

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 ^r ¹
Fy_Vc_240	Fy_Vc_2 mTn7-GFP, Rif ^r Gm ^r ²
Fy_Vc_234	Fy_Vc_2 Δvps -I, rugose variant with deletion of <i>vpsA-K</i> , Rif ^r ³
Fy_Vc_519	Fy_Vc_234 mTn7-GFP, Rif ^r Gm ^r
Fy_Vc_105	Fy_Vc_2 $\Delta rbmA$, Rif ^r ⁴
Fy_Vc_224	Fy_Vc_105 mTn7-GFP, Rif ^r Gm ^r ⁴
Fy_Vc_1400	Fy_Vc_2 $\Delta rbmC \Delta bap1$, Rif ^r ⁵
Fy_Vc_1395	Fy_Vc_1400 mTn7-GFP, Gm ^r ⁵
Fy_Vc_5	Fy_Vc_2 $\Delta vpsT \Delta lacZ$, Rif ^r ⁶
Fy_Vc_1956	Fy_Vc_5 mTn7-GFP, Rif ^r Gm ^r ²
Fy_Vc_6	Fy_Vc_2 $\Delta vpsR \Delta lacZ$, Rif ^r ⁶
Fy_Vc_1955	Fy_Vc_6 mTn7-GFP, Rif ^r Gm ^r ²
Fy_Vc_1745	Fy_Vc_2 $\Delta vpvC$, Rif ^r ⁷
Fy_Vc_1224	Fy_Vc_1745 mTn7-GFP, Rif ^r Gm ^r ²
Fy_Vc_345	Fy_Vc_2 $\Delta cdgC$, Rif ^r ⁷
Fy_Vc_361	Fy_Vc_345 mTn7-GFP, Gm ^r , Rif ^r ⁷

¹ 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.

² 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.

³ 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.

⁴ 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.

⁵ 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.

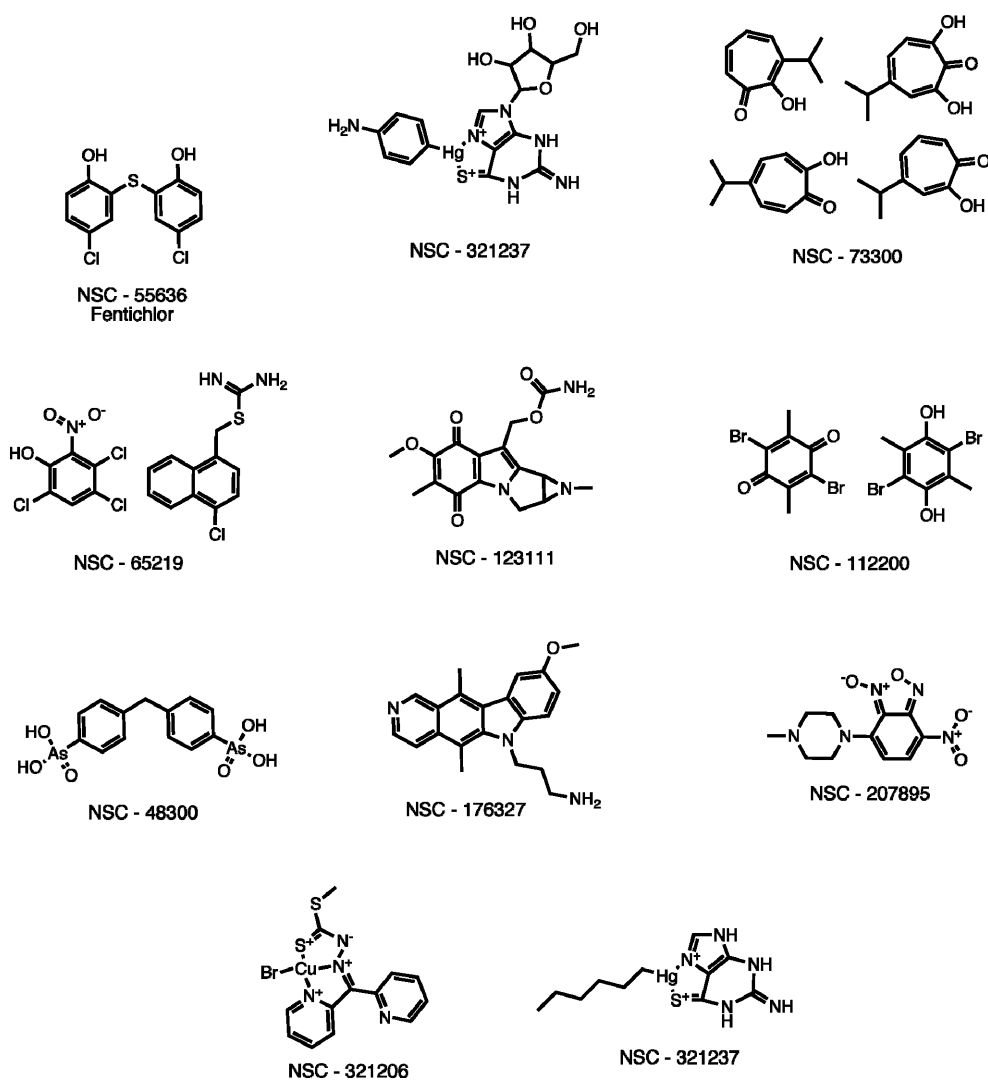
⁶ Casper-Lindley, C., Yildiz, F. H. (2004) VpsT is a transcriptional regulator required for expression of *vps* biosynthesis genes and the development of rugose colonial morphology in *Vibrio cholerae* O1 El Tor, *J. Bacteriol.* 186, 1574-1578.

⁷ 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:

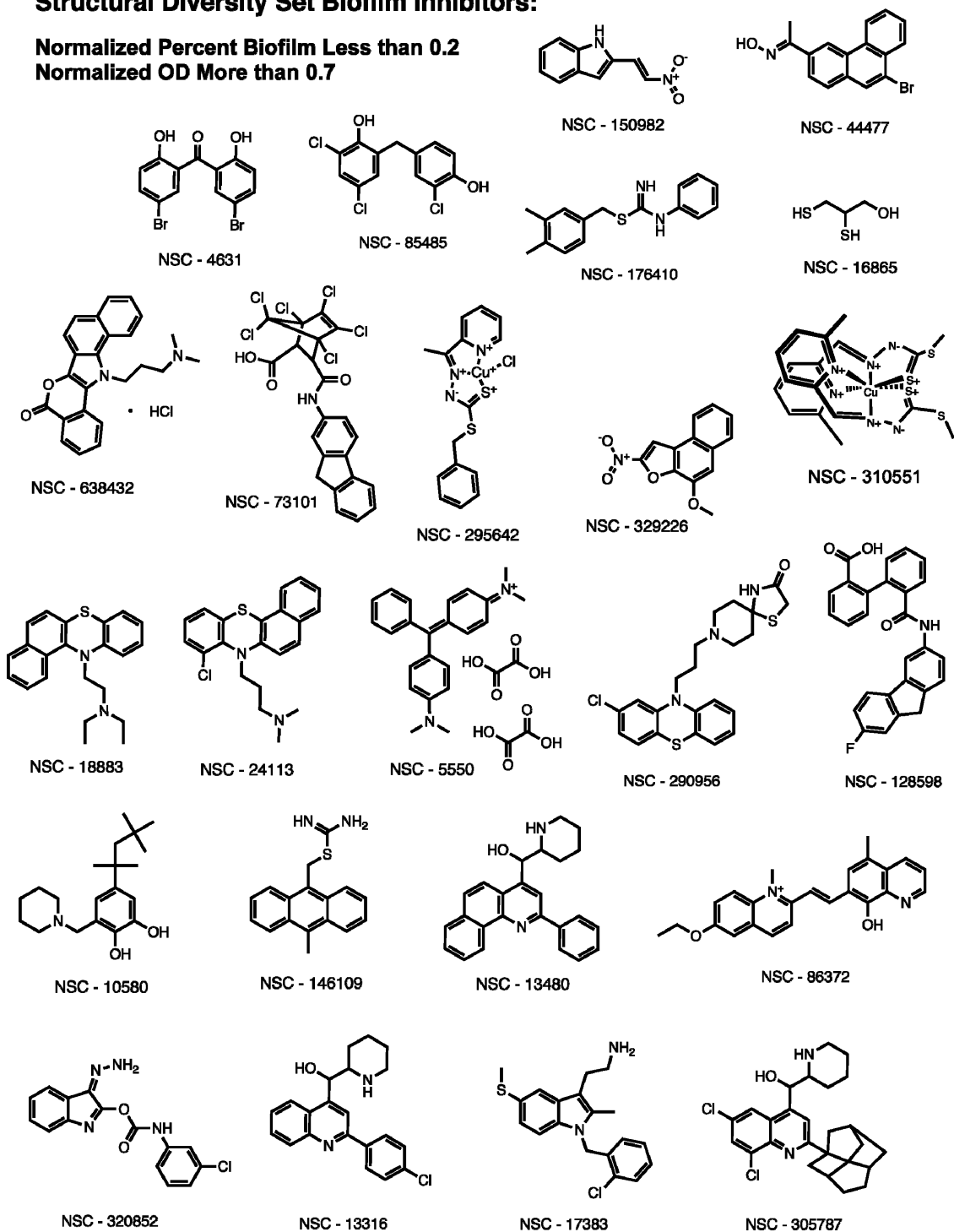
**Normalized Percent Biofilm Less than 0.2
Normalized OD Less than 0.7**



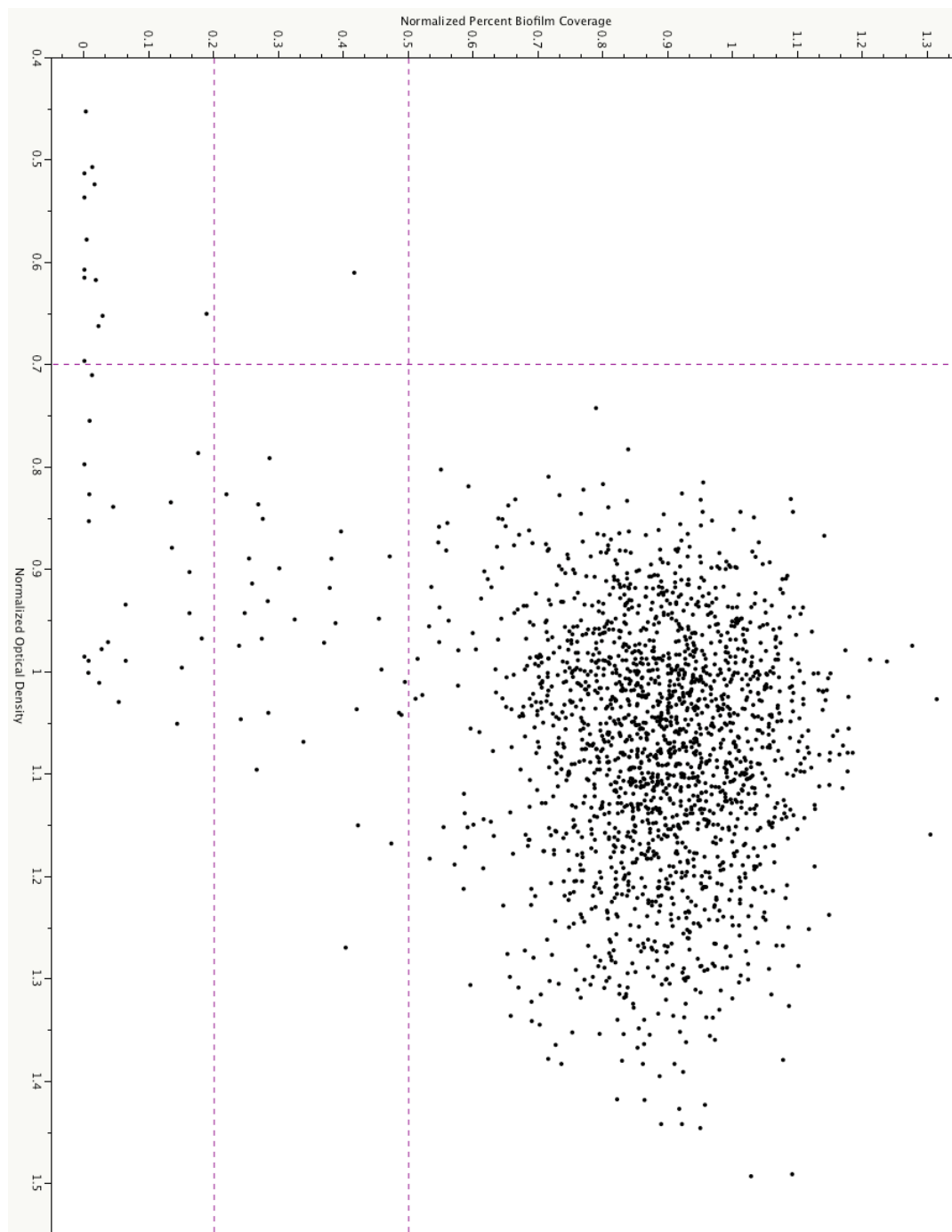
Supplementary Figure S2: Structures of Biofilm Inhibitors from the NCI Structural Diversity Set.

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.

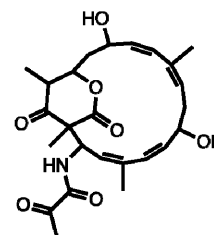
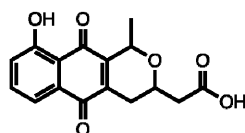
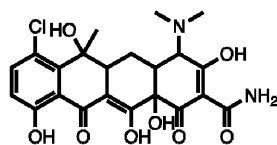
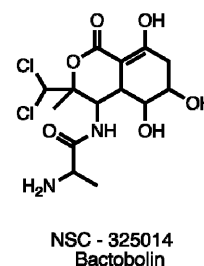
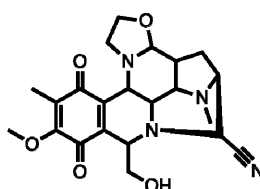
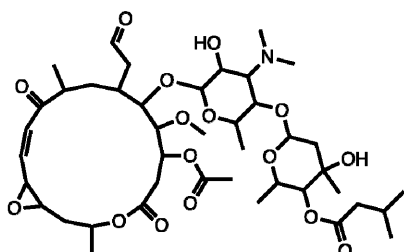
Structural Diversity Set Hits					
Normalized % biofilm coverage < 0.2			Normalized % biofilm coverage < 0.5, > than 0.2		
NSC	OD	% Biofilm Coverage	NSC	OD	% Biofilm Coverage
4631	0.986	0.000	92585	0.827	0.219
320852	0.798	0.000	177407	0.975	0.238
55636	0.513	0.000	17600	1.047	0.241
123111	0.697	0.000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

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

Natural Products Set

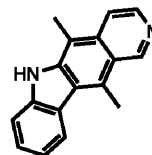
Antibiotics:

Normalized Percent Biofilm Less than 0.2
Normalized OD Less than 0.7

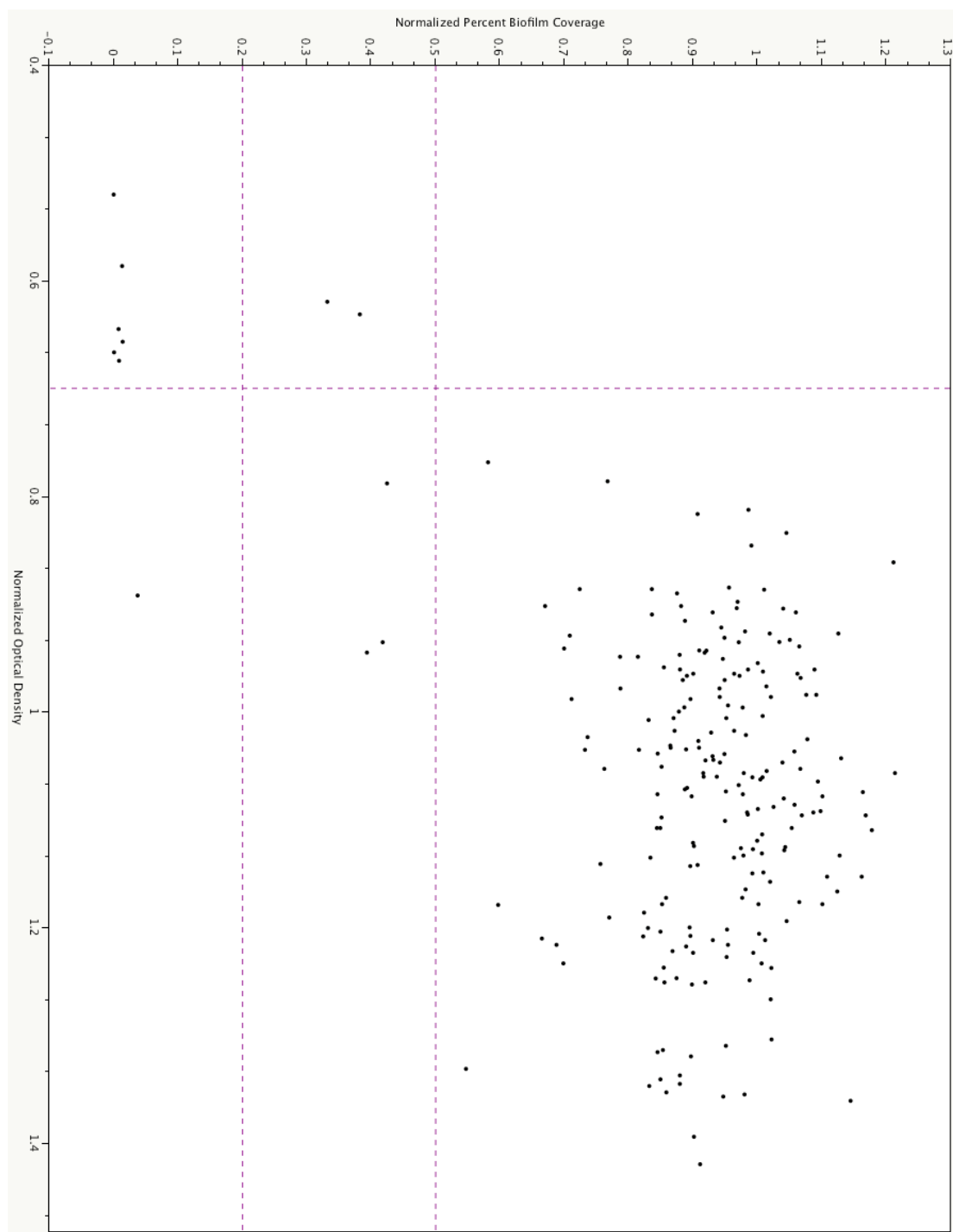


Biofilm inhibitors:

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

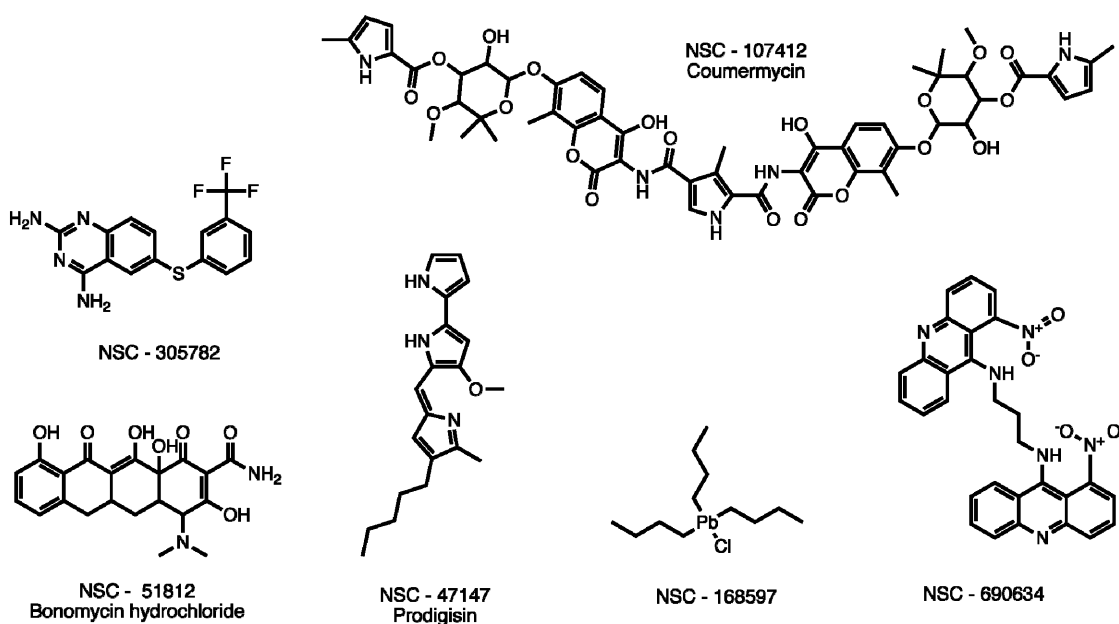
Normalized % biofilm less than 0.5, greater than 0.2		
NSC	Norm. OD	Norm. Percent Biofilm Coverage
45383	0.620	0.332
526417	0.631	0.382
312033	0.945	0.393
180973	0.936	0.418
106408	0.788	0.424
177406	1.889	0.445

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

Mechanistic Set

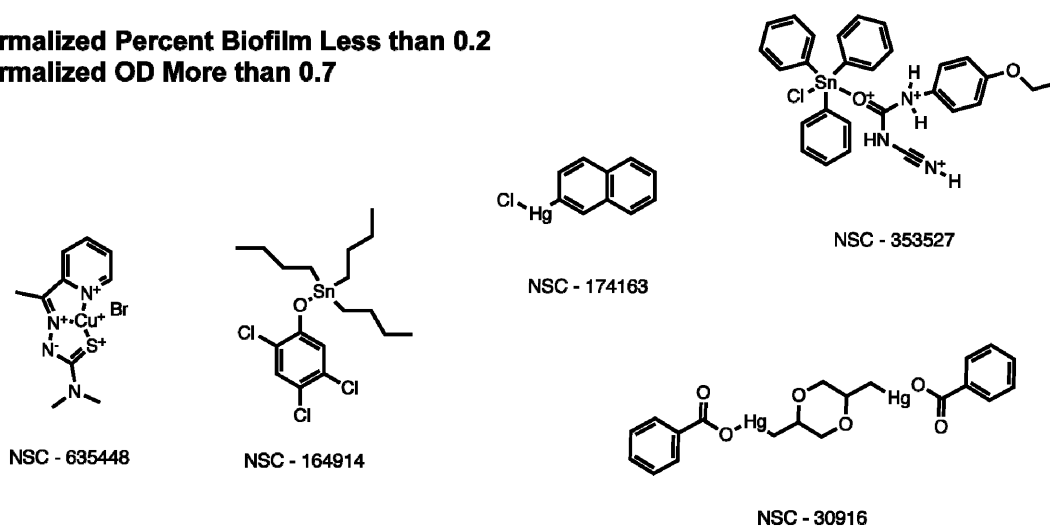
Antibiotics:

Normalized Percent Biofilm Less than 0.2
Normalized OD Less than 0.7

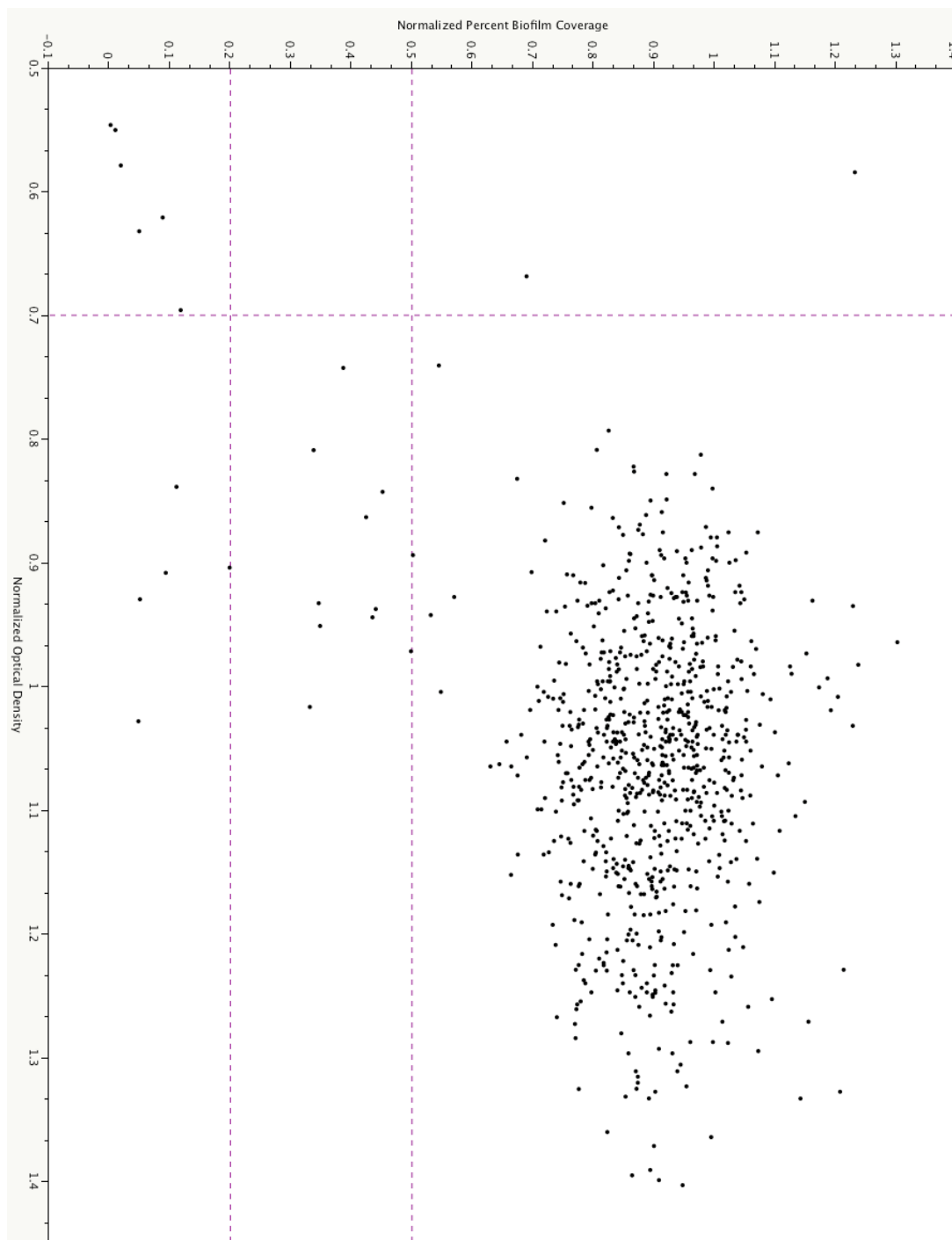


Biofilm inhibitors:

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

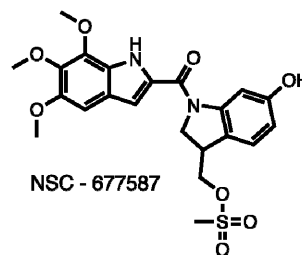
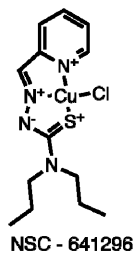
Normalized % biofilm less than 0.5, greater than 0.2		
NSC	Norm. OD	Norm. Percent Biofilm Coverage
631529	1.01662	0.331247
253995	0.80904	0.337428
313981	0.932754	0.345839
618332	0.951163	0.348146
329279	0.742521	0.386434
631521	0.863206	0.424164
96932	0.944184	0.434672
697923	0.937451	0.440122
44690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

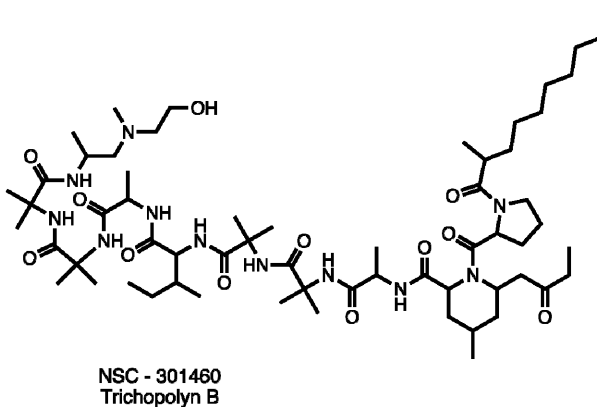
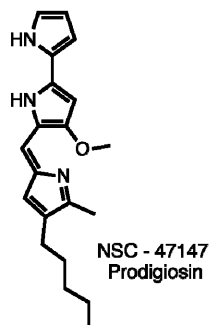
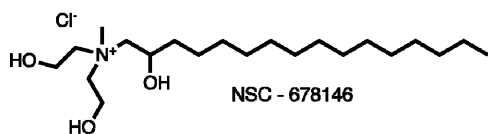
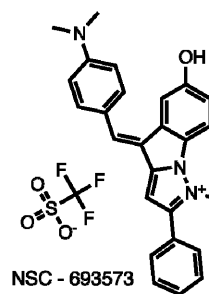
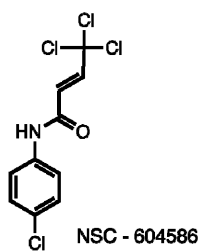
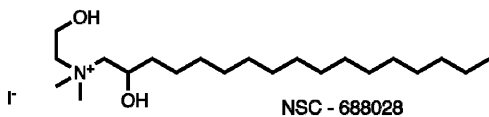
Antibiotics:

Normalized Percent Biofilm Less than 0.2
Normalized OD Less than 0.7

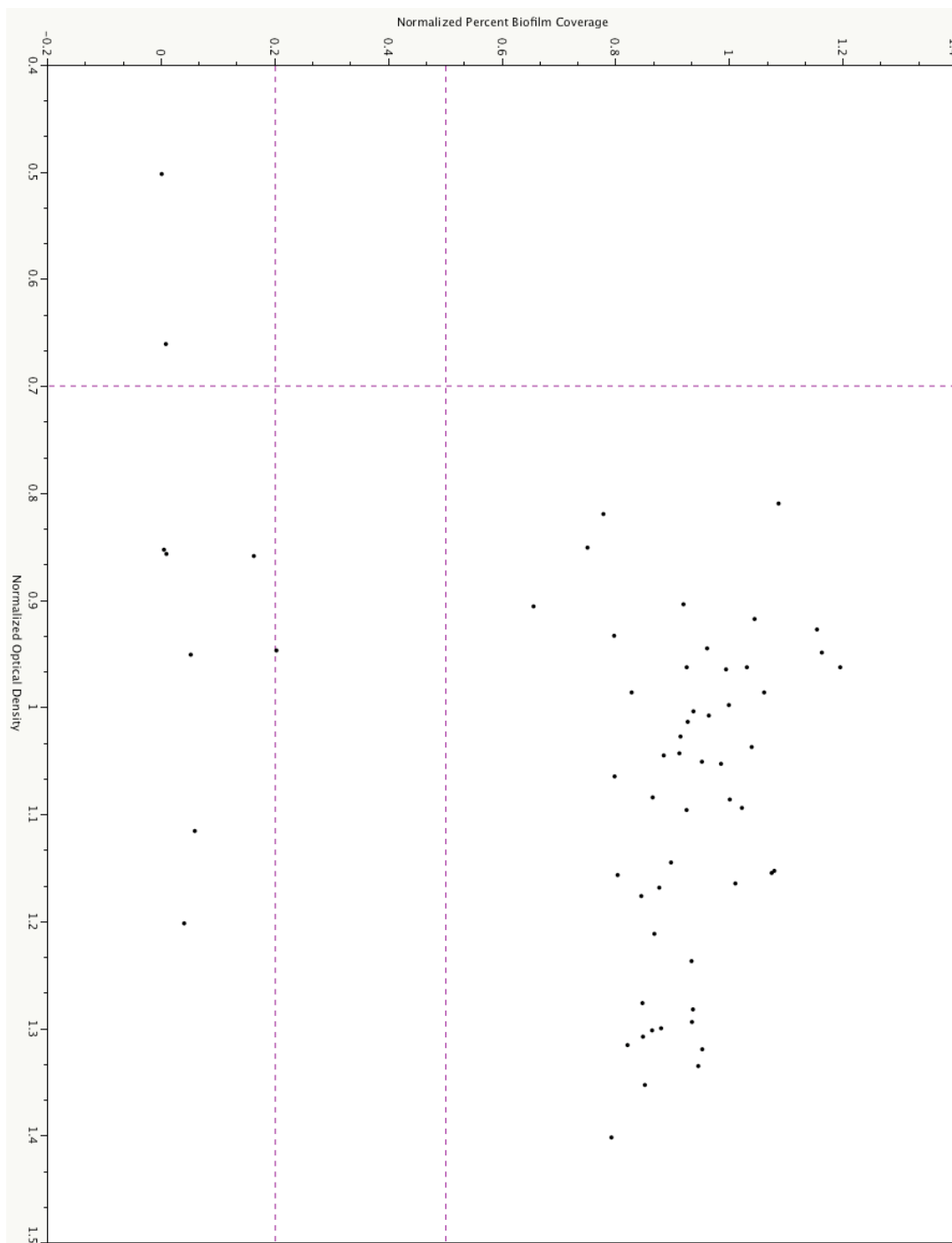


Biofilm inhibitors:

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.

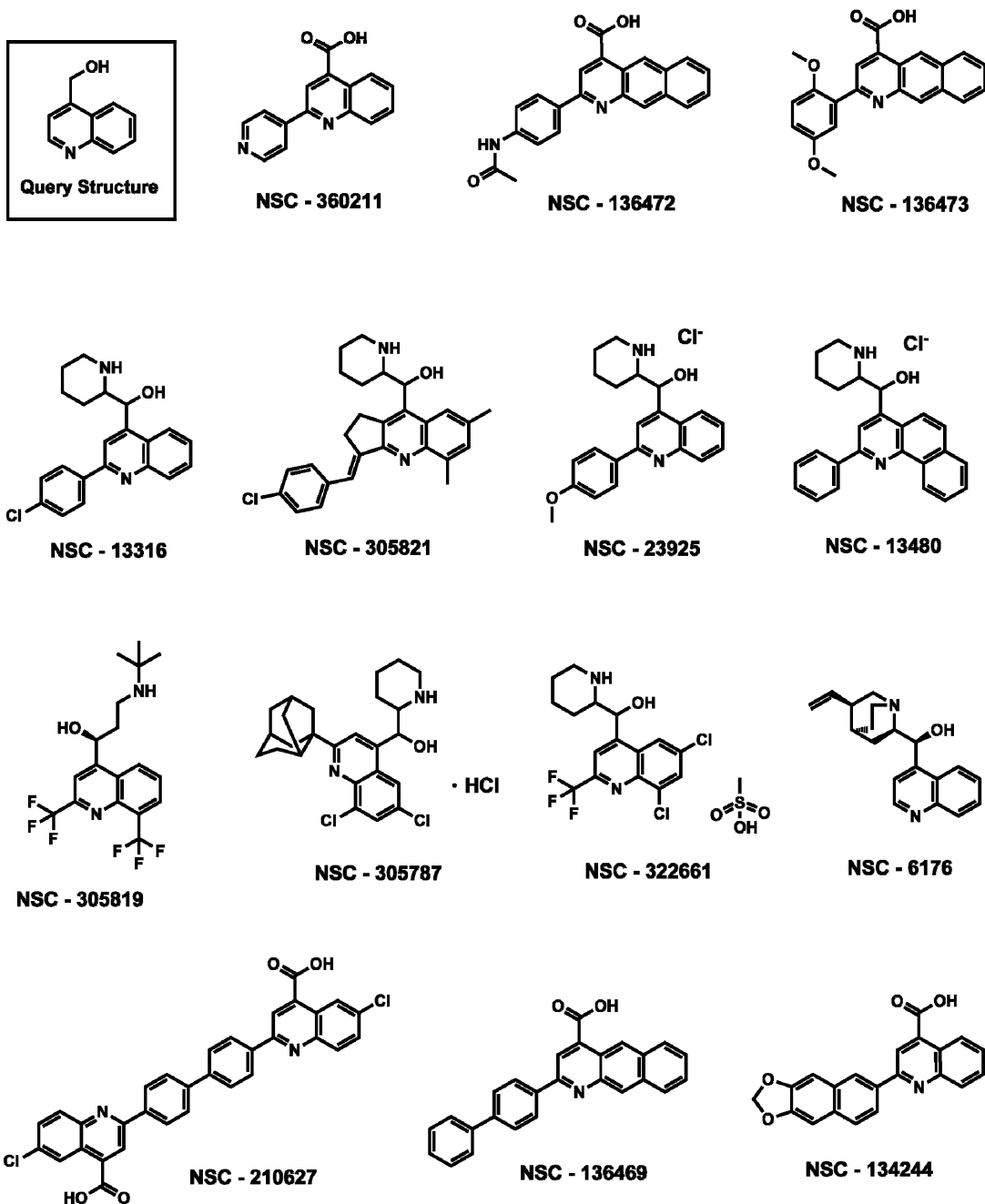
Challenge Set Hits

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

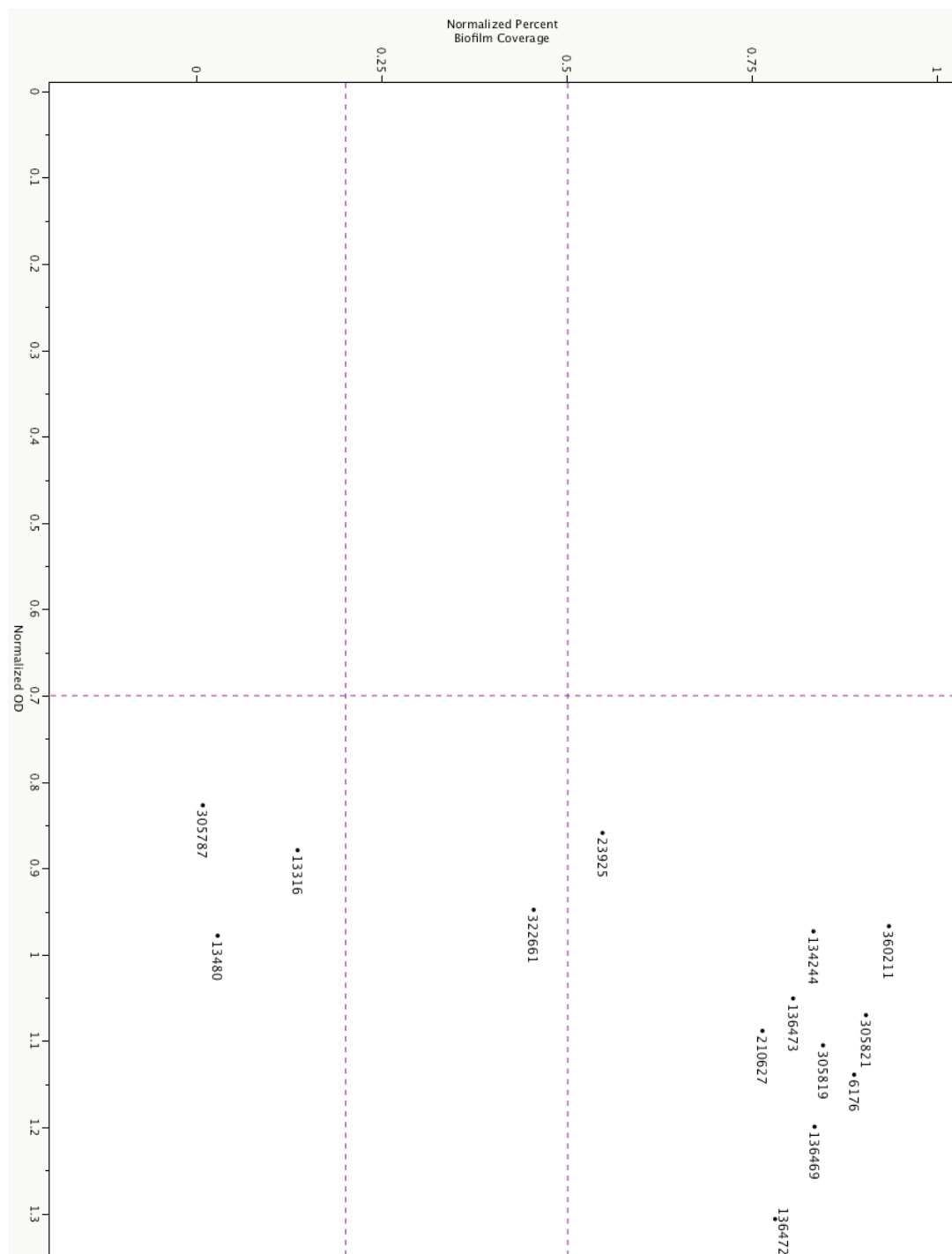
Normalized % biofilm less than 0.5, greater than 0.2		
NSC	Norm. OD	Norm. Percent Biofilm Coverage
170984	0.947	0.202

Supplementary Figure S10: Substructure Search Results of the Mefloquine-Like Query Structure for all NCI Libraries.

Substructure Search of Four NCI Libraries:



Supplementary Figure S11: 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