SGC-Oxford Oxydoreductases: Initial Assay Optimization and Screening of HADH2 and HSD17β4

March, 2007

Introduction

- The five enzymes were sent to us by researchers at the Structural Genomics Consortium, Oxford University lab (PI Udo Oppermann).
- All 5 proteins, at MW of approximately 30 kDa, have been produced by E.coli fermentation as His-tagged recombinants for the purpose of X-ray structure determination, and have been mass-spec characterized.
- The five enzymes came with time-course datasets but without further validation data.

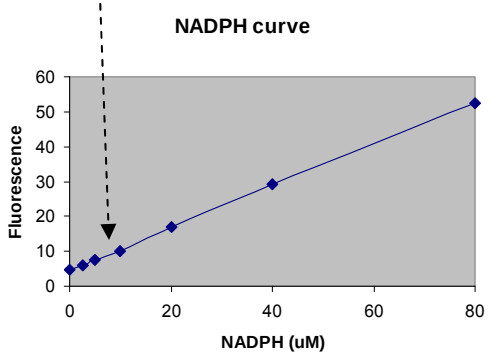
Assay Development

Parameter Tested/Modified	HADH2	HSD17β4
Enzyme Concentration	x	x
Substrate Concentration	x	x
Reaction Time	x	x
Reagent Formulation		x
Reagent Stability	x	x
Detection (reader settings, data points)	x	x
Control Inhibitor	x	x

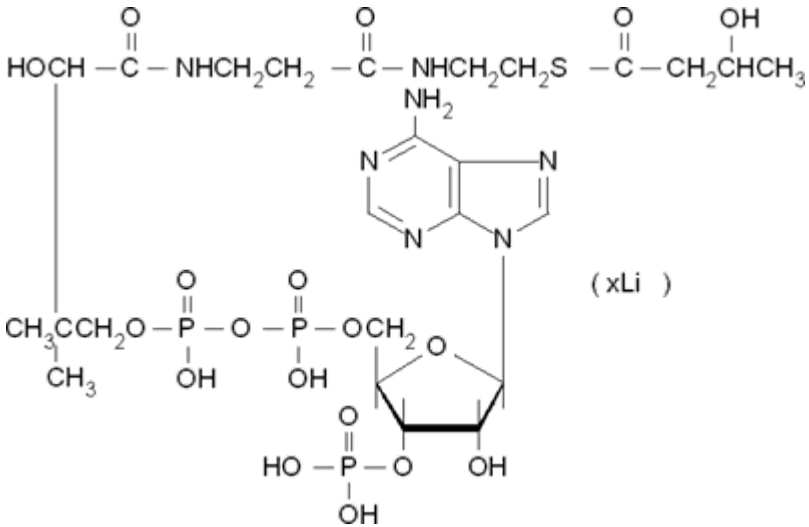
Reaction Biochemistry

Enzyme	Substrate	Cofactor	Product	Proposed Cofactor Concentration	Fluorescence Intensity Trend
HSD17β4	Estradiol	NAD+	NADH	1 mM	Increase
SPR	Sepiapterin	NADPH	NADP+	10 uM	Decrease
retSDR3	Estradiol	NAD+	NADH	1 mM	Increase
DCXR	3,4-hexanedione	NADPH	NADP+	10 uM	Decrease
HADH2	β-OH butyryl coenzyme A	NAD+	NADH	1 mM	Increase

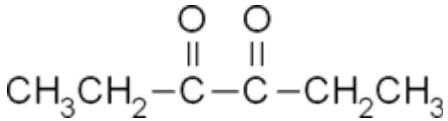
HSD17β4: hydroxysteroid dehydrogenase
SPR: Human Sepiapterin reductase
retSDR3: DHRS10 hydroxysteroid dehydrogenase (17HSDβ14)
DCXR: Dicarbonyl/L-Xylulose Reductase
HADH2: Hydroxyacyl-Coenzyme A dehydrogenase, type II



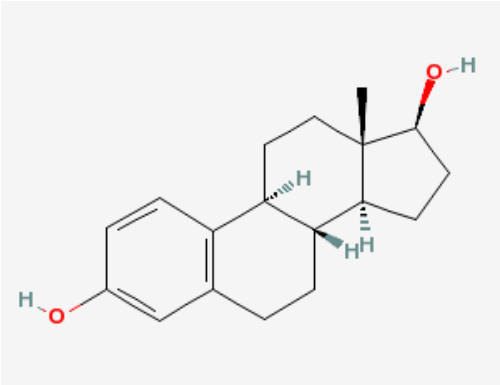
Substrates



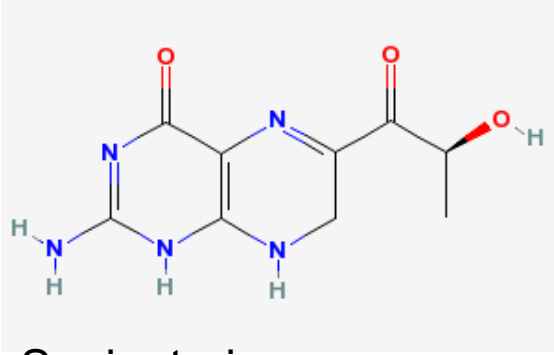
Hydroxybutyryl CoA



3,4-Hexanedione

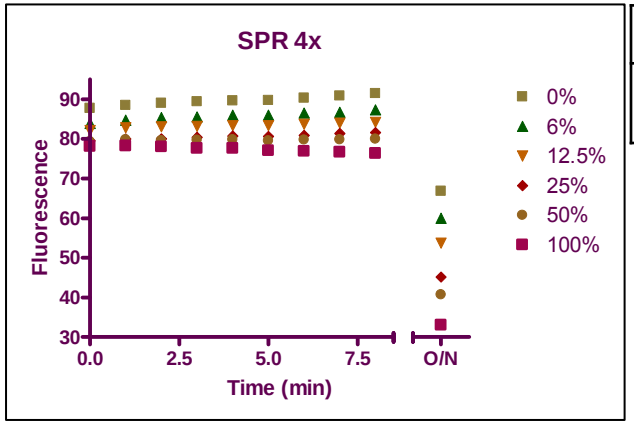


Estradiol

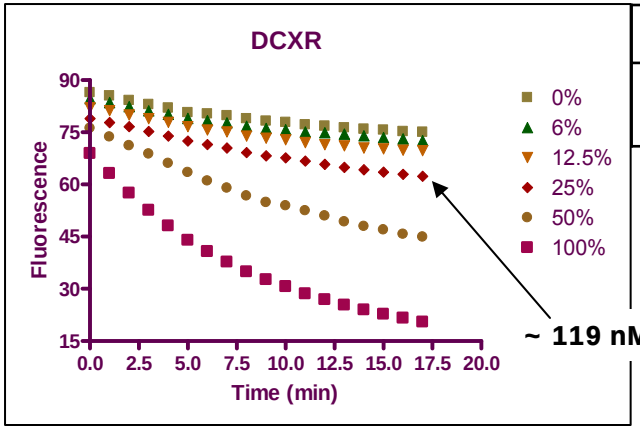


Sepiapterin

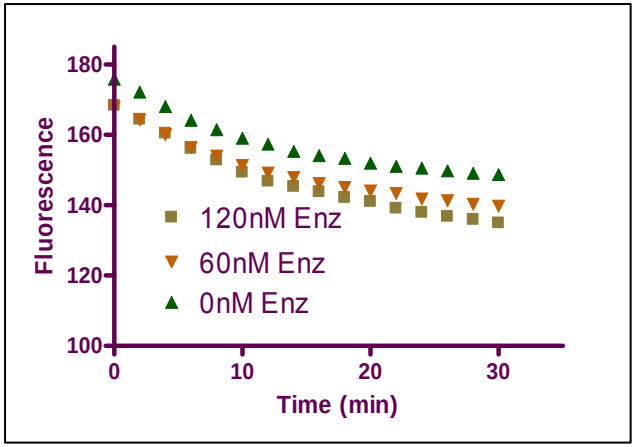
Survey of NADPH-Consuming Enzymes



E	S	NADPH
1.8 uM	90 uM	50 uM

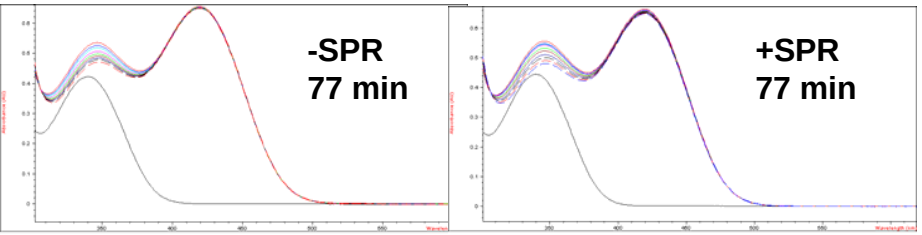


E	S	NADPH
477 nM	100 uM	50 uM



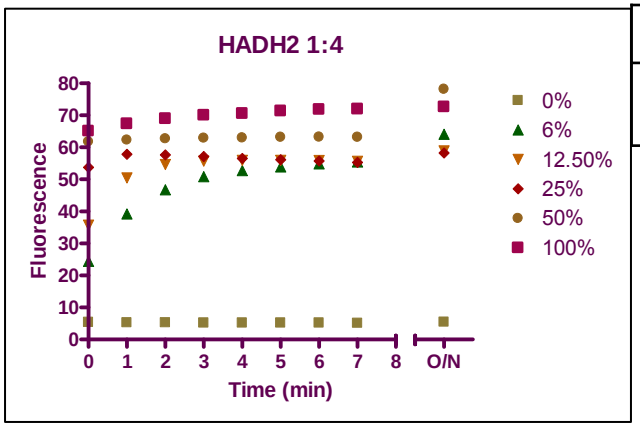
DCXR appears to work only at relatively high concentrations (>~150 nM), while at the same time providing a very limited signal-to-background (due to reaction direction and NADPH instability over extended periods of time).

Cuvette-based UV test of SPR at 445 nM

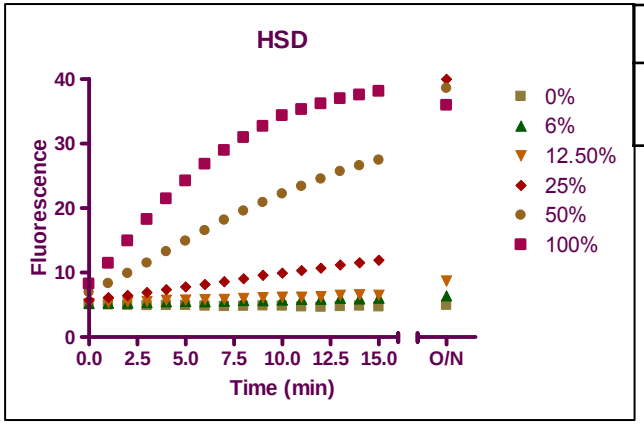


To investigate SPR further, the assay was run in quartz cuvette and the absorbance spectrum recorded as a function of time. SPR did not show detectable activity in both absorbance and fluorescence mode.

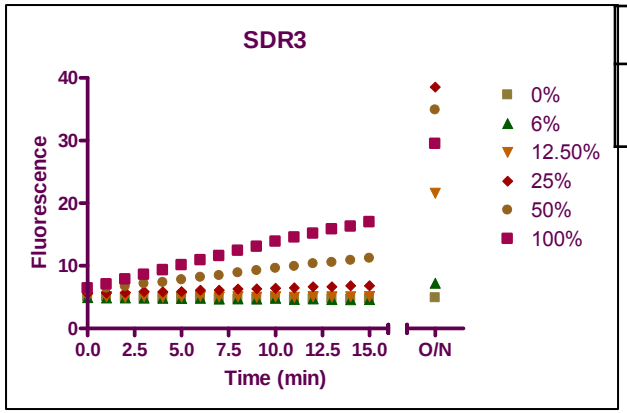
Survey of NADH-Generating Enzymes



E	S	Cf
378 nM	90 uM	1 mM



E	S	Cf
1.76 uM	90 uM	1 mM



E	S	Cf
3.4 uM	90 uM	1 mM

- HADH2: fast, potentially can be run at or below 50 nM.
- HSD: slow, potentially can be run at or below 400 nM.
- SDR3: very slow even at high concentration, needs significant further optimization.

HADH2 qHTS

Reagent Optimization

Enzyme	Substrate	Cofactor	Product	Proposed Cofactor Concentration	Fluorescence Intensity Trend
HADH2	Hydroxybutyryl CoA	NAD+	NADH	1 mM	Increase
1.5 uM	90 uM	← Proposed concentrations			
20 nM	90 uM	← NCGC screening concentrations			

The dramatic decrease (over 70-fold) in the HADH2 assay concentration results in improved dynamic range with respect to observable compound potency and has ultimately enabled the screening of the entire collection.

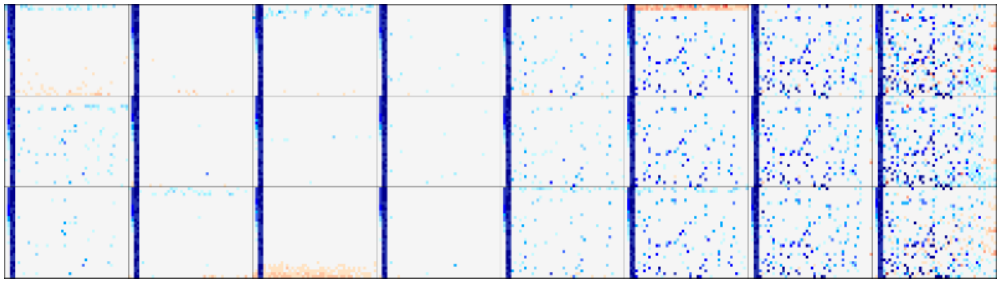
qHTS Protocol

HADH2 1536-well qHTS Protocol	NCGC ID: HADH560	PubChem AID: *unassigned*	
Step	**Parameter**	**Value**	**Description**
1	Reagent	3 uL	20 nM HADH2 enzyme, Column 1,2 and 5-48
2	Reagent	3 uL	100 mM pH 9 Tris-HCl buffer, Column 3,4
3	Compound	23 nL	Entire collection
4	Control	23 nL	Control plate
5	Time	15 min	Room temperature
6	Reagent	1uL	90 uM Butyryl CoA substrate/1mM NAD co-factor, Entire plate
7	Detector	EX DUG11/EM 450/20	Viewlux fluorescence read, Protocol 18

HADH2 1536-well qHTS Protocol	NCGC ID: HADH560	PubChem AID: *unassigned*
Step	**Notes**	
1	Medium-binding black solid-bottom Kalypsys plates with tip 1 dispenser 2 / head B with 1L glass bottle kept at 5°C using recirculating bath (protected from light), 80 ul pre-dispense.	
2	Tip 2 dispenser 2 / head B with 250 mL plastic bottle.	
3	3x 8pt Lopac at beginning and 1x 8pt Lopac in middle and at end.	
4	500 uM Apomorphine 1:2 dilution duplicate (column 2) – 16pt	
5	Auxiliary hotel 2 (near VL)	
6	Tips 1-4 dispenser 1 / head C with 1L glass bottle on ice (protected from light), 60 ul pre-dispense (per tip)	
7	Priority 1, In VL for 4 min., read every 30 sec.	

Lopac Reproducibility in Online Validation Runs

Plate Heatmaps Day 1

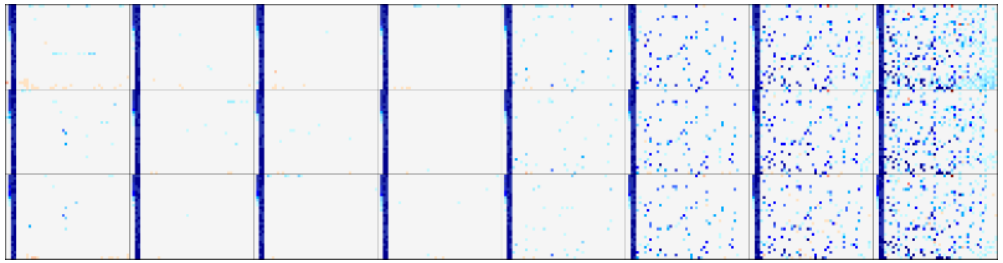


Set 1

Set 2

Set 3

Plate Heatmaps Day 2

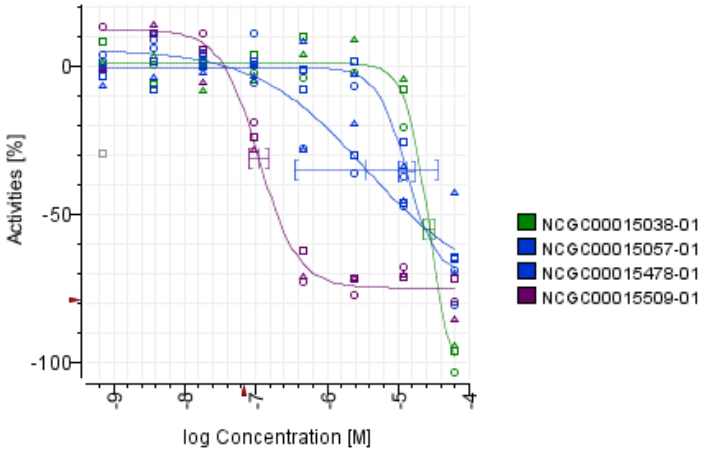


Set 1

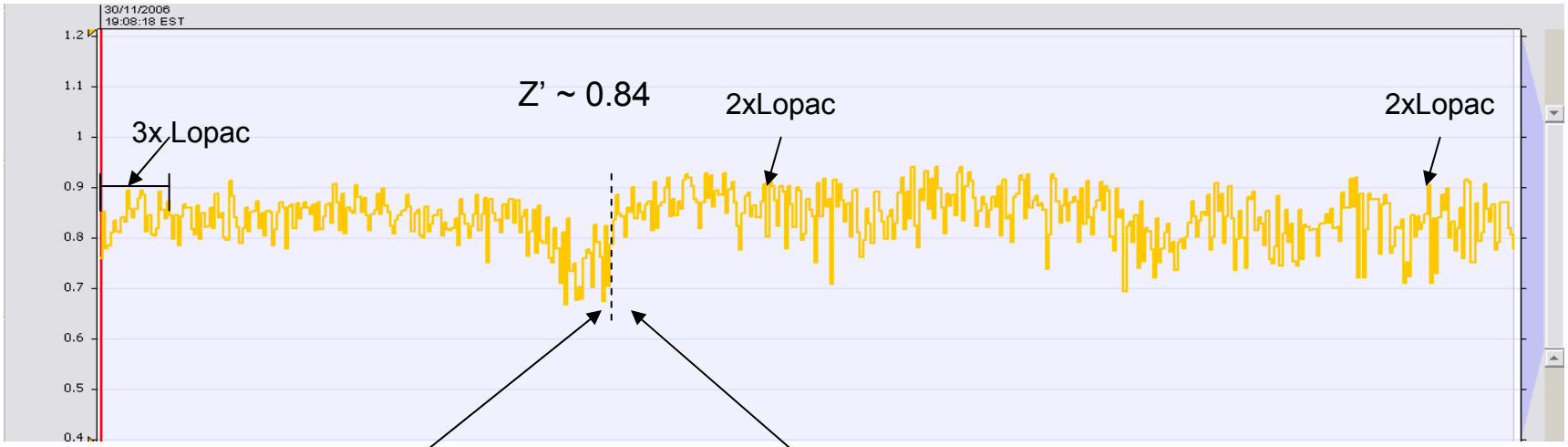
Set 2

Set 3

Example Triplicate Curves



Solid Assay Performance Despite Robotic Problems



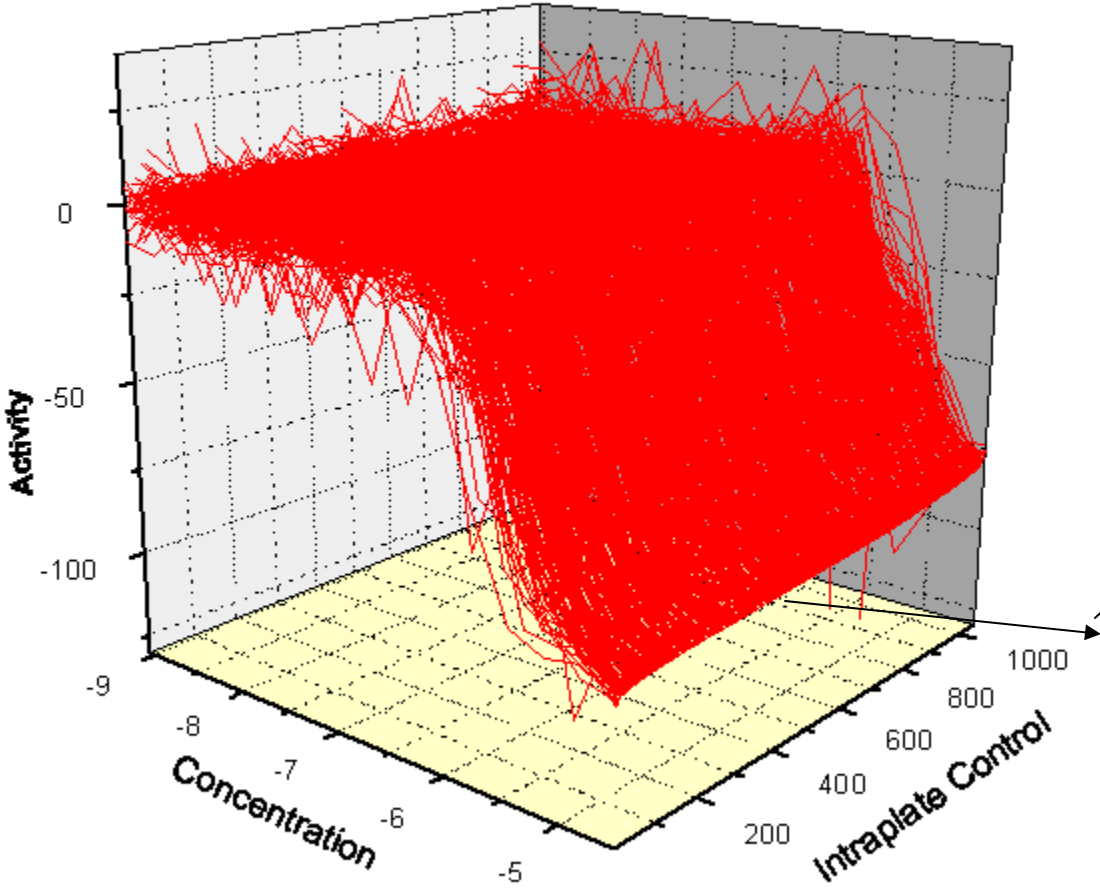
12/01/2006: Equipment failure; screen stopped at plate 190 (DPI) as re-ordered substrate failed to arrive in time

12/21/2006: Screen resumed

Assay Performance Summary

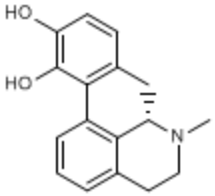
Hydroxyacyl-Coenzyme A Dehydrogenase, Type II 2	NCGC ID: HADH560	PubChem AID: *unassigned*	
Parameter	***Assay opt.***	***Validation***	***qHTS***
Date	2006-09	2006-10-04	2006-11-30; 2006-12-21
System	Off-line	Validation	Kalypsys robot
Plates Screened	N/A	8	625 (527 accepted)
Plates Failed QC	N/A	0	98 (DMSO overflow)
Compound (total # tested)	N/A	1,375	76,431
Samples (total wells)	N/A	10,521	702,592
Number of Data Points	N/A	126,252	5,620,736
% 7 pt-titrations	N/A	100%	90%
% 15 pt-titrations	N/A	0%	5%
Data Layers	Activity (delta) Time-courses	Read 1 (Background Fl.) Read 2 through 12 Delta (Activity Layer)	Read 1 (Background Fluorescence) Read 2 through 8 Delta (Activity Layer)
Average Z' [Delta Activity]	0.85	0.89	0.84
Average Signal : Background (S:B)	N/A	154	172

qHTS Performance of Intraplate Control

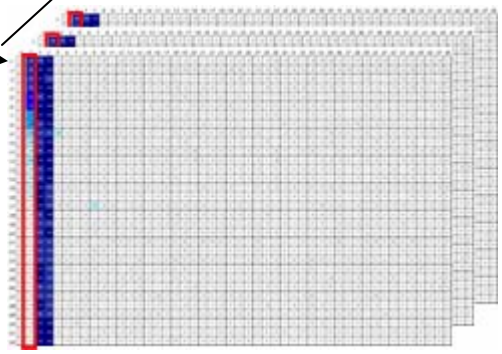


Lopac-A-4393
Apomorphin

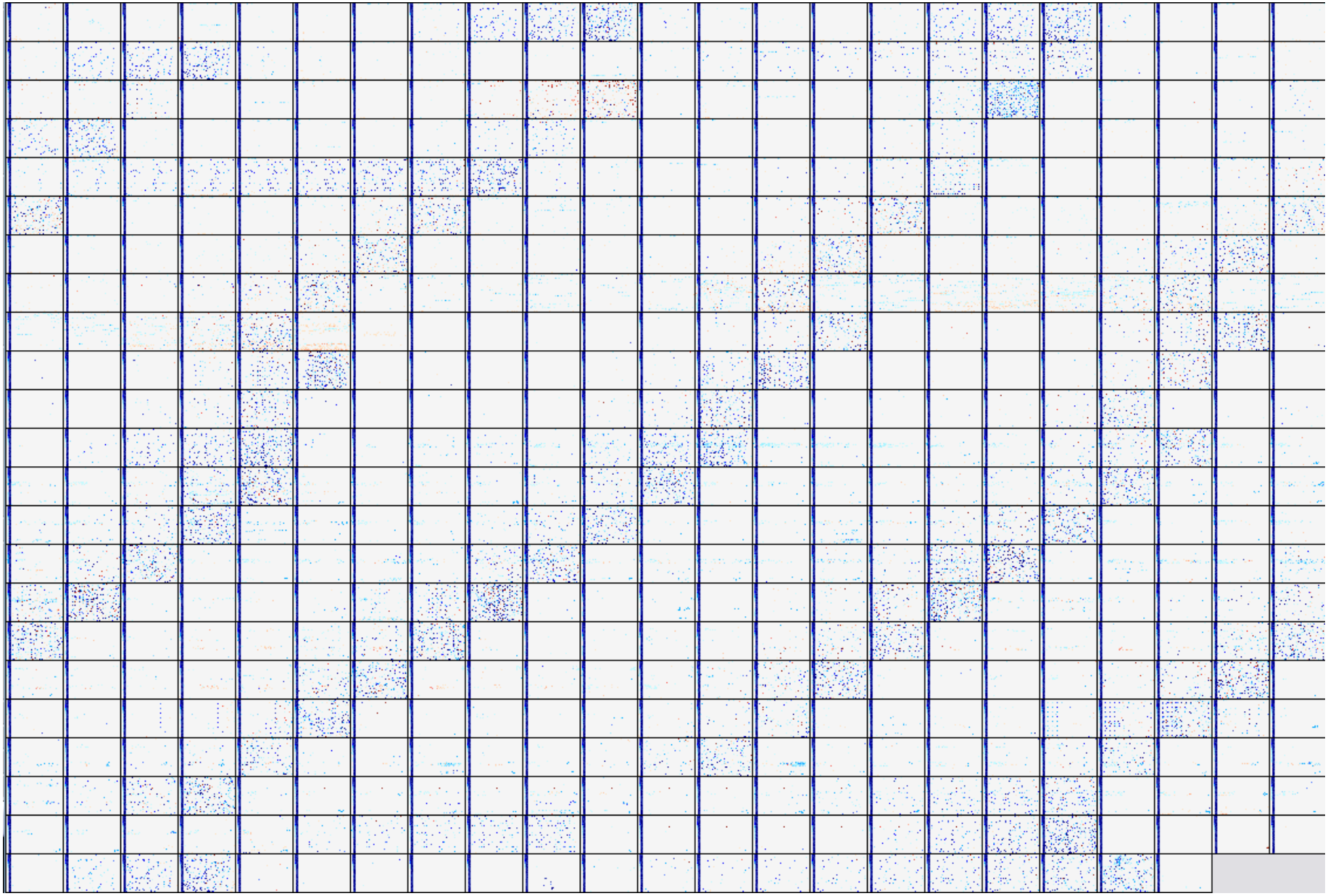
IC50 = 3.76 uM +/- 0.65
MSR = 2.5
Num of Titrations: 1054



… 527 plates, 2 control titrations on each plate

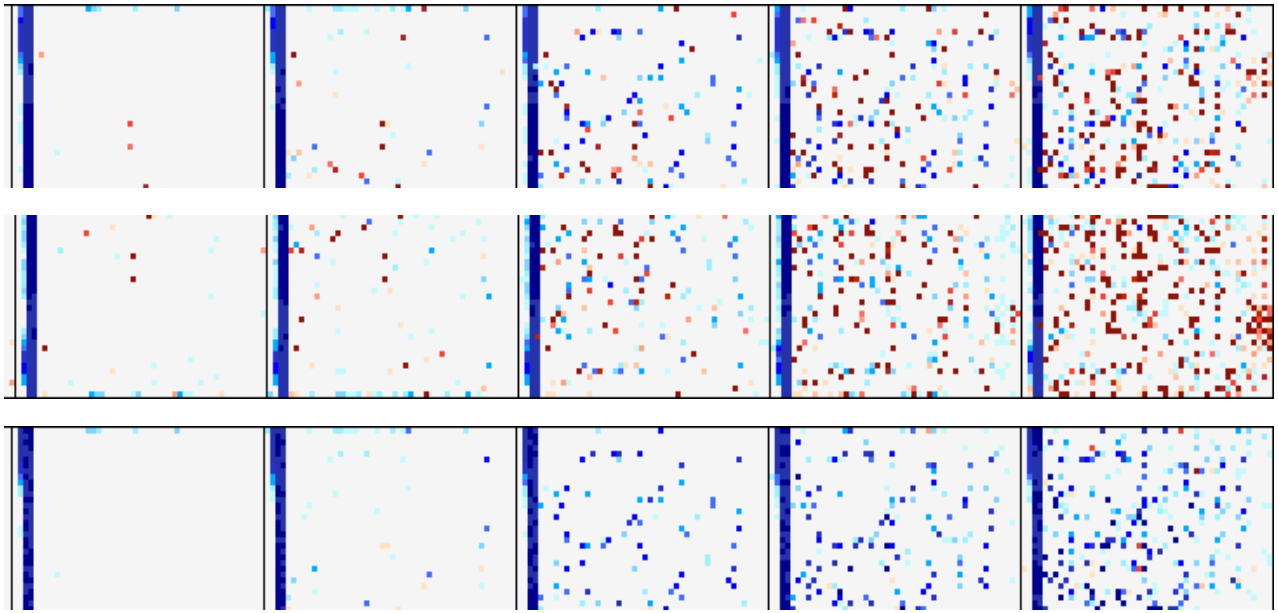


Entire-collection qHTS Heatmap: 527 plates



Activity Calculation from Kinetic Data

Multi-Read Data Heatmaps:
Lopac (Concentrations 1, 3, 5, 7, 9)



Read 1:
Increased signal shows background fluorescence

Read 8:
Same, increasing, or decreasing fluorescence observed and used for triaging compounds

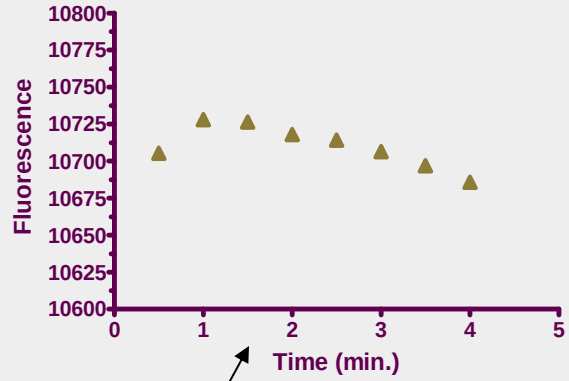
Calculated Activity:
Derived from Read 8 – Read 1 and normalized to neutral and negative controls.

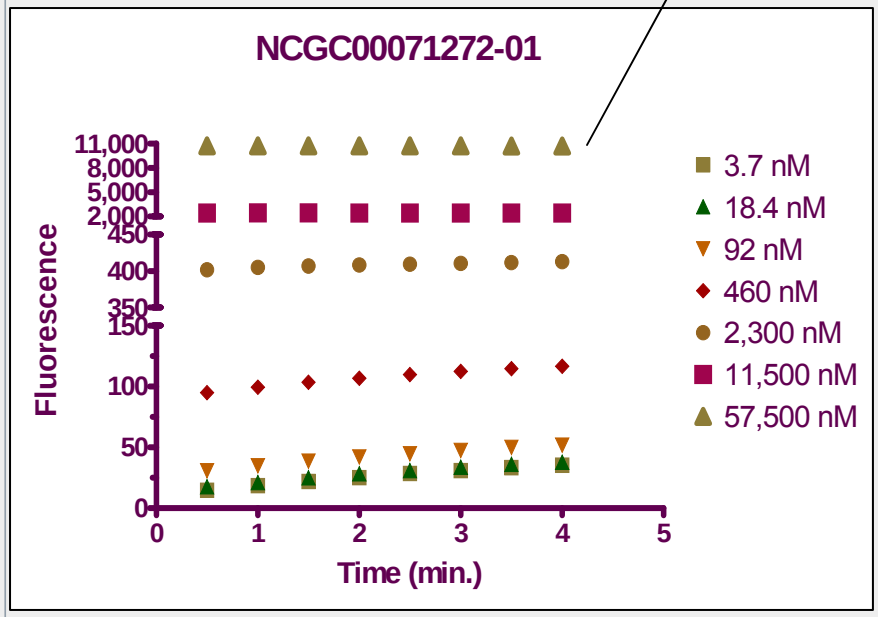
Constant fluorescence eliminated. However, *some potential artifacts remain (see next slides)*

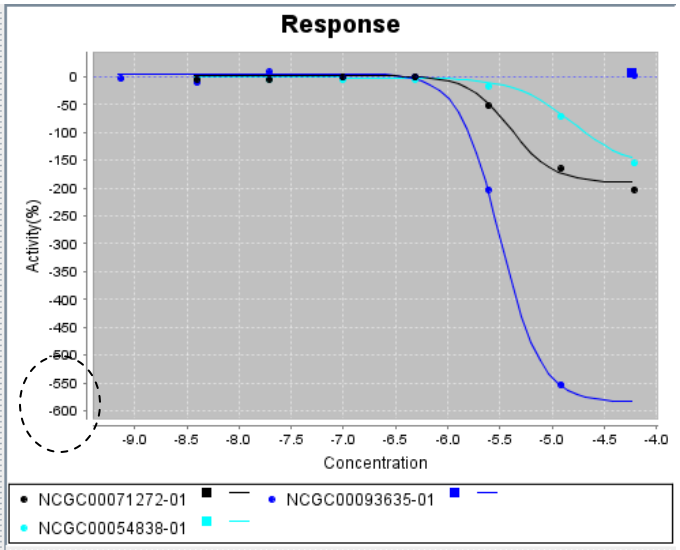
Fluorescent Artifacts: “Super” Inhibitors

X | Query | Save | Rollback | Delete | Copy | Duplicate | Export | Plot | MCS | Info | R Group | PubChem

Assay Name	Sample ID	AC50(um)	Hill Coef	Inf Activity	Curve Class	Supplier ID	Supplier	Sample Dat...
hadh560	NCGC00054838-01	15.849	1.539	-165.869	-2.1	4254770	DPISMR	delta
hadh560	NCGC00071272-01	3.981	2.094	-190.298	-1.1	866133	DPISMR	delta
hadh560	NCGC00093635-01	3.162	2.188	-585.143	-2.1	Lopac-A-022		delta
hadh560	NCGC00093635-01	2.512	1.885	-436.879	-2.1	Lopac-A-022		delta



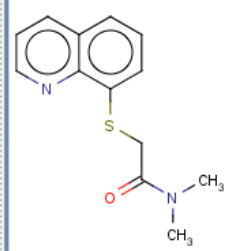


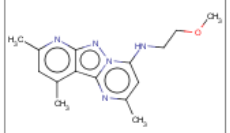


corrected ratio | normalized delta | ratio | Read 1

Structure | NAD 1 mM Carleen | normalized ratio | corrected delta | delta

NCGC00054838-01	NCGC00071272-01	NCGC00093635-01

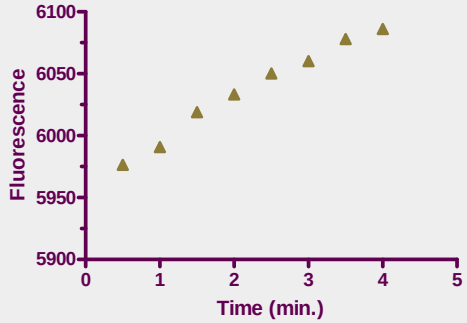


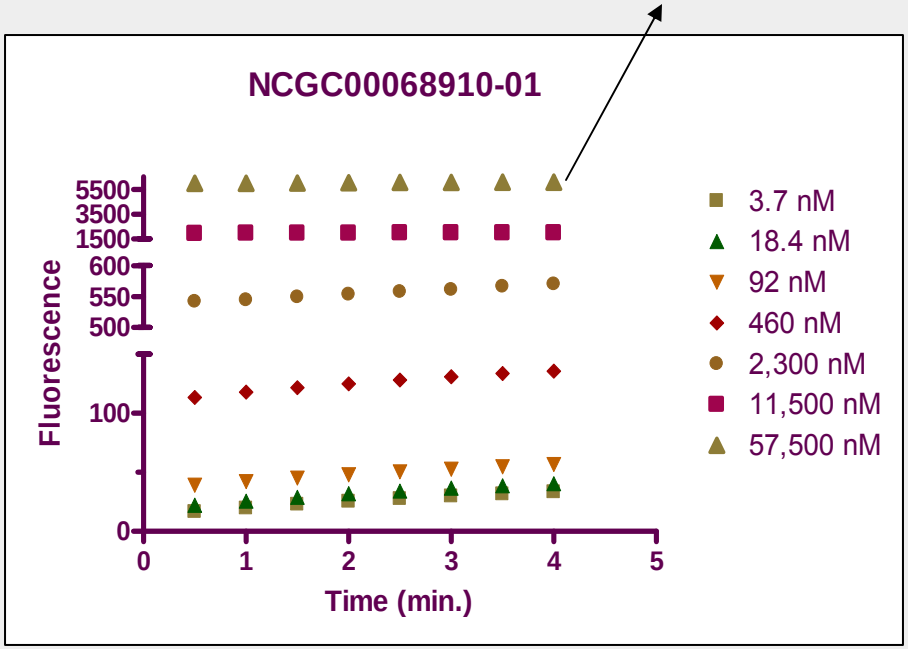


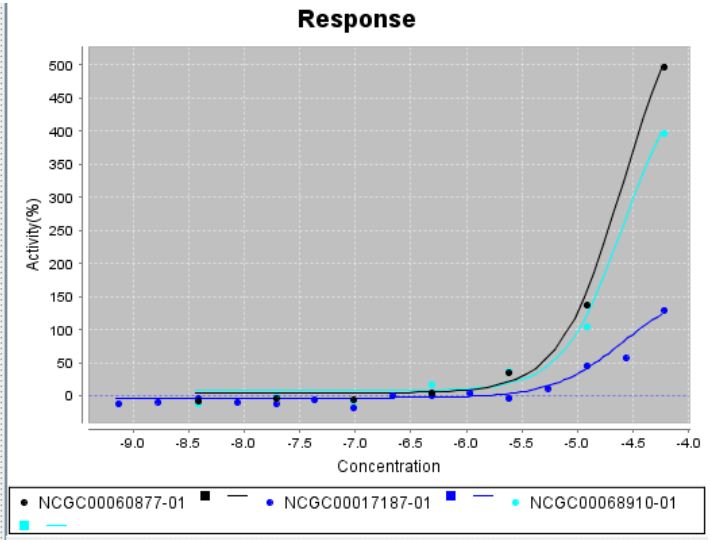
Fluorescent Artifacts: “Super” Activators

X | Query | Save | Rollback | Delete | Copy | Duplicate | Export | Plot | MCS | Info | R Group | PubChem

Assay Name	Sample ID	AC50(um)	Hill Coef	Inf Activity	Curve Class	Supplier ID	Supplier	Sample D
hadh560	NCGC00017187-01	25.119	1.372	164.674	2.1	TNP00051	Timtec	delta
hadh560	NCGC00068910-01	25.119	1.626	500.393	2.1	863112	DPISMR	delta
hadh560	NCGC00060877-01	25.119	1.539	632.149	2.1	4255688	DPISMR	delta



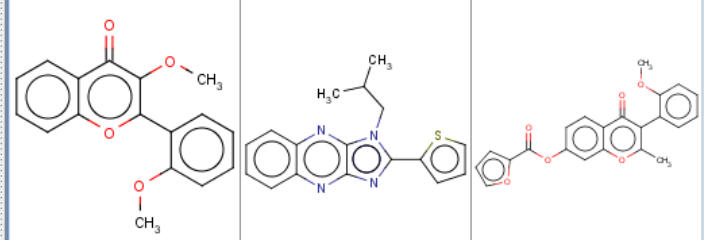




delta | corrected ratio | normalized delta | ratio | Read 1

Structure | NAD 1 mM Carleen | normalized ratio | corrected delta

NCGC00017187-01	NCGC00068910-01	NCGC00060877-01



HADH2 Curve Class Distribution

Table 1: Curve Class Definitions:

Curve Class	*Description*	*Efficacy*	*r^2*	*Asymptotes*	*Inflection*
1*	Complete response (a) Partial response (b)	> 80% (1.1) Min** - 80% (1.2)	$\geq$ 0.9	2	yes
2†	Incomplete curve	> 80% (2.1) < 80% (2.2)	> 0.9 (2.1) < 0.9 (2.2)	1	yes
3	Single pt activity	> Min	NA	1	no
4	Inactive	NA	NA	0	no

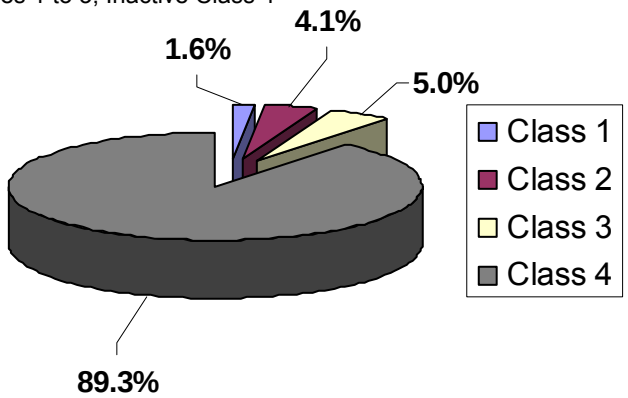
Note: in addition one can have noisy curves: Class 1.3 = >80% and r^2 > 0.9 and Class 1.4 = min – 80% and r^2 > 0.9. Analogously there can be Classes 2.3 and 2.4; Class 5 are unclassified curves

Table 2: Curve Class Activity Summary:

Activity	Distribution	Curve Classification										
		1.1	1.2	1.3	1.4	2.1	2.2	2.3	2.4	3	4	5
Inhibition	Cmpd No	386	663	1	147	1298	1697	5	67	3707	65821 88.40%	72 0.09%
	% library	0.51	0.89	0.00	0.19	1.74	2.27	0.00	0.08	4.97		
Activation	Cmpd No	22	32	0	23	255	252	0	13	506		
	% library	0.02	0.04	0.00	0.03	0.34	0.33	0.00	0.01	0.67		

Activity Summary Chart:

Active Classes 1 to 3, Inactive Class 4

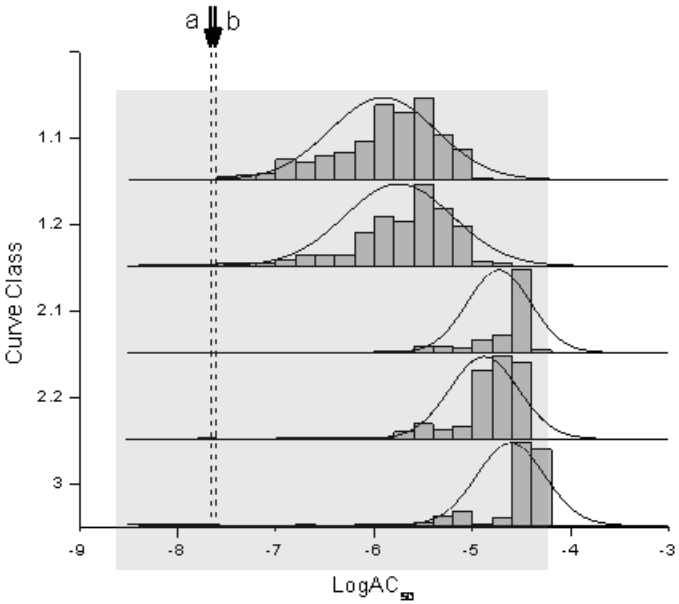


HADH2 Potency and Curve Class Distributions

Table 3: Potency Distribution by Curve Class

Potency range	Acivity	Curve Classification			
		1.1	1.2	2.1	2.2
< 100 nm	Inhibition	20 (# cmpds) 0.03%	23 0.03%	0 0.00%	2 0.00%
	Activation	1 0.00%	0 0.00%	0 0.00%	0 0.00%
1 um - 100 nm	Inhibition	141 0.19%	177 0.24%	9 0.01%	23 0.03%
	Activation	2 0.00%	9 0.01%	0 0.00%	2 0.00%
10 um - 1 um	Inhibition	247 0.33%	474 0.64%	221 0.30%	452 0.61%
	Activation	19 0.03%	23 0.03%	29 0.04%	95 0.13%
100 um - 10 um	Inhibition	0 0.00%	9 0.01%	1079 1.45%	1253 1.68%
	Activation	0 0.00%	0 0.00%	227 0.30%	154 0.21%

HADH2 Pharmacological Profile



AC50 distribution of inhibitors in each curve class.

Regions 1, 2, and ***3*** indicate concentration ranges of absent, observed, and extrapolated potencies, respectively.

Arrow A indicates the enzyme concentration at which the lowest AC50 can be observed while ***Arrow B*** is the NOEL.

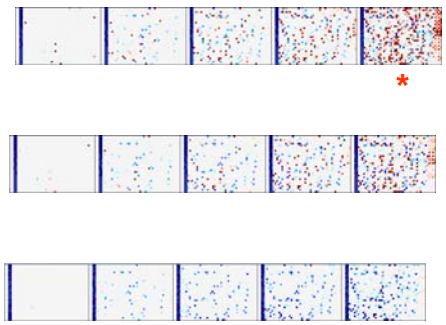
HADH2 – Elimination of Fluorescent Artifacts [entire screening collection]

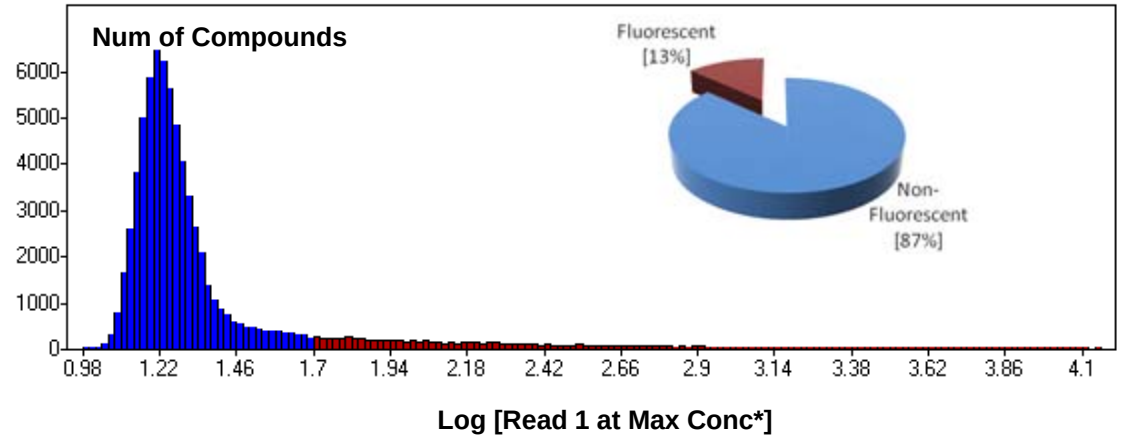
Use of Read 1 Background Fluorescence

Read 1*
Initial read

Read 8
4 min later

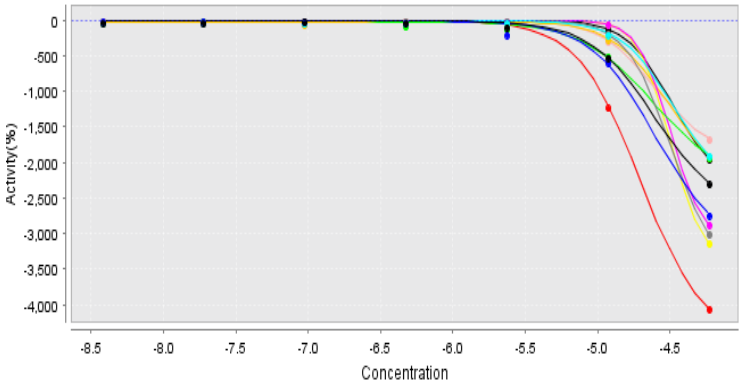
Activity
Read 8 – Read 1



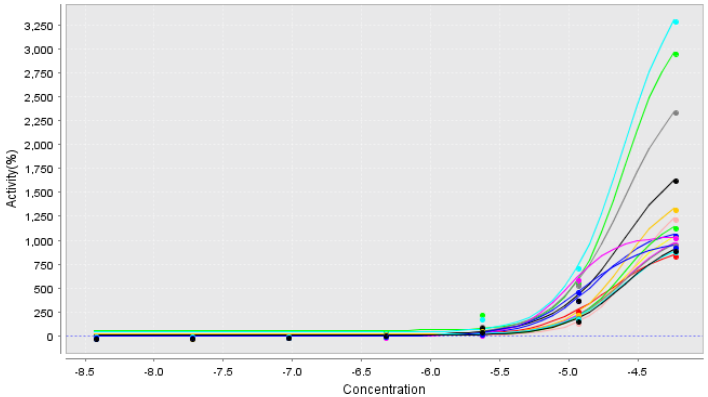


Use of Activity at Highest Concentration Tested

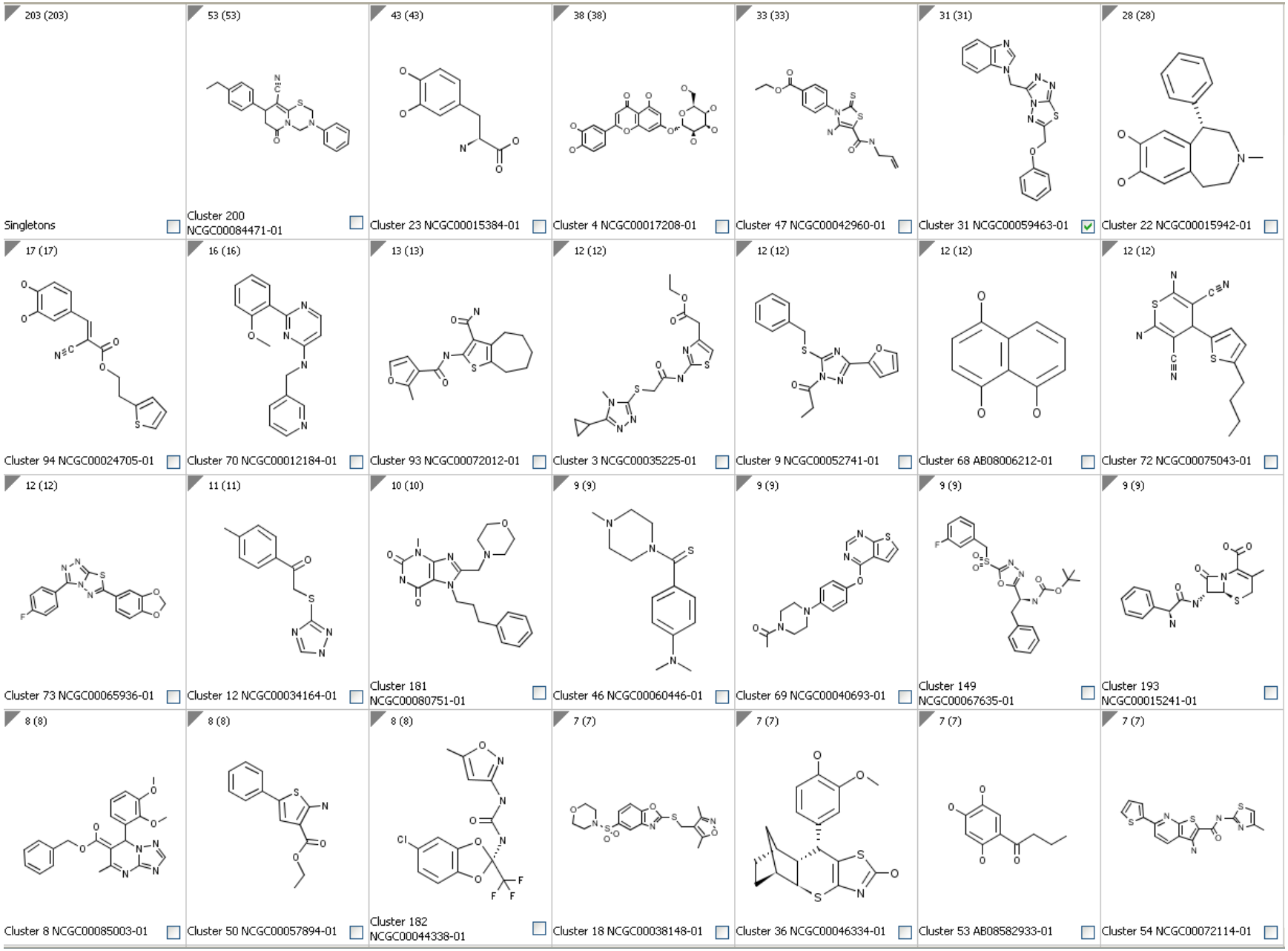
Example "Super Inhibitors"



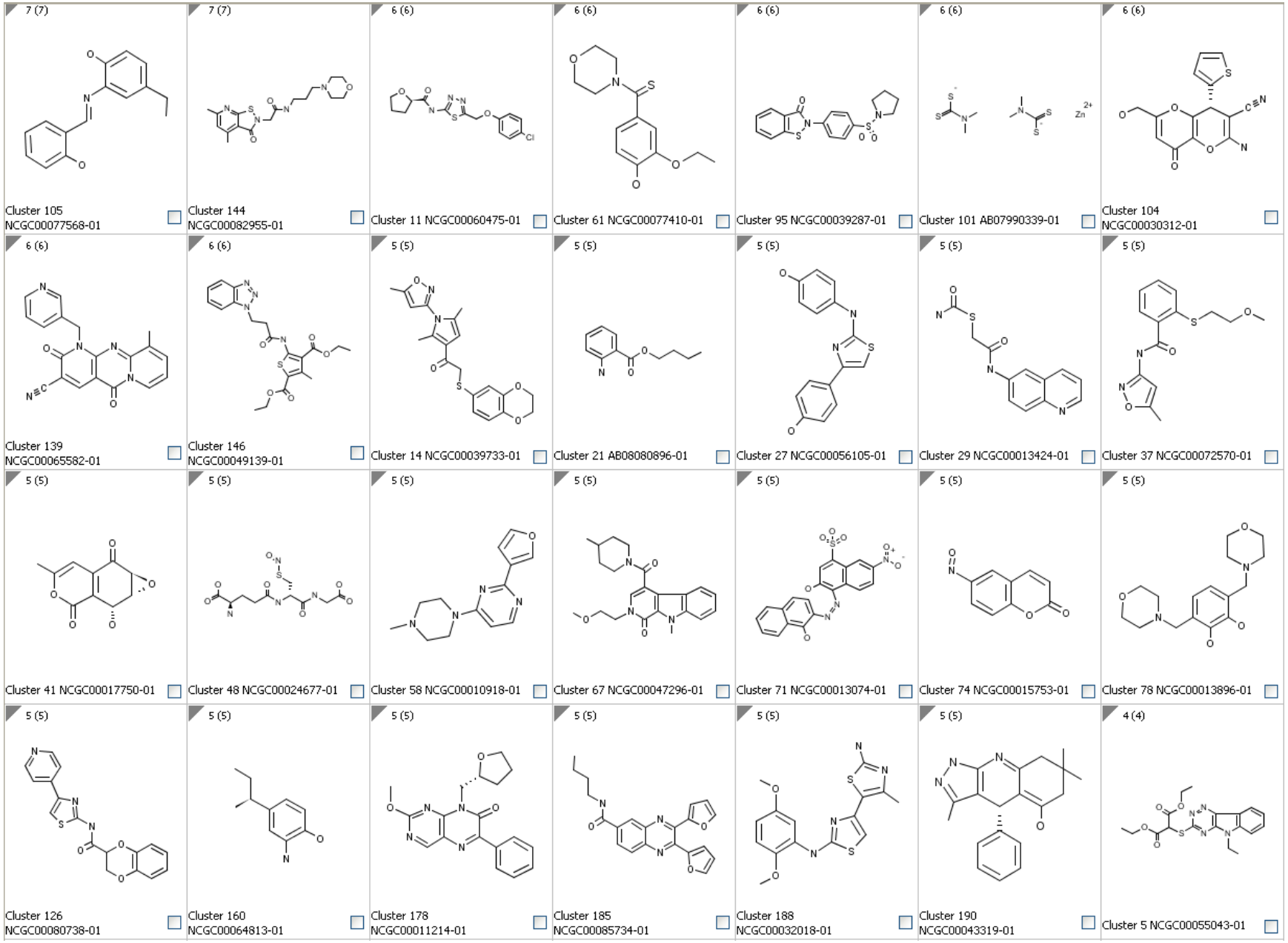
Example "Activators"



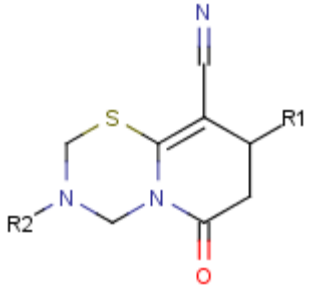
HADH2 Top Active Clusters (Pg 1)



HADH2 Top Active Clusters (Pg 2)

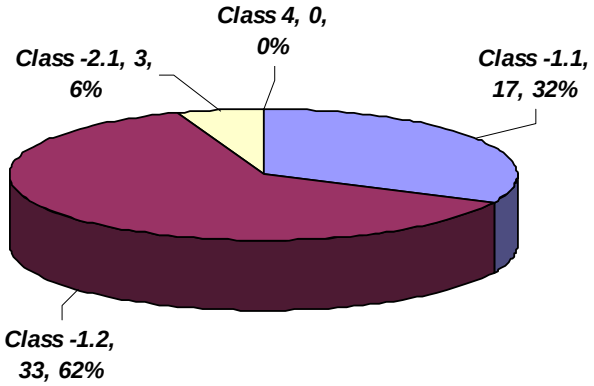


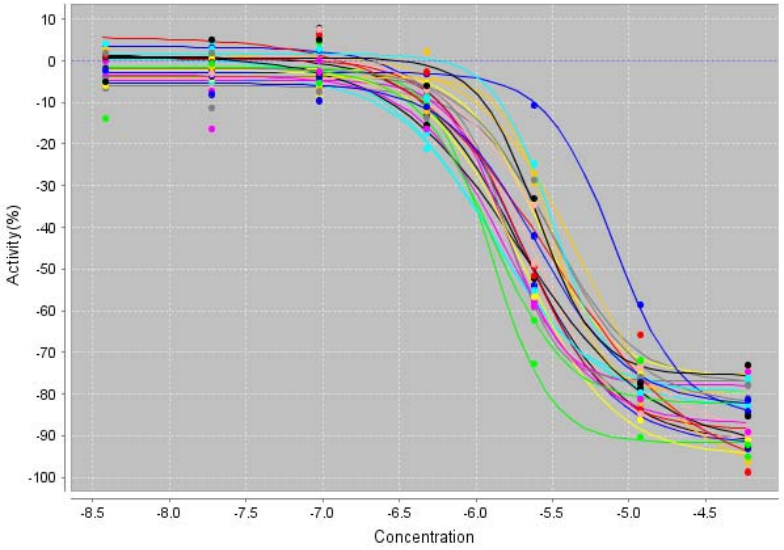
HADH2 SAR Series 1: *6-oxo, thiadiazine-9-carbonitrile series (Pg 1)*



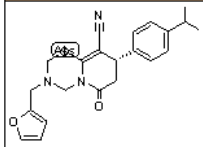
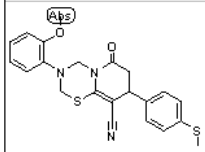
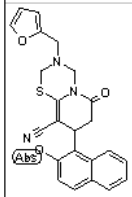
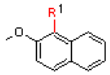
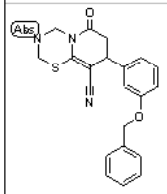
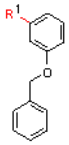
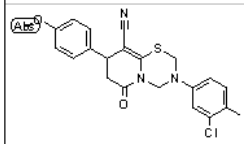
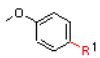
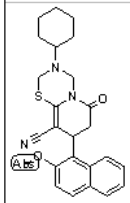
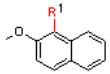
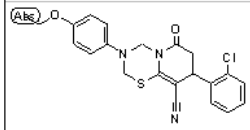
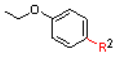
Number of actives: 53 of 53
MW Range: 327 to 497
ALogP Range: 2.36 to 5.47
Polar Surface Area: 47.3 to 93.5
Highest observed potency: 0.63 uM
Activity mode: Inhibitor
Hadh2 Background Read: Non-Fluorescent

Hadh2 background reads indicate no screening artifact
However, all members of series show up as actives

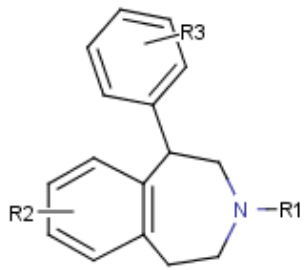




HADH2 SAR Series 1: *6-oxo, thiadiazine-9-carbonitrile series (Pg 2)*

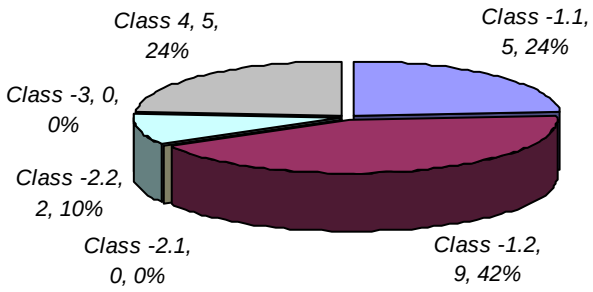
Structure	R1	R2	NCGC ID Pubchem SID Supplier ID	Hadh2 CurveClass	AC50 [uM]	Activity @ Max Conc	Purity	MW	ALogP	PSA
			NCGC00066582-01; Pubchem_SID: 865277; InterBioScreen: STOCK5S-40600	-1.1	1.26	-83.57	100%; LC-MS-ELS	393.5	4.62	60.5
			NCGC00084509-01; Pubchem_SID: 7965458; Chem Div: C499-0437	-1.1	1.26	-79.87	100%; LC-MS-ELS	423.5	4.64	56.6
			NCGC00084569-01; Pubchem_SID: 7965156; Chem Div: C499-0919	-1.1	1.26	-82.42	100%; LC-MS-ELS	431.5	4.66	69.7
			NCGC00084575-01; Pubchem_SID: 7964694; Chem Div: C499-0958	-1.1	1.26	-91.78	100%; LC-MS-UV	391.5	3.91	56.6
			NCGC00084507-01; Pubchem_SID: 7965507; Chem Div: C499-0392	-1.1	1.58	-73.26	100%; LC-MS-UV	425.9	4.88	56.6
			NCGC00084573-01; Pubchem_SID: 7965560; Chem Div: C499-0938	-1.1	1.58	-87.29	100%; LC-MS-UV	433.6	5.20	56.6
			NCGC00084579-01; Pubchem_SID: 7968902; Chem Div: C499-1007	-1.1	1.58	-77.95	100%; LC-MS-UV	425.9	4.96	56.6

HADH2 SAR Series 2: *Benzo[d]azepine series (Pg 1)*

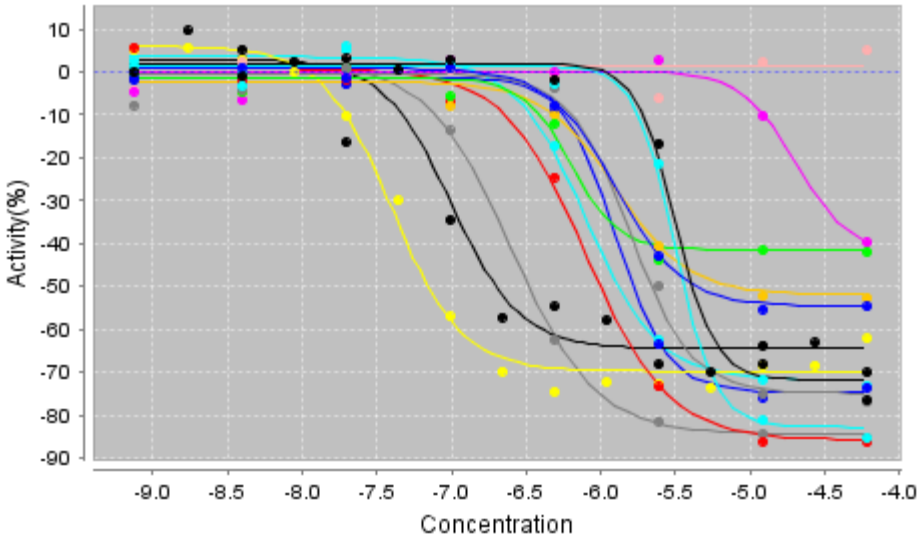


Number of actives: 16 of 21
MW Range: 255 to 374
ALogP Range: 2.38 to 4.24
Highest observed potency: 0.04 uM
Activity mode: Inhibitor
Hadh2 Background Read: Non-Fluorescent

Curve Class Distribution

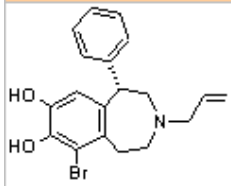
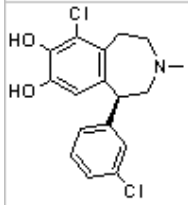
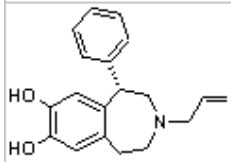
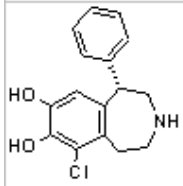
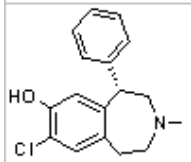
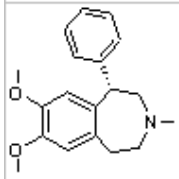


Titration Curves

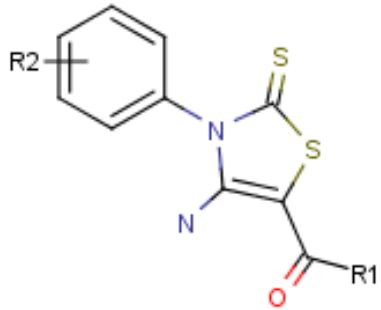


HADH2 SAR Series 2: *Benzo[d]azepine series (Pg 2)*

Representative actives and an inactive

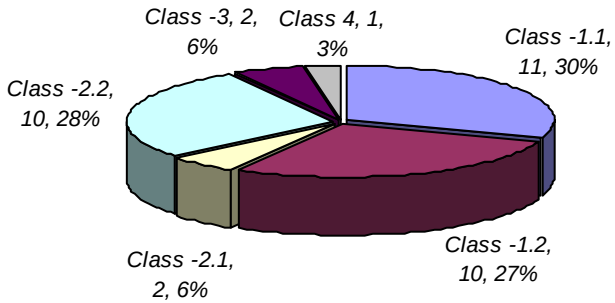
Structure	NCGC ID	Curve Class	LogAC50	Hill Slope	Compound Name	PubchemSID	Supplier
	NCGC00015127-01	-1.1	-5.7	0.80	R(+)-6-Bromo-APB hydrobromide	11110817	SigmaAldrich
	NCGC00015945-01	-1.1	-5.5	3.25	SKF 83565 hydrobromide	11111795	SigmaAldrich
	NCGC00015915-01	-1.2	-5.9	1.63	(±)-SKF 38393, N-allyl-, hydrobromide	11111764	SigmaAldrich
	NCGC00015910-01	-1.2	-5.9	2.72	(±)-6-Chloro-PB hydrobromide	11111759	SigmaAldrich
	NCGC00015301-01	-2.2	-4.7	2.25	R(+)-SCH-23390 hydrochloride	11111023	SigmaAldrich
	NCGC00015914-01	4			R(-)-SCH-12679 maleate	11111763	SigmaAldrich

HADH2 SAR Series 3: *Thioxo dihydrothiazole series (Pg 1)*

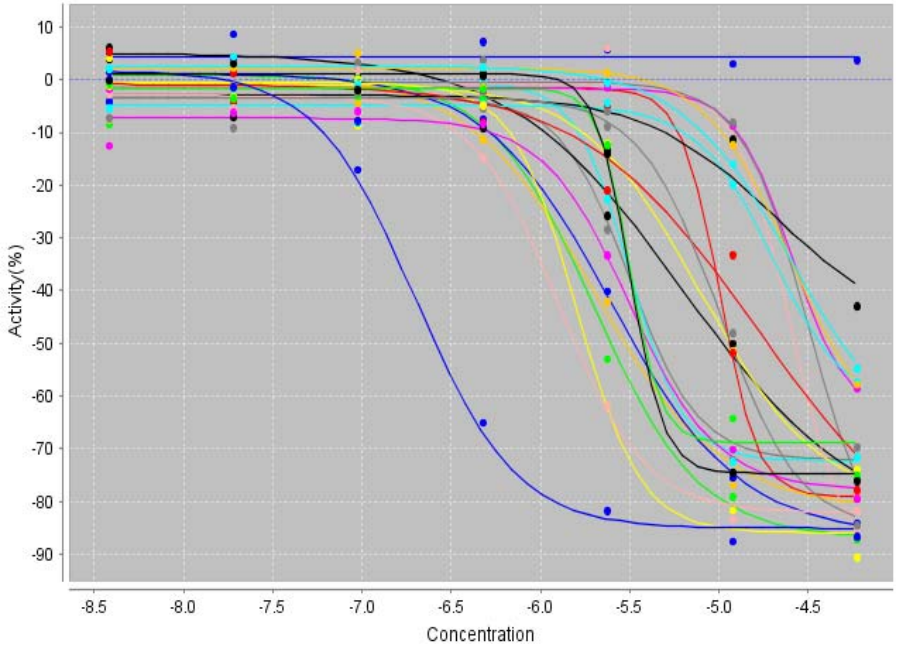


Number of actives: 35 of 36
MW Range: 266 to 474
ALogP Range: 1.15 to 4.06
Highest observed potency: 0.20 uM
Activity mode: Inhibitor
Hadh2 Background Read: Non-Fluorescent

Curve Class Distribution

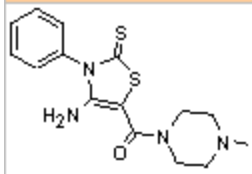
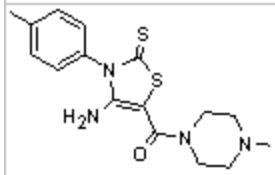
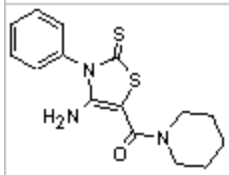
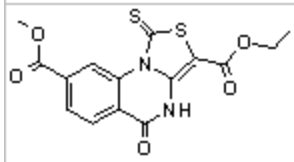
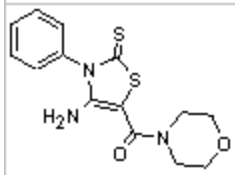
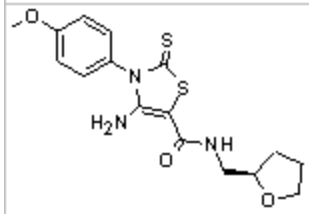


Titration Curves

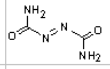
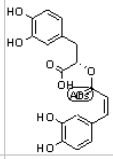
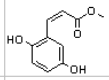
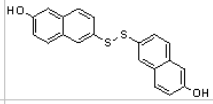
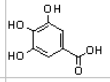
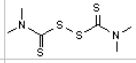
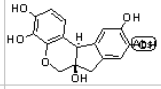
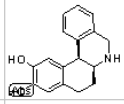
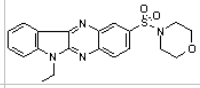
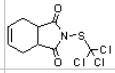
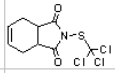
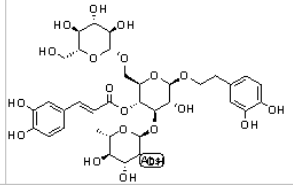


HADH2 SAR Series 3: *Thioxo dihydrothiazole series (Pg 2)*

Representative actives and an inactive

Structure	NCGC ID	Curve Class	LogAC50	Hill Slope	PubchemSID	Supplier
	NCGC00060352-01	-1.1	-6.7	1.558	4260621	MLSMR
	NCGC00061008-01	-1.1	-6.2	1.967	4254845	MLSMR
	NCGC00053637-01	-1.1	-6.1	1.626	4255874	MLSMR
	NCGC00052143-01	-1.1	-6	2.248	4246228	MLSMR
	NCGC00060246-01	-1.1	-5.9	1.788	4260544	MLSMR
	NCGC00053461-01	4			4259307	MLSMR

HADH2 Top 100 Actives *(Pg 1)*

No	Structure	NCGC ID	Curve Class	LogAC50	Hill Slope	Compound Name	Known Activity Class	Known Mode of Action	PubchemSID; SupplierID	Supplier
1		AB08582939-01	-1.1	-7.6	1.692	Azodicarbonamide			SID: 17389172;	NIEHS
2		NCGC00024695-01	-1.1	-7.6	0.6	Rosmarinic acid	Miscellaneous		SID: 11113609; Tocris-0630	Tocris
3		NCGC00024661-01	-1.1	-7.5	3.512	Methyl 2,5-dihydroxycinnamate	Signal Transduction Agent		SID: 11113575; Tocris-0577	Tocris
4		AB08080842-01	-1.1	-7.4	1.714	6-Hydroxy-2-naphthyl disulfide (DDD)			SID: 17389219;	NIEHS
5		AB07948947-01	-1.1	-7.3	3.065	Gallic acid			SID: 17389871;	NIEHS
6		AB08080834-01	-1.1	-7.3	1.967	Tetramethylthiuram disulfide			SID: 17389717;	NIEHS
7		AB07975480-01	-1.1	-7.2	2.406	Hematoxylin (C.I. 75290)			SID: 17389864;	NIEHS
8		NCGC00024845-01	-1.1	-7.2	1	Dihydrexidine hydrochloride	Dopaminergic		SID: 11113759; Tocris-0884	Tocris
9		NCGC00039105-01	-1.1	-7.2	1.967				SID: 3717614; 3717614	DPISMR
10		AB07944404-01	-1.1	-7.1	2.253	Captan 90-concentrate (solid)			SID: 17389124;	NIEHS
11		AB07985386-01	-1.1	-7.1	2.72	Captan			SID: 17389995;	NIEHS
12		AB13681063-01	-1.1	-7.0	1.222	Echinacoside			SID: 17389021;	NIEHS

HSD17β4 qHTS

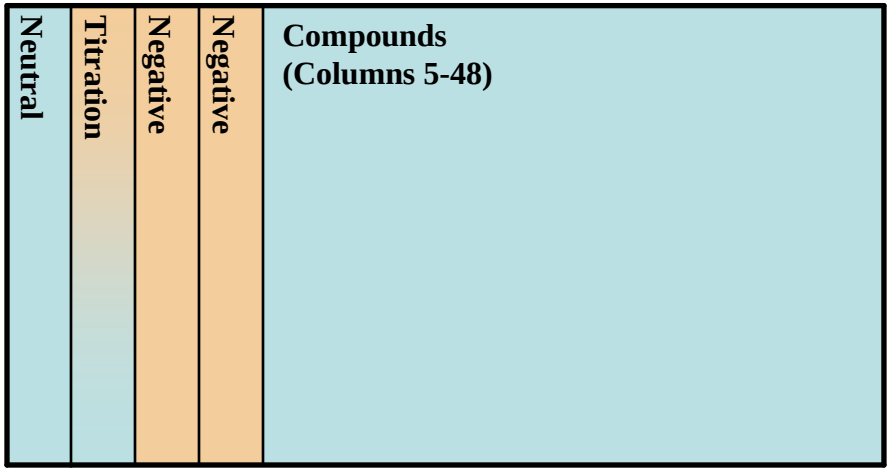
Reagent Optimization

Enzyme	Substrate	Cofactor	Product	Proposed Cofactor Concentration	Fluorescence Intensity Trend
HSD17β4	Estradiol	NAD+	NADH	1 mM	Increase
840 nM	90 uM	← Proposed concentrations			
100 nM	50 uM	← NCGC concentrations			

- Establishing the final estradiol formulation required extensive testing.
- ~ 35 mg enzyme available (enough for 8 L of assay reaction at the optimized concentration).

qHTS Plate Map

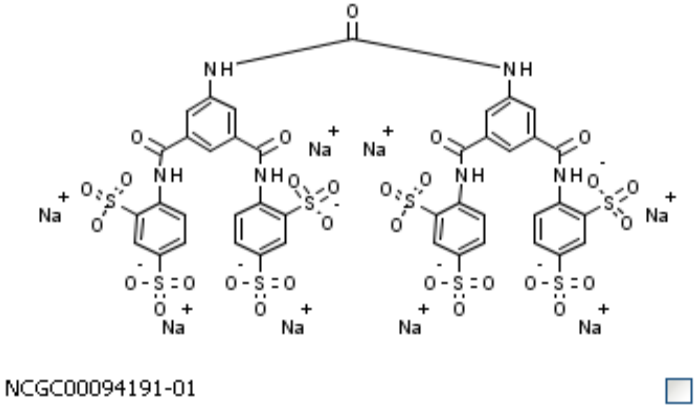
Plate Layout



Column 2 titration: control inhibitor NF449 octasodium salt (Lopac-N-4784) to col 2 of all assay plates. Two-fold, 16 pt dilution in duplicate: 11.5 μM – 0.35 nM.

NF449 octasodium salt

Potent G protein antagonist, selective for Gs-alpha



AC_{50} 142 nM online validation

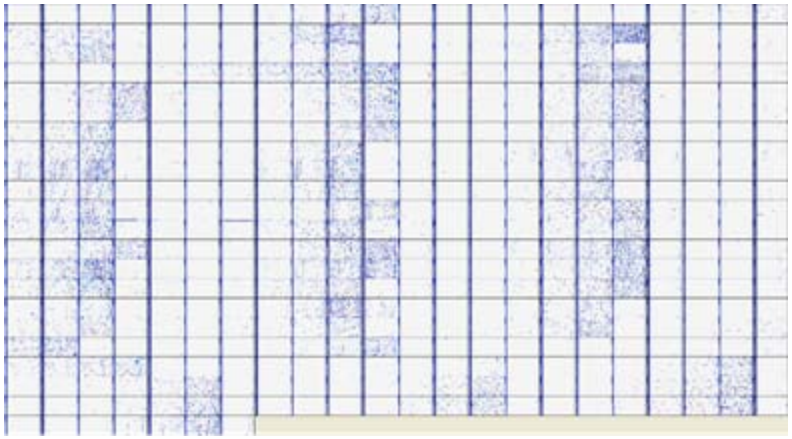
NF449 was selected as an intraplate control after a LOPAC validation screen.

HSD 17β4 qHTS Protocol			NCGC ID: hsdb130 PubChem AID: *unassigned*
Step	**Parameter**	**Value**	**Description**
1	Reagent	3 μL	Enzyme: 100 nM HSD 17β4
2	Compound	23 nL	Compound library
3	Control	23 nL	Control plate
4	Time	15 min	RT Incubation
5	Reagent	1 μL	Substrate and cofactor: 50 μM estradiol and 1 mM NAD
6	Time	15 sec, 1000 rpm	Centrifugation
7	Detector	EX 360 / EM 450	ViewLux fluorescence intensity read 1
8	Time	60 min	RT Incubation
9	Detector	EX 360 / EM 450	ViewLux fluorescence intensity read 2

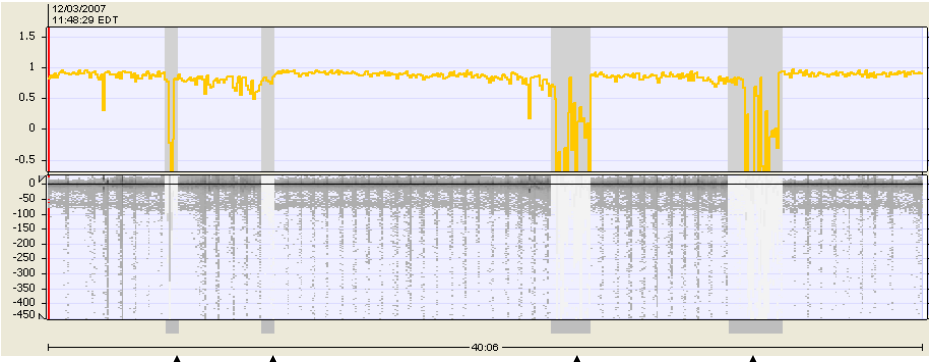
Step	Notes
1	Black solid plates, 1 tip dispense, Dispenser 2 head B, 80 μL pre-dispense, 1L glass bottle (+4°C), no enzyme in col 3-4
2	Pintool transfer of compounds to assay plate (col 5-48)
3	Pintool transfer of control inhibitor NF449 octasodium salt (Lopac-N-4784) to col 2 of all assay plates. Two-fold, 16 pt dilution in duplicate: 11.5 μM – 0.35 nM.
4	RT incubation in auxiliary hotel
5	1 tip dispense, Dispenser 1 head C, 80-100 μL pre-dispense, 0.5 L glass bottle RT. DMSO concentration 15%. (fac 3.75%)
6	Centrifugation to obtain a smooth liquid surface
7	ViewLux program 38, emission filter wheel A, Excitation DUG 11 / Emission 450/20
8	RT incubation in auxiliary hotel
9	ViewLux program 39. Priority 1. Difference read 2 – read 1 is calculated.

HSD17β4 qHTS Performance

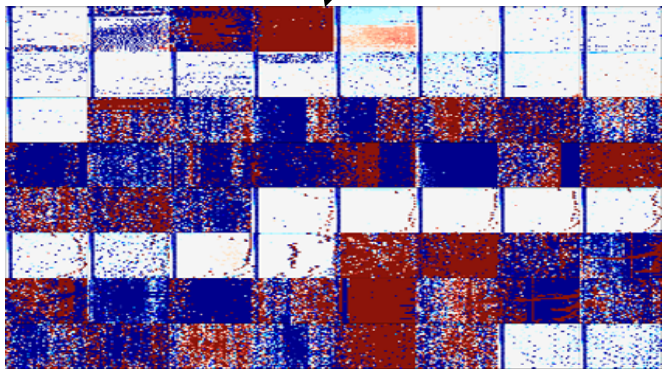
Screen job: 469 accepted plates



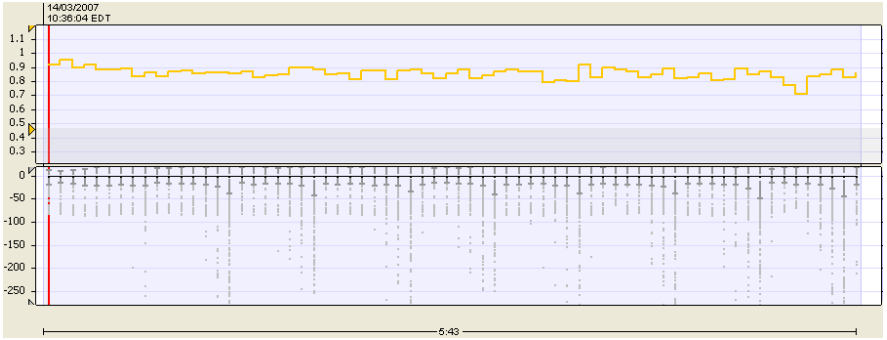
Overall Z' ~0.85



As a result of slow crystallization/buildup of estradiol in the dispenser tip/valve, the screen was interrupted several times, and 63 plates were rejected & re-screened:



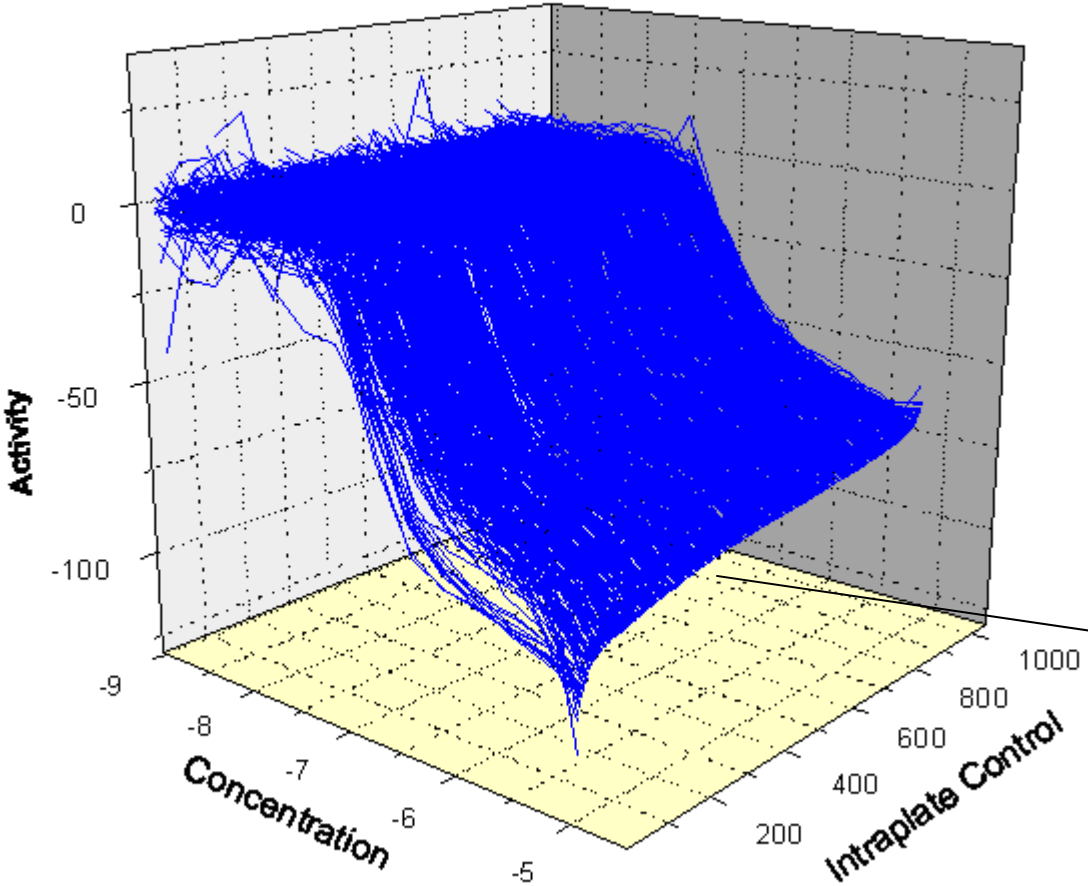
Z' for the 63-plate re-screen ~0.85



Performance Summary

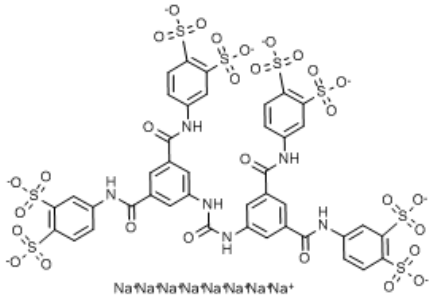
Hydroxysteroid (17-beta) dehydrogenase 4 NCGC ID: HSDB130 PubChem AID: *unassigned*			
Parameter	***Assay opt.***	***Validation***	***qHTS***
Date		2007-02-22	2007-03-12 to 2007-03-14
System	Off-line	Validation	Kalypsys robot
Plates Screened		30	532 (469 accepted)
Plates Failed QC		0	63
Compound (total # tested)		1,375 (x 3)	75,552
Samples (total wells)		33,000	583,460
Number of Data Points		66,000	1,166,921
% 7 pt-titrations		100%	95%
% 15 pt-titrations		0%	5%
Data Layers		Read 1 Read 2 Delta (Activity Layer)	Read 1 Read 2 Delta (Activity Layer)
Average Z' [Delta Activity]		0.87	0.86
Average Signal: Background (S:B)		205	241

qHTS Performance of Intraplate Control

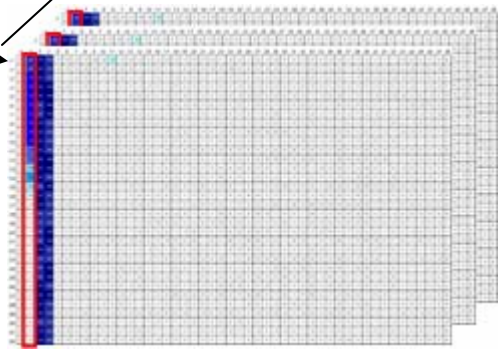


NF449

IC50 = 0.283 uM +/- 0.09
MSR = 1.9
Num of Titrations: 938



… 469 plates, 2 control titrations on each plate



HSD17β4 Curve Class Distribution

Table 1: Curve Class Definitions:

Curve Class	*Description*	*Efficacy*	*r^2*	*Asymptotes*	*Inflection*
1*	Complete response (a) Partial response (b)	> 80% (1.1) Min** - 80% (1.2)	≥ 0.9	2	yes
2†	Incomplete curve	> 80% (2.1) < 80% (2.2)	> 0.9 (2.1) < 0.9 (2.2)	1	yes
3	Single pt activity	> Min	NA	1	no
4	Inactive	NA	NA	0	no

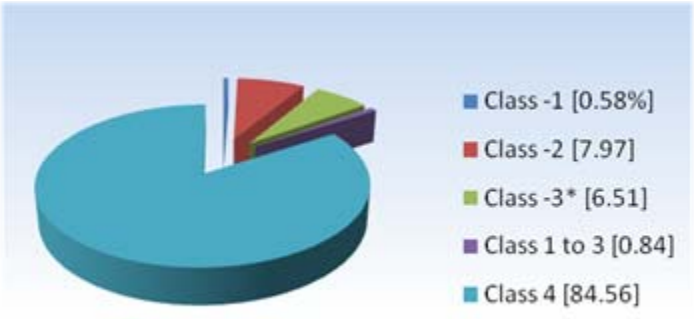
Note: in addition one can have noisy curves: Class 1.3 = >80% and r^2 > 0.9 and Class 1.4 = min – 80% and r^2 > 0.9. Analogously there can be Classes 2.3 and 2.4; Class 5 are unclassified curves

Table 2: Curve Class Activity Summary:

Activity	Distribution	Curve Classification										
		1.1	1.2	1.3	1.4	2.1	2.2	2.3	2.4	3	4	5
Inhibition	No. Cmpds.	327	114	1	147	5010	1048	1	21	4779	63893 84.56%	44 0.05%
	% library	0.43	0.15	0.00	0.03	6.63	1.38	0.00	0.02	4.97		
Activation	No. Cmpds.	12	8	0	4	215	72	0	1	326		
	% library	0.01	0.01	0.00	0.00	0.28	0.09	0.00	0.00	0.43		

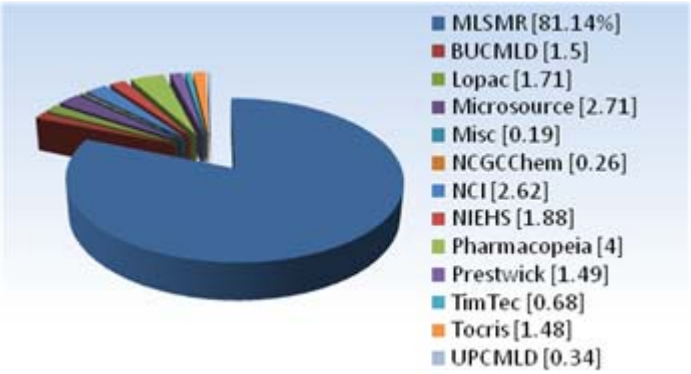
Activity Summary Chart:

Active Classes 1 to 3, Inactive Class 4



Screening Collection:

Chemical Library Distribution

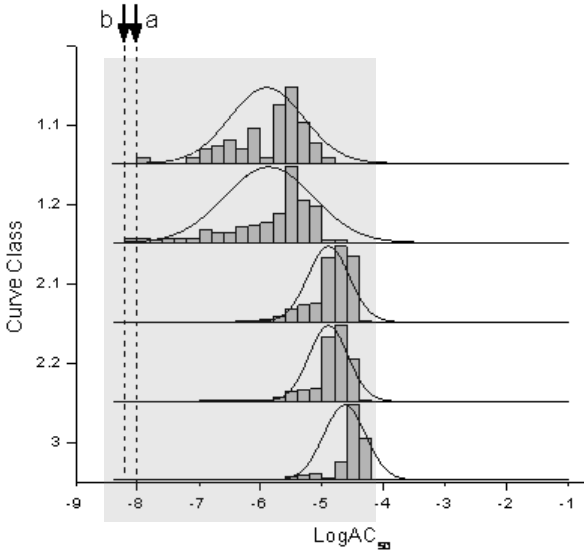


HSD17β4 Potency and Curve Class Distributions

Table 3: Potency Distribution by Curve Class

Potency range	Activity	Curve Classification			
		1.1	1.2	2.1	2.2
< 100 nm	Inhibition	1 (# cmpds) 0.00%	0 0.00%	0 0.00%	0 0.00%
	Activation	0 0.00%	0 0.00%	0 0.00%	0 0.00%
1 um - 100 nm	Inhibition	45 0.06%	12 0.02%	9 0.01%	2 0.00%
	Activation	2 0.00%	1 0.00%	0 0.00%	0 0.00%
10 um - 1 um	Inhibition	261 0.35%	97 0.13%	359 0.48%	181 0.24%
	Activation	10 0.01%	23 0.01%	16 0.02%	17 0.02%
100 um - 10 um	Inhibition	21 0.03%	5 0.01%	4655 6.16%	866 1.15%
	Activation	0 0.00%	0 0.00%	199 0.26%	54 0.07%

HSDb17 Pharmacological Profile



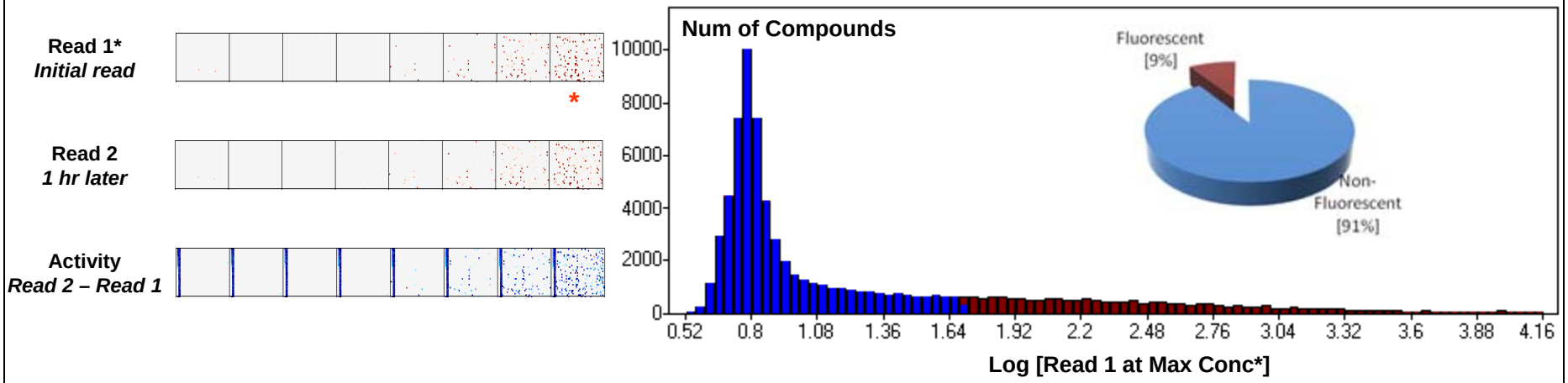
AC50 distribution of *inhibitors* in each curve class.

Regions 1, 2, and ***3*** indicate concentration ranges of absent, observed, and extrapolated potencies, respectively.

Arrow A indicates the enzyme concentration at which the lowest AC50 can be observed while ***Arrow B*** is the NOEL.

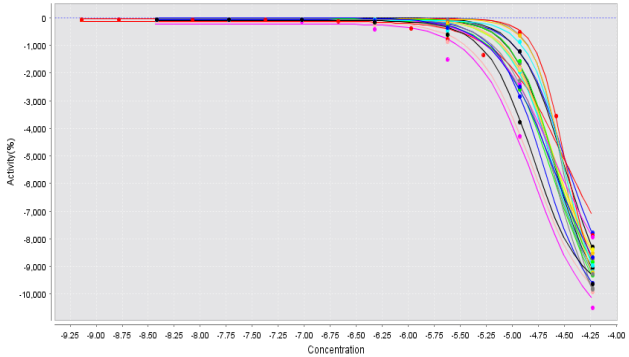
HSD17b4 – Elimination of Fluorescent Artifacts [entire screening collection]

Use of Read 1 Background Fluorescence

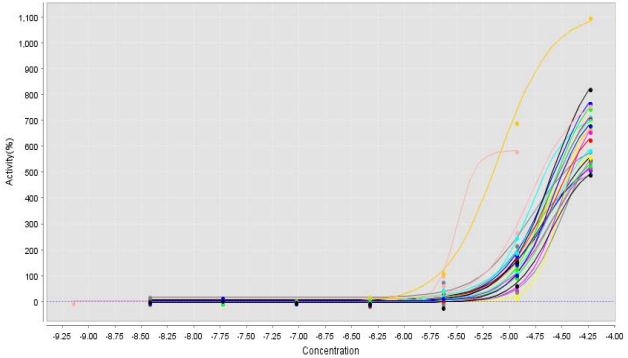


Use of Activity at Highest Concentration Tested

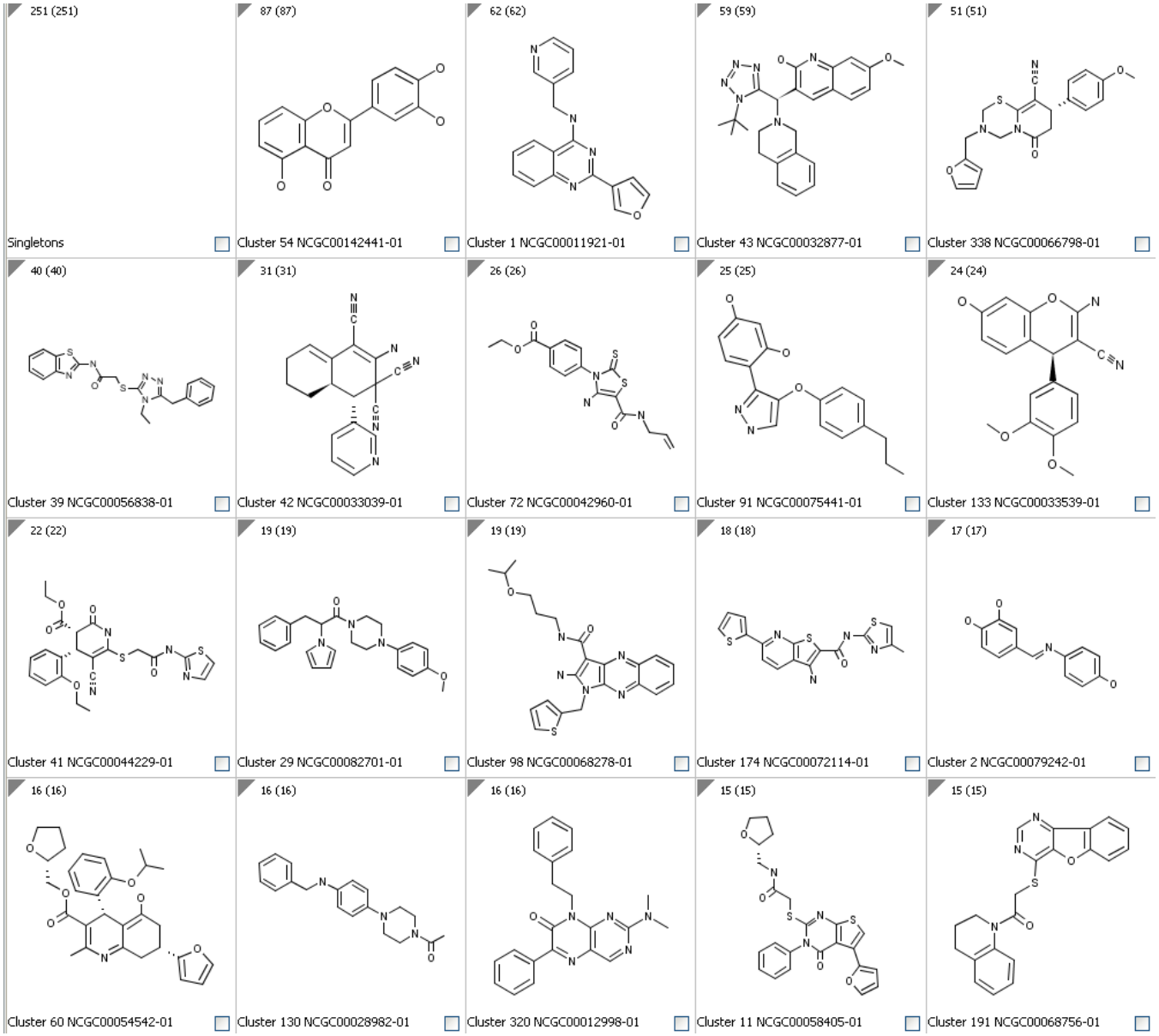
Example "Super Inhibitors"



Example "Activators"

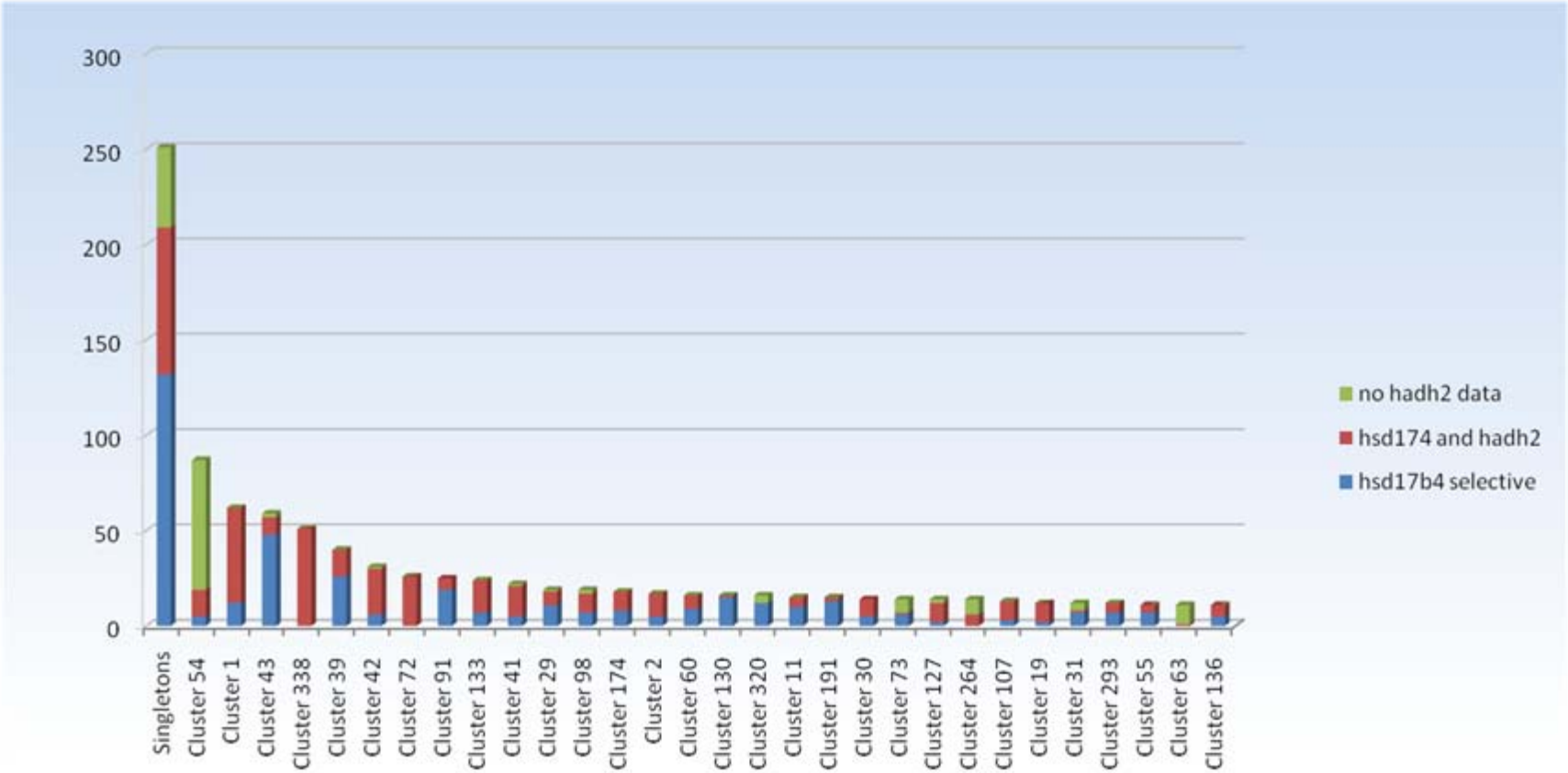


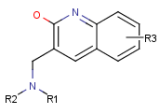
HSD17b4 – Active Clusters of Apparent Inhibitors – Pg 1



155 Clusters with >4 Actives

HSD17β4 – Active Clusters Overlap with HADH2



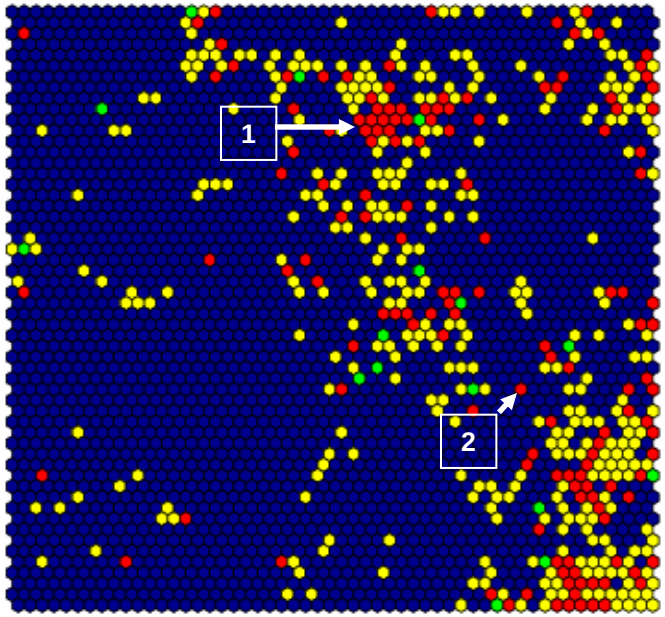


3-(aminomethyl)quinolin-2-ol
hsd17b4 selective

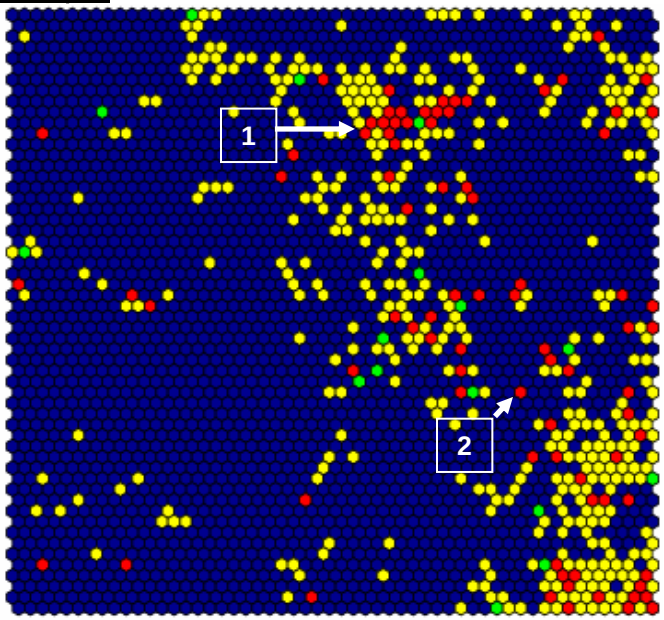
6-oxo, thiadiazine-9-carbonitrile series
non-selective or assay artifact

HADH2 vs HSD17β4: Lopac Activity Profile

HADH2:



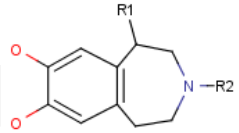
HSD17β4:



- NCGC Screening Collection
- Lopac Inactives
- Lopac Actives
- Fluorescence Artifact

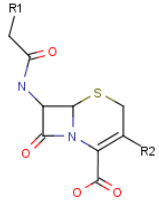
Representative Actives

1



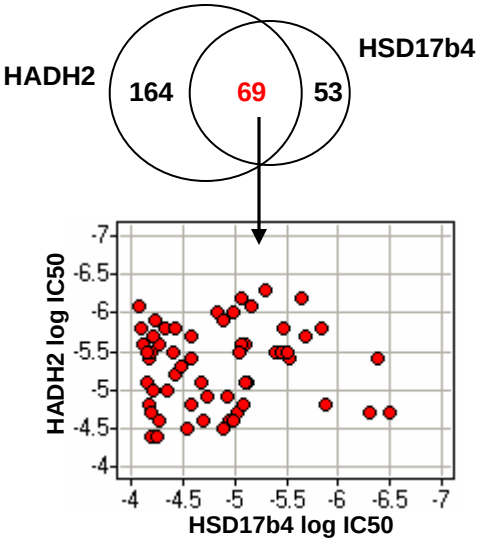
15 Benzodiazepine Dopamine agonists

2



3 Betalactam antibiotics

Actives Overlap



Self Organizing Maps Background

The SOM method is designed for the analysis of multidimensional data having the aim of identifying pattern similarities between diverse measurements that can be used to reveal underlying biological phenomena. The SOMs on the left represent the NCGC chemical library. They are organized by structural similarity where each hexagon is a cluster of structurally similar compounds, and compounds in neighboring clusters also share cluster similarity. Daylight fingerprints were used as structural descriptors. Colored hexagons represent whether those clusters contained a Lopac (N=1280) inactive, active or compounds that showed up as fluorescent artifacts in the assay.

The “Dark Matter” of the Genome

BJ www.biochemj.org

BJ Energy

Biochem. J. (2007) 402, 419–427 (Printed in Great Britain) doi:10.1042/BJ20061319 419

Structural and biochemical characterization of human orphan DHRS10 reveals a novel cytosolic enzyme with steroid dehydrogenase activity

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Abstract

To this day, a significant proportion of the human genome remains devoid of functional characterization. In this study, we present evidence that the previously functionally uncharacterized product of the human *DHRS10* gene is endowed with 17β-HSD (17β-hydroxysteroid dehydrogenase) activity. 17β-HSD enzymes are primarily involved in the metabolism of steroids at the C-17 position and also of other substrates such as fatty acids, prosta-

Conclusions

Taken together, we have provided a structural and functional characterization of an as yet poorly documented mammalian gene product. Further studies concerning temporal and spatial expression patterns, animal studies, as well as a search for other possible substrate activities will be mandatory for the additional characterization of this SDR member.

The Structural Genomics Consortium is a registered charity (number 1097737) funded by the Wellcome Trust, GlaxoSmithKline, Genome Canada, the Canadian Institutes of Health Research, the Ontario Innovation Trust, the Ontario Research and Development Challenge Fund, the Canadian Foundation for Innovation; Vinnova, Knut and Alice Wallenberg Foundation; and Karolinska Institutet. Data collection by Dr F. von Delft (Structural Genomics Consortium, University of Oxford, Oxford, U.K.) is gratefully acknowledged.