Kinetic mechanism and parameters of inhibition of serine KPC-2, CTX-M15, p99 AmpC and metallo VIM-2 by the broad-spectrum β-lactamase inhibitor VNRX-5133

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Background

VNRX-5133 is a cyclic boronate broad-spectrum β-lactamase inhibitor (BS-BLI) currently in clinical development with cefepime for the treatment of ESBL- and carbapenemase-producing Enterobacteriaceae and *P. aeruginosa*. VNRX-5133 represents a new generation of BLI with direct inhibitory activity against serine-active site β-lactamases (SBL) and emerging metallo-β-lactamases (MBLs), for example NDM and VIM. Here we describe the kinetic mechanism of inhibition in both SBLs and MBLs and provide kinetic parameters of inhibition of representative and clinically important enzymes exploiting both mechanisms.

Methods

IC_{50} determinations with a broad collection of Class A, B, C and D BLs were used to define the spectrum of inhibitory activity. The kinetic parameters of reversible inactivation of SBLs were assessed from inactivation studies to generate a rate of complex formation (k_2/K_i), equilibrium dissociation constant (K_D) and inhibitor potency (K_i). Recovery of activity upon rapid step dilution with SBLs established the active-site residency time ($t_{1/2}$, k_{-2}) and turnover number of VNRX-5133. The kinetic mechanism of inhibition of the Class B MBL VIM-2 was established by steady-state inhibition studies.

Results

The spectrum of inhibitory activity of VNRX-5133 includes Class A, C and D SBLs as well as clinically important VIM- and NDM-type Class B MBLs. In SBLs, VNRX-5133 is characterized by potent reversible inactivation through a two-step inhibition model with highly efficient rates of covalent bond formation (10^4-10^5 $M^{-1}s^{-1}$) and slow dissociation from the active site(s) ($t^{1/2}$ = 30-58 min). The turnover number against KPC-2, representing the number of inhibitor molecules required to inhibit one active site, was 3 for VNRX-5133, 2 for avibactam, 21 for vaborbactam and 483 for tazobactam. Steady-state inhibition studies established that VNRX-5133 is also a highly potent (K_i = 21.6 nM) competitive inhibitor of substrate binding in the MBL VIM-2.

Conclusions

VNRX-5133 is a potent broad-spectrum BLI possessing a unique spectrum of activity that includes not only Class A, C and D serine active site enzymes, but also emerging metallo-β-lactamases. VNRX-5133 exhibits a rapid-on/slow-off behavior with serine enzymes, whereas with VIM/NDM-type metallo enzymes, it is a competitive inhibitor of substrate binding.

Authors

Denis M. Daigle[1]

Daniel C. Pevear[1]

Christopher J. Burns[1]

Affiliation

[1] VenatoRx Pharmaceuticals, Malvern, Pennsylvania, United States

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