An assay platform for mechanistic validation of small molecule inhibitors during lead compound identification and optimisation

evotec

Florian Krieger§, Annette Deul§, Osamu Ichihara†, Joachim Kraemer§, Christina Schmidt§ and Thomas Hesterkamp§

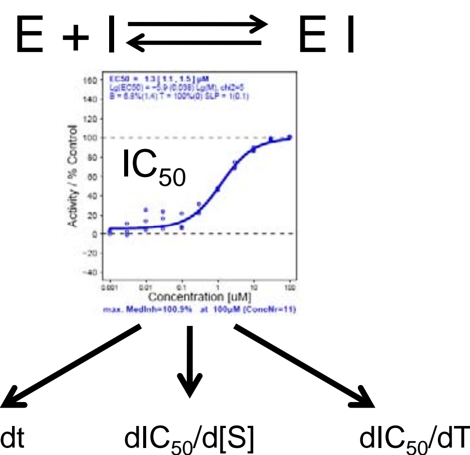
§ Evotec AG, Schnackenburgallee 114, 22525, Hamburg, Germany (Corp. HQ); † Evotec (UK) Ltd, 114 Milton Park, Abingdon, OX14 4SA, United Kingdom

Introduction

High throughput screening (HTS) of large compound collections is a frontline strategy of pharmaceutical companies to fuel their pipeline with new drug candidates. Once HTS campaigns are completed, identified hit compounds are prioritised by several filters, such as physical properties (e.g. MW, clogP) and chemical tractability. Compounds surviving this filtering process require further validation against orthogonal label-free technologies (e.g. NMR, SPR or LC/MS) and ideally the determination of their mechanism of action.

IC_{50} values obtained from concentration response curves are commonly used to rank-order the potency of hit compounds. However, since the IC_{50} value is affected by various physical parameters such as incubation time, substrate concentration or temperature (Fig.1), mechanistic studies are required to get valuable information on the mode of action. For mechanistic studies, Evotec has developed a platform of assays to evaluate the mode of compound inhibition for enzymes from different target classes suitable for medium throughput.

$E + I \rightleftharpoons EI$



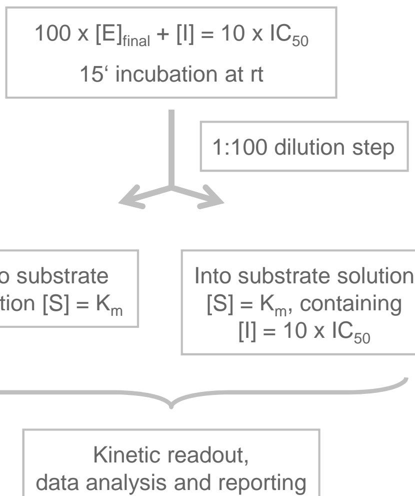
dIC_{50}/dt $dIC_{50}/d[S]$ dIC_{50}/dT

Fig. 1: The IC_{50} value is affected by various parameter, such as incubation time, substrate concentration or temperature

This platform covers an assay portfolio to classify compounds into reversible or irreversible inhibitors **(Box 1)**, further to identify competitive or non-competitive binding modes **(Box 2)** and finally, to determine enthalpy and entropy contributions of enzyme-inhibitor interactions **(Box 3)**. Case studies are presented illustrating the successful applications of the platform for lead compound identification and optimisation.

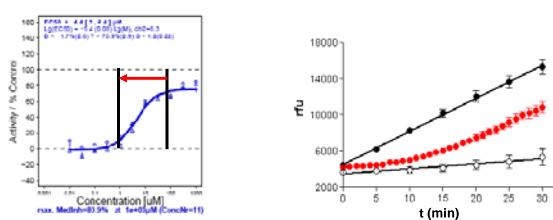
Box 1: Time dependence of the inhibition mode: Test for reversible or irreversible inhibition

A: Protocol

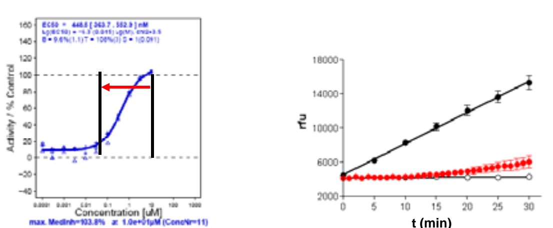


B: Examples for fragment hits

Reversible inhibition; slow recovery of enzyme activity



Most likely irreversible inhibition; extremely slow recovery of enzyme activity



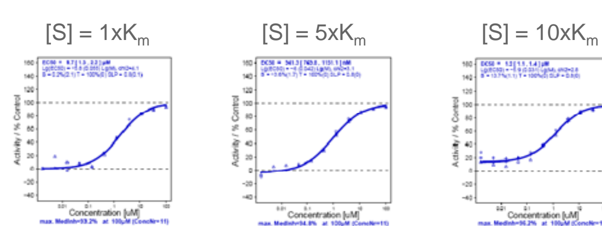
Box 2: Identifying the inhibition mode in dependence of substrate concentration

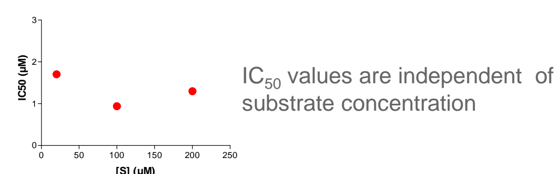
A: Protocol

- Determination of IC_{50} values at different substrate concentration, e.g. 1x, 5x, 10x K_m
- Analysis

B: Examples for fragment hits

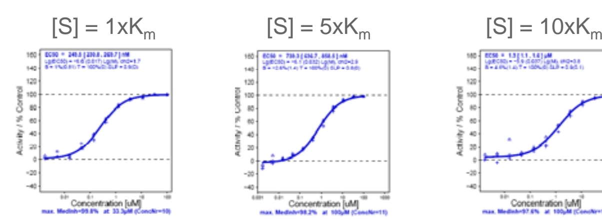
Non-competitive inhibition

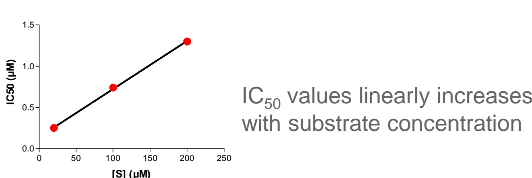




IC_{50} values are independent of substrate concentration

Competitive inhibition





IC_{50} values linearly increases with substrate concentration

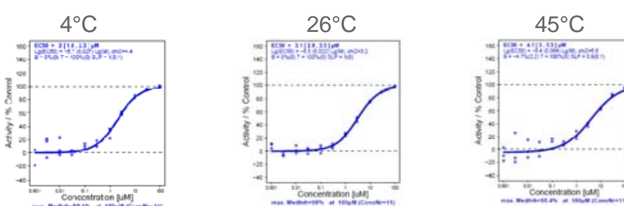
Box3: Determining enthalpic and entropic contributions of enzyme-inhibitor interactions

A: Protocol:

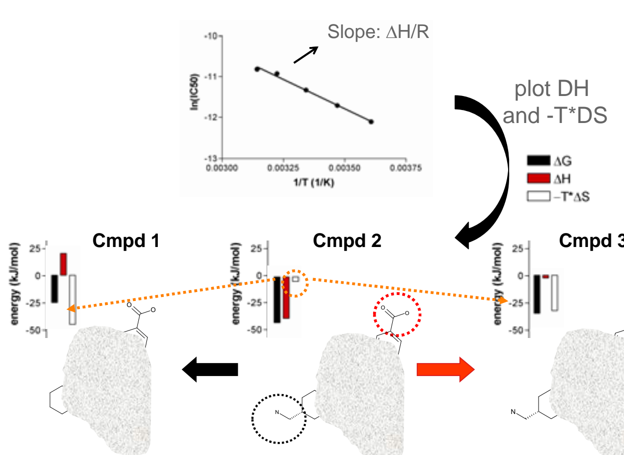
- Determination of IC_{50} at different temperatures
- Substrate concentrations correspond to the K_m value at respective temperatures
- Van't Hoff analysis: temperature dependence of IC_{50} values

B: Examples for protease inhibitors

Determination of IC_{50} values



Van't Hoff analysis and interpretation



Detection of relative changes of the thermodynamic signature in a compound series

Summary

A set of assay protocols is successfully established allowing the quantitative assessment of various physical parameters of enzyme-inhibitor interactons such as dissociation rate constants, binding enthalpy and entropy, or the mode of inhibition. The featured assay systems are routinely applied in hit-to-lead and lead optimisation programs and provide detailed information on the mechanism of action to confirm valid hits and to optimise the structure-activity relationship.