

Supporting Information

Screening an established natural product library identifies secondary metabolites that potentiate conventional antibiotics

Anne E. Mattingly¹, Karlie E. Cox¹, Richard Smith², Roberta J. Melander¹, Robert K. Ernst², Christian Melander^{1*}

¹Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

²Department of Microbial Pathogenesis, University of Maryland-Baltimore, Baltimore, MD 21201 USA

*to whom correspondence should be addressed: cmelande@nd.edu

Contents

Table S1: Re-sensitization of MRSA 1556 to Oxacillin with NCI Natural Product Library Set IV	2
Table S2: Antibiotic activities of compounds in NCI Natural Products Set IV Plate 13160330. All compounds inhibited growth at a concentration of 20 μ M in the strains specified.	3
Table S3: Antibiotic activities of compounds in NCI Natural Products Set IV Plate 13160331.....	4
Table S4: Full list of natural products active with colistin in sensitive and resistant strains of <i>A. baumannii</i> and <i>K. pneumoniae</i>	6
Table S5: Colistin potentiation activity of prodigiosin, obatoclax, rifampicin, and novobiocin in additional strains of colistin-resistant <i>A. baumannii</i> and <i>K. pneumoniae</i>	9
Table S6: Fold-change differences in the lowest effective concentration of novobiocin or prodigiosin concentration required to potentiate colistin in <i>A. baumannii</i> 4106 and <i>K. pneumoniae</i> B9 upon the addition of fetal bovine serum	10
Table S7: Minimum inhibitory concentrations of novobiocin and prodigiosin in the presence of the membrane-permeabilizing polymyxin B nonpeptide (PMBN) at 2, 4, and 8 μ g/mL	11
Figure S1: Checkerboard assays for colistin with prodigiosin, obatoclax, rifampicin, or novobiocin in <i>A. baumannii</i> 4106.	12
Figure S2: Checkerboard assays for colistin with prodigiosin, obatoclax, rifampicin, or novobiocin in <i>K. pneumoniae</i> B9	13
Table S8: Fractional inhibitory concentration indices (FICI) for antibiotic/adjuvant combination in <i>A. baumannii</i> 4106 and <i>K. pneumoniae</i> B9.....	14
Figure S3: Growth curves of <i>Klebsiella pneumoniae</i> B9.	15
Figure S4: m/z shifts in lipid A composition of an untreated <i>K. pneumoniae</i> B9 control (A) compared to cells treated with 10 μ M novobiocin (B) or 5 μ M prodigiosin (C).	16
Figure S5: DCFH-DA fluorescence assay of <i>K. pneumoniae</i> B9 cells treated with hydrogen peroxide and colistin.	17

Table S1: Re-sensitization of MRSA 1556 to Oxacillin with NCI Natural Product Library Set IV

	Compound Name	Oxacillin MIC ($\mu\text{g}/\text{mL}$)
Control	-	32
Plate 13160330		
D8	Fastigilin B	8 (4)
E12	Michellamine B diacetic acid salt	2* (16)
F13	Cryptopimaric acid	2* (16)
G9	(7AR-CIS)-7A,12A-DIHYDRO-13-METHOXY-3,3-DIMETHYL-11-(3-METHYL-2-BUTENYL)-3H,7H-BENZOFURO[3,2-C]PYRANO[3,2-G][1]BENZOPYRAN-10-OL	2* (16)
G16	Cinerubin A	8 (4)
H8	Staphylomycin S	8 (4)
I3	Pomiferin	8 (4)
L7	Vumon	8 (4)
N10	Chaetochromin	8 (4)
Plate 13160331		
C3	Shodomycin	8 (4)
C4	Doxyppyromycin hydrochloride	8 (4)
D12	Albacarcin V	4 (8)
G9	Prodiginine HCl	8 (4)
H4	Lomofungin	8 (4)
I11	Sirodesmin A	2* (16)
L16	Variabiline dyhydrochloride	2* (16)

Natural products were tested at 20 μM . Fold-change as compared to the control oxacillin MIC is expressed in parenthesis. * indicates the lowest oxacillin concentration tested.

Table S2: Antibiotic activities of compounds in NCI Natural Products Set IV Plate 13160330. All compounds inhibited growth at a concentration of 20 μ M in the strains specified.

WELL ID	NSC	COMPOUND NAME	PUBCHEM SID	BACTERIAL STRAIN OF OBSERVED ACTIVITY
C08	133100	Rifamycin sodium salt	558026	MRSA 1556
D06	71795	Ellipticine	26732720	MRSA 1556
E04	13252	Aureomycin	540479	<i>A. baumannii</i> 5075 <i>A. baumannii</i> 4106 <i>K. pneumoniae</i> B9
E05	31867	Decarboxy-norlobaric acid	90601	MRSA 1556
E08	145118	Lankacidin C	559683	MRSA 1556
F08	177406	Antibiotic X-536A	446199	MRSA 1556
F16	19990	Streptovaricin C	82709, 26664290	MRSA 1556
F20	56410	Porfiromycin	105809, 26732566	MRSA 1556 <i>K. pneumoniae</i> B9
G03	3053	Dactinomycin D	538571	MRSA 1556
G20	47147	Prodigiosin	26732718	MRSA 1556 <i>A. baumannii</i> 4106
H09	285116	Siomycin A	571338, 92764142	MRSA 1556
I10	325014	Bactobolin	457886, 92763388	MRSA 1556
I21	69187	9-Methoxyellipticine	113202, 26666180	MRSA 1556
J05	45383	Streptonigrin	99123, 26732715	MRSA 1556 <i>A. baumannii</i> 5075 <i>K. pneumoniae</i> B9
J06	82151	Daunorubicin hydrochloride	301154	MRSA 1556
K21	70931	Celastrol	114102, 92763934	MRSA 1556
L09	292567	Nigericin sodium salt	572042	MRSA 1556
L20	59729	Sparsomycin	107825	MRSA 1556
M04	22842	Cumostrol	84879, 26732716	MRSA 1556
N04	30552	Mangostin	89727	MRSA 1556

Table S3: Antibiotic activities of compounds in NCI Natural Products Set IV Plate 13160331. All compounds inhibited growth at 20 μ M in the strains specified

WELL ID	NSC	NAME	PUBCHEM SID	BACTERIAL STRAIN OF OBSERVED ACTIVITY
C09	237671	Actinomycin X4357g - methoxime	567819	MRSA 1556
C14	526417	Levomycin	580231, 92763358	MRSA 1556 <i>A. baumannii</i> 5075 <i>A. baumannii</i> 4106 <i>K. pneumoniae</i> B9
C15	785154	Dehydronuciferine	--	MRSA 1556
D07	156219	Streptovaricin G	434167, 26732585	MRSA 1556
E14	785143	Subsessiline	--	MRSA 1556
F06	136044	Rhodomyacin A	558415	MRSA 1556
F08	208734	Aclarubicin	125750	MRSA 1556
F14	785149	O-Methylmoschatoline	--	MRSA 1556
G07	145612	Rifamycin, 3- [[dimethylamino)methyl]- (9Cl)	559725	MRSA 1556
G10	269146	Variamycin	301230, 92763436	MRSA 1556
H06	138320	Dianemycin	558614, 92764026	MRSA 1556
H10	276382	Pepleomycin Sulfate	301234	MRSA 1556
I04	102816	5-Azacytidine	405114	<i>K. pneumoniae</i> 2146
I08	179834	Withanolide E	446985	MRSA 1556 <i>A. baumannii</i> 5075 <i>A. baumannii</i> 4106
I09	248605	Julimycin B2	568775, 92764128	MRSA 1556
J04	107041	Scopafungin	--	MRSA 1556
K12	343256	Stubomycin	461879, 121283285	MRSA 1556
L08	227186	Chlorobiocin	131861, 26664947	MRSA 1556 <i>A. baumannii</i> 5075 <i>A. baumannii</i> 4106
L13	256942	4'-Epiadriamycin	569372, 92764132	MRSA 1556

WELL ID	NSC	NAME	PUBCHEM SID	BACTERIAL STRAIN OF OBSERVED ACTIVITY
M08	184398	Aquayamycin	447441	MRSA 1556
M16	785171	7- Benzoyldehydronuciferine	--	MRSA 1556
N07	170365	Thiactin	563007	MRSA 1556
N15	785165	Variabiline monohydrochloride	--	MRSA 1556

Table S4: Full list of natural products active with colistin in sensitive and resistant strains of *A. baumannii* and *K. pneumoniae*. Natural products were tested at 20 μ M. Fold-change as compared to the control antibiotic MIC is expressed in parenthesis. * Indicates the lowest antibiotic concentration tested.

	Compound Name	Colistin MIC (μ g/mL)			
		<i>A. baumannii</i> 5075	<i>A. baumannii</i> 4106	<i>K. pneumoniae</i> 2146	<i>K. pneumoniae</i> B9
Control	-	1	1024	1	512
Plate 13160330					
C8	rifamycin sodium salt	0.125 (8)	-	-	4 (128)
C9	Rapamycin	0.25 (4)	-	-	-
D6	Ellipticine	0.125 (8)	-	-	4 (128)
E5	Decarboxy-norlobaric acid	-	-	-	32 (16)
E21	Erythromycin cyclopentylpropionate	-	-	0.25 (4)	-
F7	valinomycin	0.125 (8)	-	0.25 (4)	-
F8	antibiotic X-536A	-	-	-	32 (16)
F13	Cryptopimaric acid	-	-	-	64 (8)
F16	Streptovaricin C	-	-	-	1* (512)
F20	Porfiromycin	-	256 (4)	-	-
G3	Dactinomycin D	0.25 (4)	-	-	-
G9	(7 α -cis)-7 α ,12 α -dihydro- 13-methoxy-3,3-dimethyl- 11-(3-methyl-2-butenyl)- 3h,7h-benzofuro[3,2- c]pyrano[3,2- g][1]benzopyran-10-ol	0.25 (4)	-	-	-
G10	Lonchocarpic acid	-	-	-	32 (16)
G20	Prodigiosin	0.125 (8)	-	0.25 (4)	2 (256)
H9	Siomycin A	0.25 (4)	-	-	-

	Compound Name	Colistin MIC ($\mu\text{g}/\text{mL}$)			
		<i>A. baumannii</i> 5075	<i>A. baumannii</i> 4106	<i>K. pneumoniae</i> 2146	<i>K. pneumoniae</i> B9
Control	-	1	1024	1	512
I21	Methoxyelliptione	-	64 (16)	-	-
J6	Daunorubicin hydrochloride	-	-	-	32 (16)
K21	Celastrol	-	64 (16)	-	4 (128)
L9	Nigericin sodium salt	0.125 (8)	64 (16)	-	2 (256)
M4	Cumostrol	-	256 (4)	-	32 (16)
M8	Streptoal C	-	-	-	32 (16)
N4	Mangostin	0.25 (4)	128 (8)	-	4 (128)
N5	Carbomycin	0.25 (4)	64 (16)	-	1* (512)
Plate 13160331					
C4	Aklavin hydrochloride	0.25 (4)	-	-	32 (16)
C9	Actinomycin X4357g - methoxime	0.25 (4)	-	0.25 (4)	-
C15	Dehydronuciferine	-	256 (4)	-	-
D7	Streptovaricin G	0.125 (8)	2* (512)	-	2* (256)
D9	19-Formylgeldanamycin N- morpholinoimine	-	-	0.25 (4)	-
E14	Subsessiline	-	256 (4)	-	-
F4	Centaureidin	0.25 (4)	-	-	-
F6	Rhodomycin A	0.25 (4)	64 (16)	0.25 (4)	32 (16)
F8	Aclarubicin	0.25 (4)	128 (8)	0.125 (8)	4 (128)
F14	O-Methylmoschatoline	-	256 (4)	-	-
G3	Aromoline	0.25 (4)	-	-	-
G7	Rifamycin, 3- [(dimethylamino)methyl]- (9Cl)	0.25 (4)	2* (512)	-	2* (256)

	Compound Name	Colistin MIC ($\mu\text{g/mL}$)			
		<i>A. baumannii</i>	<i>A. baumannii</i>	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>
		5075	4106	2146	B9
Control	-	1	1024	1	512
G9	Prodiginine HCl	0.125 (8)	64 (16)	0.25 (4)	4 (128)
G10	Variamycin	0.25 (4)	-	-	-
H6	Dianemycin	0.0625* (16)	64 (16)	0.0625* (16)	4 (128)
H8	Borrelidin	0.25 (4)	2* (512)	-	2* (256)
H10	Pepleomycin Sulfate	0.125 (8)	4 (256)	-	2* (256)
H12	Rebeccamycin	0.25 (4)	-	-	-
I8	Withanolide E	-	-	0.25 (4)	2* (256)
I9	Julimycin B2	0.25 (4)	64 (16)	0.25 (4)	32 (16)
I11	Sirodesmin A	-	-	-	32 (16)
J4	Scopafungin	0.125 (8)	-	0.0625* (16)	-
K12	Stubomycin	-	64 (16)	-	2* (256)
L8	Clorobiocin	-	-	-	2* (256)
L15	Roemarine hydrochloride	0.25 (4)	-	-	-
L16	Variabiline dihydrochloride	0.125 (8)	256 (4)	-	-
M8	Aquayamycin	-	-	-	64 (8)
N7	Thiactin	0.125 (8)	4 (256)	0.25 (4)	-
N15	Variabiline monohydrochloride	0.125 (8)	128 (8)	0.0625* (16)	-

Table S5: Colistin potentiation activity of prodigiosin, obatoclax, rifampicin, and novobiocin in additional strains of colistin-resistant *A. baumannii* and *K. pneumoniae*

Bacterial Strain	<i>A. baumannii</i>				<i>K. pneumoniae</i>	
	3941	3942	4112	4119	C3	
Colistin MIC*	512	512	1024	1024	1024	
Prodigiosin MIC (μM)	10	20	10	160	> 160	
Lowest Effective Concentration	Prodigiosin (μM)	5	5	5	5	10
	Colistin MIC*	2	4	4	8	0.25
	(Fold-Reduction)	(256)	(128)	(256)	(128)	(4096)
Obatoclax MIC (μM)	> 160	> 160	> 160	> 160	> 160	
Lowest Effective Concentration	Obatoclax (μM)	20	20	20	20	5
	Colistin MIC*	4	8	4	8	4
	(Fold-Reduction)	(128)	(64)	(256)	(128)	(256)
Rifampicin MIC (μM)	2.5	2.5	1.25	1.25	40	
Lowest Effective Concentration	Rifampicin (μM)	0.156	0.3125	0.156	5	0.156
	Colistin MIC*	2	2	8	8	4
	(Fold-Reduction)	(256)	(256)	(128)	(128)	(256)
Novobiocin MIC (μM)	20	20	20	20	40	
Lowest Effective Concentration	Novobiocin (μM)	5	5	5	5	0.625
	Colistin MIC*	2	2	2	0.5	2
	(Fold-Reduction)	(256)	(256)	(512)	(2048)	(512)

Table S6: Fold-change differences in the lowest effective concentration of novobiocin or prodigiosin concentration required to potentiate colistin in *A. baumannii* 4106 and *K. pneumoniae* B9 upon the addition of fetal bovine serum.

	<i>A. baumannii</i> 4106			<i>K. pneumoniae</i> B9		
	5% FBS	10% FBS	20% FBS	5% FBS	10% FBS	20% FBS
2 µg/mL colistin + Novobiocin	2	2	4	1	1	1
2 µg/mL colistin + Prodigiosin	1	2	2	0.25	1	0.5

Table S7: Minimum inhibitory concentrations of novobiocin and prodigiosin in the presence of the membrane-permeabilizing polymyxin B nonpeptide (PMBN) at 2, 4, and 8 µg/mL.

	Novobiocin MIC (µM)			
	0 µg/ml PMBN	2 µg/ml PMBN	4 µg/ml PMBN	8 µg/ml PMBN
<i>A. baumannii</i> 5075	>20	2.5	1.25	2.5
<i>A. baumannii</i> 4106	20	20	20	20
<i>K. pneumoniae</i> 2146	>20	>20	>20	20
<i>K. pneumoniae</i> B9	>20	>20	>20	>20
	Prodigiosin MIC (µM)			
	0 µg/ml PMBN	2 µg/ml PMBN	4 µg/ml PMBN	8 µg/ml PMBN
<i>A. baumannii</i> 5075	>20	>20	>20	10
<i>A. baumannii</i> 4106	20	20	20	10
<i>K. pneumoniae</i> 2146	>20	>20	>20	20
<i>K. pneumoniae</i> B9	>20	>20	>20	>20

		Prodigiosin (μM)										
		80	40	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0
Colistin (μg/mL)	16	3.8	1.1	0.4	0.2	0.0	39.4	67.7	68.7	72.7	73.8	73.7
	8	3.7	1.0	0.3	0.0	1.2	67.1	74.1	76.3	82.4	83.9	84.5
	4	4.2	1.0	0.3	0.0	24.6	77.8	82.4	85.6	90.1	92.0	92.4
	2	4.2	1.4	0.3	0.8	53.3	84.9	84.1	89.9	92.4	94.6	95.5
	1	4.1	1.0	0.4	0.7	69.1	88.9	87.5	90.8	94.6	96.4	98.8
	0.5	3.8	0.9	0.3	2.5	81.7	91.5	91.5	93.8	96.7	99.3	101.7
	0.25	3.6	0.8	0.2	14.3	88.0	96.2	95.0	96.5	99.4	101.5	103.9
	0	5.0	3.5	6.6	56.3	97.3	95.7	92.4	94.0	98.2	98.0	100.0

		Obatoclox (μM)										
		80	40	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0
Colistin (μg/mL)	16	15.4	5.1	2.0	1.3	0.3	26.7	82.1	81.6	83.1	81.3	81.0
	8	13.7	6.2	1.8	1.0	3.0	65.8	85.8	88.4	89.5	88.9	86.8
	4	11.6	4.9	2.8	3.0	35.7	81.8	94.3	96.7	97.3	96.6	95.0
	2	9.8	7.1	15.5	31.2	74.2	89.0	99.0	99.3	99.6	99.1	97.8
	1	20.0	31.7	63.9	75.9	90.1	97.1	101.7	102.4	102.2	101.7	101.1
	0.5	60.9	93.0	94.7	92.8	95.4	98.3	101.8	102.5	102.5	102.2	101.5
	0.25	84.9	109.3	103.4	97.5	98.7	101.5	102.5	103.1	103.7	104.3	104.1
	0	107.1	134.1	107.8	102.1	97.6	96.7	97.9	99.9	97.8	98.8	100.0

		Rifampicin (μM)										
		20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125	0.039063	0
Colistin (μg/mL)	16	1.0	1.0	0.7	0.5	0.3	0.2	0.4	0.5	32.2	68.6	78.2
	8	1.1	0.9	0.7	0.5	0.5	0.5	0.4	26.6	70.2	84.6	88.1
	4	1.3	1.1	0.8	0.7	0.6	0.5	14.4	57.0	88.0	92.5	97.5
	2	1.1	1.1	0.6	0.7	0.8	3.7	43.5	87.8	93.9	95.7	100.3
	1	1.3	0.9	0.7	0.7	0.3	34.6	84.3	92.8	97.7	99.9	101.9
	0.5	1.2	0.8	0.5	0.3	10.2	66.8	90.4	95.7	100.2	102.1	103.5
	0.25	0.9	0.9	0.5	0.3	39.2	81.8	90.3	93.5	98.6	99.3	102.3
	0	0.9	0.9	0.8	0.6	64.9	86.8	89.2	90.7	94.4	97.4	100.0

		Novobiocin (μM)										
		40	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125	0
Colistin (μg/mL)	16	0.7	0.9	1.0	1.1	1.1	29.1	68.3	78.0	81.4	78.7	77.3
	8	0.8	0.9	1.0	1.1	1.5	57.3	81.0	84.3	87.0	83.7	84.2
	4	1.0	1.1	1.1	1.2	5.7	80.7	90.5	92.5	93.0	93.1	92.8
	2	0.9	1.0	1.2	1.2	42.6	89.7	94.8	95.6	95.9	95.7	95.4
	1	0.8	1.0	1.1	3.0	84.9	94.3	96.2	96.5	96.4	97.2	97.4
	0.5	0.7	0.7	0.3	59.7	92.6	96.6	97.4	99.0	99.5	100.1	100.7
	0.25	0.0	0.3	0.6	83.2	95.5	99.3	99.9	100.3	101.4	102.2	101.8
	0	0.0	0.3	75.4	89.6	94.3	96.6	95.5	97.5	95.7	95.9	100.0

Figure S1: Checkerboard assays for colistin with prodigiosin, obatoclox, rifampicin, or novobiocin in *A. baumannii* 4106. Cells in black indicate < 10% growth compared to the untreated control well as determined by OD600 measured on a Synergy HTX Multimode plate reader

		Prodigiosin (μM)										
		80	40	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0
Colistin (μg/mL)	16	2.2	0.8	0.3	0.1	0.0	0.0	78.3	93.7	95.6	97.0	97.1
	8	2.8	1.3	0.3	0.1	0.0	0.0	2.9	82.8	94.6	96.3	94.7
	4	2.9	1.1	1.2	0.2	0.1	0.3	36.1	91.6	97.7	98.7	97.3
	2	2.9	1.1	0.4	0.3	0.6	0.5	77.0	95.6	98.5	99.5	97.5
	1	2.9	1.3	0.3	0.2	6.5	61.2	94.1	97.1	99.0	99.4	98.3
	0.5	3.1	1.0	0.3	2.0	77.9	96.6	98.1	99.3	100.8	101.5	100.1
	0.25	2.9	1.0	1.1	18.7	95.7	100.3	98.6	100.1	101.2	101.9	101.6
	0	96.9	93.4	93.0	95.9	97.9	99.2	98.3	99.8	100.4	100.3	100.0

		Obatoclox (μM)										
		80	40	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0
Colistin (μg/mL)	16	17.7	2.9	1.2	0.5	0.2	0.0	0.1	100.4	97.1	94.9	94.9
	8	15.6	5.5	1.5	0.7	0.3	0.2	23.1	94.3	95.7	96.5	95.9
	4	26.6	3.6	1.9	1.2	0.7	0.6	79.2	98.6	98.3	98.6	99.3
	2	20.5	3.4	1.8	1.2	0.7	0.6	89.2	97.2	98.9	98.7	100.8
	1	10.3	3.0	1.8	0.9	0.4	71.4	99.7	100.2	102.1	102.1	103.0
	0.5	5.5	2.7	1.8	1.0	60.0	99.9	102.0	102.4	103.0	103.0	102.5
	0.25	6.3	2.5	1.9	77.7	101.2	104.0	101.6	102.1	103.2	103.1	103.4
	0	147.3	121.2	107.3	98.9	97.6	100.1	97.0	99.4	100.8	100.7	100.0

		Rifampicin (μM)											
		40	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125	0.039063	0
Colistin (μg/ml)	16	0.5	0.5	0.5	0.2	0.1	0.2	0.3	0.3	2.1	4.7	20.0	94.4
	8	0.5	0.7	0.6	0.3	0.1	0.3	0.3	1.0	3.3	7.7	41.1	93.0
	4	0.7	0.8	0.6	0.6	0.5	0.4	0.7	1.8	4.5	20.1	69.4	94.9
	2	0.8	1.0	0.7	0.7	0.5	0.5	1.1	25.4	45.1	81.0	90.3	98.1
	1	0.7	0.5	0.7	0.5	0.5	0.4	30.5	86.0	94.6	98.7	101.3	100.4
	0.5	0.7	0.7	0.6	41.9	61.9	82.8	95.5	100.3	102.6	102.9	102.9	103.0
	0.25	0.8	0.6	16.8	72.5	93.8	98.1	100.7	103.1	103.3	103.3	103.4	103.6
	0	0.8	14.6	60.5	85.0	99.6	98.2	96.8	95.6	99.0	98.9	100.3	100.0

		Novobiocin (μM)											
		160	80	40	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0
Colistin (μg/ml)	16	-0.1	-0.1	0.1	1.4	2.5	4.4	6.8	11.8	18.2	14.8	44.3	92.7
	8	0.0	0.1	1.4	3.8	5.7	9.1	13.9	18.7	20.0	17.1	65.8	93.1
	4	0.2	1.7	3.6	6.2	9.1	17.0	18.3	19.7	18.4	21.9	85.5	95.6
	2	1.6	3.6	5.3	7.6	12.9	17.0	18.2	18.2	29.4	58.5	92.0	96.9
	1	3.8	5.7	6.3	10.5	15.0	17.6	17.0	32.1	37.0	94.5	98.8	100.0
	0.5	6.2	7.5	8.7	15.4	17.1	16.5	44.3	48.3	95.4	100.7	102.4	102.9
	0.25	7.8	10.2	12.9	18.6	18.0	51.4	63.8	89.8	100.9	102.1	103.2	103.3
	0	10.0	12.2	16.0	23.2	31.0	38.3	79.2	93.0	95.5	97.5	99.4	100.0

Figure S2: Checkerboard assays for colistin with prodigiosin, obatoclox, rifampicin, or novobiocin in *K. pneumoniae* B9. Cells in black indicate < 10% growth compared to the untreated control well as determined by OD600 measured on a Synergy HTX Multimode plate reader

Table S8: Fractional inhibitory concentration indices (FICI) for antibiotic/adjuvant combination in *A. baumannii* 4106 and *K. pneumoniae* B9.

	<i>A. baumannii</i> 4106		<i>K. pneumoniae</i> B9	
Prodigiosin Concentration (μM)	5	10	2.5	5
Prodigiosin MIC (μM)	20	20	160	160
Colistin Concentration ($\mu\text{g/mL}$)	8	0.5	2	1
Colistin MIC ($\mu\text{g/mL}$)	1024	1024	512	512
FICI	0.26	0.50	0.02	0.03
Obatoclox Concentration (μM)	5	10	2.5	5
Obatoclox MIC (μM)	160	160	160	160
Colistin Concentration ($\mu\text{g/mL}$)	8	4	2	1
Colistin MIC ($\mu\text{g/mL}$)	1024	1024	512	512
FICI	0.04	0.07	0.02	0.03
Rifampicin Concentration (μM)	0.625	0.625	0.625	1.25
Rifampicin MIC (μM)	2.5	2.5	40	40.0
Colistin Concentration ($\mu\text{g/mL}$)	2	1	2.0	1
Colistin MIC ($\mu\text{g/mL}$)	1024	1024	512	512
FICI	0.25	0.25	0.02	0.03
Novobiocin Concentration (μM)	2.5	5	2.5	5
Novobiocin MIC (μM)	20	20	160	160
Colistin Concentration ($\mu\text{g/mL}$)	4	1	16	8
Colistin MIC ($\mu\text{g/mL}$)	1024	1024	512	512
FICI	0.13	0.25	0.05	0.05

Two lowest combinations for each condition reported. Cells highlighted in yellow indicate synergy; cell highlighted in red indicates indifference.

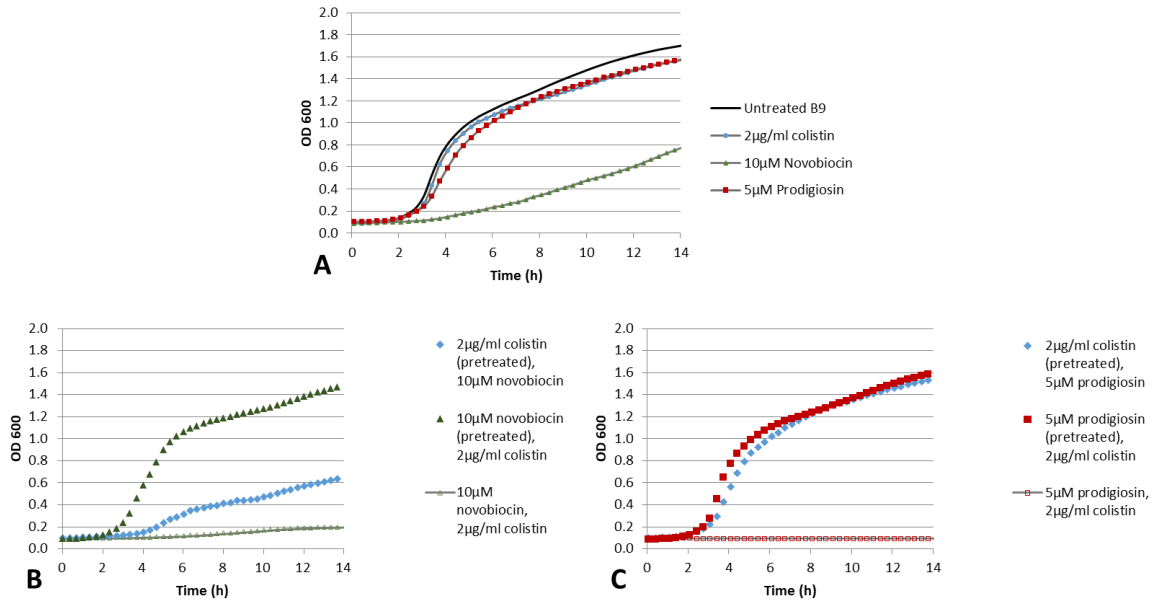


Figure S3: Growth curves of *Klebsiella pneumoniae* B9. (A) Growth of untreated B9 and B9 treated for 14 hours with 2 µg/mL colistin (MIC 512 µg/mL), 10 µM novobiocin (MIC 20 µM), or 5 µM prodigiosin (MIC > 160 µM). (B) Growth of B9 pretreated with either 2 µg/mL colistin, then treated for 14 hours with 10 µM novobiocin; B9 pretreated with 10 µM novobiocin, then treated for 14 hours with 2 µg/mL colistin; and B9 treated with colistin and novobiocin concurrently. Novobiocin at 10 µM causes growth delays, but pretreated with either antibiotic does not restore lack of growth seen with both compounds. (C) Growth of B9 pretreated with either 2 µg/mL colistin, then treated for 14 hours with 5 µM prodigiosin; B9 pretreated with 5 µM prodigiosin, then treated for 14 hours with 2 µg/mL colistin; and B9 treated with colistin and prodigiosin concurrently. Pretreatment with either compound has no major effect on growth.

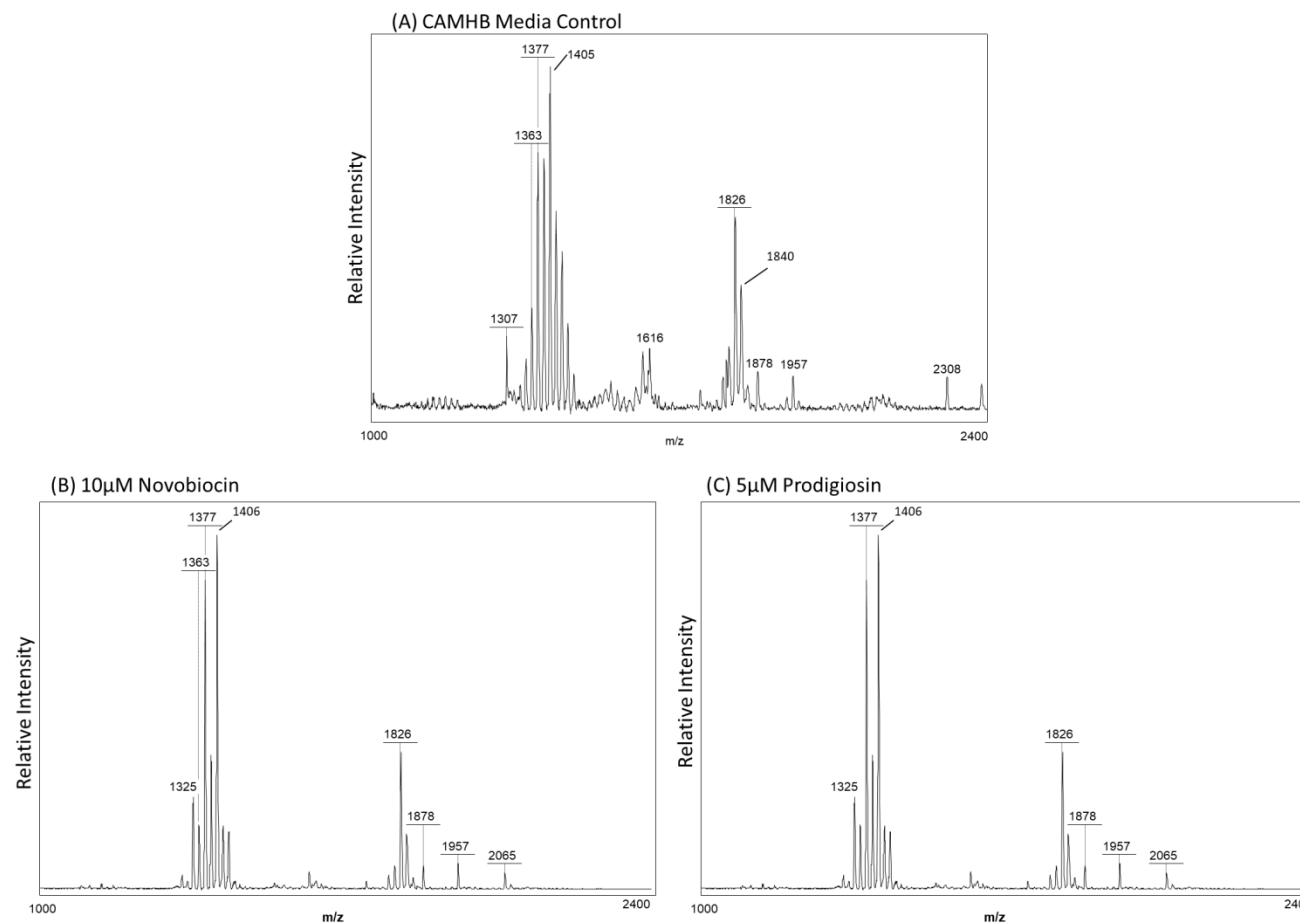


Figure S4: m/z shifts in lipid A composition of an untreated *K. pneumoniae* B9 control (A) compared to cells treated with 10 µM novobiocin (B) or 5 µM prodigiosin (C). The peak at 1826 is representative of *K. pneumoniae*. There is no indication of major changes in modifications to lipid A upon treatment with either compound. Enrichment with C16 palmitate is indicated by m/z shift ~ 238. Data is representative of 2 biologically independent samples.

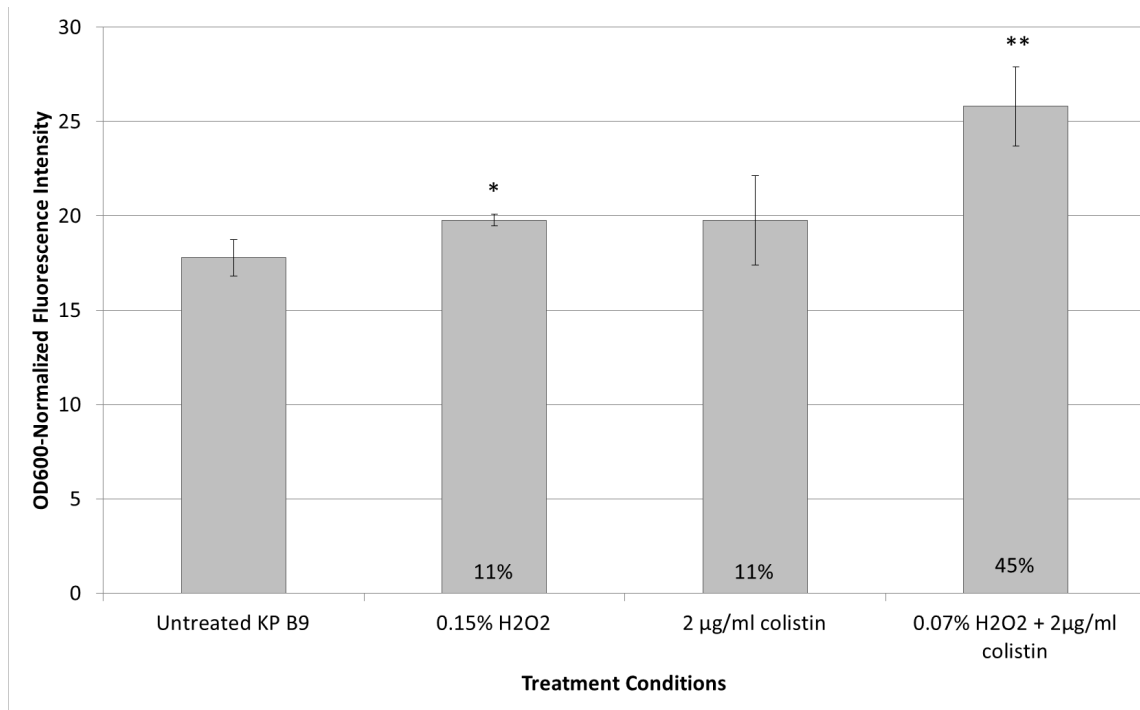


Figure S5: DCFH-DA fluorescence assay of *K. pneumoniae* B9 cells treated with hydrogen peroxide and colistin. Combination of hydrogen peroxide and colistin results in an increase in ROS fluorescence, but on a much smaller scale than prodigiosin and colistin. Data are representative of 5 fluorescence measurements in 6 independent biological samples all conducted on the same day. Note: the increase in ROS fluorescence with hydrogen peroxide was highly variable, depending on the day. Increases could range from the 11% increase shown here to 1050%. * p-value < 0.05 **p-value < 0.001