Supplemental information for

Cyclic boronates as versatile scaffolds for KPC-2 β-lactamase inhibition

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Author Contributions

CLT purified the enzyme and collected kinetic, microbiological and crystallographic data; CLT and PH solved and refined the crystal structures; AK synthesized taniborbactam; all authors analysed the data; CLT, PH and JS wrote the manuscript; CLT, PH, AJM, JB, CJS and JS designed the research; all authors edited the manuscript.

Supplementary Figures

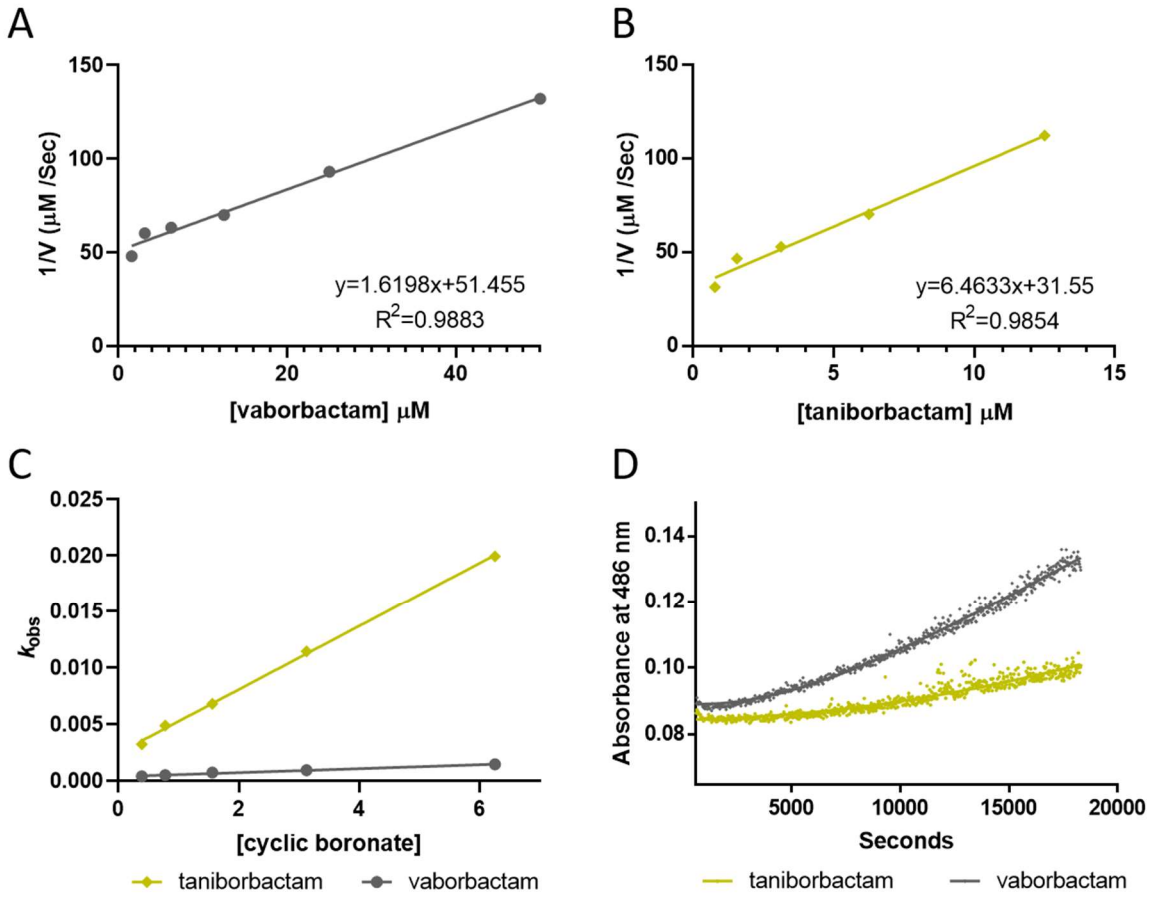

Figure S1. Kinetic characterisation of cyclic boronate inhibitors with KPC-2. (A and B) Dixon plots of reciprocals of initial nitrocefin hydrolysis rates ($1/V$) by enzyme:cyclic boronate mixtures plotted against inhibitor (A, vaborbactam and B, taniborbactam) concentration. The apparent inhibition constant K_{iapp} is obtained from the slope of the fitted straight line. (C) Plots of k_{obs} (pseudo-first-order rate constant for inactivation) against inhibitor concentration. The apparent second-order rate constant k_2/K is obtained from the slope of the fitted straight line. (D) Progress curves representing recovery of nitrocefin hydrolysis following 30-minute preincubation of enzyme (1 µM) with 8 µM cyclic boronate, followed by dilution to a final concentration of 500 pM enzyme. The rates of recovery of free enzyme, k_{off}, were obtained as described in Reference 32 of the main text. Data points shown are means of three replicates.

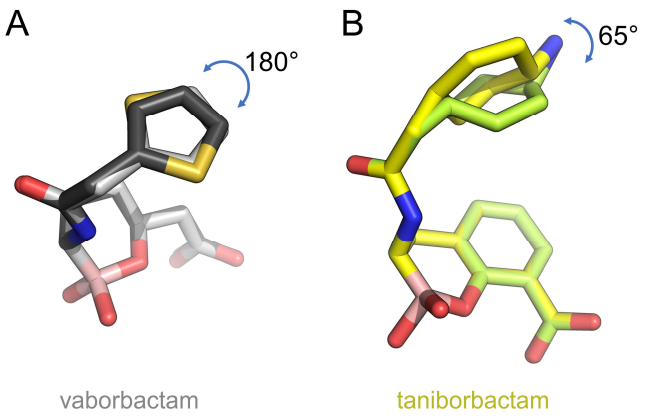

Figure S2. Conformations of the C-3 substituent of vaborbactam and taniborbactam in KPC-2. **(A)** Two conformers of vaborbactam modelled into KPC-2 with 0.62 (light grey) and 0.38 (dark grey) occupancy, showing a 180° rotation of the thiophene moiety of the C-3 substituent. **(B)** Two conformers of taniborbactam modelled into KPC-2 with 0.89 (yellow) and 0.11 (green) occupancy, showing a ~65° rotation of the cyclohexane ring of the C-3 substituent.

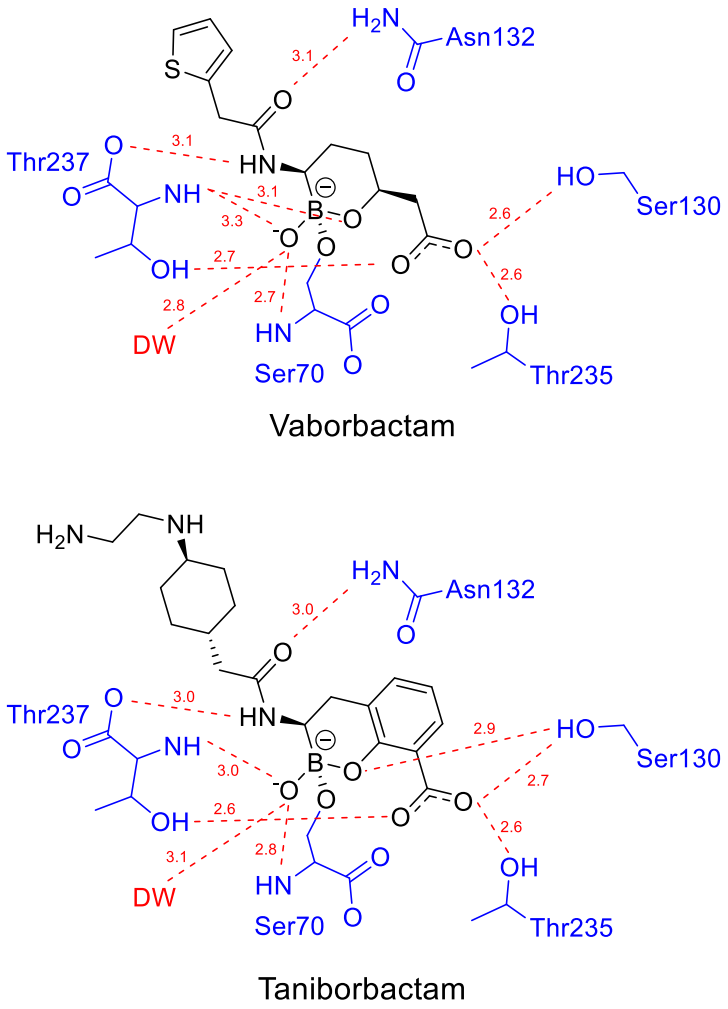

Figure S3. Interactions of vaborbactam and taniborbactam in KPC-2. Main chain protein residues are coloured blue, and the water in the deacylating position (DW) is in red. Interactions are shown as red dashes with corresponding distances in Å.

Supplementary Tables

Table S1. MICs of cefepime/meropenem against KPC-2 producing *K. pneumoniae* in the presence of cyclic boronates.

Strain	Plasmid	MIC (ug/ml)					
		cefepime			meropenem		
		No inhibitor	+VAB (4mg/L)	+TAN (4mg/L)	No inhibitor	+VAB (4mg/L)	+TAN (4mg/L)
Ecl8	pUBYT	≤0.125	-	-	≤0.125	-	-
Ecl8	pUBYT KPC-2	≥256	≤0.125	≤0.125	16	≤0.125	≤0.125

VAB: vaborbactam
TAN: taniborbactam

Table S2. Data collection and refinement statistics.

	KPC-2: vaborbactam	**KPC-2: VNRX-5133**
PDB accession	6TD0	6TD1
Data collection		
Beamline	ESRF ID23-1	ALBA BL13-XALOC
Space group	$P2_12_12$	$P2_12_12$
Molecules/ASU	1	1
Cell dimensions		
a, *b*, *c* (Å)	59.92, 77.94, 55.73	60.56, 79.76, 56.02
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Wavelength	0.92	0.90
Resolution (Å)	45.33 – 0.99 (1.01 – 0.99)	45.84 – 1.20 (1.22 – 1.20)
R_{pim}	0.027 (0.444)	0.030 (0.520)
CC 1/2	0.999 (0.552)	0.999 (0.708)
I / σI	12.2 (2.0)	11.4 (1.1)
Completeness (%)	99.3 (99.1)	100.0 (100.0)
Redundancy	12.5 (12.3)	13.0 (11.9)
Refinement		
Resolution (Å)	45.33 – 0.99	41.12 – 1.20
No. reflections	144249	85424
R_{work} / R_{free}	13.82 / 15.32	13.60 / 15.21
No. non-H atoms		
Protein	2121	2195
Solvent	331	404
Inhibitor	40[#]	50[#]
B-factors		
Protein	15.02	17.19
Solvent	28.72	35.23
Inhibitor	18.48	17.00
R.m.s. deviations		
Bond lengths (Å)	0.012	0.013
Bond angles (°)	1.502	1.425
Ramachandran (%)		
Outliers	0.0	0.0
Favoured	98.88	98.88

*Values in parentheses are for highest-resolution shell.
[#]Inhibitor modelled as dual occupancy

Table S3. Interaction distances (Å) of cyclic boronates in complex with KPC-2.

Inhibitor group/atom	Protein residue/atom	vaborbactam	taniborbactam	difference
Acetamido O	Asn132, sidechain N	3.05	3.02	0.03
Carboxylate O1	Ser130, sidechain O	2.64	2.72	0.08
Carboxylate O1	Thr235, sidechain O	2.57	2.58	0.01
Carboxylate O2	Thr237, sidechain O	2.65	2.60	0.05
Amide N	Thr237, backbone O	3.06	2.97	0.09
Boron-bound OH	Thr237, backbone N	3.27	3.04	0.23
Boron-bound OH	Ser70, backbone N	2.72	2.82	0.10
Endocyclic ester O	Ser130, sidechain O	-	2.93	-
Endocyclic ester O	Thr237, backbone N	3.06	-	-
Boron-bound OH	'deacylation' water	2.75	3.10	0.35