

Determination of IC₅₀ values using GraphPad Prism

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Presentation outline

- ▶ What is the IC_{50} ?
- ▶ How to determine the IC_{50} value of an inhibitor?
- ▶ How to calculate the IC_{50} value of an inhibitor in GraphPad Prism?

What is the IC_{50} ?

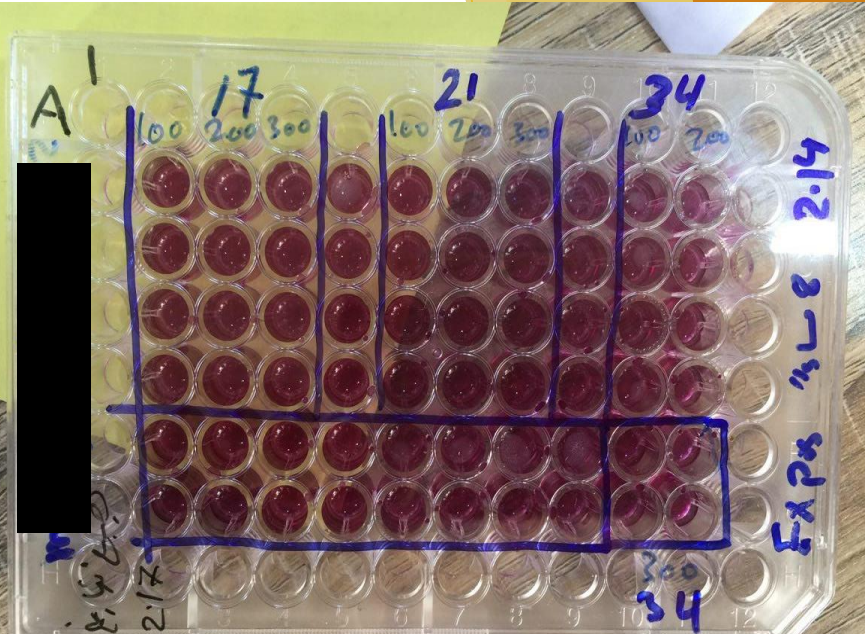
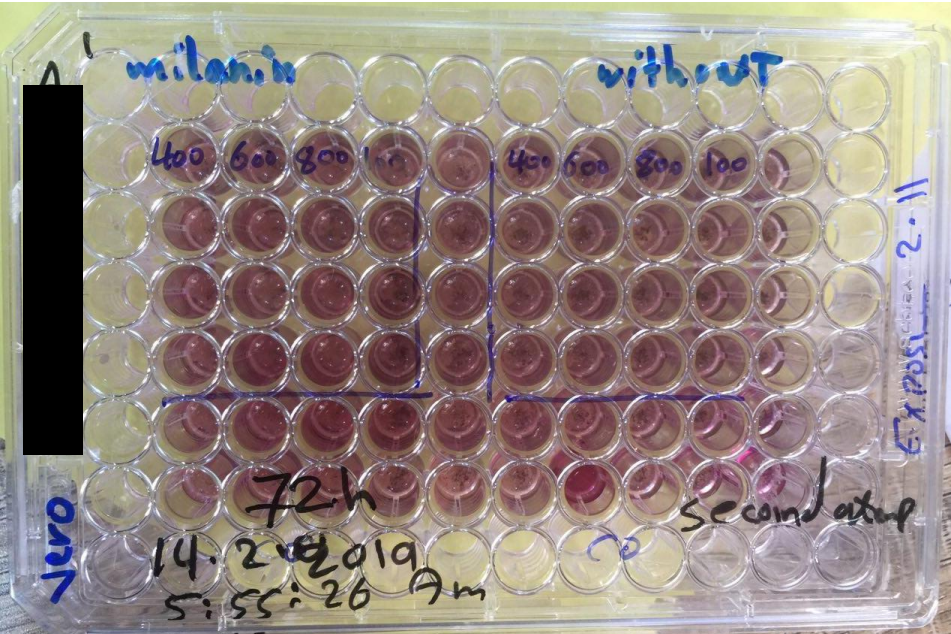
- ▶ Half maximal inhibitory concentration (IC_{50})
- ▶ Half maximal effective concentration (EC_{50})

Application

- ▶ Cell proliferation
- ▶ Cytotoxicity
- ▶ Enzyme activity

Determining IC₅₀ values

- ▶ To determine IC₅₀ values, the concentration range used of each **Inhibitor** should be determined properly.
- ▶ Concentration range: E.g. 10, 20, 30, 40, 50 and 60 μ M **Inhibitor**
- ▶ At least **three** or **four** Biological replicates for each concentration used



Absorbances Filter 1: 620nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.042	0.036	0.029	0.045	0.038	0.036	0.044	0.037	0.042	0.308	0.401	0.039
B	0.035	0.258	0.299	0.265	0.304	0.290	0.303	0.290	0.295	0.281	0.197	0.039
C	0.042	0.296	0.263	0.236	0.294	0.294	0.312	0.595	0.326	0.368	0.246	0.037
D	0.036	0.262	0.115	0.149	0.153	0.141	0.186	0.307	0.171	0.158	0.165	0.046
E	0.040	0.266	0.119	0.122	0.148	0.177	0.147	0.266	0.173	0.157	0.155	0.038
F	0.039	0.313	0.134	0.128	0.161	0.146	0.146	0.284	0.177	0.166	0.154	0.040
G	0.041	0.300	0.131	0.139	0.155	0.554	0.164	0.298	0.166	0.157	0.170	0.035
H	0.036	0.038	175	155	135	115	95	CO	3	2	1	

IC₅₀ Calculation



Welcome to GraphPad Prism



New table & graph

XY **1**

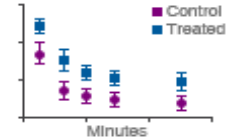
- Column
- Grouped
- Contingency
- Survival
- Parts of whole

Existing file

- Open a file
- LabArchives
- Clone a graph
- Graph portfolio

XY tables: Each point is defined by an X and Y coordinate

	X	A			B		
	Minutes	Control			Treated		
	X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title						
2	Title						
3	Title						




[Learn more](#)

Enter/import data:

- X:**
- Numbers
 - Numbers with error values to plot horizontal error bars
 - Dates
 - Elapsed times

Y: Enter and plot a single Y value for each point

- 2** Enter  replicate values in side-by-side subcolumns
- Enter and plot error values already calculated elsewhere

Enter: Mean, SD, N

Use tutorial data:

- Linear regression - Compare slopes
- Nonlinear regression -- One phase exponential decay
- Dose-response - X is log(dose)
- Interpolate unknowns from a linear standard curve
- Correlation
- Entering dates into the X column
- Entering elapsed times into the X column
- More tutorial data...

- Family
- Search results
- Data Tables
 - Data 1
- Info
 - Project info 1
- Results
 - Transform of Data 1
- Graphs
 - Data 1
 - Transform of Data 1
- Layouts
- Floating Notes

Table format: XY		Group A				Group B				Group C				
		Data Set-A				Title				Title				
	X	A:Y1	A:Y2	A:Y3	A:Y4	B:Y1	B:Y2	B:Y3	B:Y4	C:Y1	C:Y2	C:Y3	C:Y4	D:Y1
1	Title	60	5.00000											
2	Title	50	22.63727											
3	Title	40	49.98478											
4	Title	30	79.29260											
5	Title	20	98.80000											
6	Title	0	100.00000											
7	Title													
8	Title													
9	Title													
10	Title													
11	Title													
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29	Title													
30	Title													

Analyze Data

Built-in analysis

Which analysis?

- Transform, Normalize... 2
 - Transform Concentrations (X)
 - Normalize
 - Prune rows
 - Remove baseline and column math
 - Transpose X and Y
 - Fraction of total
- XY analyses
 - Nonlinear regression (curve fit)
 - Linear regression
 - Fit spline/LOWESS
 - Smooth, differentiate or integrate curve
 - Area under curve
 - Deming (Model II) linear regression
 - Column statistics
 - Row means with SD or SEM
 - Correlation
 - Interpolate a standard curve
- Column analyses
- Grouped analyses
- Contingency table analyses

Analyze which data sets?

A

When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.

Select All Deselect All

Help Cancel OK 3

- Family
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Table format: XY		Group A		Group B		Group C				
	X Title					Title				
	X	A:Y1				C:Y1	C:Y2	C:Y3	C:Y4	D:Y1
1	Title	60	5.00000							
2	Title	50	22.63727							
3	Title	40	49.98478							
4	Title	30	79.29260							
5	Title	20	98.80000							
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30	Title									

Parameters: Transform

Function List

- Standard functions
- Pharmacology and biochemistry transforms
- User-defined X functions
- User-defined Y functions

Interchange X and Y (then transform as specified below).

Transform X values using **X=Log[X]** K= **1**

Transform Y values using

- Same K for all data sets
- Different K for each data set

Data: Data Set-A

When it is impossible to transform

- Erase SD or SEM.
- Convert to an asymmetric distribution

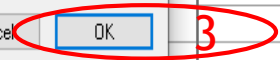
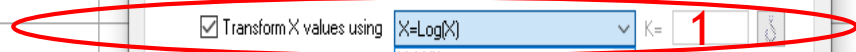
Replicates

- Transform individual Y values
- Transform the average of replicates

New graph

Create a new graph of the results

Cancel OK



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 - Transform of Data 1
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	X	A				B	C
	X Title	Data Set-A				Title	Title
	X	A:Y1	A:Y2	A:Y3	A:Y4		
1	1.778	5.000	5.500	5.600	4.300		
2	1.699	22.637	22.838	23.852	22.321		
3	1.602	49.985	49.334	52.710	48.000		
4	1.477	79.293	73.676	61.823	65.000		
5	1.301	98.900	98.000	98.216	99.000		
6	1	0.000	100.000	100.000	100.000		
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Analyze which data sets?

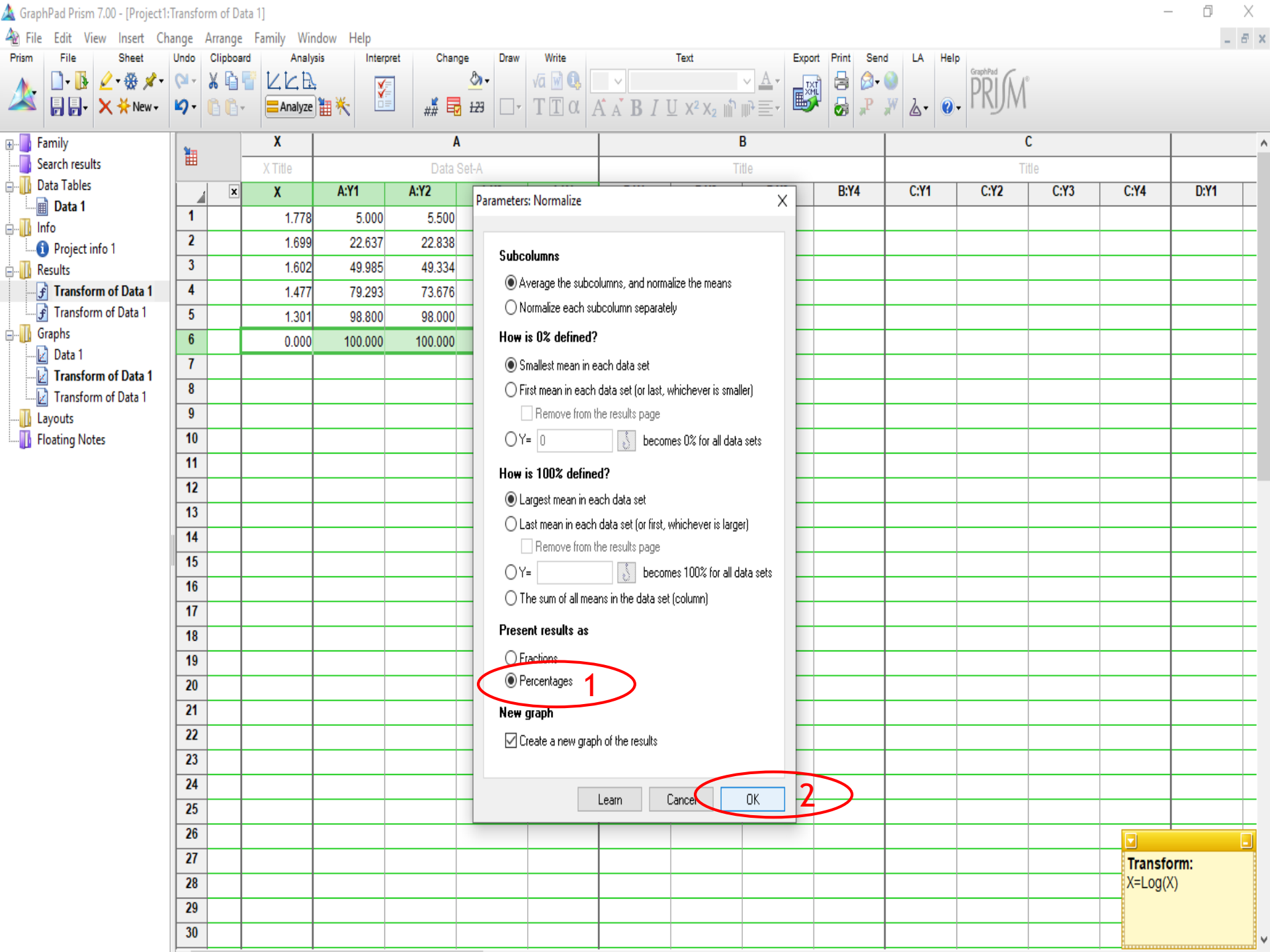
A

When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.

Select All Deselect All

Help Cancel OK

Transform:
X=Log(X)



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	X	A		B	C					
	X Title	Data Set-A		Title	Title					
	X	A:Y1	A:Y2		B:Y4	C:Y1	C:Y2	C:Y3	C:Y4	D:Y1
1	1.778	5.000	5.500							
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3	1.602	49.985	49.334							
4	1.477	79.293	73.676							
5	1.301	98.800	98.000							
6	0.000	100.000	100.000							
7										
8										
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Parameters: Normalize

Subcolumns

- Average the subcolumns, and normalize the means
- Normalize each subcolumn separately

How is 0% defined?

- Smallest mean in each data set
- First mean in each data set (or last, whichever is smaller)
 - Remove from the results page
- Y= becomes 0% for all data sets

How is 100% defined?

- Largest mean in each data set
- Last mean in each data set (or first, whichever is larger)
 - Remove from the results page
- Y= becomes 100% for all data sets
- The sum of all means in the data set (column)

Present results as

- Fractions
- Percentages 1

New graph

- Create a new graph of the results

Learn Cancel OK 2

Transform:
X=Log(X)

X	A	B
X Title	Data Set-A	Title
X	A:Y1	A:Y2
1.778	-0.105	
1.699	18.480	
1.602	47.297	
1.477	78.180	
1.301	98.736	
0.000	100.000	

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Help Cancel OK

Parameters: Nonlinear Regression

Fit Compare Constrain Weights Initial values Range Output Confidence Diagnostics Flag

Choose an equation

- Recently used
 - log(inhibitor) vs. normalized response -- Variable slope
 - log(inhibitor) vs. normalized response
 - [Inhibitor] vs. normalized response -- Variable slope
- Standard curves to interpolate
- Dose-response - Stimulation
- Dose-response - Inhibition
 - log(inhibitor) vs. response (three parameters)
 - log(inhibitor) vs. response -- Variable slope (four parameters)
 - log(inhibitor) vs. normalized response -- Variable slope**
 - [Inhibitor] vs. response (three parameters)
 - [Inhibitor] vs. response -- Variable slope (four parameters)
 - [Inhibitor] vs. normalized response
 - [Inhibitor] vs. normalized response -- Variable slope
- Dose-response - Special
- Binding - Saturation
- Binding - Competitive
- Binding - Kinetics

-If X is not already the log of dose, go back and transform your data.
-The Y values of the curve will go from 100 down to 0.

log(inhibitor) vs. normalized response -- Variable slope [Learn about this equation](#)

Fitting method

Least squares (ordinary) fit Robust fit Automatic outlier elimination

Interpolate

Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel **OK 4**

Family

Search results

Data with Results

Data 1

- ...Transform of Data
-Normalize of Tra
-Nonlin fit of No
- ...Transform of Data 1

Data Tables

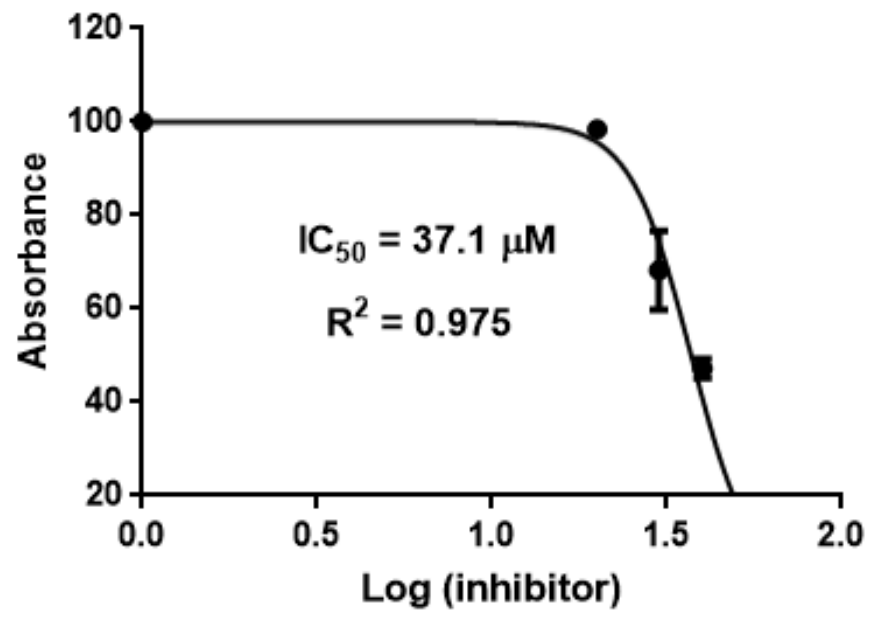
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- Transform of Data 1
- Normalize of Transform

Layouts

Floating Notes

Nonlin fit		A	B	C	D	E	F	G	H	I	J
		Data Set-A	Title	Title	Title	Title	Title	Title	Title	Title	Title
		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
1	log(inhibitor) vs. normalized response -- Variable slope										
2	Best-fit values										
3	LogIC50	1.569									
4	HillSlope	-4.991									
5	IC50	37.1									
6	Std. Error										
7	LogIC50	0.007805									
8	HillSlope	0.4092									
9	95% CI (profile likelihood)										
10	LogIC50	1.552 to 1.586									
11	HillSlope	-5.852 to -4.275									
12	IC50	35.68 to 38.52									
13	Goodness of Fit										
14	Degrees of Freedom	22									
15	R square	0.975									
16	Absolute Sum of Squares	853.8									
17	Sy,x	6.23									
18											
19	Number of points										
20	# of X values	24									
21	# Y values analyzed	24									
22											
23											
24											
25											
26											
27											
28											
29											
30											



log(inhibitor) vs. normalized response -- Variable slope

[Equation help](#)