Test

Screening Report COMPOUND PLATFORM

blabla

Type: Cytotoxicity screen
Cell viability of HeLa cells after exposition to compounds for three days

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Introduction

This report summarizes the result of the cytotoxicity test routinely conducted at our insitut *IBCS-FMS* in the working group *ComPlat* at the *KIT*.

In order to measure the cytotoxicity for mammalian organisms, *HeLa* cells are used within the assay. First, 100 µL of a solution containing 10^5 cells/mL are sowed into 96 well plates. Subsequently, after 24 h, different concentrations (0.5 µM, 5µM, 10µM and 25 µM; diluted in cell media) of the respective compounds are added to the cells. After incubation of three days, MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazoliumbromid)] is added. The cells reduce MTT to a formazan by oxidizing the reductant equivalents NADH or NADPH. Likewise, electrons from succinate are used for the reduction of MTT. This step is catalyzed by the enzyme succinate-dehydrogenase. The reaction is stopped after 3 hours, using an aqueous solution consisting of 10% SDS and roughly 0.3% HCl. The last step is the absorbance measurement of the formazan at 595 nm.

As a positiv control cells were lysed using a 20% triton solution, directly before adding MTT. Cells incubated exclusively in cell medium served as a negative control.

The cell viability is calculated using the following equation:

$$cell\ viability_{well_i} = \frac{absorbance_{well_i} - mean(positive\ control)}{mean(negative\ control)}$$

Representation of data analysis

The screening results are shown in the next section. The data is represented in one plots shown at the right column. The normalized data is depicted in the Boxplots which also include the corresponding fit. The graphics are produced by using ggplot2 (for details see Wickham (2016)). The fit is calculated based on the equation below. For further details please refer to Ritz et al. (2015) and Seber et al. (1989). As a side note, the outliers were detected using a method described by Motulsky and Brown (2006).

$$\sum_{i=1}^{N}[(y_i - f_i)/w_i]^2$$

where:

2

- y_i is the ith observation of the measured data
- w_i is the weight for the ith observation
- $f(i) = c + \frac{d-c}{1+\exp(b(\log(i)-\log(e)))}$ is the expected ith value

The following parameter are listed in the table beneath the plot:

- The estimated residual standard error (RSE)
- The response at the lowest and highest concentration
- The $-log10(IC50/1000000)$ value ($pIC50$)
- The p value compares the dose-response model with a simple linear regression model with slope 0 (a horizontal regression line corresponding to no dose effect)
- The $IC50\_relative\_higher/lower$ arre the $IC50 \pm 95\%$ confidence intervall
- The $relative\ IC50$ (e in the equation above)
- The Hill coefficent (b in the equation above)
- The first and second asymptote (parameter c and d respecitvly in the equation above)

Compounds and results

RSE	0.07062273
Response_lowestdose	0.777
Response_highestdose	0.597
pIC50	6.19
p_value	0.000346
IC50_relative_lower	−12.9
IC50_relative_higher	14.2
IC50_relative	0.645
HillCoefficient	4.96
asymptote_two	0.8282411
asymptote_one	0.5973585

Note: Response Difference lower than 25%
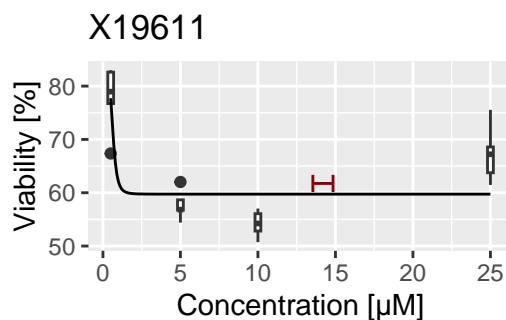
RSE	0.05054661
Response_lowestdose	0.874
Response_highestdose	0.956
pIC50	4.52
p_value	0.0247
IC50_relative_lower	
IC50_relative_higher	
IC50_relative	29.9
HillCoefficient	−4.26
asymptote_two	1.131484
asymptote_one	0.8743631

Note: Response Difference lower than 25%
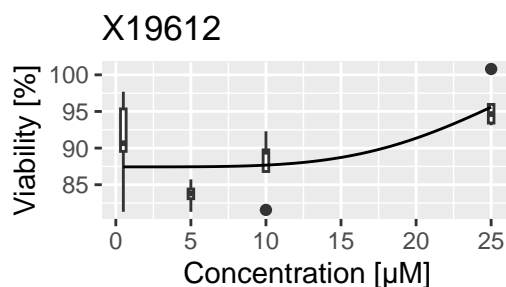
Confidence intervall could not be calculated

RSE	0.07336625
Response_lowestdose	0.859
Response_highestdose	1.01
pIC50	4.48
p_value	0.00292
IC50_relative_lower	−471
IC50_relative_higher	538
IC50_relative	33.4
HillCoefficient	−9.23
asymptote_two	3.227958
asymptote_one	0.8593316

Note: Response Difference lower than 25%
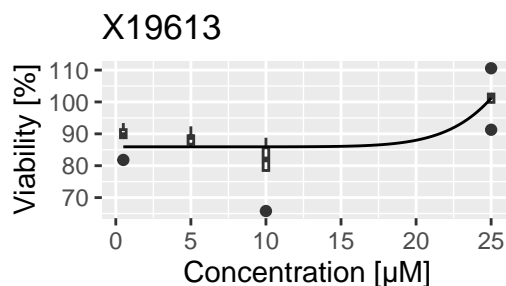

Confidence intervall not in conc. range

RSE	0.03889559
Response_lowestdose	0.958
Response_highestdose	0.738
pIC50	5.27
p_value	3.98e−08
IC50_relative_lower	−0.981
IC50_relative_higher	11.7
IC50_relative	5.37
HillCoefficient	1.06
asymptote_two	0.9790008
asymptote_one	0.6914785

Note: Response Difference lower than 25%
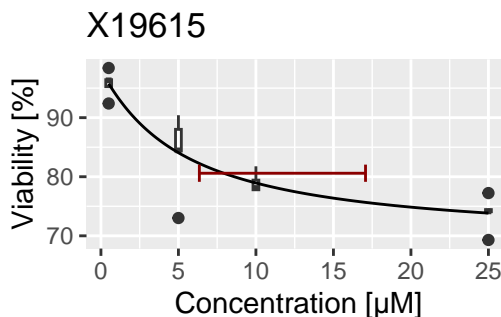
RSE	0.05684555
Response_lowestdose	0.911
Response_highestdose	0.391
pIC50	4.67
p_value	4.72e−13
IC50_relative_lower	−97.5
IC50_relative_higher	140
IC50_relative	21.5
HillCoefficient	13.9
asymptote_two	0.9105367
asymptote_one	0.3294355

Confidence intervall not in conc. range
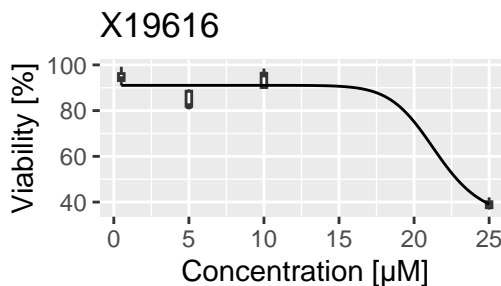
RSE	0.06596228
Response_lowestdose	0.763
Response_highestdose	0.847
pIC50	6.46
p_value	0.136
IC50_relative_lower	
IC50_relative_higher	
IC50_relative	0.348
HillCoefficient	−4.32
asymptote_two	0.8471666
asymptote_one	0.3623655

Note: Response Difference lower than 25%
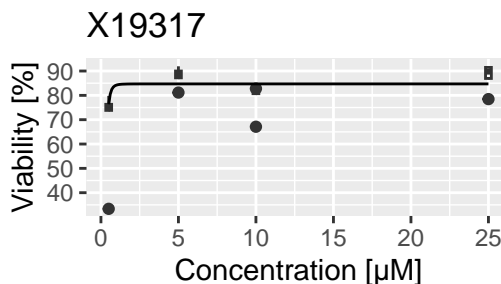
Confidence intervall could not be calculated

RSE	0.03912835
Response_lowestdose	0.847
Response_highestdose	0.892
pIC50	5.14
p_value	0.0795
IC50_relative_lower	−3.26
IC50_relative_higher	17.6
IC50_relative	7.19
HillCoefficient	−18
asymptote_two	0.8916742
asymptote_one	0.847149

Note: Response Difference lower than 25%
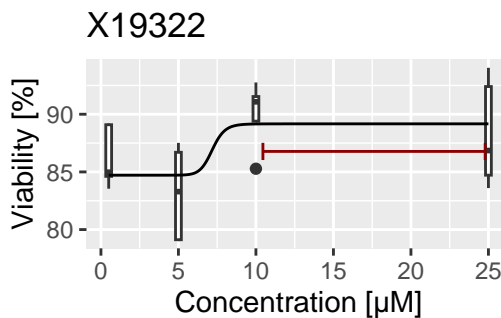
RSE	0.04684146
Response_lowestdose	0.909
Response_highestdose	0.469
pIC50	5
p_value	1.63e−12
IC50_relative_lower	8.12
IC50_relative_higher	11.8
IC50_relative	9.97
HillCoefficient	3.14
asymptote_two	0.9089781
asymptote_one	0.4449472

X19304

X19306

A warning occurred: Convergence failed
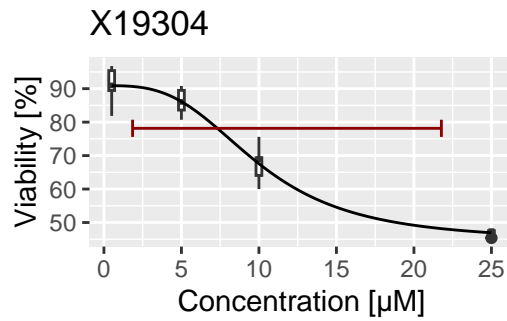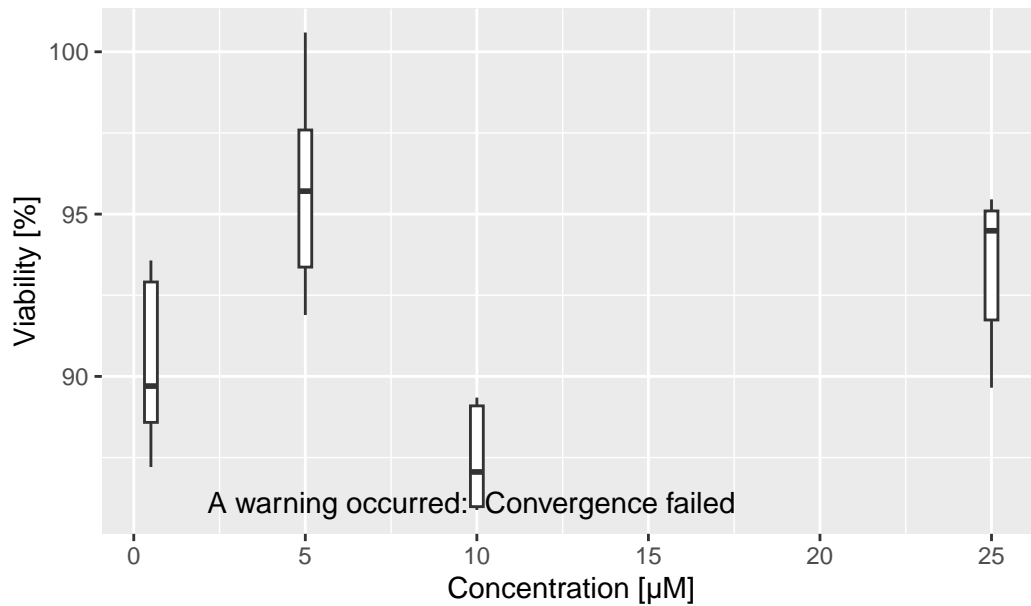
RSE	0.03799898
Response_lowestdose	0.916
Response_highestdose	0.961
pIC50	4.38
p_value	0.122
IC50_relative_lower	−842
IC50_relative_higher	925
IC50_relative	41.7
HillCoefficient	−7.23
asymptote_two	2.789289
asymptote_one	0.9155756

X19310
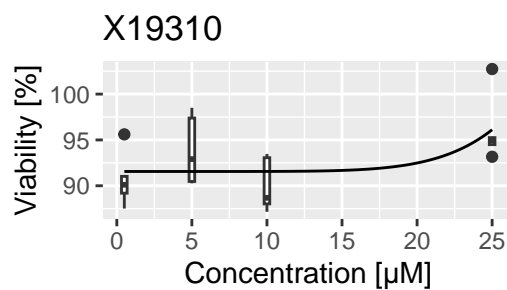
Note: Response Difference lower than 25%   *Confidence intervall not in conc. range*

RSE	0.04992366
Response_lowestdose	0.911
Response_highestdose	1.06
pIC50	5.4
p_value	0.000126
IC50_relative_lower	0.445
IC50_relative_higher	7.54
IC50_relative	3.99
HillCoefficient	−4.92
asymptote_two	1.061785
asymptote_one	0.9109509

Note: Response Difference lower than 25%

X19311
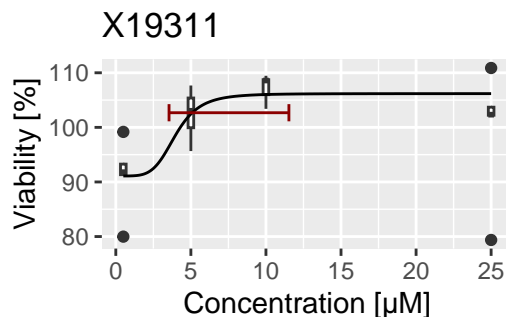
RSE	0.06963272
Response_lowestdose	0.784
Response_highestdose	0.863
pIC50	6.76
p_value	0.152
IC50_relative_lower	
IC50_relative_higher	
IC50_relative	0.172
HillCoefficient	−2.78
asymptote_two	0.8630709
asymptote_one	−0.7482009

Note: Response Difference lower than 25%

X19318
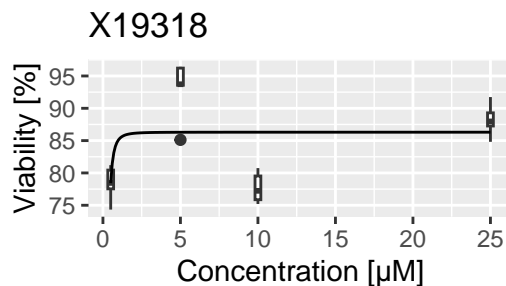
Confidence intervall could not be calculated

RSE	0.0396847
Response_lowestdose	0.845
Response_highestdose	0.859
pIC50	7.12
p_value	0.911
IC50_relative_lower	
IC50_relative_higher	
IC50_relative	0.0752
HillCoefficient	−2.12
asymptote_two	0.8590009
asymptote_one	0.09874114

Note: Response Difference lower than 25%

X19319
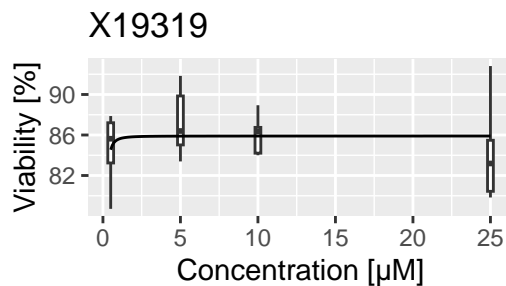
Confidence intervall could not be calculated

RSE	0.05100648
Response_lowestdose	0.83
Response_highestdose	0.916
pIC50	4.14
p_value	0.046
IC50_relative_lower	−440
IC50_relative_higher	586
IC50_relative	72.6
HillCoefficient	−0.946
asymptote_two	1.161983
asymptote_one	0.826958

Note: Response Difference lower than 25%

X19320
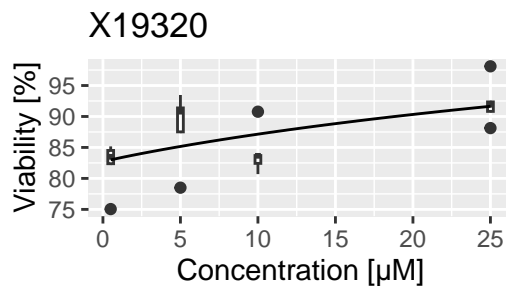
Confidence intervall not in conc. range

RSE	0.03368396
Response_lowestdose	0.903
Response_highestdose	0.984
pIC50	4.4
p_value	0.00101
IC50_relative_lower	
IC50_relative_higher	
IC50_relative	40
HillCoefficient	−2.76
asymptote_two	1.278347
asymptote_one	0.9033188

X19321
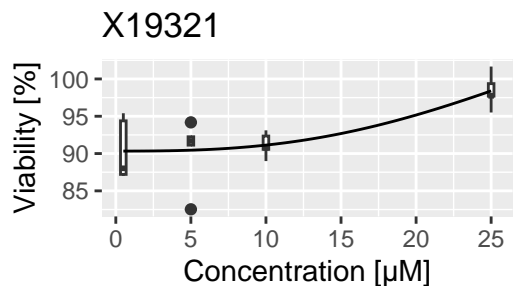
Note: Response Difference lower than 25%   *Confidence intervall could not be calculated*

RSE	0.06003911
Response_lowestdose	0.815
Response_highestdose	0.591
pIC50	4.62
p_value	2.18e−06
IC50_relative_lower	−57.4
IC50_relative_higher	105
IC50_relative	23.8
HillCoefficient	10.6
asymptote_two	0.8154458
asymptote_one	0.4594908

X19324
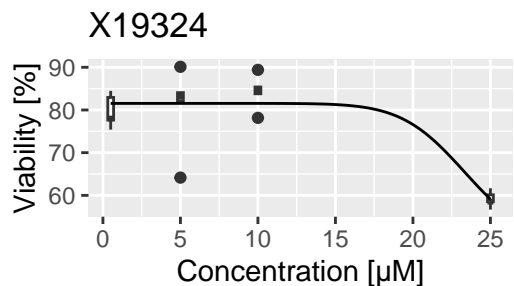
Note: Response Difference lower than 25%   *Confidence intervall not in conc. range*

RSE	0.03457023
Response_lowestdose	0.889
Response_highestdose	0.162
pIC50	4.95
p_value	0
IC50_relative_lower	2.69
IC50_relative_higher	20
IC50_relative	11.3
HillCoefficient	13.4
asymptote_two	0.8890397
asymptote_one	0.161743

X19326
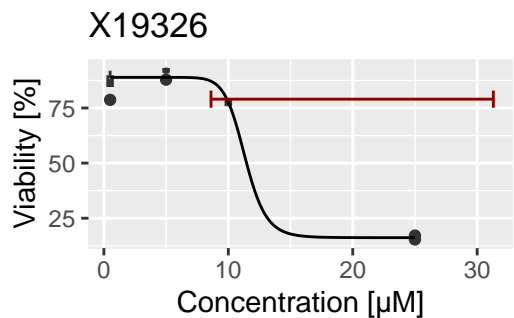
RSE	0.04356657
Response_lowestdose	0.871
Response_highestdose	0.658
pIC50	5.04
p_value	9.46e−09
IC50_relative_lower	−1.73
IC50_relative_higher	19.9
IC50_relative	9.06
HillCoefficient	18
asymptote_two	0.8709772
asymptote_one	0.6584553

X19329

Note: Response Difference lower than 25%
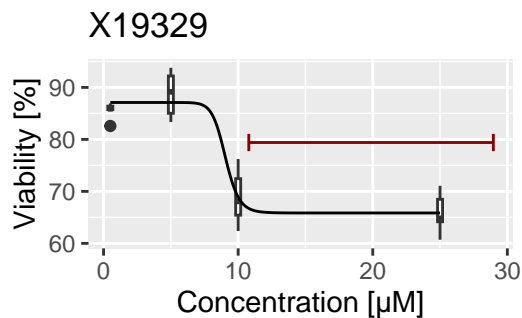
RSE	0.02341382
Response_lowestdose	0.778
Response_highestdose	0.281
pIC50	5.04
p_value	0
IC50_relative_lower	8.51
IC50_relative_higher	9.57
IC50_relative	9.04
HillCoefficient	4.21
asymptote_two	0.7781533
asymptote_one	0.2737777
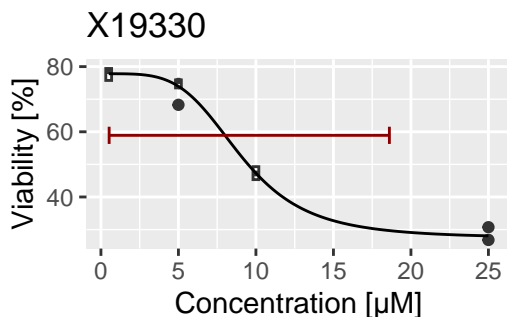
RSE	0.03320819
Response_lowestdose	0.785
Response_highestdose	0.351
pIC50	4.96
p_value	1.44e−15
IC50_relative_lower	6.58
IC50_relative_higher	15.2
IC50_relative	10.9
HillCoefficient	12.8
asymptote_two	0.7848215
asymptote_one	0.3509593
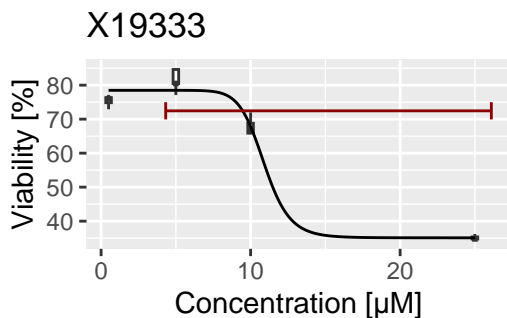
RSE	0.03870952
Response_lowestdose	0.715
Response_highestdose	0.806
pIC50	6.2
p_value	0.000886
IC50_relative_lower	−39.3
IC50_relative_higher	40.5
IC50_relative	0.631
HillCoefficient	−2.73
asymptote_two	0.805956
asymptote_one	0.6670083
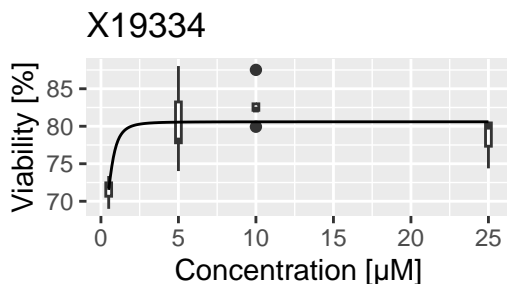
Note: Response Difference lower than 25%   *Confidence intervall not in conc. range*

|	
RSE	0.02634992
Response_lowestdose	0.816
Response_highestdose	0.574
pIC50	4.01
p_value	1.82e−12
IC50_relative_lower	−491
IC50_relative_higher	685
IC50_relative	97.1
HillCoefficient	1.73
asymptote_two	0.8167432
asymptote_one	−1.973702
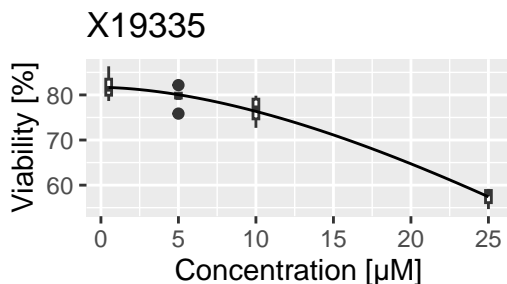
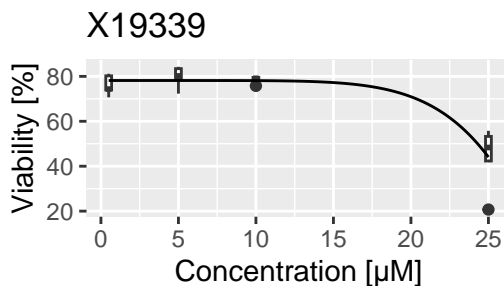*Note: Response Difference lower than 25%*   *Confidence intervall not in conc. range*

RSE | 0.01871547
Response_lowestdose | 0.624
Response_highestdose | 0.46
pIC50 | 5.33
p_value | 2.98e−12
IC50_relative_lower | 3.35
IC50_relative_higher | 6.09
IC50_relative | 4.72
HillCoefficient | 12
asymptote_two | 0.6240549
asymptote_one | 0.4598739

Note: Response Difference lower than 25%

X19338
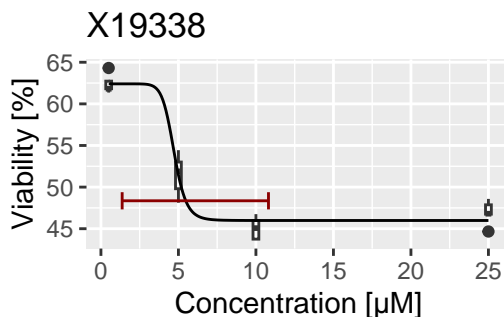
RSE | 0.07931081
Response_lowestdose | 0.782
Response_highestdose | 0.441
pIC50 | 4.45
p_value | 2.6e−07
IC50_relative_lower | −140
IC50_relative_higher | 211
IC50_relative | 35.7
HillCoefficient | 7.22
asymptote_two | 0.7815112
asymptote_one | −3.996934

Note: IC50 larger than highest measured concentration

X19339

Confidence intervall not in conc. range

RSE | 0.03027321
Response_lowestdose | 0.877
Response_highestdose | 0.878
pIC50 | 4.92
p_value | 1
IC50_relative_lower |
IC50_relative_higher |
IC50_relative | 12.1
HillCoefficient | −0.0893
asymptote_two | 0.8848514
asymptote_one | 0.8716144

Note: Response Difference lower than 25%


X19340
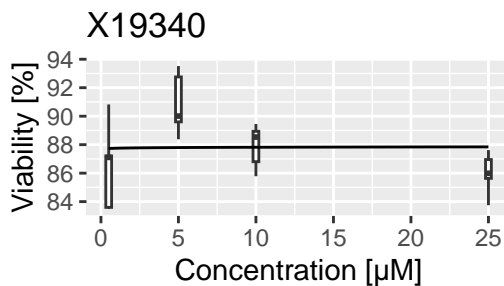
Confidence intervall could not be calculated

RSE | 0.03616457
Response_lowestdose | 0.671
Response_highestdose | 0.754
pIC50 | 6.37
p_value | 0.00113
IC50_relative_lower | −4.05
IC50_relative_higher | 4.89
IC50_relative | 0.422
HillCoefficient | −4.37
asymptote_two | 0.7542343
asymptote_one | 0.4983305

Note: Response Difference lower than 25%

X19341

X19342
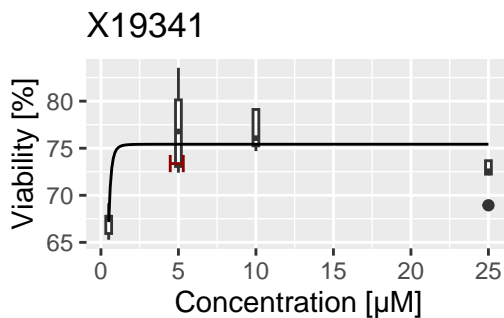
RSE	0.02273579
Response_lowestdose	0.71
Response_highestdose	0.745
pIC50	5.32
p_value	0.0428
IC50_relative_lower	2.37
IC50_relative_higher	7.14
IC50_relative	4.75
HillCoefficient	−9.72
asymptote_two	0.7452793
asymptote_one	0.7099544

Note: Response Difference lower than 25%

X19343
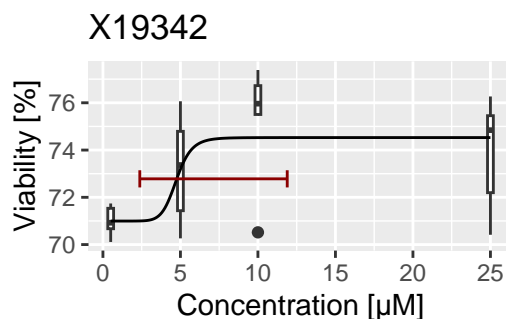
RSE	0.06020797
Response_lowestdose	0.676
Response_highestdose	0.802
pIC50	6.15
p_value	0.00286
IC50_relative_lower	−20.7
IC50_relative_higher	22.1
IC50_relative	0.716
HillCoefficient	−5.44
asymptote_two	0.801594
asymptote_one	0.6583473

Note: Response Difference lower than 25%

X19344
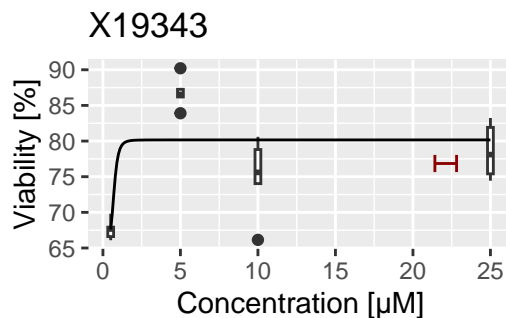
RSE	0.03958419
Response_lowestdose	0.793
Response_highestdose	0.801
pIC50	6.14
p_value	0.987
IC50_relative_lower	
IC50_relative_higher	
IC50_relative	0.721
HillCoefficient	−0.169
asymptote_two	0.8187335
asymptote_one	0.7694794

Note: Response Difference lower than 25%   *Confidence intervall could not be calculated*

X19345
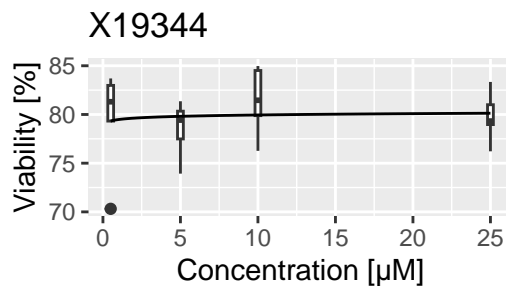
RSE	0.05761183
Response_lowestdose	0.782
Response_highestdose	0.764
pIC50	2.49
p_value	0.953
IC50_relative_lower	
IC50_relative_higher	
IC50_relative	3220
HillCoefficient	0.0172
asymptote_two	1.283614
asymptote_one	0.1998623

Note: Response Difference lower than 25%   *Confidence intervall could not be calculated*

11
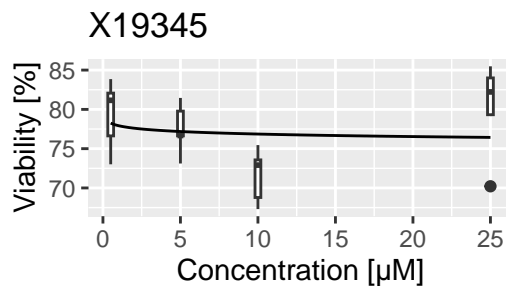
References

Motulsky, Harvey J, and Ronald E Brown. 2006. "Detecting Outliers When Fitting Data with Nonlinear Regression – a New Method Based on Robust Nonlinear Regression and the False Discovery Rate." *BMC Bioinformatics* 7 (1): 123. https://doi.org/10.1186/1471-2105-7-123.

Ritz, C., F. Baty, J. C. Streibig, and D. Gerhard. 2015. "Dose-Response Analysis Using r." *PLOS ONE* 10 (e0146021, 12). http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0146021.

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Wickham, Hadley. 2016. *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org.