SAR: how much change in activity is required for 95% probability of significance?

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Key points

- Efficiency in building SAR can increase speed and decrease cost for the development of new medicines.
- This efficiency requires knowledge of the degree of precision (scatter or variability) in activity measurements.
- Underestimation of precision causes unnecessary loss of information, because some likely genuine changes in activity are treated as noise.
- Conversely, overestimation of precision gives misleading conclusions when noise is treated as a genuine shift.
- Here, it is estimated that at least a 3.6-fold shift in IC_{50} is required for 95% confidence (minimum significant ratio, MSR) that there is a change in activity when comparing reversible inhibitors in the fluorescence dose-response assay for MPro from SARS-Cov-2 in the original assay format.
- This is a fairly typical value for MSR, but it may be changed by any modification of the assay conditions.

The MSR is an estimate of the fold-change in activity required for 95% confidence that there is a genuine change in activity (Eastwood et al, 2006; Haas et al, 2017). A change below the MSR may be significant and a change above the MSR is estimated to be significant at greater than 95% probability. For reversible inhibitors, estimation of the MSR requires independent replicate measurements of IC_{50} made on different days. This is because experimental noise between assays is greater than that within an assay, and building SAR requires comparison of potencies measured on different days.

Fluoresence dose-response data for MPro were generated by Haim Barr and Nir London (Weizmann Institute). The MSR was estimated from 9 replicate data sets for the reversible quinolone inhibitor CVD2707 (see Table 1 & Figure 2).

Figure 1. Structure of CVD2707 (MAT-POS-916a2c5a-2).

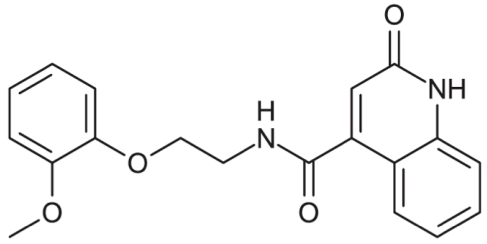
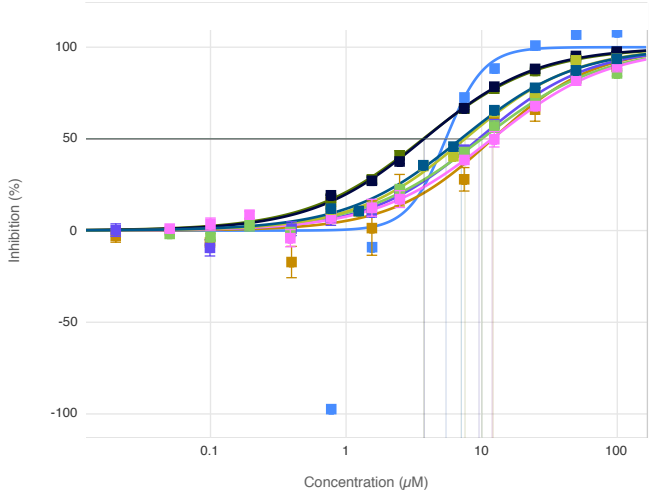


Table 1. Reported IC_{50} values for CVD2707 in the MPro fluorescence assay. Data were extracted from the CDD (Collaborative Drug Discovery) and Post Era databases. The Post Era value is a geometric mean. It is not stated what range is covered by the CIs (normally 95%), nor what is meant by the ± figure (it could be SE or SD, but these usually cover a fold range, not an arithmetic range). The structure of CVD2707 is shown.

Date	IC_{50} (CIs) (μM)
12/07/20	5.44 (1.06-27.9)
05/07/20	12.1 (8.80 -16.5)
25/06/20	9.48 (8.2-10.9)
21/06/20	9.99 (8.95-11.2)
17/06/20	11.9 (10.3-13.7)
09/06/20	3.70 (3.49-3.93)
03/06/20	3.76 (3.54-3.99)
10/05/20	7.52 (6.73-8.41)
06/05/20	7.02 (6.57-7.51)
Post Era database value (17/07/20)	7.23 ± 1.57

Figure 2. Dose-response curves. These data were used to calculate the IC_{50}s shown in Table 1.



When building SAR, activity measurements are compared between compounds, which often have been characterised on different days. There is noise in each of the values, so only changes in excess of this noise are considered likely to be significant. CIs cannot be used to determine whether differences in IC_{50} are likely to be genuine, because they reflect only the uncertainty within, not between, datasets. Repeating the experiment can give a different IC_{50}, with CIs that do not overlap (see Table 1).

If there are replicate experiments, then the geometric (not arithmetic) mean should be taken, because measurements of IC_{50} are correct to within a multiple. Replicates, however, are not usually available. The arithmetic mean pIC50 is in agreement with geometric mean IC_{50}.

A widely used approach is to estimate the minimum fold-change in activity which is likely to be significant at the 95% confidence interval, known as the minimum significant ratio or MSR (Eastwood et al, 2006; Haas et al, 2017). The scatter of results is measured in a minimum of 6 replicate assays for a reference compound. It is assumed that the variation for the test compound (which may be assayed only once) is similar to that for the reference compound. The formula for MSR is

$$MSR \approx 10^{2SD\sqrt{2}} \qquad (1)$$

SD is used rather than SE, because SD is a measure of the variability of the observed values, whereas SE is an estimate of how far the sample value is likely to be from the true population value (Altman & Bland, 2005). In dose-response studies, precision in estimated IC_{50} is to within a multiple, not an arithmetic number. The figure for SD accordingly should be calculated using pIC50 values, not IC_{50}s. The data in Table 1 give

pIC50 = 5.1
IC_{50} = 7.2 μM
Sample SD σ_{N-1} = 0.20
SE = $\sigma_{N-1}/\sqrt{N}$ = 0.066
MSR = 3.6
95% CIs = 5.4 to 9.8 μM (1.9-fold)

In calculating the MSR, it is assumed that student's *t*-value is 2.0, which corresponds to 60 degrees of freedom. This is reasonable for 6 replicate measurements. Further increasing the replicates could give a minimum value of t = 1.96, so has little effect on the estimated MSR. For this comparison, the same value of t = 2 was used to estimate the 95% CIs.

The reported IC_{50} values for CVD2707 cover a 3.3-fold range between 3.70-12.1 μM and the CIs do not overlap (Table 1). This range is slightly less than the calculated MSR = 3.6. This value is typical for assays on reversible inhibitors. The MSR may be higher for irreversible inhibitors in the same assay, because of the additional variable, with k_{inact} and K_i replacing IC_{50}.

Any change to the format of the fluorescence assay may modify the MSR.

Acknowedgements. I thank Lizbe Koekemoer (University of Oxford) for access to the CDD and to Anna Linusson (University of Umeå) for introducing me to MSR.

Walter Ward 27 July 2020

Walter Ward accepts no liability. Users are responsible for direct & indirect consequences of using this material

References

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