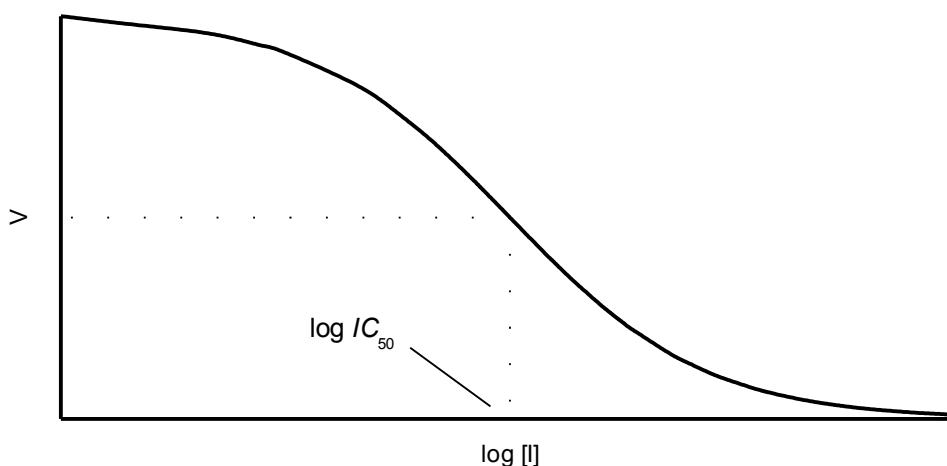


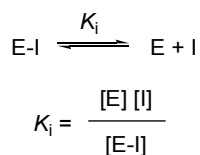
Understanding how to distinguish types of inhibitors is important, but much of the drug discovery process focuses instead upon determining whether one inhibitor is more effective than another. This determination requires quantification of an inhibitor's potency. The two most common values for quantifying 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 as  $V$  vs.  $\log [I]$  to generate a sigmoidal curve. The point of inflection of this curve occurs at the logarithm of the inhibitor concentration that decreases  $V$  by 50%, or  $log IC_{50}$ .

$IC_{50}$  Determination



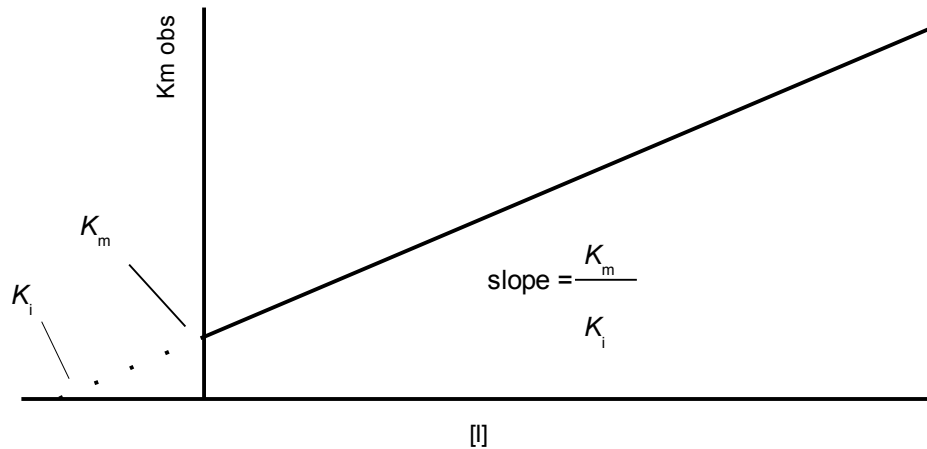
$K_i$  is the dissociation equilibrium constant of the enzyme-inhibitor complex (E-I).



$K_i$  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 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 true equilibrium constants, are considered a more pure measure of inhibition since the  $K_i$  of an enzyme-inhibitor complex is indeed 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 the 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.

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