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Selective Inhibition of USP7

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The deubiquitinating enzyme USP7 is an emerging oncology and antiviral target. Reverdy et al., in this issue of *Chemistry & Biology*, disclose the first small-molecule inhibitor selective for USP7, which recapitulates its knockdown in cancer cells and hence demonstrates the therapeutic feasibility of USP7 inhibitors.

Covalent modification of cellular proteins by ubiquitin (Ub) represents a highly versatile posttranslational mechanism that underpins proteasomal degradation and regulates a diverse array of cellular processes (Komander et al., 2009; Reyes-Turcu et al., 2009). Defects in ubiquitin-based control mechanisms have been implicated in the pathogenesis of numerous disease states including cancer, chronic diseases, and viral infections (Lindner, 2007; Singhal et al., 2008). Hence, components of the ubiquitin system emerge as attractive drug targets, reinforced by the approval of the proteasome inhibitor Velcade in oncology. Protein ubiquitination is a dynamic and reversible process controlled by the coordinated action of multiple ubiquitin-conjugating and deubiquitinating enzymes (DUBs). The human genome contains nearly 100 DUBs, which can be divided in five structural classes. USP7, which is the enzyme targeted for inhibitor discovery by Colland and coworkers (Reverdy et al., 2012), is part of the ubiquitin-specific proteases (USP) class, a group of cysteine proteases comprising approximately 60 members. Several studies have linked USP7 to diseases, particularly cancer, and the enzyme is considered a very promising target for drug discovery (Nicholson and Suresh Kumar, 2011).

A major hurdle in the discovery of inhibitors for cysteine proteases having the papain-like fold is achieving inhibitory selectivity. This is due to relatively shallow substrate-binding sites lined with residues well conserved over large families of homologous enzymes. Moreover, high-throughput screening (HTS) hits have acceptable inhibitory potency but often do not display the desired level of selectivity, requiring further optimization. Nonetheless, HTS campaigns have led to several active site-directed small-molecule USP inhibitors. A cyano-indenopyrazine derivative (HBX 41,108) was disclosed as a reversible uncompetitive low μM inhibitor of human USP7 of modest selectivity against several DUBs and other cysteine proteases (Colland et al., 2009; Reverdy et al., 2012). Another compound was found to inhibit the proteaseome-activated form of human USP14 in μM range but to inhibit IsoT (human USP5) only 20-fold weaker (Lee et al., 2010). Several structurally unrelated inhibitors of human USP1 were discovered in μM range but inhibited at least one other human USP with similar potencies (Chen et al., 2011). Other recent efforts in the field have lead to compounds with often limited assessment of selectivity. Perhaps the most successful selective inhibition of a USP was achieved for the PLpro of the SARS coronavirus. Small-molecule, non-covalent, substrate-binding-site-directed inhibitors of this viral USP were discovered in the μM range potency and with excellent selectivity profile (Ratia et al., 2008). This high selectivity was likely afforded by the relative divergence of the viral USP from human USPs both structurally and functionally. In all these cases, the inhibitors were tested against a very limited set of USPs or DUBs.

In this issue of *Chemistry & Biology*, Reverdy et al. (2012) report 9-chloro derivatives of amidotetrahydroacridine as USP7 inhibitors in the μM range, identified from an in vitro enzymatic HTS. Importantly, these compounds show good selectivity for USP7 when tested against other human USPs and DUBs. An interesting and commendable aspect is the use of an activity-based molecular probe (HAUbVS) that can label active cellular USPs, giving access to a large repertoire of USPs therefore permitting a thorough evaluation of inhibitory selectivity. The selectivity data obtained in vitro on cell lysates using HAUbVS indicate that the inhibitor (HBX

19,818) reduced USP7 labeling but not labeling of other active USPs endogenously expressed under physiological conditions. Moreover, the inhibitor specifically hits the USP7 target in living cells, as it disrupted HAUbVS competitive labeling of USP7 without any off-target activity across several different endogenous deubiquitinating activities. In cancer cells, several effects of the inhibitor were monitored aiming to recapitulate USP7 silencing. These included compromised UV-induced Chk1 phosphorylation and decreased levels of checkpoint mediator Claspin, modulation of endogenous levels of additional USP7 substrates such as Mdm2 and p53, with observed destabilization of Mdm2. Additionally, the USP7 inhibitor reduced cell proliferation, induced caspase activity and PARP cleavage, and arrested colon cancer cells in G1. All these results strongly suggest that the discovered compound displays an appreciable level of potency and selectivity toward USP7.

An irreversible active-site-directed inhibition mechanism was proposed based on mass spectrometry experiments, with a covalent bond formed with the catalytic Cys223 of USP7. Interestingly, the mass difference of 36 Da lost during covalent inhibition is consistent with displacement of the 9-Cl substituent by the active site thiolate. This proposed inhibition mechanism is unusual in that it has not been exploited in the field of cysteine protease inhibition so far. An aromatic chlorine activated by the tetrahydroacridine system may render this mechanism possible. Molecular docking of the inhibitor positions the Cl substituent in the proximity of the active site Cys, in agreement with the proposed mechanism, while a required positively charged amine substituent interacts with negatively charged residues. Intriguingly, the authors speculate that inhibition may actually occur in the inactive state of USP7 featuring a misaligned catalytic triad. The relatively limited number of USP structures solved to date show that substrate-induced conformational changes involve mostly loop movements, while the catalytic triad misalignment was confirmed only for USP7. Therefore, these compounds might indeed exploit a unique structural feature of USP7 for achieving selectivity. Structure determination of the enzyme-inhibitor complex complemented by structure-function studies are needed to confirm this and other mechanistic hypotheses. It would be interesting to see whether the reactivity of the Cl substituent can be optimized, since some weak labeling of another, solvent-exposed, Cys residue of USP7 was also noted.

The inhibitor discovery and characterization described by Reverdy et al. (2012) provides general proof-of-principle that selective small-molecule inhibitors can be identified for an enzyme family like USPs. This is a significant achievement, considering that it concentrates on the active site region perceived as intractable for achieving inhibitory selectivity. Due to the participation of USPs in complex protein networks with multiple biological functions, the tremendous possibility of off-target effects cannot be overlooked. The route from an inhibitor to a drug is certainly not trivial, and one cannot predict if the compounds will display a good safety profile. However, identification of inhibitors that bind selectively to USP7 and modulate steady-state levels of its substrates can be viewed as a step in the right direction and opens an exciting avenue toward potential therapeutic interventions.

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