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The mathematics behind quantifying the response of different chemical compounds competing with an agonist for the same biological binding site are well understood, but the process can be repetitive and tedious. Learning full use of new scientific software will give you the time to put your talents to more creative use.

Simplifying Pharmacologic Analysis with Programmable Scientific Software

Popular scientific software tools can remove the tedium from repetitive calculation tasks and free researchers to explore. Furthermore, such commercially available software can be programmed easily, allowing researchers to develop time-saving and innovative analysis routines, tailored to their own methodologies. However, in some sectors, these tools are not always used fully. For example, in the pharmaceutical industry it is commonplace to fit experimental dose-response data and then calculate resultant parameters to characterize drugs. In typical experiments, the time needed to fit each curve and to manually calculate the end results can consume hours. In the past, this was a laborious task even with sophisticated data analysis software because the software had to be applied manually to each data set. Leveraging the programmability of standard software designed for scientific research can significantly reduce the time required to carry out preclinical dose-response analyses.

Dose-Response Basics

Dose-response curves are used to illustrate biological response from differing stimulus doses. The response is measured at several increasing stimulus concentrations. Responses will either increase or decrease from initial values. Once an inflection point is reached, responses start to level off, approaching a plateau. The response curve is sigmoidal, or a slanted S-shape.

Several equations are used to fit sigmoidal data. The logistic equation, which has four parameters, is typically used to define best-fit curves. The EC_{50} parameter, which represents the effective concentration at 50% of the response range, is key to that equation. The EC_{50} can be used to compare the amounts of stimulus required to achieve the same response when competing compounds are introduced. Commercial scientific graphing and analysis software simplifies the task of fitting data to the four-

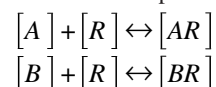
parameter logistic equation. The underlying science and mathematics of analyzing dose response curves are well known and easily applied. A particularly excellent and popular reference (and the foundation of this article) is *Pharmacologic Analysis of Drug-Receptor Interaction* by Terry Kenakin (1).

Compound concentrations are typically small, so many researchers prefer to find common logarithms of the independent values before plotting. Because of that, the center position actually corresponds to the logarithm of the EC_{50} , denoted as $\log(EC_{50})$. The EC_{50} can easily be calculated from the $\log(EC_{50})$ by raising it to the 10th power (Figure 1).

The relationship to drugs. Drugs can cause a biological change either directly or indirectly. A drug that binds to a tissue's receptor and produces stimulation is called an *agonist*, whereas a drug that blocks the effect of an agonist is called an *antagonist*. The EC_{50} parameter helps to quantify the relative effect when a different chemical compound competes with an agonist for the same biological binding sites (*receptors*). Many important drugs are antagonists, such as beta-blockers (which inhibit the effects of chemicals released during stress).

Pharmacologic Theory

To measure the affinity of an antagonist to a receptor, a series of dose-response measurements need to be taken. When agonists and antagonists bind reversibly to the same sites on the receptor, the condition is called *competitive reversible antagonism* (1). The equilibrium state in which agonist A and antagonist B form receptor complexes and disassociate can be represented as



in which AR and BR represent agonist-receptor and antagonist-receptor complexes, and brackets denote concentration values.

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When the dose-response curve measures the response of the agonist alone (so the antagonist concentration is zero), then the equilibrium diagram above can be rewritten

$$(1) \text{ as } \frac{[AR]}{[R_t]} = \frac{[A]}{[A] + K_A}$$

where $[R_t]$ is the total concentration of the free receptors plus the complexes, and K_A is the equilibrium disassociation constant for the agonist-receptor complex. After an antagonist is added to the experiment, the two equilibria representations combine to equal

$$\frac{[AR]}{[R_t]} = \frac{[A']}{[A'] + K_A \left(\frac{1 + [B]}{K_B} \right)},$$

which takes into account the increased concentration of agonist A' required to achieve the same biological response in the presence of an inhibitor with a concentration $[B]$ and an antagonist-receptor disassociation constant K_B . Essentially, this equation shows how much more agonist is needed to achieve the same occupancy, yielding an equal response.

Setting Up the Experiment

For competitive antagonism experiments, researchers measure dose-response data on a particular agonist to establish a control curve. Then researchers measure the dose-response data for several concentrations of the antagonist. Typically, dose-response curves are generated using three to five different antagonist concentrations. The dose-response data measured when the drug is in the presence of the antagonist will be shifted markedly to the right on the dose scale. A ratio of their positions can be calculated to quantify the shift by fitting the two curves and establishing their $\log(EC_{50})$ values.

The resulting curve shift, called the *concentration ratio* or historically the *dose ratio* (DR), is dependent on two factors: the affinity (attraction) of the antagonist for the receptor and the concentration of the antagonist used in the experiment. The DR can be expressed $\frac{[A']}{[A]} = \frac{[B]}{K_B} + 1$.

Because the DR can be calculated quite easily once the data sets for the agonist by itself and the agonist in the presence of an antagonist are both fit to the logistic equation, the disassociation constant K_B can

be calculated. K_B later serves as the *molecular fingerprint* for the antagonist (1).

Using Scientific Software

Modern data analysis and technical graphics packages allow pharmaceutical researchers to carry out nonlinear curve fitting quite easily (2). Built-in tools can provide researchers with a simple interface to control all facets of sigmoidal curve-fitting using the Levenberg–Marquardt algorithm. Some software can automatically carry out the nonlinear curve-fitting on several data sets with a click of a button. Such simplified interfaces allow researchers to acquire all the parameters for a large experiment quickly.

However, the average researcher still has work to do. Depending on how you denote concentration data, the DR value is determined by either dividing or subtracting the centers of the sigmoidal curves. When the graph's abscissa values (the x coordinate on an x,y graph) are the agonist concentration, then the DR is calculated by dividing the EC_{50} of the curve representing the agonist response in the presence of the antagonist by the EC_{50} of the control curve. When the graph's abscissa values are the log of the agonist concentration, then the $\log(DR)$ is calculated by subtracting the $\log(EC_{50})$ values. Such procedures can be scripted into many scientific software programs. This is the first step in simplifying the analysis.

Figure 2 shows that when the antagonist is introduced, more agonist is required to overcome its presence and achieve the same response. If a higher concentration of antagonist is used, a new dose-response curve would result, shifted even farther to the right. Clearly, DR is a function of antagonist concentration.

Analyzing Further

Arunlakshana and Schild were first to devise a mathematical model of competitive antagonism that uses the concept of dose ratios to provide the characteristic molecular parameter for the antagonist K_B (3). The relationship between the dose-ratio, antagonist concentration, and K_B is represented by the Schild equation (4):

$$\log(DR - 1) = \log[B] - \log(K_B).$$

By performing linear regression on data from three to five dose-response measurements at increasing antagonist

concentrations, you can obtain the antagonist affinity K_B , because the y-intercept is $-\log(K_B)$. Commonly, the notation of pK_B is used to denote the value of $-\log(K_B)$. A plot of such data with its linear regression is called a *Schild plot*, which pharmacological researchers use as the time-honored method for drug and receptor classifications.

The work that goes into creating a Schild plot is fairly straightforward, although it can still be quite tedious if done manually. To create the plot requires fitting dose-response data using several antagonist studies, each involving an increasing concentration of antagonist. The dose ratios must be calculated and tabulated. Later, a new table is created with Y values being set equal to

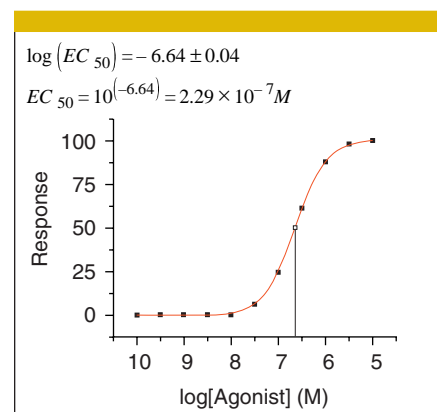


Figure 1. A four-parameter fit to dose-response; the EC_{50} parameter is marked with a drop-line symbol.

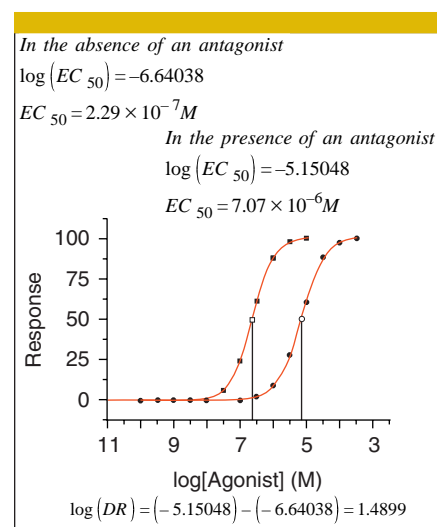


Figure 2. The response of the agonist alone (left curve) and the agonist in the presence of an antagonist (right curve); the dose-ratio can be computed as a function of their EC_{50} values.

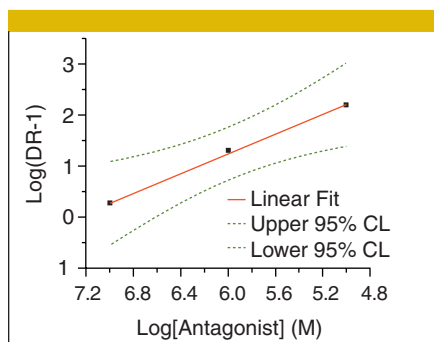


Figure 3. Schild plot analysis of dose-ratio data. At the 0.95% confidence level, the slope is not significantly different than 1. When the slope equals 1, then $[pA_2] = 7.24267 \pm 0.02867$, and the 95% confidence interval is $[pK_B] = 7.11932-7.36601$.

DR - 1, whereas X values are obtained by common logs of the antagonist concentrations.

Once the data are tabulated, a linear regression is performed. Then a statistical check must be done to determine whether the slope is significantly different from unity. Typically, if the unit slope is within the 95% confidence bands of the slope, then the experiment is consistent with the Schild competitive antagonism model. The slope can be forced to be unity, and then the y-intercept is recomputed. At that point, the value of pK_B can be read directly as the y-intercept, or the equilibrium disassociation constant can be explicitly determined by using $K_B = 10^{-1(y - intercept)}$ (Figure 3).

Leveraging the Software

Although the calculations are simple and straightforward, it is easy to see how a researcher's time can be quickly consumed when using standard analysis tools to fit all curves of an experiment consisting of about 50 traces, performing the requisite calculations, and logging the results. Researchers often underuse their software tools by using them manually.

As an example, the Origin software package uses a built-in programming language, designed for automating scientific work. The scientific software can be programmed using high-level commands to import data, create a graph, and perform analyses. Such program scripts can then be saved and reused daily. If you are performing a logistic fit for all the plotted data, only a few lines of script are needed.

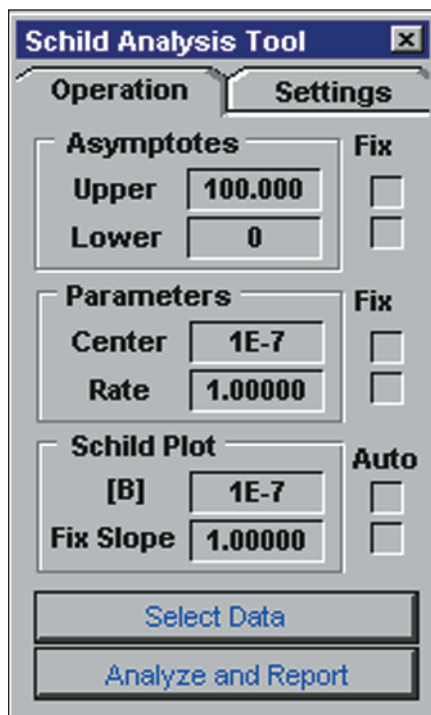


Figure 4. User-created add-on tool for automating dose response fittings and Schild plot analyses (built with OriginPro's Dialog Builder, inspired by the program's sigmoidal fitting tool).

To tabulate the results and perform a linear regression adds only a few more lines. Work can be programmed quickly because the language is specially designed for high-level instructions.

Another interesting aspect of the most recent scientific software applications is that they allow researchers to create their own interactive interfaces without having to use the more difficult Windows graphical user interface (GUI). For performing Schild analyses, some users have created their own utilities with personalized options. A custom Schild analysis tool developed using Origin Professional in Figure 4 is an example of how users can personalize their options.

Innovating Research

In addition to handling complex data manipulation based on common procedures, scientific software makes it possible to incorporate updated techniques into your personal tool set. For example, the mathematical treatment we just explored is useful only if the maximal biological response with the antagonist present is comparable to that without it. When it is not present, such as in noncompetitive

antagonism, then different techniques should be used, such as the double reciprocal analysis (1), which requires a user to find many equireactive agonist doses and then perform a linear regression on the reciprocals. Users can calculate the antagonist-receptor disassociation constant K_B based on the slope of the line in the Schild plot, similar to using the dose ratio. Such analysis techniques are easily programmed into scientific graphics and data analysis software.

Instead of using the older Schild plot method for competitive antagonism studies, scientific software can be further exploited to use multivariate nonlinear curve fitting. With only one curve fitting session the pK_B result and the corresponding statistics can be determined. User-defined fitting functions allow operators to fit data for different types of inhibition, to use an F-test or other criteria to select the most appropriate inhibition model, and to automate the process of making model decisions. Secondary plots, in which one variable is kept constant, can be generated to help illustrate the results obtained from multivariate curve fitting.

Preclinical researchers can use programmable scientific software to create tools incorporating the latest methodology and customizing it for their particular needs. Learning to create useful tools is roughly equivalent to yesterday's learning to program a good scientific calculator. However, modern scientific software couples the power of custom user interfaces with point-and-click ease, performing scientific data analyses with publication-quality graphs. Once customized, the utilities can be distributed among other researchers, then further modified to compute other parameters to investigate emerging ideas. The time saved in learning to use such software tools to their fullest extent should be of enormous value to all pharmaceutical researchers.

References

- (1) T. Kenakin, *Pharmacologic Analysis of Drug-Receptor Interaction* (Raven Press, NY, 1993).
- (2) For instance, Origin 6.1 (OriginLab Corporations, www.originlab.com).
- (3) O. Arunlakshana and H.O. Schild, "Some Quantitative Uses of Drug Antagonists," *Brit. J. Pharmacol.* 14, 48-58 (1959).
- (4) H.O. Schild, "pA₂: A New Scale for the Measurement of Drug Antagonism," *Brit. J. Pharmacol.* 2, 189-206 (1947). **BP**