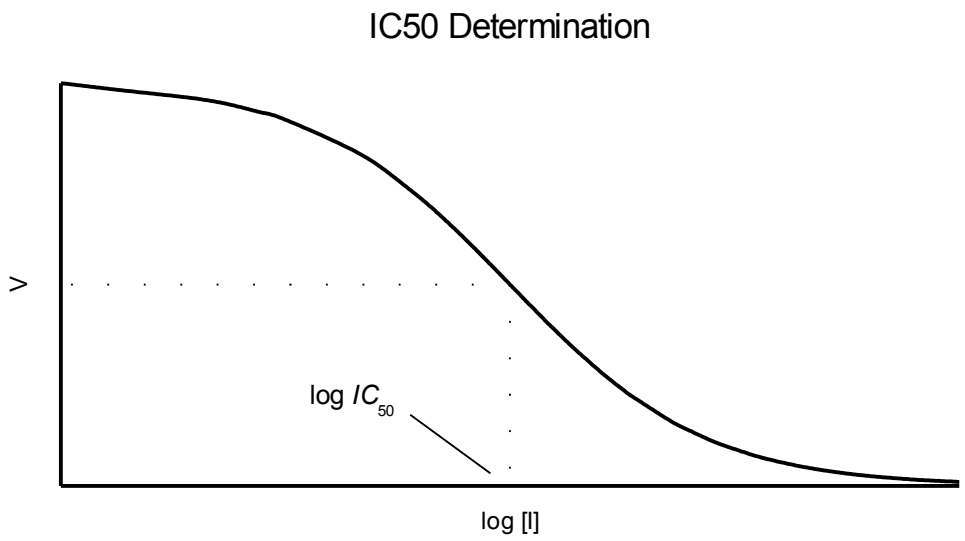
DavidsonX – D001x – Medicinal Chemistry
Chapter 4 – Enzymes
Part 3 – Measuring Inhibition
Video Clip – IC_{50} and K_i

Understanding how to distinguish types of inhibitors is important, but much of the drug discovery process focuses instead upon determining whether one inhibitor is more effective than another. This determination requires quantification of an inhibitor's potency. The two most common values for quantifying an inhibitor's effect are ***IC_{50}*** and ***K_i***. While these values can be determined for different types of inhibitors, we will be discussing specifically reversible competitive inhibitors.

IC_{50} is the concentration of an inhibitor required to reduce the rate of an enzymatic reaction by 50%. IC_{50} values are determined through a series of experiments. For all experiments, a high, constant concentration of substrate is present so that the enzyme can react at an appreciable rate. In each experiment, the amount of inhibitor (log [I]) is steadily increased, and the observed rate of the reaction (V) decreases accordingly. The various experiments are plotted at V vs. log [I] to generate a sigmoidal curve. The point of inflection of this curve corresponds to the logarithm of the inhibitor concentration that decreases V by 50%.



K_i is the dissociation equilibrium constant of the enzyme-inhibitor complex (E-I).

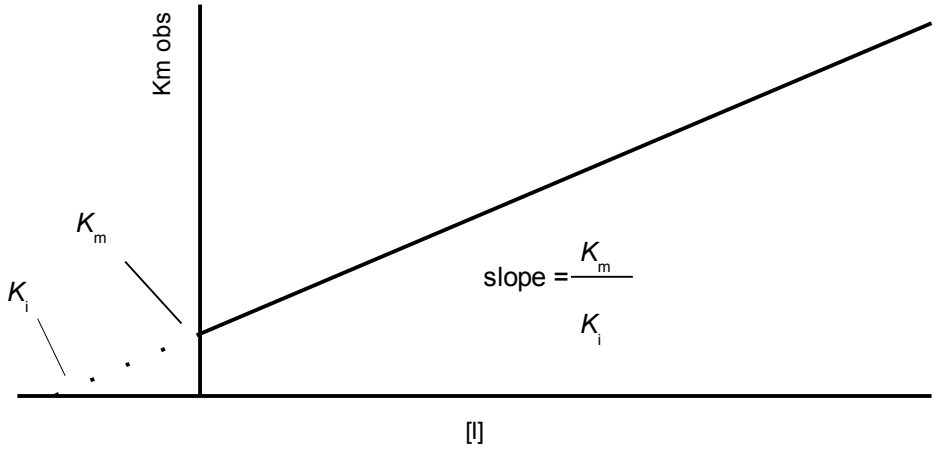
$$E\text{-}I \overset{K_i}{\rightleftharpoons} E + I$$

$$K_i = \frac{[E]\,[I]}{[E\text{-}I]}$$

K_i values are determined through a series of experiments with varying amounts of inhibitor present. Each experiment allows a K_m (K_m^{obs}) for that particular concentration of inhibitor ([I]). If it seems odd to think that K_m (the Michaelis *constant*) can vary, remember that in the presence of a competitive inhibitor, the affinity of an enzyme for a substrate decreases. That is to say, K_m increases. Plotting K_m^{obs} values against [I] generates a line with a slope of K_m/K_i and a y-intercept of K_m.

$$K_m^{obs} = \frac{K_m}{K_i}[I] + K_m$$

Ki Determination



[Note that the K_i determination plot very closely resembles Lineweaver-Burk plot in terms of its general shape and the intercepts. Be careful to remember that these are completely different relationships.]

While IC_{50} and K_i are both measures of an inhibitor's ability to block the action of an enzyme, they are not equivalent. K_i values, which are a true equilibrium constant, are considered a more pure measure since the K_i of an enzyme-inhibitor complex is a constant. IC_{50} values, in contrast, can vary since they depend on the substrate concentration used in the IC_{50} determination. Comparing IC_{50} values of the same inhibitor between different research laboratories can be problematic. Fortunately, the **Cheng-Prussoff equation** allows conversion of IC_{50} values to a more universal K_i for direct comparison of data. One needs to know the K_m of the uninhibited enzyme for its substrate, the IC_{50} value of the inhibitor, and the substrate concentration at which the IC_{50} value was determined.

$$K_i = \frac{IC_{50}}{\left(1 + \frac{[S]}{K_m}\right)}$$