

# An Image-Based 384-Well High-Throughput Screening Method for the Discovery of Biofilm Inhibitors in *Vibrio cholerae*.

## SUPPORTING INFORMATION

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**Supplementary Table S1:** List of All Bacterial Mutant Strains.

Strain	Genotype
Fy Vc 2	<i>Vibrio cholerae</i> O1 El Tor A1552, rugose variant, Rif <sup>r</sup> <sup>1</sup>
Fy Vc 240	Fy Vc 2 mTn7-GFP, Rif <sup>r</sup> Gm <sup>r</sup> <sup>2</sup>
Fy Vc 234	Fy Vc 2 Δvps-I, rugose variant with deletion of vpsA-K, Rif <sup>r</sup> <sup>3</sup>
Fy Vc 519	Fy Vc 234 mTn7-GFP, Rif <sup>r</sup> Gm <sup>r</sup>
Fy Vc 105	Fy Vc 2 ΔrbmA, Rif <sup>r</sup> <sup>4</sup>
Fy Vc 224	Fy Vc 105 mTn7-GFP, Rif <sup>r</sup> Gm <sup>r</sup> <sup>4</sup>
Fy Vc 1400	Fy Vc 2 ΔrbmC Δbap1, Rif <sup>r</sup> <sup>5</sup>
Fy Vc 1395	Fy Vc 1400 mTn7-GFP, Gm <sup>r</sup> <sup>5</sup>
Fy Vc 5	Fy Vc 2 ΔvpsT ΔlacZ, Rif <sup>r</sup> <sup>6</sup>
Fy Vc 1956	Fy Vc 5 mTn7-GFP, Rif <sup>r</sup> Gm <sup>r</sup> <sup>2</sup>
Fy Vc 6	Fy Vc 2 ΔvpsR ΔlacZ, Rif <sup>r</sup> <sup>6</sup>
Fy Vc 1955	Fy Vc 6 mTn7-GFP, Rif <sup>r</sup> Gm <sup>r</sup> <sup>2</sup>
Fy Vc 1745	Fy Vc 2 ΔvpvC, Rif <sup>r</sup> <sup>7</sup>
Fy Vc 1224	Fy Vc 1745 mTn7-GFP, Rif <sup>r</sup> Gm <sup>r</sup> <sup>2</sup>
Fy Vc 345	Fy Vc 2 ΔcdgC, Rif <sup>r</sup> <sup>7</sup>
Fy Vc 361	Fy Vc 345 mTn7-GFP, Gm <sup>r</sup> , Rif <sup>r</sup> <sup>7</sup>

<sup>1</sup> Yildiz, F. H., Schoolnik, G. K. (1999) *Vibrio cholerae* O1 El Tor: identification of a gene cluster required for the rugose colony type, exopolysaccharide production, chlorine resistance, and biofilm formation. *Proc. Natl. Acad. Sci. USA* 96, 4028-4033.

<sup>2</sup> Beyhan, S., Bilecen, K., Salama, S. R., Casper-Lindley, C., Yildiz, F. H. (2007) Regulation of rugosity and biofilm formation in *Vibrio cholerae*: comparison of VpsT and VpsR regulons and epistasis analysis of vpsT, vpsR, and hapR. *J. Bacteriol.* 189, 388-402.

<sup>3</sup> Beyhan, S., Yildiz, F. H. (2007) Smooth to rugose phase variation in *Vibrio cholerae* can be mediated by a single nucleotide change that targets c-di-GMP signalling pathway. *Mol. Microbiol.* 63, 995-1007.

<sup>4</sup> Fong, J. C. N., Karplus, K., Schoolnik, G. K., Yildiz, F. H. (2006) Identification and characterization of RbmA, a novel protein required for the development of rugose colony morphology and biofilm structure in *Vibrio cholerae*. *J. Bacteriol.* 188, 1049-1059.

<sup>5</sup> Fong, J. C. N., Yildiz, F. H. (2007) The rbmBCDEF gene cluster modulates development of rugose colony morphology and biofilm formation in *Vibrio cholerae*. *J. Bacteriol.* 189, 2319-2330.

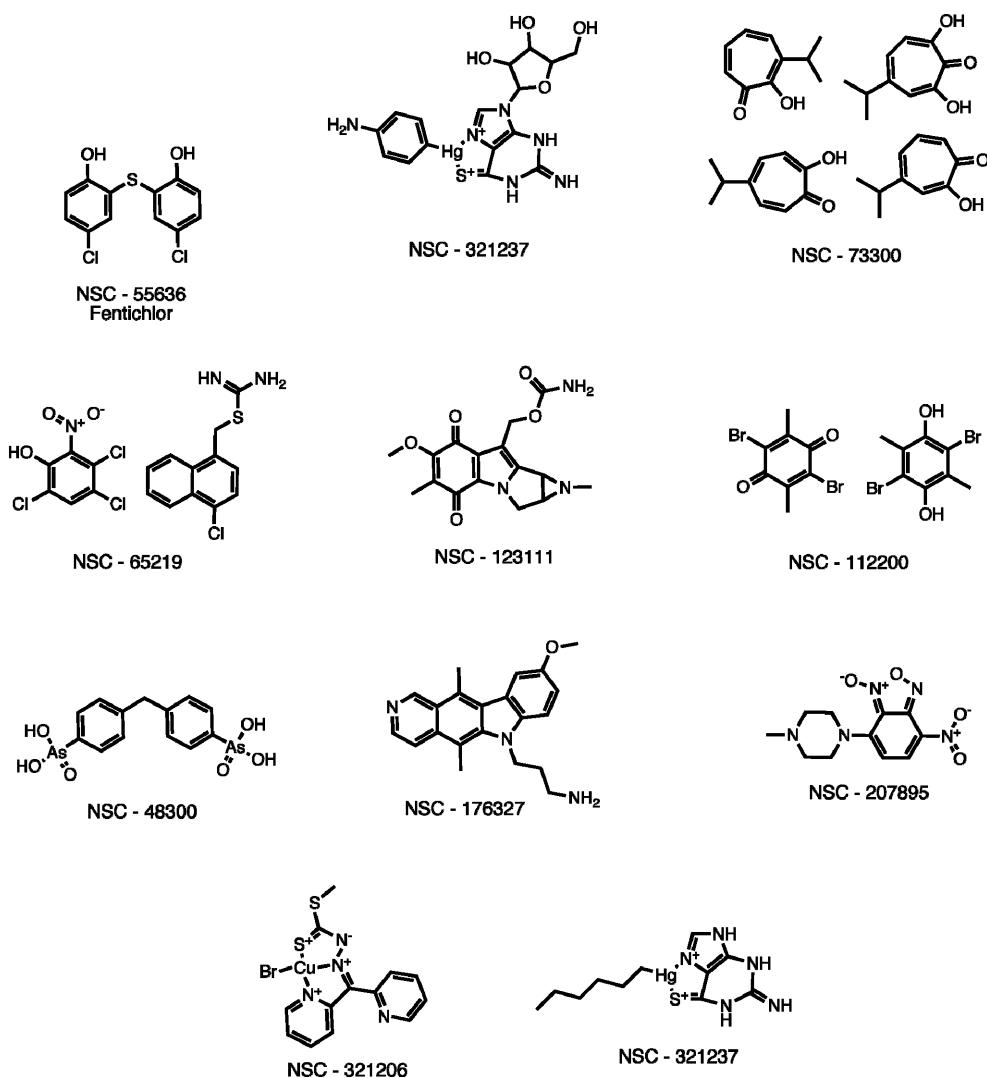
<sup>6</sup> Casper-Lindley, C., Yildiz, F. H. (2004) VpsT is a transcriptional regulator required for expression of vps biosynthesis genes and the development of rugose colonial morphology in *Vibrio cholerae* O1 El Tor. *J. Bacteriol.* 186, 1574-1578.

<sup>7</sup> Lim, B., Beyhan, S., Meir, J., Yildiz, F. H. (2006) Cyclic-di-GMP signal transduction systems in *Vibrio cholerae*: modulation of rugosity and biofilm formation. *Mol. Microbiol.* 60, 331-348.

**Supplementary Figure S1:** Structures of Antibiotic Leads from the NCI Structural Diversity Set.

**Structural Diversity Set Antibiotics:**

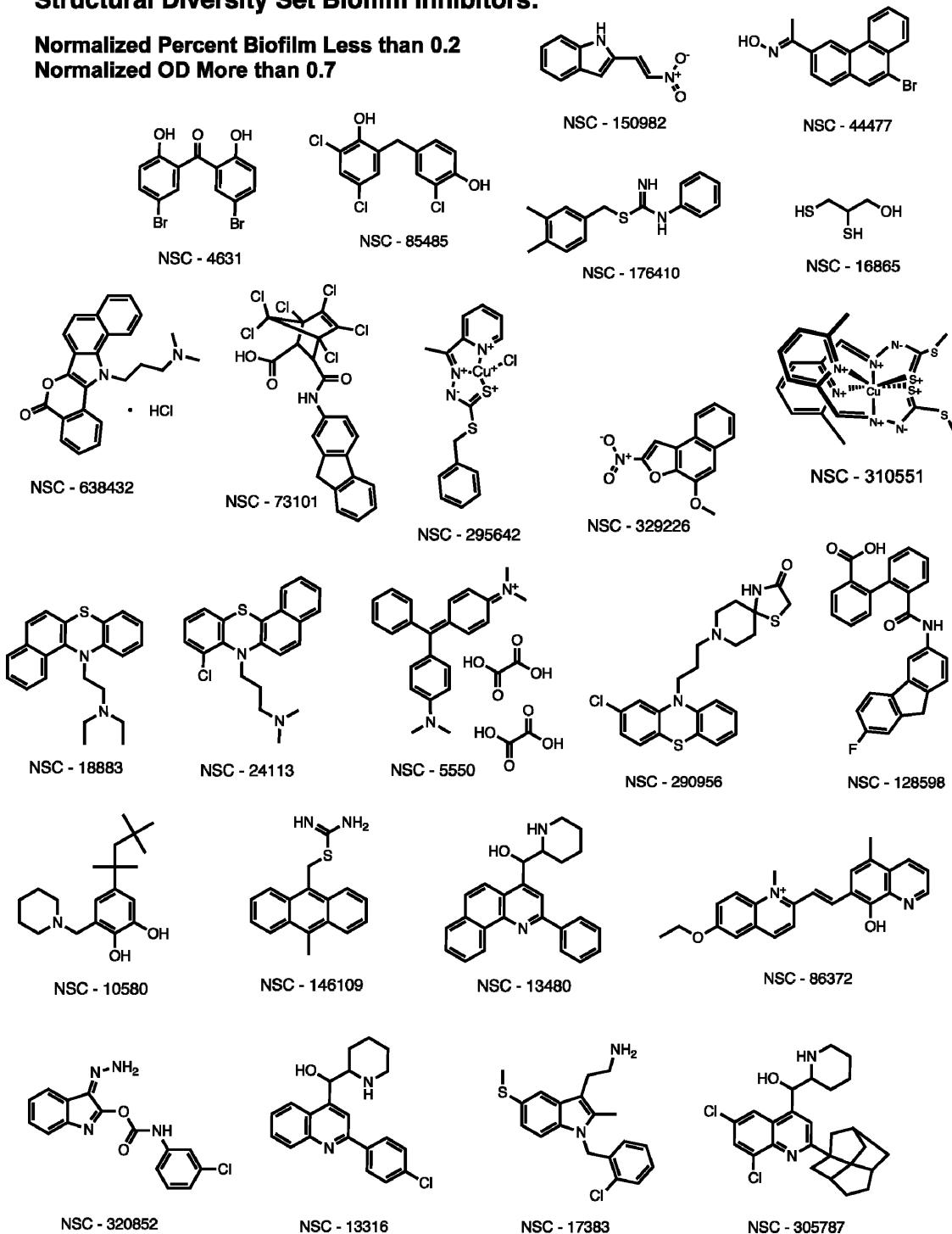
**Normalized Percent Biofilm Less than 0.2  
Normalized OD Less than 0.7**



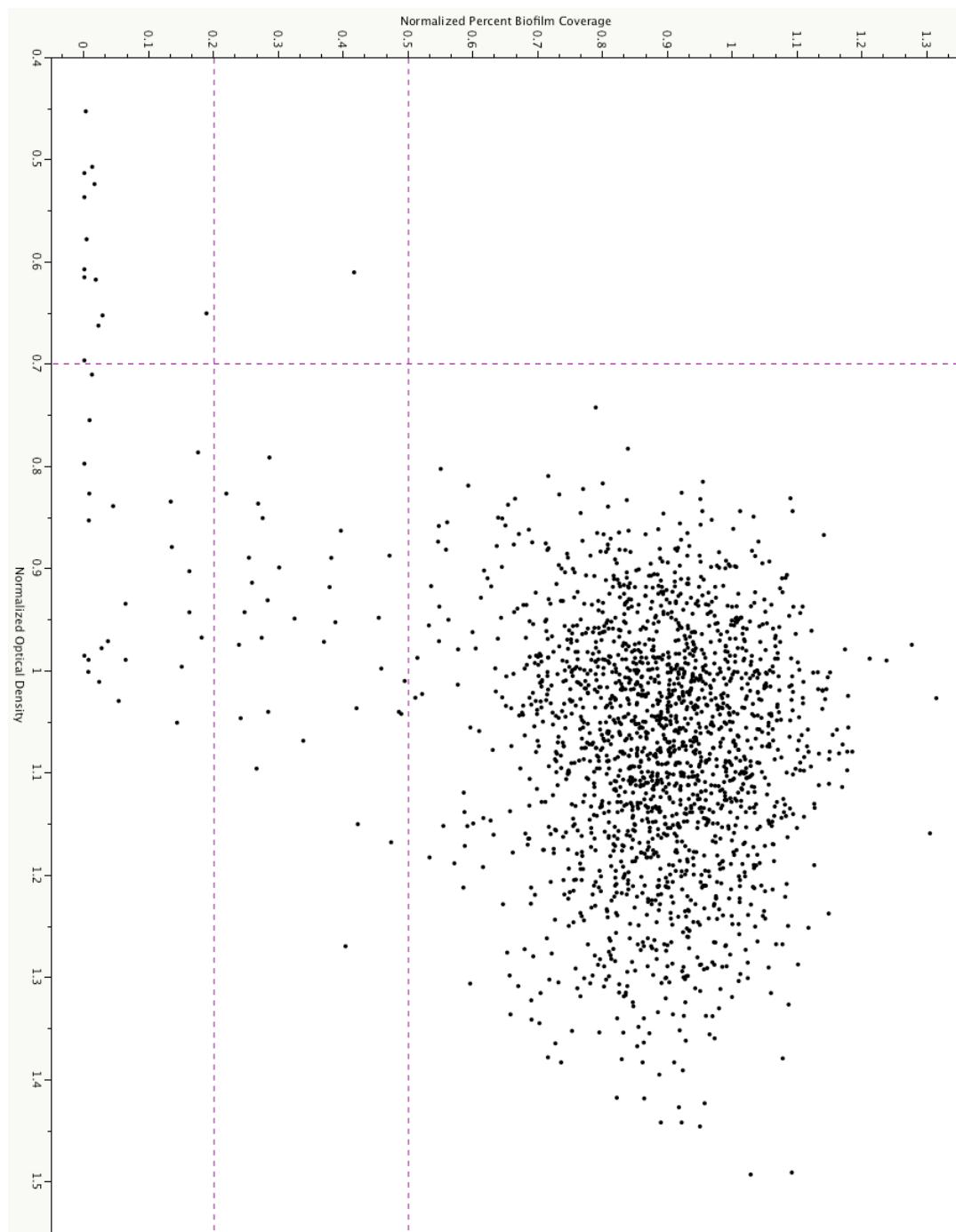
**Supplementary Figure S2:** Structures of Biofilm Inhibitors from the NCI Structural Diversity Set.

**Structural Diversity Set Biofilm Inhibitors:**

**Normalized Percent Biofilm Less than 0.2  
Normalized OD More than 0.7**



**Supplementary Figure S3:** Plot of Screening Results for the NCI Structural Diversity Set.



**Supplementary Table S2:** Table of Hits for the NCI Structural Diversity Set.

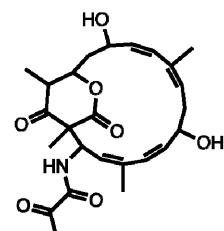
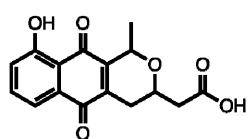
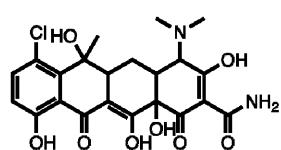
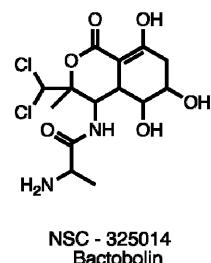
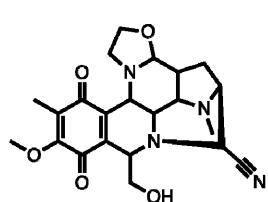
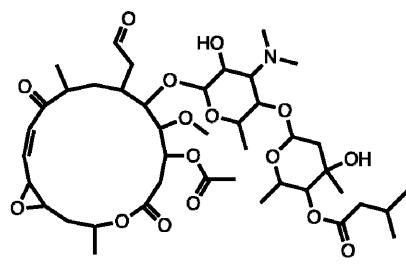
Structural Diversity Set Hits		
<b>Normalized % biofilm coverage &lt; 0.2</b>		<b>Normalized % biofilm coverage &lt; 0.5, &gt; than 0.2</b>
NSC	OD	% Biofilm Coverage
4631	0.986	0.000
320852	0.798	0.000
55636	0.513	0.000
123111	0.697	0.000
268879	0.608	0.000
321206	0.615	0.000
321237	0.537	0.000
321239	0.453	0.002
176327	0.578	0.003
310551	1.001	0.006
290956	0.990	0.006
17385	0.853	0.007
305787	0.827	0.008
146109	0.755	0.008
16865	0.711	0.012
48300	0.507	0.012
329226	0.524	0.016
112200	0.618	0.018
207895	0.663	0.022
128598	1.011	0.023
13480	0.978	0.027
73300	0.653	0.028
86372	1.728	0.028
85485	0.971	0.036
10580	0.839	0.044
348401	1.030	0.053
24113	0.935	0.064
5550	0.990	0.064
638432	0.835	0.133
13316	0.879	0.135
44477	1.051	0.143
295642	0.996	0.150
73101	0.943	0.162
18883	0.903	0.162
150982	0.787	0.175
176410	0.968	0.181
65219	0.651	0.188
92585	0.827	0.219
177407	0.975	0.238
17600	1.047	0.241
205842	0.943	0.247
97345	0.890	0.254
2805	0.914	0.259
159628	1.096	0.266
65238	0.837	0.268
19630	0.968	0.273
48171	0.851	0.275
309881	0.931	0.283
228148	1.041	0.284
40787	0.792	0.285
169804	0.899	0.300
77552	0.949	0.324
117028	1.069	0.338
48881	0.972	0.370
40792	0.918	0.378
169466	0.890	0.381
48708	0.953	0.387
371777	0.863	0.396
96255	1.270	0.403
121145	0.611	0.416
69575	1.037	0.420
150432	1.150	0.422
322661	0.948	0.454
77554	0.998	0.458
35950	0.888	0.471
201868	1.168	0.473
403822	1.041	0.485
35489	1.043	0.489
306960	1.010	0.494

**Supplementary Figure S4:** Structures of Antibiotic Leads and Biofilm Inhibitors from the NCI Natural Products Set.

## Natural Products Set

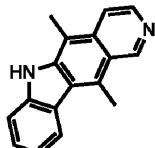
### Antibiotics:

**Normalized Percent Biofilm Less than 0.2  
Normalized OD Less than 0.7**

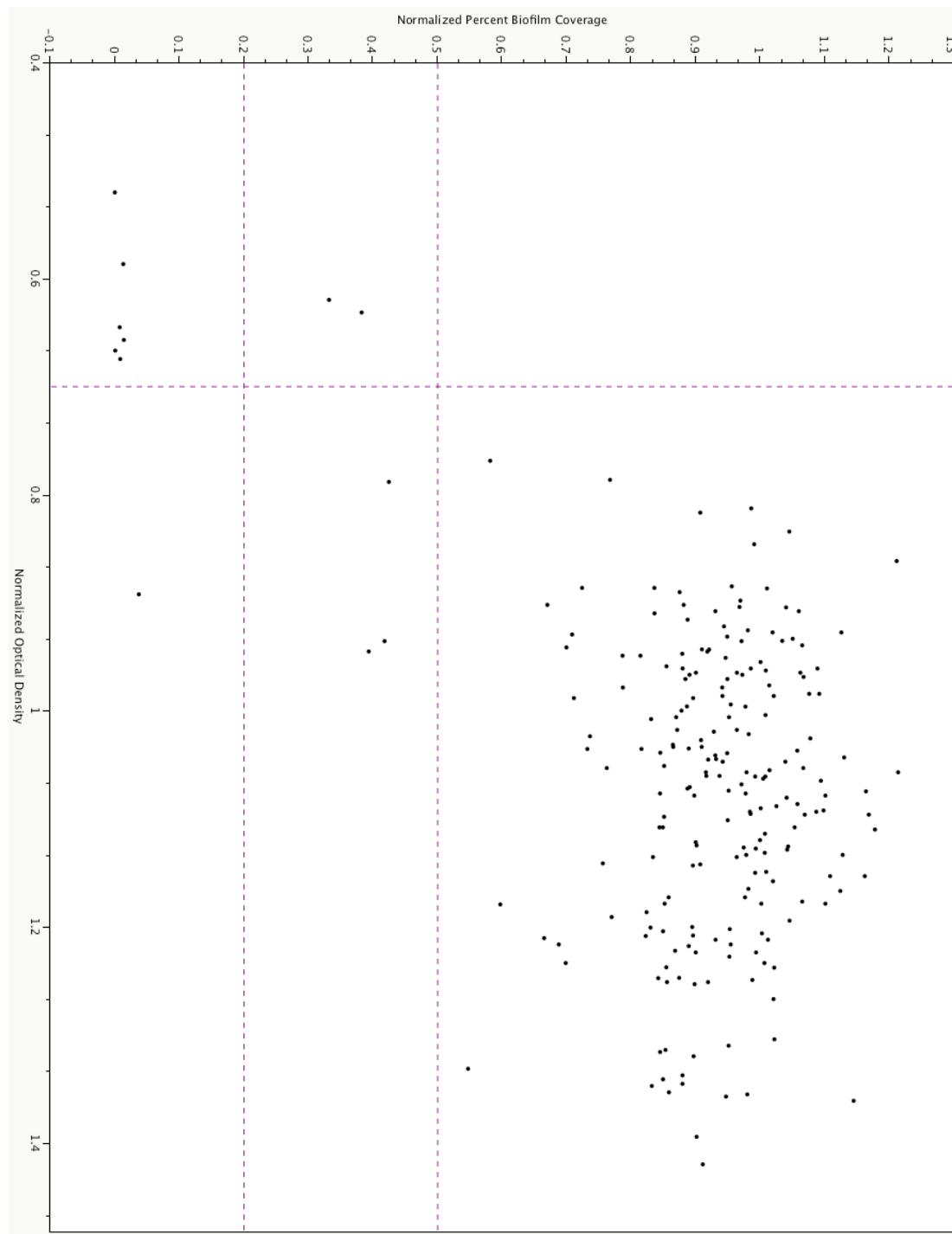


### Biofilm inhibitors:

**Normalized Percent Biofilm Less than 0.2  
Normalized OD More than 0.7**



**Supplementary Figure S5:** Plot of Screening Results for the NCI Structural Diversity Set.



**Supplementary Table S3:** Table of Hits for the NCI Natural Products Set.

Natural Product Set Hits		
Normalized % biofilm less than 0.2		
NSC	Norm. OD	Norm. Percent Biofilm Coverage
13252	0.520	0.000
267461	0.667	0.001
325014	0.645	0.008
145118	0.675	0.008
51001	0.587	0.013
349644	0.657	0.014
71795	0.892	0.037

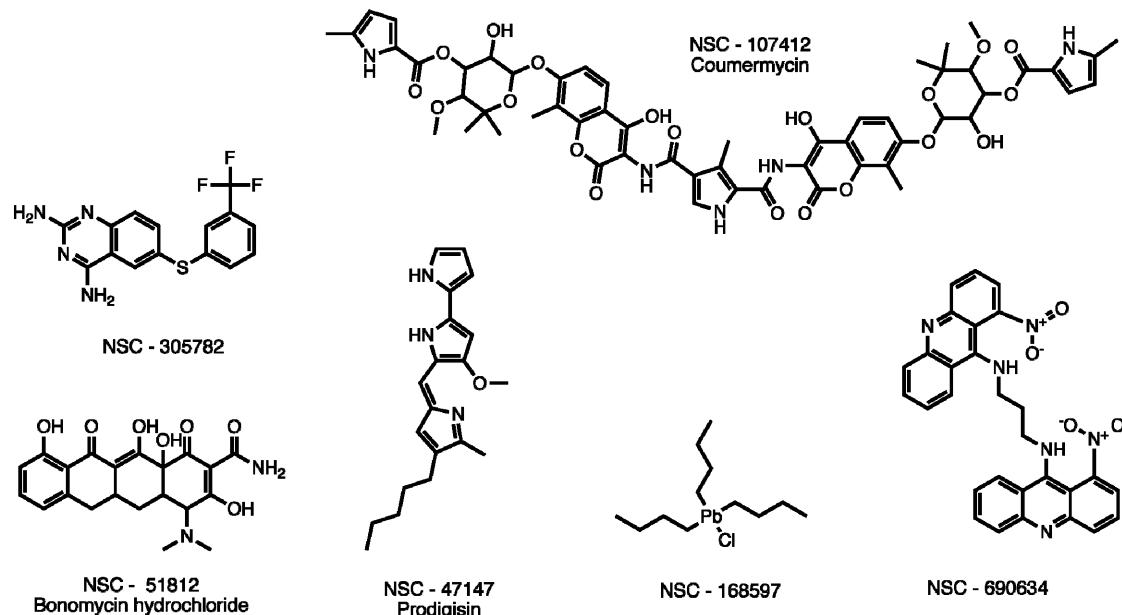
Normalized % biofilm less than 0.5, greater than 0.2		
NSC	Norm. OD	Norm. Percent Biofilm Coverage
45383	0.620	0.332
526417	0.631	0.382
312033	0.945	0.393
180973	0.936	0.418
106408	0.788	0.424
177406	1.889	0.445

**Supplementary Figure S6:** Structures of Antibiotic Leads and Biofilm Inhibitors from the NCI Mechanistic Set.

## Mechanistic Set

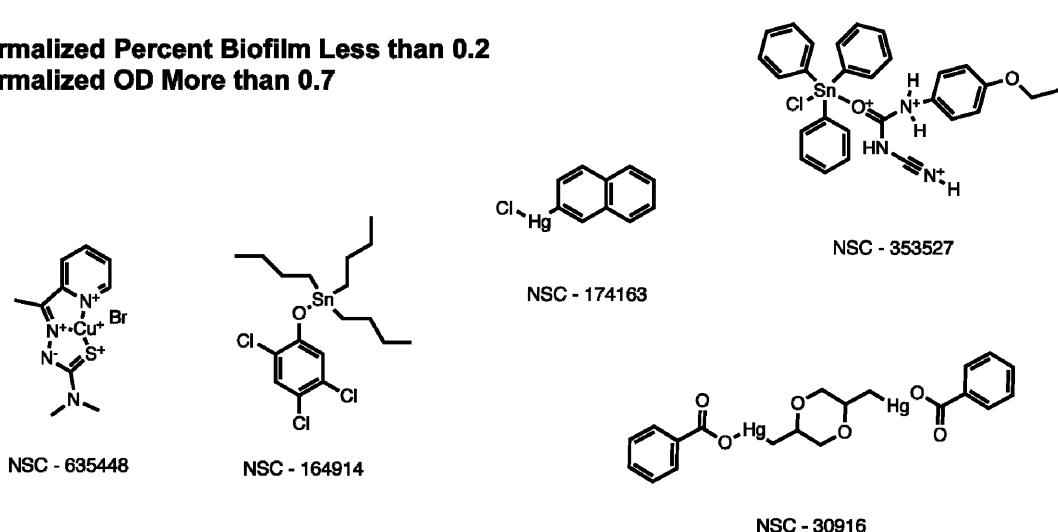
### Antibiotics:

**Normalized Percent Biofilm Less than 0.2  
Normalized OD Less than 0.7**

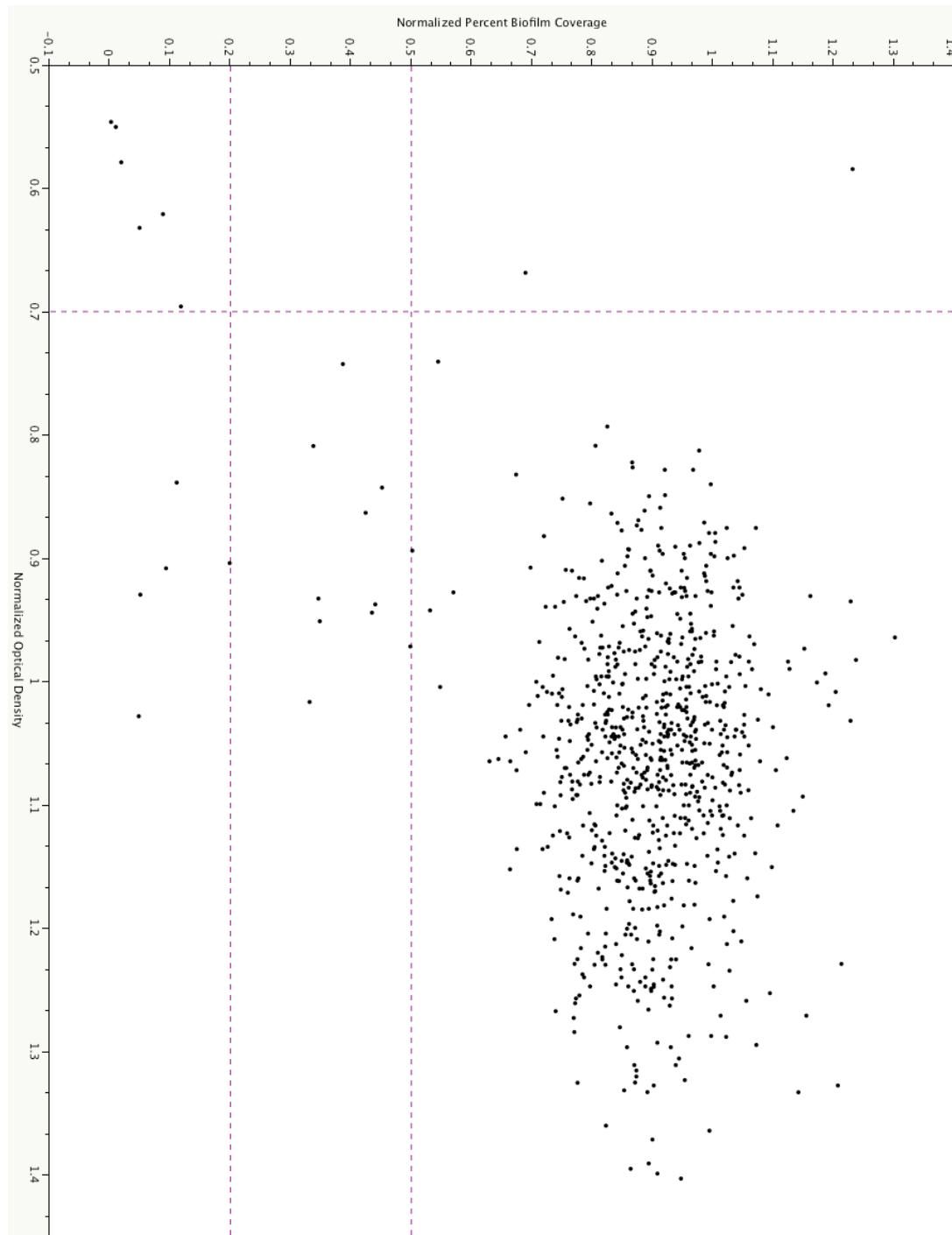


### Biofilm inhibitors:

**Normalized Percent Biofilm Less than 0.2  
Normalized OD More than 0.7**



**Supplementary Figure S7:** Plot of Screening Results for the NCI Mechanistic Set.



**Supplementary Table S4:** Table of Hits for the NCI Mechanistic Set.

Mechanistic Set Hits		
Normalized % biofilm less than 0.2		
NSC	Norm. OD	Norm. Percent Biofilm Coverage
168597	0.546152	0.002056
305782	0.550243	0.009938
51812	0.57888	0.01893
353527	1.028193	0.047967
47147	0.632063	0.049282
164914	0.929574	0.05057
107412	0.620934	0.088093
174163	0.908208	0.093183
635448	0.83866	0.110862
690634	0.695811	0.117842
30916	0.904006	0.198673

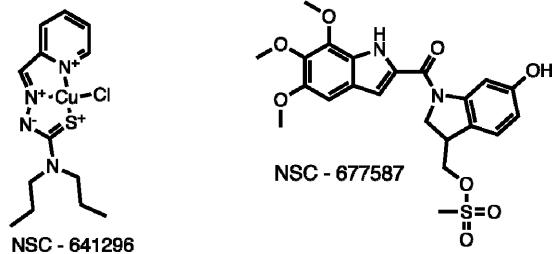
Normalized % biofilm less than 0.5, greater than 0.2		
NSC	Norm. OD	Norm. Percent Biofilm Coverage
631529	1.01662	0.331247
253995	0.80904	0.337428
313981	0.932754	0.345839
618332	0.951163	0.348146
329279	0.742521	0.386434
631521	0.863206	0.424164
96932	0.944184	0.434672
697923	0.937451	0.440122
44690	0.842751	0.45136
634503	0.971619	0.498237

**Supplementary Figure S8:** Structures of Antibiotic Leads and Biofilm Inhibitors from the NCI Challenge Set.

## Challenge Set

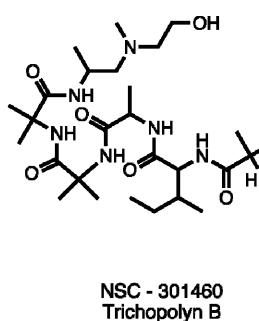
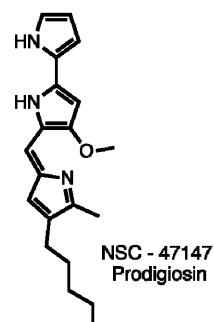
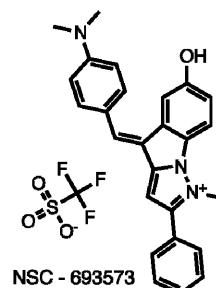
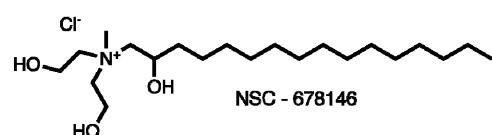
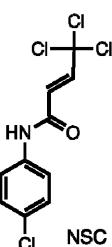
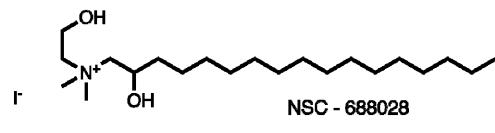
### Antibiotics:

**Normalized Percent Biofilm Less than 0.2  
Normalized OD Less than 0.7**

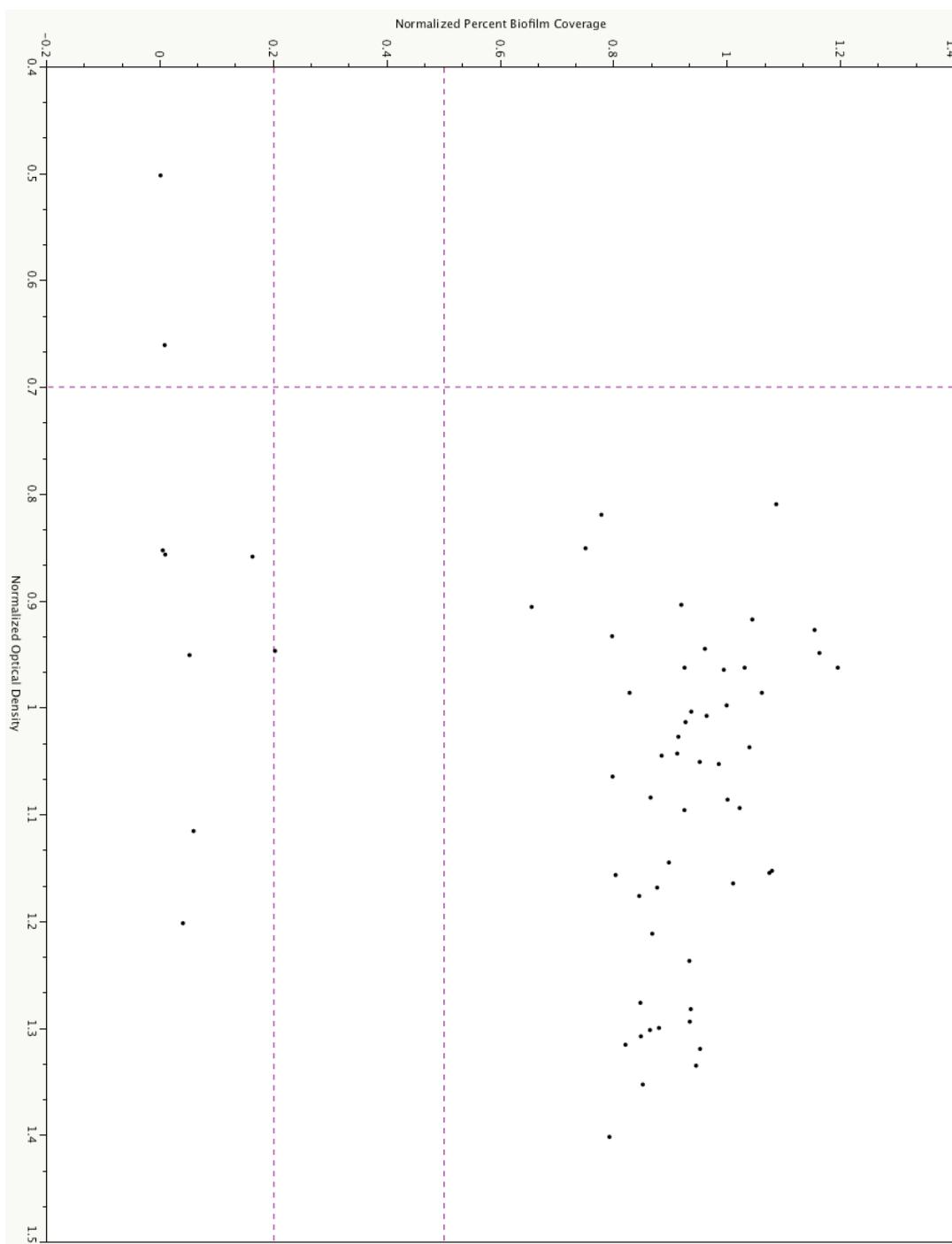


### Biofilm Inhibitors:

**Normalized Percent Biofilm Less than 0.2  
Normalized OD More than 0.7**



**Supplementary Figure S9:** Plot of Screening Results for the NCI Challenge Set.



**Supplementary Table S5:** Table of Hits for the NCI Challenge Set.

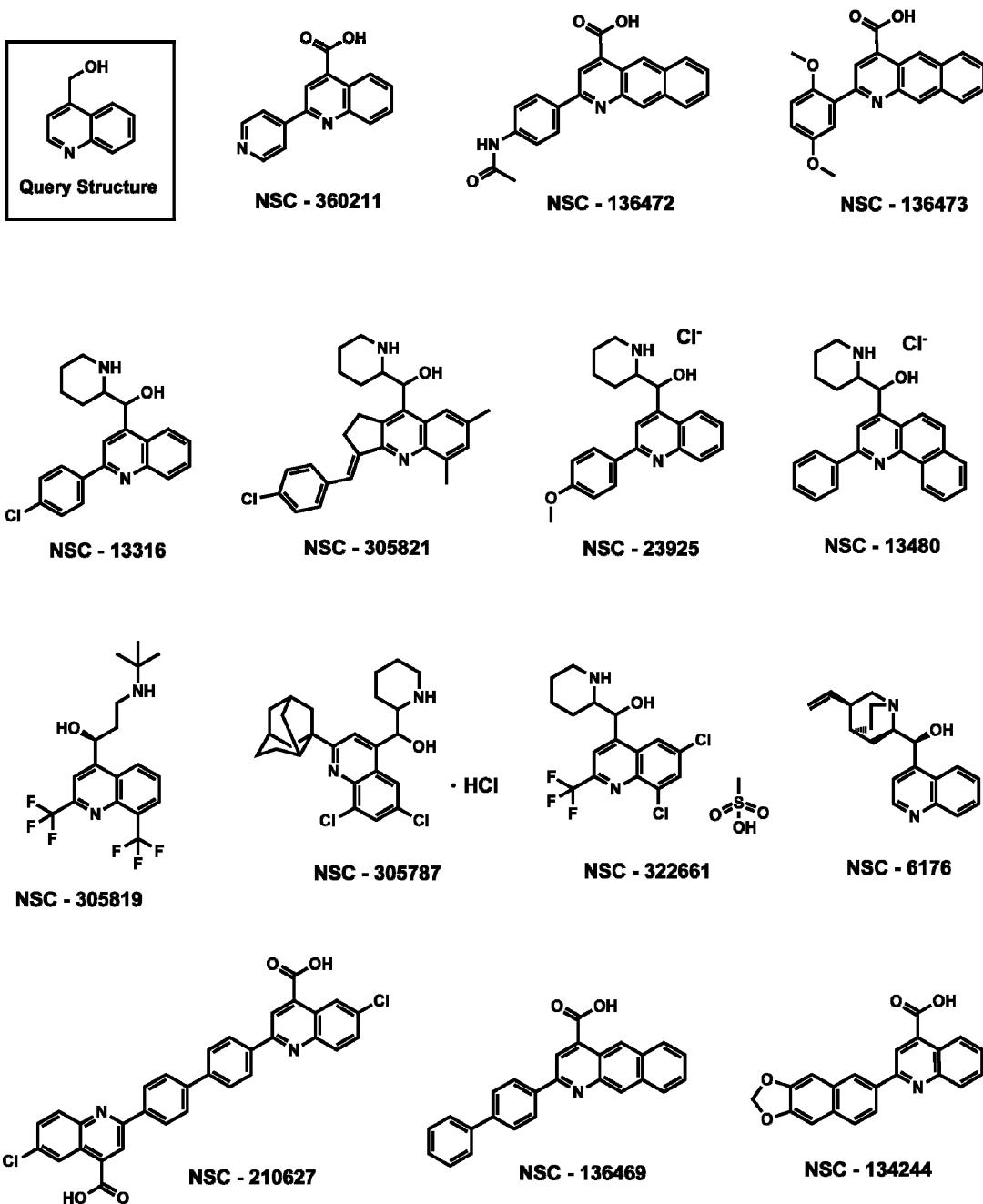
Challenge Set Hits		
Normalized % biofilm less than 0.2		
NSC	Norm. OD	Norm. Percent Biofilm Coverage
641296	0.502	0.000
47147	0.859	0.004
677587	0.661	0.007
688028	0.857	0.008
301460	1.202	0.040
678146	0.951	0.051
693573	1.116	0.058
604586	0.859	0.162

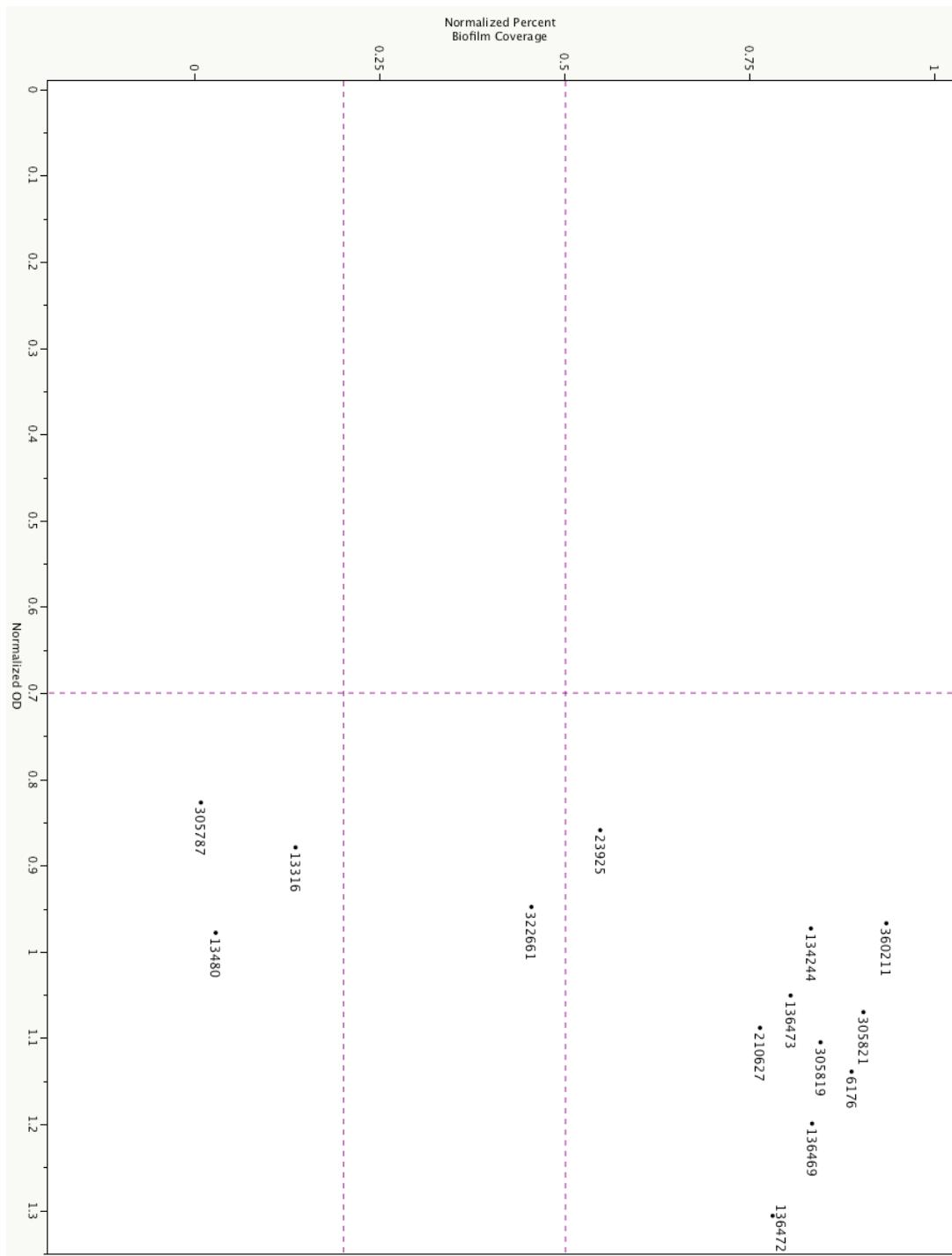
Normalized % biofilm less than 0.5, greater than 0.2		
NSC	Norm. OD	Norm. Percent Biofilm Coverage
170984	0.947	0.202

**Supplementary Figure S9:** Substructure Search Results of the Mefloquine-Like Query Structure for all NCI Libraries.

**Substructure Search of Four NCI Libraries:**



## **Supplementary Figure S10:** Plot of Screening Results for Substructure Search Compounds.



**Supplementary Table S6:** Table of Hits from the Substructure Search Results Compound Set.

Screening Data For Substructure Search Results		
NSC	Normalized Percent Biofilm Coverage	Normalized OD
360211	0.934	0.967
6176	0.887	1.139
13316	0.135	0.879
134244	0.832	0.973
23925	0.547	0.859
136472	0.780	1.306
136473	0.805	1.051
136469	0.834	1.199
13480	0.027	0.978
305821	0.903	1.070
305787	0.007	0.827
305819	0.845	1.105
322661	0.454	0.948
210627	0.763	1.088