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Evaluating the kinetic profile of your drugs

Kinetic data increases the chances of clinical effectiveness

The kinetics of drug binding and particularly residence time is increasingly recognized to play a pivotal role in clinical effectiveness. In most cases a long residence time of the drug-target complex results in an extended duration of pharmacodynamic activity, even when systemic concentrations of drug have been notably reduced through the elimination routes. Hence, long residence times can increase the duration of drug efficacy *in vivo* and can significantly diminish the potential for off-target-mediated toxicities. Furthermore, a compound with a slower dissociation rate may require a reduced dosing schedule relative to a compound with a rapid dissociation rate [1-4]

The residence time of kinase inhibitors has been demonstrated to be a primary driver of *in vivo* efficacy. The long residence time of the marketed EGFR inhibitor lapatinib has been proposed to explain the *in vivo* drug efficacy as compared to the related inhibitors gefitinib and erlotinib. The irreversible inhibitors neratinib and afatinib, which are currently undergoing clinical trials, has been shown to be active against the T790M/L858R EGFR double mutant that renders most reversible inhibitors ineffective [5].

The use of binding kinetics during the lead optimization process can not only enhance in vivo efficacy but also give access to a more diverse chemical space and more scope for industrial property.

Enzymlogic offers the analysis of the kinetic binding profile of your compounds to your kinase of interest. We use the LanthaScreen® kinase binding technology and our proprietary methodology to determine accurate kinetic data in a HTS format.

FEATURES & BENEFITS

- Determination of affinity, k_{on}, k_{off} and residence time in one assay format
- Broad K_i dynamic range: <1 nM to >10 μM
- 240 ready-to-use assays
- Parallel SAR and SKR studies
- Quantify binding to inactive or low activity kinases
- HTS format
- Rapid turnaround time: ≤ 2 weeks
- Accurate and reproducible data

Structure-kinetic relationship studies are a successful strategy for the discovery of novel drugs

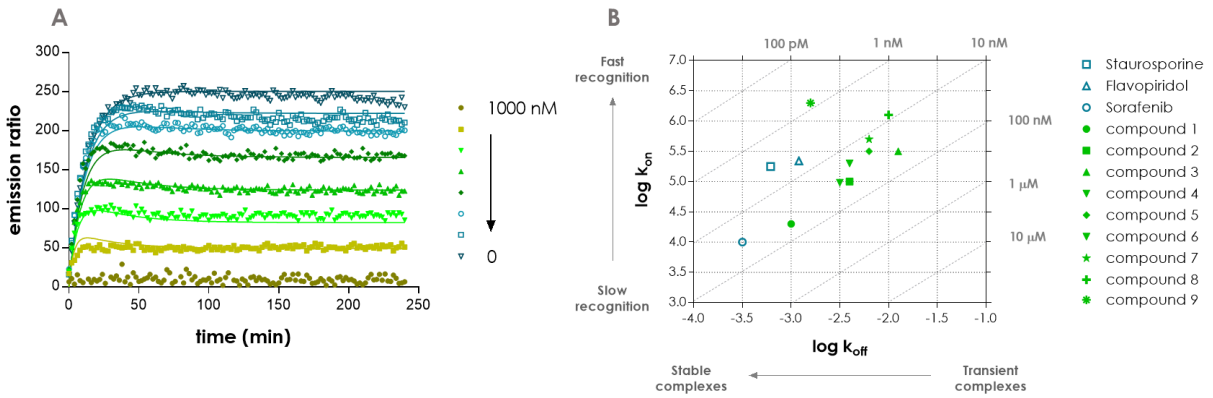

Figure 1. Competition kinetic binding assay. 2 nM CDK8/Cyclin C was incubated with a fixed concentration of an ATP-site directed tracer and increasing concentrations of the compounds. The tracer association to CDK8, which causes an increase in the TR-FRET signal, was monitored over time. K_i, k_{on}, k_{off} and residence time values were simultaneously determined. (A) Effect of Sorafenib's binding kinetics on the tracer association to CDK8 (B) Kinetic profile plot of reference compounds and nine compounds sharing the same chemotype.

Compounds with identical affinity for a given target can exhibit very different kinetic profiles, which may result in the differentiation of the clinical responses. This phenomenon is exemplified with inhibitors of CDK8/Cyclin C.

Staurosporine, Flavopiridol and Sorafenib are well known CDK8 inhibitors. Observed kinetic affinity values of reference compounds are in accordance with the equilibrium affinity data published elsewhere[6]. Sorafenib is a marketed oncology drug that binds the inactive DFG-out conformation of various kinase targets with an extended residence time[7]. As expected, Sorafenib has a long residence time on CDK8 (Table 1).

Table 1. Inhibition of CDK8/Cyclin C by reference compounds

References	K_i (nM)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	Residence time (min)
Sorafenib	30	$1.0x10^4$	$3.1x10^{-4}$	55
Flavopiridol	5.3	$2.2x10^5$	$1.2x10^{-3}$	15
Staurosporine	3.4	$1.8x10^5$	$6.1x10^{-4}$	28

The kinetic profile plot clearly shows that structural changes in the same chemical series may dramatically affect the kinetic rates constants while the affinity remains unchanged (Figure 1.B). The affinity values of compounds 1 to 8 demonstrate a modest deviation (5-fold) whereas the association and dissociation rates vary largely, 57-fold and 15-fold respectively (Table 2). These results reveal that simple affinity measurements may mask the true impact of a structural modification.

A detailed evaluation of the compound structures and kinetic parameters leads to the development of better in class drugs. Table 2 shows that compound 9 is 11 times more potent than compound 8 due to its slower dissociation rate. By contrast, compound 9 is 56 times more potent than compound 1 due to its higher association rate.

Table 2. Inhibition of CDK8/Cyclin C by nine compounds from the same chemical series

Series	K_i (nM)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	Residence time (min)
compound 1	45	$2.1x10^4$	$9.6x10^{-4}$	17
compound 2	40	$9.7x10^4$	$3.9x10^{-3}$	4
compound 3	39	$3.5x10^5$	$1.4x10^{-2}$	1
compound 4	33	$9.6x10^4$	$3.2x10^{-3}$	5
compound 5	22	$3.1x10^5$	$6.9x10^{-3}$	2
compound 6	17	$2.2x10^5$	$3.7x10^{-3}$	5
compound 7	12	$4.9x10^5$	$5.9x10^{-3}$	3
compound 8	9	$1.2x10^6$	$1.0x10^{-2}$	2
compound 9	0.8	$1.8x10^6$	$1.4x10^{-3}$	12

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