IC\textsubscript{50}-to-\(K_i\): a web-based tool for converting IC\textsubscript{50} to \(K_i\) values for inhibitors of enzyme activity and ligand binding

R. Z. Cer\textsuperscript{1}, U. Mudunuri\textsuperscript{1}, R. Stephens\textsuperscript{1} and F. J. Lebeda\textsuperscript{2,*}

\textsuperscript{1}Advanced Biomedical Computing Center, Advanced Technology Program, SAIC-Frederick Inc., NCI-Frederick, Frederick, MD 21702, USA and \textsuperscript{2}US Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD 21702-5011, USA

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ABSTRACT

A new web-server tool estimates \(K_i\) values from experimentally determined IC\textsubscript{50} values for inhibitors of enzymes and of binding reactions between macromolecules (e.g. proteins, polynucleic acids) and ligands. This converter was developed to enable end users to help gauge the quality of the underlying assumptions used in these calculations which depend on the type of mechanism of inhibitor action and the concentrations of the interacting molecular species. Additional calculations are performed for nonclassical, tightly bound inhibitors of enzyme-substrate or of macromolecule-ligand systems in which free, rather than total concentrations of the reacting species are required. Required user-defined input values include the total enzyme (or another target molecule) and substrate (or ligand) concentrations, the \(K_m\) of the enzyme-substrate (or the \(K_d\) of the target-ligand) reaction, and the IC\textsubscript{50} value. Assumptions and caveats for these calculations are discussed along with examples taken from the literature. The host database for this converter contains kinetic constants and other data for inhibitors of the proteolytic clostridial neurotoxins (http://botdb.abcc.ncifcrf.gov/toxin/kiConverter.jsp).

INTRODUCTION

Some analyses of networks, pathways and metagenomics focus on identifying key proteins or polynucleic acids as targets for inhibitory compounds. Typically, high-throughput screening assays are initially used to compare and down-select potential inhibitors of enzymatic activity or macromolecule-ligand binding. Many functional assays seek a total inhibitor concentration that reduces these activities by 50\% (IC\textsubscript{50}). However, the IC\textsubscript{50} value depends on concentrations of the enzyme (or target molecule), the inhibitor, and the substrate (or ligand) along with other experimental conditions. What is required is an accurate determination of the \(K_i\) value, an intrinsic, thermodynamic quantity that is independent of the substrate (ligand) but depends on the enzyme (target) and inhibitor. Thus, comparisons can be more readily made among different laboratories to characterize the inhibitors. While these more time-consuming assays are usually done with the most promising candidates, accurate, initial estimates of \(K_i\) values for more of the candidates would be beneficial.

A much discussed problem in the literature (1–8) is converting IC\textsubscript{50} to \(K_i\) values because even the simplest types of inhibitory mechanisms (e.g. competitive, uncompetitive and noncompetitive) will influence the calculation.

To help address this problem, our web-server tool calculates \(K_i\) values from IC\textsubscript{50} values using equations for enzyme-substrate and target-ligand interactions by different inhibitory mechanisms (http://botdb.abcc.ncifcrf.gov/toxin/kiConverter.jsp). Additional calculations are performed for tightly bound inhibitors of enzyme-substrate reactions in which free, rather than total, concentrations of the molecular species are calculated for nonclassic Michaelis–Menten kinetics. Similar calculations can be performed for target molecule-ligand systems. User-defined input values include total concentrations of the enzyme (or target molecule) and substrate (or ligand), the \(K_m\) of the enzyme-substrate (or the \(K_d\) of the target-ligand) reaction and the IC\textsubscript{50} value. The outputs include tabulations of the \(K_i\) values under different kinetic schemes, extensive tabulations of the results, summary histograms and the corresponding equations. Help buttons are available for Background, Assumptions, Literature, Links and Equations along with examples taken from the host database-server that contains kinetic information on neurotoxin inhibitors. An example calculation is included here for a tight-binding inhibitor of an enzyme–substrate reaction, while other enzyme inhibitor and protein–ligand–inhibitor examples are also provided.

*To whom correspondence should be addressed. Tel: +1 301 619 4279; Fax: +1 301 619 2348; Email: frank.lebeda@amedd.army.mil

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METHODS

Reactions and equations

The website cited in (9) served as an initial design template for our IC₅₀-to-Kᵢ converter. Equations (1–4) were adapted from refs. (3), (6) and (9) whereas we derived Equation (5) for this study. The analytic expressions for Kᵢ that are shown below were verified numerically by methods used in a previous kinetic analysis (10).

The derivations for converting IC₅₀ to Kᵢ values published by Brandt et al. (3) include three types of classic inhibitor mechanisms in which different relations may exist between inhibitor mechanisms in which different relations may exist between the inhibition and for tightly bound inhibitors (5,6).

For competitive inhibition

\[
K_i = \frac{(IC_{50} - E/2)}{(K_m/S + 1)} \tag{2a}
\]

For noncompetitive inhibition (2)

\[
K_i = IC_{50} \quad \text{when } S = K_m \text{ or } S >> K_m \text{ or } S << K_m \tag{3a}
\]

and for tightly bound inhibitors (5,6)

\[
K_i = IC_{50} - E/2 \tag{3b}
\]

This noncompetitive reaction also assumes that the inhibitor dissociation constants are equal: Kᵢₐ = Kᵢₖ, Kᵢₐₜ, Kᵢₖₜ, and Kᵢₜₜ. Mixed inhibition, where Kᵢₐ ≈ Kᵢₖ, is not considered here.

P–L–I reactions

For total concentrations, E is replaced by P and S is replaced by L. Additional reaction schemes are located at this tool’s website. As in classic enzyme–substrate systems the relation of Kᵢ and IC₅₀ in competitive inhibition is:

\[
K_i = \frac{IC_{50}}{(L/K_d + 1)} \tag{4a}
\]

For protein–ligand experiments with tight-binding inhibitors, the free rather than the total concentrations of the reactants need to be used as modified from ref. 9

\[
K_i = \frac{I_{50}}{K_d + P_0/L_{50}} + 1 \tag{4b}
\]

where I₅₀ and L₅₀ are the free concentrations of the inhibitor and ligand, respectively, at 50% inhibition, and P₀ is the free concentration of the protein in the absence of inhibitor. The concentration of the free inhibitor species is given by

\[
I_{50} = IC_{50} - P_0 + PL_{50}(1 + \frac{K_d}{L_{50}}) \tag{4c}
\]

where P₀ = −((Kₜ + L – P) + [(Kₜ + L – P)² + 4PKₜ]¹/²)/2, PL₀ = P – P₀, PL₅₀ = PL₄₀/2, L₀ = L – PL₀ and L₅₀ = L – PL₅₀.
For this study, we derived a corresponding value of $K_i$ for uncompetitive inhibition

$$K_i = \frac{I_{S0} - L_{S0}}{P - P_{0} - L_{S0} + 1} \quad (5)$$

in which the variables are the same as in Equation (4) except that $L_{S0} = -((P-L) + (P-L)^2 + 4(PL_0K_i/2)^{1/2})/2$. Although in this study we use the term $K_i$ to quantify an antagonist’s effect, the pharmacology-derived EC$_{50}$ value is more appropriate when functional experiments are performed (11).

General assumptions and caveats

It is assumed that all of the substrate- and inhibitor-binding reactions are reversible and that they all have a one-to-one stoichiometry, i.e. no multiple binding of inhibitor molecules or any form of cooperativity, or other complex mechanisms of inhibition such as partial or mixed types (3). It is also assumed that in the enzymatic reactions enzyme autocleavage did not occur and that when substrates for fluorescence resonance energy transfer were used, appropriate corrections for inner filter effects were performed. Comparison of $K_m$ or IC$_{50}$ values for a set of inhibitor candidates is only assumed to be valid when they are evaluated under identical experimental conditions. In most experimental studies of enzyme kinetics, the total concentrations of substrate and inhibitor used are in excess of the enzyme concentration to make their free and total concentrations essentially the same (1). Under the conditions of some ligand-receptor (e.g. protein)-binding studies, the free concentrations also become sufficiently important to require modifications of these equations (1, 2), and (9).

Description of the web server

The IC$_{50}$-to-$K_i$ tool is implemented as a web resource using an Oracle database (Oracle9i Enterprise Edition Release 9.2.0.4.0), Java (JDK 1.5.0) and Apache web server components including Tomcat 4.1. Information on candidate inhibitors of the botulinum neurotoxins was collected by mining the biomedical literature including searches with botXminer (12) using the National Library of Medicine’s MEDLINE®/PubMed® (13). Experimental data (IC$_{50}$ values) and accompanying assay information were manually extracted from primary literature results and other relevant databases: JCVI-Pathema-Clostridium (13), Brenda (14) and Protein Data Bank (15).

 USAGE

An internal link to the user-accessible converter is also located on the left side of the BotDB home page. The four required inputs for $E$, $S$, $K_m$ and IC$_{50}$ are indicated with default settings for several examples. After submitting these values by using the ‘calculate’ button, these input data are returned along with the $K_i$ results for the example cases.

An illustration is provided for a tight-binding inhibitor of an enzyme–substrate (E–S) reaction (Figure 1). The values for this example are from data using cimoxatone, a tight-binding inhibitor of monoamine oxidase (16). The four inputs for $E$, $S$, $K_m$ and IC$_{50}$ are 0.021, 100, 108 and 0.017, respectively, in micromolar units. The $K_i$ results for three modes of inhibition are returned on a new page. The top block of results corresponds to the solutions for a classic inhibitor (i.e. Michaelis–Menten kinetics). The second block represents the corrections made to the first set of equations [Equations (1b–3b)] for tightly bound inhibitors when there is substantial inhibitor depletion (5,6). Equations can be viewed by clicking on a label for a mode of inhibition. Below these two tables, histograms plotting the six results are shown for a visual comparison. In this example, the results from the classic and corrected equations are quite different. This difference in $K_i$ values enables the user to conclude that not all of the assumptions underlying classic Michaelis–Menten equations are being obeyed and that the data are consistent with the kinetics of a tight-binding inhibitor.

Two other examples of enzyme inhibitors are also available for users to examine at the IC$_{50}$-to-$K_i$ tool website. For classic inhibition, data values using a candidate inhibitor of botulinum neurotoxin type A (17) are used as inputs: $E$, $S$, $K_m$ and IC$_{50}$ (in micromolar units) 0.0067, 300, 1300 and 3.2, respectively. In contrast to the tight-binding inhibitor example, the returned values for $K_i$ are similar for classic and tight-binding kinetics indicating that this data set is consistent with classical kinetics.

In another example of a potentially cooperative inhibitor of CYP3A4 (18), the input data for $E$, $S$, $K_m$ and IC$_{50}$, in micromolar units, are 0.1, 50, 51 and 0.05, respectively. This example returns an error message from the converter that states that the 1:1 stoichiometry assumption may have been violated and requests the user to enter different values.

Finally, Equations (4) and (5) are used to calculate $K_i$ values for reactions involving inhibitors of P–L–binding reactions. For this case, a user interface similar to the enzyme–substrate page is produced (see website). The values for a tight-binding inhibitor of an apoptosis-related protein from ref. 9 are used as an example calculation. The inputs are labeled $P$, $L$, $K_d$ and IC$_{50}$. In this case, only the competitive and uncompetitive modes of inhibition are considered. The tabulated output includes the free concentrations of protein and ligand species in the absence of an inhibitor ($P_0$ and $L_0$, respectively). The free concentrations at 50% inhibition are also returned for the protein, ligand, inhibitor, protein–ligand complex and P–L–I complex ($P_{50}$, $L_{50}$, etc.). As with the tight-binding enzyme inhibitor calculations, the summary histograms again indicate that these data are consistent with the kinetics of a tight-binding inhibitor.

It is our intent for this general tool to provide results for classic and tight-binding inhibitors of enzyme activity and ligand-binding reactions that are assumed to follow relatively simple kinetic schemes. These different sets of kinetic results will allow investigators to decide whether additional experiments are required to understand better
the kinetic behaviors of their candidate inhibitors for further research or therapeutic product development.

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