BIOMOLECULES & THERAPEUTICS

Review
Biomol Ther 31(2), 141-147 (2023)

Structural Insights for β-Lactam Antibiotics

Dogyeoung Kim[1,†], Sumin Kim[1,†], Yongdae Kwon[1,†], Yeseul Kim[1,†], Hyunjae Park[1], Kiwoong Kwak[1], Hyeonmin Lee[1], Jung Hun Lee[2], Kyung-Min Jang[2], Donghak Kim[1], Sang Hee Lee[2,*] and Lin-Woo Kang[1,*]

[1]Department of Biological Sciences, Konkuk University, Seoul 05029,
[2]National Leading Research Laboratory of Drug Resistance Proteomics, Department of Biological Sciences, Myongji University, Yongin 17058, Republic of Korea

Abstract

Antibiotic resistance has emerged as a global threat to modern healthcare systems and has nullified many commonly used antibiotics. β-Lactam antibiotics are among the most successful and occupy approximately two-thirds of the prescription antibiotic market. They inhibit the synthesis of the peptidoglycan layer in the bacterial cell wall by mimicking the D-Ala-D-Ala in the pentapeptide crosslinking neighboring glycan chains. To date, various β-lactam antibiotics have been developed to increase the spectrum of activity and evade drug resistance. This review emphasizes the three-dimensional structural characteristics of β-lactam antibiotics regarding the overall scaffold, working mechanism, chemical diversity, and hydrolysis mechanism by β-lactamases. The structural insight into various β-lactams will provide an in-depth understanding of the antibacterial efficacy and susceptibility to drug resistance in multidrug-resistant bacteria and help to develop better β-lactam antibiotics and inhibitors.

Key Words: Antibiotics, β-Lactams, Peptidoglycan, Antibiotic resistance, Serine β-lactamases, Metallo-β-lactamases

INTRODUCTION

Antibacterials play a crucial role in modern health systems (Laxminarayan *et al*., 2013; Yan *et al*., 2020). In hospitals, almost all surgeries require the prescription of antibiotics to prevent infections. In addition to medical treatments, prevalent antibiotic use in food production, including animal husbandry and agriculture, and the resulting environmental contamination with antibiotics put selective pressure on bacterial communities to develop antibiotic resistance and cause the proliferation of multidrug-resistant (MDR) bacteria (Hou *et al*., 2017). In contrast to the rapid proliferation of MDR bacteria, the number of new antibiotics with different modes of action has diminished dramatically in recent decades. The hardship of developing a new antibacterial agent and the rapid occurrence of resistant bacterial strains have forced large pharmaceutical firms to leave the market for antibiotic drug development in favor of more profitable lines of drug development, such as cancer treatments. If the current situation continues for 50 years, deaths from MDR bacterial infections are predicted to surpass those from cancer (de Kraker *et al*., 2016).

Antibacterials aim to inhibit essential pathways or structures of bacteria, which do not exist in or are different from those in humans. However, the large population of bacteria and ease of generating new mutated strains due to rapid replication upon frequent and consistent antibiotic exposure give rise to new MDR bacterial strains (Berendonk *et al*., 2015; Blair *et al*., 2015). Pathogenic bacteria can mutate spontaneously and acquire resistance genes horizontally via mobile elements from other bacteria (Laxminarayan *et al*., 2013; Berendonk *et al*., 2015; Lee *et al*., 2016). The ease of genetic mutation and resistance gene transfer make it difficult to respond to bacterial drug resistance. These processes quickly devalue a successful new antibacterial drug and impair investment in novel antibacterial drugs.

The World Health Organization published a priority pathogen list to promote the research and development of new antibiotics for urgent caution, including the most critical MDR pathogens of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and various *Enterobacteriaceae* (Mancuso *et al*., 2021). Most of the listed MDR bacteria are Gram-negative (Breijyeh *et al*., 2020), including all the critical pathogens. The most

Open Access **https://doi.org/10.4062/biomolther.2023.008**

Received Jan 11, 2023 **Revised** Jan 19, 2023 **Accepted** Jan 20, 2023
Published Online Feb 15, 2023

***Corresponding Authors**
E-mail: lkang@konkuk.ac.kr (Kang LW), sangheelee@mju.ac.kr (Lee SH)
Tel: +82-2-450-4090 (Kang LW), +82-31-330-6195 (Lee SH)
Fax: +82-2-444-6707 (Kang LW), +82-31-335-8249 (Lee SH)
[†]The first four authors contributed equally to this work.

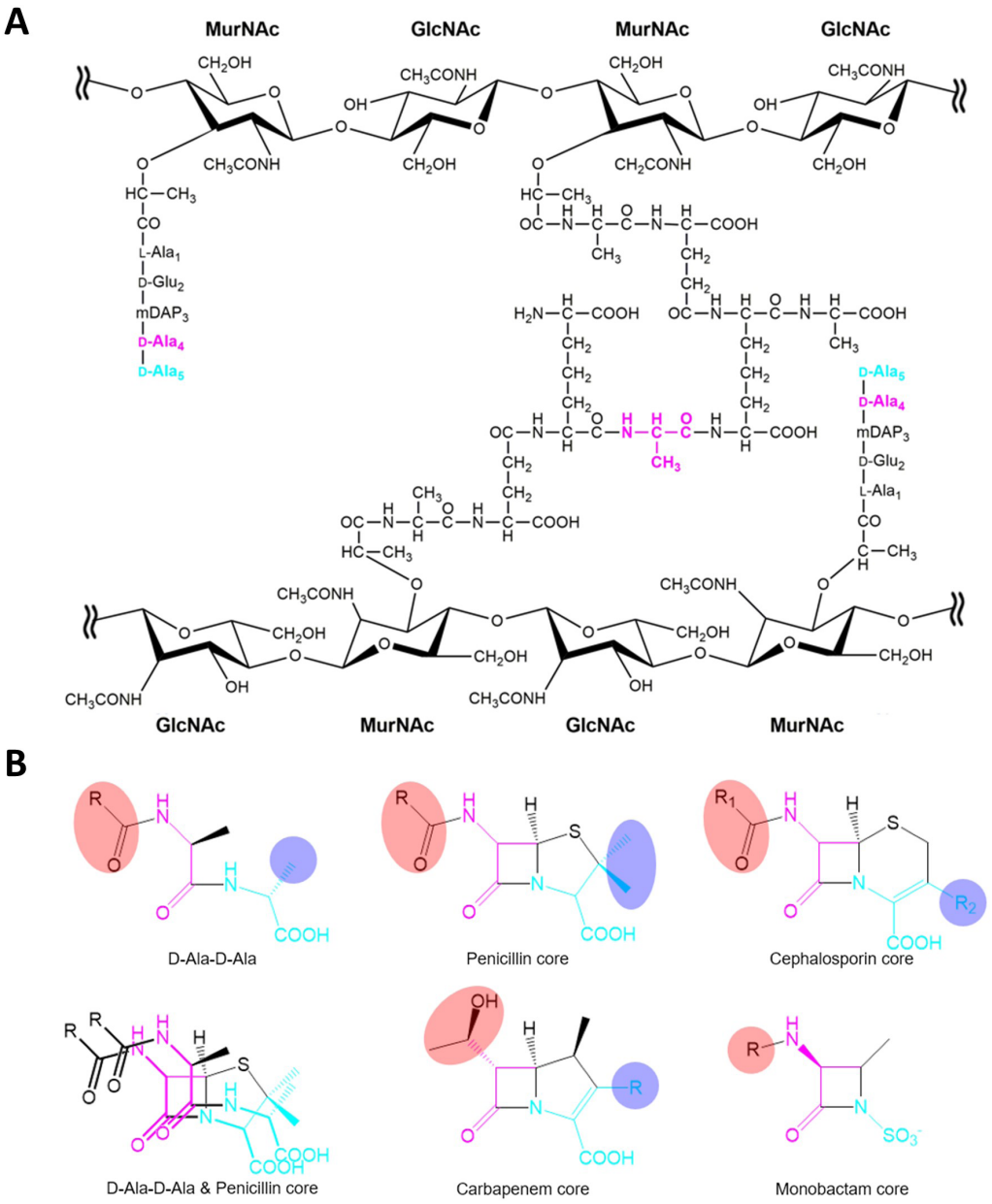

Fig. 1. Schematic drawings of representative peptidoglycan layers and D-Ala-D-Ala and β-lactams. (A) The representative structure of the peptidoglycan of *E. coli*. The glycan strands consist of alternating β(1→4) linked GlcNAc and MurNAc residues. Each MurNAc residue has a pentapeptide stem whose composition is most often L-Ala_1-D-Glu_2-$mDAP_3$-D-Ala_4-D-Ala_5. The terminal residues of the D-Ala_4-D-Ala_5 dipeptide are marked as magenta and cyan, respectively. (B) The core chemical structures of the D-Ala-D-Ala dipeptide part and four major classes of β-lactams of penicillin, cephalosporin, carbapenem, and monobactam. Structurally corresponding parts in the D-Ala-D-Ala and β-lactams are marked in the same colors as (A). The additional chemical R parts attached to the D-Ala_4 or β-lactam ring are shaded in red, and those to the D-Ala_5 or other ring are in blue.

noticeable difference between Gram-negative and Gram-positive bacteria is the presence of an additional outer membrane with lipopolysaccharides (LPS) and a thinner peptidoglycan cell wall in the periplasmic space. LPS includes a hydrophobic moiety of lipid A, an endotoxin, which is one of the most potent bacterial inducers of cytokine storms in patients (Greenfield *et al*., 2021).

The deployment of antibacterials depends on their targets. Various antibacterial agents target different bacterial-specific essential pathways, including the central dogma of DNA replication, RNA transcription, and protein translation; the metabolic pathways of nucleic and amino acid and lipid synthesis; and the bacterial cell wall and membrane structures (Silver, 2016). Although each antibacterial class has its own merits, all antibacterial agents must first reach their target area. Drugs targeting the bacterial cell wall have easier access to their target than those targeting the cytoplasm (Fig. 1A).

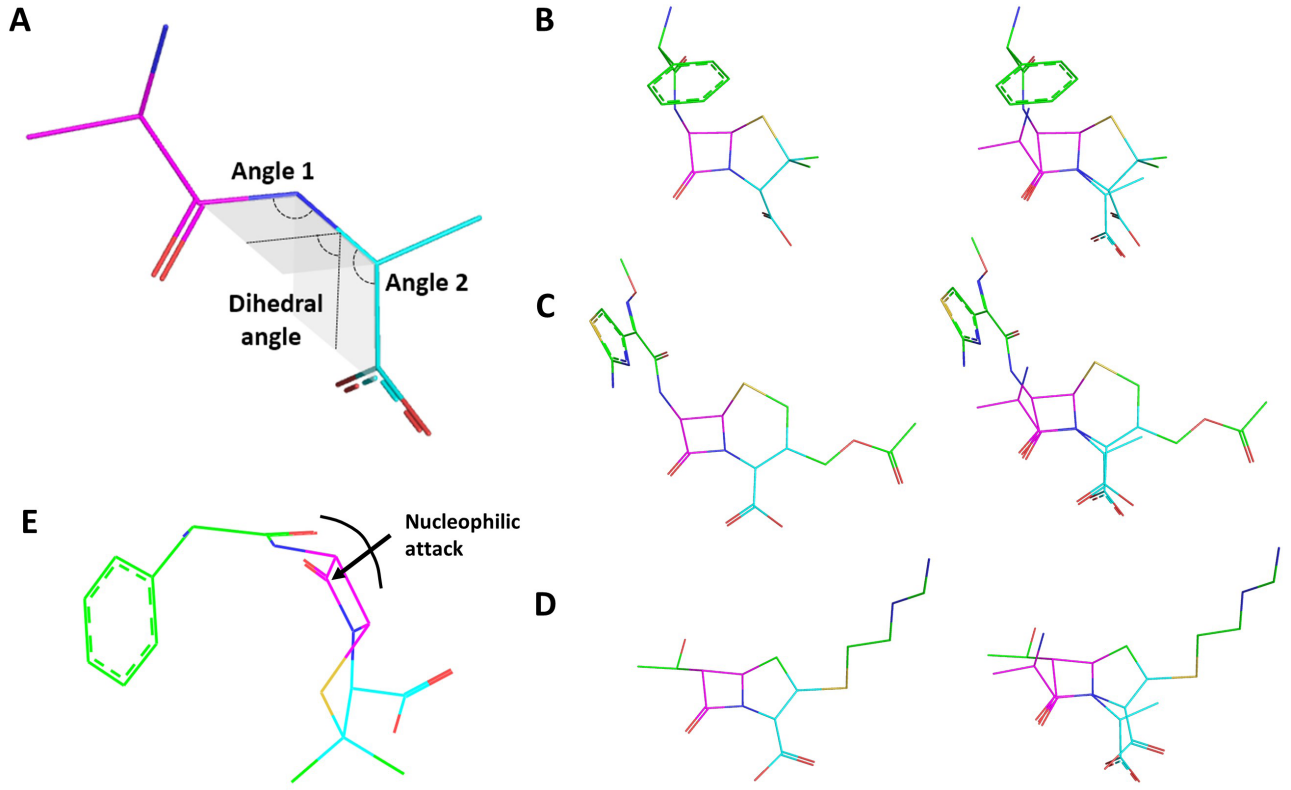

Fig. 2. The structural comparisons of the D-Ala-D-Ala and three β-lactam antibiotics. (A) The crystal structure of D-Ala-D-Ala (PDB ID: 3ITB) is labeled with the constituting two angles and a dihedral angle. The three-dimensional modeled structures (left) of (B) ampicillin, (C) cefotaxime, and (D) imipenem with the superimposed structures (right) to D-Ala-D-Ala. (E) The top view shows the bent angle between two rings of the ampicillin core scaffold. The convex side of ampicillin is labeled as a black line, and the direction of nucleophilic attack on the β-lactam ring by penicillin-binding proteins (PBPs) or β-lactamases is shown as a black arrow. Structurally corresponding parts in the D-Ala-D-Ala and β-lactams are marked in the same colors as Fig. 1.

β-LACTAM STRUCTURE

β-Lactams are the most effective antibiotics, and constitute almost two-thirds of the most commonly prescribed antibiotics (Ozturk *et al*., 2015). β-Lactam antibiotics are classified into four classes: penicillin, cephalosporins, carbapenems, and monobactams (Fig. 1B) (Yan *et al*., 2020). All members share the β-lactam ring as the essential core structure and have an additional ring structure directly connected to the β-lactam ring, except for monobactams.

The β-lactam ring is a four-membered ring with a carbonyl group. The structure of a square ring has a higher inner tension, with a sharp 90° turn at each atom, than a common five- or six-membered ring with a standard range of 109.5° to 120° angle in the atomic orbitals of sp^3 or sp^2, and the carbonyl group provides an excellent nucleophilic attack site for hydrolysis. The structural characteristics of a β-lactam ring provide higher reactivity for β-lactam antibiotics to inactivate penicillin-binding proteins (PBPs) than a linear, five- or six-membered ring and stable benzene ring with conjugated double bonds.

The conserved scaffold of β-lactam antibiotics, including the β-lactam ring at the center, contains the same chemical structure as that of the D-Ala-D-Ala dipeptide (Fig. 2). The dipeptide is involved in crosslinking the glycan chains in the peptidoglycan layer (Vollmer *et al*., 2008). Although the cross-linking peptide moiety varies among bacterial species, the D-Ala-D-Ala dipeptide is the most conserved. Compared to the linear covalent linkage of freely rotatable bonds in the backbone of D-Ala-D-Ala, β-lactam antibiotics have ring structures at the core scaffold, which restrains the flexibility of the corresponding part. The β-lactam and other rings of β-lactam antibiotics share three atoms from each D-Ala residue and have carbonyl and carboxylate groups on the same side parallel to each ring plane.

β-LACTAM WORKING MECHANISM

The peptidoglycan layer consists of the carbohydrate component of alternatively repeating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), and the cross-linking peptide component linked to MurNAc, which is diverse in composition and sequence in different bacterial species (Vollmer *et al*., 2008). In *E. coli*, a representative bacterium, PBPs recognize the terminal D-Ala-D-Ala dipeptide moiety from a glycan chain and crosslink it to the peptide of the other glycan chain. The catalytic serine residue in the active site of PBPs performs a nucleophilic attack on the $D\text{-}Ala_4$ of the pentapeptide attached to MurNAc, which cleaves the terminal $D\text{-}Ala_5$ and forms an acyl catalytic intermediate (Fig. 3). The

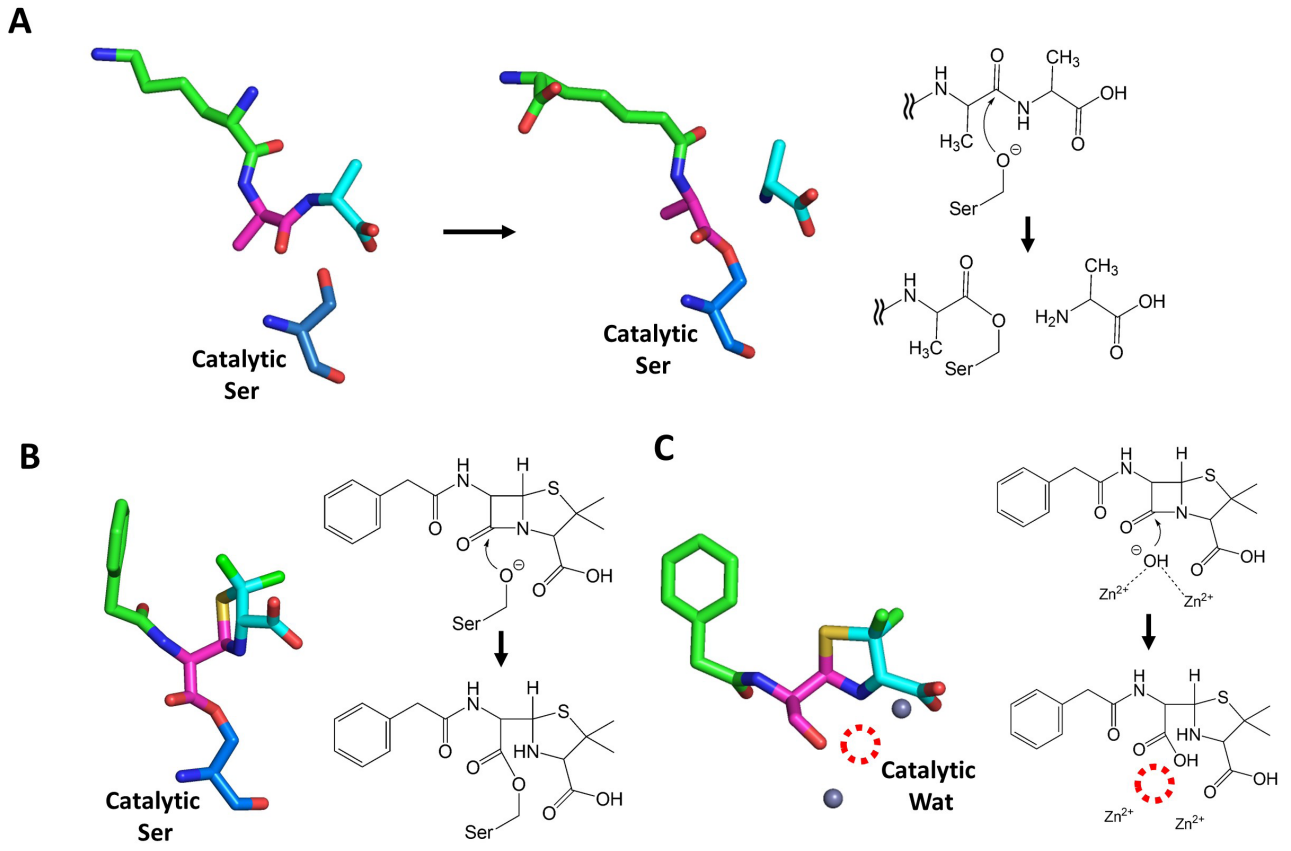

Fig. 3. The intermediate catalytic structures of penicillin-binding proteins, a serine β-lactamase, and a metallo-β-lactamase in complex with the D-Ala-D-Ala analogs and penicillin G and schematic representations of catalytic mechanisms. (A) The crystal structures of D-Ala-D-Ala-mimicking substrates and a catalytic serine residue of before (PDB ID: 3ITB) and after (PDB ID: 2J9P) nucleophilic attack on the D-Ala_4 in penicillin-binding proteins. (B) The crystal structure of hydrolyzed penicillin G attached to the catalytic serine of serine β-lactamase OXA-10 (PDB ID: 2WGI). (C) The crystal structure of hydrolyzed penicillin G with the zinc ions from metallo-β-lactamase NDM-1 (PDB ID: 4RAM). The proposed position of a catalytic water molecule is labeled with a dotted red circle. Structurally corresponding parts in the D-Ala-D-Ala and β-lactams are marked in the same colors as Fig. 1.

amino group of $mDAP_3$ (*meso*-2,6-diaminopimelic acid) in the pentapeptide from another glycan strand attacks the intermediate acyl group of D-Ala_4 and forms a crosslink between peptides (Goffin and Ghuysen, 1998; Sauvage *et al*., 2007, 2008; Chen *et al*., 2009).

The core scaffold of β-lactam antibiotics includes almost the same three-dimensional structure as that of the D-Ala-D-Ala dipeptide backbone. The nucleophilic attack on D-Ala_4 is conserved in β-lactam antibiotics as the carbonyl group of the β-lactam rings. The main difference between β-lactam antibiotics and the dipeptides is that β-lactam antibiotics have a more rigid and bulkier core structure due to the core ring structures, and various outside parts are connected to the core (Fig. 4).

β-Lactam antibiotics with two consecutive rings have a bent conformation at the center of the nitrogen atom. In the bent conformation, one side of the ring surface is widely exposed, and the other is more secluded from the solvent. Upon binding to PBPs, the convex side of the β-lactam rings faces the active site of PBPs, which exposes the target carbonyl group of β-lactams to have a better position for the nucleophilic attack of the catalytic serine of PBPs (Chen *et al*., 2009). Accordingly, the covalent bond-forming efficiency between β-lactams and PBPs is increased. On the opposite concave side of the β-lactam rings, PBPs have hydrogen bond donors to stabilize the acyl group of newly bound β-lactams.

DIVERSE β-LACTAM ANTIBIOTICS

In addition to the β-lactam ring as the core structure, penicillins, cephalosporins, and carbapenems have an additional ring with a carboxylate group on the same side of the carbonyl group in the β-lactam ring (Fig. 4). Monobactams have a sulfonate group instead of a carboxylate group that is directly attached to the nitrogen atom of the β-lactam ring without an additional ring structure.

Various additional moieties are attached to both sides of the core ring structures. The modifications directly bound to the β-lactam ring correspond to the peptide backbone from D-Ala_4 towards the third amino acid and so on in the pentapeptide. The opposite modifications of the additional ring correspond to the side chain of the terminal D-Ala_5 residue. The carboxylate group is relevant to the terminal carboxylate of D-Ala_5 in the pentapeptide crosslinker. All modifications of the β-lactam ring exist at the same position. However, five- or six-membered rings can provide additional structural diversity to

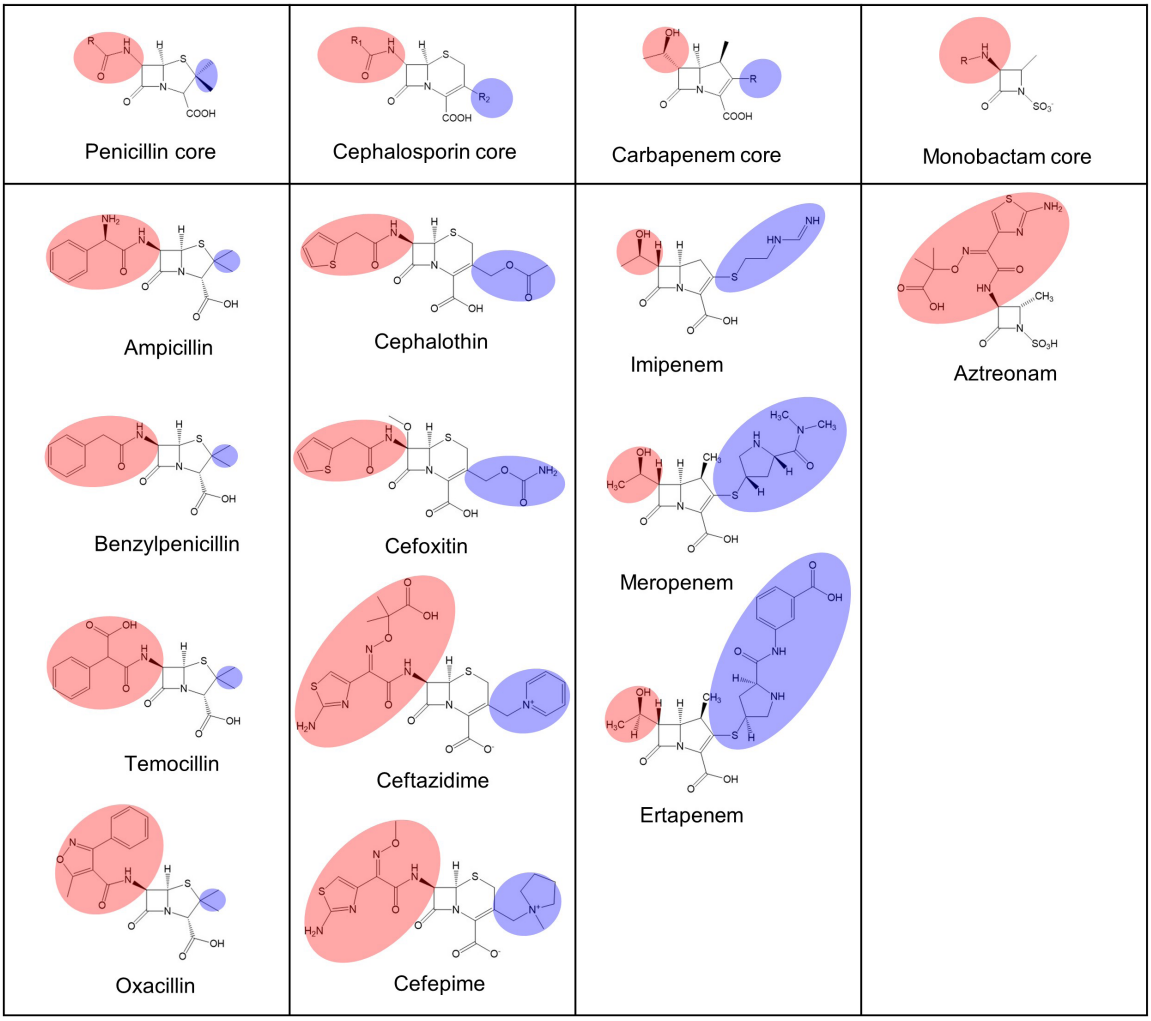

Fig. 4. The chemical structures of four main classes of β-lactam antibiotics. The conserved core scaffolds of each class of β-lactam antibiotics and the chemical structures of belonging members. The modification moieties attached to the β-lactam ring are shaded in red, and those at the other ring in blue are shown in Fig. 1.

β-lactam antibiotics due to the existence of several atoms for the modifications. The bent angles between the two β-lactam core rings have subtle differences among the three classes and change the attached positions and directions of the modification groups (Table 1).

In terms of modification groups, members of each class show overall conserved structural characteristics (Fig. 4). Penicillins have a bulkier group attached to the β-lactam ring, part of which is referred to as the red moiety and a simpler group attached to the five-member ring, as the blue moiety. Cephalosporins similarly have a bulkier red moiety but a larger blue moiety than penicillins. Moreover, due to the six-member ring, the orientation of the attached blue moiety is different from that of the penicillins. Carbapenems have structural characteristics opposite to those of penicillins, such as a smaller red moiety and bulkier blue moiety. In the five-member ring of carbapenems, two carbon atoms are available for modification of the blue moiety, and the red moiety has a simpler valine-like aliphatic structure. Monobactams are quite different from the other class members. The other three class members have a negatively charged carboxylate group on the other ring. Monobactams have a sulfonate group that is directly attached to the nitrogen atom of the β-lactam ring. Therefore, the negatively charged sulfonate groups of monobactams are closer to the β-lactam ring than the carboxylate groups of other β-lactam classes.

β-LACTAMASES

The mechanisms of bacterial resistance to β-lactams mainly fall into four categories: decreasing uptake of a drug, pumping a drug out of bacterial cells, modifying a drug target, and inactivating a drug. In Gram-negative MDR bacteria, inactivation of β-lactams by the hydrolysis of β-lactamases is a common mechanism (Paterson *et al*., 2020; Bahr *et al*., 2021).

β-Lactamases include a large group of hydrolyzing enzymes. β-Lactamase resistance genes commonly exist in mobile genetic elements, such as plasmids and transposons, which allow easy horizontal transfer to neighboring bacteria

Table 1. Comparison of angles and a dihedral angle between D-Ala-D-Ala and β-lactams.

	D-Ala-D-Ala (PDB ID: 3ITB)	Ampicillin	Cefotaxime	Imipenem
Dihedral angle (°)	63.0	98.7	30.5	29.6
Angle 1 (°)	127.1	130.6	135.7	141.2
Angle 2 (°)	121.1	111.6	120.1	127.5

The chemical structures of ampicillin, cefotaxime, and imipenem were prepared using ChemDraw Professional 16.0 (Perkin Elmer, Waltham, MA, USA). After drawing the chemical structure of each molecule in ChemDraw Professional 16.0, 3D molecular models were prepared using Chem3D 16.0 (Perkin Elmer), by calculating MM2 to minimize the steric energy and optimize the structures.

and vertical transfer to the offspring. β-Lactamases inactivate β-lactams by hydrolyzing the carbonyl group of the β-lactam ring, which corresponds to the peptide bond of the D-Ala D-Ala dipeptide (Fig. 3). β-Lactamases can be classified as serine β-lactamases and metallo-β-lactamases based on their catalytic mechanism: serine β-lactamases perform a nucleophilic attack by a catalytic serine residue, and metallo-β-lactamases by a catalytic water molecule coordinated by zinc ions in the active site (Naas *et al*., 2017). Serine β-lactamases are classified into classes A, C, and D. Metallo-β-lactamases belong to class B, which are further classified into B1, B2, and B3 subclasses, according to the characteristics of the amino acid sequences and protein structures (Crowder *et al*., 2006; Palacios *et al*., 2019; Behzadi *et al*., 2020; Park *et al*., 2020). The conserved active site of β-lactamases is required to recognize the hydrolyzing β-lactam ring of the substrate β-lactams.

The catalytic mechanism of serine β-lactamases is almost identical to that of PBPs: a catalytic serine residue attacks the carbonyl group of β-lactams, and an acyl intermediate is formed (Bush and Bradford, 2019). The acyl intermediate is released from the enzyme by consecutive nucleophilic attack of a hydroxyl group from a water molecule, and the active form of the enzyme is regenerated. In metallo-β-lactamases, a catalytic water molecule is coordinated by zinc ion(s) in the active site, and the direct nucleophilic attack on the carbonyl group of β-lactams hydrolyzes the β-lactam ring without forming an intermediate acyl form (Bahr *et al*., 2021). After cleavage of the C-N bond in the β-lactam ring, a higher degree of freedom with a freely rotatable corresponding bond can facilitate the easy release of the product from β-lactamases.

DISCUSSION

The increasing rate of MDR bacterial infections and the diminishing number of new antibacterial drugs have been among the biggest global threats to the modern healthcare system. Antibiotics have changed almost all medical practices by significantly lowering morbidity and mortality from bacterial infections. We are currently investigating effective antibiotics for the treatment of MDR bacteria. Persistent failure to develop new antibacterials with a unique mechanism of action discourages the development of a new resistance-focused drug, which is not an antibiotic but can be co-administered with antibiotics. β-lactams are among the most successful antibacterial drugs and include many different classes and generations of molecules. Currently, β-lactamase inhibitors have been systematically investigated. Despite efforts to develop β-lactam antibiotics and inhibitors, most studies have focused on identifying them rather than understanding their structure and mechanism.

In this review, we attempt to interpret the structural features of β-lactams to mimic the D-Ala-D-Ala dipeptide as the target structure and inhibit peptidoglycan synthesis in bacterial cell walls. In addition, the mechanism by which β-lactamases hydrolyze β-lactams and cause drug resistance is based on the structural features of β-lactams. Thus far, diverse β-lactams have been developed, mainly with variations of an additional ring and red and blue moieties attached to the core scaffold. From a structural viewpoint, the red moiety attached to the β-lactam ring exists at the same position corresponding to the upper amino acids before D-Ala_4-D-Ala_5 in the pentapeptide. The opposite blue moiety attached to the other ring corresponds to the side chain of terminal D-Ala_5, which is cleaved during the crosslinking of glycan chains.

As the first developed class of β-lactam antibiotics, penicillins maintain the most similar form to mimic pentapeptides, including the drug-targeting D-Ala-D-Ala dipeptide. As the second most developed class of β-lactam antibiotics, cephalosporins have a bulkier blue moiety but still maintain a bigger red moiety. Carbapenems, the last resort of β-lactam antibiotics, are the most different from penicillins because they have opposite structures, such as bulkier blue and smaller red moieties. The characteristics of the chemical and three-dimensional structures of various β-lactams provide in-depth insight into β-lactam antibiotics, such as the mimicry of the substrate and inhibition of cell wall synthesis, as well as the mechanism of drug resistance due to β-lactamases. A better understanding of the structures of β-lactam antibiotics will help us develop the next generation of β-lactam drugs and inhibitors against MDR bacteria.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the project ‘Development of biomedical materials based on marine proteins’ (Project No. 20170305) funded by the Ministry of Oceans and Fisheries, Korea (J.-H.L.).

This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (NRF-2017M3A9E4078014 and NRF-2017M3A9E4078017).

REFERENCES

Bahr, G., Gonzalez, L. J. and Vila, A. J. (2021) Metallo-beta-lactamases in the age of multidrug resistance: from structure and mechanism to evolution, dissemination, and inhibitor design. *Chem. Rev.* **121**, 7957-8094.

Behzadi, P., Garcia-Perdomo, H. A., Karpinski, T. M. and Issakhanian, L. (2020) Metallo-ss-lactamases: a review. *Mol. Biol. Rep.* **47**, 6281-6294.

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Burgmann, H., Sorum, H., Norstrom, M., Pons, M. N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F. and Martinez, J. L. (2015) Tackling antibiotic resistance: the environmental framework. *Nat. Rev. Microbiol.* **13**, 310-317.

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O. and Piddock, L. J. (2015) Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* **13**, 42-51.

Breijyeh, Z., Jubeh, B. and Karaman, R. (2020) Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules* **25**, 1340.

Bush, K. and Bradford, P. A. (2019) Interplay between beta-lactamases and new beta-lactamase inhibitors. *Nat. Rev. Microbiol.* **17**, 295-306.

Chen, Y., Zhang, W., Shi, Q., Hesek, D., Lee, M., Mobashery, S. and Shoichet, B. K. (2009) Crystal structures of penicillin-binding protein 6 from Escherichia coli. *J. Am. Chem. Soc.* **131**, 14345-14354.

Crowder, M. W., Spencer, J. and Vila, A. J. (2006) Metallo-beta-lactamases: novel weaponry for antibiotic resistance in bacteria. *Acc. Chem. Res.* **39**, 721-728.

de Kraker, M. E., Stewardson, A. J. and Harbarth, S. (2016) Will 10 million people die a year due to antimicrobial resistance by 2050? *PLoS Med.* **13**, e1002184.

Goffin, C. and Ghuysen, J. M. (1998) Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. *Microbiol. Mol. Biol. Rev.* **62**, 1079-1093.

Greenfield, K. G., Badovinac, V. P., Griffith, T. S. and Knoop, K. A. (2021) Sepsis, cytokine storms, and immunopathology: the divide between neonates and adults. *Immunohorizons* **5**, 512-522..

Hou, C. D., Liu, J. W., Collyer, C., Mitic, N., Pedroso, M. M., Schenk, G. and Ollis, D. L. (2017) Insights into an evolutionary strategy leading to antibiotic resistance. *Sci. Rep.* **7**, 40357.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K., Wertheim, H. F., Sumpradit, N., Vlieghe, E., Hara, G. L., Gould, I. M., Goossens, H., Greko, C., So, A. D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A. Q., Qamar, F. N., Mir, F., Kariuki, S., Bhutta, Z. A., Coates, A., Bergstrom, R., Wright, G. D., Brown, E. D. and Cars, O. (2013) Antibiotic resistance-the need for global solutions. *Lancet Infect. Dis.* **13**, 1057-1098.

Lee, J. H., Park, K. S., Karim, A. M., Lee, C. R. and Lee, S. H. (2016) How to minimise antibiotic resistance. *Lancet Infect. Dis.* **16**, 17-18.

Mancuso, G., Midiri, A., Gerace, E. and Biondo, C. (2021) Bacterial antibiotic resistance: the most critical pathogens. *Pathogens* **10**, 1310.

Naas, T., Oueslati, S., Bonnin, R. A., Dabos, M. L., Zavala, A., Dortet, L., Retailleau, P. and Iorga, B. I. (2017) Beta-lactamase database (BLDB) - structure and function. *J. Enzyme Inhib. Med. Chem.* **32**, 917-919.

Ozturk, H., Ozkirimli, E. and Ozgur, A. (2015) Classification of Beta-lactamases and penicillin binding proteins using ligand-centric network models. *PLoS One* **10**, e0117874.

Palacios, A. R., Mojica, M. F., Giannini, E., Taracila, M. A., Bethel, C. R., Alzari, P. M., Otero, L. H., Klinke, S., Llarrull, L. I., Bonomo, R. A. and Vila, A. J. (2019) The reaction mechanism of metallo-beta-lactamases is tuned by the conformation of an active-site mobile loop. *Antimicrob. Agents Chemother.* **63**, e01754-18.

Park, Y. S., Kim, T. Y., Park, H., Lee, J. H., Nguyen, D. Q., Hong, M. K., Lee, S. H. and Kang, L. W. (2020) Structural study of metal binding and coordination in ancient metallo-beta-lactamase PNGM-1 variants. *Int. J. Mol. Sci.* **21**, 4926.

Paterson, D. L., Isler, B. and Stewart, A. (2020) New treatment options for multiresistant gram negatives. *Curr. Opin. Infect. Dis.* **33**, 214-223.

Sauvage, E., Duez, C., Herman, R., Kerff, F., Petrella, S., Anderson, J. W., Adediran, S. A., Pratt, R. F., Frere, J. M. and Charlier, P. (2007) Crystal structure of the Bacillus subtilis penicillin-binding protein 4a, and its complex with a peptidoglycan mimetic peptide. *J. Mol. Biol.* **371**, 528-539.

Sauvage, E., Kerff, F., Terrak, M., Ayala, J.A. and Charlier, P. (2008) The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol. Rev.* **32**, 234-258.

Silver, L. L. (2016) Appropriate targets for antibacterial drugs. *Cold Spring Harb. Perspect. Med.* **6**, a030239.

Vollmer, W., Blanot, D. and de Pedro, M. A. (2008) Peptidoglycan structure and architecture. *FEMS Microbiol. Rev.* **32**, 149-167.

Yan, Y. H., Li, G. and Li, G. B. (2020) Principles and current strategies targeting metallo-beta-lactamase mediated antibacterial resistance. *Med. Res. Rev.* **40**, 1558-1592.