

# The Next Step Forward in Ubiquitin-Specific Protease 7 Selective Inhibition

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Ubiquitin-specific protease 7 is a validated anticancer target; thus, selective USP7 inhibitors are of great interest. In this issue of *Cell Chemical Biology*, Lamberto et al. (2017) and Pozhidaeva et al. (2017) report important insights into the structural inhibitor-enzyme interplay, lighting the way toward the development of selective inhibitors.

Ubiquitination or ubiquitin-like (Ubl) processes are posttranslational modifications that are strictly regulated in stepwise fashion through the ubiquitin (Ub) or Ubl-conjugating enzyme cascade. Components E1, E2, and E3 encoded by 7, 35, and around 650 genes, respectively, attach ubiquitin or Ubls to target proteins, whereas deconjugation is performed by a family of cysteine- or metallo-proteases collectively known as deubiquitinating enzymes (DUBs; around 100 different genes). Different ubiquitin conjugations will cause protein degradation through the ubiquitin proteasome system (UPS) or confer a variety of signals that can alter protein activity or localization or modulate protein interactions of the target protein (D'Arcy et al., 2015; Ronau et al., 2016).

UPS-based anticancer drug development has focused on five molecular target classes: E1, E2, E3, DUBs, and proteasome. Fourteen years ago, bortezomib, the first FDA-approved proteasome inhibitor, validated the UPS as a target for cancer therapeutics. Although significant progress has been made during the past decade, the development of UPS inhibitors has fallen behind. Only four additional ubiquitin pathway-associated drugs have been approved: the proteasome inhibitors carfilzomib and ixazomib (Manasanch and Orlowski, 2017), as well as lenalidomide and pomalidomide—derivatives of thalidomide that target the cereblon (CRBN) part of the cullin 4-containing E3 ubiquitin ligase complex (CRL4<sup>CRBN</sup>) (Huang and Dixit, 2016). Thus, finding novel small molecules as drug candidates within the UPS, and especially DUBs, is still an active pursuit within academia and the pharmaceutical industry.

Due to its critical role in regulating p53 function, ubiquitin-specific protease 7 (USP7), also known as herpes-associated ubiquitin-specific protease (HAUSP), is the most widely studied DUB. The stability and functional regulation of USP7-dependent substrates have been reported to be essential for the initiation, progression, or recurrence of a series of tumors (e.g., leukemia [Chauhan et al., 2012]). Nevertheless, a key problem with earlier USP7 inhibitors, and with DUB inhibitors in general, is their lack of specificity across the DUB family, which limits their usefulness as drugs. And, to add to the difficulty, until recently, no cocrystal structures of USP7 had been solved with small-molecule inhibitors. This is now likely to change. During the last year, a series of papers (Kategaya et al., 2017; Lamberto et al., 2017; Pozhidaeva et al., 2017; Turnbull et al., 2017) reported new selective USP7 inhibitors that possess a structurally defined mechanism of inhibition.

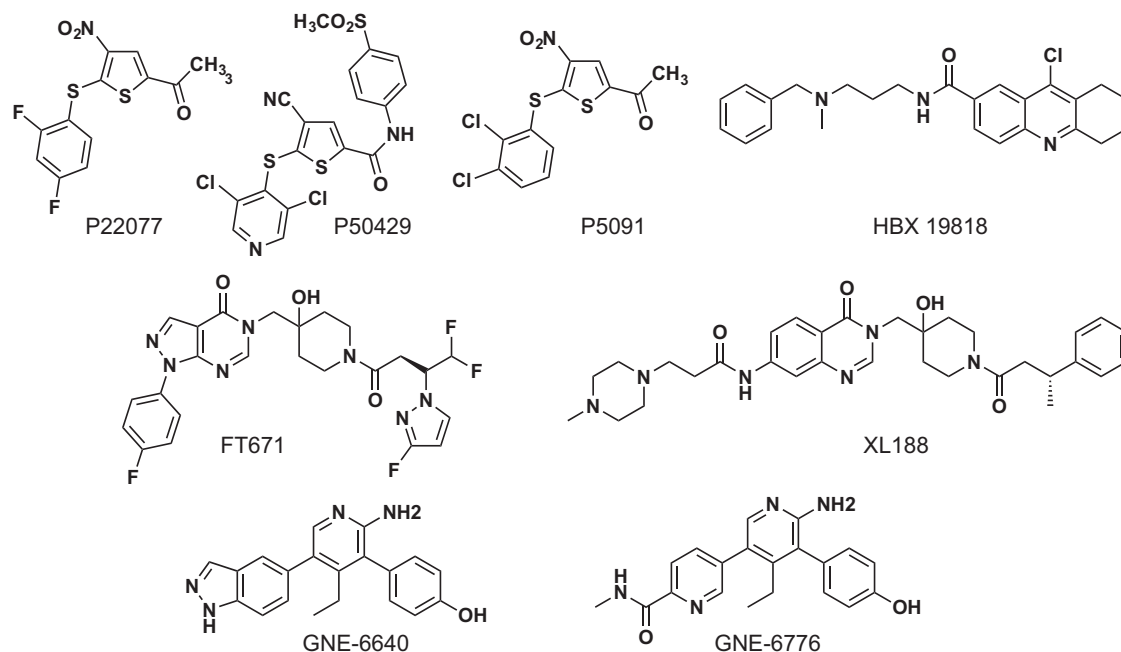
By profiling the inhibitory activity of peer-reviewed and patent-described DUB inhibitors against a large panel of DUBs, Lamberto et al. (2017) identified a low-potency (Molecule 1, IC<sub>50</sub> = 10.2 μM) and noncovalent, but selective, inhibitor of USP7 (Figure 1), features lacking in previously characterized inhibitors. The lack of cocrystal structures of targeted DUBs and small-molecule inhibitors is a limiting factor in the development of potent and selective DUB inhibitors. The strength of this work resides in solving high-resolution USP7-small molecule cocrystal structures. Thus, these cocrystal structures (1) provide insights into the localization and the binding

mode of the compounds, (2) allowed the rapid development of low nM USP7 inhibitor (XL188, IC<sub>50</sub> = 90 nM, Table 1), and (3) combined with mutagenesis studies reveal determinants of selectivity, which is invaluable for the design of the next generation of USP7 inhibitors.

Importantly, the authors demonstrate that potent and selective DUB inhibitors can be achieved and that structural characterization of chemical leads with their DUB target could accelerate probe and drug discovery programs. However, the biggest potential limitation that emerges from this paper is that all of the known USP7 inhibitors (until recently) also interact with USP47 (Altun et al., 2011). It is therefore surprising that USP47 was not included in the selectivity panel conducted across the USP members (Table 1). Future studies and development of this series must include the evaluation of the inhibitors against USP47.

The method developed by Pozhidaeva et al. (2017) nicely complements the work from Lamberto et al. (2017). USP7 is unique among USPs in that its active site is catalytically incompetent and is postulated to rearrange into a productive conformation only upon binding to ubiquitin. By assigning nuclear magnetic resonances (NMRs) of the catalytic domain of USP7, the authors provide structural and mechanistic understandings into USP7 inhibition. P22077 and P50429 (Figure 1) were the most specific USP7 inhibitors available to date and were thus chosen to investigate the molecular mechanism of USP7 inhibition. By combining NMR, mass spectrometry, and mutagenesis methods, Pozhidaeva





**Figure 1. Chemical Structures of Selected USP7 Inhibitors**

et al. (2017) demonstrated that both inhibitors target the active site of USP7 and covalently modify only the catalytic Cys223 residue. Remarkably, the inhibitors appear to mimic the interaction between the ubiquitin flexible tail and the active site, causing conformational changes in the active site confirming the X-ray crystallography studies (Kategaya et al., 2017; Pozhidaeva et al., 2017; Figure 2). Furthermore, mass spectrometry experiments revealed that, even if both compounds are chemically similar, the different substructure of each inhibitor is transferred to the protein. The proposed

mechanism of action was then confirmed *in vitro* and in cells.

P22077 and P50429 were the most specific USP7 inhibitors available; however, they both inhibit the close homolog USP47 (Table 1). The protein sequence alignment confirmed that the residues in the catalytic cavity are highly conserved within the USP family. Interestingly, only five residues are found in both enzymes and represent a potential access to the selectivity between USP7 and USP47. Earlier this year, two papers reported the discovery of new USP7 inhibitors (Kategaya et al., 2017; Turnbull et al., 2017).

The study conducted by Pozhidaeva et al. (2017) presents insights into dynamic conformational changes and transient interactions with and within USP7 inhibitors, because it used NMR rather than crystal structure, which gives only a snapshot of a low-energy conformation of a protein structure. By using the same kind of approach, it would be interesting to decipher the mechanism of action of the latest described three noncovalent USP7 inhibitors. The NMR-based approach could give novel insights that may have been overlooked, fully confirm, or in part provide new information regarding the small-molecule protein interaction useful for future inhibitor design.

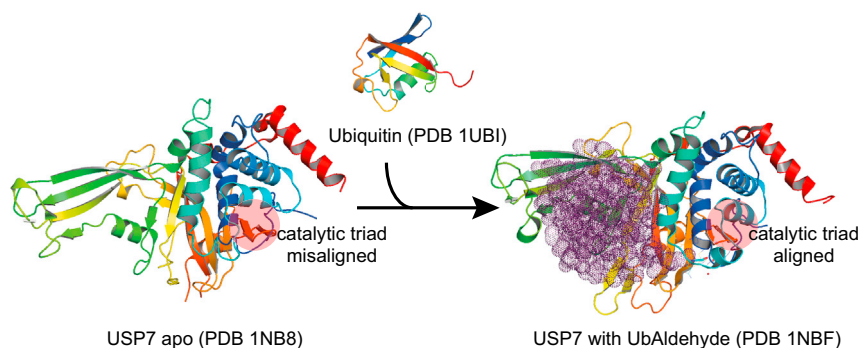
The UPS has been implicated in a very wide range of human diseases and, similarly to kinases in the past, represents the next class of drug targets. The growing availability of indispensable new tools and assays is paving the way for developing new promising anticancer therapeutics. Since the first publications of a USP7 inhibitor six years ago (Altun et al., 2011; Reverdy et al., 2012), which drove the academic understanding of USP7, three novel inhibitors have been published within the last three months. The understanding of the mechanisms around the interaction between the molecule(s) and enzyme in conjunction with the

**Table 1. Features of Selected USP7 Inhibitors**

Compound ID	Binding Mode	Selectivity (# Recombinant DUBs Tested)	USP7 / USP47 (IC <sub>50</sub> , μM)	Year of Publication
P22077	Covalent	17	8 / 8.7	2011
P050429	Covalent	7	0.42 / 1.0	2012
P0591	Covalent	9	4.2 / 4.3	2012
HBX 19,818	Covalent	8	28.1 / ?	2012
XL188	Non-covalent	41	0.09 / ?	2017
FT671	Non-covalent	41	0.052 <sup>a</sup> / >50	2017
GNE-6640	Non-covalent	37	0.75 / 20.3	2017
GNE-6776	Non-covalent	37	1.34 / >200	2017

Summary table including the number of recombinant DUBs tested in a ubiquitin-based substrate turnover assay, the compared inhibitory capacity (IC<sub>50</sub>) of USP7 and USP47, and the year of publication.

<sup>a</sup>Catalytic domain was assayed.



**Figure 2. Schematic Illustration of the Apo-structure of USP7's Catalytic Domain (PDB 1NB8) Having Its Catalytic Triad Misaligned before Ubiquitin Binding (PDB 1UBI) and Aligned upon Binding of Ubiquitin (PDB 1NBF)**

crystal structure of USP7 and NMR method could give the final push toward finding molecules that could make the leap into clinical trials and future therapies for patients.

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## A Rhomboid in the Rough: Potent Inhibitors for a Previously Undruggable Target

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In this issue of *Cell Chemical Biology*, Tichá et al. (2017) report a novel class of highly potent and selective rhomboid inhibitors. Rhomboids are implicated in several devastating human afflictions including malaria, diabetes, cancer, and Parkinson's disease (PD), making them important drug targets for future therapeutics.

The rhomboid protein family comprises a unique class of active intramembrane serine-type proteases. Rhomboids were first identified through the genetic screening efforts that won Edward Lewis, Eric Wieschaus, and Christiane Nüsslein-

Volhard the 1995 Nobel Prize in Physiology or Medicine, for uncovering the genes that control embryonic development in *Drosophila*. The name “rhomboid” refers to the diamond-shaped heads of mutant embryos. Subsequently,

it became clear that rhomboids were responsible for regulating epidermal growth factor receptor (EGFR) signaling, but the mechanism was not revealed until two breakthrough back-to-back papers published in *Cell* in 2001 from the group

