enzymlogic

Beyond IC_{50}: the importance of kinetics in drug design

Beyond IC_{50}: the importance of kinetics in drug design

Drugs with similar affinity can exhibit very different kinetic profiles, which may contribute to efficacy, safety, duration of action and differentiation from other similar therapies. Therefore, the early evaluation of the kinetics of drug-target interactions helps to improve decision-making to focus on the most promising compounds[1-8].

A clear understanding of binding kinetics provides a more complete picture of what drives target occupancy in vivo and ultimately clinical efficacy:

- The onset of the drug action is influenced by the association rate (k_{on}): the faster a drug binds to the target, the faster the occupation will occur.
- Conversely, the duration of drug action is dependent on the dissociation rate (k_{off}): the longer the duration of binding, the longer the pharmacodynamic effect.

KINETICfinder® is the ideal tool for quick prediction of drug clinical efficacy

Features and benefits

- Accurate.
- Robust.
- Reproducible.
- Sensitive.
- Broad dynamic range.
- Activated and non-activated targets.
- HTS.
- Rapid turnaround.

Applications

- Modify the on- and off-target kinetics (k_{on}, k_{off}, residence time and K_d).
- Predict clinical efficacy.
- Modulate the therapeutic index and safety profile.
- Understand PK/PD disconnects.
- Build better PK/PD models.
- Differentiate between similar therapies.

How KINETICfinder® assays work

KINETICfinder® is a highly tuned TR-FRET Kinetic Assay. Real-time binding of an active-site directed fluorescent probe is detected using a labeled anti-tag antibody, which binds to the target of interest. The binding of the probe and antibody to the target increases the TR-FET signal, whereas the displacement of the probe with a compound decreases the TR-FRET signal.

Assay process

1. **Performance:**
    - 384 microplates containing the target of interest, a fluorescent probe and labelled antibody.

- 4-point 10-fold serial dilutions of test compounds.
- A reference compound.
- Up to 32 total binding and non-specific binding controls.

2. **Detection:** Reaction is monitored over time at room temperature.
3. **Analysis:** Specific TR-FRET signals are fitted to the Motulsky-Mahan equation. Association (k_{on}) and dissociation (k_{off}) rate constants of test and reference compounds are determined and the K_d and residence time (τ) values are calculated:

$$K_d = \frac{k_{off}}{k_{on}} \qquad \tau = \frac{1}{k_{off}}$$

4. **Quality control:** S/B, Z-value and MSR (within and between-run variability) for each assay.

Approved KIT inhibitors show longer residence time

Many approved kinase inhibitors target KIT kinase, an oncogene implicated in cancer cell growth, proliferation, invasion and metastasis. Figure 1 shows the kinetic profile of 10 approved drugs along with 6 clinical and preclinical compounds against KIT using KINETICfinder®.

The kinetic plot and table 1 reveal that longer residence time contributes to the clinical success of inhibitors that targets KIT kinase:

- 70% of the clinically efficacious drugs show a long residence time, with a median of 100 minutes, compared to the 16% of the clinical and preclinical compounds.
- Development compounds dissociate 13 times faster than marketed drugs, with a median of 8 minutes.
- Approved drugs associate slightly faster to KIT compared to development compounds.

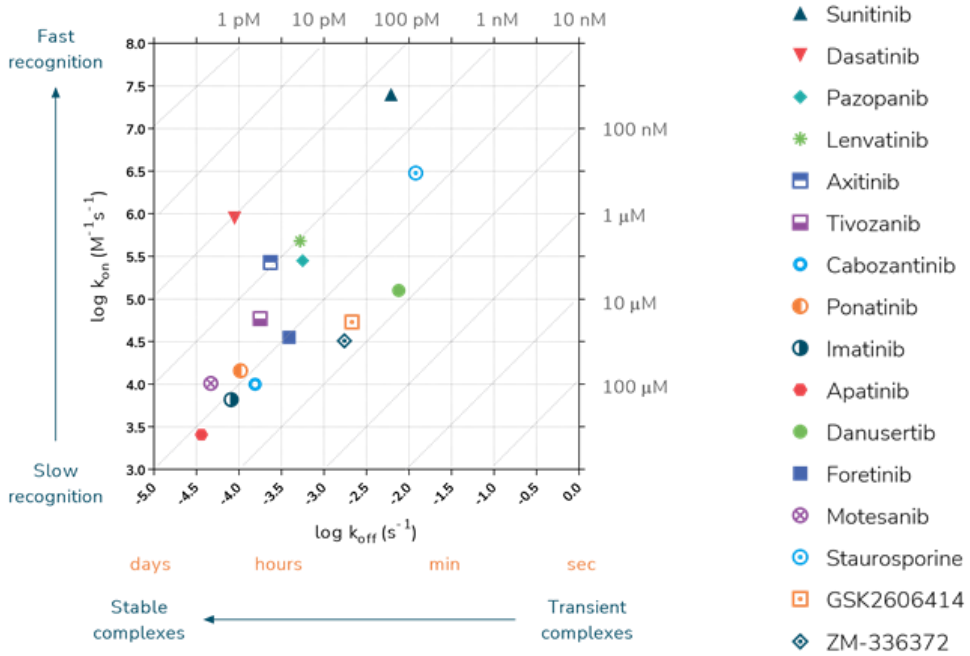
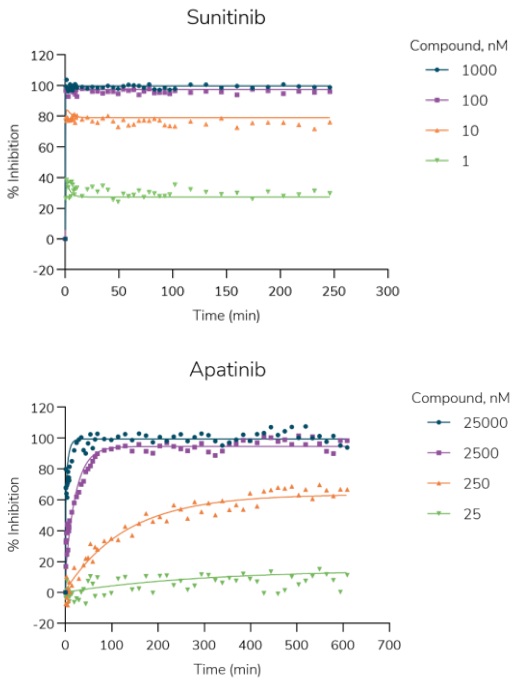


Figure 1. Kinetic plot for 16 drugs of KIT kinase using KINETICfinder®. The on-rate (k_{on}) is shown in the y-axis and the off-rate (k_{off}) in the x-axis, both in logarithmic scale. The dashed lines refer to the K_d values.



	Approved drugs	Development drugs
Association rate ($M^{-1}s^{-1}$)	$1.6x10^5$	$4.5x10^4$
Residence time (min)	100	8
Affinity (nM)	2.5	25
Rapid associating drugs (≥$1x10^6$ $M^{-1}s^{-1}$)	20%	16%
Slow dissociating drugs (≥1 h)	70%	16%

Figure 2. Graphs: Kinetic curves for two representative KIT inhibitors obtained with KINETICfinder®. **Table:** Median of the k_{on}, residence time, K_d values and % of rapid associating and slow dissociating drugs for approved drugs and clinical/preclinical compounds.

Our results are in agreement with those shown by Georgi et al. in a survey of binding kinetics for 270 compounds targeting 40 clinically relevant kinases. They found that k_{on} values were nearly unchanged between preclinical and approved drugs while k_{off} values shifted toward longer residence time for approved drugs[12].

References

1. Copeland R.A. (2016) The drug-target residence time model: a 10-year retrospective. Nat Rev Drug Discov.15(2):87-95.
2. Doris A Schuetz D.A et al. (2017) Kinetics for Drug Discovery: an industry-driven effort to target drug residence time. Drug Discov Today. 22(6):896-911.
3. Daryaee F, Tonge P.J. (2019) Pharmacokinetic-pharmacodynamic models that incorporate drug-target binding kinetics. Curr Opin Chem Biol. 50:120-127.
4. Tonge P.J. (2018) Drug-Target Kinetics in Drug Discovery. ACS Chem Neurosci. 9(1):29-39.
5. Swinney D.C. (2004) Biochemical mechanisms of drug action: what does it take for success? Nat Rev Drug Discov. 3(9):27–41.
6. Tummino P.J. et al. (2008) Residence time of receptor-ligand complexes and its effect on biological function. Biochemistry. 47(20):5481–5492.
7. Lu H. et al. (2010) Drug-target residence time: critical information for lead optimization. Curr Opin Chem Biol. 14(4):467–474.
8. Georgi V. et al. (2017) Binding kinetics in drug discovery - A current perspective. Front Biosci (Landmark Ed) 22(1):21-47.
9. Guo D. et al (2015) The Role of Target Binding Kinetics in Drug Discovery. ChemMedChem. 10(11):1793-6. doi: 10.1002/cmdc.201500310. Epub 2015 Aug 25.
10. Pan A.C. (2013) Molecular determinants of drug-receptor binding kinetics. Drug Discov Today. 18(13-14):667-73.
11. Vauquelin G., Charlton S.J. (2010) Long-lasting target binding and rebinding as mechanisms to prolong in vivo drug action Br J Pharmacol. 161(3):488-508.
12. Georgi V. et al (2018) Binding kinetics survey of the drugged kinome. J Am Chem Soc. 140(46):15774–15782.