Supporting Information

Small molecule carboxylates inhibit metallo-β-lactamases and resensitize carbapenem-resistant bacteria to meropenem

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General notes. The compounds used in the present study were obtained from the following suppliers: Compounds **1** (98%, Sigma-Aldrich), **2** (96%, Sigma-Aldrich), **3** (99%, Fisher scientific), **4** (98%, Sigma-Aldrich), **5** (95%, Sigma-Aldrich), **6** (99%, Brunschwig Chemie), **7** (98%, Brunschwig Chemie), **8** (99%, Sigma-Aldrich), DPA (98%, Combi-Blocks). Enzyme activity assays were performed on a Tecan Spark microplate reader. The ITC thermograms were obtained on an automated PEAQ-ITC calorimeter (Malvern).

Enzyme production and purification. For the production of VIM-2 and NDM-1, pOPINF NDM-1 and pTriEx-based pOPINF plasmids (ampicillin resistant) were used. The constructs were a generous gift from Dr. Christopher J. Schofield (Oxford university). In the case of IMP-28, the construct was designed in pET28b with a 6-His tag at the C-terminus. The plasmids of IMP-28, VIM-2, NDM-1 were transformed in BL21 competent *E. coli* using standard heat shock transformation method. The single colonies were grown overnight at 37 °C in LB medium containing 1% glucose and appropriate antibiotic (100 µg/mL ampicillin or 300 µg/mL amikacin). The cell suspension was diluted 100 times in YT2x supplemented with 0.1% glucose and antibiotic, shaking at 37 °C for about 4 h to reach OD_{600} of 0.5-0.7. The expression of the enzymes was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG, final concentration 0.5 mM). The cells were incubated overnight at 25 °C with shaking and then harvested by centrifugation for 20 min at 6000 rpm. The pellet was resuspended in lysis buffer (PBS, 150 mM NaCl, 0.05% TritonX-100, protease inhibitor cocktail). After two freeze-thaw cycles the cell suspension was incubated with 1 mg/ml lysozyme for 30 min at 37 °C followed by 3 cycles of sonication (30-s pulse and 30-s rest each cycle). The cellular debris were removed by centrifugation at 12000 rpm for 20 min at 4 °C. Äkta Xpress chromatography system was used to purify the enzymes. Briefly, the supernatant was loaded on 1 mL HisTrap HP column and the enzymes were eluted with 300 mM imidazole. The fractions were then loaded on HiTrap desalting column to exchange the buffer. In case of IMP-28, the fractions were collected in 20 mM Tris, 150 mM NaCl, 10% glycerol, 20 µM $ZnCl_2$. VIM-2 and NDM-1 fractions were buffer exchanged to 20 mM Tris, 200 mM NaCl. The purity of the fractions was determined on 15% SDS-PAGE gel. The concentration of the enzymes was measured by Nanodrop at 280 nm. To remove the His tag at the N-termini of VIM-2 and NDM-1, the proteins were incubated overnight at 4 °C with HRV-3C protease (1:100 w/w). The digestion mixture was passed through a HisTrap column to separate cleaved from uncleaved enzymes. The cleavage of His tag was confirmed by western blot technique. Both enzymes were buffer exchanged to 50 mM Tris (pH 7.5) containing 500 mM NaCl.

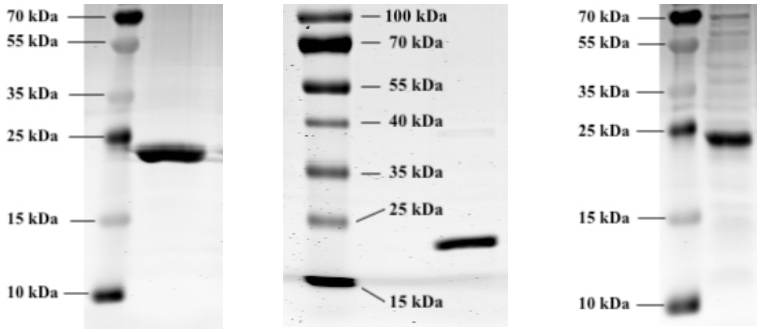


Figure S1. SDS-PAGE gels of purified NDM-1 (A), VIM-2 (B), and IMP-28 (C).

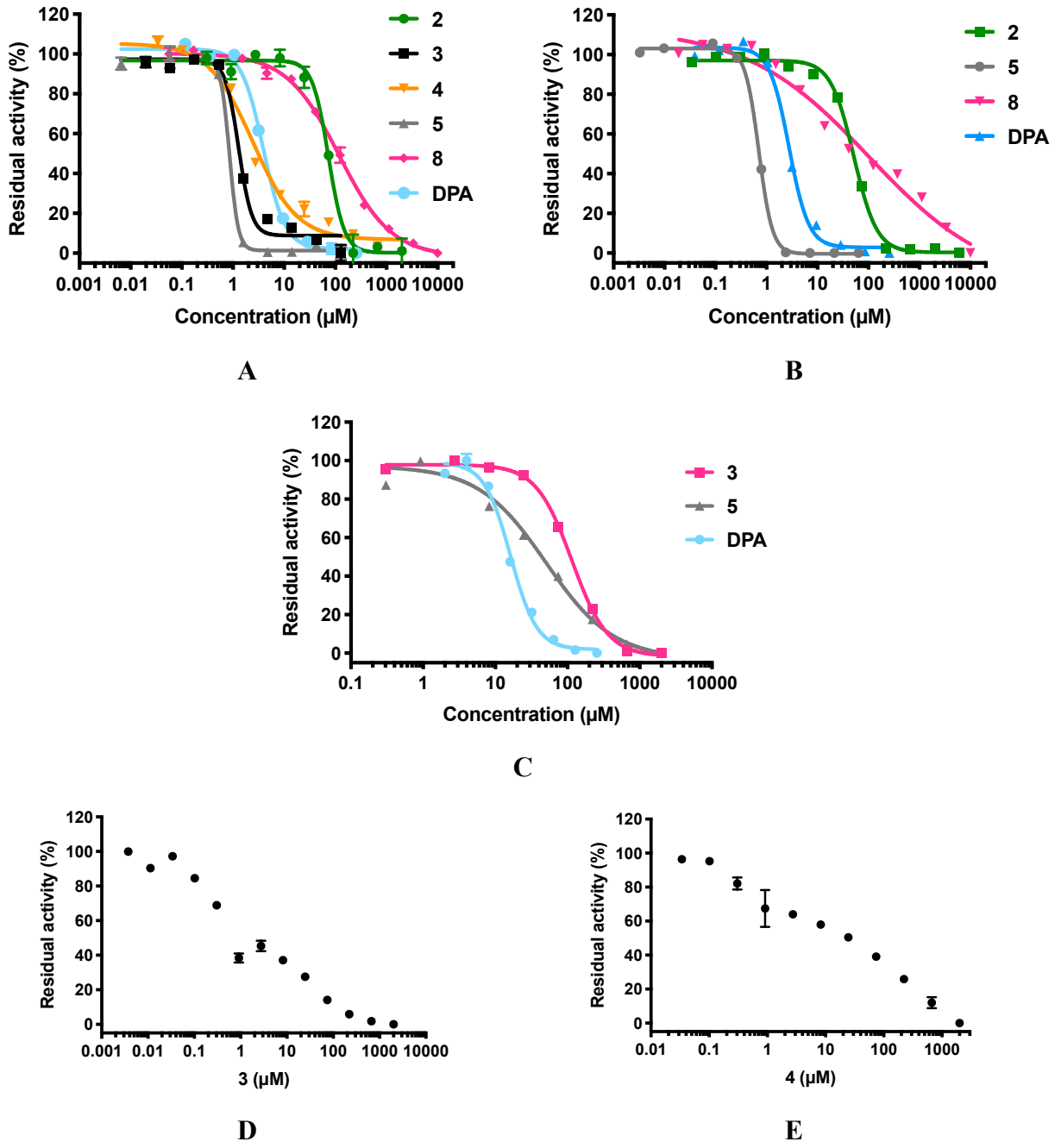


Figure S2. IC_{50} curves of the test compounds against NDM-1 (**A**), VIM-2 (**B**), and IMP-28 (**C**). The activity plot of compounds **3** (**D**) and **4** (**E**) against VIM-2 did not have a sigmoidal shape.

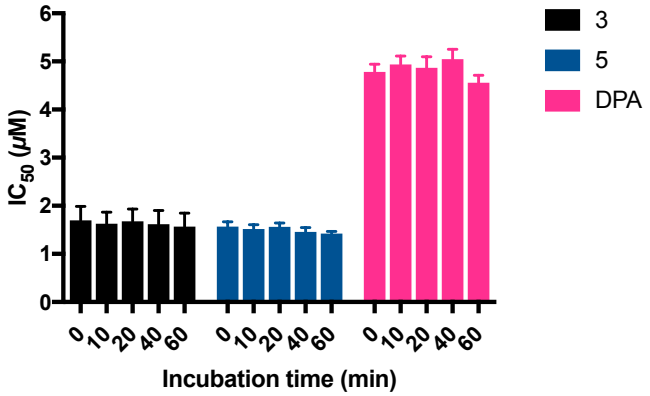
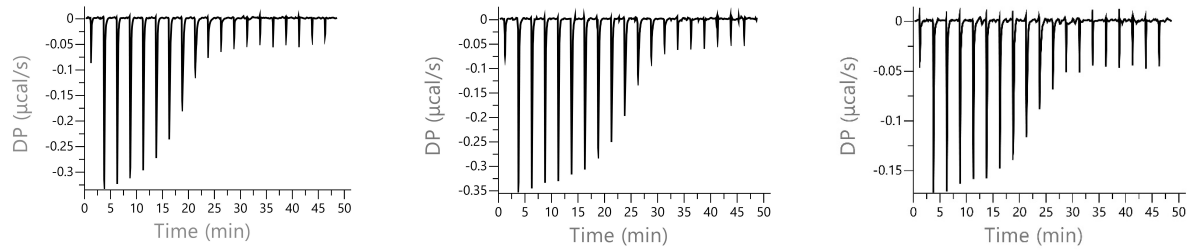


Figure S3. IC_{50} of **3**, **5**, and DPA determined after incubation with NMD-1 in multiple time-points.

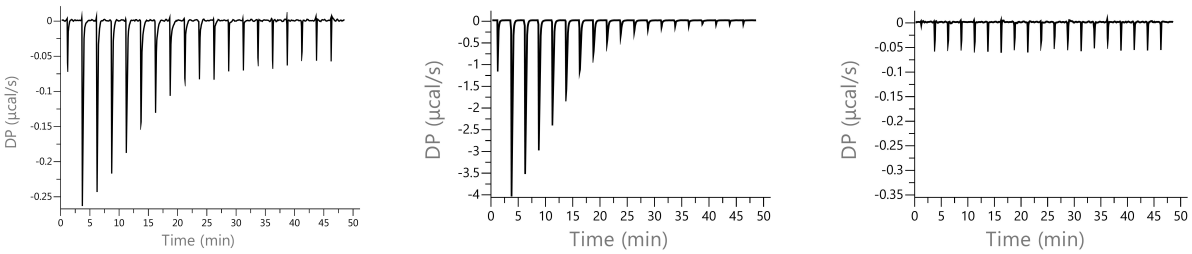
Table S1. The ITC data determined for the metal-binding affinity of the test compounds.

Compound		K_d (nM)	ΔH (kcal/mol)	-TΔS (kcal/mol)	ΔG (kcal/mol)
3[a]	Zn^{2+}	121 ± 8	-4.89 ± 0.22	-4.55 ± 0.25	-9.40 ± 0.04
4[a]	Zn^{2+}	231 ± 10	-2.96 ± 0.07	-6.10 ± 0.08	-9.06 ± 0.03
5[a]	Zn^{2+}	56 ± 15	-3.08 ± 0.11	-6.84 ± 0.28	-9.91 ±0.16
DPA[b]	Zn^{2+}	2373 ± 367	-2.46 ± 0.18	-5.21 ± 0.27	-7.68 ± 0.09
	Ca^{2+}	34233 ± 525	-5.503 ± 0.05	-0.589 ± 0.05	-6.09 ± 0.01

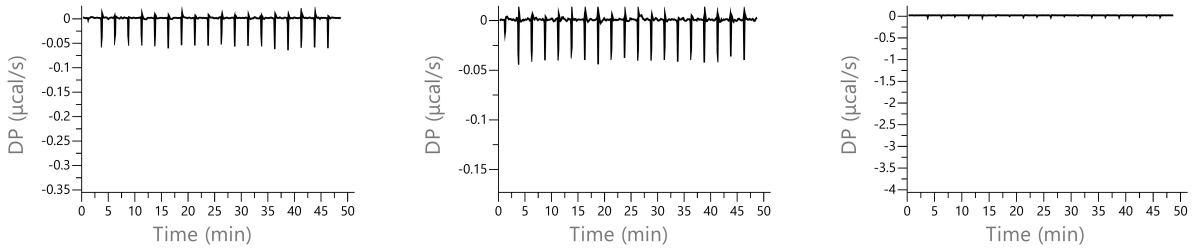
[a] No appreciable binding to Ca^{2+} and Mg^{2+} was observed.
[b] No appreciable binding to Mg^{2+} was observed.



Zn^{2+} (300 µM) : **3** (25 µM) | Zn^{2+} (500 µM) : **4** (45 µM) | Zn^{2+} (200 µM) : **5** (18 µM)

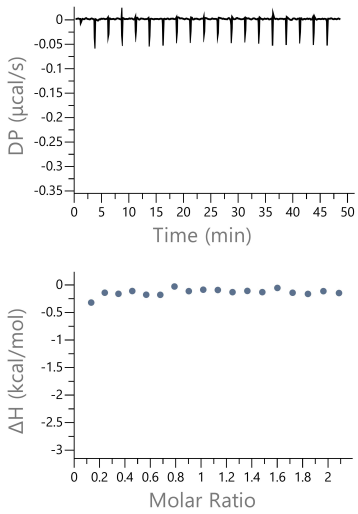


Zn^{2+} (500 µM) : DPA (50 µM) | Ca^{2+} (5 mM) : DPA (500 µM) | Buffer : **3** (25 µM)

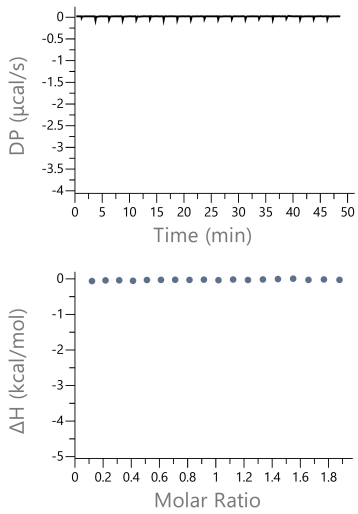


Buffer : **4** (45 µM) | Buffer : **5** (18 µM) | Buffer : DPA (500 µM)

Figure S4. ITC thermograms



Zn^{2+} (500 µM) : Buffer



Ca^{2+} (5 mM) : Buffer

Figure S4. *continued*

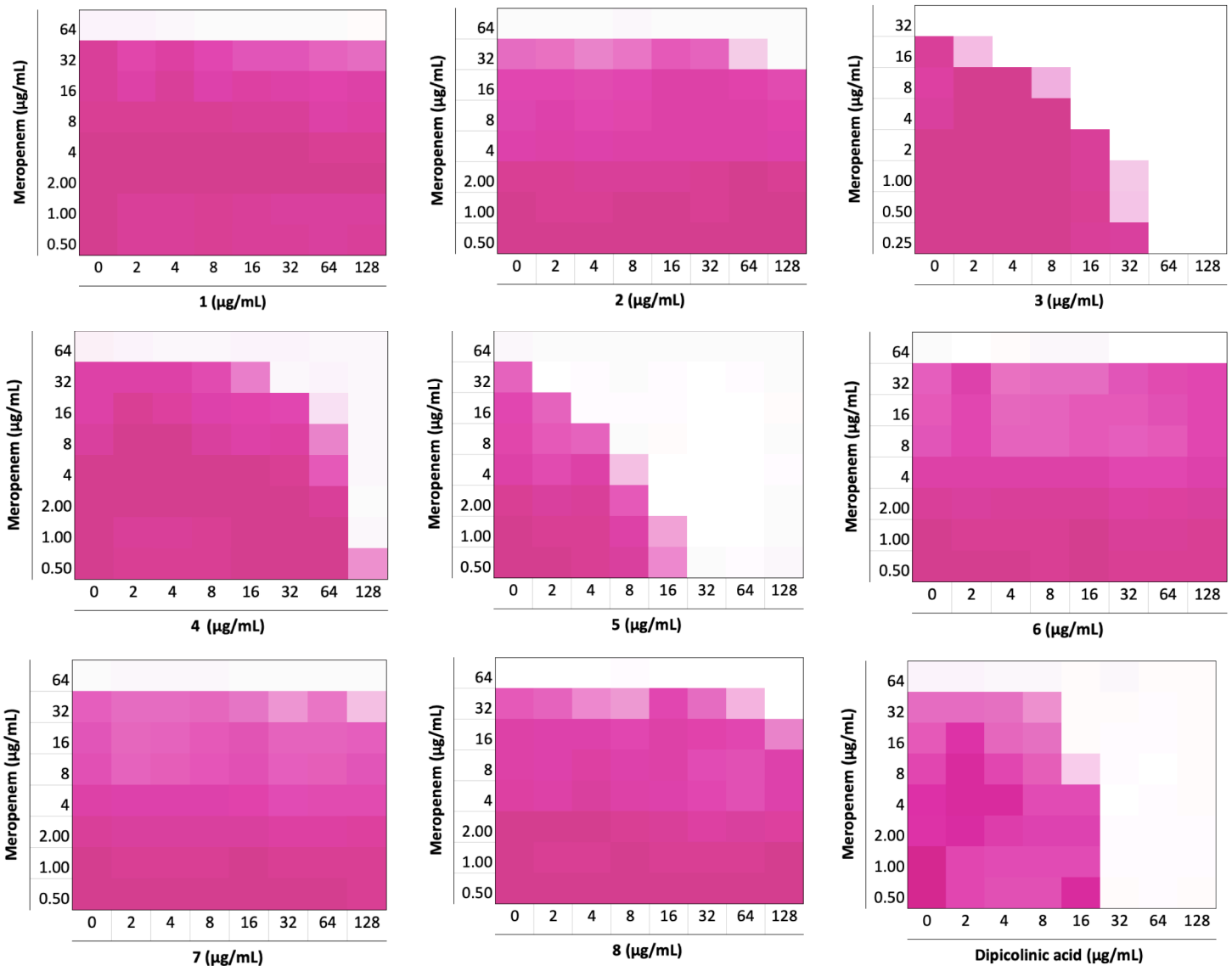


Figure S5. Checkerboard assays of the tested small molecules in combination with meropenem against an NDM-1 producing clinical isolate of *E. coli*. The optical density of the bacteria at 600 nm (OD_{600}) has been shown as color gradient between white (no bacterial growth) and magenta (maximum growth)

Table S2. MIC of meropenem alone or in combination with compound **3**, **5,** and DPA against a panel of carbapenem-resistant clinical isolates of Gram-negative bacteria.

Bacterial isolates	β-lactamase	MIC (μg/mL)			
		Mer	**Mer + 3**[a]	**Mer + 5**[a]	**Mer + DPA**[a]
E. coli [1]	NDM-1	8	0.5 (16)	0.125 (64)	0.25 (32)
E. coli [1]	NDM-1	16	≤0.125 (≥128)	≤0.125 (≥128)	0.25 (64)
E. coli [2]	NDM-1	16	0.5 (32)	0.25 (64)	0.5 (32)
E. coli [3]	NDM-1	128	4 (32)	0.5 (256)	1 (128)
K. pneumoniae [3]	NDM-1	32	1 (32)	0.125 (256)	0.5 (64)
K. pneumoniae [3]	NDM-1	64	4 (16)	≤0.5 (≥128)	1 (64)
K. pneumoniae [3]	NDM-1	16	1 (16)	0.25 (64)	0.25 (64)
P. aeruginosa [4]	NDM-1	128	16 (8)	8 (16)	8 (16)
P. stuartii [1]	NDM-1	32	0.25 (128)	0.25 (128)	0.25 (128)
A. baumannii [4]	NDM-2	32	4 (8)	2 (16)	2 (16)
E. coli [2]	NDM-4	64	2 (32)	≤0.5 (≥128)	1 (64)
E. coli [1]	NDM-5	32	4 (8)	0.5 (64)	2 (16)
E. coli [2]	NDM-5	128	16 (8)	8 (16)	8 (16)
E. coli [2]	NDM-6	128	32 (4)	8 (16)	8 (16)
E. coli [2]	NDM-7	32	≤0.5 (≥64)	≤0.5 (≥64)	≤0.5 (≥64)
E. coli [2]	NDM-15	128	64 (2)	32 (4)	64 (2)
E. aerogenes [3]	VIM-1	16	1 (16)	≤0.25 (≥64)	0.5 (32)
K. pneumoniae [1]	VIM-1	256	16 (16)	≤2 (≥128)	4 (64)
K. pneumoniae [3]	VIM-1	32	2 (16)	≤0.5 (≥64)	≤0.5 (≥64)
K. pneumoniae [3]	VIM-1	256	8 (32)	≤2 (≥128)	4 (64)
K. pneumoniae [3]	VIM-1	64	≤0.5 (≥128)	≤0.5 (≥128)	≤0.5 (≥128)
E. coli [1]	VIM-2	8	0.25 (32)	0.125 (64)	0.125 (64)
K. pneumoniae [4]	VIM-2	8	0.5 (16)	0.25 (32)	0.25 (32)
P. aeruginosa [1]	VIM-2	32	8 (4)	4 (8)	4 (8)
P. aeruginosa [1]	VIM-2	16	4 (4)	1 (16)	2 (8)
P. aeruginosa [1]	VIM-2	32	2 (16)	2 (16)	4 (8)
P. aeruginosa [3]	VIM-2, bla_{PAO}	16	2 (8)	1 (16)	2 (8)
P. aeruginosa [1]	VIM-2, OXA-50, bla_{PAO}	16	2 (8)	1 (16)	2 (8)
P. aeruginosa [2]	VIM-11	16	2 (8)	1 (16)	1 (16)
P. aeruginosa [2]	VIM-28	>256	256 (≥2)	64 (≥8)	128 (≥4)
P. aeruginosa [4]	IMP-1	>256	256 (≥2)	256 (≥2)	256 (≥2)
P. aeruginosa [2]	IMP-7	64	16 (4)	16 (4)	16 (4)
P. aeruginosa [2]	IMP-13	64	32 (2)	16 (4)	16 (4)
P. aeruginosa [3]	IMP-13, IMP-37, bla_{PAO}	64	32 (2)	16 (4)	16 (4)
K. pneumoniae [3]	IMP-28	4	0.5 (8)	0.5 (8)	2 (2)
K. pneumoniae [3]	KPC-2	256	256 (1)	256 (1)	256 (1)
K. pneumoniae [3]	OXA-48	32	32 (1)	32 (1)	32 (1)
E. coli [b]	-	≤0.0625	≤0.0625 (≥1)	≤0.0625 (≥1)	≤0.0625 (≥1)

[a] Each inhibitor was used at 32 μg/mL in combination with meropenem. None of the inhibitors showed toxicity up to 256 μg/mL against the tested strains. Fold reduction of MIC has been shown in brackets.

[b] ATCC 25922, this strain does not harbor any carbapenemase and was used as a negative control.

Strain Sources

1. Source: Vrije universiteit medical center VUmc, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.

2. Source: the Dutch national institute for public health and the environment, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands.

3. Source: Utrecht university medical center UMC, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands.

4. Source: National reference laboratory for multidrug-resistant gram-negative bacteria, department for medical microbiology, Ruhr-University Bochum, Universitaetsstr. 150, 44801 Bochum, Germany.