Poster #1182

Ceftibuten/VNRX-7145, an orally bioavailable β-lactam/β-lactamase inhibitor combination active against serine-β-lactamase-producing Enterobacteriaceae

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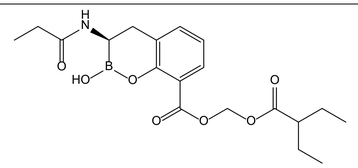
Background

Oral antibiotics are essential to treat infections in an outpatient setting and as step-down from IV therapy in the hospital. Available oral β-lactam antibiotics are rapidly becoming obsolete due to the continual evolution and proliferation of β-lactamases. VNRX-7145 is a novel cyclic boronate β-lactamase inhibitor, currently in development with ceftibuten as an oral treatment for serine-β-lactamase (SBL)-producing Enterobacteriaceae infections. *In vivo*, VNRX-7145 undergoes biotransformation to the active β-lactamase inhibitor VNRX-5236. We investigated the inhibitory properties of VNRX-5236 against a panel of clinically relevant class A, B, C and D β-lactamases.

Structure of VNRX-7145



Methods

β-lactamases were purified from *E. coli* BL21 (DE3) cells harboring expression plasmids for the various enzymes. Half-maximal inhibitory concentrations (IC_{50}s) were determined in a spectrophotometric assay with a 15 minute pre-incubation of enzyme and inhibitor using β-lactam substrates at 100 μM; CTX-M-15, SHV-5 with cefotaxime, KPC-2 with imipenem, NDM-1 with cefepime, and p99 AmpC, CMY-2, OXA-1, OXA-48, VIM-2 and IMP-1 with nitrocefin. For a subset of these enzymes, the rate of covalent complex formation (k_2/K_i) was determined under steady-state conditions by monitoring the progress of β-lactam hydrolysis in the presence of inhibitor. Dissociation rate constants (k_{off}), and associated residence times ($t_{1/2}$) were estimated using the jump-dilution method. The inhibitor constant K_i was derived from plots of fractional steady-state velocity versus inhibitor concentration.

Spectrum of inhibitory activity of VNRX-5236

IC_{50} values for VNRX-5236 relative to comparators against Ambler Class A, B, C and D β-lactamases.

Ambler Class	Enzyme	IC_{50} (μM)				
		VNRX-7145	VNRX-5236	Avibactam	Tazobactam	Clavulanic acid
A	CTX-M-15	>100	0.018	0.003	0.001	0.04
	KPC-2	>100	0.08	0.06	1.7	1.8
	SHV-5	8.98	0.368	NT	NT	NT
C	p99 AmpC	0.43	0.014	0.016	0.73	>100
	CMY-2	0.49	0.014	0.007	0.41	>100
D	OXA-1	NT	0.066	0.04	0.43	0.12
	OXA-48	9.62	0.317	0.55	3.5	14.3
B	VIM-2	>100	9.04	>100	>100	>100
	NDM-1	>100	38.1	>100	>100	>100
	IMP-1	NT	>100	>100	>100	>100

IC_{50} values are reported as the mean from duplicate measurements.
NT, not tested.

Reversible covalent inactivation of Ser-BLs by VNRX-5236

VNRX-5236 potently inhibited class A, C and D β-lactamases (IC_{50} < 0.37 μM), and demonstrated modest activity against the class B enzymes NDM-1 and VIM-2. Like other reversible β-lactamase inhibitors, the interaction between VNRX-5236 and β-lactamases is described by a 2-step model for reversible inhibition[1, 2]:

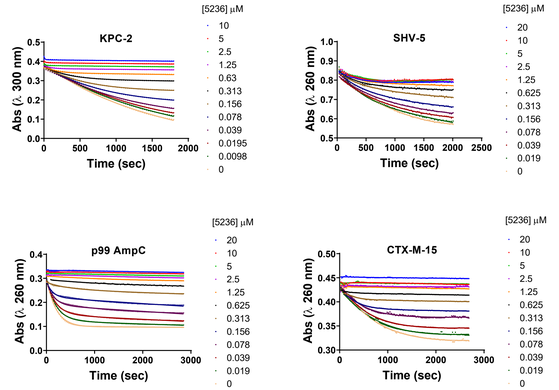
$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} E{:}I \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E\text{-}I$$

Eqn.1 $$A_i = A_0 + v_s t + (v_0 - v_s)\left[\frac{1 - e^{-k_{obs} t}}{k_{obs}}\right]$$

Eqn.2 $$k_{obs} = k_{-2} + \frac{k_2}{K_i}\left[\frac{[I]}{1 + \frac{[S]}{K_m}}\right]$$

Progress curves for β-lactam hydrolysis with increasing concentrations of VNRX-5236 were fit to equation 1. The second order rate constant k_2/K_i for β-lactamase inactivation was subsequently derived using equation 2, and showed efficient formation of covalently bound enzyme:inhibitor complexes (E-I), with k_2/K_i on the order of 10^4 $M^{-1}.s^{-1}$.

Concentration-dependent inhibition of SBLs by VNRX-5236

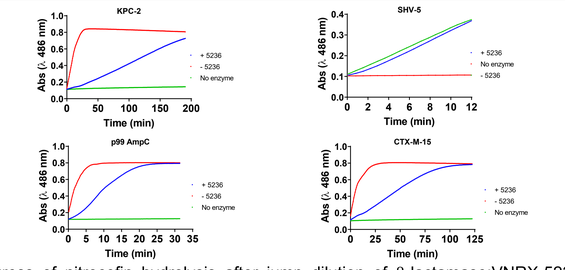


Time courses of β-lactam hydrolysis in the presence of VNRX-5236..

Dissociation kinetics of VNRX-5236

Off-rates for VNRX-5236 varied among the enzymes tested. Dissociation from KPC-2 and CTX-M-15 was slow compared to SHV-5 and p99 AmpC, resulting in longer residence times for the former pair. VNRX-5236 nevertheless possesses sufficient active site occupancy to prevent hydrolysis of β-lactams including the partner antibiotic, ceftibuten (see Poster #1181).

Off rate assessment from recovery of nitrocefinase activity



Progress of nitrocefin hydrolysis after jump dilution of β-lactamase:VNRX-5236 complexes. Data were fit to a single exponential to obtain k_{off}.

Kinetic parameters of inhibition by VNRX-5236

β-lactamase	Parameter	VNRX-5236	Avibactam
KPC-2	k_2/K_i (10^4 $M^{-1}s^{-1}$)	2.9 ± 0.07	1.2 ± 0.1
	k_{-2} (10^{-4} s^{-1})	2.5 ± 0.1	1.8 ± 0.1
	$t_{1/2}$ (min.)	46 ± 2	66 ± 4
	K_i (μM)	0.11	0.006
CTX-M-15	k_2/K_i (10^4 $M^{-1}s^{-1}$)	4.8 ± 0.9	10.8 ± 0.6
	k_{-2} (10^{-4} s^{-1})	4.5 ± 0.1	4 ± 0.1
	$t_{1/2}$ (min.)	26 ± 0.6	29 ± 1
	K_i (μM)	0.01	0.011
SHV-5	k_2/K_i (10^4 $M^{-1}s^{-1}$)	1.1 ± 0.16	NT
	k_{-2} (10^{-4} s^{-1})	12.7 ± 0.07	NT
	$t_{1/2}$ (min.)	6.5 ± 0.04	NT
	K_i (μM)	0.041	NT
p99 AmpC	k_2/K_i (10^4 $M^{-1}s^{-1}$)	6.0 ± 0.6	0.3 ± 0.1
	k_{-2} (10^{-4} s^{-1})	24 ± 0.5	0.5 ± 0.04
	$t_{1/2}$ (min.)	5 ± 0.1	249 ± 19
	K_i (μM)	0.02	0.013

Reported values for k_2/K_i include an adjustment for $(1 + [S]/K_M)$ to account for the substrate concentration used in the assays, relative to its K_M
NT, not tested.

Conclusions

VNRX-5236 exhibits potent inhibitory activity against serine class A, C and D β-lactamases, enabling the protection of partner β-lactam antibiotics with a spectrum of inhibition comparable to avibactam[2,3]. The findings support oral dosing of the ceftibuten/VNRX-7145 combination as a promising option for treatment of infections caused by multi-drug resistant gram-negative bacteria producing extended spectrum β-lactamases (ESBLs) and carbapenemases (KPC and OXA-48), both in the community and hospital settings.

References

1. Copeland (2005). Evaluation of enzyme inhibitors in drug discovery. pp 141-177. John Wiley and sons, Inc., New York, NY.
2. Ehmann et al (2013). J. Biol. Chem. 288 (39); 27960-27971
3. Ehmann et al (2012). PNAS. 109 (29); 11663-11668.

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