Supporting Information

Substrate-based Fragment Identification for the Development of Selective, Nonpeptidic Inhibitors of Striatal-Enriched Protein Tyrosine Phosphatase

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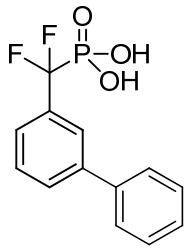
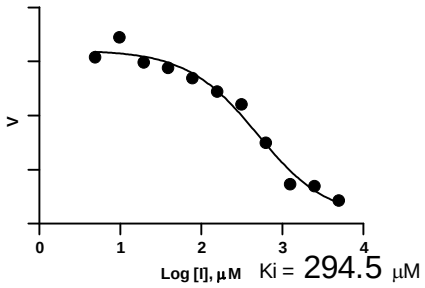
1. Analytical data for K_i determination.

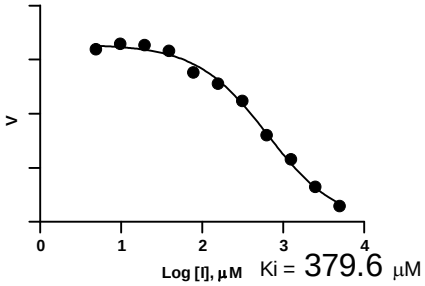
Each inhibitor K_i value was determined using at least 2 independent measurements. The same inhibitor stock solutions were used for each measurement. Dose-response curves are provided for each inhibitor. Non-linear regression analysis was accomplished in Prism (GraphPad) using single site competitive inhibition.

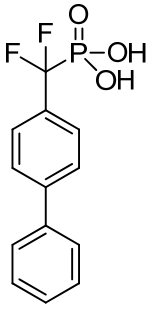


11

Average K_i = 337 μM

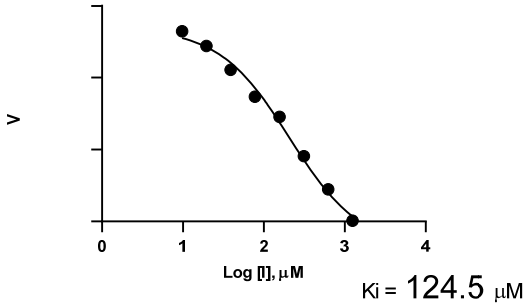


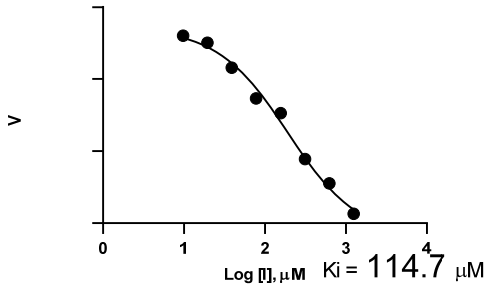




12

Average K_i = 120 μM





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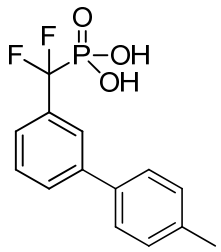
<td> 
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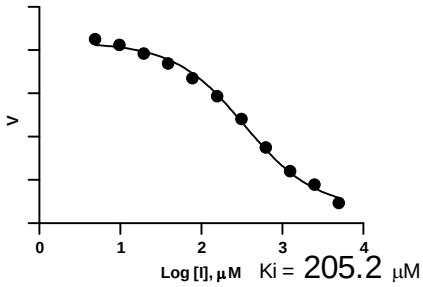
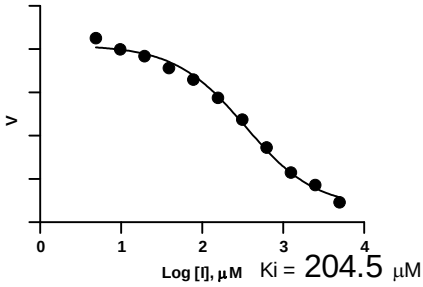
Average K_i = 205 μM</td>
<td> 
  </td>
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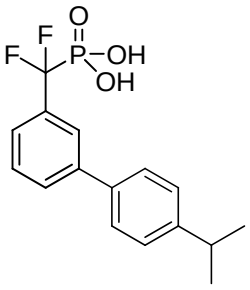
<td> 
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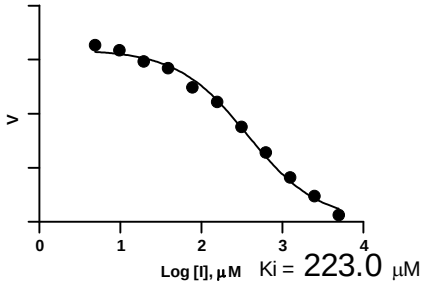
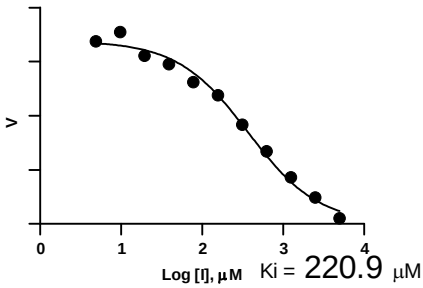
Average K_i = 222 μM</td>
<td> 
  </td>
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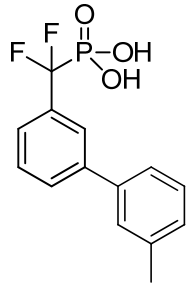
<td> 
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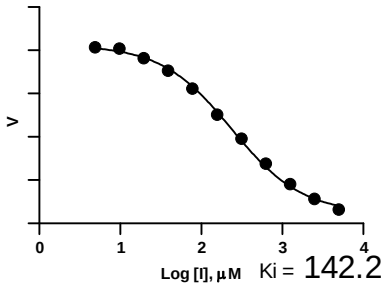
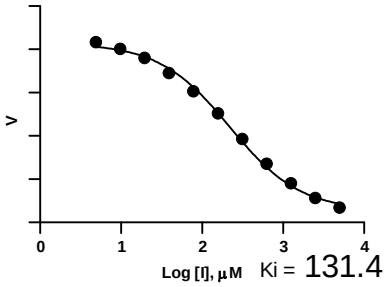
Average K_i = 137 μM</td>
<td> 
Log [I], μM Ki = 142.2 μM
 
Log [I], μM Ki = 131.4 μM</td>
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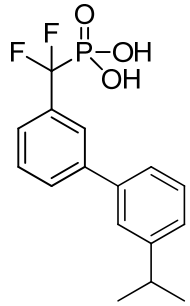
<td> 
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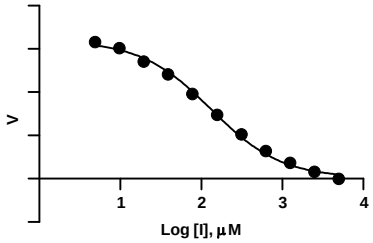
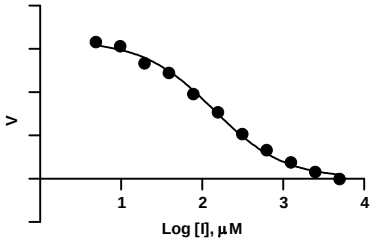
<td> 
Log [I], μM
Ki = 79.08 μM
 
Log [I], μM
Ki = 82.32 μM</td>
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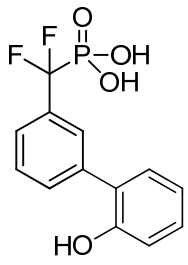
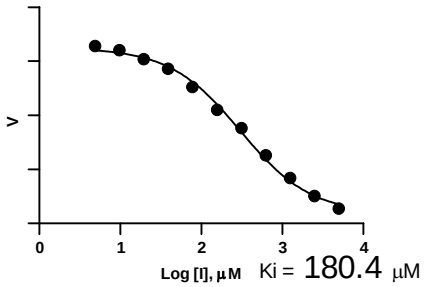
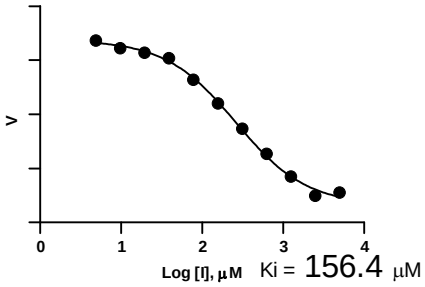
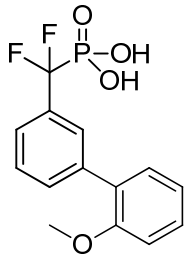


11e

Average K_i = 168 μM

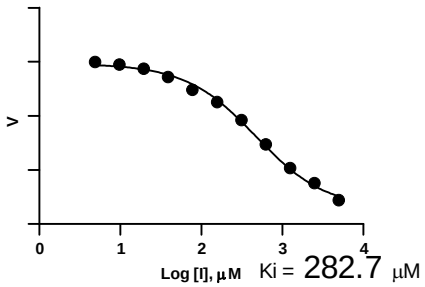


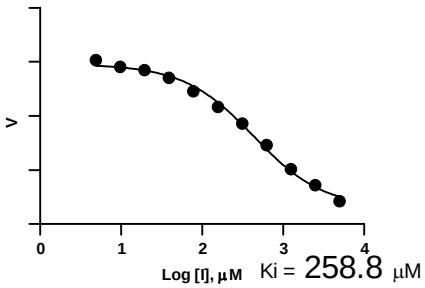




11f

Average K_i = 271 μM





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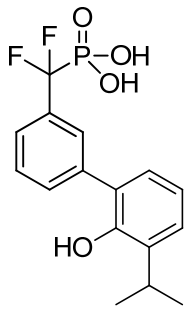
<td> 
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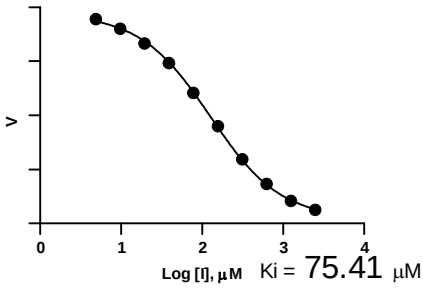
Average K_i = 69 μM</td>
<td> 
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 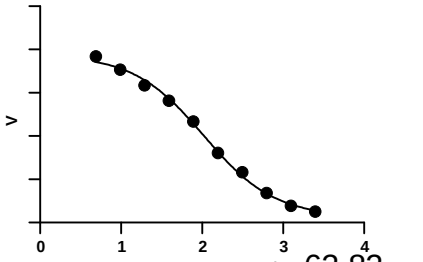
Log [I], μM Ki = 62.82 μM</td>
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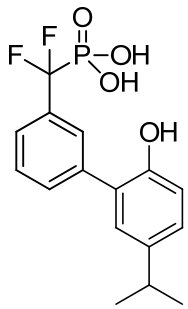
<td> 
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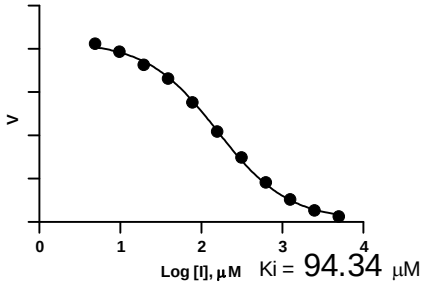
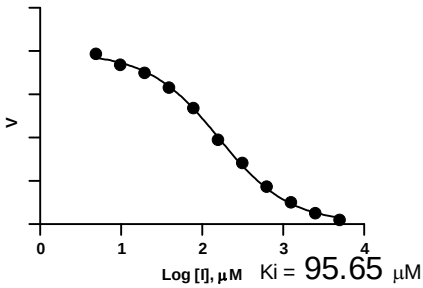
Average K_i = 95 μM</td>
<td> 
Log [I], μM Ki = 94.34 μM
 
Log [I], μM Ki = 95.65 μM</td>
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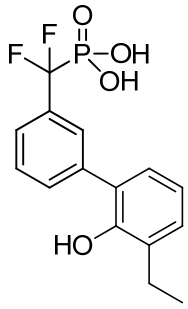
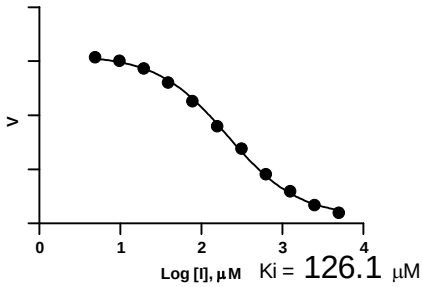
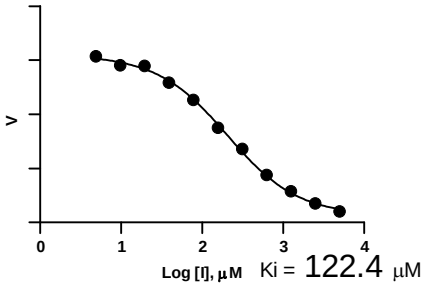
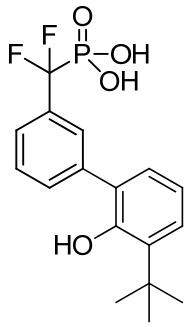
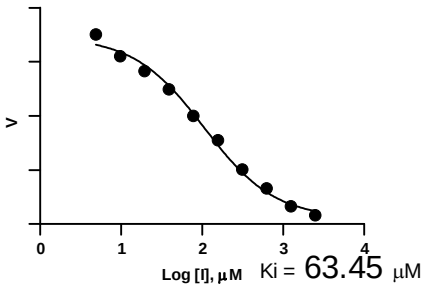
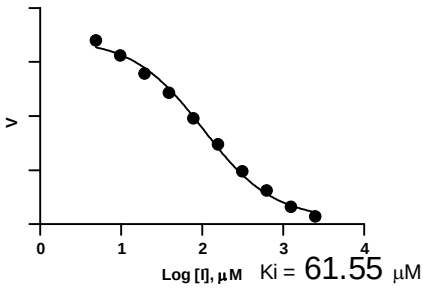
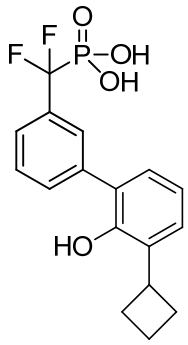
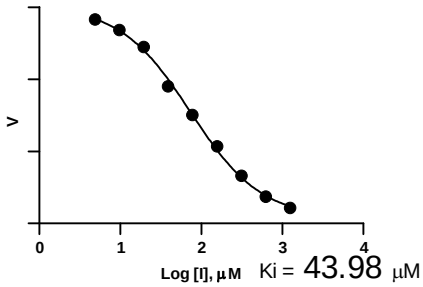
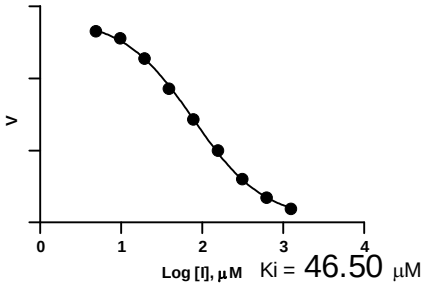
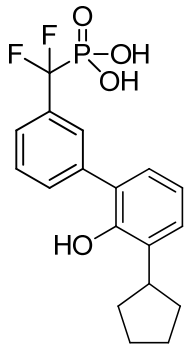
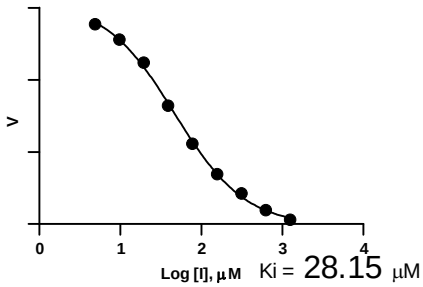
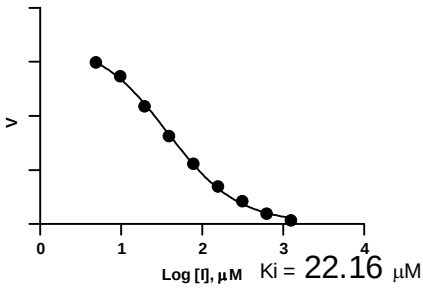
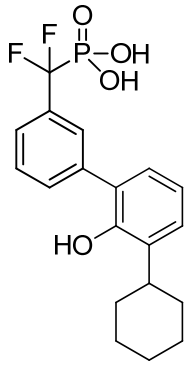
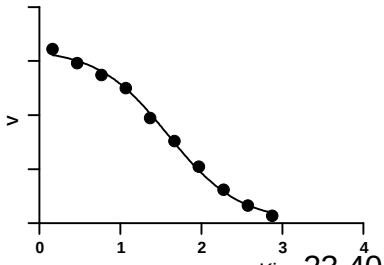
 **11i** Average K_i = 124 μM	 
 **11j** Average K_i = 63 μM	 

 **11k** Average K_i = 45 μM	 
 **11l** Average K_i = 25 μM	 

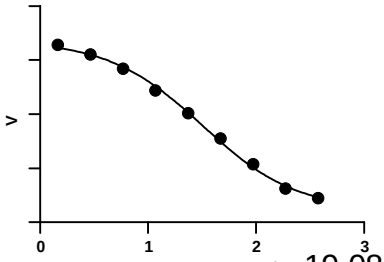


11m

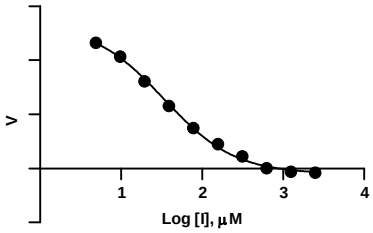
Average K_i = 20 μM



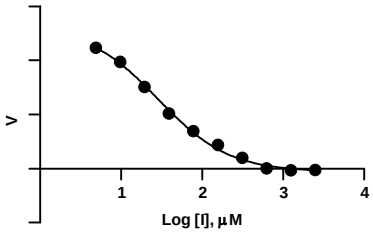
Ki = 23.40 μM



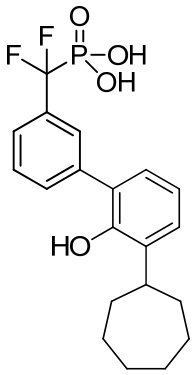
Ki = 19.08 μM



Ki = 20.40 μM

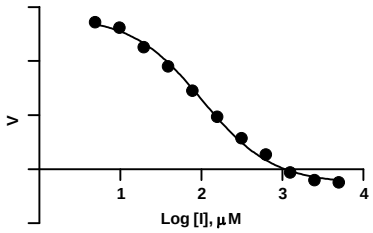


Ki = 17.23 μM

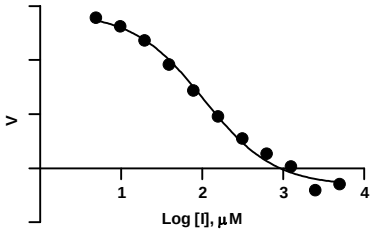


11n

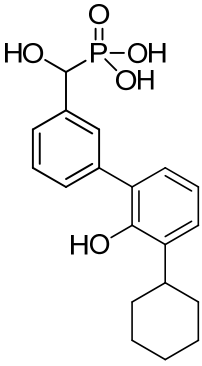
Average K_i = 62 μM



Ki = 61.34 μM

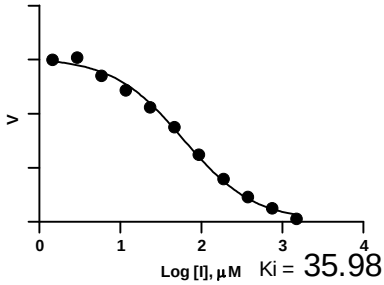


Ki = 62.50 μM

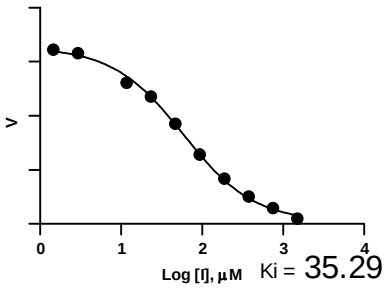


11o

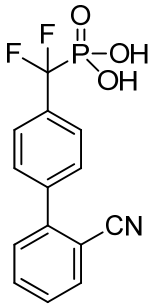
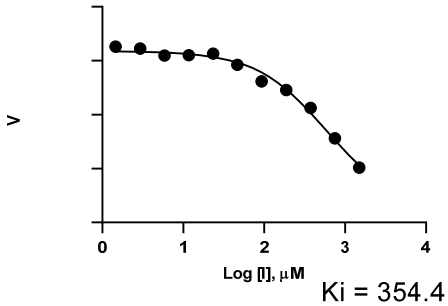
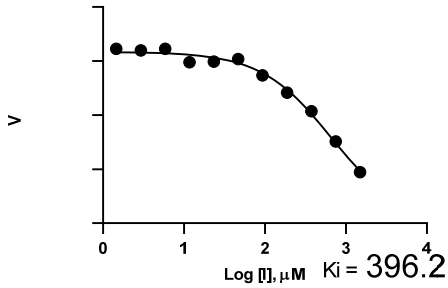
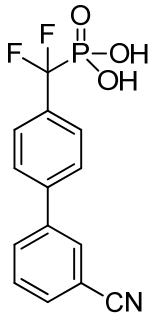
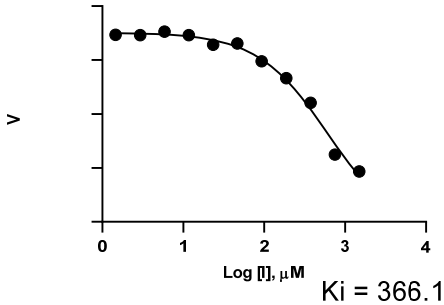
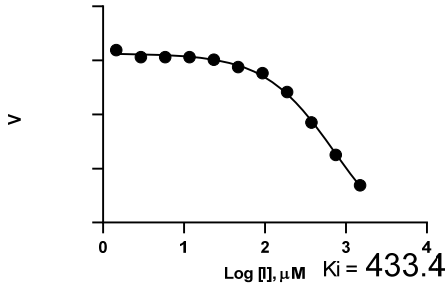
Average K_i = 36 μM

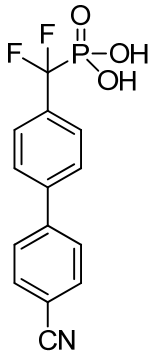


Ki = 35.98 μM



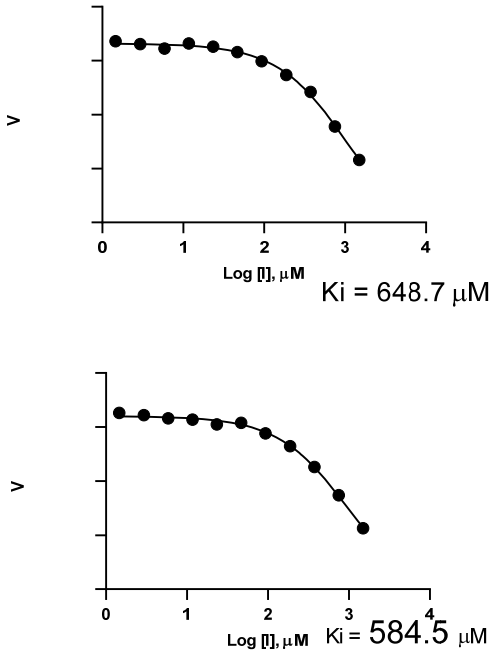
Ki = 35.29 μM

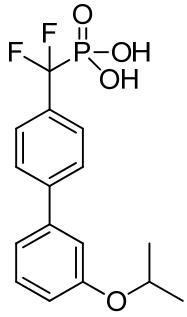
 **12a** Average K_i = 375 μM	 Ki = 354.4 μM  Ki = 396.2 μM
 **12b** Average K_i = 399 μM	 Ki = 366.1 μM  Ki = 433.4 μM



12c

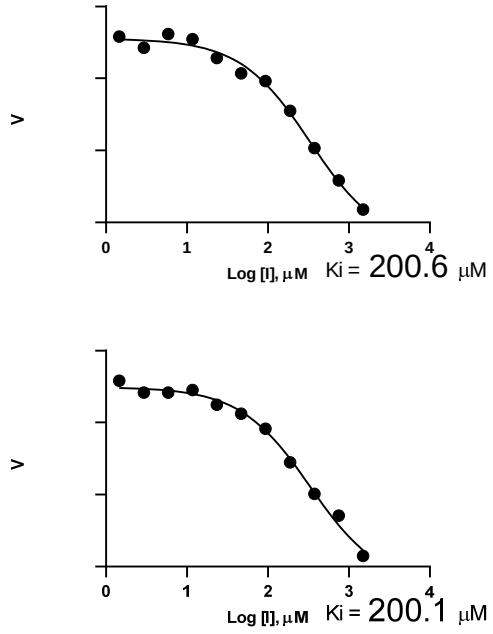
Average K_i = 617 μM





12d

Average K_i = 200 μM



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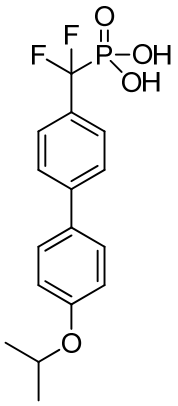
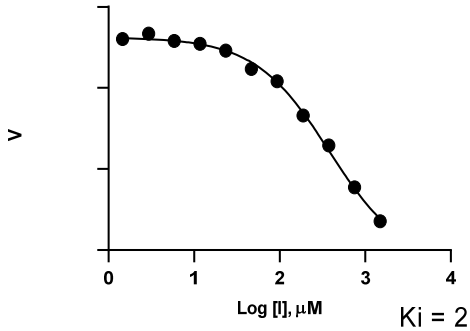
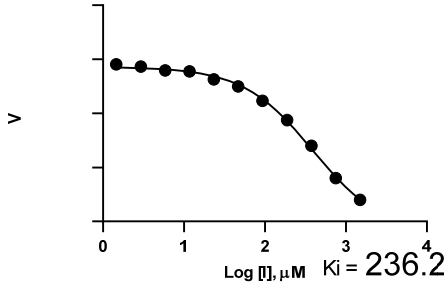
<td> 
12e
Average K_i = 231 μM</td>
<td> 

Ki = 227.5 μM
 

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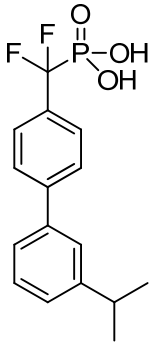
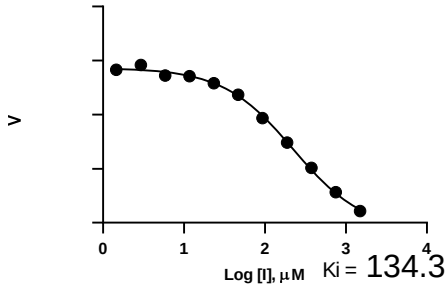
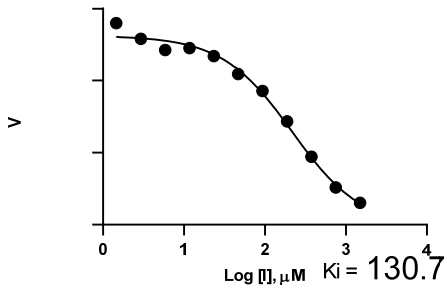
<td> 
12f
Average K_i = 132 μM</td>
<td> 

Ki = 134.3 μM
 

Ki = 130.7 μM</td>
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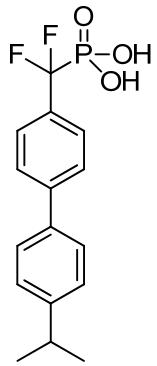
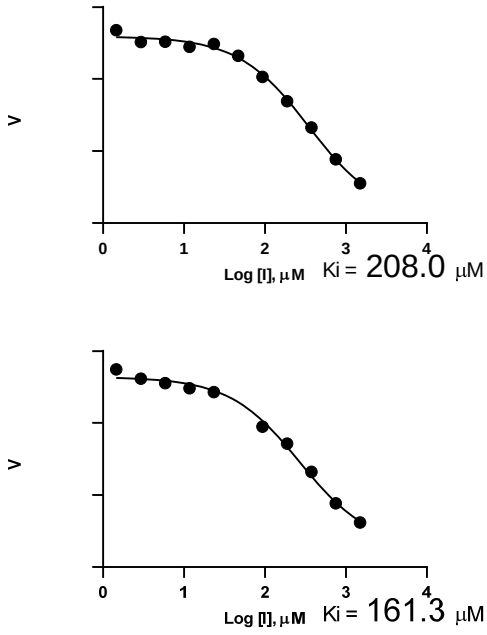
<td> 
12g
Average K_i = 184 μM</td>
<td> 
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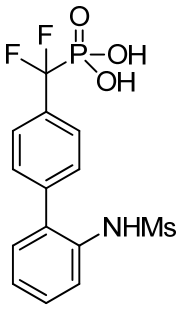
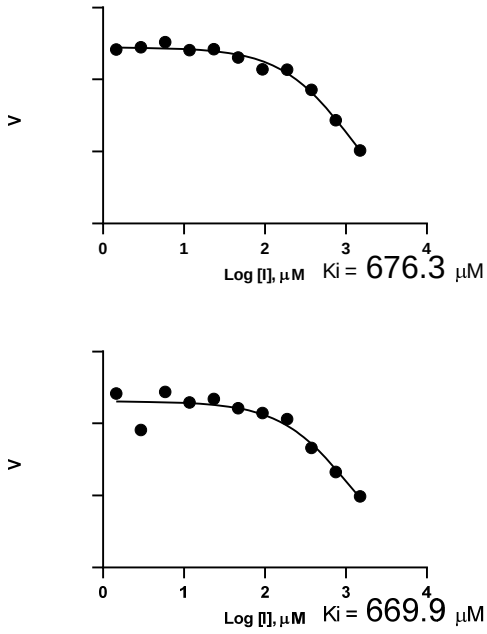
<td> 
12h
Average K_i = 673 μM</td>
<td> 
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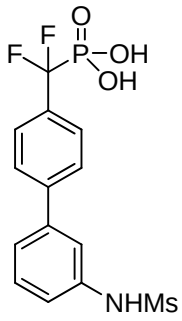
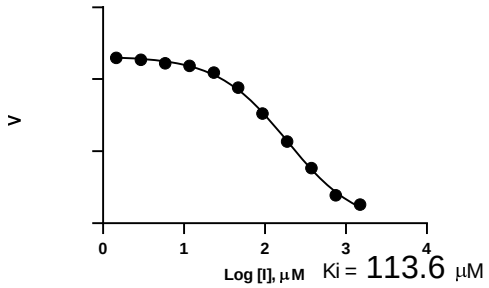
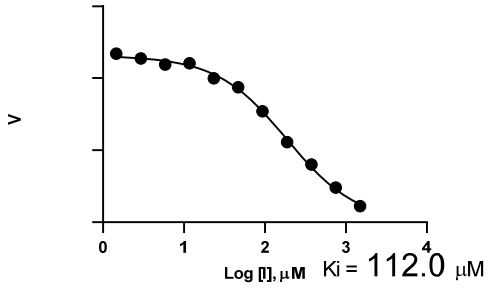
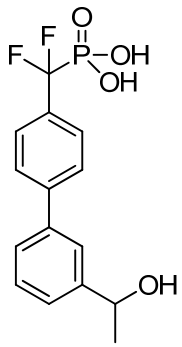


12i

Average K_i = 113 μM

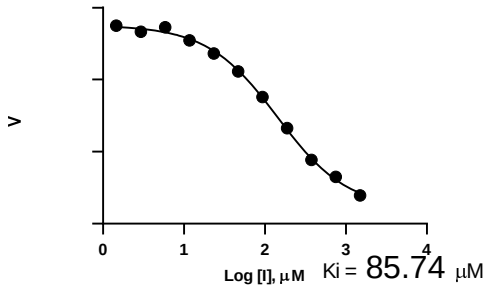


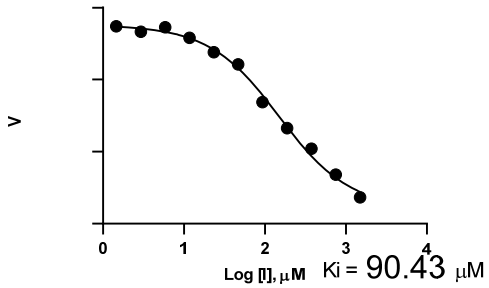


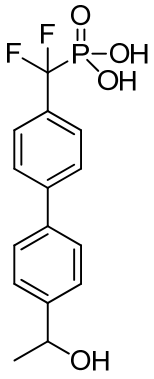


12j

Average K_i = 88 μM

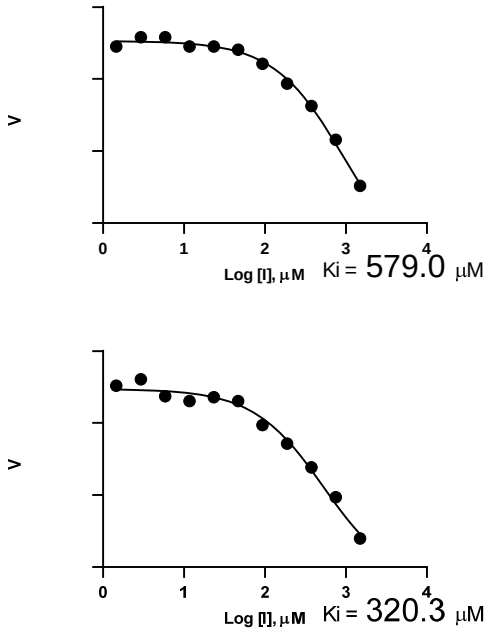


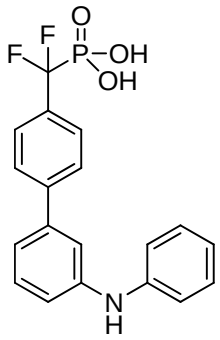




12k

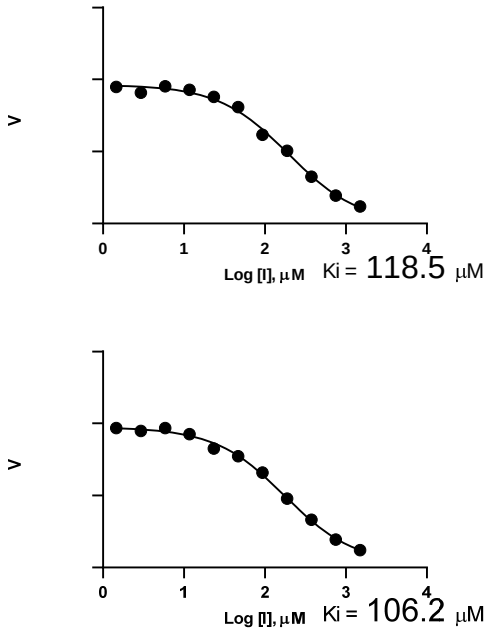
Average K_i = 450 μM

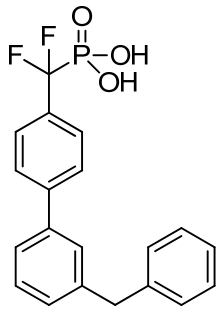




12l

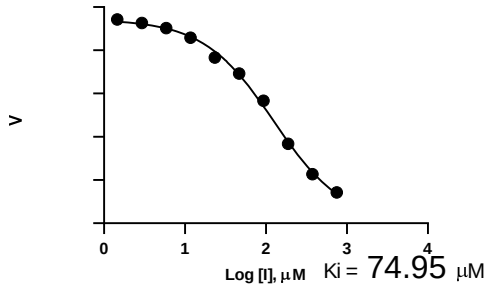
Average K_i = 112 μM

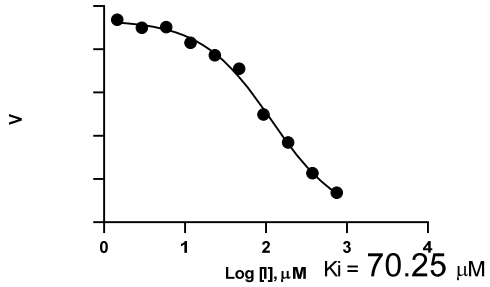


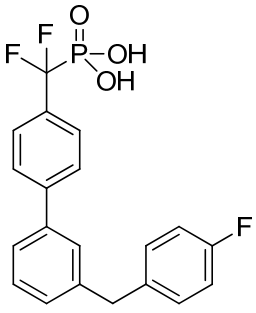


12m

Average K_i = 73 μM

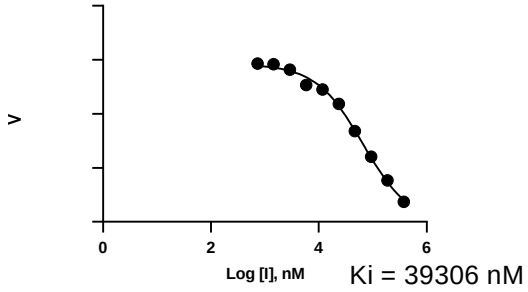


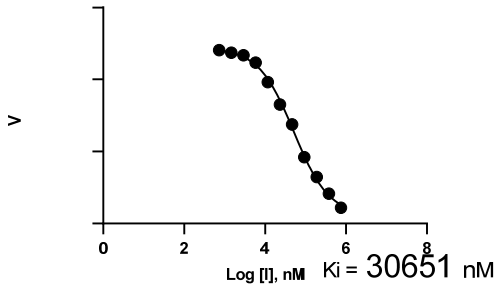


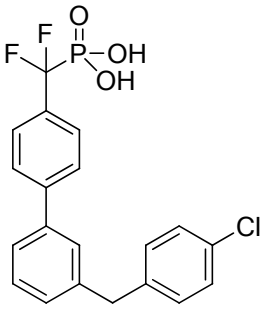


12n

Average K_i = 35 μM

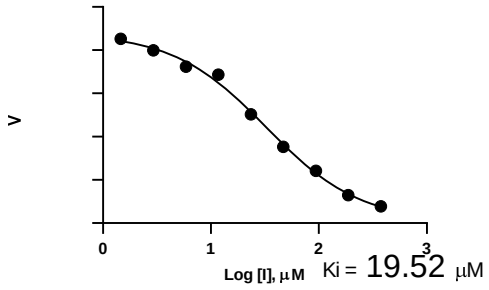


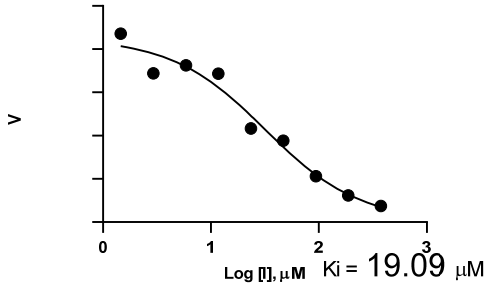


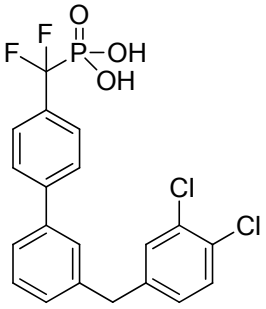


12o

Average K_i = 19 μM

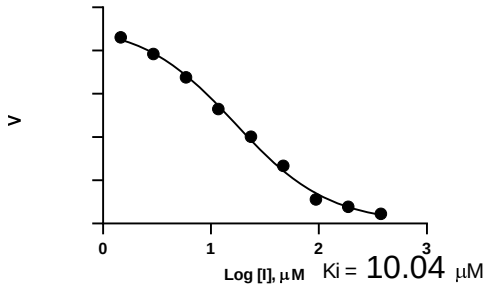


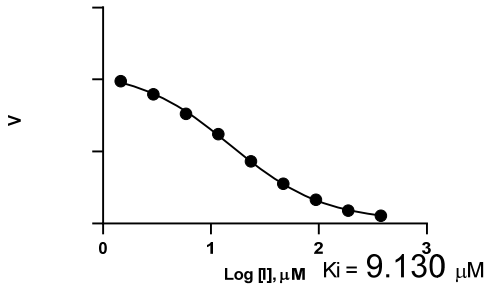




12p

Average K_i = 9.6 μM





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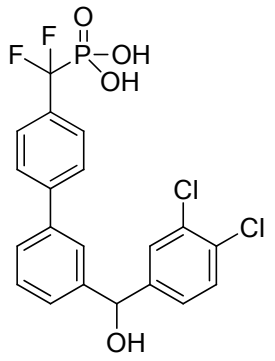
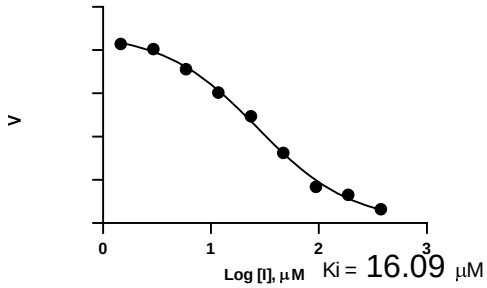
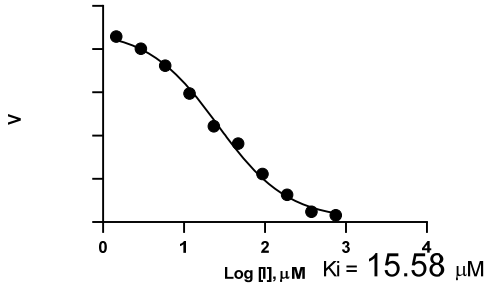
<td> 
12q
Average K_i = 16 μM</td>
<td> 
  </td>
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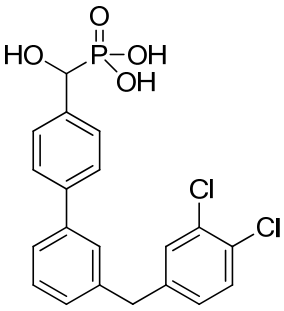
<td> 
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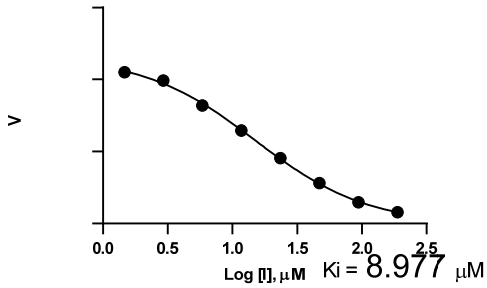
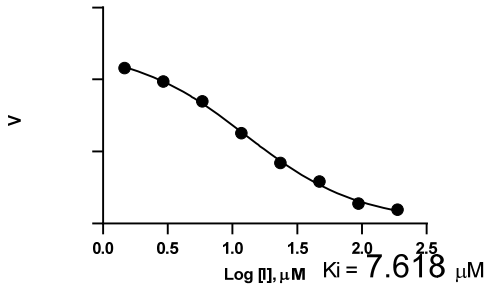
Average K_i = 8.3 μM</td>
<td> 
  </td>
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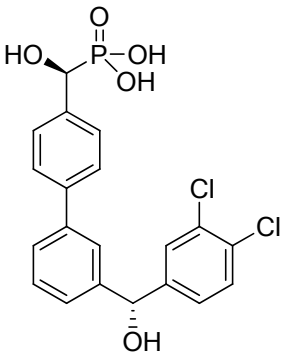
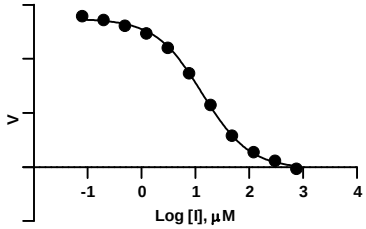
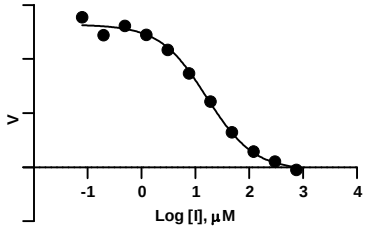


12s

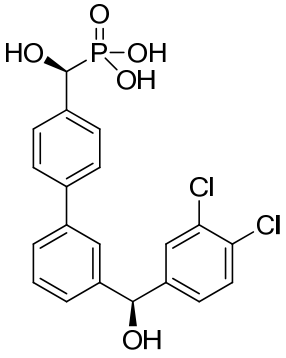
Average K_i = 8.9 μM



Ki = 8.110 μM

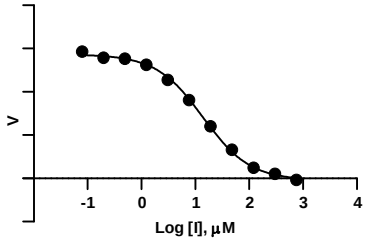


Ki = 9.758 μM

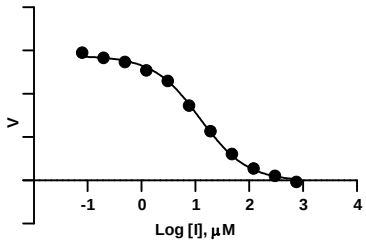


12t

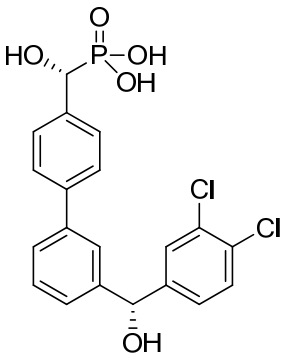
Average K_i = 7.8 μM



Ki = 8.341 μM

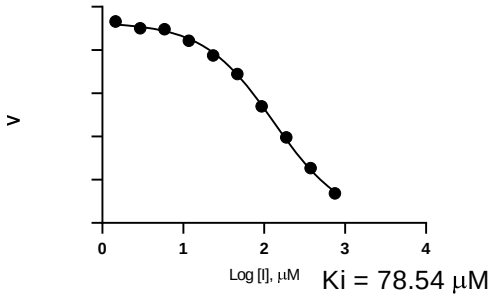


Ki = 7.349 μM

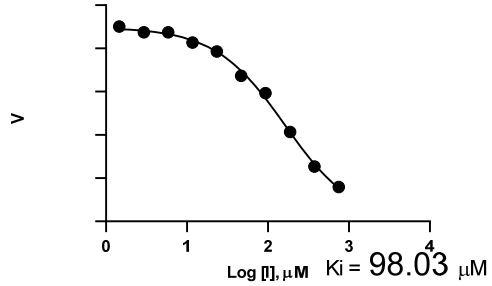


12u

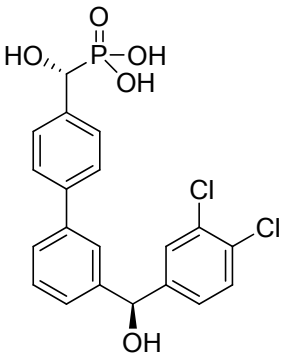
Average K_i = 82 μM



Ki = 78.54 μM

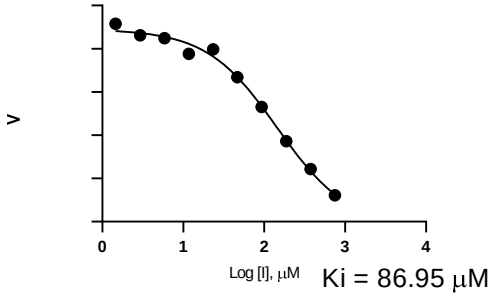


Ki = 98.03 μM

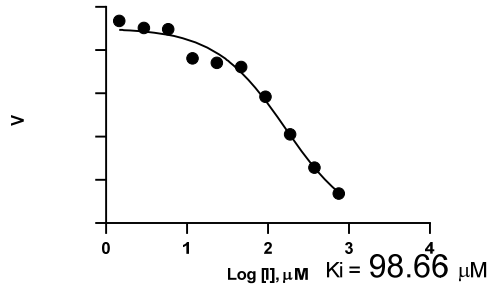


12v

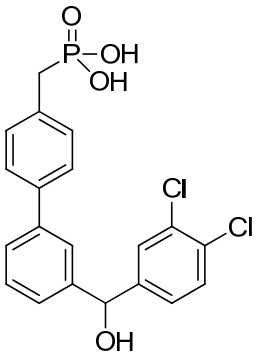
Average K_i = 93 μM



Ki = 86.95 μM

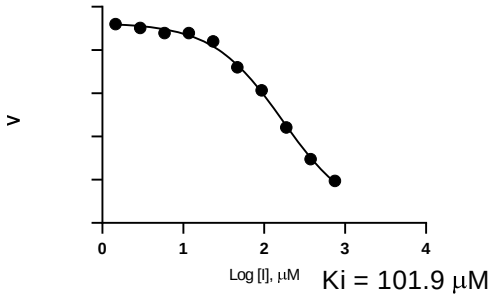


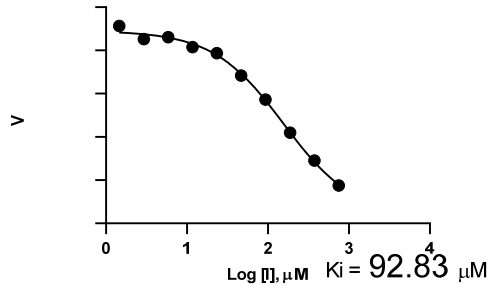
Ki = 98.66 μM



12w

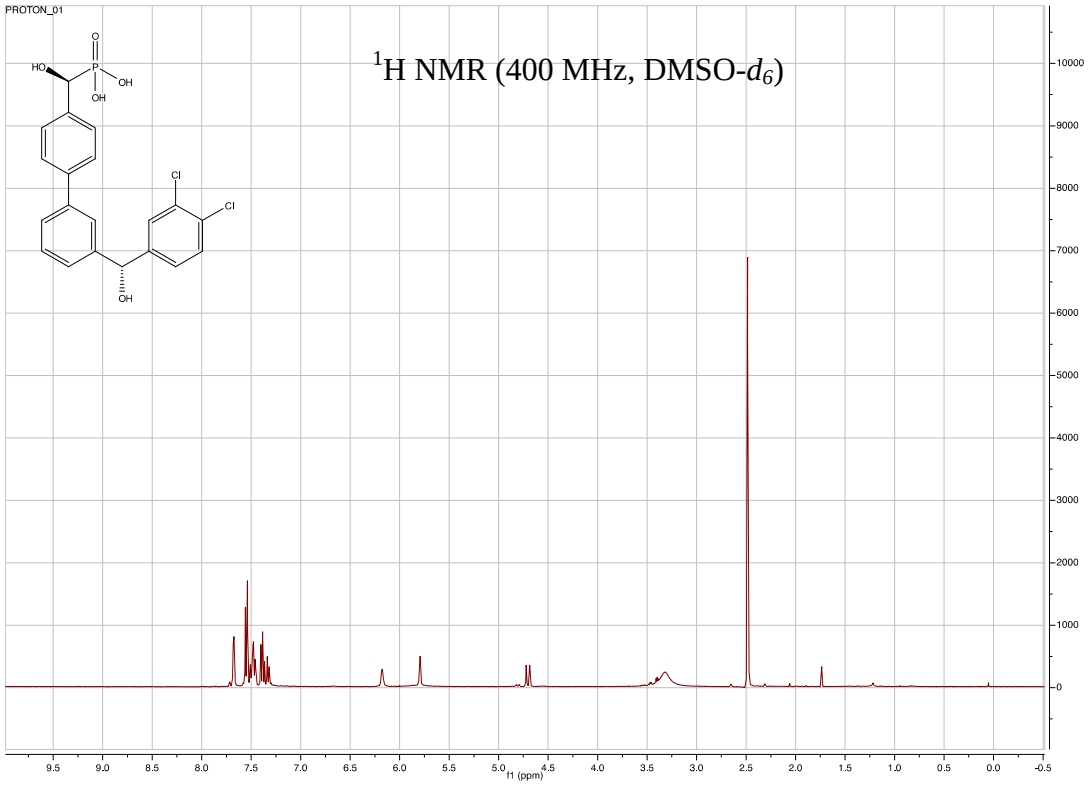
Average K_i = 97 μM

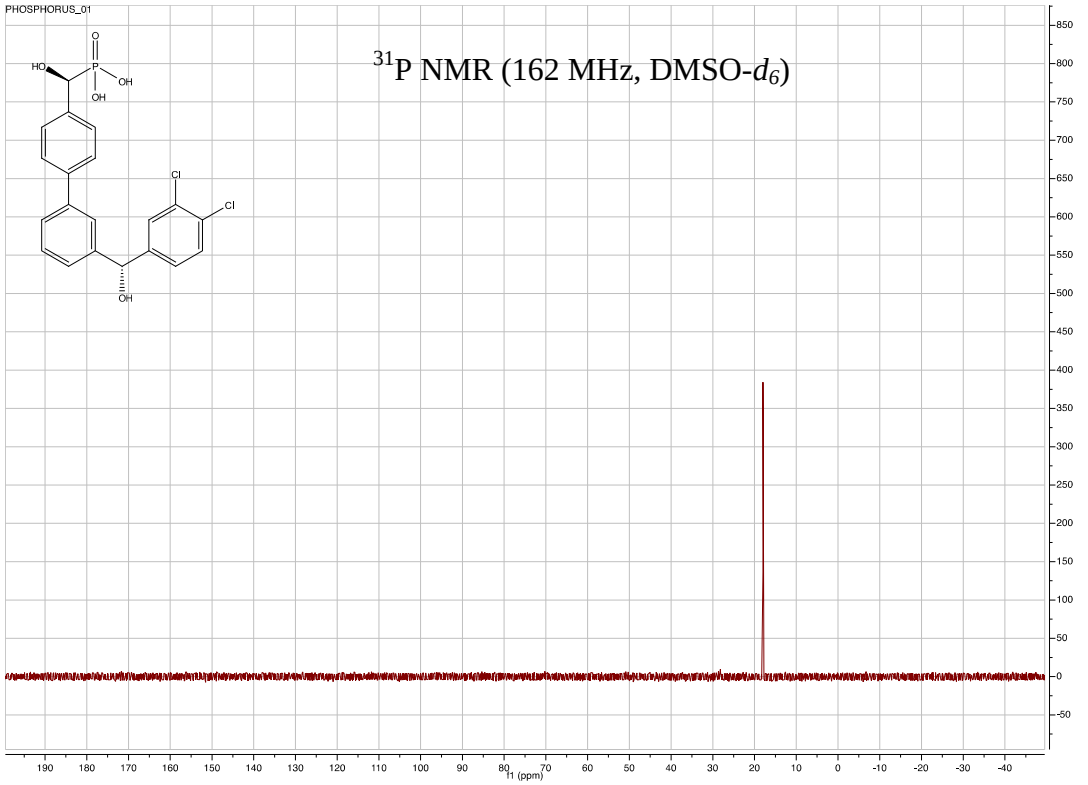




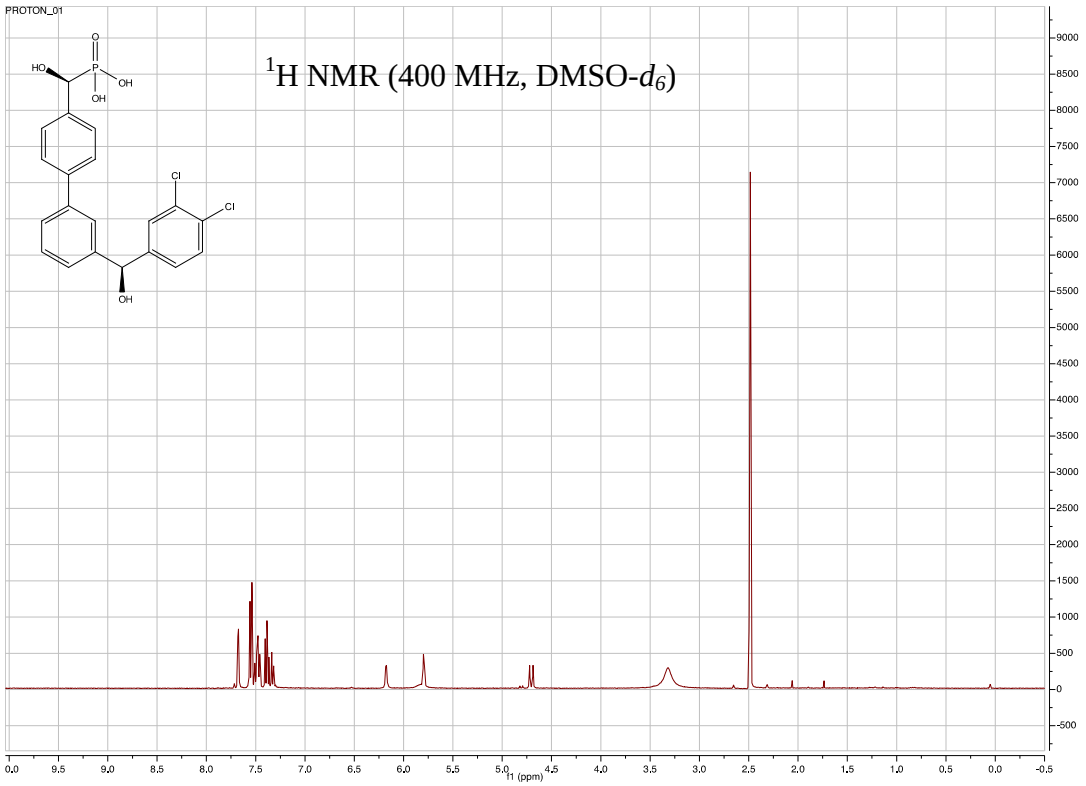
2. NMR spectra.

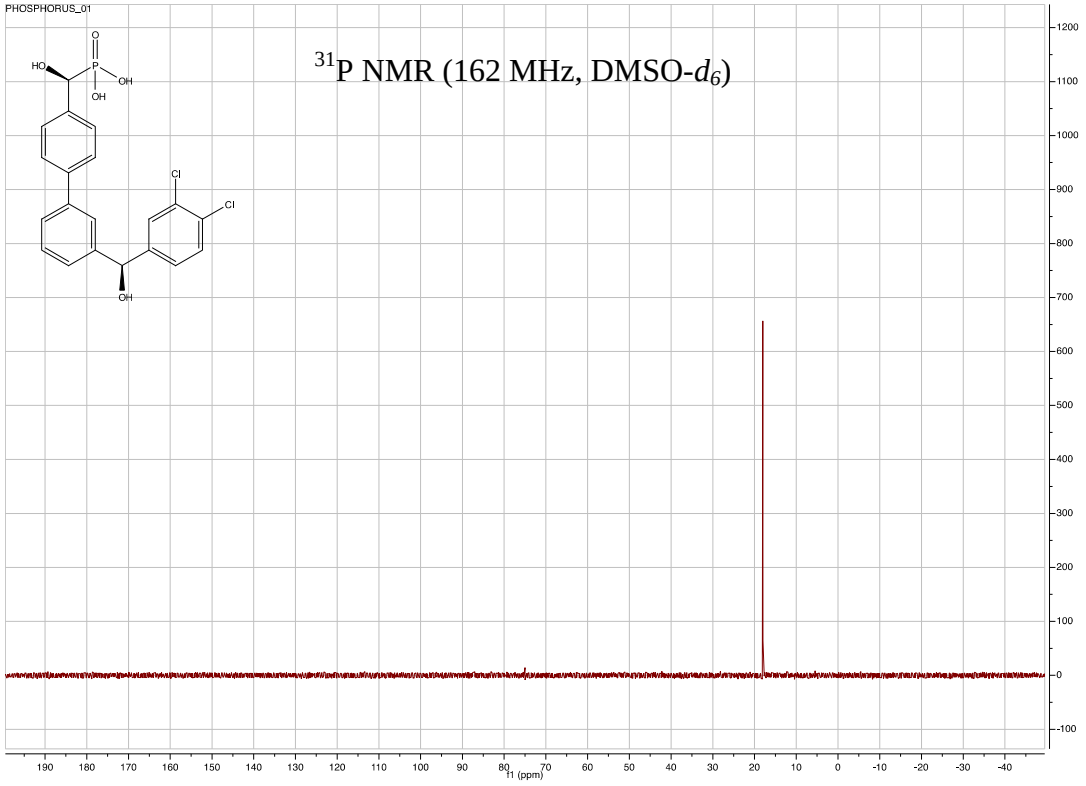
Inhibitor **12s**:





Inhibitor **12t**:





3. Table of primary and secondary antibodies used in this study.

Antibody	Format	Immunogen	Host	Dilution	Source
Anti-pTyr^{1472} GluN2B	Whole IgG, unconjugated	Synthetic phosphopeptide	Rabbit	1:1000	Cell Signaling Technology, Danvers, MA
Anti-GluN2B	Whole IgG, unconjugated	C-terminus of mouse GluN2B	Rabbit	1:1000	Cell Signaling Technology
Anti-pTyr^{402} Pyk2	Whole IgG, unconjugated	Synthetic phosphopeptide of human Pyk2	Rabbit	1:1000	Invitrogen
Anti-Pyk2	IgG2a	C-terminus of human Pyk2	Mouse	1:1000	Cell Signaling Technology
Anti-pTyr^{204} ERK1/2	Whole IgG, unconjugated	Synthetic phosphopeptide	Mouse	1:500	Santa Cruz Biotechnology
Anti-ERK2	Whole IgG, unconjugated	C-terminus of rat p44 MAP Kinase	Rabbit	1:20,000	Cell Signaling Technology
Anti-GAPDH, clone 6c5	IgG1, unconjugated	Purified protein from rabbit muscle	Mouse	1:20,000	Millipore
Anti-rabbit	Whole IgG peroxidase-conjugated	Rabbit Fc	Donkey	1:10,000	Amersham Biosciences
Anti-mouse	Whole IgG peroxidase-conjugated	Mouse Fc	Sheep	1:5,000	Amersham Biosciences

4. BBB PAMPA results.

BBB PAMPA studies were conducted by Pion, Inc (Billerica, MA) at pH 7.4. The refined effective permeability (P_e) value obtained (average of triplicates) is summarized in the table along with results for internal highly and low permeable standards – propranolol and atenolol respectively.

Compound	**Avg. P_e (10^{-6} cm/s)[a]**	**SD P_e**	**Avg. %R[b]**	**SD %R**	**Avg. logP_e**	**SD logP_e**	**Domain, nm**
Inhibitor **12s**	<0.1		4	0			260-350
propranolol	68	5	48	3	-4.17	0.03	250-498
atenolol	<0.4		1	1			250-498

[a]effective permeability measured in assay, results indicated with a "<" sign mean no quantifiable UV signal was detected in the acceptor compartments

[b]membrane retention