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RATIONAL DRUG DESIGN

Tuning kinase inhibitor residence time

On-target residence times are rarely optimized during drug development programmes, but they probably have a crucial role in determining efficacy as well as off-target and on-target adverse effects. Bradshaw *et al.* now report a new strategy to generate inhibitors of Bruton's tyrosine kinase (BTK) with prolonged residence times, based on reversible covalent binding to a non-catalytic cysteine residue. The approach could be generalizable to other drug targets that contain such residues.

Ibrutinib, an acrylamide-based inhibitor that forms an irreversible covalent bond with a non-catalytic cysteine (Cys481) in BTK, is approved for the treatment of B cell malignancies. BTK also has a role in autoimmune diseases, but irreversible covalent BTK inhibitors are unlikely to meet the long-term safety margins necessary for treating chronic conditions, owing to the formation of irreversible covalent bonds with cysteine residues on off-target proteins.

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The authors therefore sought to develop reversible covalent BTK inhibitors that targeted Cys481, and they designed molecules with a reversible covalent cyanoacrylamide electrophile coupled to a BTK-targeting scaffold. Geometrically diverse linkers between the scaffold and the cyanoacrylamide group and alkyl capping groups with increasing steric demand were then examined for their effects on BTK residence times. Using branched alkyl caps, the authors could alter the steric and electronic environment surrounding the electrophilic β-carbon, and found that a variant with a *tert*-butyl cap had prolonged BTK residence time such that 55% BTK target occupancy was maintained in cells 20 hours after inhibitor washout.

Further structural modifications were incorporated to overcome the poor physicochemical properties of the *tert*-butyl-containing compound. These efforts resulted in compounds with residency times ranging from 22–167 hours. In cells, these BTK inhibitors had IC_{50} values of 5–27 nM, and the most durable of the compounds had 59% BTK occupancy 18 hours after washout. Following oral dosing in rats, compound 9 (which had the highest residency time *in vitro*), was mostly cleared from the plasma within ~14 hours, but was still bound to 41% of BTK molecules in peripheral blood mononuclear cells 24 hours after dosing, indicating that this compound also dissociates slowly *in vivo*. The inhibitors also demonstrated strong selectivity for BTK; only BTK and the related kinase BMX were inhibited >90% by 0.1 μM of compound 9 using a 254-kinase selectivity panel.

The authors then applied this approach to design a pyridopyrimidinone series, this time to specifically bind a non-catalytic cysteine in fibroblast growth factor receptor 1. By using the same core and phenyl linker in each molecule but modifying the capping groups, they designed molecules with a variety of residence times, demonstrating the potential generalizability of this cap-modification strategy to modify the durability of the drug–target interaction.

This strategy was used to discover a proprietary reversible covalent BTK inhibitor, PRN1008, that is currently being advanced for clinical studies as a potential treatment for autoimmune and inflammatory disorders. This chemical modification strategy, and the resulting tunability of reversible covalent inhibitors, could also potentially be applied to an estimated ~40% of the kinome and also any non-kinase targets that contain an appropriate non-catalytic cysteine residue.

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ORIGINAL RESEARCH PAPER Bradshaw, M. J. *et al.* Prolonges and tunable residence time using reversible covalent kinase inhibitors. *Nat. Chem. Biol.* http://dx.doi.org/10.1038/nchembio.1817 (2015)