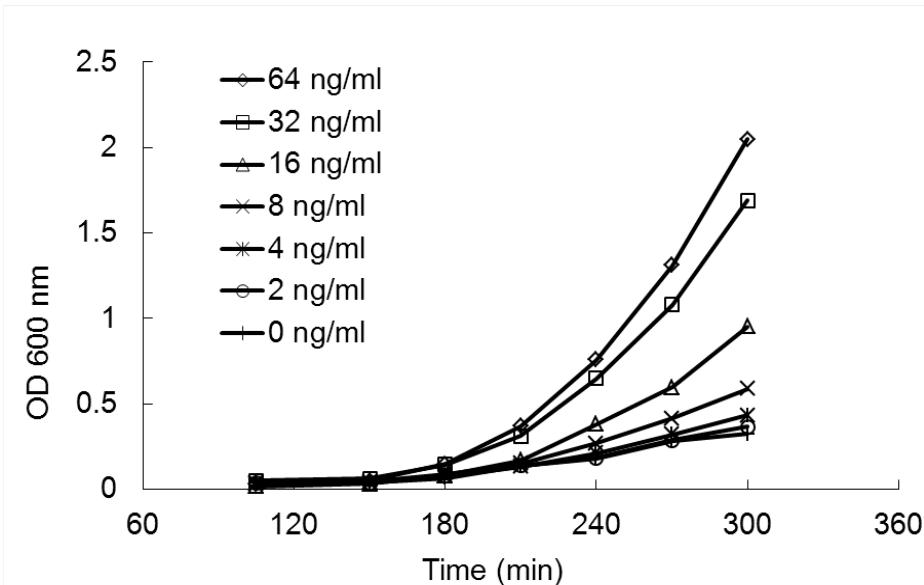


## SUPPORTING INFORMATION

### The Mechanism of Action of the Arylomycin Antibiotics and the Effects of Signal Peptidase I Inhibition

Peter A. Smith and Floyd E. Romesberg\*

Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, USA



**FIG. S1.** Regulated ectopic expression of *E. coli* SPase protein in cells lacking the chromosomally encoded SPase gene (strain PAS0162, see main text). Cells were grown overnight in Luria broth media containing 8 ng/mL aTc (to produce a minimal amount of SPase required for survival), and sub-cultured into fresh media containing the indicated concentrations of aTc. Cell growth was followed by measuring optical density at 600 nm.

(A)

gentamicin								rifampin								cephalexin										
	0	1/16	1/8	1/4	1/2	1	2	4		0	1/16	1/8	1/4	1/2	1	2	4		0	1/16	1/8	1/4	1/2	1	2	4
4	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	
2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	
1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
1/2	5	3	2	0	0	0	0	0	1/2	5	4	2	0	0	0	0	0	1/2	5	5	4	2	0	0	0	
1/4	5	5	4	3	0	0	0	0	1/4	5	5	5	0	0	0	0	0	1/4	5	5	5	4	1	0	0	
1/8	5	5	5	4	0	0	0	0	1/8	5	5	5	2	0	0	0	0	1/8	5	5	5	5	2	0	0	
1/16	5	5	5	4	2	0	0	0	1/16	5	5	5	5	3	0	0	0	1/16	5	5	5	5	3	0	0	
0	5	5	5	5	5	0	0	0	0	5	5	5	5	5	0	0	0	0	5	5	5	5	5	0	0	

## tetracycline

	0	1/16	1/8	1/4	1/2	1	2	4
4	0	0	0	0	0	0	0	0
2	0	0	1	1	0	0	0	0
1	0	5	5	4	2	0	0	0
1/2	5	5	5	5	4	0	0	0
1/4	5	5	5	5	5	0	0	0
1/8	5	5	5	5	5	0	0	0
1/16	5	5	5	5	5	0	0	0
0	5	5	5	5	5	0	0	0

### **erythromycin**

	0	1/16	1/8	1/4	1/2	1	2	4
4	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
1/2	4	5	4	4	1	0	0	0
1/4	5	5	5	5	3	0	0	0
1/8	5	5	5	5	5	0	0	0
1/16	5	5	5	5	5	0	0	0
0	5	5	5	5	5	0	0	0

## polymyxin B

	0	$1/16$	$1/8$	$1/4$	$1/2$	1	2	4
4	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
$1/2$	5	3	3	2	1	0	0	0
$1/4$	5	5	5	4	3	0	0	0
$1/8$	5	5	5	4	4	0	0	0
$1/16$	5	5	5	5	5	0	0	0
0	5	5	5	5	5	0	0	0

## trime th o prim

	0	1/16	1/8	1/4	1/2	1	2	4
4	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
1	0	1	2	0	0	0	0	0
1/2	5	5	5	3	1	0	0	0
1/4	5	5	5	5	3	0	0	0
1/8	5	5	5	5	4	0	0	0
1/16	5	5	5	5	4	0	0	0
0	5	5	5	5	5	0	0	0

## ciprofloxacin

	0	1/16	1/8	1/4	1/2	1	2	4
4	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0
1/2	5	5	5	5	0	0	0	0
1/4	5	5	5	5	1	0	0	0
1/8	5	5	5	5	5	0	0	0
1/16	5	5	5	5	5	0	0	0
0	5	5	5	5	5	0	0	0

(B)

gentamicin								rifampin								cephalexin										
	0	1/16	1/8	1/4	1/2	1	2	4		0	1/16	1/8	1/4	1/2	1	2	4		0	1/8	1/4	1/2	1	2	4	8
4	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	
2	0	0	0	0	0	0	0	0	2	0	1	1	1	0	0	0	0	2	0	0	0	0	0	0	0	
1	0	0	0	0	0	0	0	0	1	0	3	4	3	0	0	0	0	1	0	0	0	0	0	0	0	
1/2	5	0	0	0	0	0	0	0	1/2	5	5	5	5	5	0	0	0	1/2	5	2	2	0	0	0	0	
1/4	5	5	5	5	1	0	0	0	1/4	5	5	5	5	5	0	0	0	1/4	5	5	5	1	0	0	0	
1/8	5	5	5	3	0	0	0	0	1/8	5	5	5	5	5	0	0	0	1/8	5	5	5	5	1	0	0	
1/16	5	5	5	4	2	0	0	0	1/16	5	5	5	5	5	0	0	0	1/16	5	5	5	5	5	1	0	
0	5	5	5	5	5	0	0	0	0	5	5	5	5	5	0	0	0	0	5	5	5	5	0	0	0	

## tetraacycline

	0	1/16	1/8	1/4	1/2	1	2	4
4	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0
1/2	5	5	4	0	0	0	0	0
1/4	5	5	5	5	3	0	0	0
1/8	5	5	5	5	5	0	0	0
1/16	5	5	5	5	5	0	0	0
0	5	5	5	5	5	0	0	0

## erythromycin

	0	1/16	1/8	1/4	1/2	1	2	4
4	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
1/2	5	4	2	1	0	0	0	0
1/4	5	5	5	5	1	0	0	0
1/8	5	5	5	5	5	0	0	0
1/16	5	5	5	5	5	0	0	0
0	5	5	5	5	5	0	0	0

## vancomycin

	0	1/16	1/8	1/4	1/2	1	z	4
4	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
1/2	5	5	5	4	0	0	0	0
1/4	5	5	5	5	1	0	0	0
1/8	5	5	5	5	5	0	0	0
1/16	5	5	5	5	5	0	0	0
0	5	5	5	5	5	0	0	0

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	0	1/16	1/8	1/4	1/2	1	2	4
4	0	0	0	0	0	0	0	0
2	0	1	1	1	0	0	0	0
1	0	2	2	4	1	0	0	0
1/2	5	5	5	5	4	0	0	0
1/4	5	5	5	5	5	0	0	0
1/8	5	5	5	5	5	0	0	0
1/16	5	5	5	5	5	0	0	0
0	5	5	5	5	5	0	0	0

### ciprofloxacin

	0	1/16	1/8	1/4	1/2	1	2	4
4	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0
1/2	5	5	5	4	0	0	0	0
1/4	5	5	5	5	0	0	0	0
1/8	5	5	5	5	1	0	0	0
1/16	5	5	5	5	3	0	0	0
0	5	5	5	5	5	0	0	0

**FIG. S2.** Summary of checkerboard MIC experiments measuring the activity of arylomycin A-C<sub>16</sub> in combination with other antibiotics against *E. coli* (A) and *S. aureus* (B). Antibiotic concentrations are presented relative to the MIC. Each number represents the total number of replicates from five independent experiments that grew at the indicated combination of antibiotic concentrations. In each checkerboard, the arylomycin concentration is indicated on vertical axis and that of the second agent is indicated on the horizontal axis.