Review

Targeting multi-drug resistant pathogens with synergistic beta-lactams and beta-lactam inhibitor combinations

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Abstract

Background: Infections caused by multidrug-resistant organisms pose a significant global health challenge due to the limited therapeutic options available. Synergistic drug combinations are often employed to enhance treatment efficacy. Because peptidoglycan is unique to prokaryotes, many studies focus on combinations that include beta-lactam antibiotics. To address bacterial strains that produce beta-lactamases, a range of beta-lactamase inhibitors has been developed. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) regularly update clinical guidelines to include standards for new beta-lactam/beta-lactamase inhibitor combinations. **Objective:** This work provides an overview of beta-lactam and beta-lactamase inhibitor combinations, together with updated clinical guidelines and an analysis of the relevant Protein Data Bank (PDB) entries related to these synergistic combinations. First, the latest EUCAST and CLSI guidelines are reviewed to identify the most recent beta-lactam/beta-lactamase inhibitor combinations used in therapy. Next, PDB entries related to these combinations are examined and compared with new compounds under investigation. EUCAST and CLSI guidelines are valuable for guiding therapeutic strategies, not only for interpreting antibiograms. The newest beta-lactamase inhibitors include avibactam, relebactam, vaborbactam, and enmetazobactam. X-ray crystallography studies have enhanced understanding of inhibitor interactions with major beta-lactamases in Gram-negative pathogens—serine beta-lactamases and metallo-beta-lactamases. **Conclusion:** Investigating synergistic combinations of beta-lactams and beta-lactamase inhibitors is promising because beta-lactams target peptidoglycan and may enable new therapeutic strategies.

Keywords: Multi-drug resistance, Synergy, Beta-lactamase inhibitors, Beta-lactams

1. Introduction

Multi-drug resistant (MDR) infections pose a significant challenge in healthcare and can spread rapidly in hospital settings.[1,2] The rise of carbapenem-resistant strains is concerning.[3] To address this issue, a variety of strategies are available. Research has shown that targeted combinations of treatments can effectively overcome resistance and improve therapeutic outcomes. In addition, clinical trials are increasingly exploring combinations of antibiotics with other drugs and innovative antibacterial agents in bi-therapy and tri-therapy approaches, with the goal of improving interventions against MDR infections.[4]

Beta-lactams and beta-lactamase inhibitors have been utilized in combination for many years to treat infections.[5,6] Beta-lactams specifically target the final stages of peptidoglycan synthesis, particularly the transpeptidation step. Peptidoglycan is an essential component of the cell wall in most prokaryotes and is not present in eukaryotic cells. Beta-lactam antibiotics are also generally considered safe for use during pregnancy and are not associated with major fetal harm.[7] Given these factors, beta-lactam antibiotics, when used

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in conjunction with other compounds, are a reasonable option in treatment regimens for MDR infections.[8,9]

Due to the urgent need for effective treatments against MDR isolates, researchers have explored combinations of antibiotics with other drugs or compounds. Numerous studies have demonstrated that certain combinations can reverse specific resistance traits.[10] To develop effective therapeutic strategies for infections caused by MDR isolates, it is essential to implement sophisticated approaches that prevent antibiotic inactivation, including strategies that reduce antibiotic degradation.[11]

Beta-lactam/beta-lactamase inhibitor combinations can provide synergistic activity by preventing the degradation of beta-lactam antibiotics by beta-lactamases. However, clinical effectiveness can be compromised by the emergence of inhibitor-resistant beta-lactamases.[12] For example, cilastatin sodium (PubChem CID 23663403), a sodium thioether salt, inhibits renal dehydropeptidase I, the enzyme responsible for imipenem degradation. Imipenem is therefore co-administered with cilastatin to reduce renal metabolism of imipenem, and relebactam can be added as a beta-lactamase inhibitor to improve activity against selected beta-lactamase-producing organisms.[13,14] Some studies have also explored broader applications of beta-lactamase inhibitors. For instance, clavulanic acid, a beta-lactamase inhibitor used alongside amoxicillin, has been investigated for the treatment of *Helicobacter pylori* infection.[15]

Beta-lactam antibiotics are effective; however, microorganisms have developed resistance mechanisms against them. Most MDR isolates associated with hospital-acquired infections are resistant to beta-lactam antibiotics, including some beta-lactam/beta-lactamase inhibitor combinations. Despite this, combining these antibiotics with other drugs or compounds can yield synergistic effects. Masoudi *et al*.[16] reported that certain nanoantibiotics, such as titanium dioxide and zinc oxide nanoparticles, when used in conjunction with amoxicillin-clavulanic acid, can inhibit biofilm formation by MDR *Acinetobacter baumannii* and *Escherichia coli*.

Natural compounds have been used for many years to treat a variety of diseases. Today, numerous natural compounds with antibacterial, antiviral, and antifungal properties have been identified.[10] Certain environmental organisms are significant sources of compounds, particularly secondary metabolites with antibacterial activity.[17] As with other medications, it is important to explore the effects of combining natural extracts with antibiotics.[18-20] These combinations may be synergistic, antagonistic, or indifferent. In this context, we are particularly interested in investigating the synergistic effects of combining natural compounds with beta-lactam antibiotics. *Streptomyces* species are a well-known source of antibiotics and related compounds, including beta-lactams (e.g., cephalosporins, carbapenems, and monobactams) and beta-lactamase inhibitors such as clavulanic acid and olivanic acid.[17,21]

Research investigating combinations of antibiotics and natural compounds has reported promising results against bacterial species linked to periodontal disease. An *in vitro* study by Saquib *et al*.[22] examined the combined effects of three plant extracts and four antibiotics against four bacterial species associated with periodontal disease. The most significant synergistic effect was observed for *Punica granatum* combined with amoxicillin against *Aggregatibacter actinomycetemcomitans*, a Gram-negative facultative anaerobe commonly found in the oral microbiota.

The present review examines crystal structures of beta-lactam/beta-lactamase inhibitor combinations available in the Protein Data Bank (PDB; https://www.rcsb.org). The review is organized into several sections: An overview of beta-lactam/beta-lactamase inhibitor combinations; a summary of European Committee on Antimicrobial Susceptibility Testing (EUCAST; https://www.eucast.org/) and clinical and laboratory standards institute (CLSI; https://clsi.org/) guidance relevant to laboratory interpretation; and a detailed analysis of available co-crystal structures involving beta-lactams and their binding partners, including approved therapies and newer compounds under investigation.

2. Beta-lactam/beta-lactamase inhibitor combinations

Clinical laboratories depend on standardized guidelines to report resistance phenotypes of bacterial isolates. In this context, we highlight the most recent EUCAST and CLSI recommendations, including expert rules relevant to beta-lactam/beta-lactamase inhibitor combinations.

2.1. EUCAST expert rules

Antibiotics that inhibit bacterial cell wall synthesis, particularly peptidoglycan synthesis, have a high therapeutic index because peptidoglycan is not present in eukaryotic cells.

Beta-lactam/beta-lactamase inhibitor combinations are included in EUCAST Version 15.0 (2025) (Table 1). Penicillin-beta-lactamase inhibitor combinations are active against many Gram-negative bacteria and some anaerobic Gram-positive species. Cephalosporin-beta-lactamase inhibitor combinations are active against Gram-negative bacilli and *Haemophilus influenzae*. Monobactam-beta-lactamase inhibitor combinations are used primarily against *Enterobacterales*. EUCAST Version 15.0 (2025) includes four penicillin-beta-lactamase inhibitor combinations, three cephalosporin-beta-lactamase inhibitor combinations, two

Table 1. EUCAST recommendations for beta-lactam/beta-lactamase inhibitor combinations (Version 15.0, 2025)

Antibiotic class/combination	Enterobacterales, non-fermenters, and other gram-negative bacilli	Cocobacillary Gram-negative	Anaerobic bacteria
Penicillins			
Ampicillin-sulbactam	Enterobacterales	*Haemophilus influenza*[c], *Moraxella catarrhalis*[f]	*Prevotella* spp.[a,b], *Fusobacterium necrophorum*, *Clostridium perfringens*, *Cutibacterium acnes*[a,b]
Amoxicillin-clavulanic acid	Enterobacterales, *Burkholderia pseudomallei*	*Kingella kingae*[a], *Haemophilus influenzae*, *Moraxella catarrhalis*	*Bacteroides* spp.[d], *Prevotella* spp.[a,b], *Fusobacterium necrophorum*, *Clostridium perfringens*, *Cutibacterium acnes*[a,b]
Piperacillin-tazobactam	Enterobacterales, *Pseudomonas* spp., *Vibrio* spp., *Achromobacter xylosoxidans*	*Haemophilus influenzae*	*Bacteroides* spp.[d], *Prevotella* spp.[a,b], *Fusobacterium necrophorum*, *Clostridium perfringens*, *Cutibacterium acnes* [a,b]
Ticarcillin-tazobactam	*Enterobacterales, Pseudomonas* spp.	-	-
Cephalosporins			
Cefepime-enmetazobactam	Enterobacterales, *Pseudomonas* spp.[e]	*Haemophilus influenzae* [e]	-
Ceftazidime-avibactam	Enterobacterales, *Pseudomonas aeruginosa*	-	-
Ceftolozane-tazobactam	Enterobacterales, *Pseudomonas aeruginosa*	*Haemophilus influenza*	-
Carbapenems			
Imipenem-relebactam	Enterobacterales, *Pseudomonas aeruginosa, Acinetobacter* spp.[e]	*Haemophilus influenzae*[e]	-
Meropenem-vaborbactam	Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter* spp.[e]	*Haemophilus influenzae*[e]	-
Monobactams			
Aztreonam-avibactam	Enterobacterales		

Notes: [a]Zone diameter breakpoints only; [b]Only isolates resistant to benzylpenicillin should be tested for susceptibility to individual agents; [c]No zone diameter breakpoints, as susceptibility can be inferred from intravenous amocicillin-clavulanic acid; [d]Breakpoints for *Bacteroides* spp. are also valid for *Parabacteroides* spp. and *Phocaeicola dorei/vulgates*; [e]The addition of a beta-lactamase inhibitor does not add clinical benefits; [f]Susceptibility can be inferred from amoxicillin-clavulanic acid.

carbapenem-beta-lactamase inhibitor combinations, and one monobactam-beta-lactamase inhibitor combination.

2.2. The CLSI expert rules

The CLSI M100 performance standards for antimicrobial susceptibility testing are an important standard used in clinical laboratories. Each year, CLSI provides updated guidance and new additions needed for interpreting antibiogram results obtained by disk diffusion or dilution methods.

Several beta-lactam/beta-lactamase inhibitor combinations were added relatively recently to CLSI standards (Table 2). In 2024, CLSI included sulbactam-durlobactam. The European Medicines Agency issued a decision (EMEA-002807-PIP01-20-M01; 2022) related to the pediatric investigation plan for durlobactam/sulbactam (https://www.ema.europa.eu/en/medicines/human/paediatric-investigation-plans/emea-002807-pip01-20-m01). Sulbactam-durlobactam is a beta-lactam/beta-lactamase inhibitor combination with a narrow application, targeting the *Acinetobacter baumannii-calcoaceticus* complex. Its main therapeutic indication is hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia in adults.[23]

2.3. Bacterial beta-lactamases

Beta-lactamases (E.C. 3.5.2.6) are enzymes that hydrolyze beta-lactam antibiotics and may show different specificities for penicillins, cephalosporins, and carbapenems. Beta-lactamases are commonly classified by the Ambler molecular classification (classes A-D); in addition, standardized residue-numbering schemes (e.g., Ambler/ABL numbering) are used to compare homologous enzymes.[24-26] The Bush-Jacoby-Medeiros functional classification offers an additional approach based on substrate and inhibitor profiles (Table 3).[27]

One notable group is class B beta-lactamases, also known as metallo-beta-lactamases (MBLs), which require zinc for catalytic activity. Many beta-lactamases are produced by human pathogens, and these enzymes continue to evolve, making antibiotic-resistant infections increasingly challenging to treat. As a result, functional groupings are updated as new enzymes emerge and as additional information becomes available on hydrolysis spectra and inhibitor susceptibility.[28]

2.4. Beta-lactamase inhibitors

Beta-lactamase inhibitors, when used in combination with beta-lactam antibiotics, can improve activity against beta-

Table 2. Beta-lactam/beta-lactamase inhibitor combinations according to CLSI standards

Beta-lactam/beta-lactamase inhibitor combination	CLSI breakpoint additions since 2010	Bacterial species
Ceftazidime-avibactam	January 2018 (M100, 28th ed..)[a,b]	Enterobacterales, *Pseudomonas* spp.
Ceftolozane-tazobactam	January 2016 (M100-S26)[b], January 2018 (M100, 28th ed..)[a], March 2021 (M100-Ed31)[c]	Enterobacterales, *Haemophilus influenzae* and *Haemophilus parainfluenzae*, *Streptococcus* spp. (Viridans group)
Imipenem-relebactam	March 2021 (M100–Ed31)[a,b]	Enterobacterales, *Pseudomonas* spp., anaerobes
Meropenem-vaborbactam	January 2019 (M100, 29th ed..)[a,b]	Enterobacterales
Sulbactam-durlobactam	February 2024 (M100–34th ed..)[a,b]	*Acinetobacter* spp.
Piperacillin-tazobactam	January 2017 (M100, 27th ed..)[b] January 2018 (M100, 28th ed..)[b]	Anaerobes

Notes: [a]Disk diffusion breakpoints; [b]Minimal inhibitory concentration breakpoints; [c]Minimal inhibitory concentration for *Haemophilus influenzae* and *Haemophilus parainfluenzae*.

Table 3. Characteristics of beta-lactamases according to Ambler and Bush-Jacoby-Medeiros classifications

Beta-lactamases	Ambler class	Functional classification	Active site
Broad-spectrum, inhibitor-resistant, extended-spectrum beta-lactamases and serine carbapenemases	Class A	Group 2	Serine
Metallo-beta-lactamases	Class B	Group 3	Zn^{2+}
Cephalosporinases	Class C	Group 1	Serine
Broad-spectrum, inhibitor-resistant, extended-spectrum beta-lactamases and serine carbapenemases	Class D	Group 2	Serine

Note: Ambler classes are assigned according to the Ambler molecular classification,[24,25] and functional classifications follow Bush-Jacoby-Medeiros.[27,28]

lactamase-producing bacteria. Table 4 summarizes key characteristics of beta-lactamase inhibitors approved for clinical use. Notably, six of the seven approved beta-lactamase inhibitors contain sulfur in their chemical structure.

Clavulanic acid is a conjugate acid of clavulanate produced by fermentation of *Streptomyces clavuligerus*.[29] It has weak antibacterial activity alone and is used in combination with penicillins such as amoxicillin and ticarcillin. Sulbactam, tazobactam, and enmetazobactam are penicillanic acid sulfones.

Sulbactam is a beta-lactamase inhibitor and can also bind penicillin-binding proteins (PBPs) in Gram-positive and Gram-negative species.[30] Durlobactam is a diazabicyclooctane beta-lactamase inhibitor (PubChem CID 89851852) administered in combination with sulbactam (food and drug administration [FDA] approval in 2023). This inhibitor targets serine beta-lactamases (SBLs; class A, C, and D) and is indicated for hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia caused by susceptible isolates of the *A. baumannii-calcoaceticus* complex.[31]

Tazobactam is a beta-lactamase inhibitor with activity against the OHIO-1, SHV-1, and TEM groups of beta-lactamases.[32] It was approved by the FDA in 1994 in combination with piperacillin for infections caused by Gram-positive and Gram-negative species, including anaerobes. Ceftolozane-tazobactam was initially approved in 2014, and its indication was expanded in 2019 to include hospital-acquired bacterial pneumonia and ventilator-associated pneumonia with Gram-negative susceptible isolates. In combination with metronidazole, it can be used for complicated intra-abdominal infections.[33]

Avibactam is a member of the class of azabicycloalkanes approved by the FDA in 2015 in combination with ceftazidime for the treatment of complicated urinary infections (including pyelonephritis) caused by susceptible microorganisms, including *E. coli*, *Klebsiella pneumoniae*, *Citrobacter koseri*, *Enterobacter aerogenes, Enterobacter cloacae*, *Citrobacter freundii*, *Proteus* spp., and *Pseudomonas aeruginosa*. It can also be administered with metronidazole for the treatment of complicated intra-abdominal infections caused by susceptible microorganisms, including *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *Providencia stuartii*, *E. cloacae*, *Klebsiella oxytoca*, and *P. aeruginosa*.[34,35]

Relebactam is a derivative of diazabicyclooctane, approved by the FDA in 2019 in combination with imipenem and cilastatin. It inhibits Class A and Class C SBLs by forming a covalent acyl-enzyme intermediate with the active-site serine residue.[36]

Vaborbactam is a cyclic boronic acid pharmacophore derivative approved by the FDA in 2017 in combination with meropenem for the treatment of complicated urinary tract infections (including pyelonephritis) caused by susceptible microorganisms, including *E. coli*, *K. pneumoniae*, and *E. cloacae* species complex.[37] A triple-combination strategy (e.g., meropenem-vaborbactam-aztreonam) has been proposed for selected resistant phenotypes, including *K. pneumoniae* isolates resistant to ceftazidim-avibactam.[38]

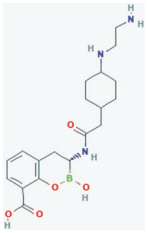
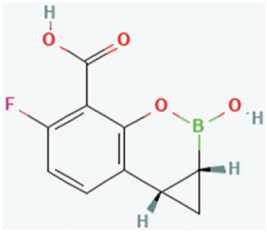
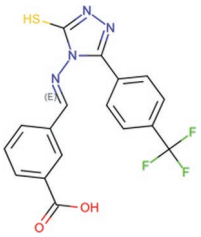
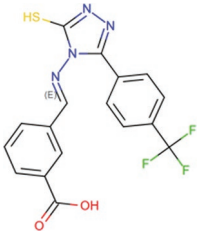
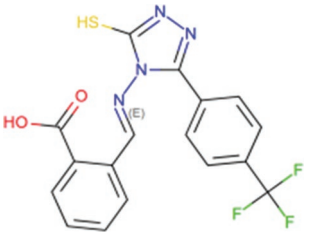
Enmetazobactam is a penicillanic acid sulfone derivative that was approved by the FDA on February 23, 2024, in combination with cefepime for the treatment of complicated urinary tract infections.[39]

Efforts to address inhibitor-resistant infections have prompted extensive research focused on developing new beta-lactamase inhibitors. Table 5 summarizes several

Table 4. Characteristics of clinically approved beta-lactamase inhibitors

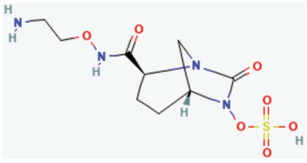
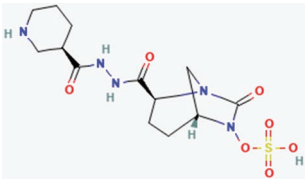
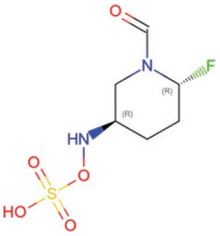
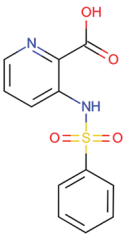
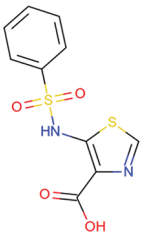
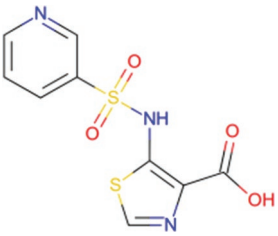
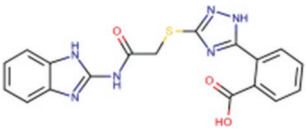
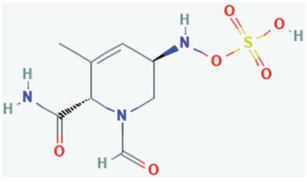
Beta-lactamase inhibitor	PubChem CID	Molecular formula	Combinations	FDA approval date (brand)
Clavulanic acid	5280980	$C_8H_9NO_5$	Amoxicillin, ticarcillin	July 22, 1985 (Augmentin); April 1, 1985 (Timentin)
Sulbactam	23663973	$C_8H_{10}NNaO_5S$	Ampicillin, durlobactam	December 31, 1986 (Unasyn); May 23, 2023 (Xacduro)
Tazobactam	123630	$C_{10}H_{12}N_4O_5S$	Piperacillin, ticarcillin, ceftolozane	October 22, 1993 (Zosyn); December 19, 2014 (Zerbaxa)
Avibactam	9835049	$C_7H_{11}N_3O_6S$	Ceftazidime, aztreonam	February 25, 2015 (Avyca); February 7, 2025 (Emblaveo)
Relebactam	44129647	$C_{12}H_{20}N_4O_6S$	Imipenem	July 16, 2019 (Recarbrio)
Vaborbactam	56649692	$C_{12}H_{16}BNO_5S$	Meropenem	August 29, 2017 (Vabomere)
Enmetazobactam	23653540	$C_{11}H_{14}N_4O_5S$	Cefepime	February 22, 2024 (Exblifep)

Table 5. New beta-lactamase inhibitors showing experimental activity

Beta-lactamase inhibitor	PubChem CID	Molecular structure	PDB IDs	References
Taniborbactam (VNRX-5133)	76902493		6SP6, 6SP7	40,41
Xeruborbactam (QPX7728)	140830474		–	41,42
Triazole-based inhibitors (CP35, CP56, CP57)	–		8B1W	43
			8B1Z	
			8B20	

(*Cont'd...*)

Table 5. (*Continued*)

Beta-lactamase inhibitor	PubChem CID	Molecular structure	PDB IDs	References
Nacubactam	73386748		9IO6, 9H18	44-47
Zidebactam (WCK 5107)	77846445		9IO7, 6T5Y	46,48-50,51
ANT3310, ANT90, ANT330, ANT431 (diazabicyclooctane derivates)	–		6ZXI	52,53
			5MXQ	
			5MXR	
			6HF5	
2-triazolylthioacetamides	–		6KW1	54
ETX2514	137349620		5VFD	55

investigational compounds with activity against SBLs and/or MBLs. X-ray structures of these inhibitors co-crystallized with beta-lactamases are discussed in the following section.

Taniborbactam is a small molecule and a cyclic boronate derivative currently under investigation in Phase III clinical trials. When combined with cefepime, it exhibits activity comparable to aztreonam-avibactam against SBLs and MBLs.[56]

Xeruborbactam is a small molecule and a cyclic boronate derivative currently under investigation. It is active against selected SBLs and MBLs.[42] When combined with meropenem, xeruborbactam shows activity against some imipenemase-producing Enterobacterales; however, meropenem alone is ineffective against imipenemase-producing *P. aeruginosa*.[41]

Nacubactam is a derivative of diazabicyclooctane and serves as an inhibitor of SBLs. It is being investigated for complicated urinary tract infections (ClinicalTrials.gov identifier: NCT03174795; EudraCT: 2021-001396-16). Misawa *et al*.[45] reported that nacubactam combined with imipenem and certain beta-lactams (e.g., cefazolin, cefotiam, cefoxitin, or cefuroxime) was effective against *Mycobacterium abscessus.*

Zidebactam is a derivative of diazabicyclooctane that has a dual mechanism: It inhibits SBLs and binds to PBP2.[46,48] It is being investigated in combination with cefepime for infections caused by Gram-negative beta-lactamase-producing organisms.[49,50]

3. Beta-lactamases catalytic mechanisms

Crystallographic studies can elucidate molecular mechanisms underlying catalysis.

A complex structure of AmpC beta-lactamase with a series of acylglycine boronic acids provides insights into inhibitor affinity for SBLs. Caselli *et al*.[57] demonstrate that the R1 side chains of beta-lactams can contribute to affinity for beta-lactamases (PDB ID: 1FSY).

Recent studies highlight the pivotal role of Ser130 in the active site of class A SBLs. X-ray crystal structures of SHV-like beta-lactamases (PDB IDs: 1TDL, 1TDG) indicate that Ser130 is crucial for interactions with beta-lactamase inhibitors such as clavulanic acid and tazobactam.[58] Site-directed mutagenesis has provided valuable insights into inhibitor-resistant beta-lactamases, revealing that certain residues affect acylation rates for specific substrates. For instance, a CTX-M-14 variant (K234R) from *E. coli* showed that substitution of Lys234 with arginine decreases the acylation rate of cefotaxime. In addition, the conformational change associated with residue 234 reduces acylation of clavulanic acid (PDB ID: 7K2X).[59] The K234R substitution in SVH-1 beta-lactamase can be addressed by derivatives such as SA2-13 ((3R)-4-[(4-carboxybutanoyl)oxy]-N-(1E)-3-sulfino-D-valine) (PDB ID: 4MBK).[60]

Mutations in the active site of beta-lactamases can affect enzyme stability. However, mutations located outside the active site can also stabilize mutated enzymes. For instance, the M182T mutation in TEM-64 beta-lactamase (PDB ID: 1JWZ) illustrates this effect.[61]

4. Crystal structures of beta-lactams and inhibitor-resistant beta-lactamases

A search of the PDB identified numerous beta-lactamase structures, including complexes co-crystallized with inhibitors, as well as enzymes involved in beta-lactam antibiotic biosynthesis. Many MDR isolates carry inhibitor-resistant beta-lactamases, which can reduce the effectiveness of beta-lactam/beta-lactamase inhibitor combinations. Structural analysis of inhibitor-resistant beta-lactamases can clarify the molecular basis of resistance.

Using the advanced search query "inhibitor-resistant" AND "beta-lactamase," 124 structures were retrieved from the PBD database on August 11, 2025. After manual curation, 35 entries corresponded to beta-lactamase structures co-crystallized with inhibitors. In addition, five entries corresponded to PBPs co-crystallized with inhibitors. Therefore, we included 35 beta-lactamase structures and five PBP structures in the present analysis. Sequence analysis and multiple alignment can provide additional insights relevant to inhibitor-resistant beta-lactamases. The effectiveness of beta-lactamase inhibitors extends to both Gram-negative and Gram-positive bacteria species.

4.1. Beta-lactamase inhibitors effective against beta-lactamases from Gram-negative bacteria

Beta-lactamases can be broadly grouped by active-site chemistry into SBLs and MBLs.

4.1.1. SBLs with beta-lactamase inhibitors crystal structures

Many inhibitor-resistant beta-lactamases are variants of broad-spectrum enzymes.[62] One of the most studied SBLs is the TEM-1 plasmid-encoded class A beta-lactamase, which belongs to the transpeptidase superfamily (UniProt/Swiss-Prot: P14677). The name "TEM" originates from the patient (Temoniera) from whom it was first isolated.[63]

The crystal structure of TEM-76 beta-lactamase (PDB ID: 1YT4) revealed a water molecule (Wat1023) in the active site that replaces the side-chain hydroxyl group of Ser130. A similar water molecule that facilitates clavulanate cross-linking has also been observed in other TEM beta-lactamases, including TEM-32 and TEM-84.[64]

Wang *et al*.[61] proposed that inhibitors structurally similar to substrates are less likely to select resistance mutations. To test this hypothesis, they examined a series of analogs against the TEM-1 class A beta-lactamase. X-ray crystallography and kinetic experiments showed that some boronic analogs mimic the transition states of acylation and deacylation (PDB IDs: 1NYY, 1NXY, and 1JWZ) (Figure 1).[65] The 3D structure of TEM-64 (PDB ID: 1JWZ) was downloaded from the PDB (https://www.rcsb.org/structure). Interactions were visualized using BIOVIA Discovery Studio Modeling Environment (Release 2017; Dassault Systèmes, USA; http://accelrys.com).

CTX-M is a class A beta-lactamase that was first identified in a cefotaxime-resistant *E. coli* isolated from an ear sample of a newborn.[66] Unlike the plasmidic CTX-M, the SHV-like beta-lactamase (sulphydryl variable) likely originated from a chromosomal penicillinase found in *K. pneumoniae*.[67] Studies by Winkler *et al*.[68] and Soeung *et al*.[59] showed that the arginine residue interacts with clavulanic acid in the class A beta-lactamases SHV from *K. pneumoniae* and CTX-M from *E. coli*. The structure of the mutated SHV-1 K234R (PDB IDs: 4FCF and 7K2W) indicates that two specific residues, Arg234 and the movement of Ser130, are responsible for resistance to clavulanate. Furthermore, Soeung *et al*.[59] suggest a distinction between penicillins and cephalosporins, recommending that coupling clavulanic acid with cephalosporins should be prioritized to reduce the development of inhibitor resistance (PDB IDs: 7K2Y and 7K2W). The importance of Ser130 has led to the development of new inhibitors such as SA-13, which are effective against SHV mutants from *K. pneumoniae* (PDB IDs: 3V50, 3V5M) (Figure 2).[69]

Combination ("tri-therapy") strategies are used clinically, and structural studies can help clarify effective combinations. The crystal structure of serine-class A beta-lactamase from *Stenotrophomonas maltophilia* (PDB IDs: 5NE1 and 5NE3) reveals that ceftazidime, avibactam, and aztreonam work synergistically. This is because SBLs do not interact with aztreonam as substrates, in contrast to MBLs.[70]

4.1.2. MBLs with beta-lactamase inhibitor crystal structures

Liu *et al*.[40] demonstrated that taniborbactam (VNRX-5133), a boronic-acid-containing broad-spectrum beta-lactamase inhibitor, can restore the efficacy of beta-lactams against carbapenem-resistant *P. aeruginosa* and carbapenem-resistant Enterobacterales (PDB IDs: 6SP6 and 6SP7). Additionally, several triazole-based compounds have shown promise for inhibiting broad-spectrum beta-lactamases based on molecular modeling and *in vitro* enzymatic studies. Bersani *et al*.[43] identified three 1,2,4-triazole-3-thione derivatives that are effective against NDM-1 (New Delhi MBL), VIM-2 (Verona integron-encoded MBL), and VIM-4 MBLs (PDB IDs: 8B1W, 8B1Z, and 8B20).

Furthermore, Davies *et al*.[52] identified new diazabicyclooctanes (including ANT3310 and ANT90) that are effective against SBLs. Notably, ANT3310 restores carbapenem activity not only against carbapenem-resistant *Enterobacterales* but also against OXA-type beta-lactamase

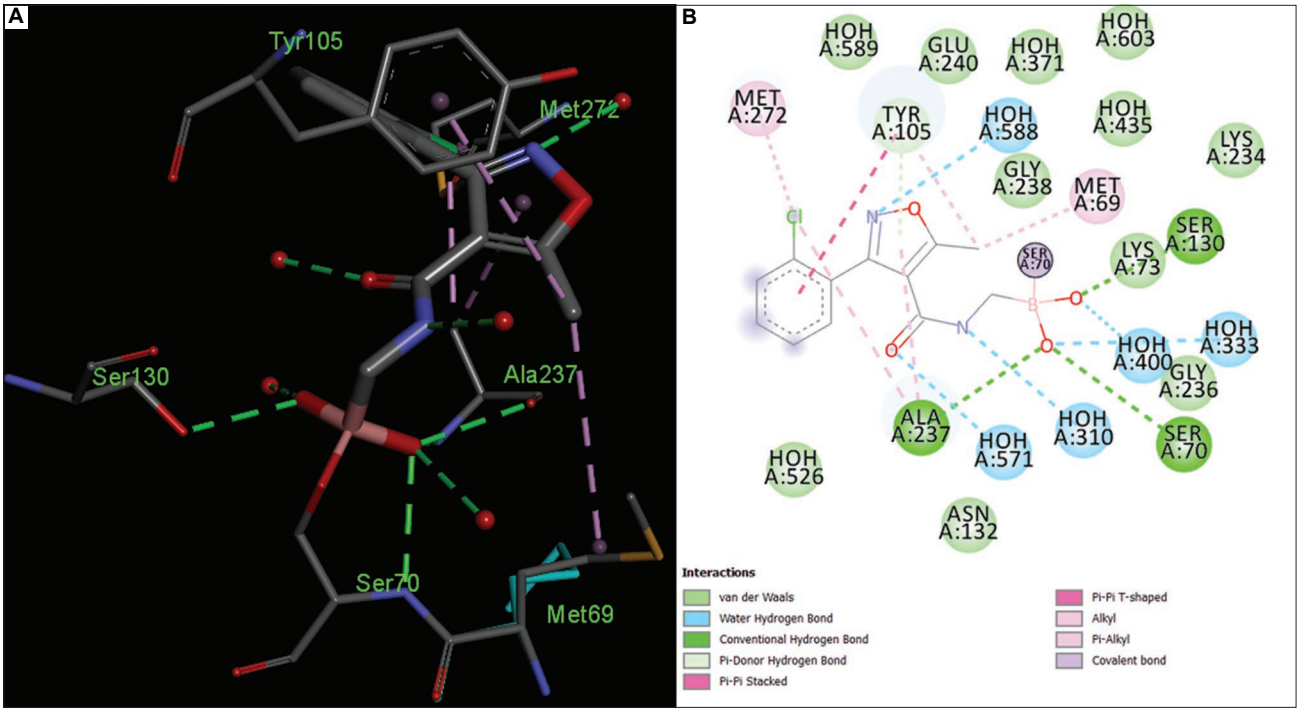


Figure 1. 3D interactions of a boronic acid derivative with TEM-64 beta-lactamase (PDB ID: 1JWZ). (A) 3D structure showing interacting residues with the ligand; (B) 2D interaction diagram highlighting Ser130 and key water-mediated hydrogen bonds.

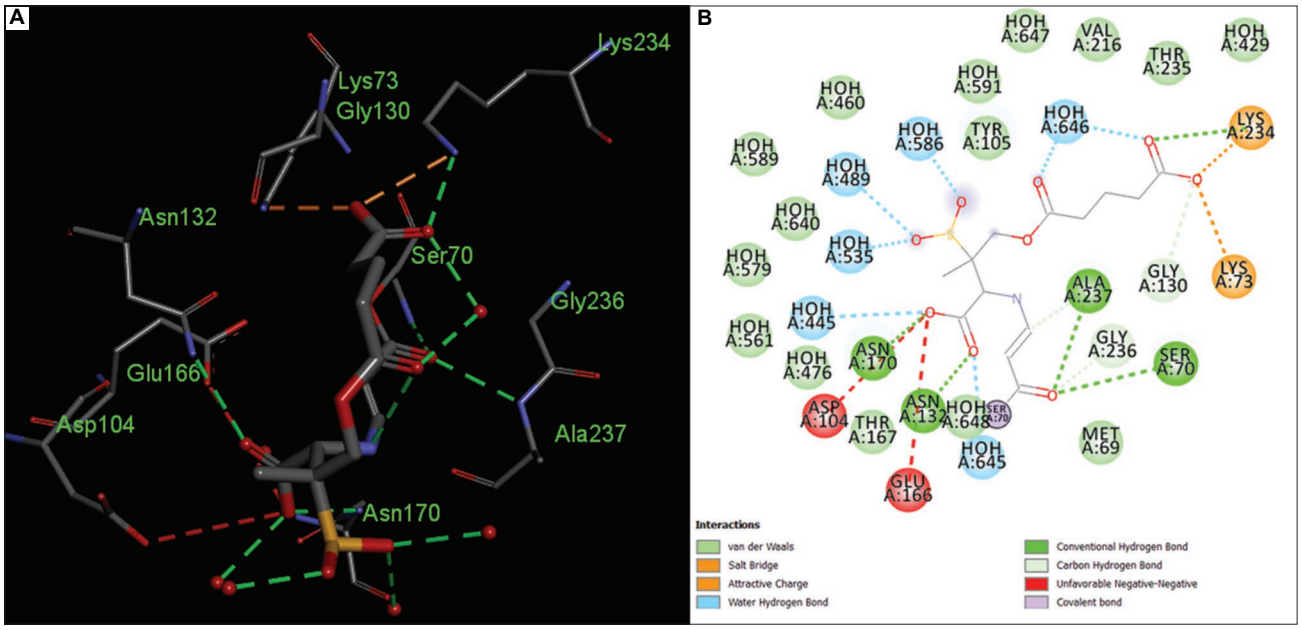


Figure 2. 3D interactions of SA-13 derivative with the SHV-S130G mutant (PDB ID: 3V50). (A) 3D structure showing interacting residues with the ligand SA-13; (B) 2D interaction diagram showing that SA2-13 can form a stable intermediate similar to the wild-type complex. The structure was retrieved from the Protein Data Bank, and interactions were visualized using BIOVIA Discovery Studio Modeling Environment.

(OXA)–carrying *A. baumannii* isolates (PDB IDs: 6ZXI). Compared to relebactam and avibactam, derivatives of ANT3310 contain a fluorine substituent, which may contribute to enhanced efficacy (Figure 3). OXA-type beta-lactamases are plasmid-mediated enzymes particularly resistant to oxacillin.

Crystal structures help clarify interactions of newer beta-lactamase inhibitors compared with older agents. Figure 4 shows interacting residues of OXA-48 from *K. pneumoniae* (PDB ID: 6ZXI) with the new diazabicyclooctane ANT3310.

Leiris *et al*.[53] designed and optimized a series of pyridine-2-carboxylic acid derivatives suitable for inhibition of MBL-producing isolates (NDM, VIM, and IMP). X-ray structures of VIM-2 carbapenem-hydrolyzing class D beta-lactamase from *P. aeruginosa* with these new inhibitors (PDB IDs: 5MXQ, 5MXR, 6HF5) show inhibitor interactions with residues from the so-called "Zn^{2+}" site (Asp, Cys, His).

The compound ETX2514 (PubChem CID: 137349620), in combination with sulbactam, is a potent inhibitor targeting the class D OXA-24 from *A. baumanii* (PDB ID: 5VFD).[55]

Crystallographic studies, along with thermodynamic experiments, identified a 2-triazolylthioacetamide derivative that interacts with key residues in the active site of VIM-2 MBL from *P. aeruginosa* (PDB ID: 6KW1). The inhibitor binds to Zn^{2+} ions and interacts with Asn233, His263, and a water molecule (Figure 5).[54]

4.2. PBPs with inhibitor co-crystal structures

To combat antibiotic resistance in bacterial isolates producing beta-lactamases, one strategy is to target additional components of cell-wall synthesis. PBPs, which are found in both Gram-negative and Gram-positive bacteria, play a crucial role in peptidoglycan synthesis. Goldberg *et al*.[71] described a novel γ-lactam siderophore (YU253911 and YU253434) that binds to *P. aeruginosa* PBP3 through multiple hydrogen bonds at the active site (PDB IDs: 7LC4 and 6VOT). In addition, the γ-lactam siderophore showed synergy with sulbactam against MDR *Acinetobacter* spp. in an animal model.[72]

Meticillin-resistant *Staphylococcus aureus* (MRSA) expresses a PBP, PBP2a, which supports cell-wall synthesis in the presence of beta-lactam antibiotics. Quinazolinone binds to an allosteric site and can facilitate beta-lactam binding. When combined with tazobactam, this interaction demonstrated synergy in an animal model (PDB IDs: 6Q9N and 6H5O).[73]

A recent study reported that specific boronate inhibitors are effective against MDR *Neisseria gonorrhoeae*. X-ray crystal structure analysis indicates that the VNRX-14079 boronate compound binds to the transpeptidase domain of PBP2 from *N. gonorrhoeae* (PDB IDs 9MCZ and 9MD0).[74] This interaction involves a covalent bond between the boron atom and the active-site serine residue at position 310, as well as interactions involving the β3–β4 loop. These findings highlight the potential of these inhibitors in addressing this critical public health challenge.

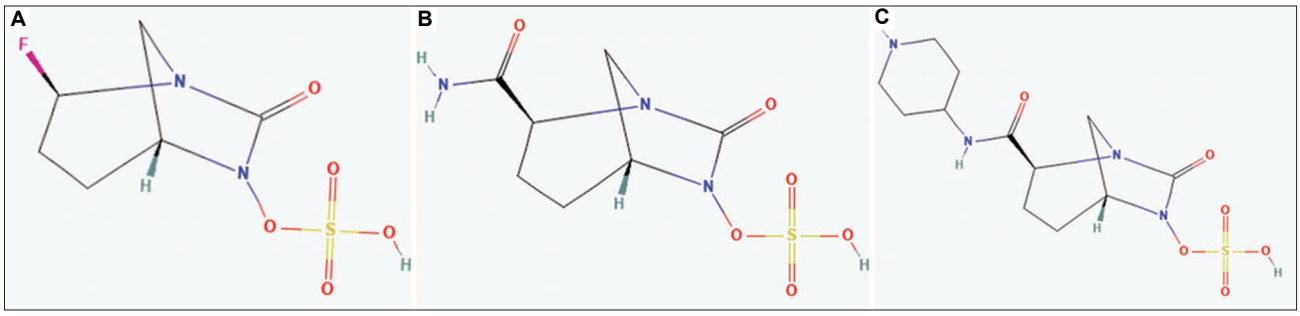


Figure 3. 2D structures of the beta-lactamase inhibitors (A) ANT3310 (PubChemCID: 146346770), (B) avibactam (PubChemCID: 9835049), and (C) relebactam (PubChemCID: 44129647).[52]

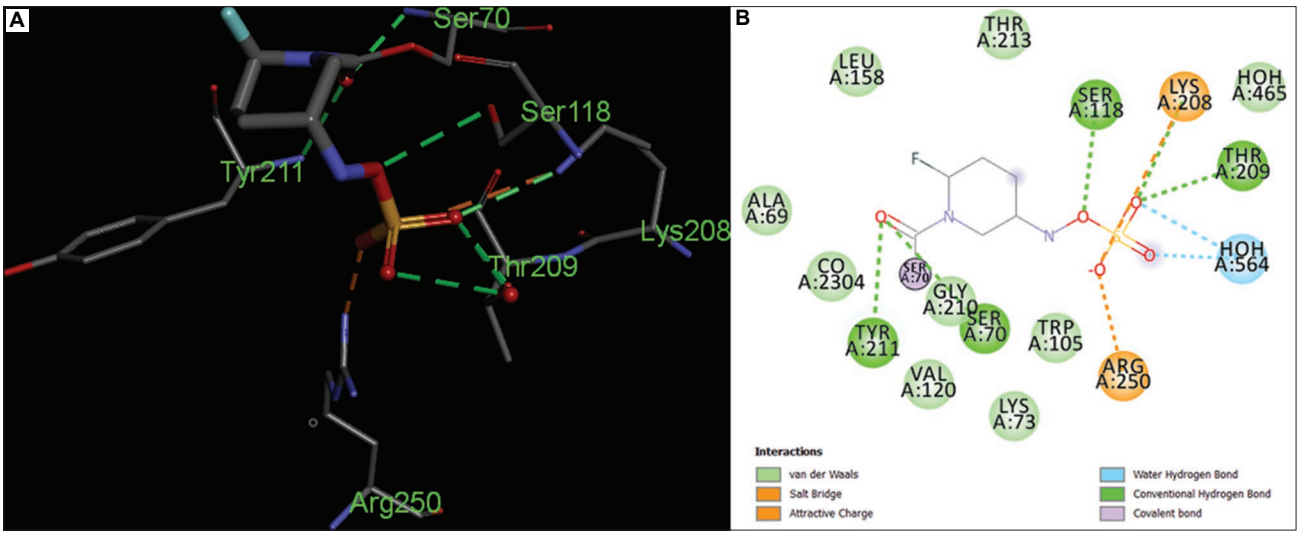


Figure 4. 3D interactions of the ANT3310 derivative with the OXA-48 carbapenem-hydrolyzing class D beta-lactamase (PDB ID: 6ZXI). (A) 3D structures showing interacting residues with the ligand ANT3310; (B) 2D interaction diagram highlighting the fluorine substituent in the active-site cavity. The structure was retrieved from the Protein Data Bank, and interactions were visualized using BIOVIA Discovery Studio Modeling Environment.

4.3. Beta-lactamase inhibitors effective against antibiotic-resistant Gram-positive species

Recent *in silico* studies have screened and identified benzimidazole-based compounds with the potential to restore beta-lactam susceptibility in MRSA. Nguyen *et al*.[75] determined the X-ray structure of the *S. aureus* BlaR1 regulatory/sensor protein (UniProt P18357) crystallized with two benzimidazole-based compounds. The first inhibitor, a boronate compound (boronate 4), is [1-[[2,4-bis(trifluoromethyl)phenyl] methyl]benzimidazol-2-yl]sulfanylmethyl-{3}-oxidanyl-bis(oxidanyl)boron] (PDB ID: 8C0P). The second inhibitor is an imidazole compound, 3-[[2,4-bis(trifluoromethyl)phenyl] methyl]-5-(hydroxymethyl)-1H-imidazole-2-thione (PDB ID: 8C0S). Boronate 4 inhibitor interacts with the active-site serine residue and shows efficacy in animal experiments when combined with oxacillin or meropenem against MRSA (Figure 6).

Caselli *et al*.[57] reported that two acylglycine boronic acids showed synergy with amoxicillin against *S. aureus* expressing a Group 2 beta-lactamase.

Mycobacterium tuberculosis is commonly classified as a high-G+C Gram-positive bacterium; however, it stains poorly with the Gram stain because of its lipid-rich cell envelope (notably mycolic acids), which underlies its acid-fast property. Some genome-based analyses have suggested that *M. tuberculosis* shares more features with Gram-negative bacteria than with typical Gram-positive bacteria.[76] BlaC is the principal beta-lactamase of *M. tuberculosis*. Resistance to beta-lactam/beta-lactamase inhibitor combinations is considered unlikely to arise primarily through structural alterations of BlaC. Kurz *et al*.[77] demonstrated that an inhibitor-resistant BlaC variant retained susceptibility to meropenem-clavulanic acid combination (PDB ID: 4JLF).

5. Discussion

Multidrug-resistant organisms are a major global concern. There is an urgent need for alternatives to existing treatments for these MDR strains, including new drug combinations. However, developing new compounds is a lengthy process. To address the critical need for effective treatments against

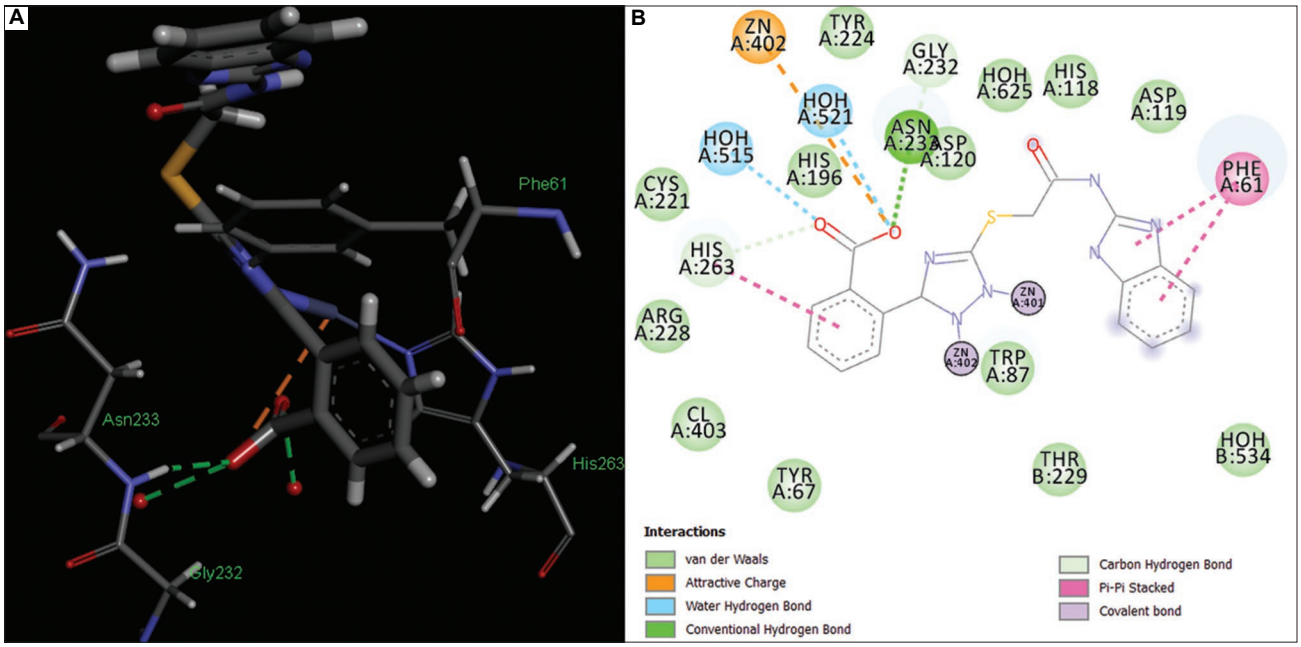


Figure 5. Interactions of the ANT90 derivative with VIM-2 class D beta-lactamase (PDB ID: 6KW1). (A) 3D structure showing interacting residues with triazolylthioacetamides derivative; (B) 2D interaction diagram. The structure was retrieved from the Protein Data Bank, and interactions were visualized using BIOVIA Discovery Studio Modeling Environment.

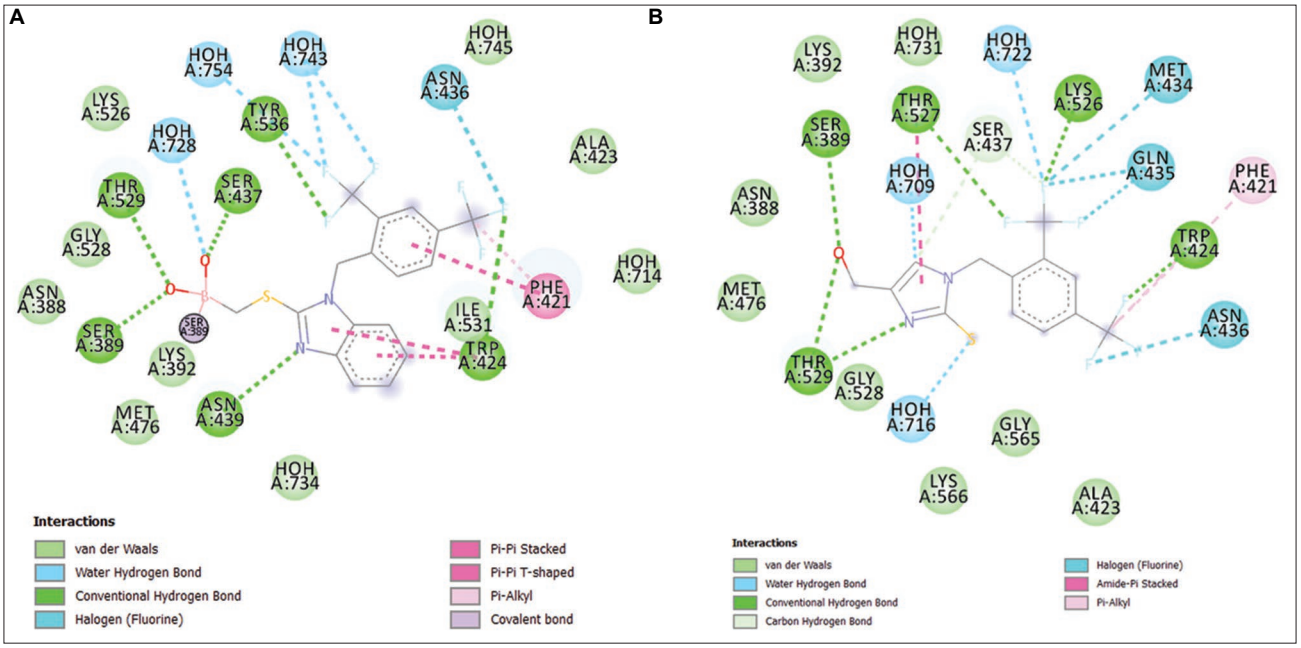


Figure 6. Interactions of benzimidazole-based compounds with *S. auresus* regulatory protein BlaR1. (A) Boronate 4 (PDB ID: 8C0P); (B) imidazole inhibitor (PDB ID: 8C0S). The structures were retrieved from the Protein Data Bank, and interactions were visualized using BIOVIA Discovery Studio Modeling Environment.

MDR clinical isolates, researchers are actively exploring innovative solutions that combine antibiotics with other drugs or compounds. Advances in *in silico* studies have significantly improved the selection and optimization of compounds that may help reverse certain antibiotic-resistant traits.[75] Artificial intelligence (AI) tools also show promise for improving the design of new inhibitors.

Infections caused by MDR organisms and the continued evolution of beta-lactamases challenge healthcare professionals to integrate clinical needs with basic science. Clinical laboratories must keep pace with newly approved synergistic combination treatments for infections caused by beta-lactamase-producing isolates. Accordingly, expert guidelines from EUCAST and CLSI support the interpretation

of antibiogram results and help ensure alignment with current standards. However, both established therapies and new drugs must be continuously reviewed in light of beta-lactamase evolution and the ongoing emergence of new mutations.

The prevalence of beta-lactamases in Gram-negative bacilli, particularly within the order Enterobacterales, is a major concern. The TEM family of beta-lactamases has been studied extensively. TEM-1-type beta-lactamases are encoded by a gene located within the transposon Tn3. The association of this gene with plasmids is crucial because it facilitates rapid and widespread transmission of antibiotic resistance. Furthermore, mutations within the active-site cavity may be accompanied by secondary mutations at positions distant from the active site, which can help restore structural stability of the enzyme.

Understanding the molecular factors driving the evolution of inhibitor-resistant beta-lactamases is essential for designing new antibacterial compounds and for re-evaluating synergistic combinations. The significance of Ser130 in the active site of SBLs has supported the development of new inhibitors, such as SA-13, which retain activity against selected SHV mutants. These inhibitors may help address challenges posed by SHV mutations and improve treatment efficacy. Key structural information is available in PDB IDs 3V50 and 3V5M, which describe interactions relevant to inhibitor activity against resistant variants.[69]

X-ray structures of intermediate forms of SHV-1 mutants in complex with inhibitors provide insight into conformational changes in inhibitor-enzyme complex (PDB IDs: 2H10, 2H0Y, and 2H0T).[78] In addition, boronic acid inhibitors have been evaluated as a strategy to address inhibitor-resistant beta-lactamases.[68]

The emergence of inhibitor-resistant beta-lactamases is reducing treatment options and increasing the need for new synergistic antibiotic combinations. Bi-therapy and tri-therapy strategies offer potential approaches for resistant infections. Although beta-lactam/beta-lactamase inhibitor combinations have been used for many years, the rapid emergence of beta-lactamases resistant to currently available inhibitors continues to complicate the treatment of infections caused by MDR isolates. As a result, tri-therapy is increasingly considered, especially for nosocomial infections. A combination of imipenem, cilastatin, and relebactam has shown activity against imipenem-non-susceptible Gram-negative pathogens.[13,14] In addition, quinazolinone combined with piperacillin–tazobactam has demonstrated efficacy against MRSA.[73]

X-ray structures of beta-lactamases in complex with inhibitors provide valuable insights relevant to inhibitor design and resistance mitigation. However, structural findings should be complemented by kinetic studies assessing acylation and deacylation rates. Promising directions include rational drug design targeting broad-spectrum MBLs and SBLs. In this context, AI may accelerate structure-based inhibitor design.

6. Conclusion

The treatment of MDR infections often presents significant challenges because of limited therapeutic options, which can necessitate bi-therapy or tri-therapy. Investigation of synergistic combinations of beta-lactams and beta-lactamase inhibitors is particularly promising because these therapies target peptidoglycan, which is exclusively found in prokaryotes. However, the emergence of beta-lactamases resistant to inhibitors highlights the need to optimize both existing and new inhibitors. X-ray crystal structures available in the PDB are valuable for understanding drug interactions and for comparing new compounds with approved inhibitors. Focusing research on specific drug combinations can support progress through coordinated efforts in both research and clinical settings.

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Conflict of interest

Mihaela Ileana Ionescu is a Guest Editor of this special issue. The author declared that she has no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions

This is a single-authored article.

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Consent for publication

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