Inhibitors

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Reversible Covalent Inhibition of a Protein Target

*Chang-Uk Lee and Tom N. Grossmann**

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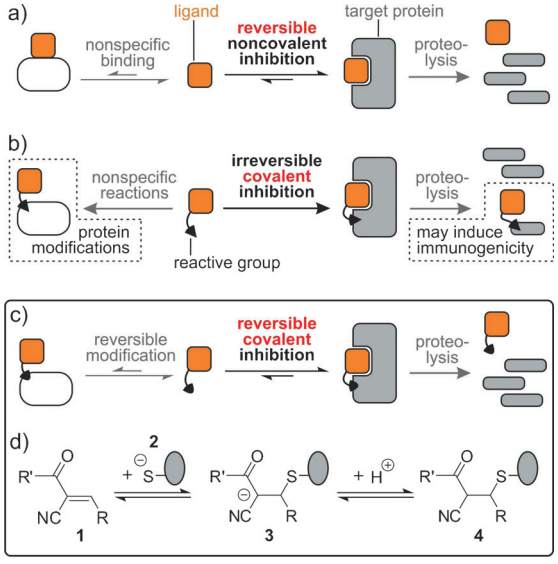
During the last decades, classical approaches in drug development such as the reversible inhibition of target proteins with inert small molecules have proved to be insufficient for the selective manipulation of a variety of disease-relevant targets. Hence, the development of compounds that, for example, target ribonucleic acids or protein–protein interactions, or selectively inhibit a single member of a large protein family can prove to be a formidable challenge. These problems initiated the search for new drug classes and delivery strategies. In one approach, ligands are modified with reactive moieties capable of forming a covalent bond with the protein of interest. Potential advantages of this strategy involve high target affinity and prolonged pharmacodynamics.[1] So far, most approved drugs that trigger the formation of covalent and irreversible adducts with their target were discovered by serendipity (e.g. acetylsalicylic acid, penicillin, and omeprazole). However, owing to toxicity issues resulting from covalent protein modifications, there has been reluctance towards the use of reactive moieties in rational drug design.[2] Given the potential benefits of covalent inhibition, a search for reactive groups with reduced toxicological risks was initiated.[1] In this context, one strategy focuses on the use of covalent but reversible inhibitors.[2b] Along these lines, Taunton and co-workers recently described the fast and reversible addition of thiols to electron-deficient olefins and its application for the design of ligands capable of forming a covalent bond with the target protein.[3] Under physiological conditions this covalent target binding proved to be reversible, therefore having the potential to minimize the risks associated with irreversible protein modifications.

Although kinases are important target proteins in drug discovery, they represent challenging targets when aiming for the selective inhibition of a single family member.[4] In a classic targeting approach, ligands are optimized for improved noncovalent interactions within the active site of the respective protein (Scheme 1 a). In structure-based approaches, such ligands are subsequently modified with electrophilic groups in order to promote coupling reactions with nonconserved cysteine residues in proximity to the active kinase site (Scheme 1 b). This covalent and irreversible attachment of the inhibitor increases its affinity for the target and additionally acts as a second selectivity filter.[5] However, one drawback of reactive moieties, such as acrylamides and halogen acetamides, is their potential toxicity arising from their irreversible reactions with off-target nucleophiles, for example, glutathione and proteins with hyperreactive cysteine residues. In addition, there is the danger that irreversibly modified proteins could cause an immune response (Scheme 1 b).[2a] The precise effects of irreversible protein modifications are difficult to predict, and the risks are most relevant for the long-term treatment of chronic diseases. Therefore, the development of targeted covalent inhibitors would benefit from strategies that minimize the occurrence of such protein modifications. Previous strategies mainly aimed at reducing the reactivity of the requisite electrophilic moiety in order to minimize off-target reactions. Encouraged by earlier examples that showed the feasibility of covalent but reversible protein inhibition (Scheme 1 c),[2b,6] Taunton and co-workers use highly reactive electrophiles that are attacked by cysteine side chains in a reversible manner (Scheme 1 d).[3] The reported kinase inhibitors are modified with electron-deficient olefins that target a nonconserved cysteine side chain adjacent to the active site.



Scheme 1. Representation of several protein-targeting strategies: a) reversible inhibition by nonreactive ligands, b) irreversible inhibition by reactive ligands, and c) reversible covalent inhibition by reactive ligands. d) Mechanism for the addition of thiols **2** to electron-deficient Michael acceptors **1** and the corresponding back reaction (elimination). R = aromatic ring, R′ = O-alkyl/NH-alkyl.

[*] M. Sc. C.-U. Lee, Dr. T. N. Grossmann
Chemical Genomics Centre of the Max Planck Society
Otto-Hahn-Strasse 15, 44227 Dortmund (Germany)
E-mail: tom.grossmann@cgc.mpg.de
Homepage: http://www.cgc.mpg.de

Based on the previously reported observation of fast and reversible reactions between thiols and 2-cyanoacrylates at neutral pH,[7] olefins with various electron-withdrawing groups were designed.[3] Kinetic investigations revealed that the reaction of thiols **2** only with doubly activated Michael acceptors **1** rapidly reaches equilibrium (Scheme 1 d). The authors suggest that the electron-withdrawing substituents in **1** render the β carbon more susceptible to nucleophilic attack and that they stabilize the resulting carbanion (**3**), thereby accelerating the addition reaction. Furthermore, electron-withdrawing groups promote the C_α–H acidity of product **4**, which supports a rapid back reaction (elimination). These activated Michael acceptors were subsequently used to design electrophilic pyrrolopyrimidines derived from a known irreversible inhibitor (FMK, Figure 1 a)[8] of p90 ribosomal protein S6 kinases (RSKs). Based on the predicted binding orientation, the pyrrolopyrimidine scaffold was modified with an electron-deficient Michael acceptor moiety designed to be in proximity to Cys436 flanking the active site of the C-terminal kinase domain of RSK2. Several reversible covalent inhibitors (RCIs) were synthesized including RCI14 and RCI16, which showed the expected inhibitory effect on RSK2. The crystal structure of RCI16 bound to the C-terminal kinase domain of RSK2 verified the anticipated binding mode and the formation of a covalent bond with Cys436 (Figure 1 b).[3]

Further experiments investigated the reversible nature of the bond formation between the inhibitor and the kinase.[3] It was shown that unfolding or proteolytic digestion of the protein after inhibitor binding promotes the elimination reaction and dissociation of the inhibitor. This indicates the importance of an intact protein ternary structure for bond formation. Although the kinase domain contains several solvent-exposed cysteine side chains, RCIs react only with Cys436, even at micromolar concentrations. Interestingly, the inhibitory effect of RCIs was not affected by millimolar concentrations of water-soluble thiols, such as glutathione, whereas the mutation of Cys436 abolished the inhibitory activity. Taken together, these observations verify the reversible nature of the reaction, and they demonstrate the importance of ligand binding for efficient and selective thiol addition (template effect). In vitro assays showed high selectivity of RCI14 for the inhibition of RSK proteins (out of a panel of 442 kinases) with an inhibitory potency exceeding that of the related irreversible inhibitor FMK (Figure 1 a). In cell-based assays, RCI14 was tested for its effect on RAF-induced epithelial cell migration and invasion,[3] a process in which RSK proteins are involved. RCI14 induced an expected inhibition of cell mobility, comparable to that of FMK and consistent with RSK inhibition. Analysis of lysates obtained from cells pretreated with a fluorescent analogue of RCI14 showed that the compound binds reversibly to RSK proteins. RCI14 and FMK showed similar cellular retention and were detectable for approximately two days after incubation.

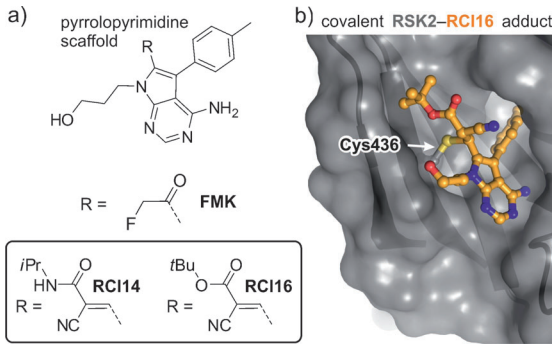


Figure 1. a) Irreversible (FMK) and reversible (RCI14, RCI16) covalent inhibitors of the C-terminal kinase domain of RSK2. b) Crystal structure of RCI16 covalently bound to RSK2 (view into the active site of the C-terminal kinase domain of RSK2; PDB 4D9U).[3]

In conclusion, Taunton and colleagues report the use of electron-deficient Michael acceptors for reversible addition/elimination reactions with thiols under physiological conditions.[3] They apply 2-cyanoacrylates and -acrylamides to convert a known irreversible kinase inhibitor into a reversible covalent inhibitor. Such an approach might lead to the improved affinity and selectivity often associated with protein inhibition through the covalent capture of nonconserved residues without suffering from toxicological risks associated with the irreversible modification of proteins. In principle, this strategy should be applicable to a large number of disease-relevant target proteins. For example, it was estimated that approximately 20% of all kinases might be accessible for cysteine-based targeting.[5] Given the potential such an approach could offer in drug discovery, it is essential to further investigate the applicability and toxicity of these pharmacophores.

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