

Figure S1

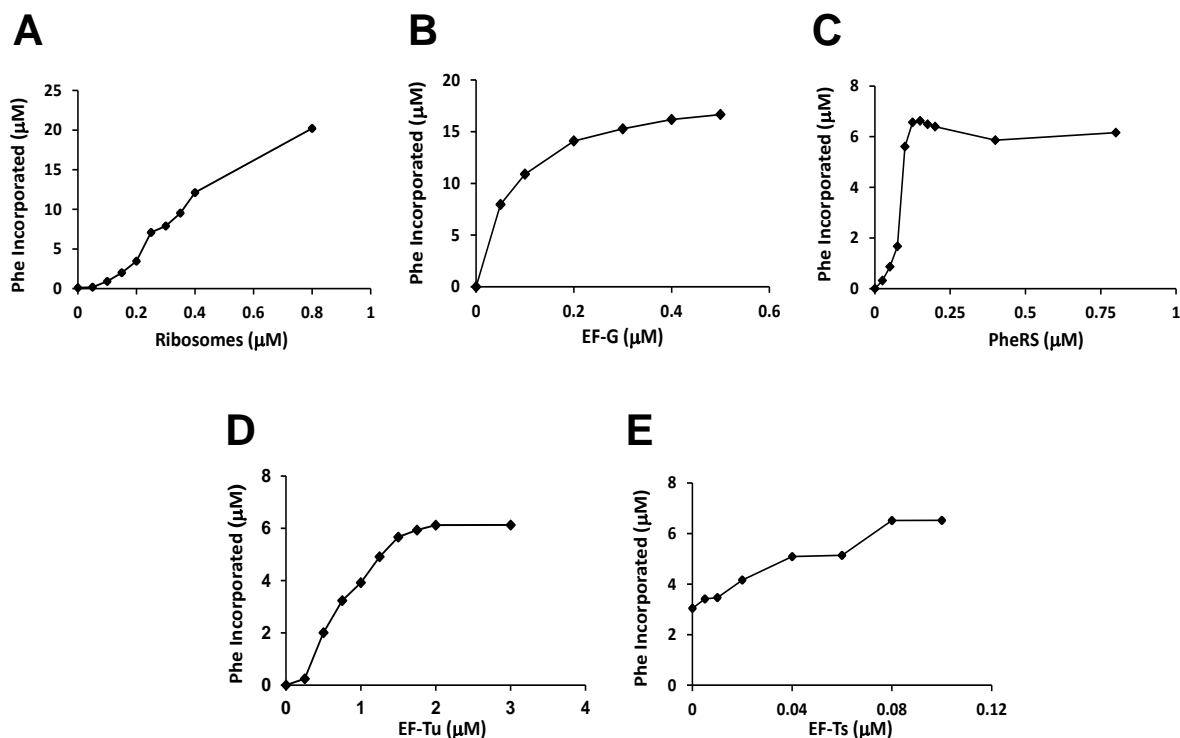


FIGURE S1. Optimization of the *P. aeruginosa* coupled aminoacylation/translation (A/T) assay. (A) Titration of *P. aeruginosa* ribosomes in the A/T protein synthesis system assay. Ribosomes (0.05 to 0.8 μM) were assayed in the presence of saturating amounts of the other components. (B) Plot of poly-Phe synthesis as a function of increasing concentrations of *P. aeruginosa* EF-G (0.05 to 0.5 μM) in the AT system. (C) Plot of poly-Phe synthesis as a function of increasing concentrations of *P. aeruginosa* PheRS (0.025 to 0.8 μM) in the AT system. (D) Plot of poly-Phe synthesis as a function of increasing concentrations of *P. aeruginosa* EF-Tu (0.25 to 3.0 μM) in the AT system. (E) Plot of poly-Phe synthesis as a function of increasing concentrations of *P. aeruginosa* EF-Ts (0.005 to 0.1 μM) in the AT system. All data points represent the average of the results of assays carried out in triplicate.

Figure S2

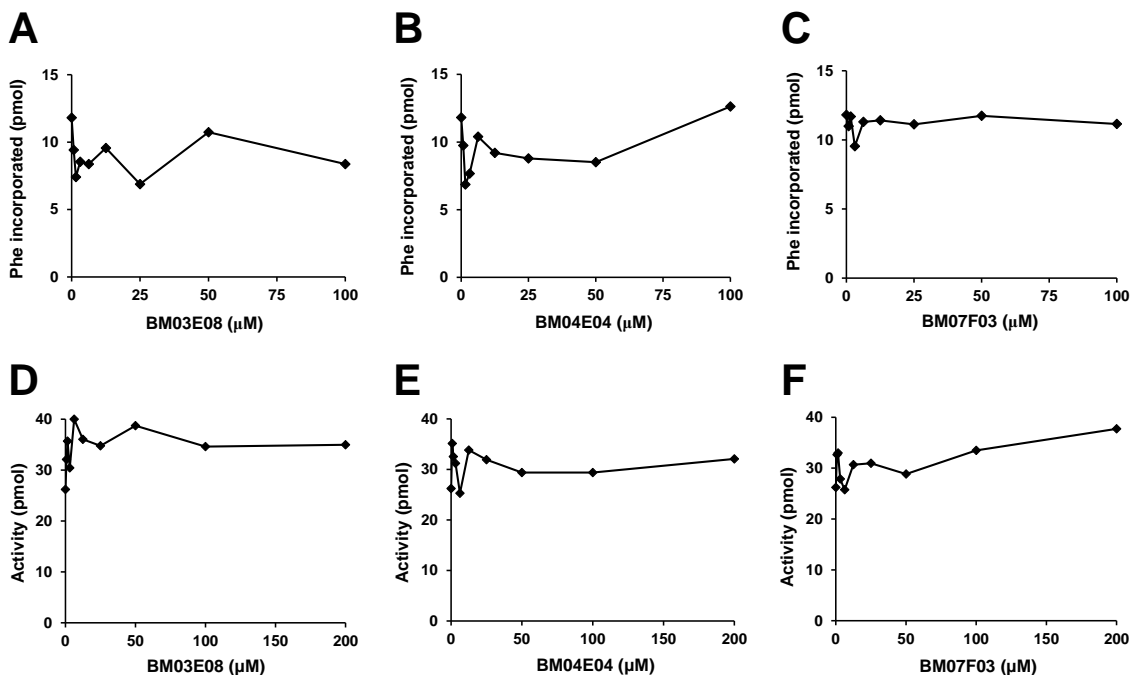


FIGURE S2. Compounds specifically inhibit bacterial protein synthesis. First, to determine if the compounds inhibit protein synthesis in eukaryotic protein synthesis, the compounds (A) BM03E08, (B) BM04E04, and (C) BM07F03 were titrated into optimized wheat germ cell extract assays. “Phe incorporated” represents the pmol of phenylalanine polymerized during protein synthesis. To determine the effect of the compounds on human mitochondrial PheRS (hmPheRS) the compounds (D) BM03E08, (E) BM04E04, and (F) BM07F03 were titrated into aminoacylation assays. The aminoacylation assays contained 50 pmol of tRNA^{Phe}. “Activity” is indicative of pmol of tRNA^{Phe} aminoacylated. The compound concentrations ranged from 0.8 to 200 μM in all assays.

Table S1. Minimum inhibitory concentration of quality control antibiotics against the panel of ten pathogenic bacteria.

Organism	QC Agent	Average MIC ($\mu\text{g/ml}$)
<i>E. coli</i>	Ampicillin	6.3 ¹
<i>E. coli tolC</i>	Ampicillin	1.7
<i>E. faecalis</i>	Vancomycin	3.8
<i>H. influenzae</i>	Ampicillin	0.5
<i>M. catarrhalis</i>	Ampicillin	1.2
<i>P. aeruginosa</i>	Tetracycline	14.7
<i>P. aeruginosa</i> PA0200	Tetracycline	0.5
<i>P. aeruginosa</i> hypersensitive	Tetracycline	0.25
<i>S. aureus</i>	Oxacillin	0.4
<i>S. pneumoniae</i>	Penicillin	1

¹ The average of MIC values for the quality control antibiotics are from numerous MIC determination completed in our laboratory.