Supporting Information

Phenylthiazole Antibacterial Agents Targeting Cell Wall Synthesis Exhibit Potent Activity *In Vitro* and *In Vivo* against Vancomycin-resistant Enterococci

Haroon Mohammad, Waleed Younis, Lu Chen, Christine E. Peters, Joe Pogliano, Kit Pogliano, Bruce Cooper, Jianan Zhang, Abdelrahman Mayhoub, Eric Oldfield, Mark Cushman, and Mohamed N. Seleem

Table of Contents

Table S1: Strains of *E. faecium* and *E. faecalis* utilized in this study.

Strain ID	Alternate Strain ID	Isolated From	Year	Source	Antimicrobial Resistance Phenotype
E. faecalis ATCC 49532	UWH 1921	Wisconsin, USA[1]	-	Blood	Gentamicin
E. faecalis ATCC 49533	UWH 1936	Wisconsin, USA	-	Blood	Streptomycin
E. faecalis NR-31887	B3336	-	1987	Blood	Gentamicin
E. faecalis NR-31975	MMH594	Wisconsin, USA	1985	Blood	Erythromycin and gentamicin
E. faecalis ATCC 29212	-	-	-	Urine	-
E. faecalis ATCC 51299 (VRE)[2]	NJ-3	Missouri, USA	-	Peritoneal fluid	Vancomycin
E. faecalis HM-201 (VRE)	TX0104	Connecticut, USA	2002	Blood of endocarditis patient	Ciprofloxacin and vancomycin
E. faecalis HM-934 (VRE)	ERV103	Bogota, Columbia	2006	Human secretion	Ciprofloxacin and vancomycin
E. faecalis NR-31972 (VRE)	SF28073	Michigan, USA	2003	Urine	Erythromycin, gentamicin, and vancomycin
E. faecalis HM-334 (VRE)	S613	-	2004	Blood	Vancomycin
E. faecalis HM-335 (VRE)	R712	-	2004	Blood	Vancomycin
E. faecium HM-204	TX1330	Texas, USA	1994	Feces	
E. faecium HM-463	TX0133a04	Texas, USA	2006	Blood of diabetic patient with endocarditis	Ampicillin and ciprofloxacin
E. faecium HM-959	513	-	-	-	Ampicillin, ciprofloxacin, and doxycycline

Table S1 continued

E. faecium NR-28979 (VRE)	E1162	France	1997	Blood	Ampicillin and vancomycin
E. faecium ATCC 700221 (VRE)	-	Connecticut, USA	-	Feces	Teicoplanin and vancomycin
E. faecium HM-968 (VRE)	ERV102	Colombia	2006	Oral sputum	Vancomycin
E. faecium NR-31914 (VRE)	E0120	Netherlands	1995	Ascites fluid	Vancomycin
E. faecium NR-31912 (VRE)	Patient #3-1	-	-	Stool	Vancomycin
E. faecium NR-31909 (VRE)	Patient #2-1	-	-	Stool	Vancomycin
E. faecium NR-31903 (VRE)	Patient #1-1	-	-	Stool	Linezolid and vancomycin
E. faecium NR-31915 (VRE)	E0164	Netherlands	1996	Turkey feces	Gentamicin and vancomycin
E. faecium E1071 (VRE)	-	Netherlands	2000	-	Vancomycin
E. faecium NR-31916 (VRE)	E0269	Netherlands	1996	Turkey feces	Gentamicin and vancomycin

[1]USA = United States of America
[2]VRE = vancomycin-resistant enterococci

Table S2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of phenylthiazole compounds **1-3**, ampicillin, ciprofloxacin, doxycycline, vancomycin, and linezolid against six vancomycin-sensitive (VSE) and eight vancomycin-resistant *Enterococcus faecalis* or *E. faecium* (VRE) strains.

	1		**2**		**3**		**Ampicillin**		**Ciprofloxacin**		**Doxycycline**		**Vancomycin**		**Linezolid**	
Strain	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis ATCC 49532	1	4	2	4	2	4	0.5	1	1	1	0.5	32	1	-	1	64
E. faecalis ATCC 49533	8	8	8	16	8	16	0.5	0.5	1	1	>64	>64	1	32	1	64
E. faecalis NR-31887	8	8	16	16	8	8	0.5	0.5	0.5	0.5	32	>64	1	8	1	16
E. faecalis NR-31975	4	4	2	2	2	4	-	-	-	-	-	-	1	64	1	128
E. faecalis ATCC 29212	4	-	16	-	8	-	-	-	-	-	-	-	4	-	4	-

Table S2 continued

E. faecalis ATCC 51299 (VRE)	2	2	2	4	2	4	-	-	-	-	-	-	16	-	1	128
E. faecalis HM-201 (VRE)	8	8	16	16	4	4	0.5	0.5	64	>64	1	32	64	64	1	16
E. faecalis HM-934 (VRE)	8	8	16	16	8	8	1	1	>64	>64	1	64	>64	>64	1	64
E. faecalis NR-31972 (VRE)	2	4	2	16	4	4	-	-	-	-	-	-	>128	ND[1]	1	64
E. faecalis HM-334 (VRE)	4	4	2	2	2	2	-	-	-	-	-	-	>64	>64	1	32
E. faecalis HM-335 (VRE)	2	2	1	1	1	1	-	-	-	-	-	-	>64	>64	1	32

Table S2 continued

E. faecium HM-204	4	16	8	32	4	8	1	1	1	1	1	32	1	16	2	32
E. faecium HM-463	4	4	8	16	4	4	16	32	>64	>64	0.5	16	0.5	0.5	0.5	8
E. faecium HM-959	4	4	8	8	4	4	>64	>64	>64	>64	>64	>64	1	16	2	16
E. faecium NR-28979 (VRE)	2	4	1	16	1	4	0.5	0.5	2	2	32	>64	>64	>64	1	16
E. faecium ATCC 700221 (VRE)	0.5	2	0.5	2	0.5	0.5	-	-	-	-	-	-	>64	ND	0.5	>128
E. faecium HM-968 (VRE)	2	4	1	16	1	4	-	-	-	-	-	-	>64	ND	0.5	128
E. faecium NR-31914 (VRE)	2	4	1	32	2	4	-	-	-	-	-	-	>128	ND	1	>128

Table S2 continued

E. faecium NR-31912 (VRE)	2	4	2	16	2	4	-	-	-	-	-	-	>64	>64	2	32
E. faecium NR-31909 (VRE)	2	2	1	1	1	1	-	-	-	-	-	-	64	-	1	8
E. faecium NR-31903 (VRE)	2	4	2	16	1	4	-	-	-	-	-	-	>64	>64	32	>64
E. faecium NR-31915 (VRE)	4	4	1	1	1	8	-	-	-	-	-	-	4	ND	2	64
E. faecium E1071 (VRE)	4	4	4	16	4	4	-	-	-	-	-	-	>64	>64	2	32
E. faecium NR-31916 (VRE)	4	4	2	2	2	2	-	-	-	-	-	-	>64	>64	1	32

[1]ND = Not determined

Table S3: Fractional inhibitory concentration (FIC) index of phenylthiazole compounds **1-3** tested in combination with ciprofloxacin against enterococci via the checkerboard assay.

Test Combination	***E. faecalis* ATCC 51299**	***E. faecalis* ATCC 49532**	***E. faecalis* ATCC 49533**
1 + Ciprofloxacin	0.50	0.75	0.63
2 + Ciprofloxacin	-	1.00	0.75
3 + Ciprofloxacin	-	1.00	1.00

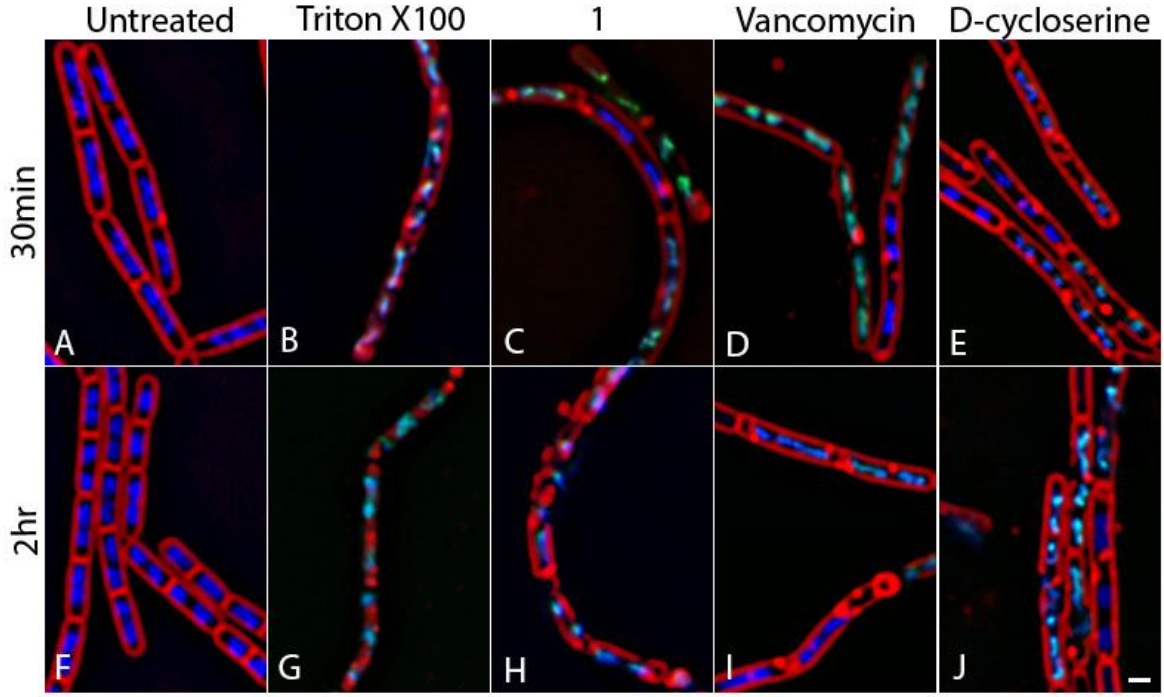

Figure S1. Profiles of membrane and cell wall active compounds in *B. subtilis* grown in LB at 37 °C. (A, F) Untreated *B. subtilis* cells show no lysis. (B, G) Cells treated with 0.1% Triton-X-100, a membrane active compound. (C, H) Cells treated with compound **1** at 5 × MIC (12.5 µg/mL). (D, I) Cells treated with Vancomycin at 5 × MIC (0.78 µg/mL). (E, J) Cells treated with D-cycloserine at 1 × MIC (37.5 µg/mL). Both cell wall inhibitors and membrane active compounds cause lysis, which is observed by the increase in staining by SYTOX. Cells were stained with FM 4−64 (red), DAPI (blue), and SYTOX Green (green).

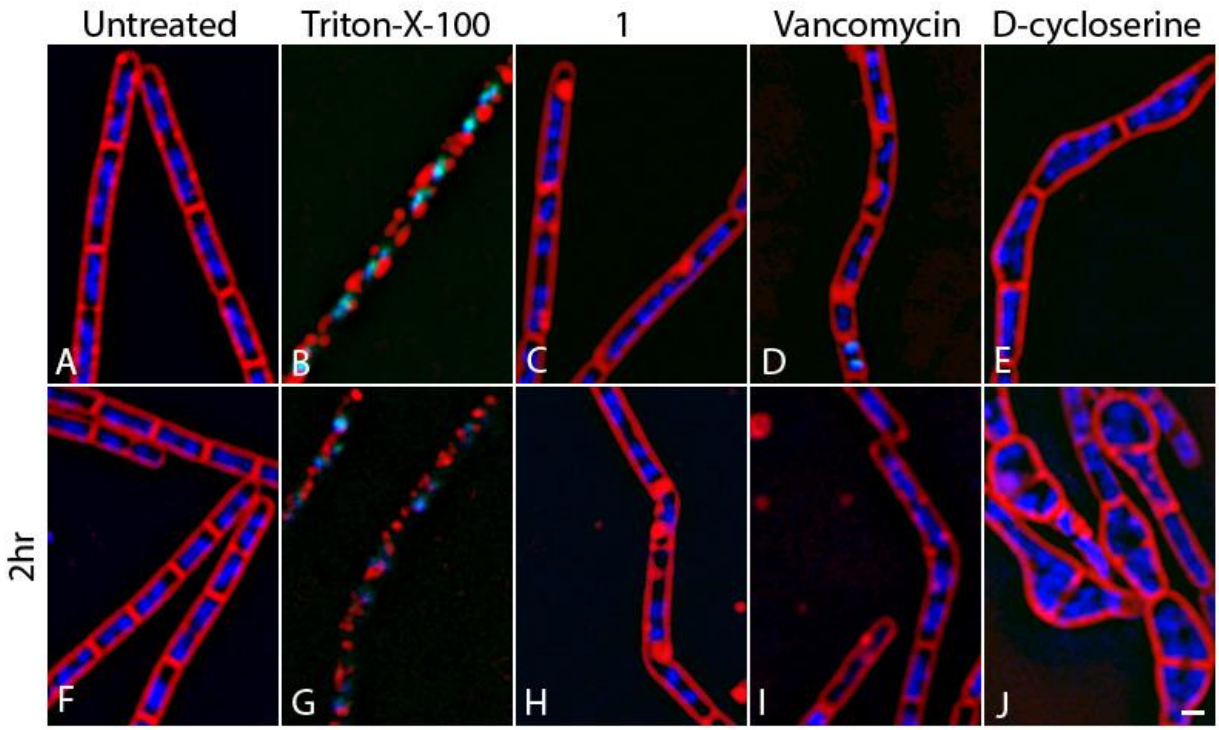

Figure S2. Profiles of membrane and cell wall active compounds in *B. subtilis* grown in LB in the presence of MSM at 37 °C. (A, F) Untreated cells show no cell shape defects or lysis. (B, G) Cells treated with 0.1% Triton-X-100. (C, H) Cells treated with compound **1** at 5 × MIC (12.5 µg/mL), show subtle cell shape defects consistent with cell wall inhibition. (D, I) Cells treated with vancomycin at 5 × MIC (0.78125 µg/mL). (E, J) Cells treated with D-cycloserine at 1 × MIC (37.5 µg/mL).

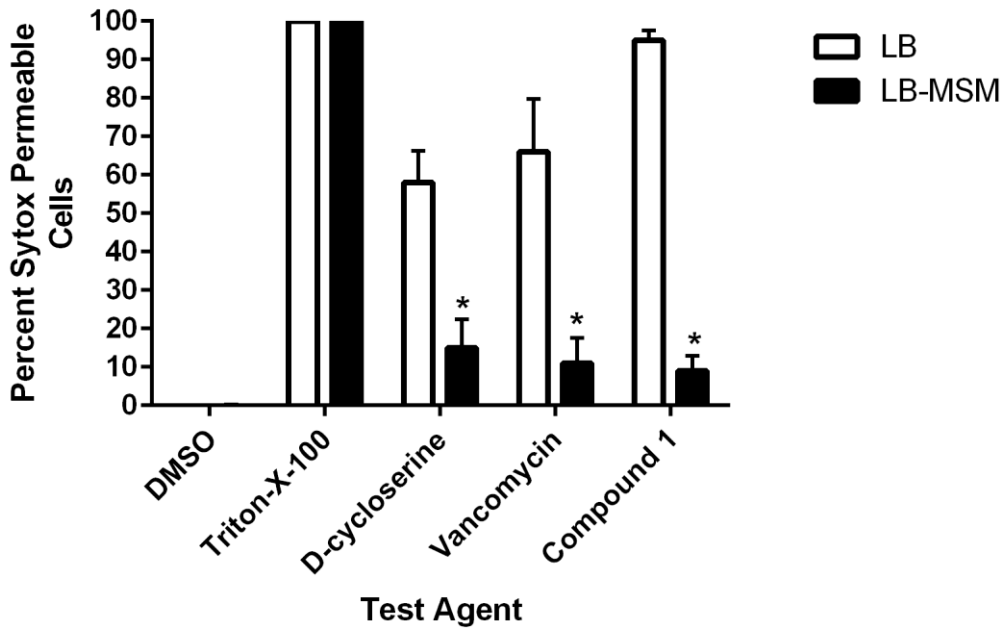

Figure S3. Percent Sytox permeable *B. subtilis* cells. Sytox permeable cells were counted at two hours. Untreated cells have no Sytox permeable cells in either LB or LBMSM, which osmotically stabilizes spheproplasts. Lysis caused by treatment with Triton X-100, a membrane active compound, is unaffected by LBMSM whereas lysis caused by cell wall inhibitors D-cycloserine and vancomycin, and by compound **1** is greatly reduced by growth in LBMSM. A two-way ANOVA with post-hoc Sidak's multiple comparisons test found statistical significance ($P < 0.05$) between LB and LB-MSM groups for cells receiving treatment with D-cycloserine, vancomycin, and phenylthiazole compound **1**.

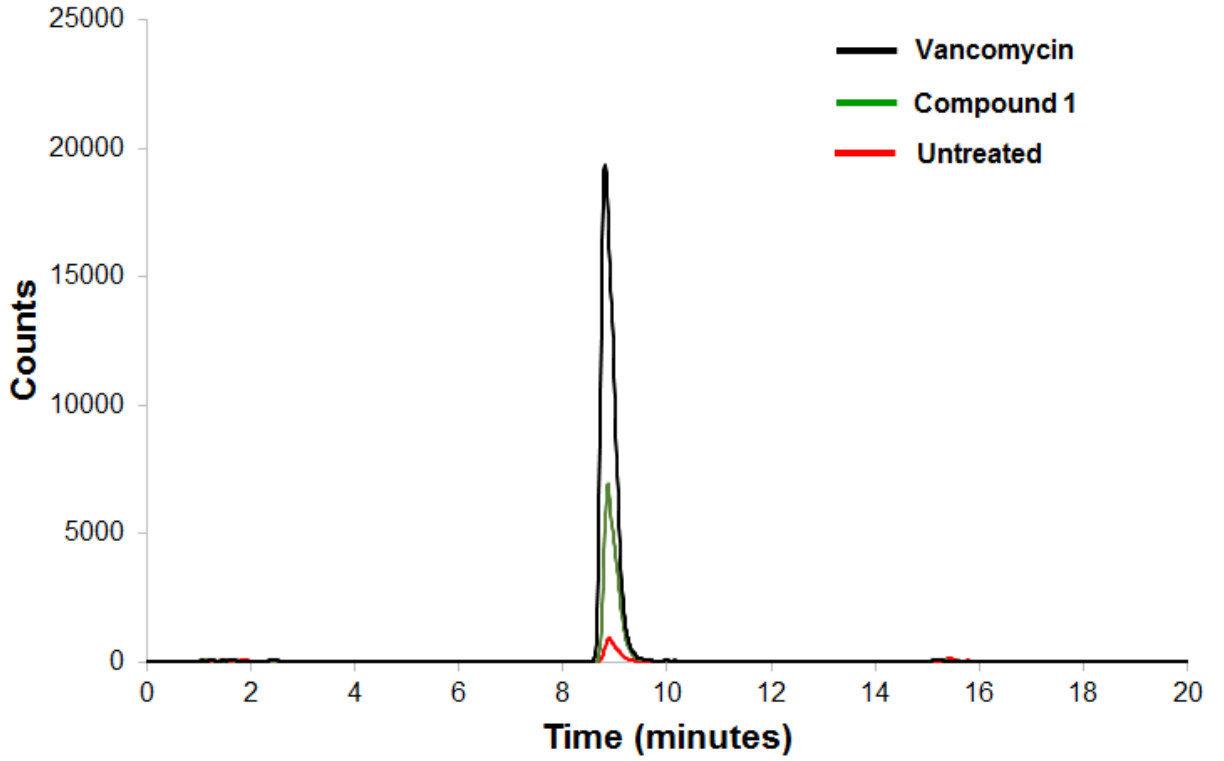

Figure S4. Detection of final soluble cell wall precursor (UDP-*N*-acetylmuramyl pentapeptide) inside bacterial cytoplasm. HPLC chromatogram of *E. faecalis* NR-31975 treated with 10 × MIC of compound **1** or vancomycin for 30 minutes. After centrifugation, the bacterial pellet was boiled for 30 minutes to release contents present in the bacterial cytoplasm. The lysate was analyzed using HPLC/MS to determine the accumulation of the final soluble precursor in cell wall synthesis, UDP-*N*-acetylmuramyl pentapeptide (designated by the black arrows).