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Microbiology Congress 2018: Structure-based virtual screening to identify the lactamase CTX-M-9 inhibitors: An in silico effort to overcome antibiotic resistance in E. Coli - Sako Mirzaie - Islamic Azad University

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Recently, the quick spreads of broad-spectrum β-lactams antibiotic resistance in pathogenic strains of bacteria has become a major global health problem. These new emerging resistances cause ineffectiveness of antibiotics and increasing the severity of diseases and treatment costs. Among different and diverse resistance targets, we chose a class-A β-lactamase, CTX-M-9, with the aim of identifying new chemical entities to be used for further rational drug design. Based on this purpose, a set of 5000 molecules from the Zinc database have been screened by docking experiments using AutoDock Vina software.

The best ranked compound with respect of the previously proved inhibitor compound 19 was further tested by molecular dynamics (MD) simulation. Our molecular modeling analysis demonstrates that ZINC33264777 has ideal characteristics a potent β-lactamase CTX-M-9 inhibitor. In the free form of β-lactamase, NMR relaxation studies showed the extensive motions near the active site and in the Ω-loop. However, our molecular dynamics studies revealed that in the compound 1: β-lactamase complex, the flexibility of Ω-loop was restricted.

The most common mechanism of resistance to β-lactam antibiotics in Gram-negative bacteria is the production of β-lactamases that hydrolyze the drugs. Class A β-lactamases are serine active-site hydrolases that include the common TEM, CTX-M, and KPC enzymes. The TEM enzymes readily hydrolyze penicillins and older cephalosporins. Oxyimino-cephalosporins, such as cefotaxime and ceftazidime, however, are poor substrates for TEM-1 and were introduced, in part, to circumvent β-lactamase-mediated resistance. Nevertheless, the use of these antibiotics has lead to evolution of numerous variants of TEM with mutations that significantly increase the hydrolysis of the newer cephalosporins. The CTX-M enzymes emerged in the late 1980s and hydrolyze penicillins and older cephalosporins and derive their name from the ability to also hydrolyze cefotaxime.

The CTX-M enzymes, however, do not efficiently hydrolyze ceftazidime. Variants of CTX-M enzymes, however, have evolved that exhibit increased hydrolysis of ceftazidime. Finally, the KPC enzyme emerged in the 1990s and is characterized by its broad specificity that includes penicillins, most cephalosporins, and carbapenems. The KPC enzyme, however, does not efficiently hydrolyze ceftazidime. As with the TEM and CTX-M enzymes, variants have recently evolved that extend the spectrum of KPC β-lactamase to include ceftazidime. This review discusses the structural and mechanistic basis for the expanded substrate specificity of each of these enzymes that result from natural mutations that confer oxyimino-cephalosporin resistance. For the TEM enzyme, extended-spectrum mutations act by establishing new interactions with the cephalosporin. These mutations increase the conformational heterogeneity of the active site to create sub-states that better accommodate the larger drugs. The mutations expanding the spectrum of CTX-M enzymes also affect the flexibility and conformation of the active site to accommodate ceftazidime. Although structural data are limited, extended-spectrum mutations in KPC may act by mediating new, direct interactions with substrate and/or altering conformations of the active site.

In many cases, mutations that expand the substrate profile of these enzymes simultaneously decrease the thermodynamic stability. This leads to the emergence of additional global suppressor mutations that help correct the stability defects leading to increased protein expression and increased antibiotic resistance. The R164S and G238S substitutions are associated with the largest increases in cefotaxime and ceftazidime hydrolysis when introduced into the TEM-1 enzyme.

These substitutions are likely the driver substitutions for clinically relevant resistance and are the most often observed in TEM ESBLs. The G238S substitution is predominantly associated with enhanced hydrolysis of cefotaxime and increased resistance. Residue 238 is situated on the β3 strand that forms a side of the active site. Because some increase in cefotaxime resistance is required for selection of intermediate mutations, mutational pathways that include a step with no selection advantage will be dead-ends. Thus, negative epistasis excludes some of the possible pathways by which complex mutations may arise.

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