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In vitro screening, molecular docking, and ADME-Tox investigations for the design of novel beta-lactam antibiotics (Ampicillin and Ceftriaxone) derivatives as PBP2a inhibitors

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ABSTRACT
Objective: In previous studies on chickens (*Gallus domesticus*) roaming in Kinshasa, the antibiotic resistance profile of their gut microbiota was established using the conventional bacteriological test of their excreta and susceptibility testing using the diffusion disc method. Several *Enterobacteriaceae* were resistant to antibiotics commonly sold in the city, including *Staphylococcus aureus*. To identify novel therapeutics effective against this pathogen, an *in-silico* study was undertaken to develop analogs of ampicillin and ceftriaxone.
Methodology and Results: Six (6) ampicillin derivatives and four (4) ceftriaxone derivatives were generated through an *in silico* pharmacochemical study and subsequent molecular docking. The corresponding molecular structures were visualized by employing specialized computer tools. Subsequently, employing advanced bioinformatics methodologies, the physicochemical properties, pharmacokinetic profile, potential toxicity, and molecular docking studies of these derivatives with PBP2a proteins were executed.
Conclusion and application of results: The investigated compounds show promising results as potential drug candidates for PBP2a inhibitors. The development of novel Ampicillin and ceftriaxone derivatives, as well as the *in* silico ADMET properties provide valuable insights for further research in the field of antibacterial drug discovery. Their potential affinity with PBP2a and convenient oral administration make them candidates for clinical use. Their favorable pharmacokinetic properties and limited toxicity reinforce their appeal as therapeutic options. In addition, some ceftriaxone derivatives have demonstrated significant inhibition of the PBP2a enzyme, which is implicated in antibiotic resistance. Although ampicillin derivatives did not show greater inhibition capacity than ampicillin itself, one specific derivative revealed

comparable inhibition. These results provide valuable insights into the discovery of new antibacterial drugs and pave the way for future in vivo animal studies and development in this field.

Keywords: Commensal birds, MRSA, Cross-resistance, Antibiotic-resistance, Antibiotic discovery.

INTRODUCTION

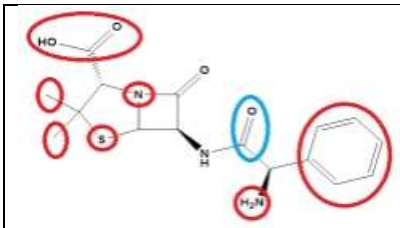
Infectious diseases continue to present a significant global health challenge, leading to substantial morbidity and mortality (Institute of Medicine (US), 2003; Fonkwo, 2008; Baker, Mahmud, and Miller, 2022). Bacterial infections encompass a wide spectrum of illnesses, varying in severity, and can be transmitted through direct contact, by air, or contaminated water (Kotra, 2007; Doron and Gorbach, 2008). Approximately five years ago, the ornithological research unit within the Biology Department of the Faculty of Sciences of the University of Kinshasa embarked on a collaborative effort with the Microbiology laboratory of the Faculty of Medicine and the medicinal chemistry laboratory of the Faculty of Pharmaceutical Sciences at the same university. This multidisciplinary team aimed to identify *enterobacteria* originating from the intestinal tracts of commensal birds and to investigate the antibiotic resistance patterns of these *enterobacteria*, which were collected from the fecal matter of stray chickens within the Kinshasa city province. *Enterobacteria*, including *Proteus vulgaris*, *Klebsiella pneumoniae*, *Salmonella sp.*, *Pseudomonas aeruginosa*, *Citrobacter spp*, and *Escherichia coli*, have been identified in bird droppings. Birds are infected with the bacteria in the feces of poultry, livestock, and humans that are thrown as garbage in public landfills (Kisasa *et al.*, 2020; Kisasa *et al.*, 2021). Studies conducted on stray chickens have provided insights into the antibiotic resistance profile of these *enterobacteria* in the city of Kinshasa. The analysis revealed that the resistance of these bacteria to certain commonly used antibiotics, obtained without veterinary medical prescription, varies across different areas. Notably, Salmonella, among the identified enterobacteria, exhibited the highest level of resistance. The most prevalent pattern of microbial resistance was observed for ampicillin and tetracycline (Ekumbo *et al.*, 2023). β-lactam antibiotics, such as penicillin and cephalosporin, are extensively employed in the treatment of bacterial infections. However, their efficacy is hindered by the emergence of resistance, particularly in methicillin-resistant *Staphylococcus aureus* (MRSA) strains (Tattevin, 2011; Green *et al.*,2012). The survival of bacteria is intricately linked to the integrity of their cell wall, which plays a crucial role in providing structural support, mechanical stability, and protection against environmental variations and external threats (Chapot-Chartier and Kulakauskas, 2014; Dörr *et al.*, 2019). Cell wall biosynthesis, a biological process in bacteria, involves the formation of peptidoglycan, a predominant polymer that serves as the building unit (Liu and Breukink, 2016). Peptidoglycan backbone biosynthesis is facilitated by transglycosylases and transpeptidases, also known as penicillin-binding proteins (PBPs) (Popham and Young, 2003; Miyachiro, Contreras-Martel, and Dessen, 2019; Shambhavi *et al.*, 2021). These PBPs play a crucial role in bacterial survival and have become prime targets for antibiotics, particularly β-lactams (Strominger and Tipper, 1965). In the case of Staphylococcus *aureus*, four native PBPs (PBP1, PBP2, PBP3, and PBP4) have been identified, while a fifth PBP, PBP2a, is present in methicillin-resistant *S. aureus* (MRSA) strains,

contributing to their antibiotic resistance (Lim and Strynadka, 2002; Fergestad *et al.,* 2020). The clinical qualification of a strain as resistant is determined by its ability to survive the antibiotic therapy administered (Alonso *et al.,* 2017; WHO, 2018; Ekumbo *et al.,* 2023). However, it is worth noting that the statistics concerning this global issue may be underestimated (WHO, 2018; Burnham *et al.,* 2020). About 700,000 cases of antibiotic resistance-related deaths have been recorded worldwide (Shankar, 2016). This alarming figure, coupled with the undeniable burden placed on society, both from a socioeconomic and financial perspective, further exacerbates existing social inequalities (Tadesse *et al.,* 2017; Yam *et al.,* 2019; Assani Sabiti *et al.,* 2023). Despite the advancements made in the discovery of new antibiotics for treating methicillin-resistant *Staphylococcus aureus* (MRSA) infections, resistance to these therapeutic agents continues to emerge. The persistent evolution of antibiotic resistance presents an ongoing challenge, underscoring the urgent need to explore new avenues for antibiotic discovery. The focus of our study involved two antibiotics: Ampicillin and Ceftriaxone. Our first experiment entailed the *in-silico* development of molecules possessing similarities to Ampicillin and Ceftriaxone. This involved pharmacomodulation or changes in the positions of groups on the outer nuclei of both antibiotics, to determine their pharmacokinetic and toxicity profiles. Pharmacokinetics, often described as the interaction between the body and a drug, encompasses the fate of the drug from its introduction to its elimination, as well as the temporal evolution of its absorption (Cutler, 1984; Turfus *et al.,* 2017; Currie, 2018). The pharmacokinetic profile and toxicity of a drug play a pivotal role in its development. The second experiment involved docking, a numerical approach to molecular modelling. The objective was to identify the optimal orientation between beta-lactams or β-lactam antibiotics and the presumed bacterial targets responsible for their resistance to antibiotic therapy. These two methodologies aim to develop more potent molecules capable of inhibiting the proteins accountable for bacterial resistance to the tested antibiotics.

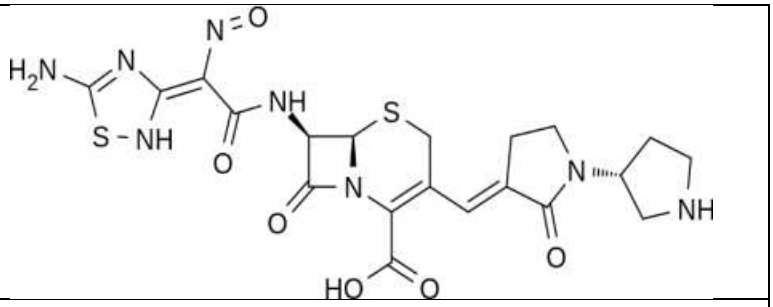
MATERIALS AND METHODS

Pharmacokinetics, ADMET profile: Bioinformatics tools were employed in this study. The computational tasks were carried out using an HP laptop, specifically a model equipped with a 2.4 GHz AMD processor and 4 GB of RAM. Various application software packages were utilized, including ChemDraw 16.0 Professional by CambridgeSoft, SwissADME (Daina *et al.,* 2017), pkCSM (Pires *et al.,* 2015), and ADMETlab2.0 (Xiong *et al.,* 2021). ChemDraw 16.0 Professional is a software tool designed for the depiction of chemical and biological structures in 2D. It offers a range of functionalities, such as the conversion between structure and IUPAC nomenclature. Additionally, due to its compatibility with multiple ADMET property calculation software programs, 2D structures generated in ChemDraw can be seamlessly imported into calculation software for further analysis. Web-based tools, namely SwissADME, pkCSM, and ADMETlab2.0, were employed to evaluate the physicochemical properties, pharmacokinetics, drug-likeness, and toxicology of compounds with potential therapeutic applications. Figure 1 illustrates the six Ampicillin derivatives generated through *in silico* pharmacomodulation. Modification of the carbonyl group in the main chain of Ampicillin resulted in the introduction of ester and ether functionalities, along with the addition of chlorine (Cl) and fluorine (F) atoms. Similarly, four

ceftriaxone derivatives were generated by modifying various groups within the ceftriaxone structure. To ensure the novelty of these derivatives, their structures were converted into SMILES codes (Polanski and Gasteiger, 2017; Mvondo et al, 2021) using the SwissADME server. Furthermore, to verify that these compounds had not been previously synthesized, their structures were converted into IUPAC names using ChemDraw software, followed by a comprehensive search of the scientific literature for matching compounds based on both their IUPAC names and/or SMILES codes.



Ampicillin with the possibility of pharmacomodulation

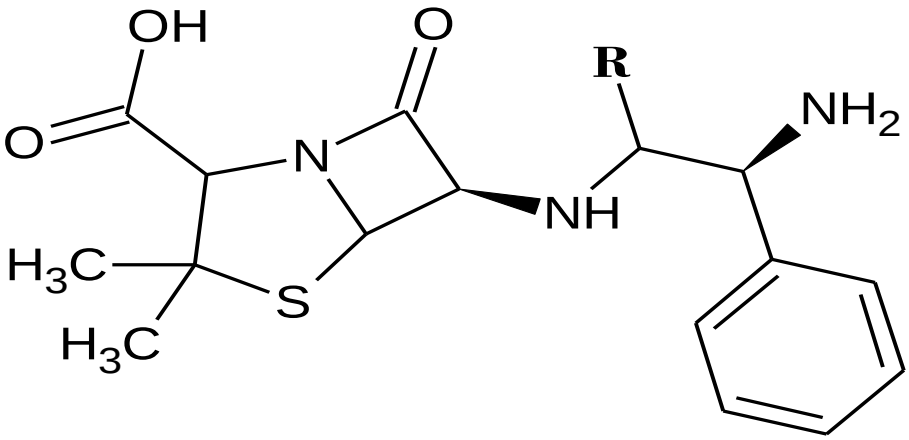


Ceftobiprole

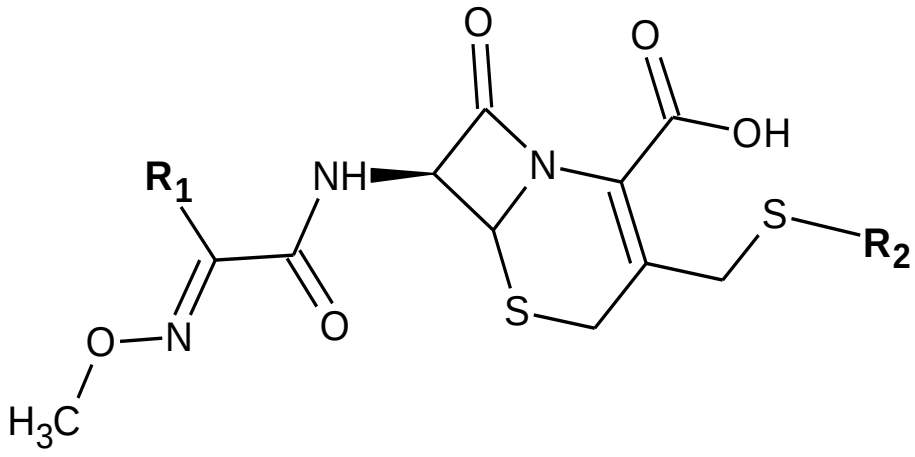
Figure 1: a) Ampicillin with the possibility of pharmacomodulation. b) Ceftobiprole.

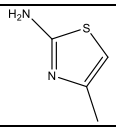
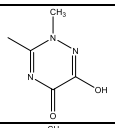
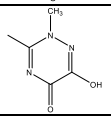
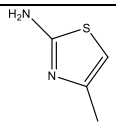
Painting: Structure of Ampicillin derivatives

Structure of ceftriaxone derivatives



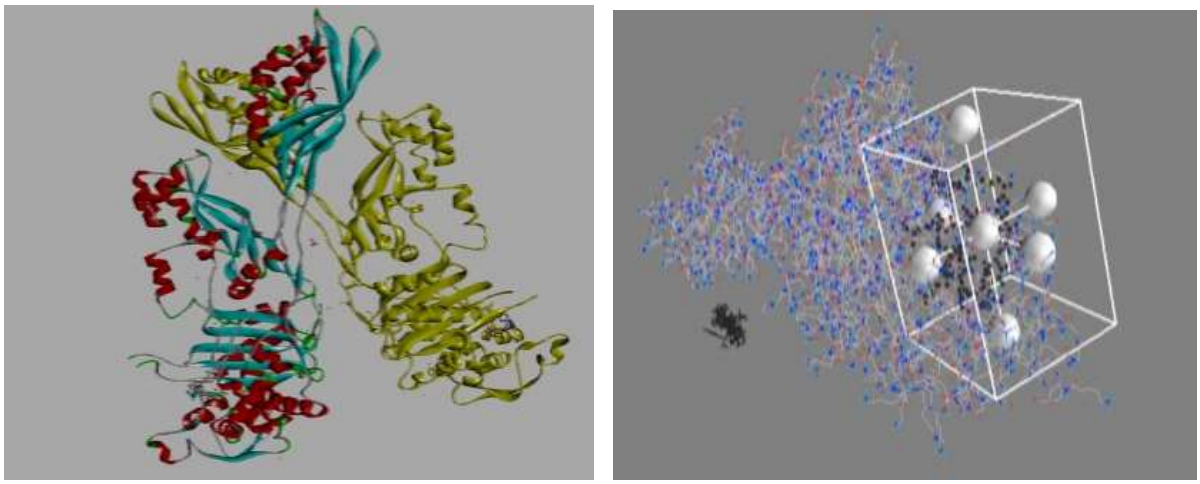
Ampicillin	R=O
Ampicillin-derivative 1	R = OCH_3
Ampicillin-derivative 2	R = CH_2Cl
Ampicillin-derivative 3	R = $OCOCH_3$
Ampicillin-derivative 4	R = OCH_2Cl
Ampicillin-derivative 5	R = $OCOCH_2F$
Ampicillin-derivative 6	R = OH



	R1	R2
Ceftria-derivative 1		CH_3
Ceftria-derivative 2	CH_3	
Ceftria-derivative 3	NH_2	
Ceftria-derivative 4		

Docking: Bioinformatics tools were used in this research, including the online Protein Data Bank (PDB) database provided by RCSB (Research Collaboratory for Structural Bioinformatics). The crystal structure of the PBP2 protein (ID: 4DKI) was obtained from the PDB database. The Discovery Studio Client 2021 tool allowed us to visualize the structure of the protein before and after docking, as well as to prepare the protein and visualize the type of interaction between the ligands and the receptor. ChemDraw 16 was used for designing and optimizing the structures of the ligands. The Pyrex tool was employed for calculating the docking score. To generate the ligands, six structures derived from ampicillin and four structures derived from ceftriaxone were designed using ChemDraw 16.0. The structures of ampicillin and ceftriaxone were obtained from the PubChem online chemical library before docking. The ligands were prepared by minimizing the free energies associated with molecular geometry and atom repulsions using the MM2 energy minimization function. The ligands were then saved in PDB format. The PBP2a protein structure obtained from the PDB database was prepared for docking by removing the B chain, water molecules, and co-crystallized ligand. The amino acids constituting the active site of the protein were noted before removing the co-crystallized ligand, as well as the interactions between the ligand and the protein receptor Hydrogens were added, and the CHARMn force field was applied using the Discovery Studio 2021 software. The prepared protein structure was saved in PDB format. The

ligand structures and the prepared protein structure in PDB format were loaded into the PyRx software. The protein was designated as a macromolecule, and each ligand structure was considered a ligand. The active site residues of the protein were indicated, and the coordinates of the active site center were adjusted during grid setting (Figure 2b). Subsequently, the docking calculations were initiated.



a) **b)**

Figure 2: a) 3D structure of PBP2a (chain B in yellow). b) Grid setting

RESULTS AND DISCUSSION

Pharmacokinetics: The SMILES codes obtained were submitted to different prediction tools. Firstly, the physicochemical properties were analyzed. The parameters taken into account include the molecular weight MW (g/mol), the logarithm of the octanol-water partition coefficient (log P), the number of acceptor hydrogen bonds (nHA), the number of donor hydrogen bonds (nHD), the number of rotatable bonds (nRot), the number of rigid bonds (nRig), the molar refractivity (in $m^3.mol^{-1}$) and the polar topological surface area (TPSA, in Å²). The number of rotatable bonds (nRot) and the number of rigid bonds were used to calculate the flexibility (Flx) of each molecule, which is defined as the ratio nRot/nRig. The results of these parameters, obtained using the SwissADME software, are presented in Table 1, except for nRig and nRot, whose values were obtained using the ADMETlab2.0 tool. The pharmacokinetic profile, including absorption, distribution, metabolism, elimination as well as toxicity, was predicted using the online tools SwissADME, pkCSM, and ADMETlab2.0, as previously described. The results of these predictions are presented in Table 2.

Table 1: Physicochemical parameters of the 6 Ampicillin derivatives

No.	Formula	MW	Log P	nHA	nHD	nRot	nRig	MR	Flx	TPSA
1	$C_{17}H_{23}N_3O_4S$	365.45	2.09	6	3	6	16	98.26	0.375	104.89
2	$C_{17}H_{22}ClN_3O_4S$	399.89	1.81	6	3	7	16	103.05	0.438	104.89
3	$C_{18}H_{23}N_3O_5S$	393.46	2.02	7	3	7	17	103.26	0.412	121.96
4	$C_{18}H_{24}ClN_3O_5S$	429.92	1.31	8	4	8	16	109.02	0.500	125.12
5	$C_{18}H_{22}FN_3O_5S$	411.15	2.04	8	3	8	17	103.31	0.471	121.96
6	$C_{16}H_{23}N_3O_4S$	353.44	1.69	7	5	5	15	94.49	0.333	119.05

Table 2: Physicochemical parameters of the 4 ceftriaxone derivatives

Compound	Formula	PM	Log P	nHA	nHD	nRot	MR	TPSA
0	$C_{18}H_{18}N_8O_7S_3$	554.58	1.07	11	4	9	133.37	213.96
1	$C_{15}H_{17}N_5O_5S_3$	443.52	1.84	7	3	8	111.40	172.11
2	$C_{16}H_{18}N_6O_7S_2$	470.48	1.25	10	3	8	113.62	183.07
3	$C_{15}H_{17}N_7O_7S_2$	471.47	0.89	10	4	8	111.52	182.04
4	$C_{16}H_{20}N_6O_5S_3$	472.56	1.62	8	4	9	118.91	183.81

Table 3: Pharmacokinetic and toxicological profile of the 6 ampicillin derivatives

Absorption	Ligand 1	Ligand 2	Ligand 3	Ligand 4	Ligand 5	Ligand 6
AGI	High	High	Weak	Weak	Weak	Weak
Log *S*	-1.98	-1.94	-2.09	-2.05	-1.94	-1.92
HIA	46.9	45.1	46.1	37.9	41.8	33.1
Distribution						
BVG	27.2	27.6	25.6	26.2	34.1	29.5
BBB	-1.0/No	-1.2/No	-1.1/No	-1.6/No	-1.3/No	-1.8/No
Metabolism						
CYP2D6	Yes	No	Yes	Yes	No	Yes
CYP3A4	No	No	No	No	No	No
Excretion						
Total clearance	0.4	0.5	0.4	0.7	0.5	0.6
SR OCT2	No	No	No	No	No	No
Toxicity _						
Mutagenicity	No	No	No	No	No	No
Hepatotoxicity	Yes	Yes	Yes	Yes	Yes	Yes
Carcinogenicity	No	No	No	No	No	No
LD_{50}, in mol/kg and mg/kg/day for values in bold)	2,198 **1099**	1,650 **825**	2,262 **1131**	2,179 **1090**	1,662 **831**	2,229 **1115**

Table4: Pharmacokinetic and toxicological profile of the 4 Ceftriaxone derivatives

	Compound				
Setting	0	1	2	3	4
Absorption					
Solubility (log mol/L)	-2.853	-2,446	-2,700	-2.685	-2,520
Human Intestinal Absorption (%)	28.03	43,371	26,398	17,173	25,806
Bioavailability score	0.11	0.11	0.11	0.55	0.55
Distribution					
BBB permeability (pkCSM) BHE permeability (SwissADME)	-2,291 No	-1.731 No	-1.943 No	-1.975 No	-1,817 No
Metabolism					
CYP2D6	No	Yes	No	No	Yes
CYP3A4	No	No	No	No	No
Excretion					
Total clearance	-0.062	0.175	0.039	-0.083	0.403
Renal OCT2 Substrate	No	No	No	No	No
Toxicity					
Mutagenicity (Ames Test)	No	No	No	No	No
Hepatotoxicity	Yes	Yes	Yes	Yes	Yes
Acute oral toxicity in rats (LD_{50}, in mol/kg and mg/kg/day for values in bold)	2,326 **1163**	2,122 **1061**	1,854 **927**	1,840 **920**	2,132 **1066**

Docking: The energies obtained after minimization of flexibility energy for each molecule are given in the table below.

Table 5. Ligand flexibility energy

Ligand	Energy calculated
Ampicillin	47.96 kcal/mol
Ampicillin-derivative 1	61.79 kcal/mol
Ampicillin-derivative 2	66.84 kcal/mol
Ampicillin-derivative 3	60.03 kcal/mol
Ampicillin-derivative 4	49.61 kcal/mol
Ampicillin-derivative 5	64.33 kcal/mol
Ampicillin-derivative 6	39.58 kcal/mol
Ceftriaxone	97.26 kcal/mol
Ceftriaxone-derivative 1	84.75 kcal/mol
Ceftriaxone-derivative 2	79.81 kcal/mol
Ceftriaxone-derivative 3	83.46 kcal/mol
Ceftriaxone-derivative 4	85.25 kcal/mol

Table 6.\ Binding energy between ligands and receptor (PBP2a)

Ligand	Binding Affinity (Kcal/Mol)
Ceftriaxone	-7.5
Ceftriaxone derivative 2	-7.1
Ceftriaxone derivative 3	-7.0
Ampicillin	-6.6
Ampicillin derivative 6	-6.6
Ampicillin derivative 1	-6.5
Ampicillin derivative 2	-6.4
Ampicillin derivative 4	-6.4
Ampicillin derivative 3	-6.3
Ceftriaxone derivative 4	-6.2
Ampicillin derivative 5	-6.2
Ceftriaxone derivative 1	-6.0

DISCUSSION

The focus of our study involved two antibiotics: Ampicillin and Ceftriaxone. Our first experiment entailed the *in-silico* development of molecules possessing similarities to Ampicillin and Ceftriaxone. In this part, the results on molecules developed with a similarity with these two antibiotics are discussed to understand their factors modulating their pharmacokinetic profiles on the one hand, and their docking on the other hand to justify their optimal orientations between beta-lactams or β-lactam antibiotics and the presumed bacterial targets responsible for their resistance to antibiotic therapy.

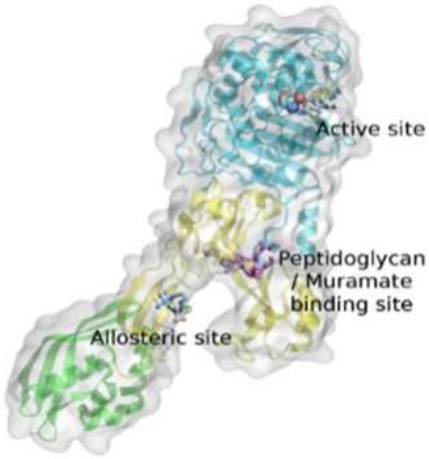
Pharmacokinetic: The initial phase of the drug absorption process entails the disintegration of tablets or capsules, followed by the dissolution of the active ingredient. However, the efficacy and completeness of oral absorption can be compromised due to limited solubility. Consequently, early evaluation of solubility is crucial in the quest for novel drugs. The solubility prediction of a compound is expressed as a logarithmic molar concentration (log mol/L). Compounds exhibiting solubility values ranging from -4.0

to 0.5 log mol/L are deemed suitable for optimal absorption (Buri, 1983). In the case of ceftriaxone derivatives resulting from pharmacomodulation, all four derivatives displayed satisfactory solubility. However, their rate of intestinal absorption was significantly low, ranging from 17% to 43%. This parameter proves to be more significant than solubility alone. Relying solely on solubility would inaccurately classify ceftriaxone as a compound with favourable oral absorption, which is not the case. Conversely, the percentage of intestinal absorption in humans accurately reflects the reality. Among all the derivatives, only compound 1 exhibited higher absorption (43%) compared to ceftriaxone (28%). The bioavailability scores are consistent with the likelihood of intestinal absorption in humans, except for compounds 3 and 4 (Denis, 1996). Compound 1 demonstrated a bioavailability score of 0.55, indicating potential good absorption, as it could achieve a bioavailability greater than 10% in rats (Alamri, 2020). However, this contradicts the probability of intestinal absorption in humans (Isabelle St-Jean, 2015). The permeability of the blood-brain barrier (BBB) plays a crucial role in determining whether a molecule will exert a beneficial or harmful effect on the brain (Leterrier *et al.,* 2023). The BBB permeability values obtained using the pkCSM software were all negative and below the recommended threshold of 0.3, (Tamaian *et al. 2023*) indicating that the compounds are unable to cross the BBB. These findings are further supported by the SwissADME software, which provides a binary response (yes/no). Xenobiotic metabolism, facilitated by specific enzymes such as cytochromes P450, plays a central role in the biotransformation, metabolism, and detoxification of foreign compounds in the body. CYP2D6 and CYP3A4 enzymes have been reported to be primarily involved in drug metabolism (Shin *et al.,* 2011). Encouragingly, the metabolism prediction results indicate that all derivatives are classified as non-CYP3A4 inhibitors. Derivatives 1 and 4 show particular promise as CYP2D6 inhibitors. Excretion parameters in pharmacokinetics encompass total clearance and organic cation transporter 2 (OCT2) substrate. OCT2 serves as a molecular transporter that plays a crucial role in drug compound absorption, elimination, and renal clearance. Consequently, renal OCT2 has a direct correlation with total clearance. Moreover, OCT2 provides valuable insights not only into clearance but also into the renal toxicity of a molecule (Fourrier and Seidowsky, 2010). All ceftriaxone derivatives are anticipated to be OCT2 inhibitors based on predictions from the pkCSM software. This implies that these derivatives may undergo renal absorption and not persist in the body for an extended duration. In terms of toxicity assessment, the Ames mutagenicity test is employed to evaluate the mutagenic potential of chemical compounds (Pélissier, 1996). For all the derivatives investigated, this test yielded negative results. Compounds exhibiting an LD50 (median lethal dose) between 500 and 2000 mg/kg are considered safe with minimal toxicity. (Van Den, 1990) Conversely, compounds with an LD50 below 300 are deemed toxic. In our case, the studied derivatives possess LD50 values ranging from 920 to 1163 mg/kg, classifying them as non-toxic. According to Pfizer's established rule, compounds with a high log P (> 3) and low TPSA (< 75) are more likely to be toxic (Monge, 2006). Analysing the log P and TPSA results presented in Table 2, we can conclude that these compounds are not toxic. However, it is important to note that all these compounds exhibit hepatotoxicity. The obtained results indicate that the four pharmaco-modulated ceftriaxone derivatives possess a favourable ADMET (absorption, distribution, metabolism, elimination, and

toxicity) profile, albeit with limited oral absorption and hepatotoxicity molecules. As for the ampicillin derivatives, their molecular weights range from 353 to 430 g/mol, all falling below 500 g/mol. The log P values, varying between 1 and 2, are also below 5. The number of acceptor hydrogen bonds (nHA) for these derivatives ranges from 6 to 8 (less than 10), while the number of donor hydrogen bonds (nHD) is between 3 and 5 (≤ 5). None of these molecules violates Lipinski's rule, indicating their potential as promising oral drug candidates. Log S results suggest that all six ligands exhibit good solubility. SwissADME results on gastrointestinal absorption (AGI) reveal that only compounds 1 and 2 demonstrate good gastrointestinal absorption, while the other molecules exhibit low but not negligible absorption. The percentage absorption of these six compounds by the human gut ranges from 33% to 47%, with compound 1 exhibiting the highest absorption and compound 6 the lowest, in accordance with absorption predictions for derivatives 1 and 6. The binding of a drug to plasma proteins significantly influences its pharmacodynamic behaviour. This binding directly affects the drug's efficacy, as only the free fraction of a molecule exerts pharmacological effects (Ozier, 1996). Predicted plasma protein binding (LPP) values range from 27.6% to 34.1%, well below the 90% threshold for drugs with high plasma protein binding. Consequently, our derivatives are expected to have minimal binding to plasma proteins, allowing a significant portion of free molecules to exert pharmacological activity. The blood-brain barrier (BBB) values obtained using pkCSM are consistently negative and below the recommended threshold of 0.3, indicating that the derivatives studied cannot penetrate the BBB. Regarding metabolism, the results indicate that two derivatives (compounds 2 and 5) do not inhibit CYP2D6 compared to the other derivatives. Conversely, the findings are promising for CYP3A4, as none of the derivatives inhibits this enzyme. Clearance results reveal that all six derivatives exhibit poor clearance, ranging from 0.4 to 0.7 mL/min/kg. However, all analysed derivatives are predicted to be OCT2 inhibitors. The predicted LD50 values for all studied derivatives range from 825 to 1131 mg/kg, classifying them as non-toxic. In general, the predicted results for the six ampicillin derivatives indicate that these compounds possess favourable pharmacokinetic and toxicological profiles, except for exhibiting hepatotoxicity.

Molecular docking: The molecules' free energies were minimized using the previously described method, and the results are summarized in Table 5. By minimizing the energy, the molecules achieve a stable conformation, reducing atomic repulsions and electronic interactions. This stable conformation enhances the molecule's solubility in the biological medium and its ability to traverse the target protein's cavities. The molecule's size influences its flexibility, promoting interactions with the protein's amino acids. Table V shows that ceftriaxone exhibits the highest flexibility energy, followed by derivatives 4, 1, and 3, respectively. Derivative 2 has the lowest flexibility energy. Among the ampicillin derivatives, derivative 2 has the highest flexibility energy, followed by derivatives 5, 1, 3, 4, and 6, respectively. Ampicillin itself has an energy of 47.9680 kcal/mol. The choice of the PBP2 protein for docking is based on its crucial role in the synthesis of the bacterial wall in methicillin-resistant staphylococci (MRSA). Docking was used to calculate the binding energy of ampicillin, ceftriaxone, and their derivatives, as shown in Table VI. The results indicate that ceftriaxone has a higher binding energy than all the designed derivatives, suggesting its potential as a more effective inhibitor of PBP2a.

Among the ceftriaxone derivatives, derivatives 2 and 3 exhibit similar levels of inhibition towards the PBP2a enzyme due to their close binding energies. Derivatives 1 and 4 have significantly lower binding energies compared to derivatives 2 and 3, which may explain the observed difference in inhibition levels during in vitro tests. In the case of ampicillin derivatives, none of the designed derivatives has a higher binding energy than ampicillin itself. Therefore, none of these derivatives is expected to have a greater inhibition capacity than ampicillin. Derivative 6 shows a similar level of inhibition as ampicillin, with an equal binding energy. All the designed derivatives have lower binding energies compared to ampicillin, albeit with minimal differences. These observations suggest that all molecules may have a similar level of inhibition. Compared to the fifth-generation cephalosporin ceftobiprole, known for its effectiveness against MRSA and its main target being PBP2a, which is also the co-crystalized ligand of the protein used in this study, none of the designed derivatives have binding energy higher than, equal to, or even close to that of ceftobiprole, whose binding energy is 9.7 kcal. If the biological activity of a molecule towards a biological target were solely determined by the binding energy, then the designed derivatives would be considered inactive compared to PBP2a, as their binding energies are lower than that of ceftobiprole, which serves as a reference in this study. The analysis of interactions between ceftobiprole and the target protein PBP2a reveals hydrogen bonds with residues Ser403, Ser462, Asn464, Gly520, Ser598, Thr600, and Glu602. Hydrogen bonds are essential for molecular recognition between a receptor and a ligand. Additionally, other electrostatic interactions such as Van der Waals interactions, salt bridges, unconventional hydrogen bonds, and Pi-alkyl interactions are observed between ceftobiprole and PBP2a. The literature suggests that residues Gly402, Ser403, Lys406, Ser462, Gly520, Lys597, Ser598, and Tyr600 are among the functional residues of PBP2a. Particularly, residues Ser400, Gly402, Ser403, and Thr404 play a crucial role, with Ser403 being a catalytic residue of the protein.The analysis of interactions between the designed derivatives and the molecular target, compared to the interactions between ceftobiprole and the literature data, reveals that all the derivatives interact with at least one of the mentioned residues through hydrogen bonds or other electrostatic interactions such as Van der Waals interactions, pi-sulphur interactions, pi-alkyl interactions, carbon-hydrogen bonds, pi-pi shaped, and pi-anion interactions (Figure 3). It should be noted that some ligands form unfavourable interactions with the protein, which destabilize the ligand-receptor complex compared to the previously mentioned hydrogen bonds and interactions.



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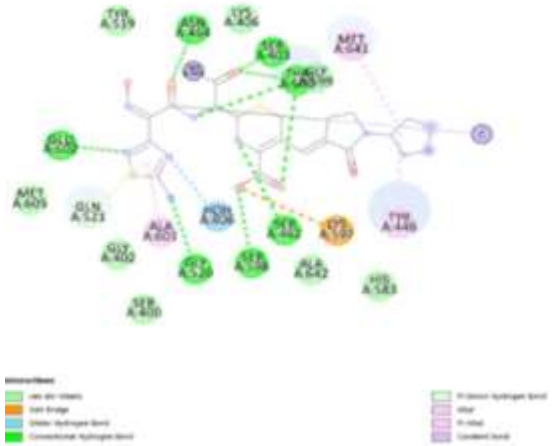
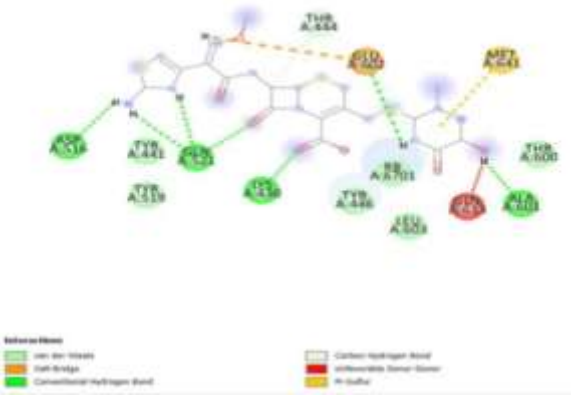
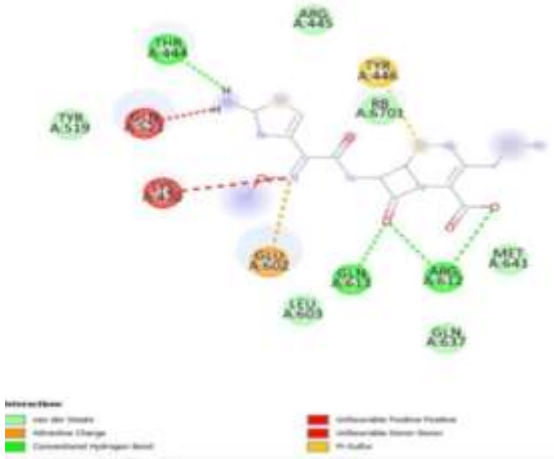


b

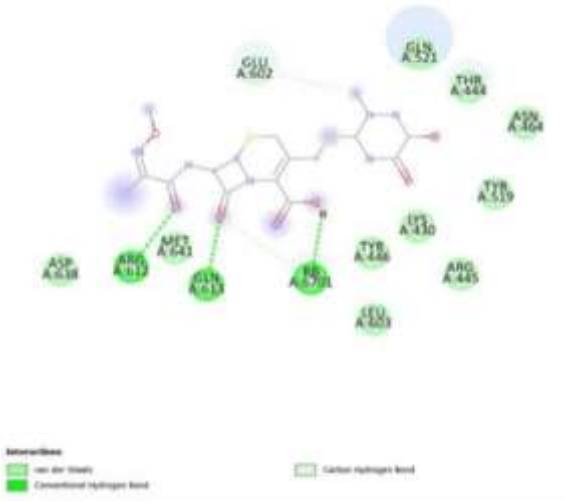
Figure 3. a) PBP2a protein. b) Interaction between Ceftobiprole and the PBP2a protein.



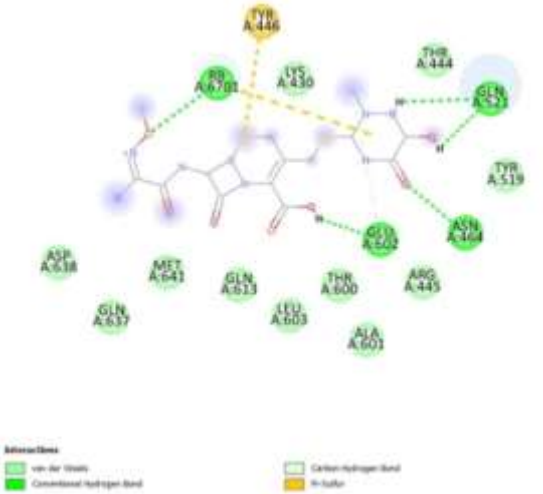
Ceftriaxone



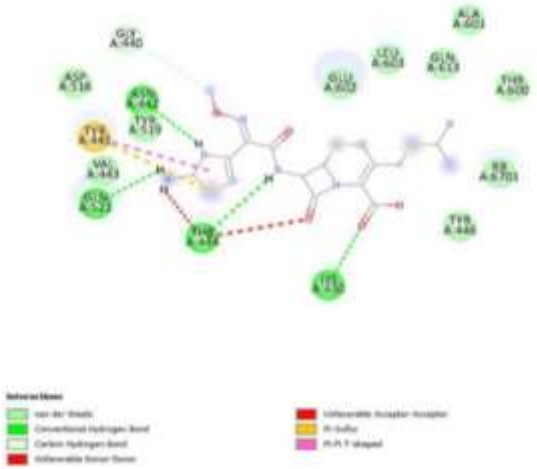
Ceftriaxone derivative 1



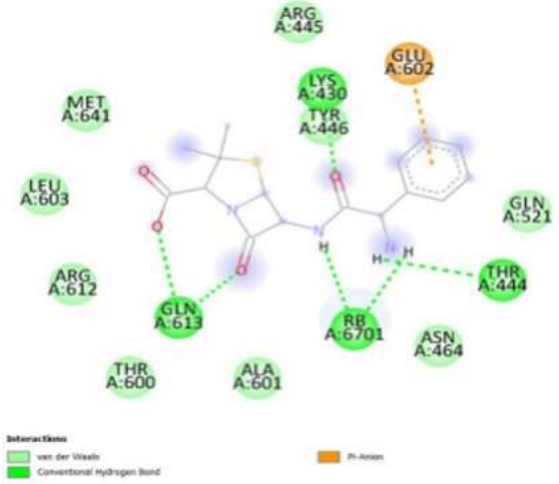
Ceftriaxone derivative 2



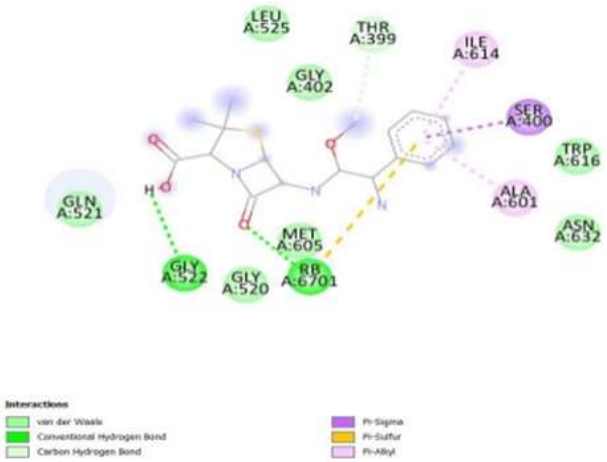
Ceftriaxone derivative 3



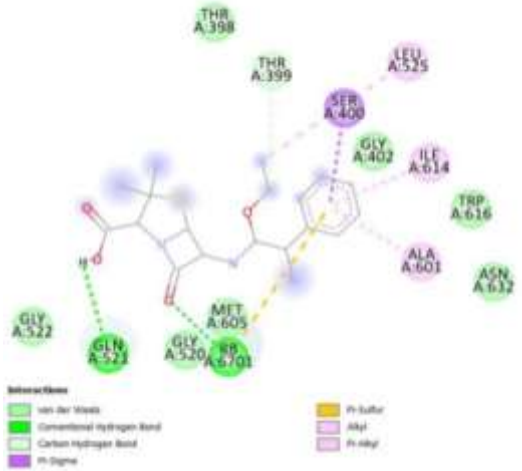
Ceftriaxone derivative 4



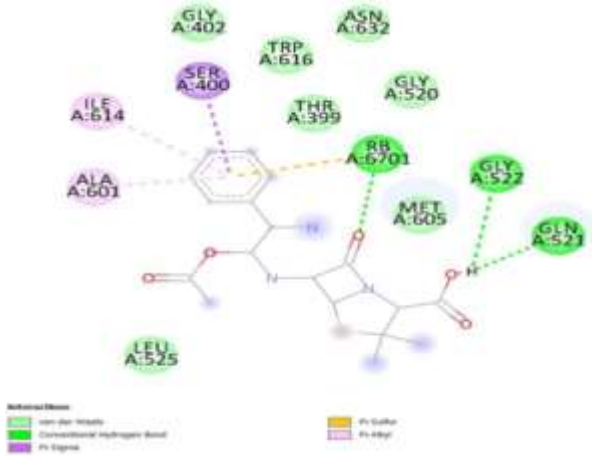
Ampicillin



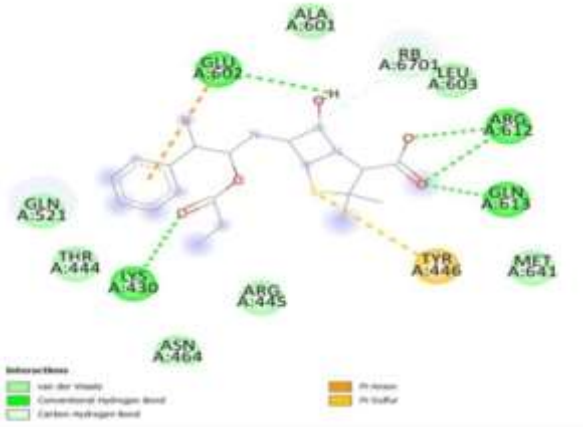
Ampicillin-derivative 1



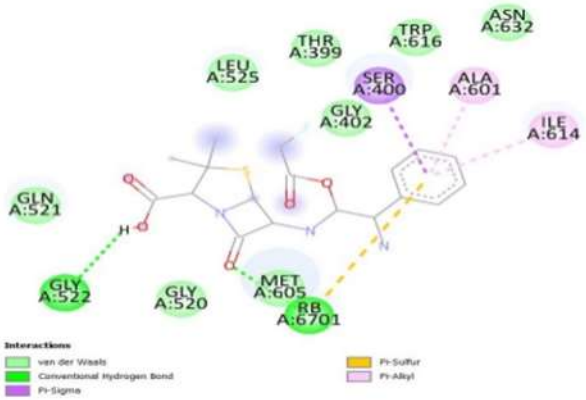
Ampicillin-derivative 2



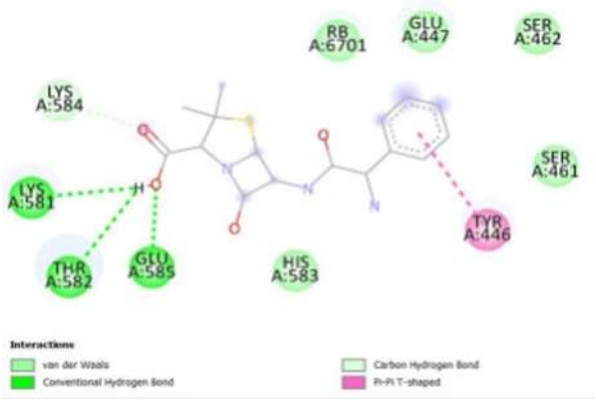
Ampicillin-derivative 3



Ampicillin-derivative 4



Ampicillin-derivative 5



Ampicillin-derivative 6

Figure 4: Interaction between the designed derivative and the PBP2a protein

CONCLUSION AND APPLICATION OF RESULTS

In the current investigation, the focus was on two specific antibiotics, namely Ampicillin and Ceftriaxone. The objective of this study was to develop novel compounds derived from these antibiotics, with the potential to possess an intriguing antibacterial profile and elicit minimal resistance from microorganisms. To achieve this, six analogs of Ampicillin and four analogs of Ceftriaxone were generated utilizing bioinformatics resources. The evaluation of the newly derived compounds involved assessing their pharmacokinetics and toxicology using SwissADME, pkCSM, and ADMETlab2.0 software. The pharmacodynamic profile of these derivatives was predicted through advanced bioinformatics methods. The results revealed that, overall, these novel molecules exhibited favourable pharmacokinetic properties, making them suitable for oral administration. It was observed that they do not cross the blood-brain barrier. Additionally, these compounds demonstrated a satisfactory toxicological profile, albeit with the exception of limited hepatotoxicity. In terms of molecular docking analysis, it was discovered that Ceftriaxone showed promising potential as an inhibitor of PBP2a when compared to other molecules. Among the four derivatives of Ceftriaxone, derivative 2 and derivative 3 displayed similar levels of inhibition against the PBP2a enzyme due to their closely matched binding energies. Conversely, derivatives 1 and 4 exhibited lower binding energies compared to derivatives 2 and 3, which could explain the observed differences in inhibition levels during in vitro tests. Regarding the series of Ampicillin derivatives, the results indicated that none of the derivatives designed using in silico pharmaco-modulation exhibited higher binding energies than Ampicillin itself. Therefore, it can be inferred that none of these derivatives possess a greater inhibition capacity than Ampicillin. However, derivative 6 demonstrated a comparable level of inhibition to Ampicillin, as evidenced by its equal binding energy. All the designed derivatives exhibited lower binding energies when compared to Ampicillin, although the difference was marginal. These observations suggest that all the molecules may have a similar level of inhibition.To conclude, the compounds investigated in this study show promise as potential drug candidates. The development of new molecules derived from Ampicillin and Ceftriaxone, as well as the evaluation of their pharmacokinetics, toxicology, and inhibitory properties, provide valuable insights for further research in the field of antibacterial drug discovery. Newly developed antibacterial compounds based on ampicillin and Ceftriaxone show promising potential in the treatment of bacterial infections. Their potential affinity with PBP2a and convenient oral administration make them candidates for clinical use. Their favorable pharmacokinetic properties and limited toxicity reinforce their appeal as therapeutic options. In addition, some Ceftriaxone derivatives have demonstrated significant inhibition of the PBP2a enzyme, which is implicated in antibiotic resistance. Although ampicillin derivatives did not show greater inhibition capacity than ampicillin itself, one specific derivative revealed comparable inhibition. These results provide valuable insights into the discovery of new antibacterial drugs and pave the way for future in vivo animal studies and development in this field.

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