Supplemental Table 1

Supplemental Table 1. *P. aeruginosa* strain isolate information.

***P. aeruginosa* Strain**	**Source**
PAO1	Infected wound (ATCC 15692)
PA103	Sputum (ATCC 29260)
H25815	Tracheal aspirate
H28822	Catheterized urine
M57-15	Mucoid CF isolate
M69781	Sputum
T63621	Tracheal aspirate
W40423	Sputum
W41033	Urine
W43532	Bronchial washing

Supplemental Table 2

Target	NCBI Gene ID	Number Designation	Base Sequence (5' to 3')	Location on gene relative to start site	5' End	3' End
AcpP	879895	–0206	CAT ACC TTG TT	-8 to +3	TEG	$(RXR)_4XB$
AcpP	879895	–0307	CTC ATA CCT TG	-6 to +5	H	$(RXR)_4XB$
AcpP	879895	–0445	CTC ATA CCT TG	-6 to +5	$(RXR)_4XB$	H
AcpP	879895	–0446	CTC ATA CCT TG	-6 to +5	$(RGR)_4XB$	H
AcpP	879895	–0447	CTC ATA CCT TG	-6 to +5	$(RFR)_4XB$	H
LpxC	881292	–0251	GTT GTT TGA TC	+3 to +13	$(RXR)_4XB$	H
LpxC	881292	–0505	GTT GTT TGA TC	+3 to +13	TEG	$(RXR)_4XB$
RpsJ	881717	–0066	CCT CAG ACT CC	-15 to -5	$(RXR)_4XB$	H
RpsJ	881717	–0067	CCT CAG ACT CC	-15 to -5	R6G	H
RpsJ	881717	–0207	CCT CAG ACT CC	-15 to -5	TEG	$(RGR)_4XB$
RpsJ	881717	–0208	CCT CAG ACT CC	-15 to -5	TEG	R6G
RpsJ	881717	–0209	GCA TTT GAC CT	-7 to +4	TEG	$(RXR)_4XB$
RpsJ	881717	–0249	GCA TTT GAC CT	-7 to +4	$(RXR)_4XB$	H
RpsJ	881717	–0308	CCT CAG ACT CC	-15 to -5	H	$(RXR)_4XB$
RpsJ	881717	–0371	GCA TTT GAC CT	-7 to +4	R6G	H
RpsJ	881717	–0628	CCT CAG ACT CC	-15 to -5	$(dRdFdF)_3XB$ (D amino acids)	Acetyl
RpsJ	881717	–0629	CCT CAG ACT CC	-15 to -5	$(dRdFdF)_3dRXB$ (D amino acids)	Acetyl
RpsJ	881717	–0630	CCT CAG ACT CC	-15 to -5	$(dRXdR)_4XB$	Acetyl
RpsJ	881717	–0631	CCT CAG ACT CC	-15 to -5	R8B (D amino acids)	Acetyl
RpsJ	881717	–0632	CCT CAG ACT CC	-15 to -5	dR6G (D amino acids)	Acetyl
RpsJ	881717	–1075	CCT CAG ACT CC	-15 to -5	TEG	H
Scr	N/A	–0078	ATC GTT GCA TC	N/A	$(RXR)_4XB$	H
Scr	N/A	–0949	TCT CAG ATG GT	N/A	TEG	$(RXR)_4XB$
Scr	N/A	–1073	TCT CAG ATG GT	N/A	$(RXR)_4XB$	H

Supplemental Table 2. PPMOs.
PPMOs are listed in alphabetical and numerical order. For the location relative to the start site we defined 'A' of ATG as +1. Non-common abbreviations are as follows – X: 6-aminohexanoic acid (aminocaproic acid), B: Beta-alanine (for conjugation), d: prefix for non-natural stereoisomer form of an amino acid, TEG: Triethylene glycol, Scr: Scramble.

Supplemental Figure 1

(a) **MICs of PPMOs in MH II media**

Strain ID	Species	AcpP-0206	AcpP-0445	LpxC-0251	LpxC-0505	RpsJ-0066	RpsJ-0207	RpsJ-0208	RpsJ-0209	RpsJ-0628	RpsJ-0629	RpsJ-0630	RpsJ-0631	RpsJ-0632
PAO1	*P. aeruginosa*	>16	NT	NT	>16	NT	>16	>16	>16	>16	>16	>16	>16	>16
H25815	*P. aeruginosa*	>16	NT	NT	>16	NT	>16	>16	>16	>16	>16	>16	>16	>16
H28822	*P. aeruginosa*	>16	NT	NT	>16	NT	>16	>16	>16	>16	>16	>16	>16	>16
M57-15	*P. aeruginosa*	>16	NT	NT	>16	NT	>16	>16	>16	>16	>16	>16	>16	>16
M69781	*P. aeruginosa*	>16	NT	NT	8	NT	4	>16	>16	>16	>16	>16	>16	>16
T63621	*P. aeruginosa*	>16	NT	NT	>16	NT	>16	>16	>16	>16	>16	>16	>16	>16
W40423	*P. aeruginosa*	>16	NT	NT	>16	NT	>16	>16	>16	>16	>16	>16	>16	>16
W41033	*P. aeruginosa*	>16	NT	NT	>16	NT	>16	>16	>16	>16	>16	>16	>16	>16
W43532	*P. aeruginosa*	>16	NT	NT	>16	NT	>16	>16	>16	>16	>16	>16	>16	>16
PPMO MIC_{50} [μM]		>16	NT	NT	>16	NT	>16	>16	>16	>16	>16	>16	>16	>16

Key

NT	Not Tested
>16	>16 μM
16	16 μM
8	8 μM
4	4 μM
2	2 μM
1	1 μM
≤0.5	≤0.5 µM

(b) **MICs of PPMOs in MH II media with 1μg/ml Colistin**

Strain ID	Species	AcpP-0206	AcpP-0445	LpxC-0251	LpxC-0505	RpsJ-0066	RpsJ-0207	RpsJ-0208	RpsJ-0209	RpsJ-0628	RpsJ-0629	RpsJ-0630	RpsJ-0631	RpsJ-0632	Colistin MIC (μg/mL)
PAO1	*P. aeruginosa*	8	NT	NT	2	NT	8	>16	>16	8	>16	2	8	4	4
H25815	*P. aeruginosa*	≤0.5	NT	NT	≤0.5	NT	≤0.5	16	16	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	8
H28822	*P. aeruginosa*	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	2
M57-15	*P. aeruginosa*	1	NT	NT	≤0.5	NT	>16	4	≤0.5	>16	>16	>16	>16	1	4
M69781	*P. aeruginosa*	≤0.5	NT	NT	≤0.5	NT	>16	1	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	8
T63621	*P. aeruginosa*	4	NT	NT	2	NT	>16	8	2	4	8	2	2	2	2
W40423	*P. aeruginosa*	4	NT	NT	2	NT	>16	16	2	8	16	2	2	4	8
W41033	*P. aeruginosa*	≤0.5	NT	NT	≤0.5	NT	16	2	≤0.5	≤0.5	8	≤0.5	≤0.5	1	4
W43532	*P. aeruginosa*	2	NT	NT	≤0.5	NT	≤0.5	≤0.5	≤0.5	>16	>16	>16	>16	2	4
PPMO MIC_{50} [μM]		1	NT	NT	≤0.5	NT	16	4	≤0.5	4	8	2	2	1	4

(c) **MICs of PPMOs in MH II media with 2μg/ml PMBN**

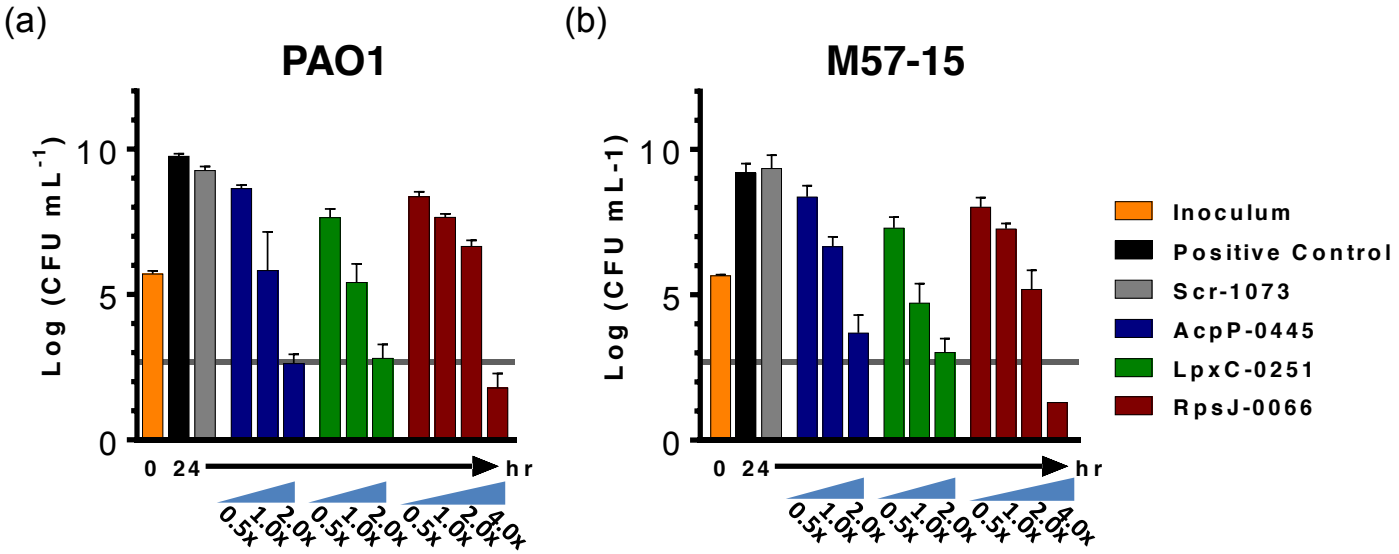
Strain ID	Species	AcpP-0206	AcpP-0445	LpxC-0251	LpxC-0505	RpsJ-0066	RpsJ-0207	RpsJ-0208	RpsJ-0209	RpsJ-0628	RpsJ-0629	RpsJ-0630	RpsJ-0631	RpsJ-0632	PMBN MIC (μg/mL)
PAO1	*P. aeruginosa*	4	2	2	2	2	>16	>16	4	>16	>16	4	8	>16	>16
H25815	*P. aeruginosa*	≤0.25	≤0.5	1	1	≤0.5	≤0.5	1	≤0.5	2	4	≤0.5	1	1	>16
H28822	*P. aeruginosa*	16	2	2	1	8	>16	>16	4	>16	>16	4	16	>16	>16
M57-15	*P. aeruginosa*	2	1	2	2	2	>16	>16	16	>16	>16	4	8	>16	>16
M69781	*P. aeruginosa*	8	1	2	≤0.5	2	1	1	≤0.5	8	16	≤0.5	≤0.5	4	4
T63621	*P. aeruginosa*	8	1	2	8	2	>16	>16	8	>16	>16	4	8	>16	4
W40423	*P. aeruginosa*	4	1	8	8	4	>16	>16	16	>16	>16	8	>16	>16	4
W41033	*P. aeruginosa*	4	1	4	16	2	4	>16	16	>16	>16	8	8	16	>16
W43532	*P. aeruginosa*	4	1	4	4	2	16	16	16	>16	>16	16	16	>16	>16
PPMO MIC_{50} [μM]		4	1	2	2	2	>16	>16	4	>16	>16	4	8	>16	>16

(d) **MICs of PPMOs in MOPS media**

Strain ID	Species	AcpP-0206	AcpP-0445	LpxC-0251	LpxC-0505	RpsJ-0066	RpsJ-0207	RpsJ-0208	RpsJ-0209	RpsJ-0628	RpsJ-0629	RpsJ-0630	RpsJ-0631	RpsJ-0632
PAO1	*P. aeruginosa*	1	1	2	4	1	16	>16	8	>16	>16	4	8	>16
H25815	*P. aeruginosa*	2	2	4	2	1	16	8	8	>16	>16	2	2	4
H28822	*P. aeruginosa*	4	1	2	4	1	8	>16	4	>16	>16	8	8	>16
M57-15	*P. aeruginosa*	2	1	2	4	≤0.5	8	>16	8	>16	>16	4	8	16
M69781	*P. aeruginosa*	4	1	1	2	≤0.5	1	4	1	>16	>16	2	1	4
T63621	*P. aeruginosa*	1	1	4	4	1	16	>16	4	>16	>16	4	2	>16
W40423	*P. aeruginosa*	≤0.5	1	1	1	≤0.5	1	2	1	>16	>16	1	2	1
W41033	*P. aeruginosa*	4	2	4	8	1	8	>16	4	>16	>16	8	2	>16
W43532	*P. aeruginosa*	2	1	2	8	≤0.5	16	>16	4	>16	>16	8	4	>16
PPMO MIC_{50} [μM]		2	1	2	4	1	8	>16	4	>16	>16	4	2	>16

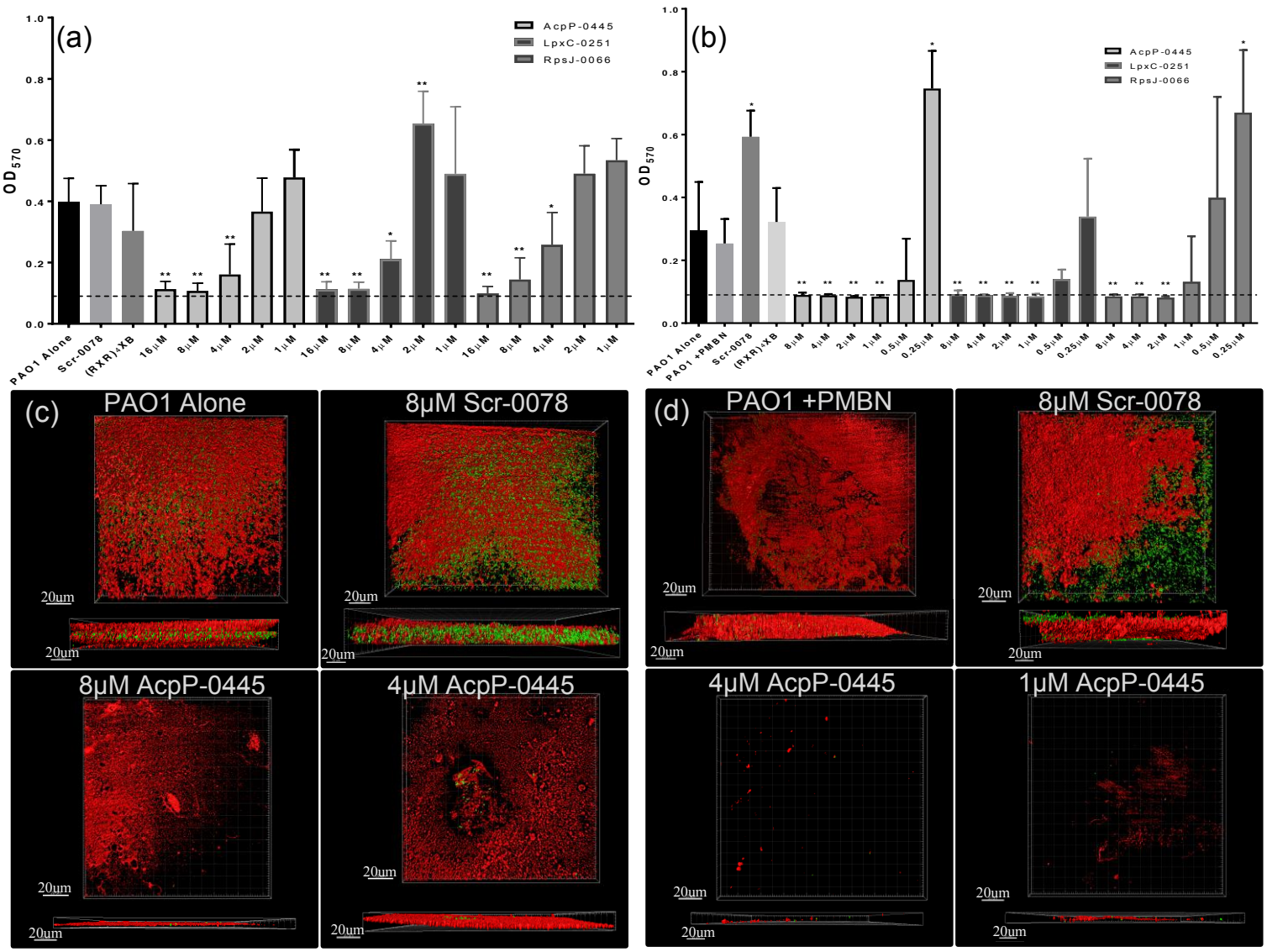
Supplemental Figure 1. Heatmaps of *P. aeruginosa* growth inhibition by PPMOs.
Heatmaps of PPMO MICs in *P. aeruginosa* strains grown in MHII (a), MHII in the presence of sub-inhibitory concentrations of colistin (b), MHII in the presence of sub-inhibitory concentrations of PMBN (c), or MOPS minimal media (d) . Tested PPMOs had minimal activity against *Pseudomonas* grown in MHII alone but the addition of sub-inhibitory concentrations of colistin, PMBN, or growth in MOPS resulted in enhanced antimicrobial inhibition.

Supplemental Figure 2



Supplemental Figure 2: Lead PPMOs against *P. aeruginosa* are bactericidal.
MBC assays of lead PPMOs against PAO1 **(a)** and M57-15 **(b)**, at 0.5-, 1-, 2-, and 4-times the MIC. Grey-line underlays represent a 3-log CFU reduction as compared to starting inoculum. Positive control is 24 hr culture growth without any treatment. These data are combined results from at least 3 experiments.

Supplemental Figure 3



Supplemental Figure 3: PPMO treatment prevents formation of *P. aeruginosa* biofilm.
PAO1-GFP was grown in MBEC plates in the conditions indicated. Optical density measurements of crystal violet stained biofilm formations grown in MHII **(a)** or in MHII with 2 μg/mL PMBN **(b)**. Dashed line represents the lower limit of detection. This data is combined from 3 independent experiments, error bars represent standard deviation, and statistics were done with a one-way ANOVA and Holm-Šídák's multiple comparisons test where ** $p < 0.0001$ and * $p < 0.05$ is indicated for **(a)** as compared to PAO1 Alone **(a)** and as compared PAO1+PMBN **(b)**. Spinning disk confocal microscopy images of MBEC pegs are depicted from above and the side incubated in MHII **(c),** or MHII with 2 μg/mL PMBN **(d)**. Green indicates PAO1-GFP and red is biofilm stained with Concanavalin A-Alexafluor 647.

Supplemental Figure 4

	P. aeruginosa Strain				*P. aeruginosa* Strain		
PPMO (µM)	**PAO1**	**M57-15**	**W43532**	**Antibiotic (µg/mL)**	**PAO1**	**M57-15**	**W43532**
AcpP-0445	8	8	4	**Pip/Tazo**	4.00	4.00	4.00
LpxC-0251	16	16	8	**Tobramycin**	0.50	0.50	0.50
RpsJ-0066	8	32	32	**Levofloxacin**	0.50	0.25	0.25
Scr-1073	>128	>128	>128	**Colistin**	4.00	4.00	4.00

Supplemental Figure 4. MICs of PPMOs and Antibiotics used in Synergy assays.
Three strains of *P. aeruginosa*, PAO1, M57-15, and W43532 were utilized to examine if PPMOs could act favorably with current therapeutic antibiotics (Figure 5); these data are the individual MICs of compounds tested. Data shown here is representative of 3 independent experiments.