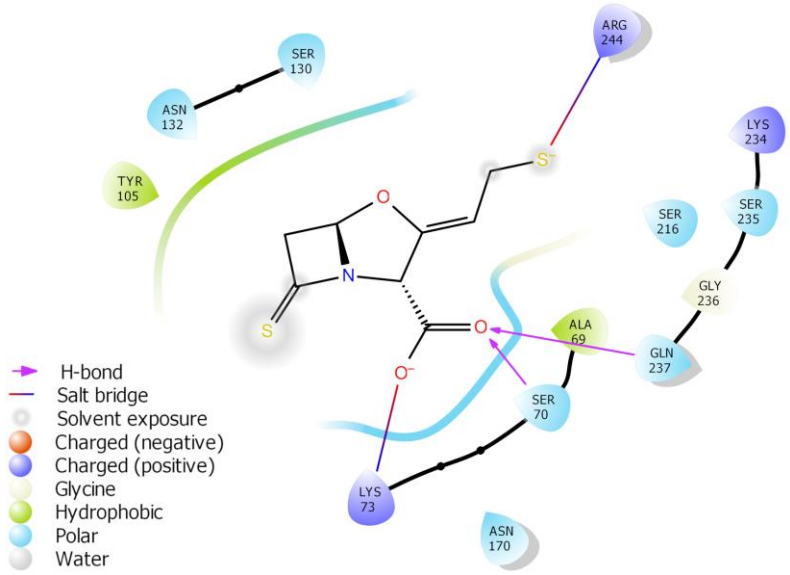
Therefore, 00-36-00-00-11 RR is the lead compound of the research, with an XP GScore of -7.214 kcal/mol, *Percentage Human Oral Absorption* of (82.976%), and values for other pharmacokinetic descriptors within their respective recommended ranges, except for the *Solute Electron Affinity* descriptor, with a value of 2.083 eV, outside the recommended range of -0.9 eV to 1.7 eV (SCHRÖDINGER, 2015c). Figures 13 and 14 illustrate details about its interactions with β-lactamase's active site.

Figure 13: 00-36-00-00-11 RR docked at the active site of β-lactamase. Optimal and ugly steric contacts are not shown.



Source: From the authors.

Figure 14: Diagram of ligand-receptor interactions of 00-36-00-00-11 RR with β-lactamase.



Source: From the authors.

In light of the promising results, further *in vitro* studies should be carried out to test the lead bioisosteres for their inhibitory effect over *S. aureus* β-lactamase, and to evaluate their safety, which could lead to the development of new drugs against *S. aureus* infections.

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