

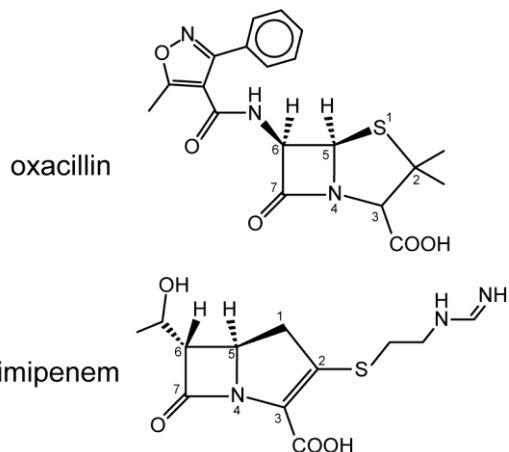
## Structural and mechanistic studies on carbapenem-hydrolysing class D serine $\beta$ -lactamases leading to improved inhibitor design

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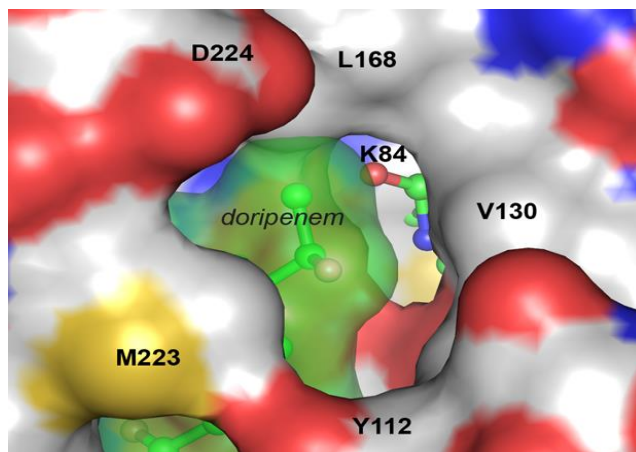
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The class D serine  $\beta$ -lactamases comprise a superfamily of almost 900 enzymes capable of conferring high-level resistance to  $\beta$ -lactam antibiotics, predominantly the penicillins including oxacillin (Fig. 1) and cloxacillin, and some early generation cephalosporins. In recent years it has been discovered that some members of the class D  $\beta$ -lactamase superfamily have evolved the ability to deactivate carbapenems (Fig. 1), *last resort*  $\beta$ -lactam antibiotics generally held in reserve for highly drug resistant bacterial infections. These enzymes are collectively known as Carbapenem-Hydrolyzing Class D serine  $\beta$ -Lactamases or CHDLs [1,2]. Most alarmingly, a large number (>500) of these CHDLs have appeared in several *Acinetobacter baumannii* strains, leading the CDC to elevate this once nosocomial infection of little clinical importance into a *major opportunistic pathogen*, now deemed to be an *urgent global threat* [3] with mortality rates from infections by resistant strains often exceeding 50% [4].

The mechanism of  $\beta$ -lactam deactivation by the class D serine  $\beta$ -lactamases involves the covalent binding of the antibiotic to an active site serine to form an acyl-enzyme intermediate (acylation). This is followed by hydrolysis of the acyl bond (deacylation), catalysed by a water molecule activated by a carboxylated lysine residue [5]. It was initially thought that the carbapenems acted as potent inhibitors of the class D enzymes since formation of the covalent acyl-enzyme intermediate expelled all water molecules from the active site, and stereochemistry of the side group at carbon 6 of the  $\beta$ -lactam ring effectively blocked access into the pocket housing the catalytic lysine, thus preventing the deacylation step. Our recent structural studies on three CHDLs (OXA-23, OXA-48 and OXA-143) [4,6,7] have indicated that their carbapenem hydrolysing ability may be due to small-scale dynamics of two surface hydrophobic residues which form a hydrophobic lid over the internal pocket housing the catalytic lysine. Movement of one or both of these residues allow for the transient opening and closing of a channel (Fig. 2) through which water molecules from the milieu can enter the lysine pocket to facilitate the deacylation reaction. Although the hydrophobic residues responsible for the channel formation are present in *all* class D  $\beta$ -lactamases, sequence and structural differences nearby may be responsible for the evolution of carbapenemase activity in the CHDLs. Current and future work aimed at non-covalent inhibitor development in OXA-23, and improved covalent inhibitor design focused on blocking access to the catalytic lysine pocket in OXA-23 and OXA-48 will be presented.



**Figure 1.** The first-generation  $\beta$ -lactam oxacillin (top) and the carbapenem imipenem (bottom). The stereochemistry at carbon 6 is important for differences in their reactivity towards the  $\beta$ -lactamases.



**Figure 2.** The surface of the CHDL OXA-143 calculated with Val130 in an open conformation, showing a hole which opens into the internal pocket housing the catalytic lysine (Lys85).

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