*Why Using **pIC50** Instead of **IC50** Will Change Your Life*

A CDD Webinar by

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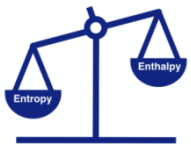

Others in the Series: Recording of Lipinski's *"Entropic and Enthalpic Propensities Inherent in SBDD and HTS"* available online here: https://www.collaborativedrug.com/recordings

Questions: Please ask your questions in the chat box, and we will try to answer them at the end

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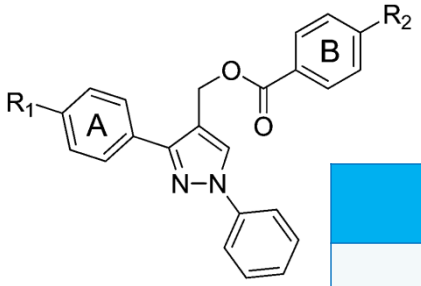
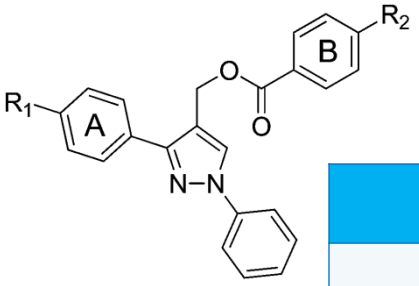
Is this what it looks like when you review assay data?



This week's SAR update

R1	R2	IC50 (μM)
-H	-H	30
-OH	-H	> 30
$-CF_3$	-H	0.000682
$-CH_2NH_2$	-H	1.011
-H	$-CH_2CH_2OH$	0.99
-H	$-CH_2NH_2$	0.90
-OH	-OH	0.8
-OH	-CF3	0.00502
-OH	$-CH_2NH_2$	0.002
$-CF_3$	$-CH_2NH_2$	0.001
$-CF_3$	-OH	0.0013

Why are these data hard to understand?



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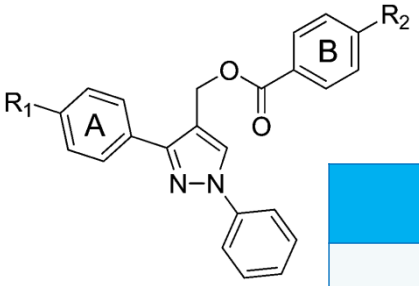
Are these compounds equipotent?

Do these pairs of compounds have similar potency?

Is the second compound "two times better" than the first one?

What's wrong with this table?



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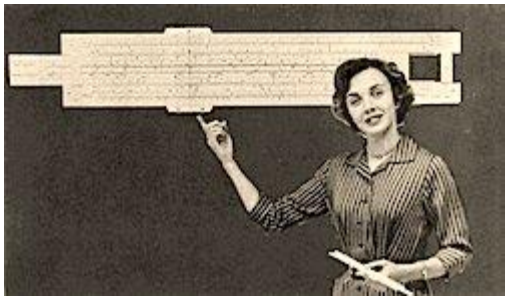
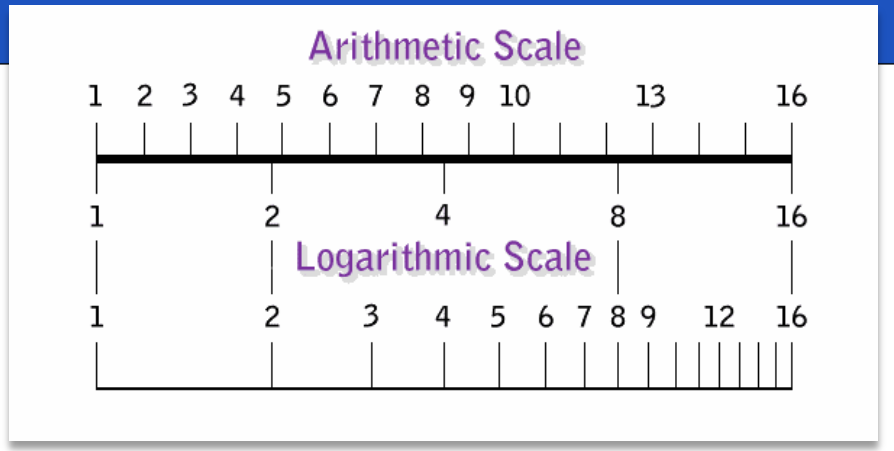
$-CF_3$	$-CH_2NH_2$	0.001
$-CF_3$	-OH	0.0013

Issues with using IC50 to report data

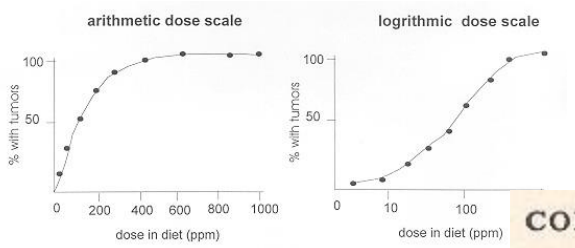
- **Too many digits**
  - Difficult to use consistent significant figures
- **Reporting IC50 values encourages linear thinking about an exponential value**
  - Implies zero or negative values are possible
  - Encourages arithmetic vs. geometric averaging
  - Implies cutting the IC50 in half means you are doubling potency
  - Encourages non-optimal experimental design

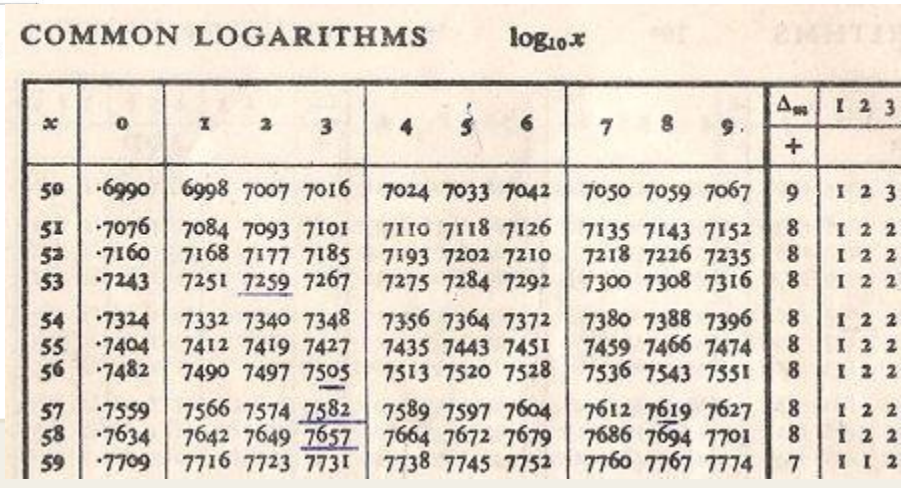
So how can I discourage linear thinking?





Start thinking logarithmically!



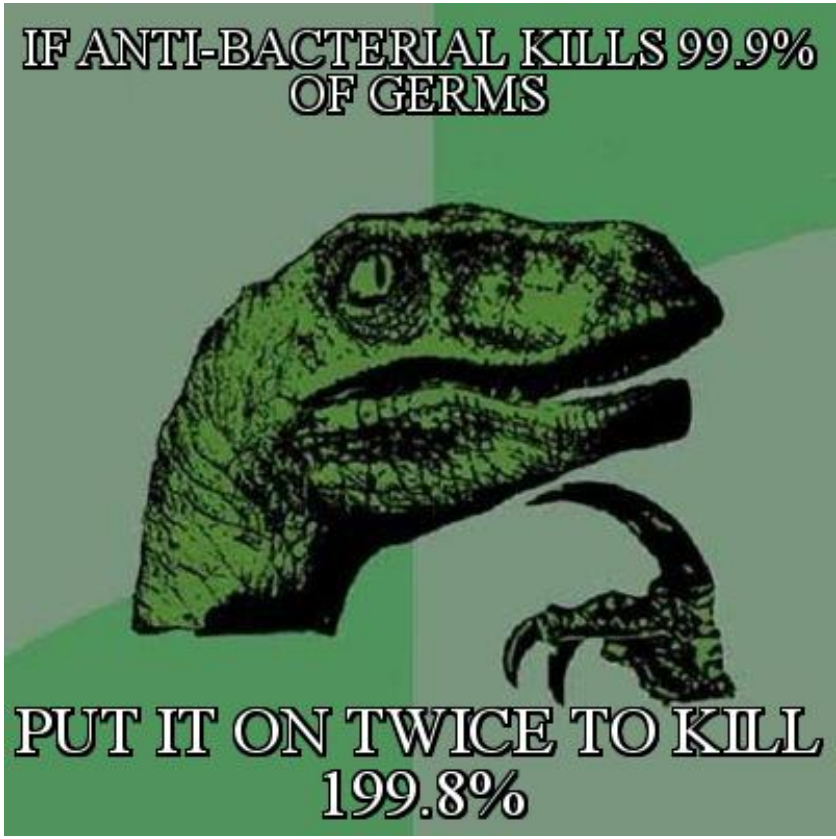


x	0	1	2	3	4	5	6	7	8	9	Δ	1	2	3
50	·6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	9	1	2	3
51	·7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	8	1	2	2
52	·7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	8	1	2	2
53	·7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	8	1	2	2
54	·7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	8	1	2	2
55	·7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	8	1	2	2
56	·7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	8	1	2	2
57	·7559	7566	7574	7582	7589	7597	7604	7612	7619	7627	8	1	2	2
58	·7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	8	1	2	2
59	·7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	7	1	1	2

Start thinking logarithmically!

So would a product that kills 99.99% instead of 99.9% really be only 0.09% better?

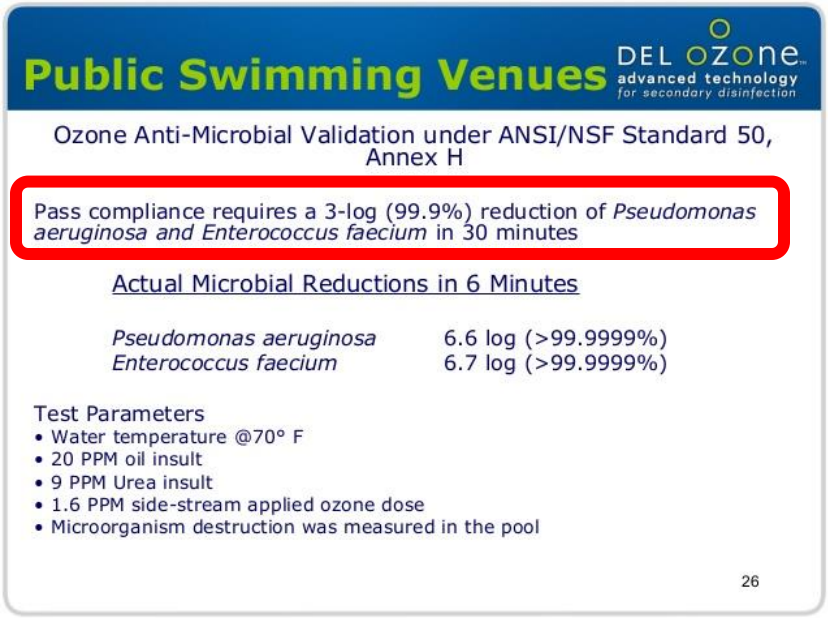
or



Start thinking logarithmically!

In anti-infective circles, 99.9% killing is usually referred to as a “3-log kill”

Why?



Kills:	Fold reduction	Log kill
90%	10x	1
99%	100x	2

Start thinking logarithmically!

You've always been doing this...

Concentration of Hydrogen ions compared to distilled water		Examples
10,000,000	pH 0	Battery acid
1,000,000	pH 1	Hydrochloric acid
100,000	pH 2	Lemon juice, vinegar
10,000	pH 3	Grapefruit, soft drink
1,000	pH 4	Tomato juice, acid rain
100	pH 5	Black coffee
10	pH 6	Urine, saliva
1	pH 7	"Pure" water
1/10	pH 8	Sea water
1/100	pH 9	Baking soda,
1/1,000	pH 10	Great Salt Lake
1/10,000	pH 11	Ammonia solution
1/100,000	pH 12	Soapy water
1/1,000,000	pH 13	Bleach
1/10,000,000	pH 14	Liquid drain cleaner

So you have been thinking logarithmically!

So what is pIC50 ?

Survey Question #1

Do you use IC50/EC50 and/or pIC50/pEC50 to report in vitro assay data where you work?

1. **Only IC50**
2. **Mostly IC50, some pIC50**
3. **Mostly pIC50, some IC50**
4. **Only pIC50**

What the heck is a “pIC50”

Note: no units! pIC50 is dimensionless

- pIC50 is the negative log of the IC50 in Molar
- An IC50 of 1 uM is 10^{-6} M, which is pIC50 = 6.0
- An IC50 of 1 nM is 10^{-9} M, which is pIC50 = 9.0
- An IC50 of 10 nM is 10^{-8} M, which is pIC50 = 8.0
- An IC50 of 100 nM is 10^{-7} M, which is pIC50 = 7.0
- An IC50 of 30 nM is 3 x10^{-7} M,
  which is also $10^{-7.5}$ M, which is pIC50 = 7.5

Do you see a pattern?

This should look familiar

- pH is the negative log of the [H+] in M
- pIC50 is the negative log of the IC50 in M

It's not too different from what you're used to!

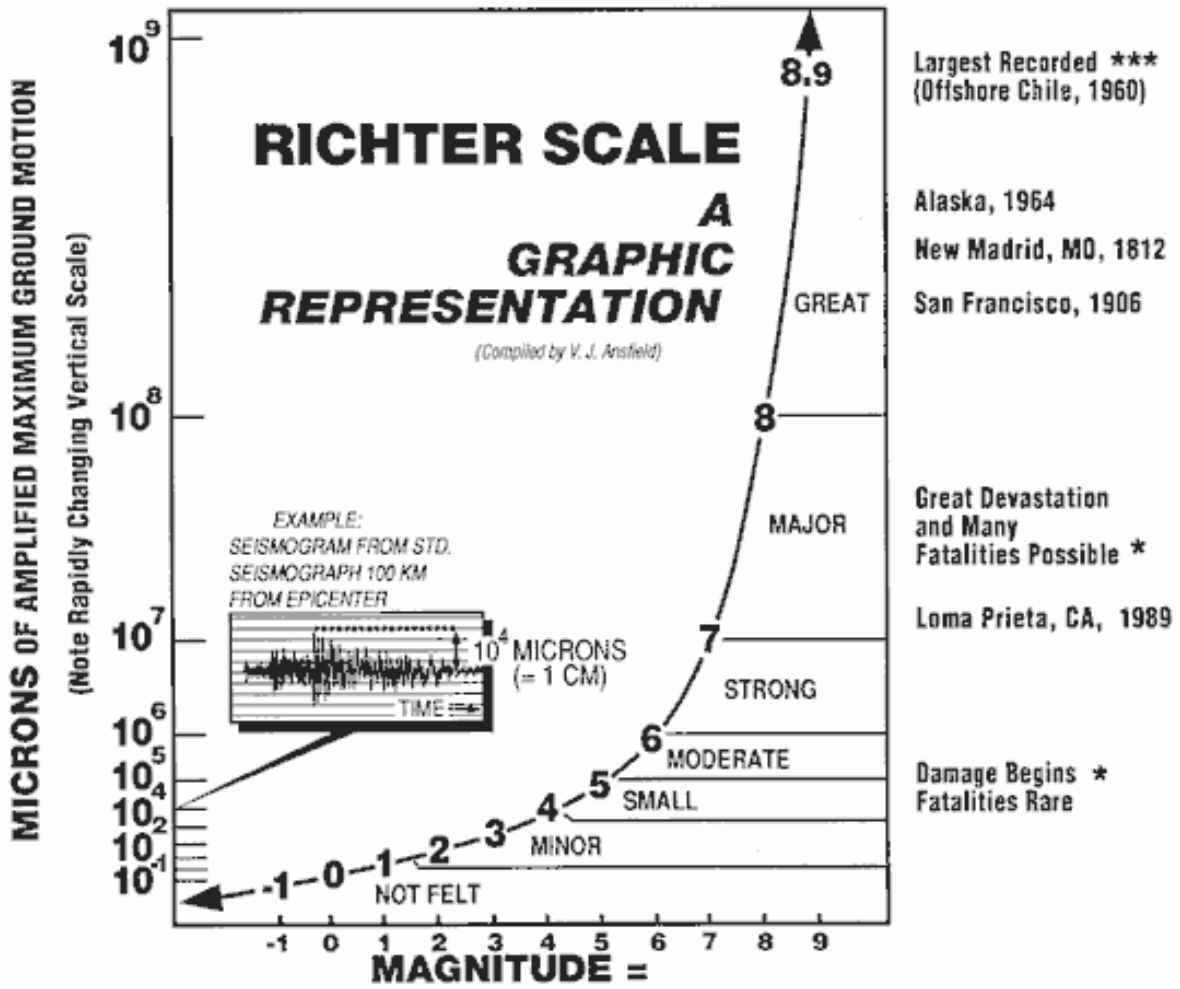
Inhibitor Potency

IC50, μM	pIC50
30	4.5
> 30	< 4.5
0.000682	9.2
1.011	6.0
0.99	6.0
0.90	6.0
0.8	6.1
0.00502	8.3
0.002	8.7
0.001	9.0
0.0013	8.9

pH

[H+], mM	pH
1000	0
100	1.0
10	2.0
1	3.0
0.1	4.0
0.01	5.0
0.001	6.0
0.0001	7.0
0.00001	8.0
0.000001	9.0
0.0000001	10.0

Other log scales you know and love...



Think of it as the Richter scale for drug discovery

Richter Scale	Value	pIC50 scale
Not felt by many people; no damage	**3.0**	**1 mM?** Only if we're doing fragment based discovery
Felt by all; minor breakage of objects	**4.0**	Don't bother resynthesizing
Some damage to weak structures	**5.0**	Are you sure???
Moderate damage in populated areas	**6.0**	**1 μM?** It's a hit, not a lead
Serious damage over large areas; loss of life	**7.0**	OK, we're making progress
Severe destruction, loss of life over large areas	**8.0**	Getting nice potency
Epic destruction; time to move back to Kansas	**9.0**	**1 nM?** Call J. Med Chem!
Never recorded in modern history; Welcome to New Atlantis!	**10.0**	**100 pM?** Call Nature Reviews Drug Discovery!
Repent...	**11.0**	**10 pM?** Call Stockholm!

So, why will using
pIC50
instead of
IC50
will change my life?

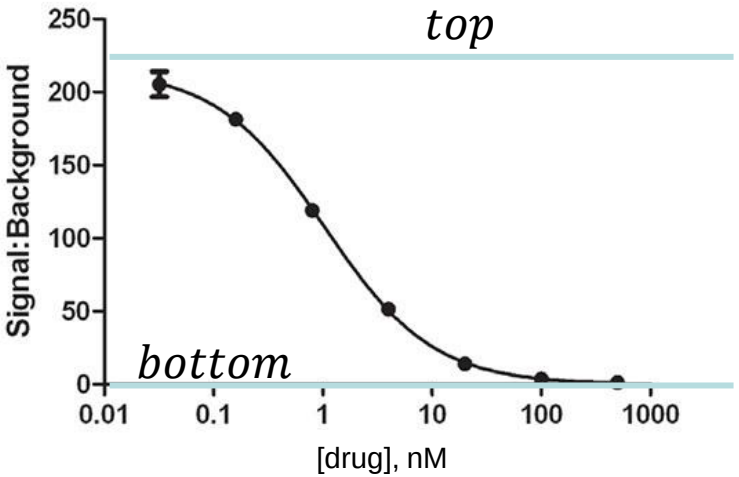
#1
pIC50 will encourage you to look at in vitro assay data logarithmically

IC50 is calculated logarithmically!

THE "IC50 EQUATION": ALSO KNOWN AS THE "FOUR-PARAMETER LOGISTIC", "HILL" OR "SIGMOID" EQUATION

$$y = bottom + \frac{top - bottom}{1 + 10^{(\log IC50 - \log x) * HillSlope}}$$

Notice that curve fitting programs actually work with **log** IC50 and **log** [compound], and **not** the linear forms.



Dose dependent inhibition is a logarithmic phenomenon, so it makes more sense to think about the data this way

Some older software still uses this non-log form of the equation.

$$y = bottom + \frac{top - bottom}{1 + \left(\frac{x}{IC50}\right)^{hillslope}}$$

Don't use it!

pIC50 encourages logarithmic thinking

ID	IC50 (μM)	pIC50
NewCo-100	30	4.5
NewCo-101	> 30	< 4.5
NewCo-102	0.000682	9.2
NewCo-103	1.011	6.0
NewCo-104	0.99	6.0
NewCo-105	0.90	6.0
NewCo-106	0.8	6.1
NewCo-107	0.00502	8.3
NewCo-108	0.002	8.7
NewCo-109	0.001	9.0
NewCo-110	0.0013	8.9

- The transition from μM to nM is smoother
- "Spacing" between IC50 values is more relevant

NO, NewCo-109 is **not** twice as "good" as NewCo-108.

#2

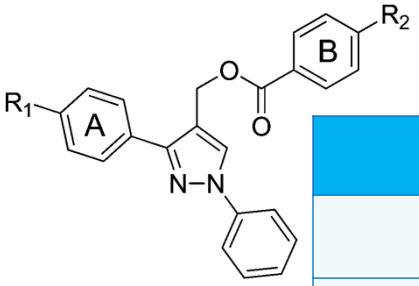
pIC50 will allow you to present in vitro assay data cleanly and in an easy to read form

pIC50 encourages consistent data presentation

ID	IC50 (μM)	pIC50
NewCo-100	30	4.5
NewCo-101	> 30	< 4.5
NewCo-102	0.000682	9.2
NewCo-103	1.011	6.0
NewCo-104	0.99	6.0
NewCo-105	0.90	6.0
NewCo-106	0.8	6.1
NewCo-107	0.00502	8.3
NewCo-108	0.002	8.7
NewCo-109	0.001	9.0
NewCo-110	0.0013	8.9

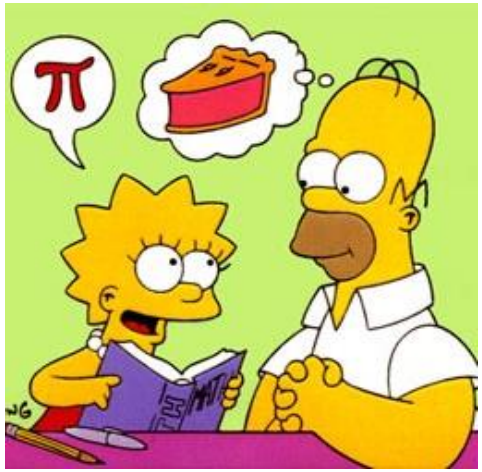
- A consistent number of digits *and* significant figures

Now your audience can focus on the SAR!



R1	R2	pIC50
-H	-H	4.5
-OH	-H	< 4.5
$-CF_3$	-H	9.2
$-CH_2NH_2$	-H	6.0
-H	$-CH_2CH_20H$	6.0
-H	$-CH_2NH_2$	6.0
-OH	-OH	8.3
-OH	-CF3	8.7
-OH	$-CH_2NH_2$	8.7
$-CF_3$	$-CH_2NH_2$	9.0
$-CF_3$	-OH	8.9

NOW WE'RE COMMUNICATING CLEARLY!



#3
pIC50 will make it easy and intuitive to average your in vitro assay data

Survey Question #2

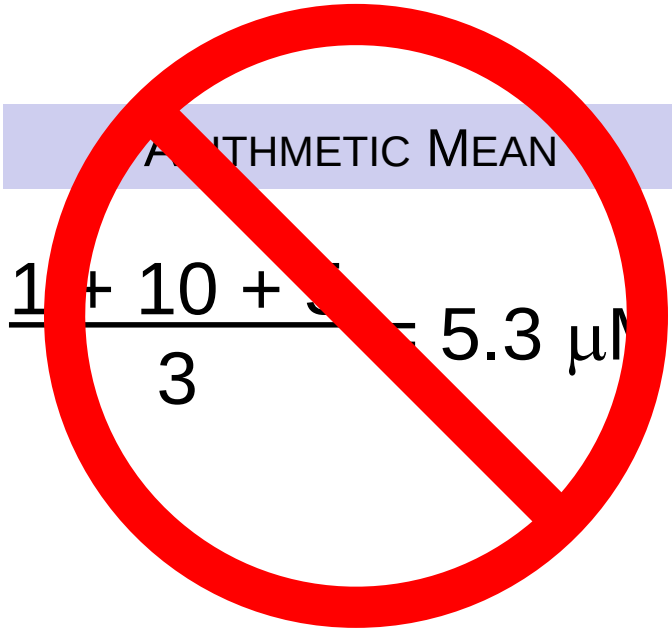
Do you average your replicate potency data (IC50/EC50 or pIC50/pEC50) using arithmetic or geometric means?

1. **Arithmetic**
2. **Geometric**
3. **Don't know**

IC50 averages should be geometric

Example Data

IC50 determination # 1: 1 μM
IC50 determination # 2: 10 μM
IC50 determination # 3: 5 μM



Arithmetic Mean

$$\frac{1 + 10 + 5}{3} = 5.3\ \mu M$$

Don't average IC50 values using arimetic means!

Geometric Mean

$$\sqrt[3]{1 * 10 * 5} = 3.7\ \mu M$$

Arithmetic Mean of pIC50 values

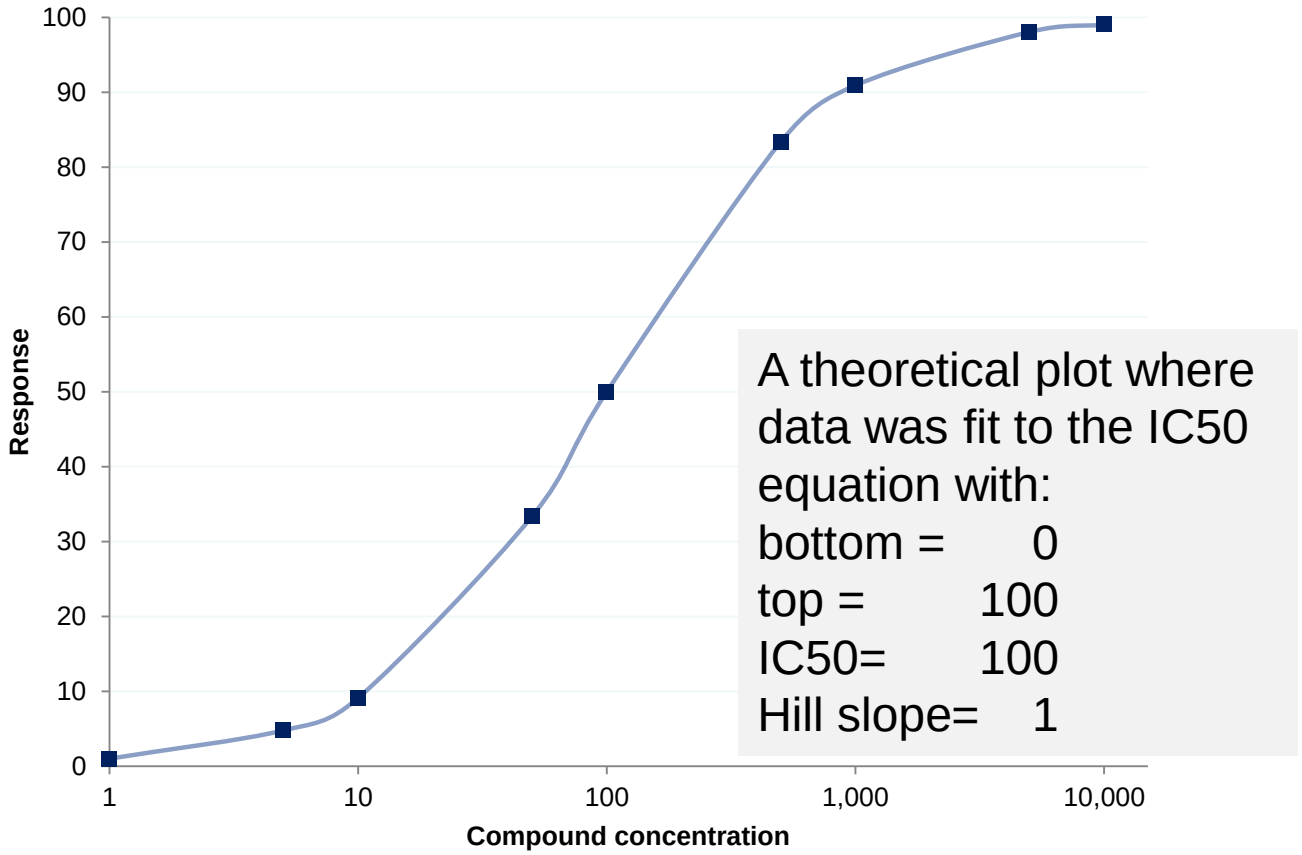
$$\frac{6 + 5 + 5.3}{3} = 5.4$$

→ 3.7 μM

4
pIC50 and logarithmic thinking will improve how you plan your experiments

How do you set up your dilution series?

- Do you typically set up “half-decade” dilution curves?
  - The classic 1000, 500, 100, 50, 10, 5, 1 nM, etc?

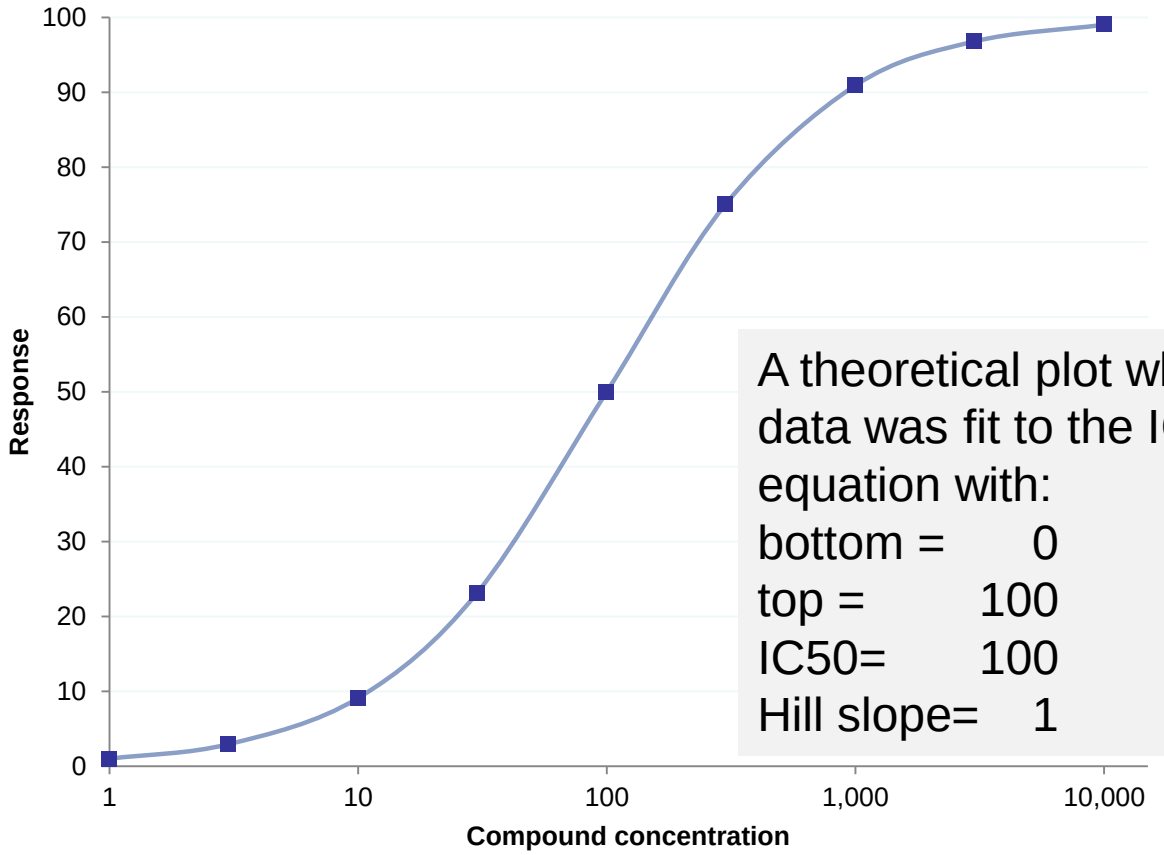


A theoretical plot where data was fit to the IC50 equation with:
bottom = 0
top = 100
IC50= 100
Hill slope= 1

Now think logarithmically

- Set up “half-log” dilution curves:
  - 1000, 300, 100, 30, 10, 3, 1 nM, etc?

Note how the points on this plot are evenly spaced, while on the previous slide are clumped.



A theoretical plot where data was fit to the IC50 equation with:
bottom = 0
top = 100
IC50= 100
Hill slope= 1

5
pIC50 and logarithmic thinking will improve how you look at the reliability of your data

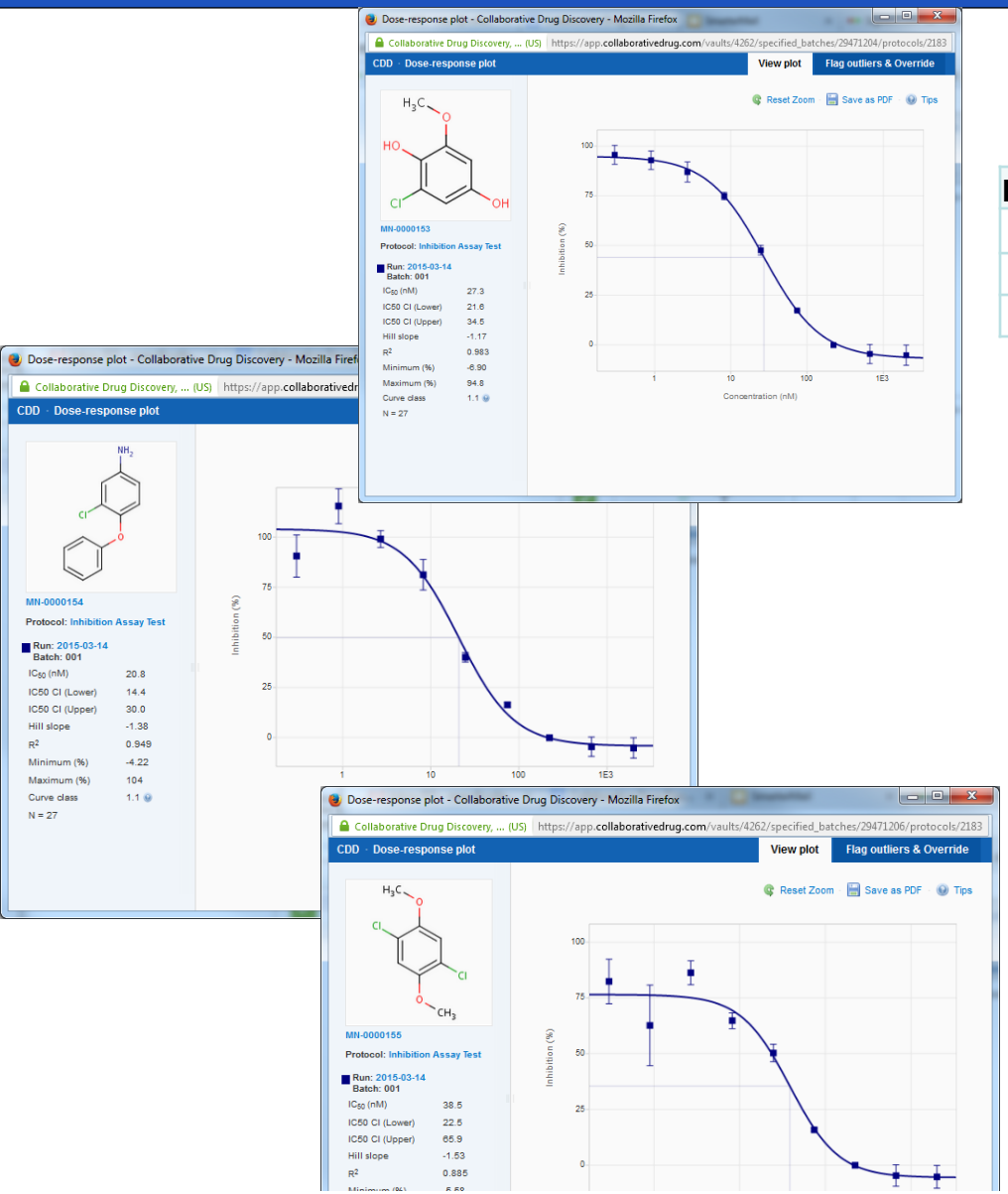
The two common forms of reporting data “confidence”

- **Standard error of the calculated value**:
  - the standard deviation of that parameter if you repeated the experiment many times

- **95% Confidence Interval**
  - Estimate of the precision of a measurement
  - If the experiment were repeated 100 times, there is a 95 percent chance that your true value will be in this range
  - If a confidence interval is very wide, your data don't define that parameter very well.
  - Is **approximately** 2x the SE above and below the mean

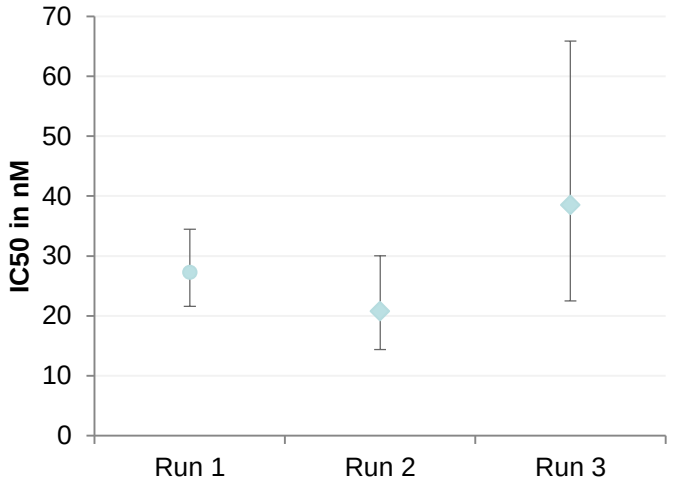
- Modern software will report the 95% CI of an IC50, but NOT the SE.

Analysis of the 95% confidence intervals



Some test data: “tight run” “less tight” “messy”

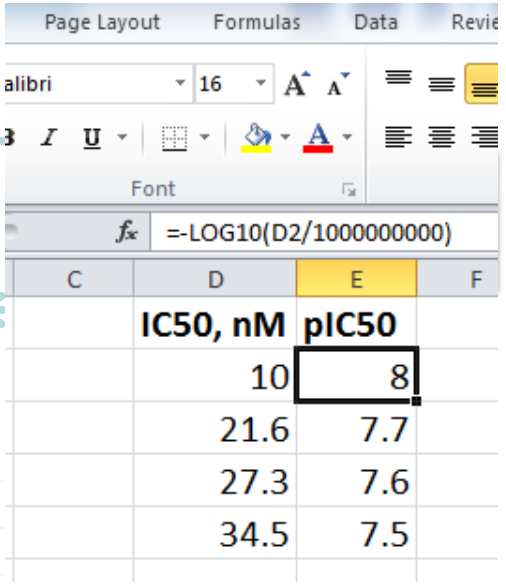
Parameter	Run 1	Run 2	Run 3
95% CI Low, nM	21.6	14.4	22.5
IC50, nM	**27.3**	**20.8**	**38.5**
95% CI Hi, nM	34.5	30.0	65.9

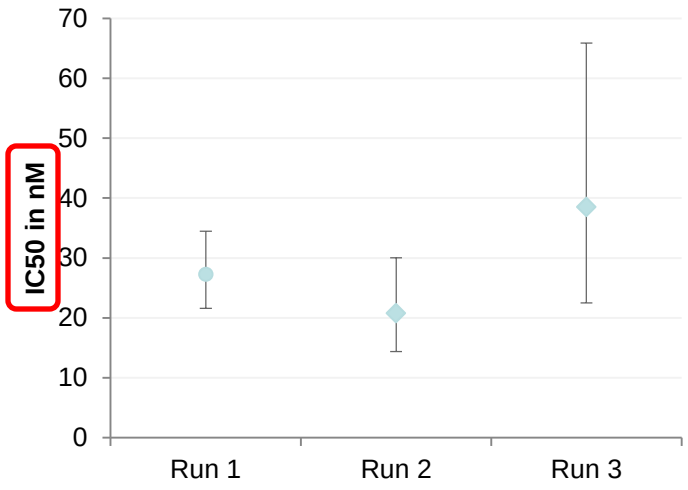


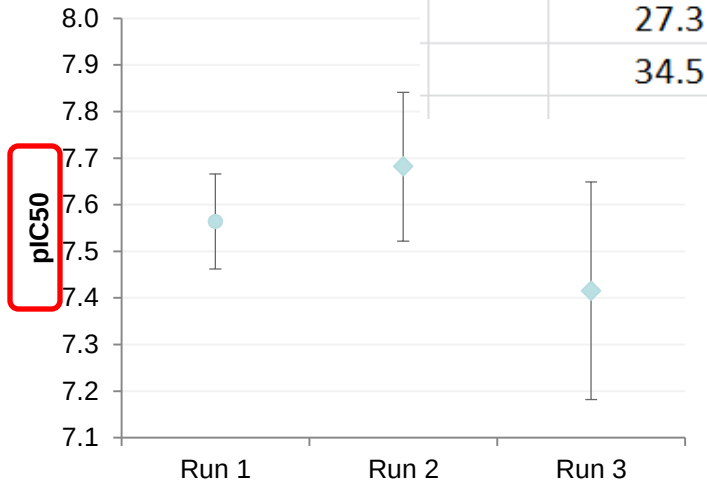
The error bars are not symmetrical! What does that mean?

Looking at the 95% CI of the pIC50

Parameter	Run 1	Run 2	Run 3
95% CI Low, nM	21.6	14.4	22.5
IC50, nM	**27.3**	**20.8**	**38.5**
95% CI Hi, nM	34.5	30.0	65.9

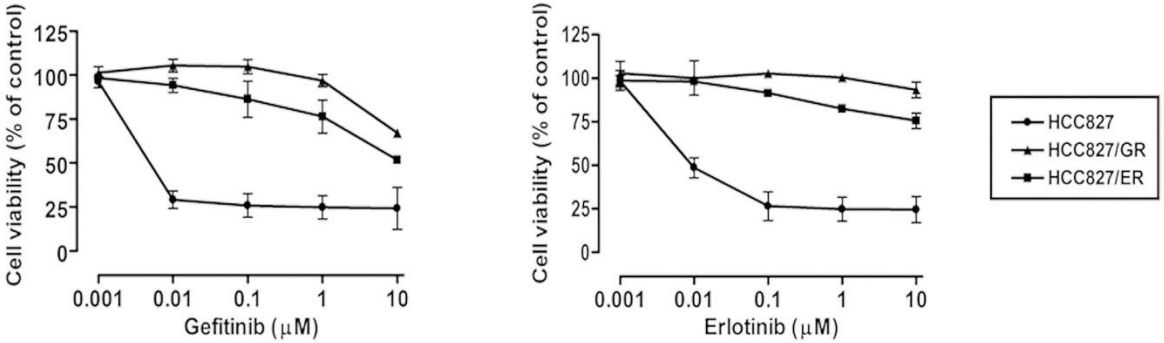




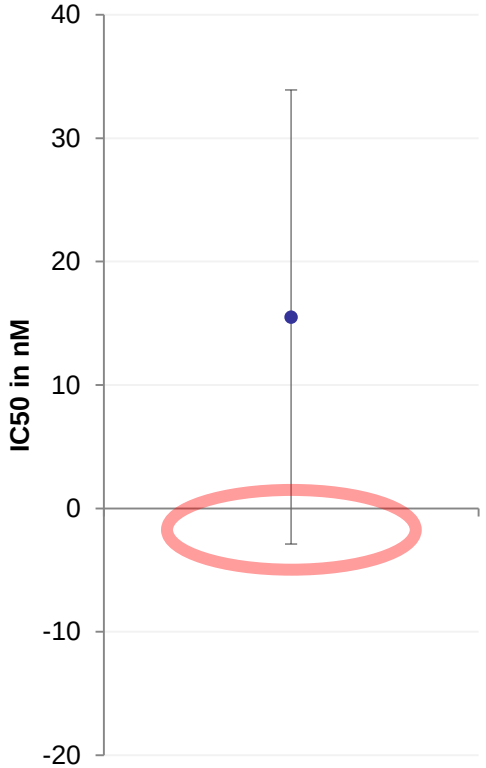


By using pIC50, your error bars are properly symmetrical, and it's easy to see which run had the "tighter" data

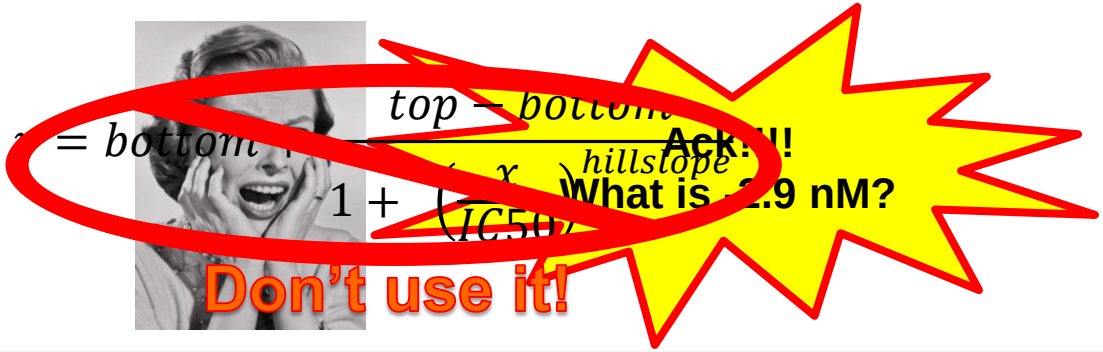
An example from the literature...



IC_{50} (nM)	Gefitinib	Erlotinib
HCC827	7.2 ± 0.3	15.5 ± 9.2
HCC827/GR	> 10000	> 10000
HCC827/ER	> 10000	> 10000



If we estimate the 95% confidence interval as ~2x the SE above and below the mean, does this mean the 95% CI is **-2.9 to 33.9 nM**?



Summary

How can using pIC50 instead of IC50 can change your life?

Conclusion

- Using pIC50 instead of IC50 will force/encourage you to think about your assays logarithmically
- Reviews of assay data will be easier to present (only 2 significant digits to deal with, even over a large range of potencies)
- You'll average potency properly by using simple arithmetic means of the pIC50 values
  - instead of Geomeans of the IC50 values
- Reliability ranges will be correct, symmetric and you will never encounter negative IC50 values!

For those of you that use CDD…

- Using CDD Calculations, you can convert

  IC50 and the IC50 Confidence Intervals
  to
  pIC50 and **p**IC50 Confidence Intervals

Bonus slide... It works for micro data too!

ID	IC50 (μM)	MIC (μg/mL)
NewCo-200	4.4	16
NewCo-201	> 30	>128
NewCo-202	0.012	1
NewCo-203	0.018	1
NewCo-204	0.018	0.125

pMIC:

The negative log of the MIC **in Molar!**

ID	pIC50	pMIC
NewCo-200	5.4	5.4
NewCo-201	<4.5	<4.4
NewCo-202	7.9	6.5
NewCo-203	7.7	6.7
NewCo-204	7.7	7.5

*Why Using **pIC50** Instead of **IC50** Will Change Your Life*

A CDD Webinar by

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Thank You. Any Questions?

More info:

CDD Vault, our hosted biological and chemical data management solution:
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