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.