

Supplementary information

A Niclosamide-Tobramycin hybrid adjuvant potentiates cefiderocol against *P. aeruginosa*

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NMR spectra

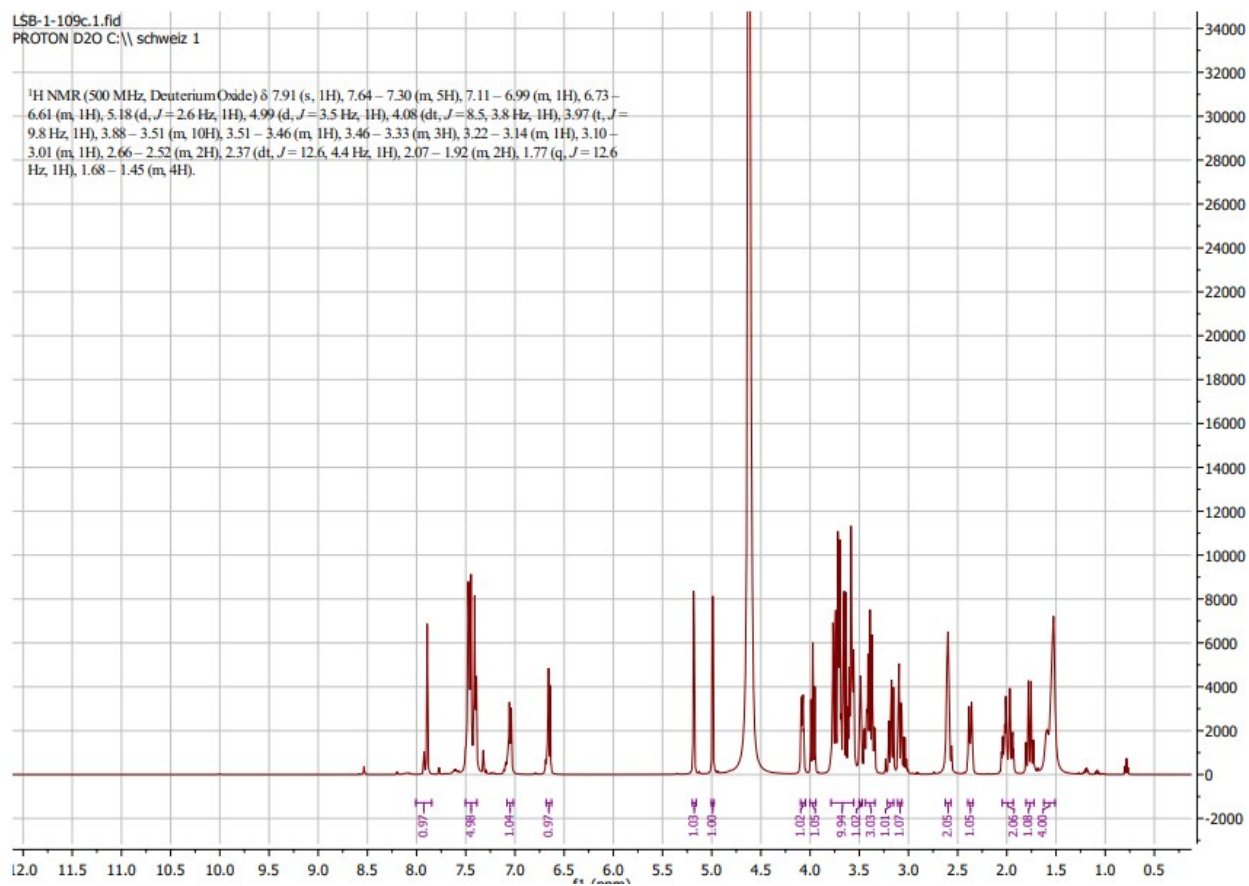


Figure S1. Hybrid 7 ^1H NMR spectrum

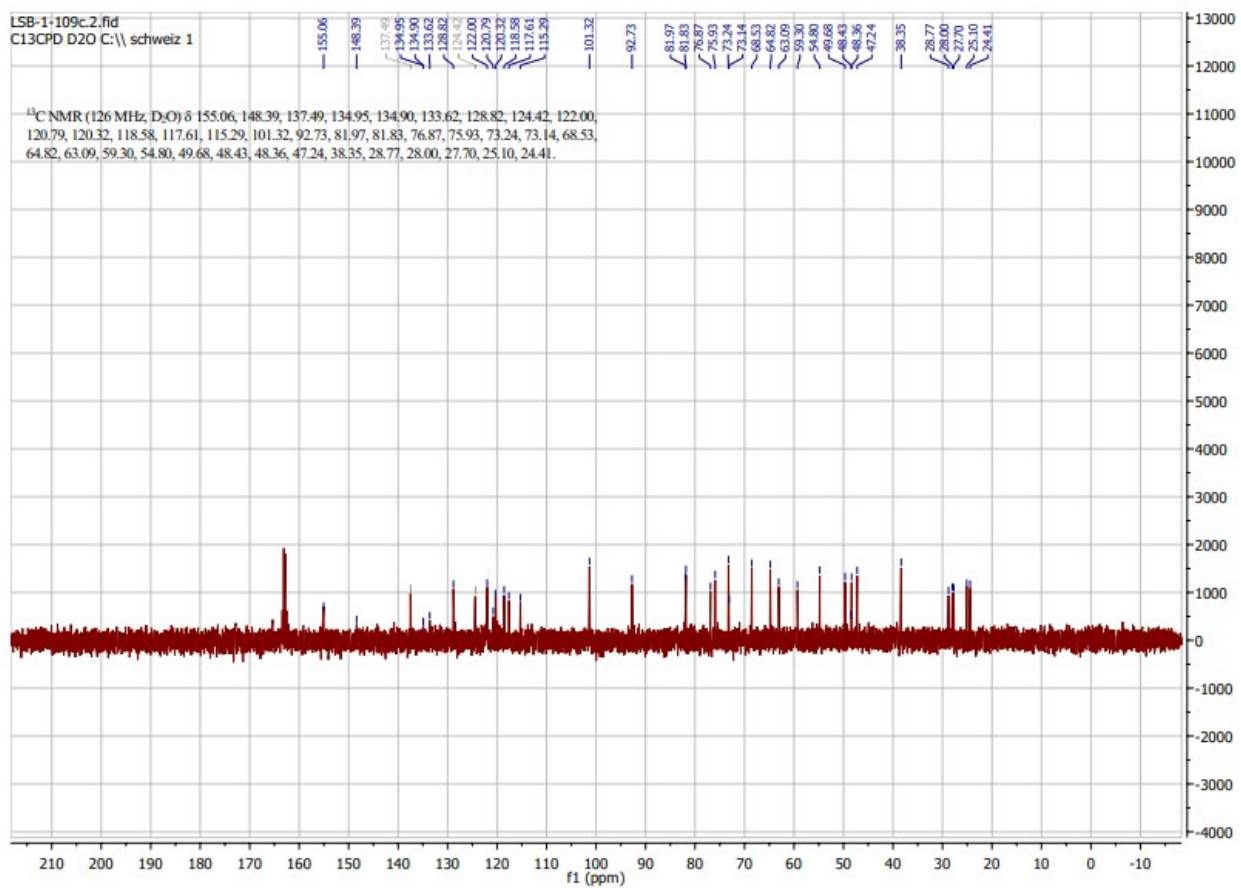


Figure S2. Hybrid 7 ¹³C NMR spectrum

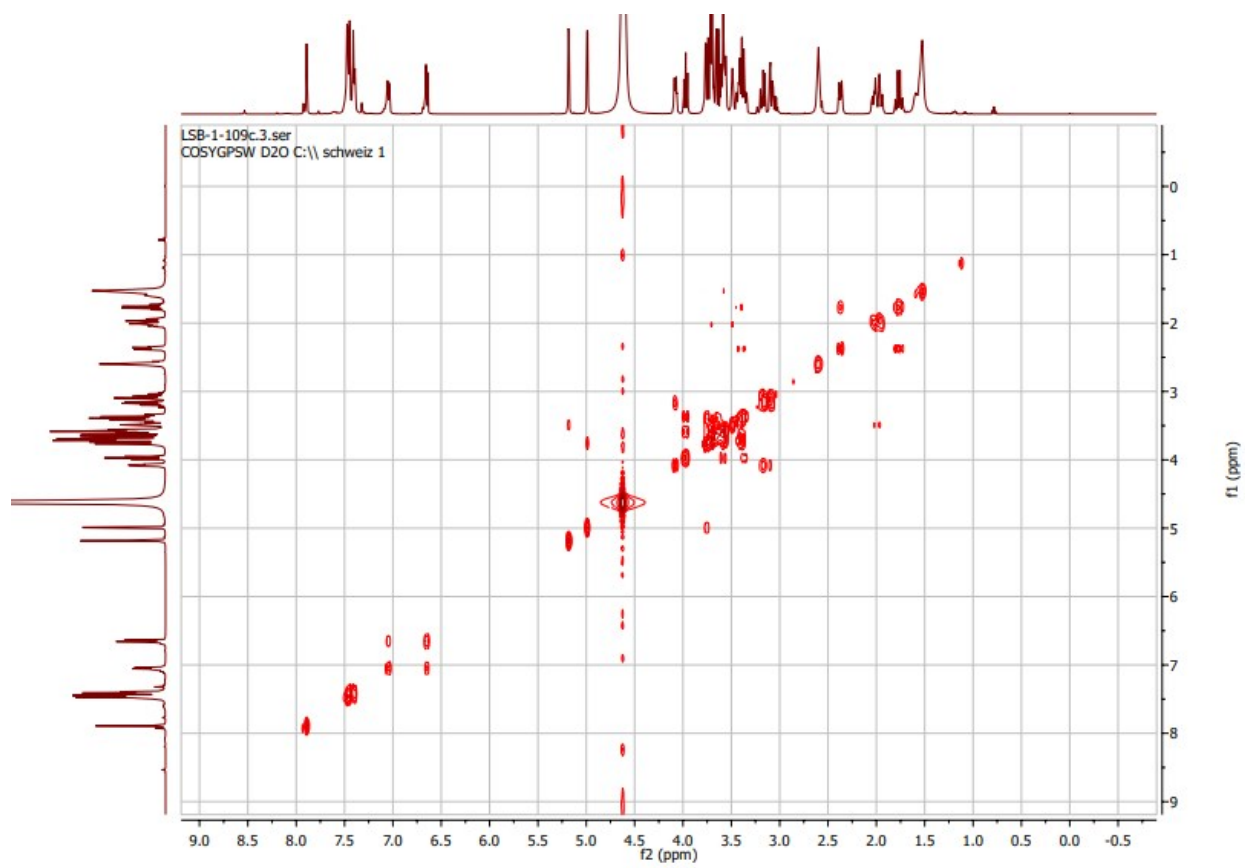


Figure S3. Hybrid 7 COSY NMR spectrum

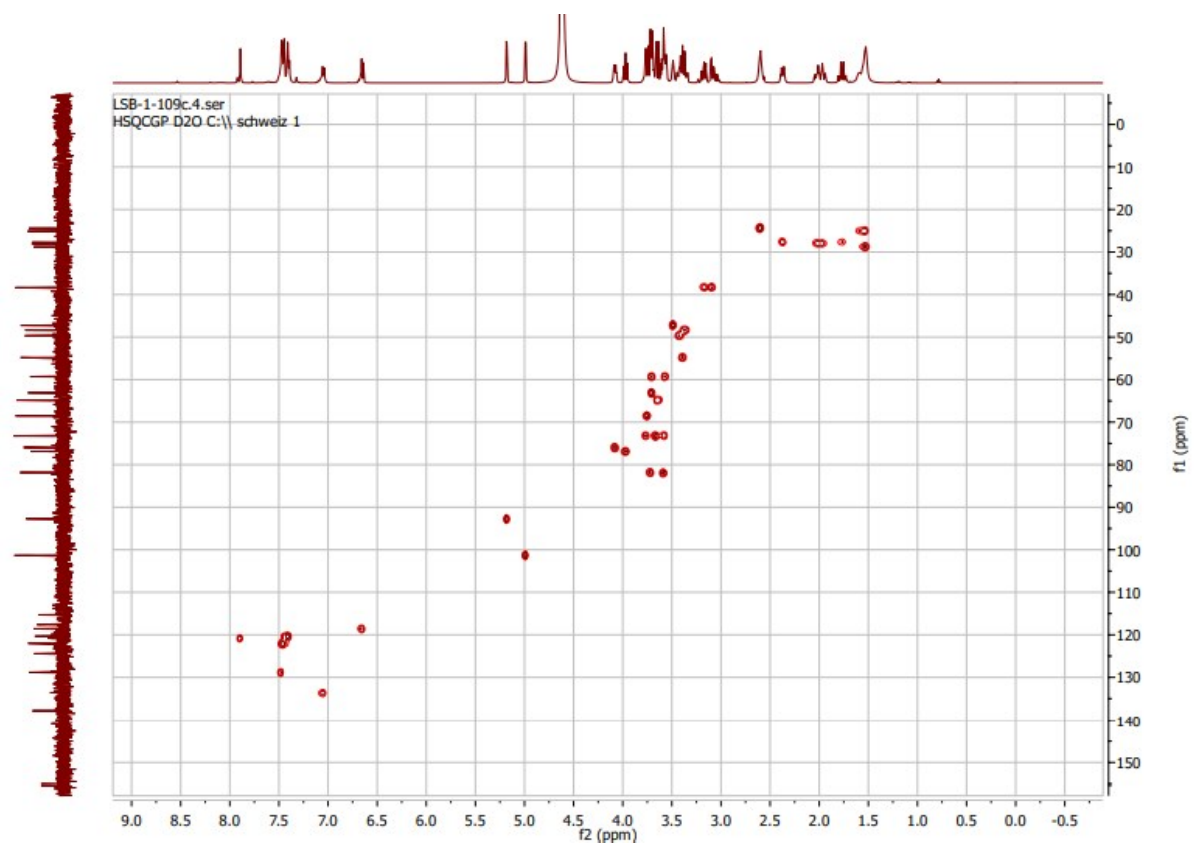


Figure S4. Hybrid 7 HSQC NMR spectrum

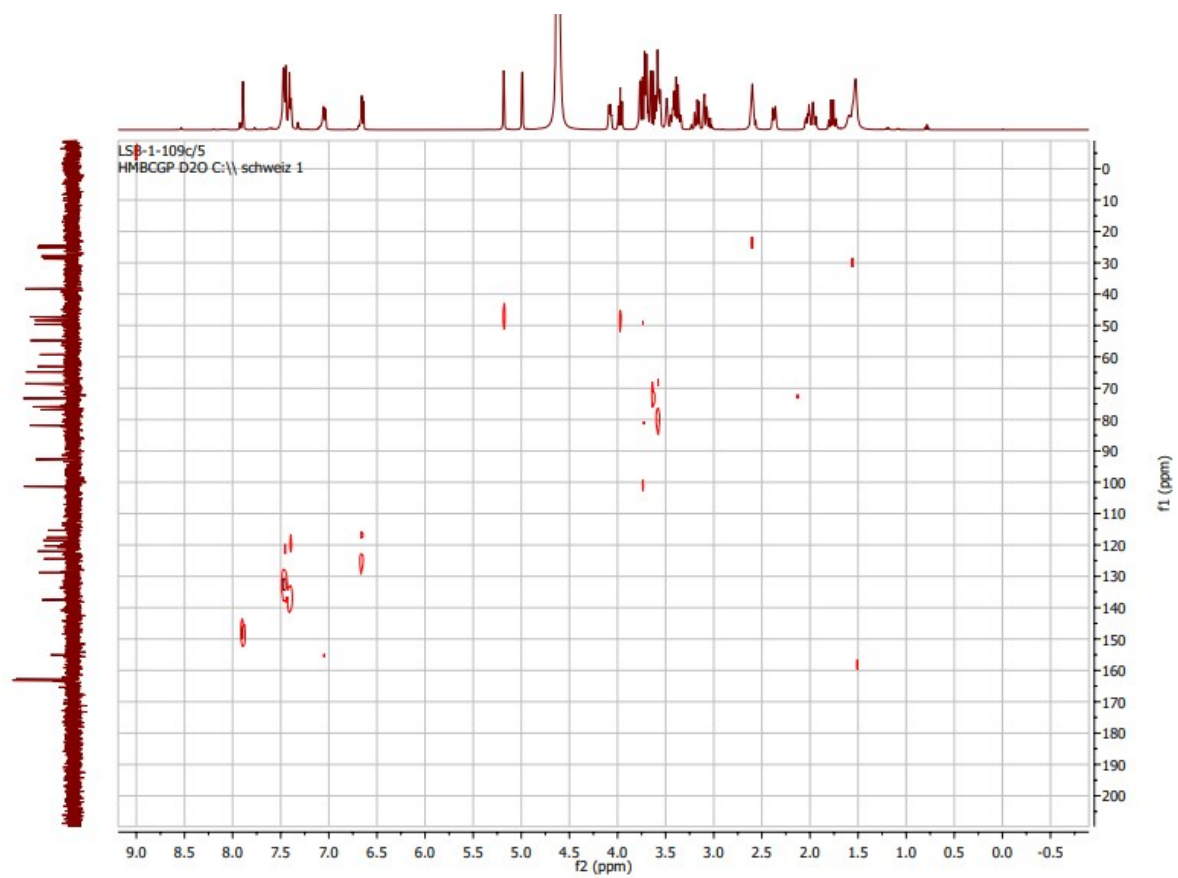


Figure S5. Hybrid 7 HMBC NMR spectrum

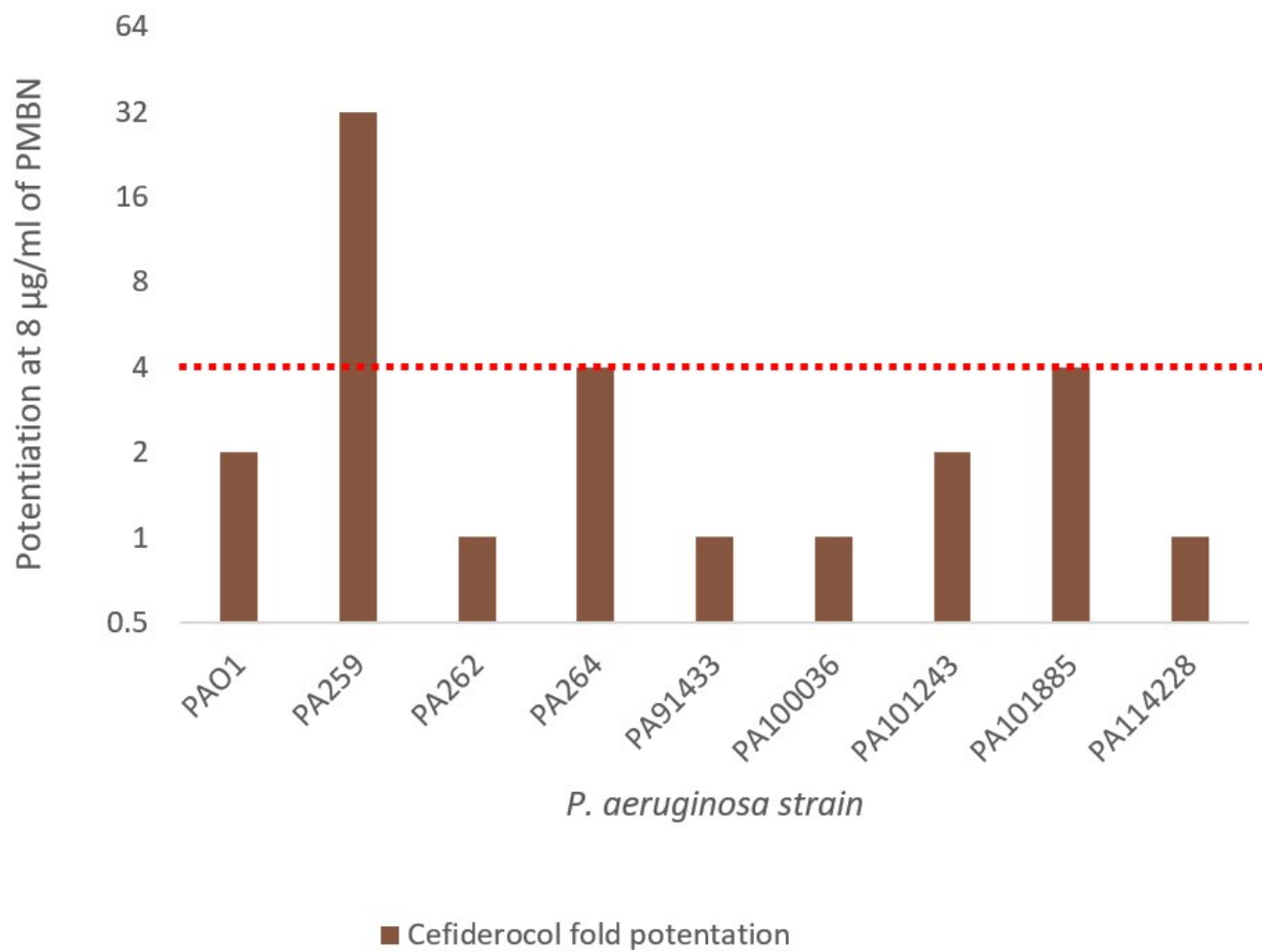


Figure S6. Fold potentiation of cefiderocol in combination with 8 µg/ml PMBN against wild-type and MDR clinical isolates of *P. aeruginosa* in MHB. Red dotted line denotes a 4-fold potentiation. MIC of PMBN was higher than 128 mg/mL for all strains.

Biological Data

Table S1. Activity of niclosamide and niclosamide-azide analog (**3**) against Gram-negative and Gram-positive bacteria

Strain	Minimum Inhibitory Concentration ($\mu\text{g/ml}$)	
	Niclosamide	3
PAO1	512	>256
<i>A. baumannii</i> ATCC 17978	512	>256
<i>E. coli</i> ATCC 25922	1024	>256
<i>S. aureus</i> ATCC 29213	1	2
MRSA ATCC 33592	≤ 0.25	1
MRSE 61589	≤ 0.25	0.5
<i>E. faecalis</i> ATCC 29212	2	64
<i>E. faecium</i> ATCC 27270	2	16

Table S2. Checkerboard studies of colistin and **3** against wild-type *P. aeruginosa* PAO1 and colistin-resistant clinical isolates

Strain	MIC _{Colistin}	MIC _{Combi}	MIC ₃	MIC _{Combi}	FIC index	Interpretation
PAO1	1	0.125	>256	1	0.063<x<0.067	Synergy
PA91433	16	0.25	>256	1	0.016<x<0.019	Synergy
<i>K. pneumoniae</i> 113250	256	0.25	>256	2	0.002<x<0.010	Synergy
<i>K. pneumoniae</i> 113254	256	0.5	>256	1	0.002<x<0.006	Synergy
<i>E. coli</i> 94393	4	0.015625	>256	4	0.004<x<0.020	Synergy
<i>E. coli</i> 94474	16	0.25	>256	2	0.016<x<0.018	Synergy

Table S3. Potentiation of antibiotics with adjuvant 7 against *P. aeruginosa* PAO1 in MHB

Drug	MIC _{Drug}	MIC at 8 µg/ml of 7	Potentiation at 8 µg/ml of 7
Moxifloxacin	1	0.25	4
Ciprofloxacin	0.25	0.125	2
Vancomycin	256	64	4
Tobramycin	2	2	1
Colistin	1	1	1
Niclosamide	512	0.5	1024
Piperacillin	8	4	2
Cefotaxime	16	8	2
Ceftazidime	4	1	4
Cefiderocol	0.125	0.0313	4
Ceftolozane	0.5	0.125	4
Aztreonam	4	0.5	8
Minocycline	16	2	8
Doxycycline	8	1	8
Rifampicin	16	1	16
Chloramphenicol	32	2	16
Erythromycin	256	16	16
Novobiocin	1024	16	64

Fosfomycin	64	32	2
Linezolid	1024	64	16
Clindamycin	1024	256	64
Pleuromutilin	512	128	4
Trimethoprim	128	16	4
Sulfamethoxazole	256	64	4
Cotrimoxazole	2:38	0.25:4.75	4

Table S4. Potentiation of antibiotics with adjuvant **7** against *A. baumannii* ATCC 17978 in MHB

Drug	MIC _{Drug}	MIC at 8 µg/ml of 7	Potentiation at 8 µg/ml of 7
Moxifloxacin	0.0313	0.033	1
Ciprofloxacin	1	1	1
Vancomycin	128	128	1
Tobramycin	1	1	1
Colistin	0.5	0.5	1
Niclosamide	512	2	256
Piperacillin	64	64	1
Cefotaxime	16	16	1
Ceftazidime	4	1	4
Cefiderocol	1	0.5	2

Ceftolozane	2	2	1
Aztreonam	64	32	2
Minocycline	0.25	0.25	1
Doxycycline	0.125	0.125	1
Rifampicin	2	1	2
Chloramphenicol	128	64	2
Erythromycin	16	8	2
Novobiocin	8	2	4
Fosfomicin	128	128	1
Linezolid	256	128	2
Clindamycin	512	256	2
Pleuromutilin	1024	128	8
Trimethoprim	64	64	1
Sulfamethoxazole	>608	>608	1
Cotrimoxazole	32:608	32:608	1

Table S5. Potentiation of antibiotics with adjuvant **7** against *E. coli* ATCC 25922 in MHB

Drug	MIC _{Drug}	MIC at 8 µg/ml of 7	Potentiation at 8 µg/ml of 7
Moxifloxacin	0.016	0.008	2
Ciprofloxacin	0.016	0.004	4
Vancomycin	128	16	8
Tobramycin	2	1	2
Colistin	0.25	0.125	2
Niclosamide	512	1	512
Piperacillin	2	0.25	8
Cefotaxime	0.25	0.0313	8
Ceftazidime	0.25	0.063	4
Cefiderocol	0.125	0.063	2
Ceftolozane	1	0.5	2
Aztreonam	0.25	0.063	4
Minocycline	1	0.25	4
Doxycycline	0.5	0.25	2
Rifampicin	4	0.031	128
Chloramphenicol	4	1	4
Erythromycin	32	1	32
Novobiocin	32	0.5	64

Fosfomicin	16	16	1
Linezolid	128	32	4
Clindamycin	64	4	16
Pleuromutilin	64	8	8
Trimethoprim	256	1	256
Sulfamethoxazole	304	76	4
Cotrimoxazole	8:152	0.5:9.5	16

Table S6. Neither niclosamide nor tobramycin alone potentiate cefiderocol against wild-type *P. aeruginosa* PAO1 in MHB

Drug ₁	MIC _{Drug1}	MIC _{Combi}	Drug ₂	MIC _{Drug2}	MIC _{Combi}	FIC index
Cefiderocol	0.125	0.125	Tobramycin	1	0.5	1.5
Cefiderocol	0.125	0.125	Niclosamide	512	0.25	1.031<x<1.063

Table S7. Checkerboard studies of cefotaxime and **7** against wild-type and multidrug-resistant *P. aeruginosa* strains in MHB

Strain	MIC _{Drug}	MIC _{Combi}	MIC _{Adjuvant}	MIC _{Combi}	FIC index
PAO1	16	4	64	16	0.5
PA259	2048	1024	>128	1	$0.5 < x < 0.508$
PA260	1024	128	>128	8	$0.125 < x < 0.188$
PA264	2048	1024	>128	16	$0.5 < x < 0.625$

Table S8. Checkerboard studies of ceftazidime and **7** against wild-type and multidrug-resistant *P. aeruginosa* strains in MHB

Strain	MIC _{Drug}	MIC _{Combi}	MIC _{Adjuvant}	MIC _{Combi}	FIC index
PAO1	4	0.5	64	16	0.375
PA259	512	256	>128	0.25	$0.5 < x < 0.502$
PA260	64	16	>128	8	$0.25 < x < 0.313$
PA264	128	32	>128	2	$0.25 < x < 0.266$

Table S9. Checkerboard studies of ceftolozane and **7** against wild-type and multidrug-resistant *P. aeruginosa* strains in MHB. ND = Not determined

Strain	MIC _{Drug}	MIC _{Combi}	MIC _{Adjuvant}	MIC _{Combi}	FIC index
PAO1	0.5	0.125	64	1	0.266
PA259	>16	ND	>128	ND	ND
PA260	1	0.5	>128	16	0.5<x<0.625
PA264	2	2	>128	0.25	1<x<1.002

Table S10. Checkerboard studies of cefiderocol and 7 against wild-type and multidrug-resistant *P. aeruginosa* strains in ID-CAMHB

Strain	MIC _{Drug}	MIC _{Combi}	MIC _{Adjuvant}	MIC _{Combi}	FIC index	Interpretation	Potential at 8 µg/ml of adjuvant
PAO1	0.0313	0.008	64	2	0.281	Synergy	4
PA259	4	4	>128	0.5	1<x<1.002	Additive	1
PA260	0.063	0.063	>128	0.25	1<x<1.002	Additive	1
PA262	0.0313	0.0156	>128	0.25	0.5<x<0.502	Additive	2
PA264	0.0156	0.0156	>128	0.25	1<x<1.002	Additive	1

Table S11. MICs (in µg/mL) of various antibiotics against MDR clinical isolates.*

a) *Pseudomonas aeruginosa* isolates

<i>P. aeruginosa</i>	PTZ	A/C	AZT	FOX	CFZ	CTR	CPM	CTX	CAZ	IMI	MER	DOR	ETP	CIP	LEV	MOX	TOB	GEN	AMK	TGC	MIN	DOX	ERC	OMC	CST	CAM
259-96918	64	>32	32	>32	>128	>64	>64	2048	512	32	1024	>1024	>32	>16	256	>16	256	>32	>64	32	32	32	8	64	1	1024
262-101856	64	>32	32	>32	>128	64	32	128	16	32	32	16	>32	>16	64	>16	1024	>32	>64	32	64	1024	8	64	1	2048
264-104354	256	>32	64	>32	>128	>64	32	2048	128	32	64	16	>32	>16	64	>16	128	>32	8	32	32	64	8	64	1	4096
91433	64	>32	512	>32	>128	>64	16	1024	1024	32	16	16	>32	2	ND	16	16	32	>32	32	16	32	16	128	4	8
114228	ND	ND	>32	ND	ND	ND	ND	128	8	ND	8	8	ND	ND	ND	ND	2	ND	ND	ND	32	16	8	16	4	ND

b) *Acinetobacter baumannii* isolates

<i>A. baumannii</i>	PTZ	FOX	CFZ	CPM	CTX	CAZ	C/T	IMI	MER	CIP	LEV	MOX	TOB	GEN	AMK	TGC	MIN	DOX	ERC	OMC	CST	CAM
AB027	512	ND	>128	>128	>256	ND	>16	32	16	>16	8	8	ND	32	>64	4	0.25	ND	0.5	1	0.25	128
AB031	4	ND	>128	4	16	ND	>16	0.25	1	0.25	0.25	0.12	ND	<0.5	2	8	0.25	ND	0.25	2	0.25	128
LAC-4	ND	ND	ND	ND	8	>16	8	<1	<1	>4	2	ND	>4	>4	4	<4	4	<4	0.06	1	0.125	32
92247	<1	32	128	4	ND	ND	2	ND	4	≤0.06	ND	ND	ND	ND	<1	0.25	0.125	ND	ND	ND	4	ND

c) *Escherichia coli* isolates

<i>E. coli</i>	PTZ	A/C	AZT	FOX	CFZ	CPM	CAZ	C/T	IMI	MER	ETP	CIP	LEV	MOX	TOB	GEN	AMK	TGC	MIN	DOX	ERC	OMC	CST	CAM
94393 (mcr-1 +)	≤1	4	≤0.12	4	1	≤0.25	≤0.25	0.25	0.25	≤0.03	≤0.03	0.5	1	1	≤0.5	≤0.5	2	0.25	2	4	0.5	4	4	4
94474 (mcr-1 +)	16	>32	≤0.12	16	4	≤0.25	0.5	0.5	0.25	≤0.03	≤0.03	>16	32	16	32	16	2	1	64	>32	1	16	16	4
107115	>512	>32	>64	>32	>128	>64	>32	>6	8	32	>32	>16	32	16	8	>32	2	0.25	32	>32	0.125	4	0.5	512

d) *Klebsiella pneumoniae* isolates

<i>K. pneumoniae</i>	PTZ	A/C	AZT	FOX	CFZ	CPM	CAZ	C/T	IMI	MER	ETP	CIP	LEV	MOX	TOB	GEN	AMK	TGC	MIN	DOX	ERC	OMC	CST	CAM
113250	4	4	≤0.12	1	1	1	0.5	2	0.25	≤0.03	≤0.03	≤0.06	0.125	≤0.06	≤0.5	≤0.5	≤1	ND	2	2	1	2	>16	4
113254	<1	2	≤0.12	1	1	1	≤0.2	0.5	0.12	≤0.03	≤0.03	≤0.06	0.0625	≤0.06	≤0.5	≤0.5	≤1	ND	2	2	0.5	4	>16	2
116381	8	16	16	16	>128	16	8	1	0.5	<0.03	0.12	>16	128	>16	4	≤0.5	≤1	1	64	>32	0.5	4	0.5	>512

e) *E. cloacae* isolates

<i>E. cloacae</i>	PTZ	A/C	AZT	FOX	CFZ	CPM	CAZ	C/T	IMI	MER	ETP	CIP	MOX	TOB	GEN	AMK	TGC	MIN	DOX	ERC	OMC	CST	CAM
117029	2	16	≤0.12	>32	>128	≤0.25	0.5	0.25	0.25	≤0.03	≤0.03	≤0.06	≤0.06	2	≤0.5	2	0.5	32	>32	0.5	4	0.25	8
118564	2	>32	≤0.12	>32	>128	0.25	0.5	ND	ND	0.12	ND	0.06	0.12	1	1	2	ND	ND	4	ND	ND	>16	ND
121187	1	8	≤0.12	>32	32	0.25	0.5	ND	ND	0.06	ND	0.25	1	32	>32	1	ND	ND	>32	ND	ND	>16	ND

* Complete susceptibility data was not available for all isolates used. PTZ: piperacillin-tazobactam, A/C: amoxicillin-clavulanic acid, AZT: aztreonam, FOX: ceftiofloxacin, CFZ: cefazolin, CTR: ceftriaxone, CPM: ceftazidime, CTX: cefotaxime, CAZ: ceftazidime, C/T: ceftolozane-tazobactam, IMI: imipenem, MER: meropenem, DOR: doripenem, ETP: ertapenem, CIP: ciprofloxacin, LEV: levofloxacin, MOX: moxifloxacin, TOB: tobramycin, GEN: gentamicin, AMK: amikacin, TGC: tigecycline, MIN: minocycline, DOX: doxycycline, ERC: eravacycline, OMC: omadacycline, CST: colistin, CAM: chloramphenicol, ND: not determined.