Structural basis for serine- and metallo-β-lactamase inhibition by VNRX-5133, a new β-lactamase inhibitor (BLI) in clinical development

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Background

Acquired resistance to carbapenems significantly narrows therapeutic options. Recently introduced combinations (ceftazidime/avibactam and meropenem/vaborbactam) are not effective against metallo-carbapenemases, such as VIM-2 or NDM-1, that are of great concern in clinical settings. VNRX-5133 is a new broad-spectrum BLI in clinical development with direct inhibitory activity against Class A, C and D serine-active site and VIM/NDM Class B metallo-β-lactamases in CREs and *P. aeruginosa*. We hereby report the X-ray structure of two clinically-relevant enzymes, CTX-M-15 and VIM-2 in complex with the cyclic boronate VNRX-5133.

Methods

β-lactamase crystals were obtained using the sitting drop method, and subsequently soaked with VNRX-5133. Diffraction data were collected at the ESRF synchrotron radiation facility. The structures were determined by molecular replacement, using the coordinates of the native structures (PDB 4HBT and 1KO3) as the search model.

Results

The structures of CTX-M-15 and VIM-2 β-lactamases in complex with VNRX-5133 were obtained at a resolution of 1.1 and 1.8 angstroms, respectively. In the CTX-M-15 complex, VNRX-5133 was covalently bound to the catalytic serine residue (Ser Oγ boron distance, 1.53 ang) and the boron atom adopted a tetrahedral conformation. One boron hydroxyl group was located in the oxyanion hole, indicating that the inhibitor mimics the transition state intermediate formed during the acylation step. The inhibitor created a set of substrate-like interactions with conserved residues of serine-β-lactamases, but none involving the inhibitor carboxamide substituent. In VIM-2, the inhibitor boron was also found in the sp3 hybridization state. The boron hydroxyl groups interacted with Zn1 and the conserved Asn233, while the carboxylate and O atom of the oxaborinane cycle interacted with Zn2. Inhibitor binding also induced the narrowing of the active site cleft, due to the approach of conserved Asn233 and Phe61. The substituted amino group of the inhibitor side chain interacted with Glu149, which is conserved in NDM-1 and where an Asp residue is found in IMP-1.

Conclusions

Our data provide a structural basis for broad-spectrum inhibition of β-lactamases by VNRX-5133, which is able to mimic the substrate transition state intermediates in the mechanistically divergent class A (serine) and B (metallo) enzymes.

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