Reversibility of cysteine protease inhibition in the context of drug design

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Given the recent publication of a crystal structure of SARS-CoV-2 main protease with a covalently bound ligand and the results of the crystal-based fragment screen conducted at the XChem facility (UK Diamond Light Source), it might be helpful to say something about reversibility of covalent binding to the catalytic thiol of a cysteine protease.

Reversibility of covalent bond formation between the catalytic cysteine thiol and an atom (usually carbon) in the inhibitor structure is typically determined by the nature of the warhead. Nitrile-based inhibitors (e.g. odanacatib) tend to bind reversibly while vinylsulfones (e.g. K777) are typically irreversible inhibitors.

If the covalent bond formation is reversible, then the covalent nature of the ligand-protein does not present any special challenges for design. For example, affinity can be measured and the structure of the bound complex is directly relevant to affinity. In particular, non-covalent ligand-protein contacts can be treated as if the ligand had bound non-covalently and IC$_{50}$ is a direct measure of activity.

Design becomes more complicated if covalent bond formation is irreversible because the affinity of the ligand is neither measurable nor particularly relevant. Activity of irreversible inhibitors is typically quantified as the ratio of the inactivation rate constant (k$_{\text{inac}}$) to the inhibition constant (K$_i$) and this means that the transition state structure is more relevant to activity than the structure of the covalently-bound complex. An IC$_{50}$ value measured for an irreversible inhibitor reflects both the rate at which it reacts with the enzyme as well as how long it is in contact with enzyme during the assay and is a relatively indirect measure of activity.

Many drug discovery scientists in the cysteine protease field would prefer to work with reversible inhibitors than with irreversible inhibitors. Some of this stems from a belief that irreversible inhibitors are inherently less selective than reversible inhibitors. I keep an open mind on that question and actually see the principal challenges in the design of irreversible inhibitors as stemming from the ‘kinetic’ nature of the inhibitory activity. In particular, it is more difficult to relate activity to structure for irreversible inhibitors and the need to account for binding kinetics adds a layer of complexity to PK/PD models. While irreversible inhibition may be advantageous in some situations, irreversibility to the extent that ligand remains bound to peptide fragments after protein degradation would generally be undesirable because of potential for immunogenicity.

If aiming to target the catalytic cysteine reversibly, models for covalently-bound fragments can easily be extracted from structures of relevant protein-ligand complexes (e.g. cathepsin K with nitrile-based inhibitor). Models like these are arguably more relevant to design of reversible inhibitors than the structures that have been determined for irreversibly-bound fragments. If aiming to target the catalytic cysteine irreversibly, a model for the relevant transition state may be more relevant to design than the structure that has been determined experimentally for an irreversibly-bound fragment.