Supporting Information

Selective permeabilization of Gram-negative bacterial membranes using multivalent peptide constructs for antibiotic sensitization

Authors:

Leslie W. Chan[1], Kelsey E. Hern[2], Chayanon Ngambenjawong[2], Katie Lee[3], Ester J. Kwon[1], Deborah T. Hung[3-5], Sangeeta N. Bhatia[1-3,6-8]*

Affiliations:

1. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States
2. Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States
3. Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, Massachusetts 02139, United States
4. Department of Molecular Biology, Center for Computational and Integrative Biology, Massachusetts General Hospital, Boston, Massachusetts 02114, United States
5. Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA
6. Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States
7. Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, United States
8. Howard Hughes Medical Institute, Cambridge, Massachusetts 02139, United States

*Corresponding Author: Sangeeta N. Bhatia
Address: 500 Main Street, 76-453, Cambridge, MA 02142, USA
Phone: 617-253-0893
Fax: 617-324-0740
Email: sbhatia@mit.edu

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Table S1. Candidate peptides for peptide-antibiotic conjugates. D-amino acids are prefixed with d and in parenthesis. A cysteine was added to the C-terminal of each peptide for conjugation to linezolid-C_6-azide.

Peptide	Sequence	Structure	Cell penetration/membrane disruption mechanism
Buforin 2	TRSSRAGLQFPVGRVHRLLRK	N-terminal random coil region (residues 1-4), an extended helical region (residues 5-10), a proline hinge (residue 11), and a C-terminal *α*-helical region (residues 12-21)[1]	**Transient toroidal pore formation**; penetrates membrane without permeabilization; removal of proline hinge causes peptide localization to the cell surface and membrane permeabilization[2]
Lactoferrin (Human)	KCFQWQRNMRKVRGPPVSCIKR	N-terminal amphipathic helix connected to a β-strand via a loop; no disulfide bond included for linezolid screen[3]	Bacterial membrane depolarization via unknown mechanism[3]
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	Amphipathic α-helical peptide[4]	Forms oligomers in membranes; evidence suggest membrane disruption by peptide **"carpeting" in the membrane or toroidal pore formation**[4]
Pexiganan	GIGKFLKKAKKFGKAFVKILKK	Unstructured in solution; forms dimeric antiparallel α-helical coiled-coil structure upon association with lipid membranes[5]	Disrupts membrane through **toroidal-type pore formation**[5]
PR-26	RRRPRPPYLPRPRPPPFFPPRLPPRI	Extended[6]	Antimicrobial domain of the cathelicidin PR-39, previously shown to translocate via an **inner membrane transporter** SbmA; non-lytic transport mechanism[7]
$S4_{13}$-PV	ALWKTLLKKVLKAPKKKRKV	Unstructured in solution[8]	Undergoes conformational change into helix during interaction with membrane[8]

TAT	YGRKKRRQRRRG	Random coil[9]	**Carpet model of membrane disruption**[9]
WLBU2	RRWVRRVRRWVRRVVRVVRRWVRR	Amphipathic α-helical peptide[10]	Proposed mechanism: **domain formation** with different elasticity that leads to leakage along domain walls[11]
4D-K_5L_7	KL(dL)(dL)KLK(dL)K(dL)LK	Amphipathic α-helical peptide[12]	Inserts into membrane and induces LPS miscellization; **carpet and detergent model of membrane disruption**[12]

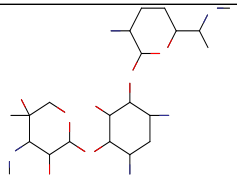
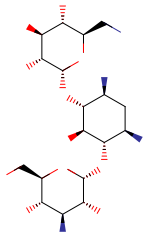
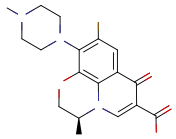
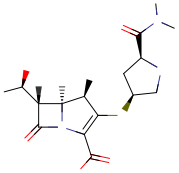
Table S2. MIC values for peptides alone and physical mixtures of peptides with a fixed concentration of linezolid (80 μM). Values were determined via microdilution assays with *P. aeruginosa* (strain PA14).

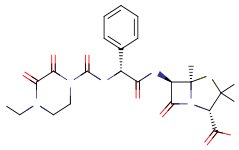
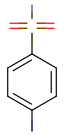
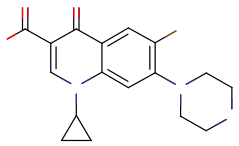
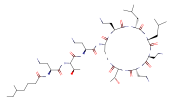
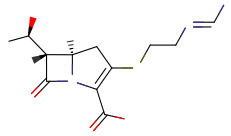
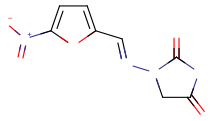
Peptide	MIC	MIC_{+LZD}
Buforin 2	10	10
Lactoferrin	>40	>40
LL-37	20	20
Pexiganan	1.25	1.25
PR-26	5	5
$S4_{13}$-PV	2.5	2.5
TAT	>40	>40
WLBU2	>40	>40
4D-K_5L_7	10	10

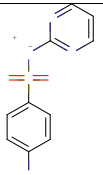
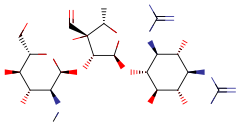
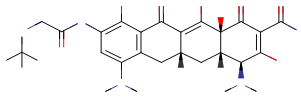
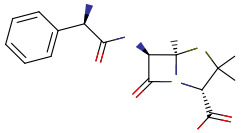
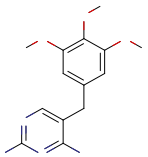
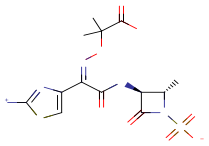
Table S3. Test reaction conditions for amine-functionalization of 0.5-g dextran quantities. *Indicates dextrans used to make WD10 and WD40 potentiator candidates.

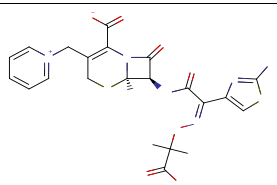
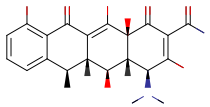
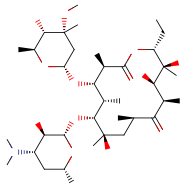
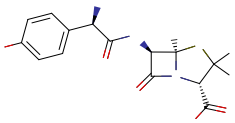
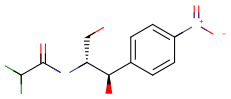
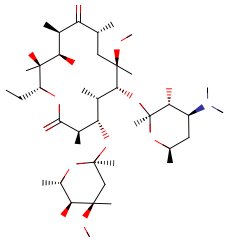
Sample ID	Dextran MW	Molar ratio sodium periodate: dextran	mL 100 mM sodium periodate	mL 3 M ethylenediamine (in 0.1 M sodium phosphate buffer, pH 7.4, 150 mM NaCl)	mL 1 M cyanoborohydride	Amine groups per dextran
Am-Dex10-100	10 kDa	12.5:1	18.75	12.5	2.5	5
Am-Dex10-200*	**10 kDa**	**25:1**	**12.5**	**12.5**	**2.5**	**27**
Am-Dex10-300	10 kDa	37.5	6.25	12.5	2.5	40
Am-Dex40-100*	**40 kDa**	**50:1**	**18.75**	**12.5**	**2.5**	**22**
Am-Dex40-200	40 kDa	100:1	12.5	12.5	2.5	34
Am-Dex40-300	40 kDa	150:1	6.25	12.5	2.5	70

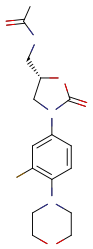
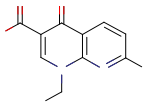
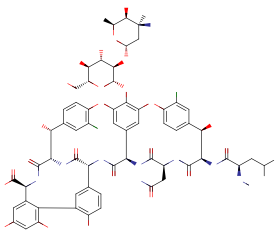
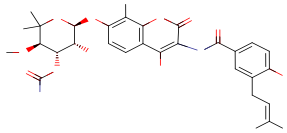
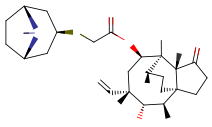
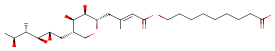
Table S4. Class, clinical breakpoints, and physicochemical properties of drugs in the tested antibiotic panel. Antibiotics with antipseudomonal activity are indicated with *. Information for rotatable bonds, globularity, PBF, and functional groups were consolidated using eNTRYway (entry-way.org). Green indicates values that fall within favorable range for intracellular accumulation as defined by Richter et. al. while red indicates those that do not. Yellow indicates values for antimicrobial peptides, which were excluded from correlation analyses. Clinical breakpoints were consolidated from CLSI or EUCAST. Blue indicates MIC values in the presence of WD40 fall at or below the clinical breakpoint.

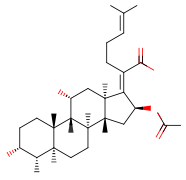
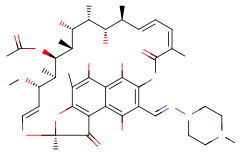
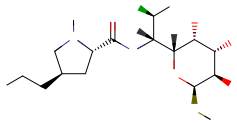
ANTIBIOTIC (Drug class: drug target)	Structure	MW	ClogP (ChemAxon)	Rotatable bonds	Globularity	PBF	Functional group	Activity with potentiator	MIC (μg/mL)	MIC_{+WD40} (μg/mL)	MIC fold change	Clinical breakpoint (μg/mL)
GENTAMICIN* (Aminoglycoside: protein synthesis)		477.6	-3.1	7	0.069	1.085	Primary amine	Additive	1	1	1	4 (Pseudomonas spp.)
KANAMYCIN* (Aminoglycoside: protein synthesis)		484.5	-3.1	6	0.122	1.169	Primary amine	Additive	32	32	1	1 (Pseudomonas spp.)
LEVOFLOXACIN* (Fluoroquinolone: nucleic acid synthesis)		361.37	-0.02	2	0.048	0.655	Tertiary amine	Additive	0.5	0.5	1	1 (Pseudomonas spp.)
MEROPENEM* (Carbapenem: cell wall)		383.46	-0.69	5	0.058	0.855	Secondary amine	Additive	0.5	0.25	2	2 (Pseudomonas spp.)

PIPERACILLIN (Beta-lactam: cell wall)		238.16	0.03	3	0.079	0.616	No amine	Additive	4	2	2	16 (Pseudomonas spp.)
SULFANILAMIDE (Sulfonamide: folate pathway)		172.2	-0.16	1	0.083	0.478	No amine	Additive	2048	1024	2	Not available
CIPROFLOXACIN* (Fluoroquinolone: nucleic acid synthesis)		331.34	-0.57	3	0.04	0.536	Secondary amine	Additive	0.25	0.0625	4	0.5 (Pseudomonas spp.)
COLISTIN* (Polymyxin: cell wall)		1155.45	0.22	28	NA	NA	Primary amine	Synergistic	1	0.25	4	2 (Pseudomonas spp.)
IMIPENEM* (Carbapenem: cell wall)		299.35	-0.19	6	0.176	0.915	No amine	Additive	0.5	0.125	4	4 (Pseudomonas spp.)
NITROFURANTOIN* (Nitrofuran: protein synthesis)		138.06	-0.86	1	0.143	0.504	No amine	Synergistic	512	128	4	64 (S. aureus)

SILVER SULFADIAZINE (Sulfa antibiotics: cell wall)		581.6	-7.7	9	0.253	1.301	Secondary amine	Synergistic	16	4	4	not available
STREPTOMYCIN* (Aminoglycoside: protein synthesis)		585.65	0.66	7	0.034	0.84	Secondary amine	Synergistic	8	2	4	16 (E. coli)
TIGECYCLINE (Glycylcycline: protein synthesis)		517.55	0.67	6	0.069	1.077	No amine	Synergistic	16	4	4	0.5 (Staphylococcus spp.)
AMPICILLIN (Beta-lactam: cell wall)		290.32	1.26	5	0.147	1.006	No amine	Synergistic	64	8	8	8 (Enterobacteriaceae)
TRIMETHOPRIM (DHFR inhibitor: folate pathway)		357.14	0.19	3	0.115	0.835	No amine	Additive	16	2	8	2 (Staphylococcus spp.)
AZTREONAM* (Beta-lactam monobactam: cell wall)		349.4	0.88	4	0.098	1.016	Primary amine	Synergistic	4	0.25	16	1 (Pseudomonas spp.)

CEFTAZIDIME* (Beta-lactam: cell wall)		435.43	0.04	7	0.098	0.907	No amine	Synergistic	2	0.125	16	8 (Pseudomonas spp.)
DOXYCYCLINE (Tetracycline: protein synthesis)		444.43	-0.72	2	0.206	1.178	Tertiary amine	Synergistic	64	2	32	1 (Staphylococcus spp.)
ERYTHROMYCIN (Macrolide: protein synthesis)		546.58	-1.2	9	0.07	1.108	No amine	Synergistic	128	4	32	1 (Staphylococcus spp.)
FOSFOMYCIN (Phosphonic acid: cell wall)		733.93	2.37	7	0.122	1.231	Tertiary amine	Synergistic	16	0.5	32	32 (Staphylococcus spp.)
AMOXICILLIN (Beta-lactam: cell wall)		365.4	0.75	4	0.154	1.218	Primary amine	Synergistic	128	2	64	8 (Enterobacteriaceae)
CHLORAMPHENICOL (Nitrobenzenes: protein synthesis)		323.13	1.15	6	0.105	0.898	No amine	Synergistic	4096	64	64	8 (Staphylococcus spp. and Enterobacteriaceae)
CLARITHROMYCIN (Macrolide: protein synthesis)		747.95	3.18	8	0.191	1.359	Tertiary amine	Synergistic	128	2	64	2 (Staphylococcus spp.)

LINEZOLID (Oxazolidinone: protein synthesis)		337.35	0.61	4	0.067	0.939	No amine	Synergistic	8192	64	128	4 (Staphylococcus spp.)
NALIDIXIC ACID (Quinolone: nucleic acid synthesis)		232.24	0.95	2	0.053	0.475	No amine	Synergistic	128	1	128	Not available
VANCOMYCIN (Glycopeptide: cell wall)		1449.3	1.11	13	NA	NA	Primary amine	Synergistic	1024	8	128	2 (Staphylococcus spp.)
NOVOBIOCIN (Aminocoumarin: nucleic acid synthesis)		612.62	3.07	9	0.04	1.114	No amine	Synergistic	256	1	256	Not available
RETAPAMULIN (Pleuromutilin: protein synthesis)		517.76	4.63	6	0.105	1.226	Tertiary amine	Synergistic	512	2	256	0.5 (Staphylococcus spp.)
MUPIROCIN (Carboxylic acid: protein synthesis)		500.62	2.25	17	0.04	1.124	No amine	Synergistic	2048	4	512	1 (Staphylococcus spp.)

FUSIDIC ACID (Fusidane: protein synthesis)		516.71	4.97	6	0.21	1.428	No amine	Synergistic	1024	0.5	2048	1 (Staphylococcus spp.)
RIFAMPIN (Antimycobacterial: nucleic acid synthesis)		822.94	3.85	5	0.256	1.841	Tertiary amine	Synergistic	512	0.25	2048	0.5 (Staphylococcus spp., S. pneumoniae, Streptococcus spp.)
CLINDAMYCIN (Lincosamide: protein synthesis)		424.98	1.59	7	0.101	1.108	Tertiary amine	Synergistic	2048	0.5	4096	0.5 (Staphylococcus spp., S. pneumoniae)

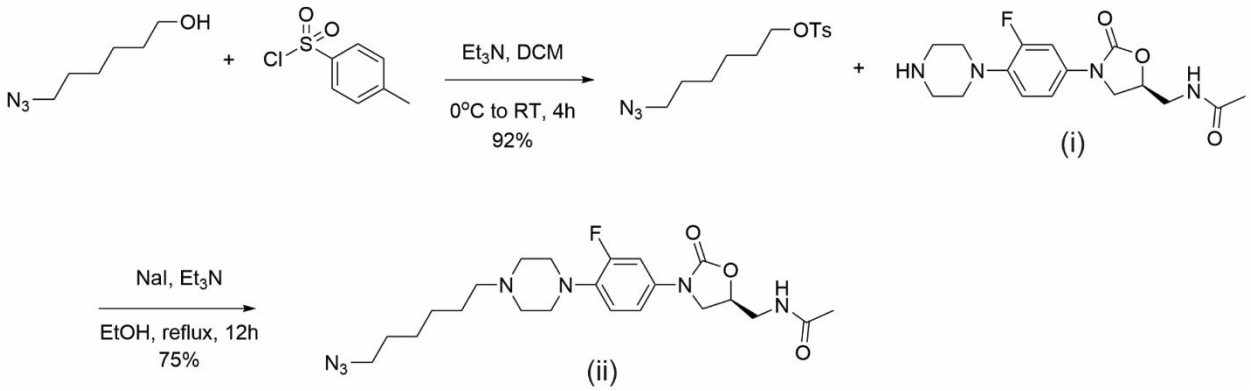
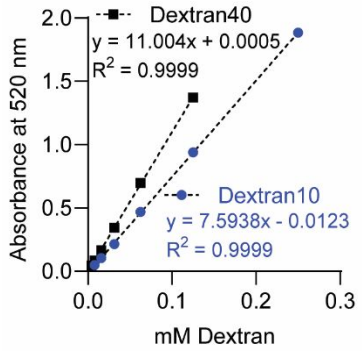


Figure S1. Synthetic scheme for azido-functionalized linezolid variant (Azido-LZDvar, compound ii). In this synthetic pathway, a piperazine variant of linezolid (compound i) is alkylated with a C_6-azide linker using reaction conditions previously reported by Phetsang et al.[13]

A



B

Scaffold type	Molar feed ratio WLBU2:Dextran	A_{280}	A_{520}	[WLBU2] (mM)	[Dextran] (mM)	WLBU2 per Dextran
Dextran 10	5	0.635	0.367	0.372	0.050	7.4
Dextran 10	10	0.591	0.190	0.346	0.027	13.0
Dextran 10	20	0.559	0.101	0.327	0.015	21.9
Dextran 40	5	0.746	1.005	0.437	0.091	4.8
Dextran 40	10	0.615	0.506	0.360	0.046	7.8
Dextran 40	20	0.544	0.245	0.319	0.022	14.3

Figure S2. Quantification of conjugated WLBU2 peptides in potentiator candidates. WLBU2 peptides were conjugated onto rhodamine-labelled Dextran 10 and Dextran 40. (**A**) Standard curves were generated via measurement of absorbance at 520 nm wavelength for serially-diluted solutions containing known concentrations of rhodamine-labelled Dextran 10 and Dextran 40 in PBS. (**B**) 3 different molar feed ratios (5, 10, or 20 WLBU2 peptides to dextran) were used per scaffold type to generate 6 potentiator candidates. After purification and lyophilization, dried samples were dissolved in PBS and absorbance at 280 nm and 520 nm wavelength were used to calculate molar concentrations of WLBU2 peptide and dextrans, respectively. Beer's Law, $A = \varepsilon c l$, was used to convert absorbance to peptide concentration ($A = A_{280}$, $\varepsilon_{WLBU2} = 17{,}070\ M^{-1} \cdot cm^{-1}$, $l = 0.1$cm). Equations for the standard curves shown in (**A**) were used to convert absorbance at 520 nm (A_{520}) to dextran concentration.

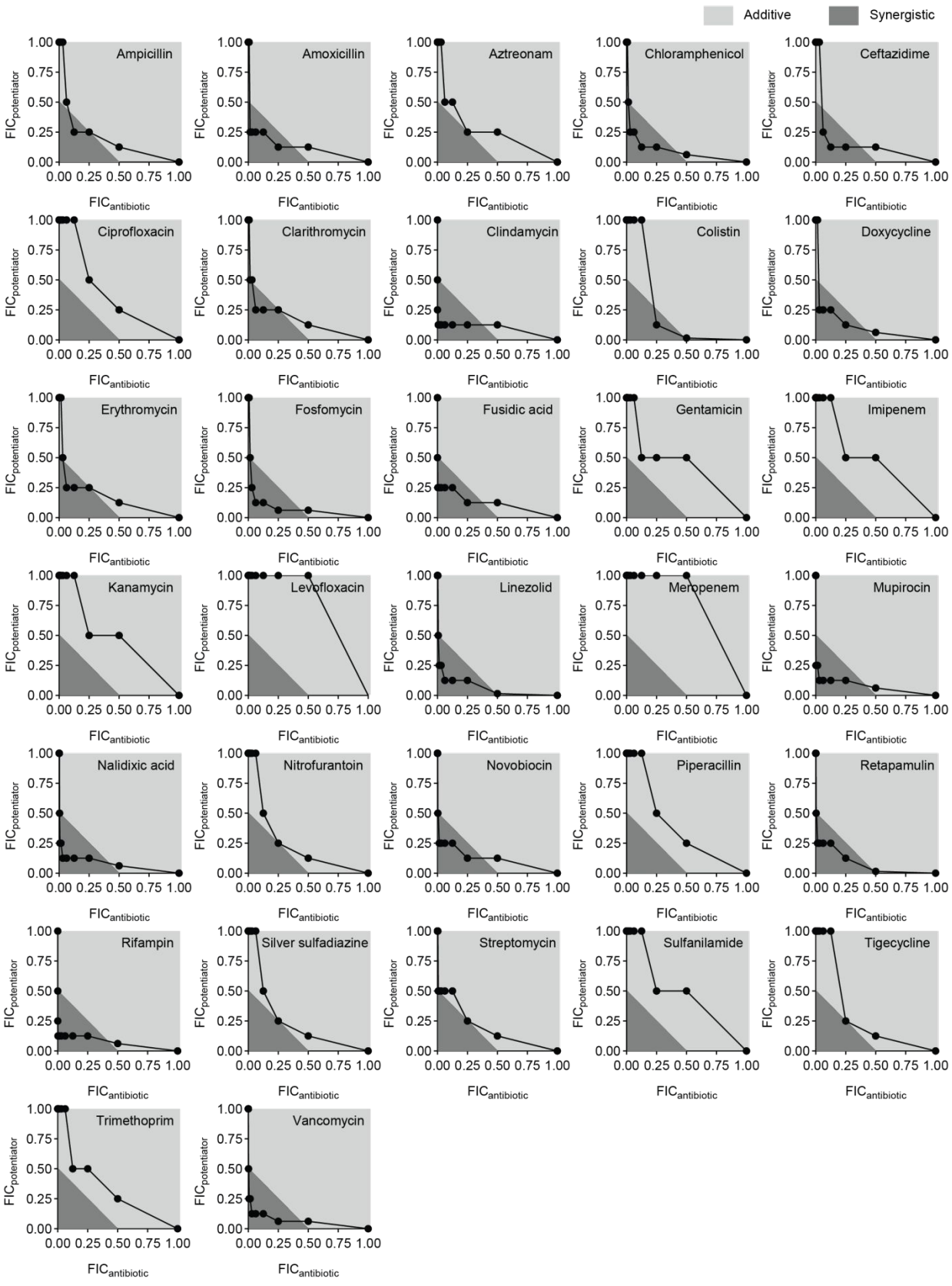

Additive
Synergistic
Ampicillin
Amoxicillin
Aztreonam
Chloramphenicol
Ceftazidime
Ciprofloxacin
Clarithromycin
Clindamycin
Colistin
Doxycycline
Erythromycin
Fosfomycin
Fusidic acid
Gentamicin
Imipenem
Kanamycin
Levofloxacin
Linezolid
Meropenem
Mupirocin
Nalidixic acid
Nitrofurantoin
Novobiocin
Piperacillin
Retapamulin
Rifampin
Silver sulfadiazine
Streptomycin
Sulfanilamide
Tigecycline
Trimethoprim
Vancomycin
FIC potentiator
FIC antibiotic
0.00
0.25
0.50
0.75
1.00

Figure S3. FIC values from checkerboard assays to characterize the activity of different antibiotics in combination with the WD40 in PA14.

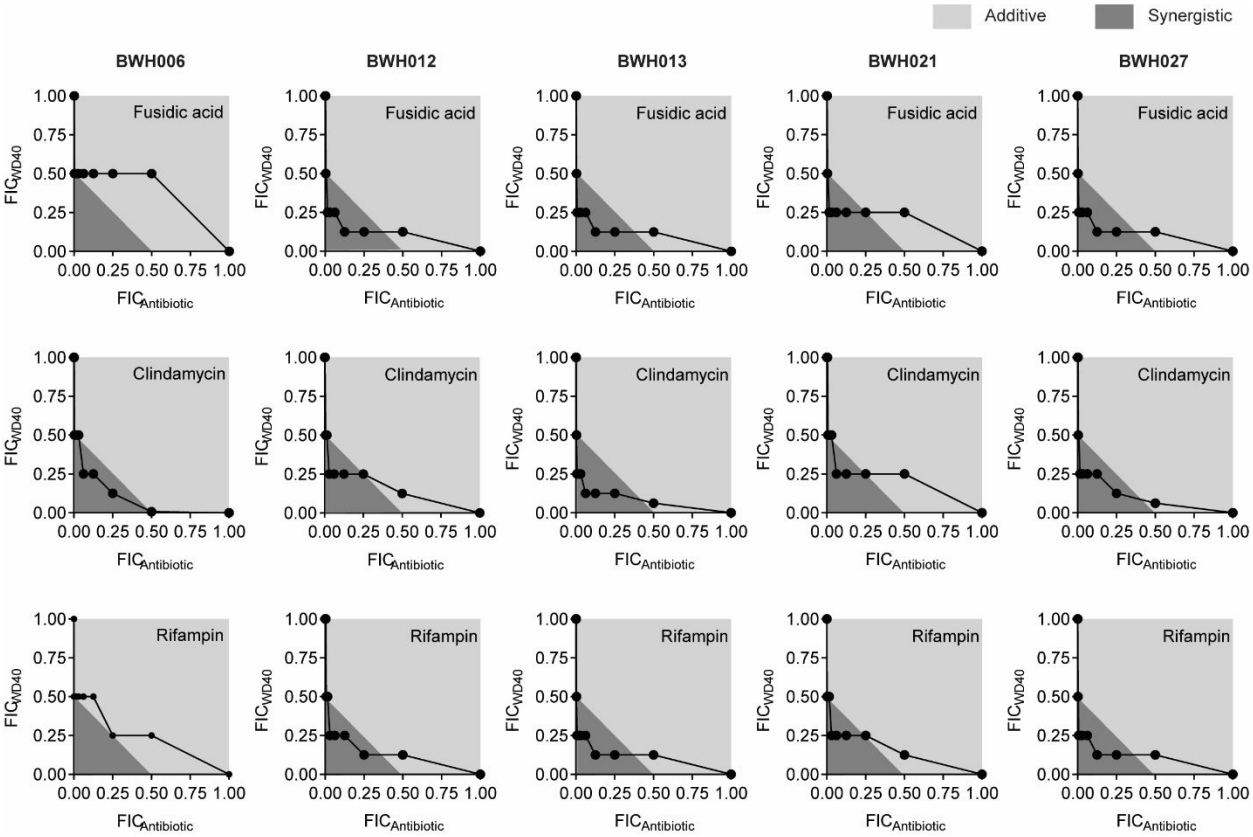


Figure S4. FIC values from checkerboard assays to characterize the activity of fusidic acid, clindamycin, and rifampin in combination with WD40 in clinical isolates BWH006, BWH012, BWH013, BWH021, and BWH027.

Supplementary References

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