Supporting Information

Strategic approaches to overcome resistance against Gram negative pathogens using β-lactamase inhibitors and β-lactam enhancers: The activity of three novel diazabicyclooctanes, WCK 5153, zidebactam (WCK 5107), and WCK 4234

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Supporting Information Figure S1.

Table S1. Data collection and refinement statistics for KPC-2 and OXA-24/40 bound to compounds **1**, **2**, and **3**.

Data collection	**KPC-2 soaked 3**	**KPC-2 soaked 2**	**KPC-2 soaked 1**	**KPC-2 co-crystallized 3**	**KPC-2 co-crystallized 2**	**KPC-2 co-crystallized 1**	**OXA-24/40 co-crystallized 3**
PDB identifier	6B1F	6B1J	6B1X	6B1H	6B1W	6B1Y	6B22
Space group	P1	P1	P1	P1	P1	P1	P41212
Cell dim. (Å,°)	34.54 37.43 82.34 92.33 90.05 95.25	34.44 37.42 82.13 92.31 90.04 95.26	34.49 37.44 82.24 92.30 90.21 95.08	34.49 37.37 81.83 87.78 89.96 84.53	34.53 37.35 82.07 87.66 89.71 84.31	34.53 37.35 82.07 87.66 89.71 84.31	102.54 102.54 84.31 90 90 90
Wavelength (Å)	1.12708	1.12708	1.12708	1.12708	1.12708	1.12708	0.97910
Resolution (Å)	1.44-37.23 (1.44-1.46)	1.60-37.23 (1.60-1.63)	1.45-37.26 (1.45-1.48)	1.80-37.17 (1.80-1.84)	1.73-37.13 (1.73-1.77)	1.80-37.13 (1.80-1.84)	1.93-38.99 (1.93-197)
Redundancy	2.0 (1.8)	2.0 (1.9)	2.0 (1.9)	2.0 (1.9)	2.0 (2.0)	2.0 (2.0)	5.8 (5.6)
Unique refl.	67,950 (1,939)	51,151 (2,437)	67,132 (2,315)	35,855 (1,977)	40,430 (2,129)	35,965 (2,083)	34,171 (2,247)
$\langle I \rangle / \langle \sigma(I) \rangle$	13.1 (2.6)	11.9 (1.9)	15.2 (3.9)	9.6 (4.9)	12.8 (2.0)	14.0 (2.6)	11.9 (2.0)
Rmerge (%)	3.5 (25.9)	4.6 (42.0)	3.0 (18.7)	6.2 (15.9)	5.1 (34.5)	4.6 (27.2)	12.7 (115.1)
Completeness (%)	91.6 (54.4)	94.9 (89.1)	92.6 (64.7)	95.1 (88.3)	95.3 (91.7)	95.5 (92.0)	99.4 (98.6)
Refinement							
Resolution range (Å)	1.44-31.78	1.60-34.29	1.45-34.46	1.80-33.36	1.73-34.35	1.80-37.00	1.93-38.99
R-factor (%)	15.0	15.0	14.5	16.1	15.2	14.8	17.8
R_{free} (%)	17.4	18.7	16.9	21.0	19.4	19.7	19.8
Median B-factor ($Å^2$)							
β-lactamase	9,11	11,13	7,10	9,9	12,13	12,13	20
DBO inhibitors	9,12	11,18	7,12	9,9	11,15	10,15	39
RMSD dev. ideality							
Bond lengths (Å)	0.009	0.014	0.013	0.015	0.013	0.015	0.013
Angles (deg)	1.91	1.79	2.35	2.06	1.65	1.69	1.87

Table S2. MICs in mg/L for laboratory-engineered *A. baumannii* knockout strains.

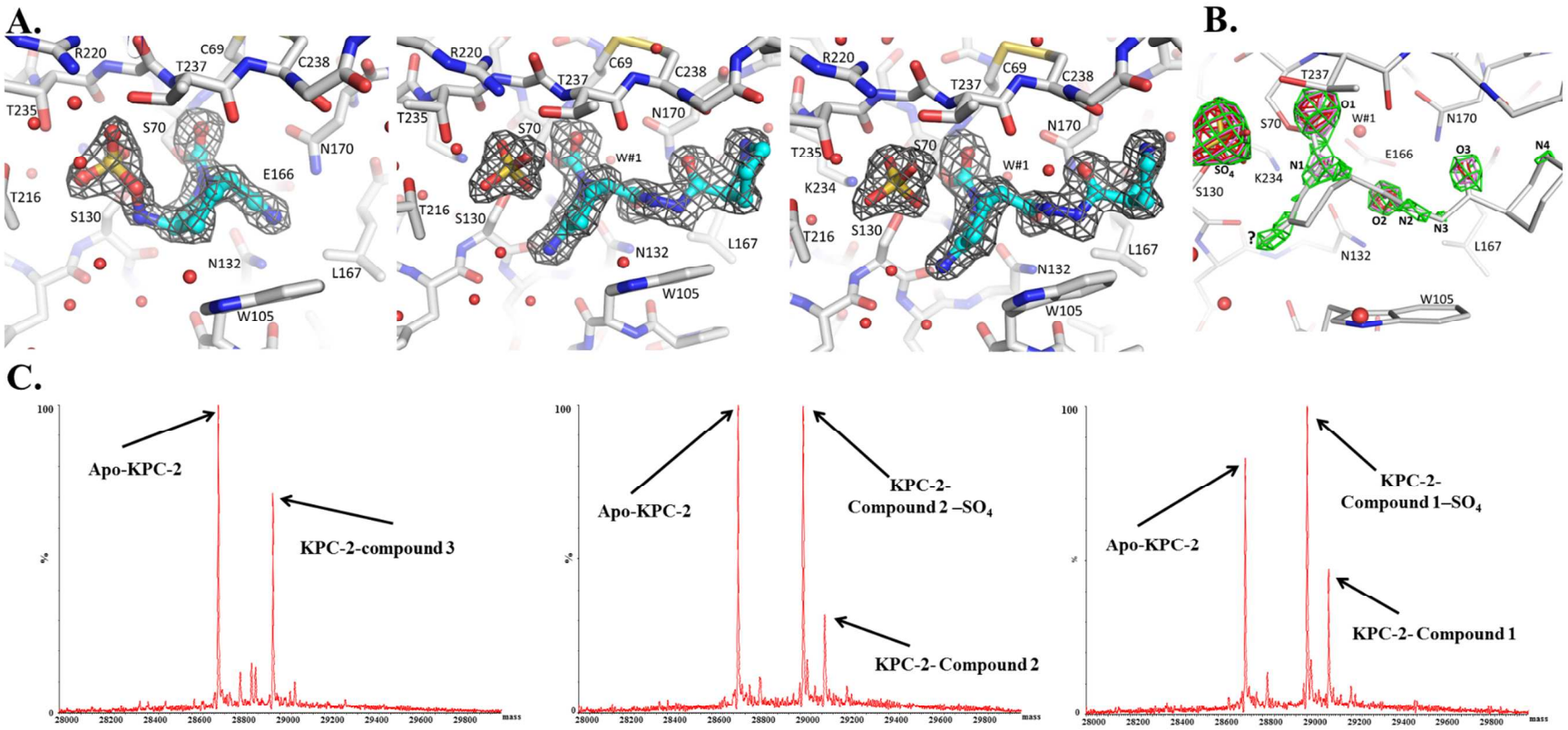
STRAIN	3	2	1	Avibactam	Relebactam	Cefepime	Cefepime/3-4*	Cefepime/3-8	Cefepime/1-4	Cefepime/1-8	Cefepime/2 -4	Cefepime/2-8	Cefepime/Avibactam-4	Cefepime/Relebactam-4	Meropenem	Meropenem/3-4	Meropenem/3-8	Meropenem/1-4	Meropenem/1-8	Meropenem/2-4	Meropenem/2-8	Meropenem/Avibactam-4	Meropenem/Relebactam-4
Acinetobacter baumannii AVAMC1 (ATCC17978)	> 16	> 16	> 16	> 16	> 16	2	4	4	1	1	1	1	2	2	<= 0.12	0.25	0.25	0.25	0.25	<= 0.12	0.25	0.25	0.25
Acinetobacter baumannii AVAMC2 *adeB*::Km	> 16	> 16	> 16	> 16	> 16	0.25	0.25	0.25	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12
Acinetobacter baumannii AVAMC3 *adeJ*::Km	> 16	> 16	> 16	> 16	> 16	0.25	0.25	0.25	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12
Acinetobacter baumannii AVAMC4 *adeG*::Km	> 16	> 16	> 16	> 16	> 16	2	2	4	1	1	1	2	2	2	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12
Acinetobacter baumannii AVAMC5 *acrA*::Km	> 16	> 16	> 16	> 16	> 16	2	2	2	0.5	1	0.5	1	2	2	<= 0.12	<= 0.12	<= 0.12	<= 0.12	0.25	0.25	<= 0.12	<= 0.12	<= 0.12
Acinetobacter baumannii AVAMC6 *acrR*::Km	> 16	> 16	> 16	> 16	> 16	2	2	2	1	1	1	1	2	2	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	0.25	<= 0.12
Acinetobacter baumannii AVAMC7 Δ*adeB*/*adeJ*,*acrR*::Km	> 16	> 16	> 16	> 16	> 16	0.25	0.25	0.25	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12
Acinetobacter baumannii AVAMC8 Δ*adeB*/*adeJ*	> 16	> 16	> 16	> 16	> 16	0.25	0.25	0.25	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12
Acinetobacter baumannii ATCC17978 *blhA*:Km	> 16	> 16	> 16	> 16	> 16	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12
Acinetobacter baumannii ATCC17978 Δ*adeB*Δ*adeJ blhA*:Km	> 16	> 16	> 16	> 16	> 16	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12	<= 0.12

Color coding of MICs: ≤0.12-1 µg/mL, 2-8 µg/mL, and ≥16 µg/mL. *DBOs were used at either 4 µg/mL (-4) or 8 µg/mL (-8).

Figure S1 Legend.

Figure S1. A. Electron density of compounds **1**, **2**, and **3** bound in the active site of KPC-2 obtained by co-crystallization experiments. Shown are unbiased omit |Fo|-|Fc| electron density with the ligands removed from refinement and map calculations. Left, **3** bound to KPC-2; center, **2** bound to KPC-2; right, **1** bound to KPC-2. Electron density is contoured at 3σ level. The electron density maps of **2** and **1** KPC-2 co-crystallized structures revealed the presence of a desulfated DBO species with its separated sulfate ion bound in close proximity. **B.** Electron density maps of **2** bound to KPC-2 obtained by co-crystallization. The protein atoms are color coded according to atom type whereas the main **2** fragment is colored grey for all its atoms for proper contrast with the differently colored density maps. The unbiased |Fo|-|Fc| electron density map is contoured at 6.5σ (green), 7.5σ (pink), and 8.5σ (red) levels. These different contours are to show the relative strength of the electron density corresponding to the nitrogen (7 electrons), oxygen (8 electrons), and sulfur (16 electrons) atoms of the ligand and sulfate ion allowing identification of a nitrogen atom at the desulfated **2** fragment position labeled by '?'. The density levels at this position are comparable to that of the nitrogen N1 in the same molecule and are not as strong as the density levels at the three oxygen positions in this DBO suggesting that imine hydrolysis of this DBO has not yet occurred [11]. **C.** Mass spectrometry of KPC-2 with **3** (left), **2** (center), and **1** (right) after incubation at 1:1 ratio in the crystallographic mother liquor (w/o PEG) for 24 hrs.

Figure S1.


A.
R220
C69
T237
C238
T235
S70
N170
E166
T216
S130
N132
L167
W105
W#1
K234
B.
O1
O2
O3
N1
N2
N3
N4
SO4
?
C.
100
%
0
Apo-KPC-2
KPC-2-compound 3
KPC-2-Compound 2 –SO4
KPC-2- Compound 2
KPC-2-Compound 1–SO4
KPC-2- Compound 1
28000
28200
28400
28600
28800
29000
29200
29400
29600
29800
mass