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Enhancing Reversible Covalent Drug Design

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Kinetic aspects of Reversible Covalent Drug Design

Irreversible covalent drugs present numerous advantages over reversible non-covalent inhibitors[1].

- Longer-lasting effect as new target synthesis is needed for activity restoration.
- Effective inhibition of challenging targets like those with a shallow pocket or targets that engage in protein–protein interactions.
- Less frequent dosing.
- Higher selectivity by targeting poorly conserved amino acids (i.e. cysteines).

Despite their advantages, irreversible covalent drugs face challenges like off-target toxicities and immunogenic risks. Introducing reversibility can mitigate these issues, as reversible covalent inhibitors are not permanently bound, they can detach from unintended proteins, reducing the risk of immune system activation and off-target toxicity (Fig. 1).

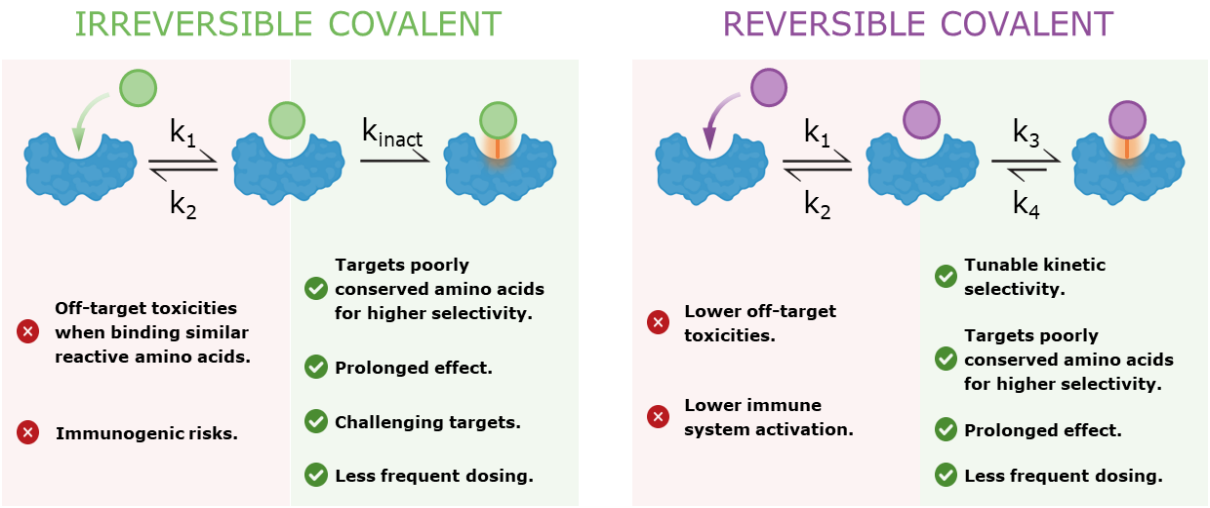


***Figure 1**. Advantages and challenges of covalent drugs.*

Fine-tuning reversible covalent warheads

By tuning the **reactivity of the warhead** (k_3) and the **residence time**, reversible covalent inhibitors can maintain the benefits of irreversible covalent inhibitors while limiting off-target toxicity. One way to achieve this is adjusting the reactivity of the covalent warhead and making structural changes around it to stabilize the reversible covalent adduct and extend its duration.[2]

Since the intention for developing reversible covalent inhibitors is to reduce off-target effects by taking advantage of reversible kinetics, the residence times of the inhibitor with its on- and off-target proteins should be optimized. A high dissociation rate towards secondary targets enables prompt reversal of any accidental covalent bonding.

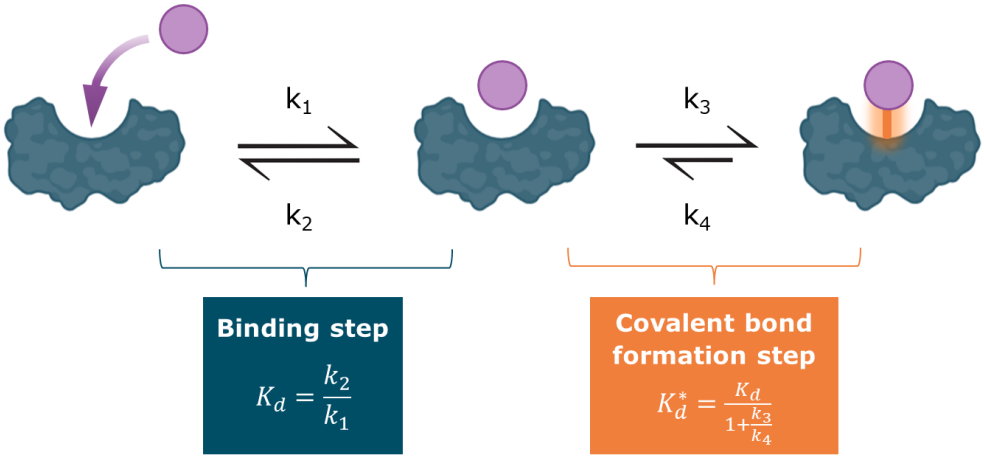
IC_{50} measurements overlooks kinetics, impacting efficacy and selectivity

While drug discovery programs often commonly optimize drugs by maximizing the half-maximal inhibitory concentration (IC_{50}), the two-step inhibition process of reversible

covalent drugs is more accurately elucidated through kinetic measurements.

This arises because IC_{50} measurements fail to adequately represent the actual binding affinity of these drugs. Whereas kinetic measurements can determine the affinity of the initial non-covalent step (K_d) which is defined by the ratio of the rate constants k_1 to k_2 and the affinity (K_d^*) of the covalent complex formation. The formation of the covalent bond between the inhibitor and the target is governed by the rate constant k_3 whereas the reverse reaction back to the non-covalent complex is governed by the rate constant k_4 (Fig. 2).

Kinetic measurements can also determine residence time, a vital piece of information for reversible covalent drugs that refers to the time a drug spends bound to its target, which can also be described as the inverse of the dissociation rate.[3] In this case, the overall dissociation rate is composed of the rate constants associated with both forward and reverse steps in the covalent bond formation, as well as the dissociation rate from the initial complex ($k_{off} = k_2 x k_4/(k_2+k_3+k_4)$).



$$Residence\ time = \frac{k_1 + k_3 + k_4}{k_2 \times k_4}$$

Figure 2. *Mechanism for reversible covalent inhibition.*

COVALfinder® and KINETICfinder® are the ideal tools for fine-tuning Reversible Covalent Drugs

Benefits

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

Applications

- Modify compound reactivity and residence time.
- Modulate the therapeutic index and safety profile.
- Guide the selection of compounds for further studies.
- Understand PK/PD disconnects.

Analysis of the Reversible Covalent BTK Inhibitor Rilzabrutinib

Bruton's tyrosine kinase (BTK), expressed in B cells and mast cells, plays a critical role in multiple immune-mediated disease processes. Rilzabrutinib is currently being evaluated in Phase III trials for adults and adolescents with persistent or chronic immune thrombocytopenia and numerous Phase II clinical trials for immune disorders[4]. Rilzabrutinib is a very potent reversible BTK inhibitor that engages a noncatalytic cysteine (Cys481) present in BTK in a covalent manner. Despite potent binding to BTK (IC_{50} =1.3 nM) and other kinases with a structurally homologous cysteine like HER4 (IC_{50}=11 nM) and to a lesser extent ITK (IC_{50}=440 nM), it has been suggested that Rilzabrutinib might dissociate faster from off-target kinases, potentially limiting side-effects.[5]

Analyzing reactivity and reversibility is crucial for the optimization of Reversible Covalent Drugs

COVALfinder® and KINETICfinder® platforms have been used in combination to provide an in-depth understanding of the binding mechanism of Rilzabrutinib with its primary target BTK and secondary targets HER4 and ITK.

Our results confirm that BTK-Rilzabrutinib, HER4-Rilzabrutinib and ITK-Rilzabrutinib complex formation each take place in reversible sequential events: such that Rilzabrutinib binding to BTK, HER4 and ITK is the initial event followed by covalent bond formation with the non-catalytic cysteine of BTK, HER4 and ITK as the second step (Fig. 3-4).

As shown in Table 1, the K_d* values obtained with COVALfinder® do not match the IC_{50} values reported in the literature. Generally, there is a 10- to 20-fold difference, which negatively impacts the accurate interpretation of a drug's efficacy and toxicity, as well as the correct assessment of the therapeutic window.

Our findings also reveal that Rilzabrutinib rapidly forms a covalent bond with the non-catalytic cysteine of BTK, HER4 and ITK. The half-life for this process is similar between the main and secondary targets (1.5, 1.7 and 2.3 minutes, respectively). Moreover, the covalent bond between Rilzabrutinib and BTK, HER4 and ITK is completely reversible (residence time of 812, 225 and 44 minutes respectively).

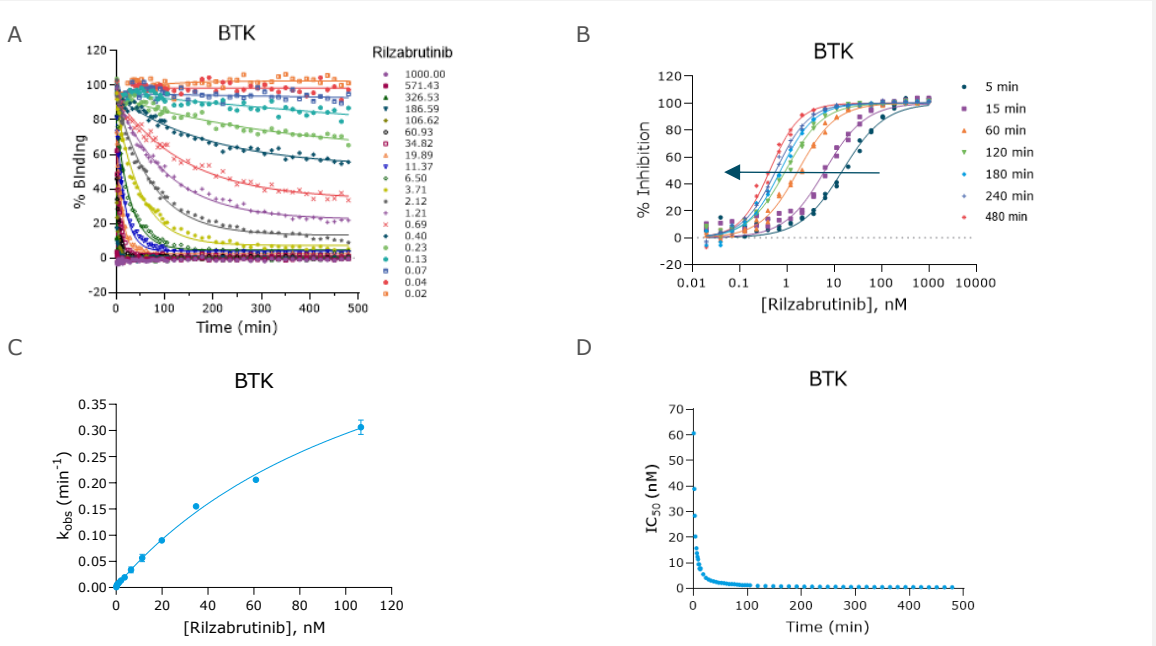


Figure 3. Characterization of the kinetics of BTK inhibition by the reversible covalent drug Rilzabrutinib. (A) Progress curve of BTK incubated with increasing compound concentrations. (B) Dependence of k_{obs} on Rilzabrutinib concentration. (C) Dose-response curves over time. (D) IC_{50} values over time.

	Binding step			Covalent bond formation step				
Target	**k_1 ($M^{-1}s^{-1}$)**	**k_2 (s^{-1})**	**K_d (nM)**	**k_3 (s^{-1})**	**k_4 (s^{-1})**	**K_d* (nM)**	**k_{off} (s^{-1})**	**Residence time (min)**
BTK	$3.30x10^5$	$1.49x10^{-2}$	45	$1.12x10^{-2}$	$3.60x10^{-5}$	0.15	$2.05x10^{-5}$	812
HER4	$2.19x10^5$	$8.24x10^{-3}$	38	$9.84x10^{-3}$	$1.64x10^{-4}$	0.62	$7.42x10^{-5}$	225
ITK	$5.61x10^4$	$2.95x10^{-2}$	527	$7.15x10^{-3}$	$4.93x10^{-4}$	34	$3.92x10^{-4}$	43

Table 1. Kinetic constants, residence time and affinity values of Rilzabrutinib for BTK, HER4 and ITK measured by COVALfinder® and KINETICfinder®

The high affinity of Rilzabrutinib to BTK (K_d*: 0.14 nM) is a result of the extraordinary stability of the BTK-Rilzabrutinib complex (k_{off}: 2.0×10^{-5} s^{-1}). This enables Rilzabrutinib to mimic the long-lasting activity of an irreversible covalent inhibitor, with its in vivo dissociation rate controlled by BTK degradation and resynthesis.

Our results support those of Bradshaw et al. who found that, in rats, BTK inhibition remained around 57% after 840 minutes (14 hours), even though the serum concentration of Rilzabrutinib dropped to less than 3 ng/mL from nearly 500 ng/mL after 1 hour at a 40 mg/kg dose.

Thus, despite being cleared from circulation, Rilzabrutinib showed significant target engagement 14 hours after oral dosing, reflecting its slow dissociation from BTK in vivo.[2]

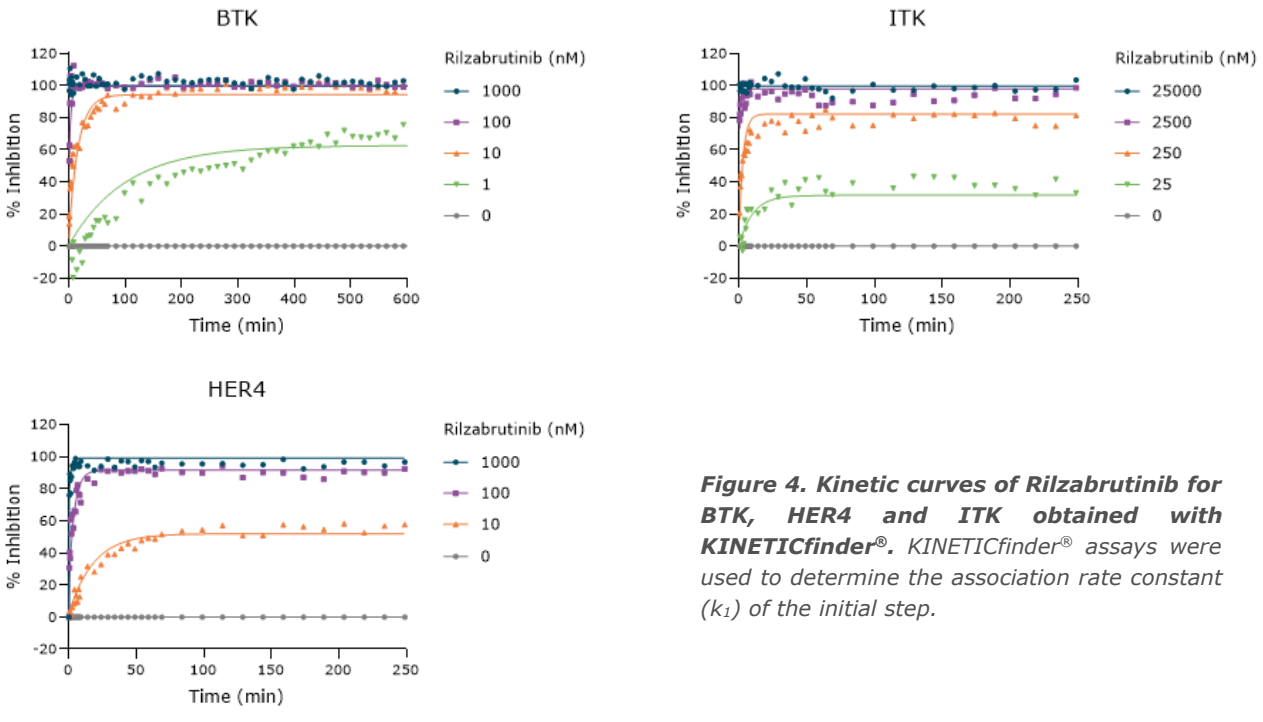


Figure 4. Kinetic curves of Rilzabrutinib for BTK, HER4 and ITK obtained with KINETICfinder®. *KINETICfinder® assays were used to determine the association rate constant (k_1) of the initial step.*

Kinetic selectivity of Rilzabrutinib maximizes its therapeutic window

Rilzabrutinib has outstanding kinome-wide selectivity, avoiding all kinases that lack the conserved cysteine as well as many physiologically important kinases that have this cysteine, including EGFR, HER2, JAK3 and MKK7.[2]

This exceptional selectivity is achieved through two distinct mechanisms utilized by reversible covalent drugs:

- Forming a bond with a reactive amino acid residue.
- Tuning inhibitor residence time against on- and off-targets.

An important benefit of fine-tuning residence time is the ability to achieve high sustained occupancy of the target without the need for extended exposure to the compound, whilst also limiting off-target toxicity.

In a phase I study, Rilzabrutinib demonstrated a rapid elimination half-life ($t_{1/2}$) of 3.20 hours[6]. Despite this rapid clearance, Rilzabrutinib's prolonged residence time on BTK (14 hours) guarantees sustained target inhibition[7].

The shorter residence times on ITK (43 minutes) and HER4 (3.75 hours) mean that any initial off-target binding is quickly reversible. This kinetic selectivity, combined with the rapid elimination, helps in reducing the duration and impact of off-target effects, improving the drug's safety profile.

Consequently, Rilzabrutinib's therapeutic window is maximized. The chance of adverse side effects is minimized, and tolerability improves while efficacy is maintained over an extended period of time.

References

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