

Supporting Information for:

Identification of Novel Non-Hydroxamate Anthrax Toxin Lethal Factor Inhibitors by Topomeric Searching, Docking and Scoring, and *In Vitro* Screening

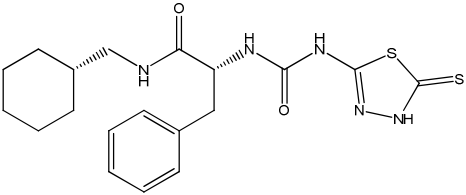
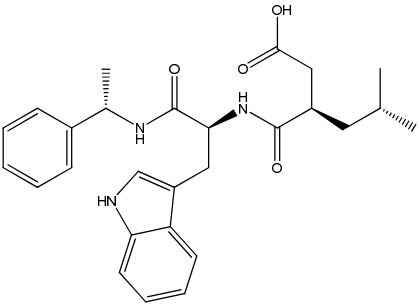
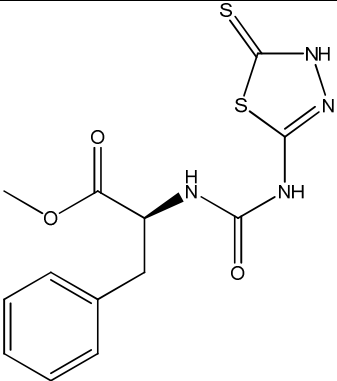
Ting-Lan Chiu, Jonathan Solberg, Satish Patil, Todd W. Geders, Xia Zhang, Subhashree Rangarajan, Rawle Francis, Barry C. Finzel, Michael A. Walters, Derek J. Hook, and Elizabeth A. Amin

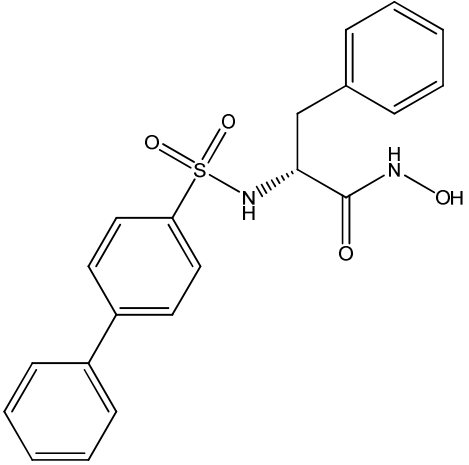
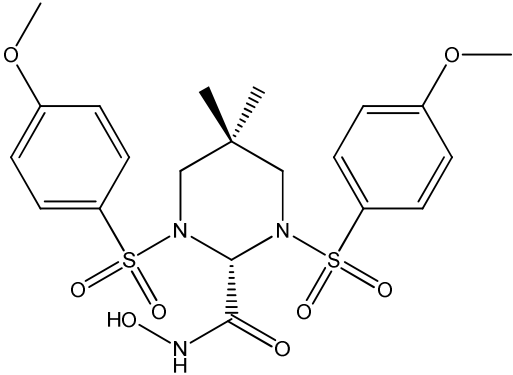
To be published in *J. Chem. Inf. Model.*

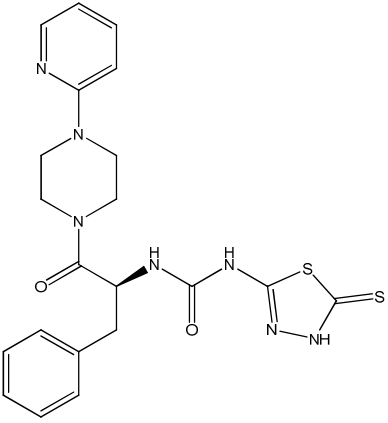
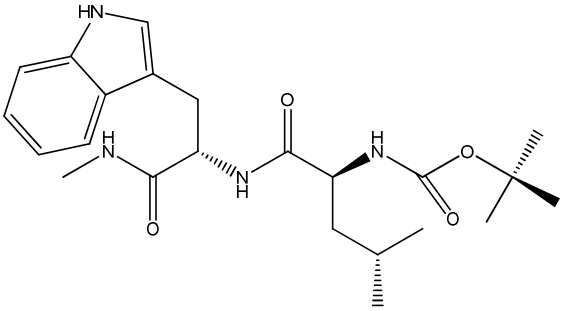
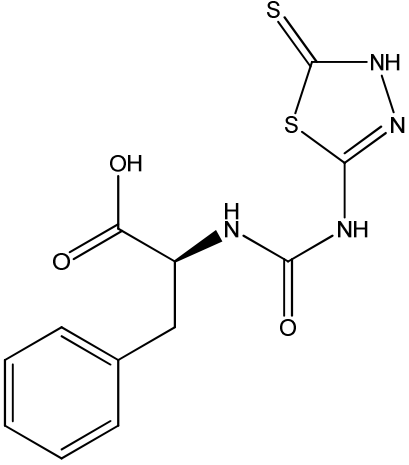
This supporting information was prepared on October 30, 2009 and consists of 22 pages.

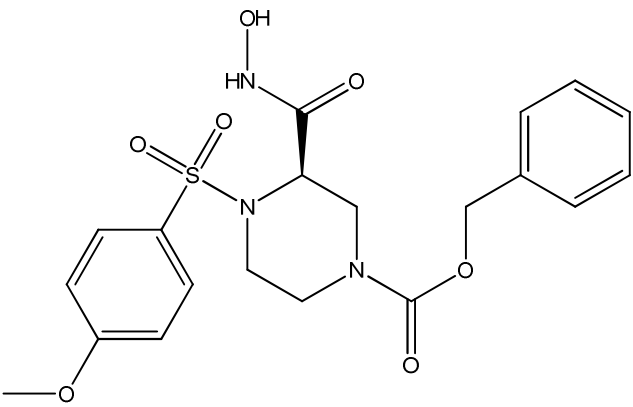
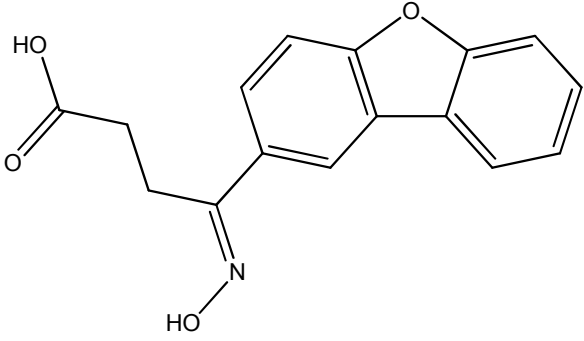
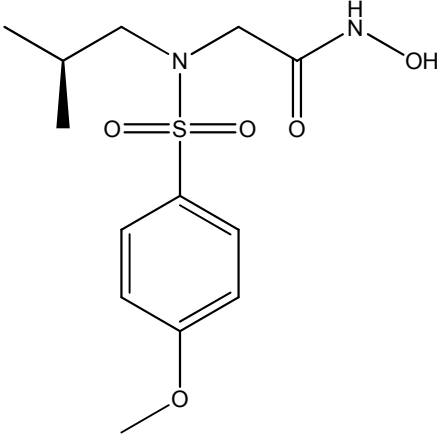
<u>Contents:</u>	<i>page</i>
Table S1. Seventeen matrix metalloproteinase inhibitors (MMPi)s found to be inactive against the anthrax toxin lethal factor, with total Surflex-Dock scores	S-2
Table S2. <i>In vitro</i> assay data for the top five anthrax toxin LF inhibitor hit compounds 5426202 , 5421384 , 5428736 , 5426345 , and 150002740	S-10
Table S3. <i>In vitro</i> assay data for two matrix metalloproteinase inhibitors active against the anthrax toxin lethal factor: GM6001 and M444264	S-14
Table S4. Identity and purity analysis data for the top three anthrax toxin LF inhibitor hit compounds: 5426202 , 5421384 , and 5428736	S-16

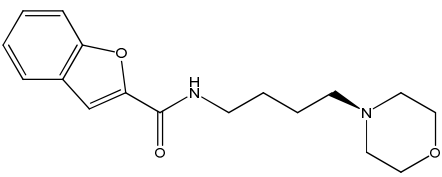
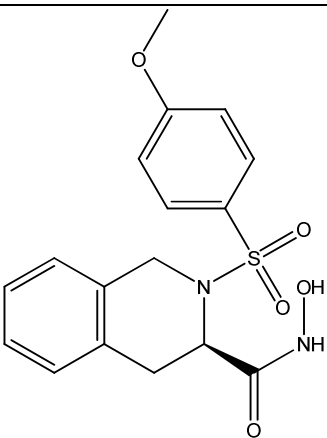
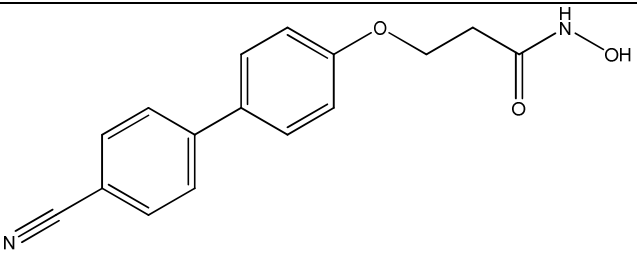
Table S1. Seventeen matrix metalloproteinase inhibitors (MMPIs) found to be inactive against the anthrax toxin lethal factor, with total Surflex-Dock scores.

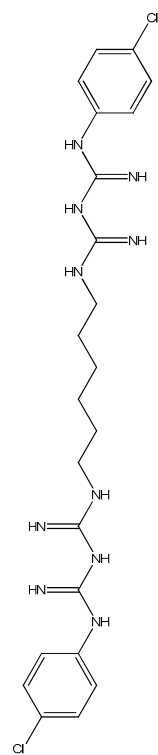
Compound	Total Surflex-Dock Score (-log K_d)
 M444243	11.48
 M364200	11.31
 M444239	10.46

 <p>M444249</p>	9.94
 <p>M444247</p>	9.82

 <p>Chemical structure of M444240: A benzyl group is attached to a chiral center (wedge bond) of a 2-oxoethylamine derivative. This chiral center is also bonded to a nitrogen atom which is part of a piperazine ring. The piperazine ring is further substituted with a pyridin-2-yl group. The nitrogen atom of the 2-oxoethylamine derivative is also bonded to a carbonyl group, which is further substituted with a 1,2,4-thiazole-5-yl group.</p>	9.38
 <p>Chemical structure of M364210: A complex bicyclic system consisting of a benzene ring fused to a six-membered ring containing a nitrogen atom and a carbonyl group. This system is linked via a carbonyl group to a chiral center (wedge bond) of a piperazine ring. The piperazine ring is further substituted with a methyl group (dashed bond) and a carbonyl group. The carbonyl group is further substituted with a methyl group (wedge bond) and an oxygen atom.</p>	9.31
 <p>Chemical structure of M444242: A benzyl group is attached to a chiral center (wedge bond) of a 2-hydroxyethylamine derivative. This chiral center is also bonded to a nitrogen atom which is part of a 1,2,4-thiazole ring system. The nitrogen atom of the 2-hydroxyethylamine derivative is also bonded to a carbonyl group.</p>	9.15

 <p>M444253</p>	8.88
 <p>M444260</p>	8.74
 <p>M444225</p>	8.73

 <p>M233105</p>	8.51
 <p>M444237</p>	8.29
 <p>M444280</p>	7.12



M220557

6.78

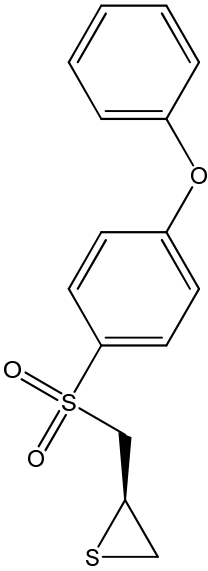
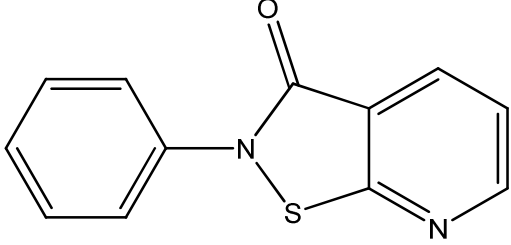
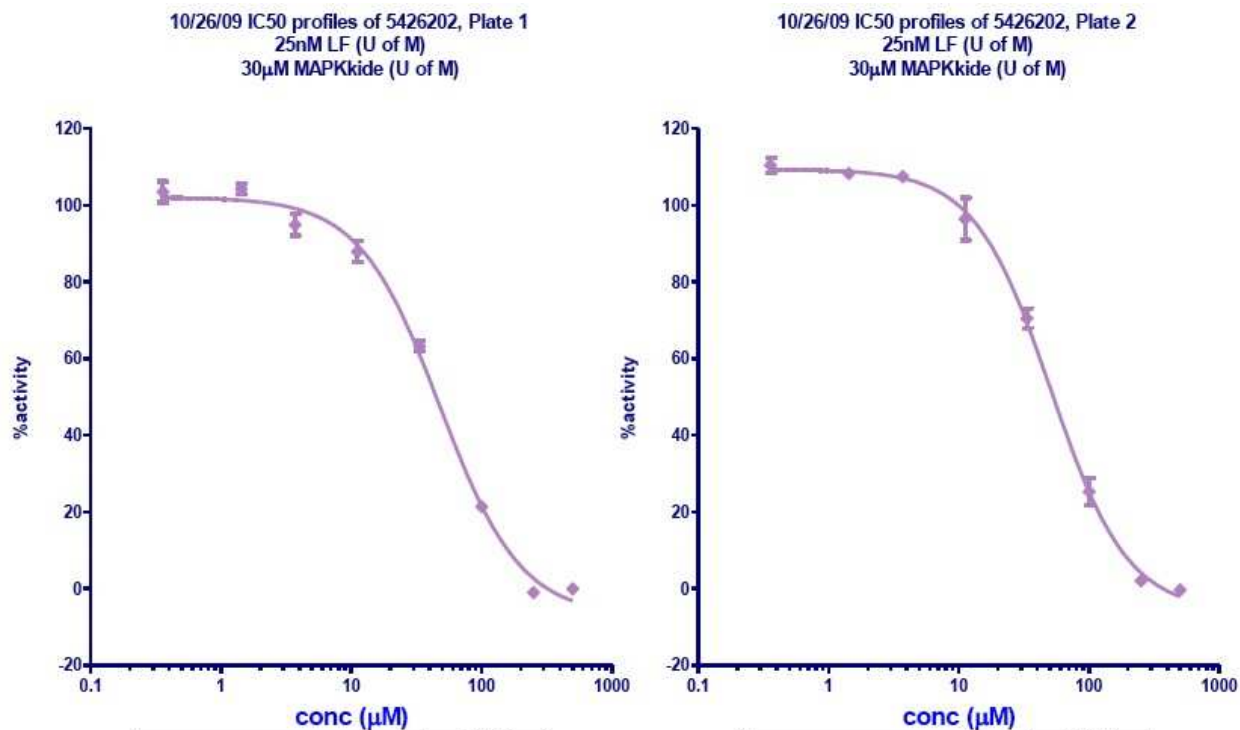
 <p>M444274</p>	5.41
 <p>M682300</p>	4.71

Table S2. *In vitro* assay data for the top five anthrax toxin LF inhibitor hit compounds: **5426202**, **5421384**, **5428736**, **5426345**, and **150002740**

(a) **5426202**

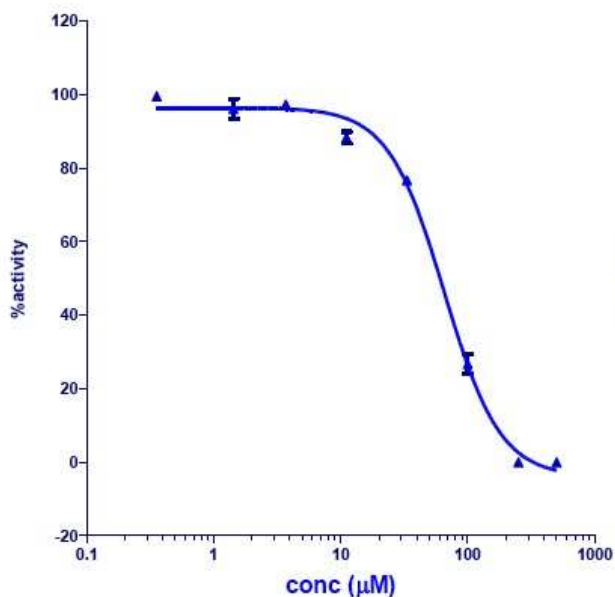


log(inhibitor) vs. response – Variable slope		5426202
Best-fit values		
BOTTOM		-7.064
TOP		102.1
LOGIC50		1.681
HILLSLOPE		-1.403
IC50		47.99
Span		109.1
Std. Error		
BOTTOM		3.820
TOP		1.803
LOGIC50		0.03808
HILLSLOPE		0.1535
Span		4.506
95% Confidence Intervals		
BOTTOM		-14.94 to 0.8345
TOP		88.16 to 106.0
LOGIC50		1.568 to 1.764
HILLSLOPE		-1.737 to -1.068
IC50		39.64 to 58.09
Span		99.32 to 119.0
Goodness of Fit		
Degrees of Freedom		12
R ²		0.9940
Absolute Sum of Squares		176.0
Sy.x		3.830
Number of points		
Analyzed		16

log(inhibitor) vs. response – Variable slope		5426202
Best-fit values		
BOTTOM		-5.783
TOP		109.4
LOGIC50		1.708
HILLSLOPE		-1.480
IC50		51.00
Span		115.2
Std. Error		
BOTTOM		3.092
TOP		1.523
LOGIC50		0.03024
HILLSLOPE		0.1333
Span		3.806
95% Confidence Intervals		
BOTTOM		-12.52 to 0.9550
TOP		106.1 to 112.7
LOGIC50		1.842 to 1.774
HILLSLOPE		-1.770 to -1.189
IC50		43.82 to 59.36
Span		106.9 to 123.5
Goodness of Fit		
Degrees of Freedom		12
R ²		0.9959
Absolute Sum of Squares		137.0
Sy.x		3.379
Number of points		
Analyzed		16

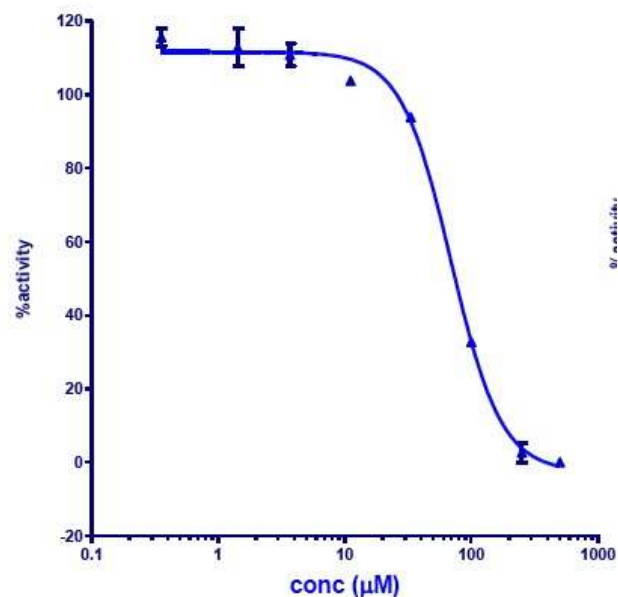
(b) 5421384

10/26/09 IC50 profile of 5421384, Plate 1
25nM LF (U of M)
30 μ M MAPKkide (U of M)



5421384	
log(inhibitor) vs. response -- Variable slope	
Best-fit values	
BOTTOM	-4.001
TOP	96.49
LOGIC50	1.816
HILLSLOPE	-1.972
IC50	65.52
Span	100.5
Std. Error	
BOTTOM	2.551
TOP	1.325
LOGIC50	0.02656
HILLSLOPE	0.1891
Span	3.055
95% Confidence Intervals	
BOTTOM	-9.561 to 1.558
TOP	93.60 to 99.37
LOGIC50	1.758 to 1.874
HILLSLOPE	-2.384 to -1.560
IC50	57.34 to 74.86
Span	93.83 to 107.1
Goodness of Fit	
Degrees of Freedom	12
R ²	0.9949
Absolute Sum of Squares	140.3
Sy.x	3.419
Number of points	
Analyzed	16

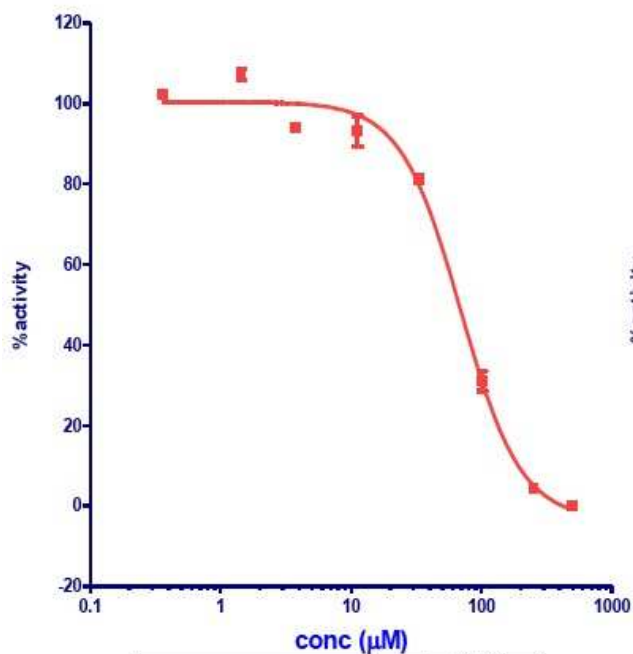
10/26/09 IC50 profile of 5421384, Plate 2
25nM LF (U of M)
30 μ M MAPKkide (U of M)



5421384	
log(inhibitor) vs. response -- Variable slope	
Best-fit values	
BOTTOM	-2.577
TOP	111.6
LOGIC50	1.842
HILLSLOPE	-2.197
IC50	69.44
Span	114.2
Std. Error	
BOTTOM	2.914
TOP	1.590
LOGIC50	0.02613
HILLSLOPE	0.2289
Span	3.503
95% Confidence Intervals	
BOTTOM	-8.927 to 3.774
TOP	108.2 to 115.1
LOGIC50	1.785 to 1.899
HILLSLOPE	-2.895 to -1.698
IC50	60.91 to 79.16
Span	106.6 to 121.9
Goodness of Fit	
Degrees of Freedom	12
R ²	0.9941
Absolute Sum of Squares	214.1
Sy.x	4.224
Number of points	
Analyzed	16

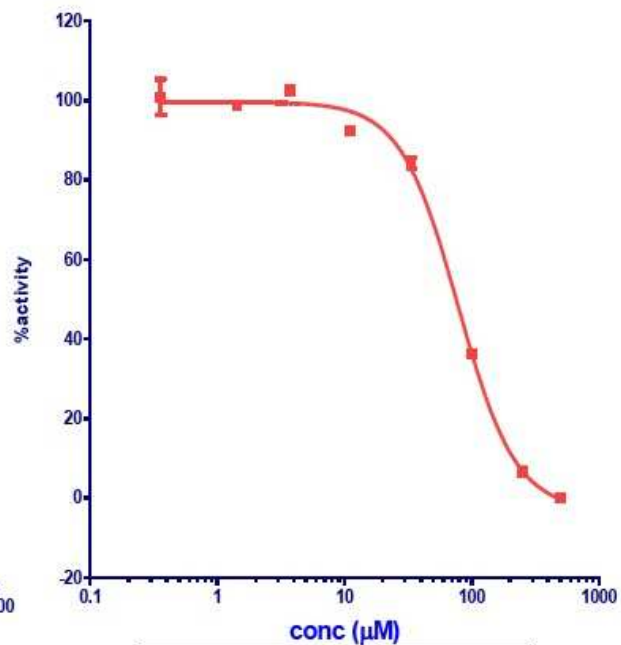
(c) 5428736

10/26/09 IC50 profile of 5428736, Plate 1
25nM LF (U of M)
30 μ M MAPKkide (U of M)



5428736	
log(inhibitor) vs. response -- Variable slope	
Best-fit values	
BOTTOM	-3.350
TOP	100.3
LOGIC50	1.842
HILLSLOPE	-1.887
IC50	89.43
Span	103.7
Std. Error	
BOTTOM	3.759
TOP	1.817
LOGIC50	0.03662
HILLSLOPE	0.2466
Span	4.463
95% Confidence Intervals	
BOTTOM	-11.54 to 4.841
TOP	96.39 to 104.3
LOGIC50	1.762 to 1.921
HILLSLOPE	-2.425 to -1.350
IC50	57.77 to 83.43
Span	93.97 to 113.4
Goodness of Fit	
Degrees of Freedom	12
R ²	0.9908
Absolute Sum of Squares	259.4
Sy,x	4.649
Number of points	
Analyzed	16

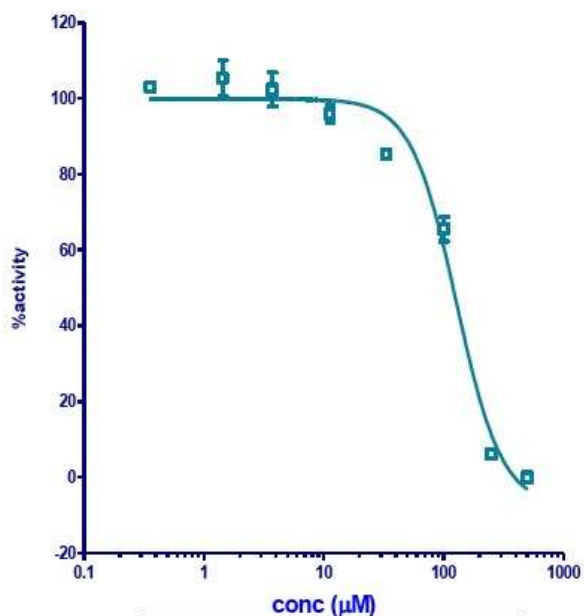
10/26/09 IC50 profile of 5428736, Plate 2
25nM LF (U of M)
30 μ M MAPKkide (U of M)



5428736	
log(inhibitor) vs. response -- Variable slope	
Best-fit values	
BOTTOM	-3.124
TOP	99.80
LOGIC50	1.894
HILLSLOPE	-1.919
IC50	78.34
Span	102.7
Std. Error	
BOTTOM	2.772
TOP	1.240
LOGIC50	0.02526
HILLSLOPE	0.1852
Span	3.256
95% Confidence Intervals	
BOTTOM	-9.164 to 2.918
TOP	96.90 to 102.3
LOGIC50	1.839 to 1.949
HILLSLOPE	-2.323 to -1.518
IC50	69.02 to 88.93
Span	95.83 to 109.8
Goodness of Fit	
Degrees of Freedom	12
R ²	0.9954
Absolute Sum of Squares	124.2
Sy,x	3.217
Number of points	
Analyzed	16

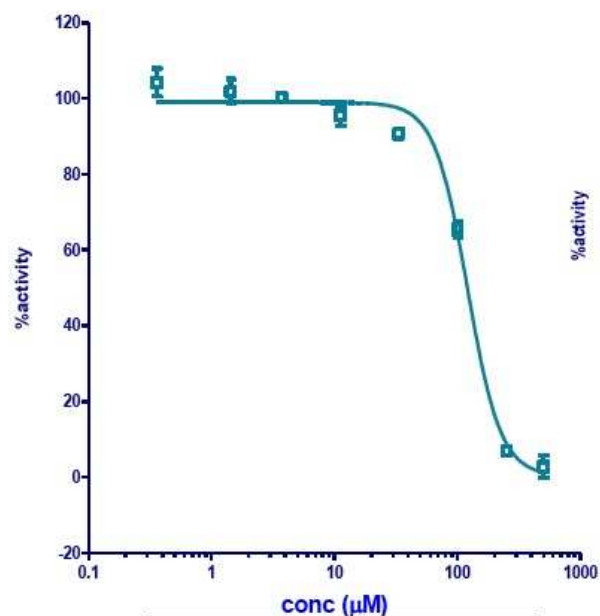
(d) 5426345

10/26/09 IC50 profile of 5426345, Plate 1
25nM LF (U of M)
30 μ M MAPKkide (U of M)



log(inhibitor) vs. response -- Variable slope		5426345
Best-fit values		
BOTTOM		-7.603
TOP		99.89
LOGIC50		2.107
HILLSLOPE		-2.303
IC50		128.0
Span		107.5
Std. Error		
BOTTOM		8.052
TOP		2.328
LOGIC50		0.05219
HILLSLOPE		0.5589
Span		8.804
95% Confidence Intervals		
BOTTOM		-25.15 to 9.942
TOP		94.82 to 105.0
LOGIC50		1.993 to 2.221
HILLSLOPE		-3.517 to -1.090
IC50		98.49 to 186.3
Span		88.31 to 126.7
Goodness of Fit		
Degrees of Freedom		12
R ²		0.9795
Absolute Sum of Squares		546.8
Sy.x		6.751
Number of points		
Analyzed		16

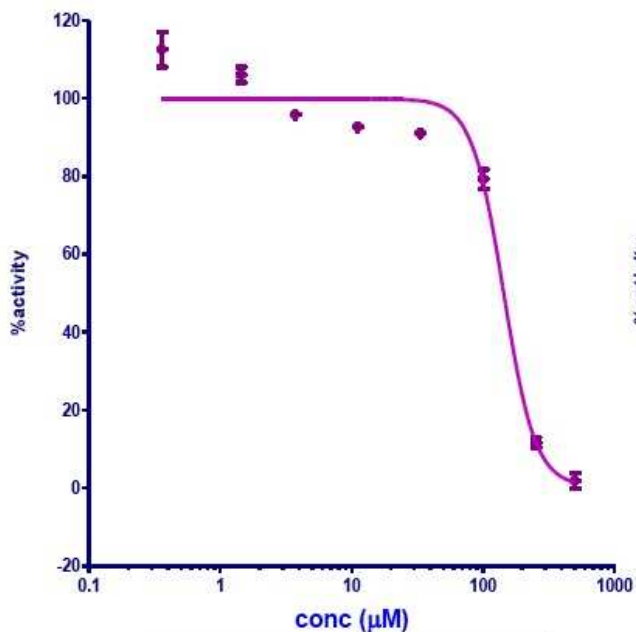
10/26/09 IC50 profile of 5426345, Plate 2
25nM LF (U of M)
30 μ M MAPKkide (U of M)



log(inhibitor) vs. response -- Variable slope		5426345
Best-fit values		
BOTTOM		0.3556
TOP		98.93
LOGIC50		2.083
HILLSLOPE		-3.232
IC50		121.0
Span		98.58
Std. Error		
BOTTOM		4.409
TOP		1.632
LOGIC50		0.03154
HILLSLOPE		0.7570
Span		4.873
95% Confidence Intervals		
BOTTOM		-9.251 to 9.962
TOP		95.38 to 102.5
LOGIC50		2.014 to 2.152
HILLSLOPE		-4.882 to -1.583
IC50		103.3 to 141.8
Span		87.96 to 109.2
Goodness of Fit		
Degrees of Freedom		12
R ²		0.9882
Absolute Sum of Squares		300.6
Sy.x		5.005
Number of points		
Analyzed		16

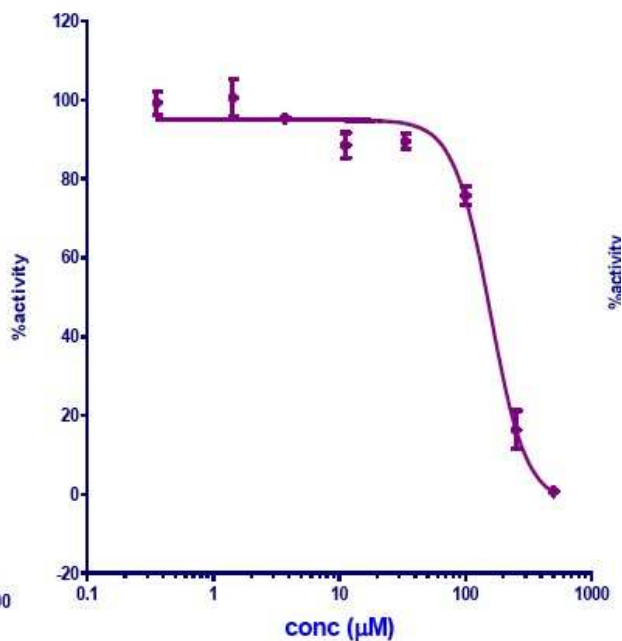
(e) 150002740

10/26/09 IC50 profile of 150002740, Plate 1
25nM LF (U of M)
30µM MAPKkide (U of M)



150002740	
log(inhibitor) vs. response -- Variable slope	
Best-fit values	
BOTTOM	0.3906
TOP	99.92
LOGIC50	2.157
HILLSLOPE	-3.613
IC50	143.7
Span	99.53
Std. Error	
BOTTOM	6.733
TOP	2.518
LOGIC50	0.05130
HILLSLOPE	0.9423
Span	7.283
95% Confidence Intervals	
BOTTOM	-14.28 to 15.08
TOP	94.43 to 105.4
LOGIC50	2.046 to 2.269
HILLSLOPE	-5.666 to -1.559
IC50	111.1 to 185.9
Span	83.66 to 115.4
Goodness of Fit	
Degrees of Freedom	12
R ²	0.9710
Absolute Sum of Squares	743.3
Sy,x	7.870
Number of points	
Analyzed	16

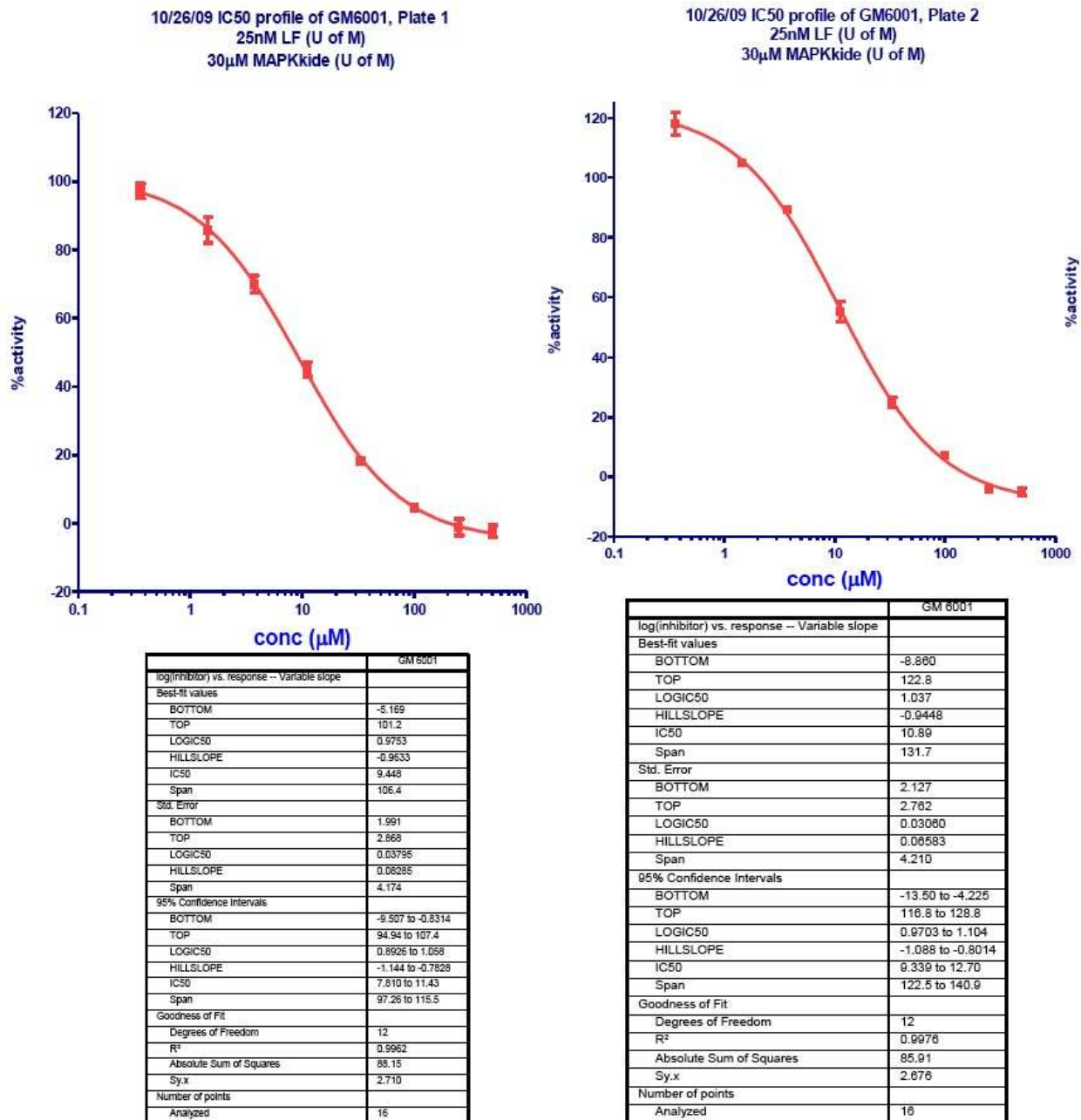
10/26/09 IC50 profile of 150002740, Plate 2
25nM LF (U of M)
30µM MAPKkide (U of M)



150002740	
log(inhibitor) vs. response -- Variable slope	
Best-fit values	
BOTTOM	-2.257
TOP	94.95
LOGIC50	2.196
HILLSLOPE	-3.023
IC50	167.0
Span	97.21
Std. Error	
BOTTOM	5.830
TOP	1.816
LOGIC50	0.03956
HILLSLOPE	0.5279
Span	6.036
95% Confidence Intervals	
BOTTOM	-14.52 to 10.01
TOP	91.00 to 98.91
LOGIC50	2.110 to 2.282
HILLSLOPE	-4.173 to -1.873
IC50	128.7 to 191.4
Span	84.05 to 110.4
Goodness of Fit	
Degrees of Freedom	12
R ²	0.9827
Absolute Sum of Squares	378.4
Sy,x	5.615
Number of points	
Analyzed	16

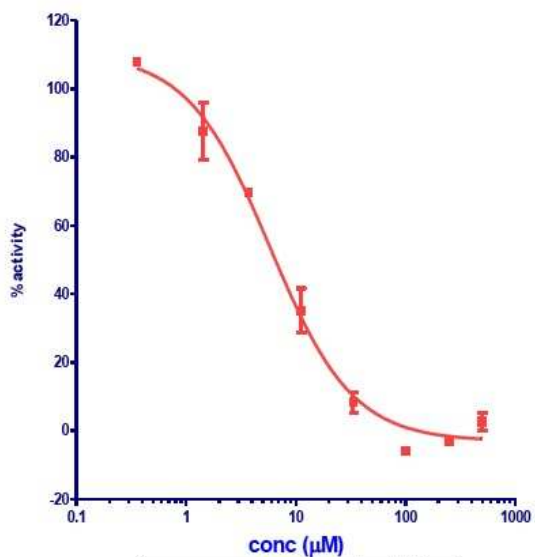
Table S3. *In vitro* assay data for two matrix metalloproteinase inhibitors active against the anthrax toxin lethal factor: **GM6001** and **M444264**

(a) **GM6001**



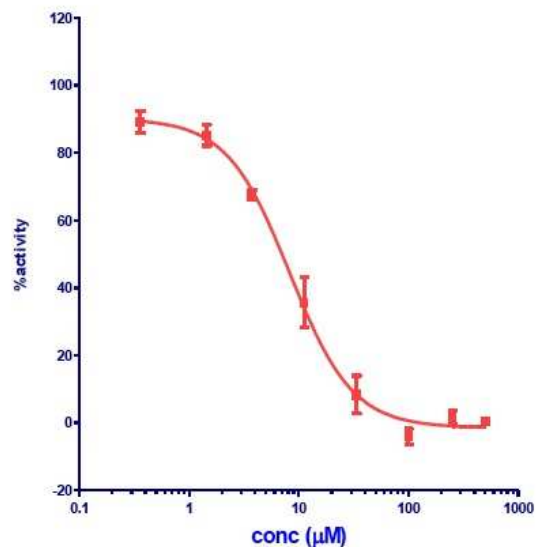
(b) M444264

10/29/09 IC50 profile of M444264, Set 1 (n = 2)
25nM LF (U of M)
30 μ M MAPKkide (U of M)



log(Inhibitor) vs. response - Variable slope		M444264
Best-fit values		
BOTTOM		-3.066
TOP		110.3
LOGIC50		0.7651
HILLSLOPE		-1.155
IC50		5.822
Span		113.4
Std. Error		
BOTTOM		3.309
TOP		6.252
LOGIC50		0.06919
HILLSLOPE		0.1914
Span		7.929
95% Confidence Intervals		
BOTTOM		-10.35 to 4.216
TOP		96.55 to 124.1
LOGIC50		0.6128 to 0.9174
HILLSLOPE		-1.576 to -0.7338
IC50		4.100 to 8.267
Span		95.91 to 130.5
Goodness of Fit		
Degrees of Freedom		11
R ²		0.9842
Absolute Sum of Squares		418.5
Sy.x		6.169
Number of points		15
Analyzed		15

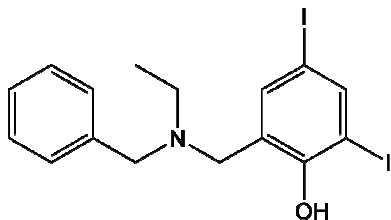
10/29/09 IC50 profile of M444264, Set 2 (n = 2)
25nM LF (U of M)
30 μ M MAPKkide (U of M)



log(Inhibitor) vs. response - Variable slope		M444264
Best-fit values		
BOTTOM		-1.434
TOP		90.15
LOGIC50		0.9182
HILLSLOPE		-1.517
IC50		8.283
Span		91.58
Std. Error		
BOTTOM		2.305
TOP		3.521
LOGIC50		0.04993
HILLSLOPE		0.2334
Span		4.610
95% Confidence Intervals		
BOTTOM		-6.457 to 3.589
TOP		82.47 to 97.82
LOGIC50		0.8096 to 1.027
HILLSLOPE		-2.026 to -1.009
IC50		5.450 to 10.63
Span		81.53 to 101.6
Goodness of Fit		
Degrees of Freedom		12
R ²		0.9663
Absolute Sum of Squares		307.1
Sy.x		5.059
Number of points		15
Analyzed		15

Table S4. Identity and purity analysis data for the top three anthrax toxin LF inhibitor hit compounds: **5426202**, **5421384**, and **5428736**

(a) **5426202**

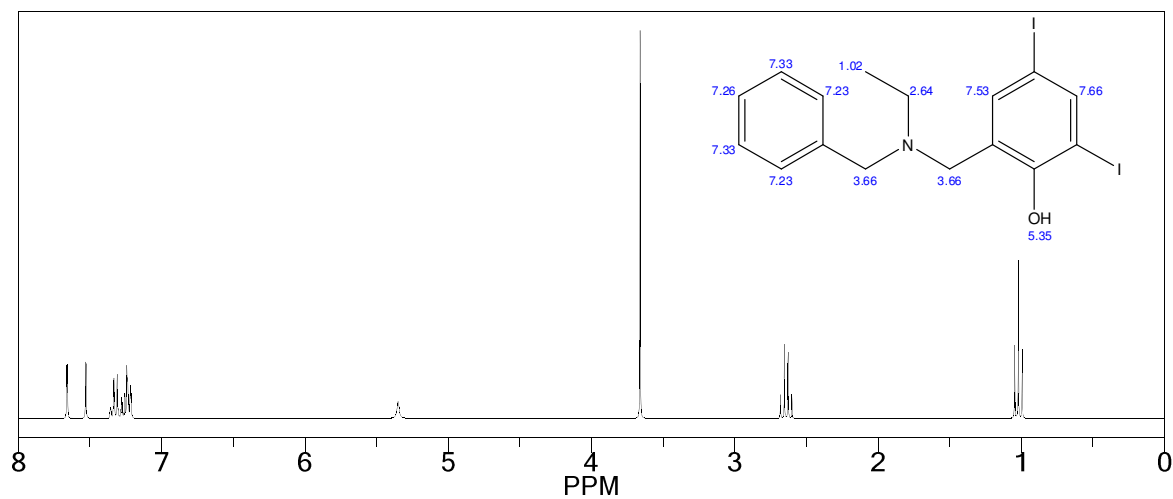


Chemical Formula: $C_{16}H_{17}I_2NO$

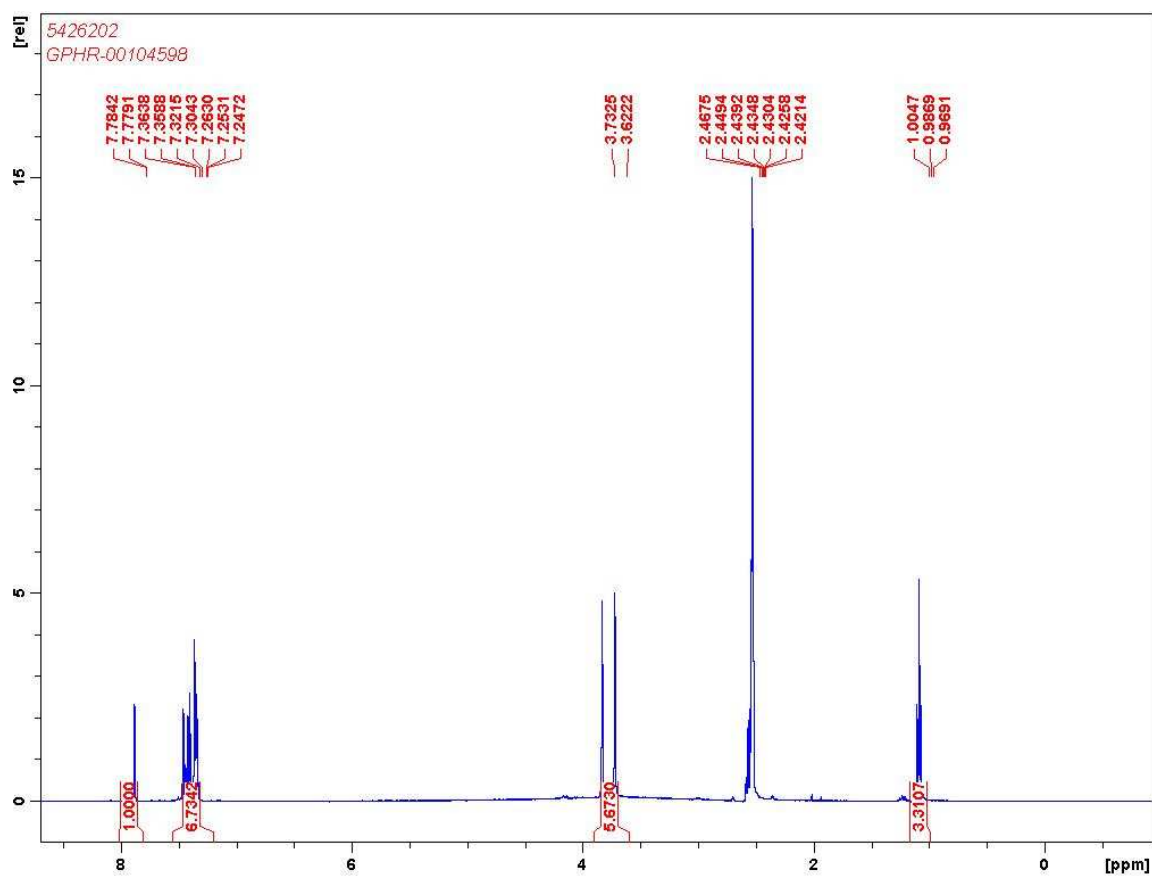
Exact Mass: 492.9400

Purity >95%

Predicted 1H NMR spectrum:



Observed ^1H NMR spectrum:

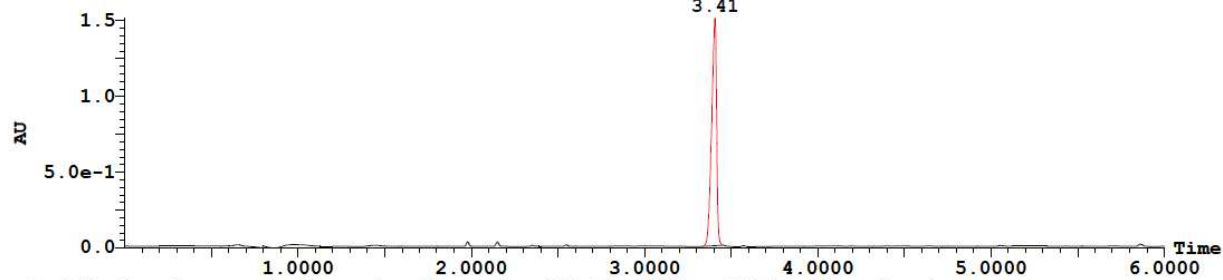


LC-MS:

Sample 12 Vial 2:8 ID File GPHR-00104598 Date 21-Jul-2009 Time 16:59:41 Description 5426202

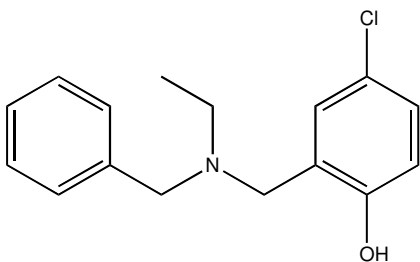
PDA Ch3 220nm@1.2nm-MBF Smooth (SG, 2x2)

(5) 1.517
494.08
3.41 Range: 1.517



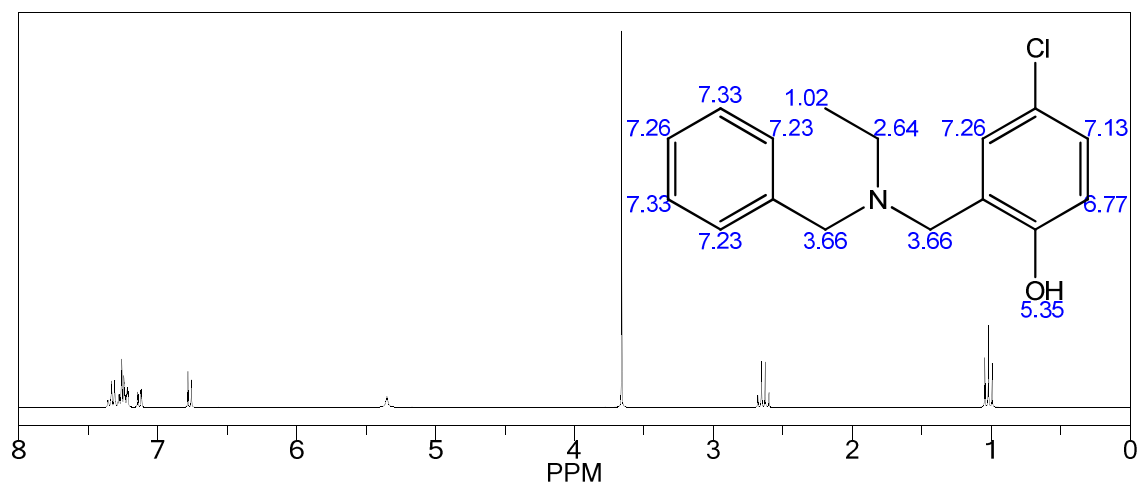
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
5		3.41	4.99e+04	100.00	0	2e+06	

(b) 5421384

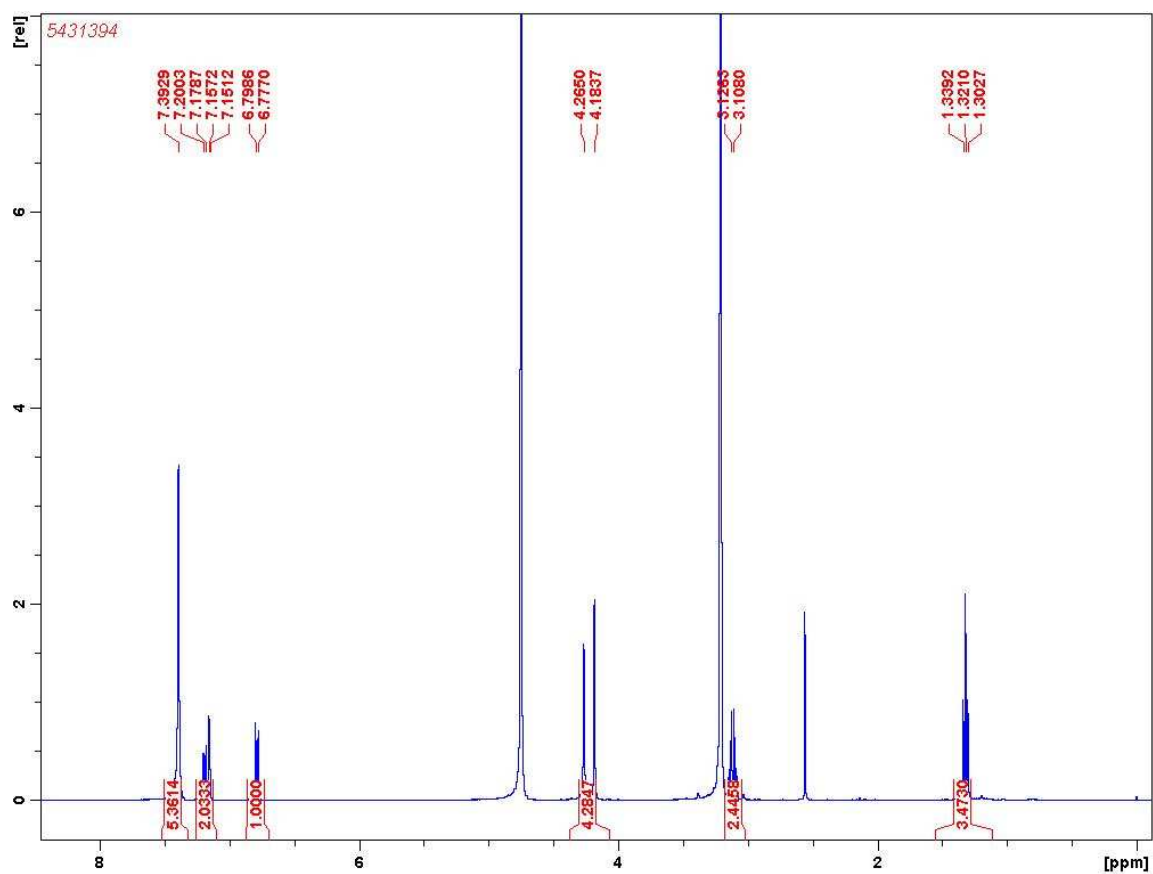
Chemical Formula: $C_{16}H_{18}ClNO$

Exact Mass: 275.1077

Purity: >95%

Predicted 1H NMR spectrum:

Observed ^1H NMR spectrum:



LC-MS:

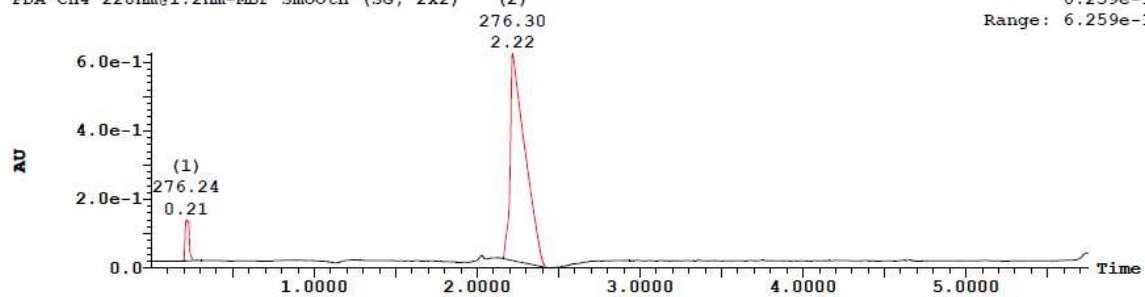
Sample 5 Vial 1:13 ID File 5421384_2 Date 30-Oct-2009 Time 16:31:29 Description

PDA Ch4 220nm@1.2nm-MBF Smooth (SG, 2x2)

(2)

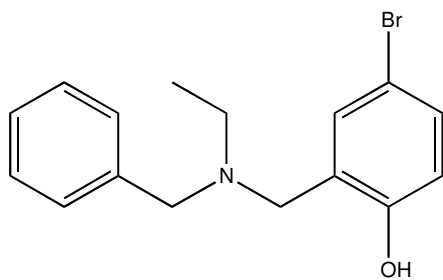
6.259e-1

Range: 6.259e-1



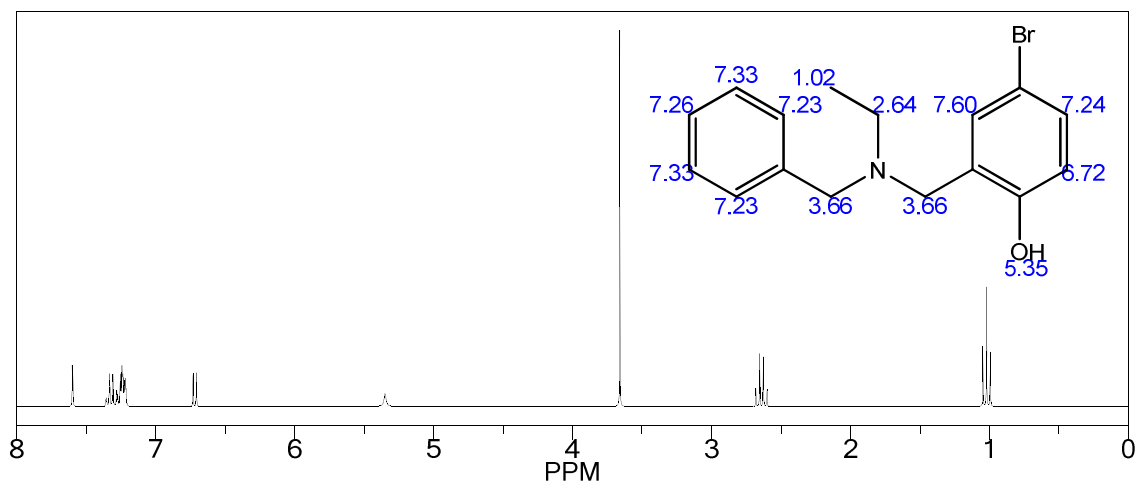
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		0.21	3.56e+003	5.28	0	1e+005	
2		2.22	6.39e+004	94.72	0	6e+005	

(c) 5428736

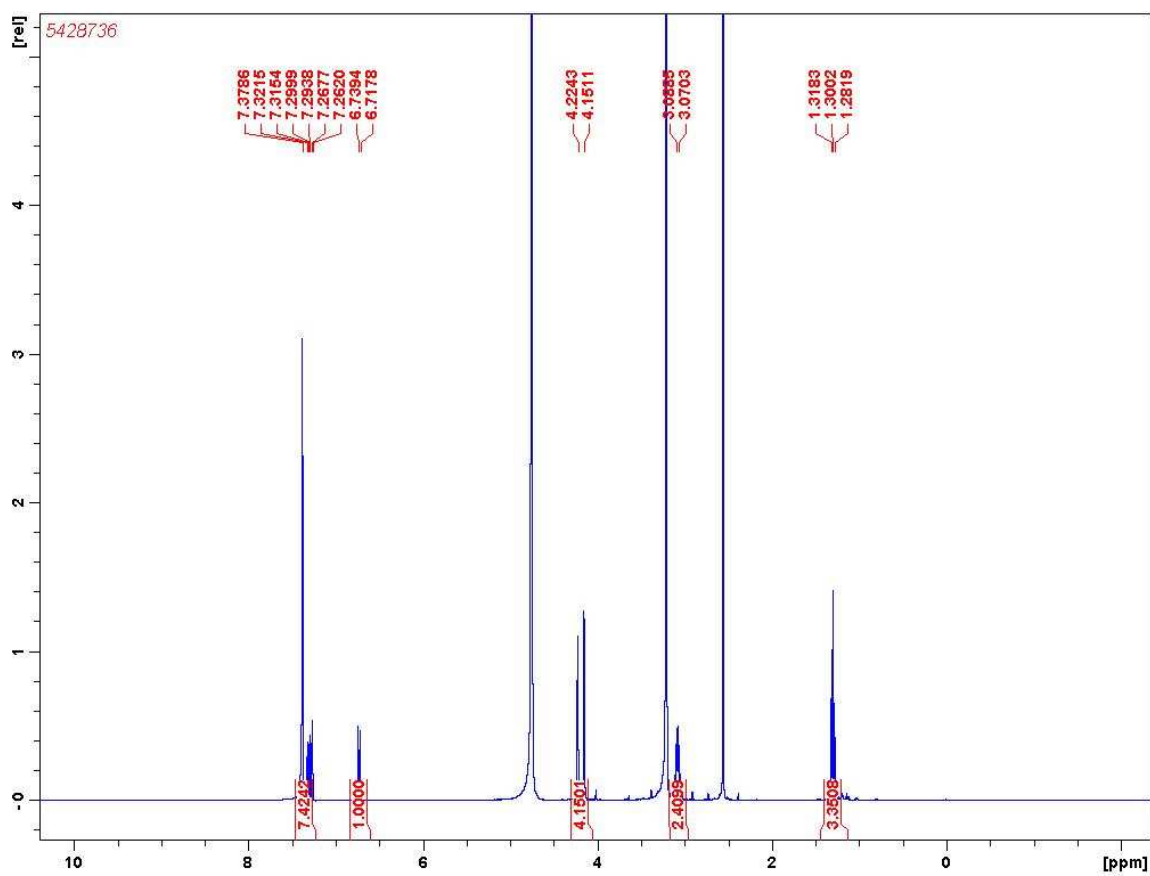
Chemical Formula: $C_{16}H_{18}BrNO$

Exact Mass: 319.0572

Purity: >95%

Predicted 1H NMR spectrum:

Observed ^1H NMR spectrum:

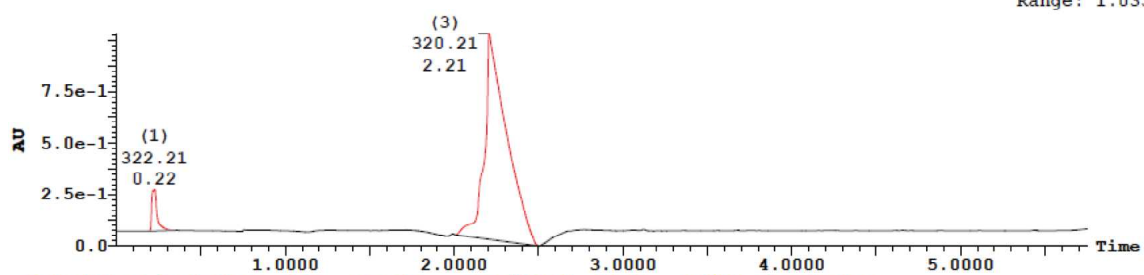


LC-MS:

PDA Ch4 220nm@1.2nm-MBF Smooth (SG, 2x2)

1.033

Range: 1.033



Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		0.22	7.51e+003	4.65	0	2e+005	
3		2.21	1.54e+005	95.35	0	1e+006	

LC-MS solvent gradient table:

Time (min)	Flow rate (mL/min)	% solvent A	% solvent B
Initial	0.25	95	5
5	0.25	5	95
5.5	0.25	5	95
6	0.25	95	5
6.25	0.25	95	5

Solvent A: 95:5 water:acetonitrile with 0.1% formic acid

Solvent B: 95:5 acetonitrile:water with 0.1% formic acid