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Ubiquitin-based anticancer therapy: Carpet bombing with proteasome inhibitors vs surgical strikes with E1, E2, E3, or DUB inhibitors ☆

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ABSTRACT

The proteasome inhibitor bortezomib remains the only ubiquitin pathway effector to become a drug (VELCADE®) and has become a successful treatment for hematological malignancies. While producing a global cellular effect, proteasome inhibitors have not triggered the catastrophe articulated initially in terms such as "buildup of cellular garbage". Proteasome inhibitors, in fact, do have a therapeutic window, although in the case of the prototype bortezomib it is small owing to peripheral neuropathy, myelosuppression and, as recently reported, cardiotoxicity [1]. Currently, several second-generation molecules are undergoing clinical evaluation to increase this window. An alternative strategy is to target ubiquitin pathway enzymes acting at non-proteasomal sites—E1, E2, and E3, associated with ubiquitin conjugation, and deubiquitylating enzymes ("DUBs")—that act locally on selected targets rather than on the whole cell. Inhibitors (or activators, in some cases) of these enzymes should be developable as selective antitumor agents with toxicity profiles superior to that of bortezomib. Various therapeutic hypotheses follow from known cellular mechanisms of these target enzymes; most hypotheses relate to cancer, reminiscent of the FDA-approved protein kinase inhibitors now marketed. Since ubiquitin tagging controls the cellular content, activity, or compartmentation of proteins associated with disease, inhibitors or activators of ubiquitin conjugation or deconjugation are predicted to have an impact on disease. For practical and empirical reasons, inhibitors of ubiquitin pathway enzymes have been the favored therapeutic avenue. In approximately the time that has elapsed since the approval of bortezomib in 2003, there has been some progress in developing potential anticancer drugs that target various ubiquitin pathway enzymes. An E1 inhibitor and inhibitors of E3 are now in clinical trial, with some objective responses reported. Appropriate assays and/or rational design may uncover improved inhibitors of these enzymes, as well as E2 and DUBs, for further development. Presently, it should become clear whether one or both of the two general strategies for ubiquitin-based drug discovery will lead to truly superior new medicines for cancer and other diseases. This article is part of a Special Issue entitled: Ubiquitin Drug Discovery and Diagnostics.

1. Introduction

Molecular-based treatment options for cancer patients have historically consisted of antimetabolites (Gemcitabine/Gemzar®), topoisomerase poisons (CPT11/Camptostar®), tubulin stabilizers (Paclitaxel/Taxol®), and, more recently, protein kinase inhibitors (Imatinib/Gleevec®), with several additional classes undergoing clinical evaluation (e.g., HDAC inhibitors, phosphatase inhibitors, PARP inhibitors). The era of molecular oncology drugs began in 2001 with the FDA approval (for chronic myelogenous leukemia) of Gleevec®, an inhibitor of the protein kinase Bcr-Abl, and, shortly thereafter, of other protein kinase inhibitors (e.g., Tykerb®, Tarceva®, Nexavar®) for various malignancies. These kinase inhibitors were the first of a new class of antitumor agent (targeted effector) that was developed to deliver efficacy accompanied by a reduced number and severity of side effects, compared with those of traditional toxic chemotherapeutic agents. The attractiveness of this therapeutic strategy resides in the existence of a multitude of kinases, each with an identifiable function critical to cell growth, suggesting that selective pharmacologic action is possible. The new, molecular targeted drugs impact various signaling pathways by post-translational modification—addition (kinases) or removal (phosphatases) of a phosphate group to or from a serine, threonine, or tyrosine of a target protein, resulting most often in activation or deactivation of an enzyme. A parallel, but more complex post translational modification strategy is afforded by the ubiquitin–proteasome pathway, in which the 76-amino acid protein tag ubiquitin is conjugated to (by sequential action of the E1 activating enzyme, E2 conjugating enzyme, and E3 ligase) or deconjugated from ε-amino groups of lysines of specific target proteins. Consequences of ubiquitylation/de-ubiquitylation include changes in cellular half-life (and thus activity) of target proteins, cellular compartmentation, and trafficking between cytosol and nucleus or between the cell membrane and vesicles containing proteases. As in the case of

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kinases/phosphatases, selective action is possible owing to the large numbers of E3 ligases (several hundreds) and DUBs (approximately eighty). However, in contrast to phosphorylation, the ubiquitin pathway includes additional levels of complexity. Firstly, a single ubiquitin may be conjugated to a target protein or to a ubiquitin already conjugated to a protein, resulting in poly-ubiquitin chains. Moreover, the poly-ubiquitin chain linkage may be linear or branched, and may involve one of several lysines contained in the ubiquitin molecule (e.g., K63, K48, K11). Consequently, the potential for selective binding of a ubiquitin pathway enzyme and/or an enzyme inhibitor to the ubiquitylated target protein must be considered in a more 3-dimensional setting. The ubiquitin-proteasome pathway offers a third target class—the proteasome itself, which receives poly-ubiquitylated proteins and degrades them utilizing various proteolytic activities. During the past two decades, these three molecular target classes (two that are relatively selective and one that is nonselective) have been studied and exploited for anticancer drug development. In fact, it was the global target, the proteasome, not the target-selective DUBs or ligases, which produced the first positive drug discovery results and the first efficacious ubiquitin pathway drug for cancer treatment, bortezomib, in 2003.

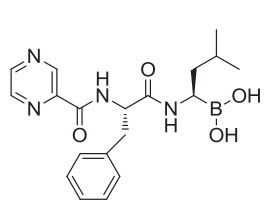
The following is not meant to be a comprehensive accounting of the current state of ubiquitin pathway-based drug development, but rather an impressionistic view of the field in 2012 with some necessary citations. It is focused on cancer, though ubiquitin is relevant to all cell processes and thus all pathologies, and it considers in order the proteasome, the E1–E2–E3 enzyme sequence, and the deubiquitylation reaction as potential or actual sources of new ubiquitin drugs.

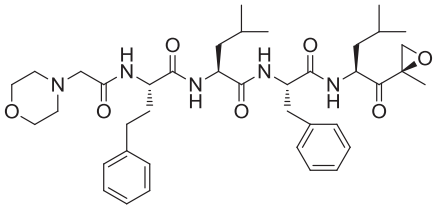
2. Proteasome inhibitors

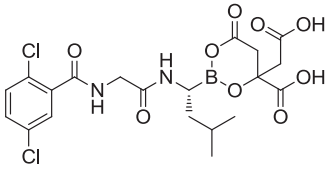
2.1. Bortezomib: an unanticipated success story

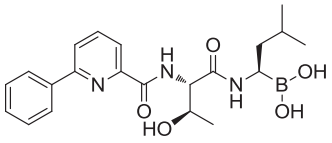
The simple consequence of blocking cellular protein degradation in the proteasome is the accumulation of ubiquitylated proteins of all sorts, which appears upon first consideration to be an intolerable consequence for any cell. The proteasome was thought of as the cell's garbage disposal unit, and its blockade assumed to result in a huge excess of unwanted proteins—a toxic event. On the contrary, however, this global response has been harnessed to provide antitumor activity against multiple myeloma and mantle cell lymphomas in patients, accompanied by manageable toxicity. In 2003 [1], bortezomib, a dipeptidylboronic acid that binds reversibly to the β5 subunit of the 20S degradation chamber of the proteasome and thereby inhibits it, was approved by the FDA for treatment of relapsed refractory multiple melanoma, following impressive activity in clinical trials [2]. Subsequently, bortezomib was approved for first line treatment of multiple myeloma and for the treatment of relapsed mantle cell lymphoma [3]. This compound had been studied extensively during preclinical and clinical development to elucidate its mechanisms of cytotoxicity and its preference for tumor *vs.* normal cells (reviewed in [4]). Because of bortezomib's clinical success, mechanism studies of this drug and other proteasome inhibitors have received less attention than the clinical studies, as bortezomib is active in numerous combinations and may have expanded clinical utility. Nevertheless, details of mechanism are emerging. As expected, numerous proteins responsible for apoptosis (e.g. p53) were found to be degraded by the proteasome and it is now clear that inhibition of proteasome activity leads to apoptosis by sparing these proteins. Proteasome inhibitors may also induce apoptosis indirectly by inhibiting NF-κB activation [5], thereby preventing transcription of various anti-apoptotic proteins. In addition, cellular mechanism studies have suggested that bortezomib inhibits angiogenesis, which could contribute to its antitumor activity [6], is efficacious in various combination therapies, and can overcome resistance to traditional cytotoxic therapies [7]. Thus, although bortezomib has a reasonable therapeutic index, it is a pleiotropic cytotoxic drug (producing peripheral neuropathy and other toxicities commonly associated with chemotherapeutic agents). Its therapeutic window is very narrow, as dose-limiting toxicities are evident just above the treatment dose. Moreover, due in part

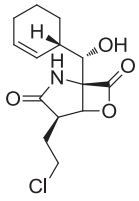
Table 1
Proteasome inhibitors currently in clinical development or approved by the FDA.

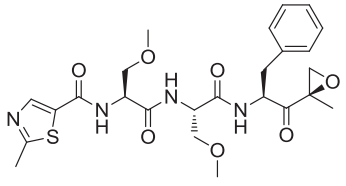

Bortezomib/VELCADE®


Carfilzomib


MLN-9708


CEP-18770


Marizomib / NPI0052


PR-047/ ONX 0912

Compound	Mechanism	Preferred Site	Phase
Bortezomib/VELCADE®	Reversible	Chymotrypsin-like	Approved by FDA
Carfilzomib	Irreversible	Chymotrypsin-like	NDA[a]
MLN-9708	Reversible	Chymotrypsin-like	Phase I-II
CEP-18770	Reversible	Chymotrypsin-like	Phase I-II
Marizomib / NPI0052	Irreversible	Chymotrypsin-like, trypsin-like	Phase I
PR-047/ ONX 0912	Irreversible	Chymotrypsin-like	Phase I

[a]FDA decision expected summer 2012

to mutations in the β5 chymotrypsin-like catalytic subunit of the proteasome, resistance to bortezomib is becoming evident [8,9].

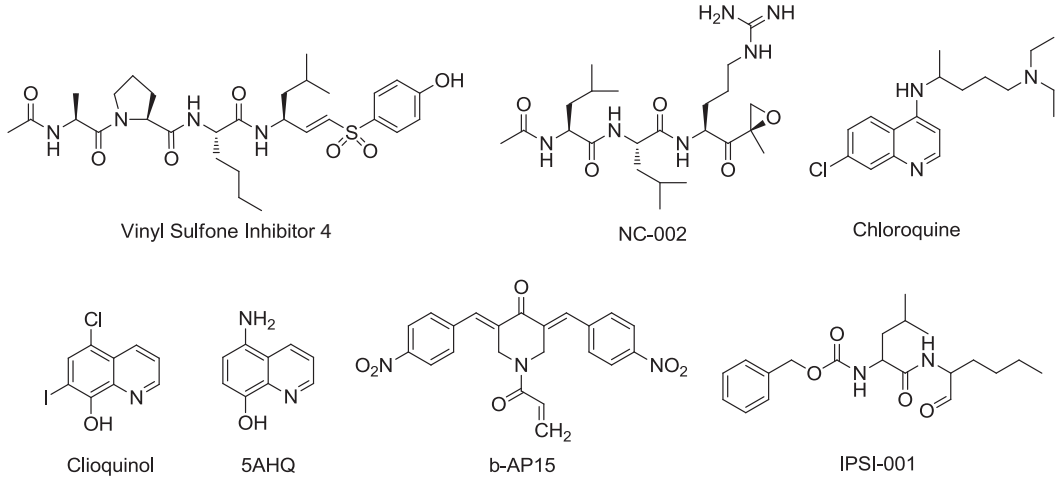
2.2. Other proteasome inhibitors in clinical trial

While MLN-9708 and CEP-18770 are both reversible inhibitors like bortezomib, they offer the potential advantage of oral administration, which may improve patient experience even though they may not be superior to bortezomib in therapeutic index or susceptibility to resistance. However, other latter-generation proteasome inhibitors now in clinical trial (Table 1) may have improved therapeutic index and in part due to a different mode of binding a diminished tendency to be compromised by resistance as compared with bortezomib [9]. To address these clinical challenges, two proteasome-related strategies have emerged. The first strategy addresses the binding mode. For example, carfilzomib, the next inhibitor scheduled for FDA review, is an *irreversible* binder to the chymotrypsin-like β5 site, in contrast to bortezomib, which binds reversibly. The epoxyketonecarfilzomib is a more potent inhibitor than bortezomib, contributing to an improved therapeutic index. The second strategy exploits proteasome heterogeneity. In addition to the classical ("constitutive") proteasome, which is present in all cells, some cells contain a second proteasome that differs from the constitutive proteasome in the β1, β2, and β5 catalytic subunits (denoted as β1i, β2i, and β5i for the immunoproteasome) and contains an 11S regulatory particle [10]). Because this second proteasome type can be induced by interferon and generates antigenic peptides for the immune response, it is known as the *immunoproteasome*. The heterogeneity strategy is also exemplified by carfilzomib, which inhibits both constitutive proteasomes and immunoproteasomes and overcomes bortezomib resistance in preclinical models [11]. It is interesting to note that the most potent experimental immunoproteasome inhibitor, IPSI-001, preferentially targets the β1i subunit of the immunoproteasome and, like carfilzomib, overcomes resistance to bortezomib [12]. An orally bioavailable truncated version of carfilzomib, ONX 0192, is in Phase I clinical trial. Another potential means of overcoming resistance to bortezomib lies in the ability of the irreversible Phase I proteasome inhibitor NPI0052 which in contrast to bortezomib inhibits the trypsin-like *as well as* chymotrypsin-like protease activity [13].

2.3. Experimental (preclinical) proteasome inhibitors

Proteasome inhibitors currently in preclinical development are addressing the issues of therapeutic index (including the need for activity in a broad spectrum of solid tumors) and resistance in various ways. Following up on an observation in the Goldberg laboratory suggesting that allosteric interactions among the proteasome subunits may offer additional therapeutic strategies [14], Kisselev et al. have developed inhibitors that are selective for β1 (caspase-like [15]) or β2 (trypsin-like [16]) subunits and have shown that trypsin site-selective inhibitors can sensitize myeloma cells to chymotrypsin-like site inhibitors such as bortezomib and, in combination with caspase-like site inhibitors, inhibit cell growth in the absence of chymotrypsin-like site inhibitors [16]. Other studies have identified various known small molecules (e.g., chloroquine and more potent substituted chloroquines such as 5AHQ) as allosteric inhibitors with clinical potential (reviewed in [10]) (Table 2). It is anticipated that allosteric proteasome inhibitors similar to these will enter clinical trial within the next few years. Another class, represented by clioquinol, may work by binding metals that are essential to the proteasome [10]. Finally, deubiquitylating activity resident in the 19S regulatory portion of the intact proteasome has recently been identified as a novel anticancer target using the small molecule b-AP15, found in a functional screen [17]. This compound selectively inhibited the deubiquitylating activity of the 19S associated DUBs UCH-L5 and

Table 2
Experimental proteasome inhibitors.



Compound	Binding Site	Mechanism
IPSI-001	β1i	Unknown
Vinyl Sulfone Inhibitor 4 [14, 15]	Caspase-like (β1, β1i); selective for β1i	Irreversible catalytic
NC-002[16]	Trypsin-like (β2, β2i); equipotent vsβ2, 2i	Irreversible catalytic
Chloroquine[b], 5AHQ [10]	Allosteric; between α and β subunits	Noncompetitive.
Clioquinol	Binds metals (Cu)	Unknown; may be several
b-AP15	Proteasome associated DUBs UCH-L5 and USP14	Inhibits 19S regulatory protein deubiquitylating activity

[b]has additional targets unrelated to the proteasome

USP14, resulting in a blockade of proteasome activity; it thus represents a very early but promising class of proteasome inhibitor.

An alternative strategy to targeting the proteasome is to target enzymes upstream of the proteasome. In general these enzymes are considered attractive targets due to the fact that there are multiple enzymes that modulate ubiquitin conjugation and deconjugation. Thus, targeting these enzymes offers a greater degree of specificity and therefore a reduced potential for side effects. Below we discuss the current status of small molecule therapeutics that target ubiquitin conjugation (E1, E2 and E3) and deconjugation (DUBs).

3. E1, E2, and E3 ligase inhibitors

The three enzymes sequentially involved in target protein ubiquitylation (E1 activating, E2 conjugating, and E3 ligase enzymes) are currently active targets in anticancer drug discovery [18]. Eight human E1s, which activate ubiquitin or a ubiquitin-like protein and transfer it to the E2 conjugating enzyme, are known [19]. Several dozen E2 enzymes and several hundred E3 ligases are known, although it is not clear how many of these are potentially therapeutically relevant. Nevertheless, data presented below support the argument that selective inhibitors of E1, E2, and E3 enzymes can be found or designed.

3.1. E1 activating enzyme inhibitors

E1 catalyses the first step in the conjugation of ubiquitin or a ubiquitin-like protein to a target protein—an ATP-dependent covalent attachment of the ubiquitin or ubiquitin-like protein molecule to its active site cysteine [18]. An adenosine sulfamate analogue, MLN4924, inhibits the E1 enzyme responsible for NEDDylation, the covalent addition of an ubiquitin-like protein, NEDD8, to specific target proteins including SCF^{Skp2} [20], an E3 ligase linked to cell cycle regulation. In the case of Skp2, NEDDylation results in pro-growth activation, and MLN4924 is currently in Phase II clinical trial for hematologic cancers (Table 3). Experimental inhibitors of E1 have also been reported, for example PYR-41, an irreversible ubiquitin E1 active site binder that enters cells and, while possibly too reactive to be a clinical candidate, is nonetheless useful as a tool compound [21] (Table 4).

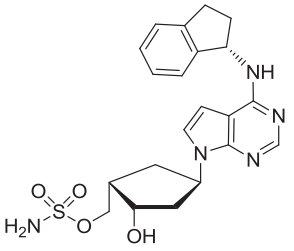
3.2. E2 conjugating enzyme

Ubiquitin activated by E1 is transferred to a cysteine of the E2 enzyme. E2 then interacts with an E3 ligase, which binds the target protein and transfers the ubiquitin from the E2 cysteine to a target protein lysine [18]. Recently a small molecule selective allosteric site inhibitor of the E2 enzyme hCdc34, named CC0651, was reported [22]. Cdc34 ubiquitylates p27, among other target proteins, and inhibition of p27 ubiquitylation and degradation is predicted to prevent tumor cell cycle progression. Thus, compounds such as CC0651 are in preclinical development as potential anticancer agents.

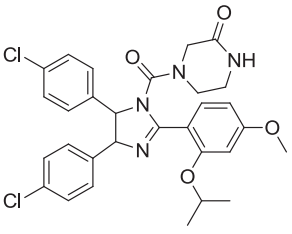
3.3. E3 ligase

The E3 ligase is responsible for determining which target proteins are ubiquitylated in concert with E1 and E2; as approximately six hundred E3 ligases are known, selective inhibition of a given E3 ligase is likely to affect a limited number of cellular proteins, which under most circumstances translates to less complicated pharmacology and potentially limited side effects. The majority of E3 ligases do not

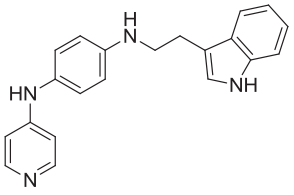
Table 3
E1, E2, or E3 inhibitors currently in clinical development.



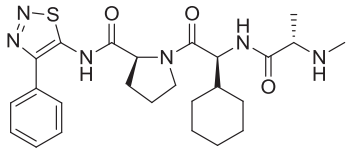
MLN4924



RO5045337 (RG7112, Nutlin-3)



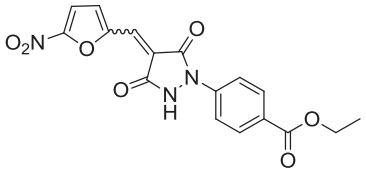
JNJ-26854165 (serdemetan)

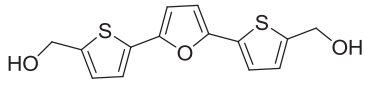


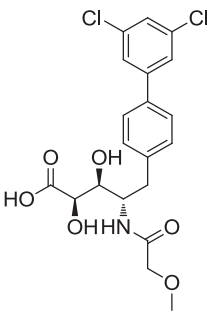
GDC-0152

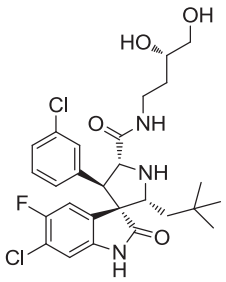
Compound	Mechanism	Phase
MLN4924	Targets E1: Forms covalent adduct with NEDD8, a ubiquitin-like protein, inhibiting E1 activating enzyme for NEDDylation	Phase II
RO5045337 (RG7112, Nutlin-3)[23]	Targets E3: Antagonizes MDM2-p53 interaction by binding in the MDM2 pocket	Phase I
JNJ-26854165 (serdemetan)[23]	Targets E3: Antagonizes regulation of p53 by MDM2	Phase I
Smac mimetics GDC-0152[24]	TargetE3: Antagonize E3 ligase IAPs, inducing self-ubiquitylation, degradation	Phase I, II

Table 4
Experimental E1, E2, or E3 inhibitors.









Compound	Enzyme	Binding Site	Mechanism
PYR-41	E1	May block active site cysteine	Irreversible inhibition of ubiquitin activation
CC0651	E2	Allosteric site on E2 enzyme Cdc34	Prevents ubiquitin transfer to target protein
RITA (NSC 652287)[23]	E3 MDM2	MDM2 binding domain of p53	Inhibits MDM2-p53 binding
MI-219[28]	E3 MDM2	p53 binding domain of MDM2	Inhibits MDM2-p53 binding

possess a classic active site, instead they mediate protein–protein interactions between the charged E2 and the protein substrate. Therefore, perhaps not surprisingly, the most advanced ligase-based drug discovery strategy to date has been the development of antagonists of E3-substrate binding. Attempts to find an antagonist of the E3 ligase MDM2/HDM2 (HDM2 being the human enzyme) were among the earliest of these exercises, as MDM2 seemed to be a perfect anticancer target, being responsible for ubiquitylating the tumor suppressor pro-apoptotic protein p53.

The prevailing view was that even though fewer than 50% of tumors possessed functional p53, that number represented a huge patient population that would benefit from therapy directed against MDM2. Inhibition of MDM2 was one of many molecular oncology strategies employed in the last 10–15 years to maximize p53 presence and activity in tumors ([28]; reviewed in [25]), and the two E3 ligase antagonists currently in clinical trial for cancer, RO5045337 (nutlin-3) and JNJ-26854165 (Table 3), are directed at MDM2, specifically, at the regulation of its substrate, p53 [23]. Several compounds inhibiting members of a family of E3 ligases known as anti-apoptotic proteins (IAPs) have also recently entered clinical trial [24]. These IAPs ubiquitylate proteins that are essential to apoptosis, eliminating their function and, thus, blocking apoptosis. Analogs of Smac, a naturally occurring protein that binds to the IAP, triggering its auto-ubiquitylation, subsume this function and restore apoptotic activity. It was speculated in an opinion piece published in 2005 [26] that the nutlin compounds might not progress to clinical trial owing to poor animal efficacy and that E3s in general are perhaps too complex for drug discovery. The fact that 7 years later there are two MDM2-p53 binding inhibitors (including a nutlin) and seven IAP antagonists in Phase I/II clinical trial suggests that E3 ligases constitute a viable, if not the most facile drug discovery area.

In addition to MDM2 and the IAPs, at least nine E3 ligases have been linked to cancer; most of them, like MDM2, act as oncoproteins by inhibiting apoptosis or promoting cell cycling, so inhibitors would be potential anticancer drugs. Although efforts have been made to find inhibitors of these and other E3s (ubiquitylation endpoint), the search is complicated by the participation of three or four enzymes in the conjugation reaction [18]. To date no drugs targeting additional E3 ligases have entered the clinic, although several such inhibitors have been reported and are useful as tool compounds (Table 4). For a comprehensive list of potential E3 ligase anticancer targets, see [27].

Thus, for all of the enzymes involved in conjugating ubiquitin to a protein, small molecule inhibitors are being developed, and, in the case of E1 and E3 enzymes, are being evaluated in the clinic for treatment of cancer. While the clinical progression of bortezomib was relatively rapid, data to date suggest that it may take longer to determine whether efficacious drugs will come from inhibitors of ubiquitin conjugation.

4. DUB inhibitors

There are approximately eighty known DUBS, proteases that hydrolyze isopeptide or α-peptide bonds linking ubiquitin to its target protein (in some cases another ubiquitin). Many of these have been validated as targets for cancer and other diseases [27,37,38]. DUBs serve to recycle ubiquitin monomers to prevent proteasomal degradation of proteins tagged with ubiquitin, and to trim ubiquitin from tagged proteins [18,39]. Proteasome-associated DUBs are a very recently described class of anticancer target; inhibitors of these enzymes are discussed above in the *Proteasome* section. It is the second function–*sparing of target proteins by the removal of conjugated ubiquitin*–that has made DUBs attractive targets for cancer and other diseases [18]. If the DUB's target protein is beneficial, for example, a tumor suppressor, DUB activity would be therapeutically advantageous as it would increase the half-life of the beneficial protein. In this case, activators of the DUB would be therapeutically useful, and there are examples of well-studied DUBs that would require activation for therapy [27]. It has been difficult, however, to discover and develop enzyme activators (as compared with receptor agonists), so the opposite strategy has been more widely pursued—the use of DUB inhibitors to prevent the sparing of a deleterious target protein (for example, an oncogenic protein) upon deubiquitylation, ensuring it is degraded, thereby decreasing its cellular half-life. In the past 10–15 years, tool compounds and/or preclinical development candidate small molecule DUB inhibitors have been reported; these compounds were identified as inhibitors of several DUBs and have a range of reported selectivities with respect to other

DUBs and other cysteine proteases [31,32,35]. Four examples of these inhibitors are given below; their story is a chronicle of the state of DUB-based anticancer drug development up to the present time (Table 5).

4.1. Pan-DUB inhibitors

Among the earliest reported DUB inhibitors are cyclopentenone prostaglandins that induce apoptosis and also increase the cellular content of poly-ubiquitylated proteins [29]. The latter suggests they are nonselective DUB inhibitors. A small molecule discovered in screening for DUB inhibitors, PR-619, is selective for DUBs over other cysteine proteases, but inhibits all DUBs tested with moderate potency. PR-619 is cell permeant and thus is useful as a tool compound [30]. More recently, HBX 41,108 which was originally reported to be a USP7 inhibitor was confirmed to be a non-selective DUB inhibitor [31,32].

4.2. UCH-L1 inhibitors

Considerable work has been performed on a series of UCH-L1C-terminal DUB inhibitors [33]. UCH-L1 is one of the earliest described DUBs and it has been of therapeutic interest in both neurodegenerative disease and cancer [40]. Like the pan-DUB inhibitors, these inhibitors are of moderate potency and enter cells; in addition, many are selective for UCH-L1, making them valuable tool compounds for translational research in cancer and neurodegenerative disease.

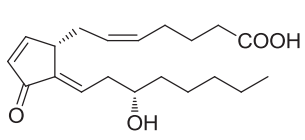
4.3. USP7/HAUSP inhibitors

A classic example of the strategy of ablating oncoproteins by inhibiting the DUBs that protect them from ubiquitylation and degradation is exemplified in the search for suitable inhibitors of USP7/HAUSP, one of the first therapeutically relevant DUBs to be described and arguably the most actively pursued DUB target in cancer drug discovery today [41–43]. Although USP7, like all DUBS, deconjugates ubiquitin from several target proteins, inhibition of USP7 promotes the degradation of its primary cellular target, HDM2, resulting in net p53 stabilization and activation [30,42,43]. Several series of small molecule USP7/HAUSP inhibitors including HBX 19,818 and the P005091 analog P050429 have achieved cellular proof of concept (anticipated cellular effects on MDM2 and p53-dependent protein activities [30,32,34]); some of these compounds are in preclinical development, but no compound has entered clinical trial. Other USP7 inhibitors that are not developable are nonetheless being used as tool compounds.

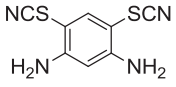
4.4. Viral DUB inhibitors

DUB inhibitor studies are not restricted to mammalian enzymes. For example, the competitive small molecule inhibitor, GRL0617

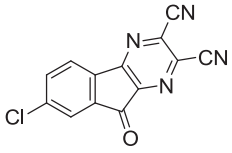
Table 5
Experimental DUB inhibitors.



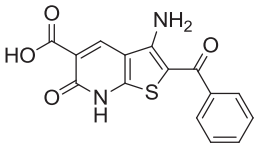
△12-PGJ$_2$



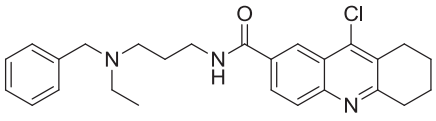
PR-619



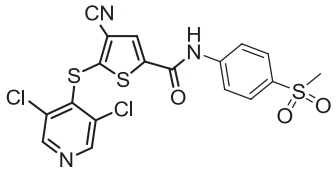
HBX 41,108



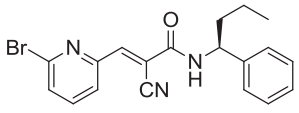
UCH-L1 inhibitor 1



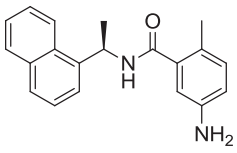
HBX 19,818



P0050429



WP1130



GRL0617

Compound	Enzyme(s)
Δ12PGJ$_2$ (J Prostaglandins)[29]	DUBs (pan)
PR-619[30]	DUBs (pan)
HBX 41,108 [31, 32]	USP7, additional DUBs
UCH-L1 inhibitor 1[33]	Ubiquitin C-terminal hydrolase-L1 (UCH-L1)
HBX 19,818[32]	USP7
P0050429[34]	USP7
WP1130 [35]	USP9X, USP5, USP14, UCH37
GRL0617 [36]	SARS Plpro

was demonstrated to inhibit the SARS Coronavirus protease and isopeptidase Papain-like protease (Plpro) *in vitro* and in cellular viral replication assays [36]. Notably the authors were able to obtain a co-crystal structure of GRL0617 bound to Plpro, which is predicted to accelerate preclinical development of this series.

Thus, while DUBs with well defined active sites appear to be simpler biochemical targets than E3 ligases, DUB-based anticancer drug development is proceeding with no more facility than is ligase-based development, to judge by compounds in the clinic or in late preclinical development.

5. Conclusions

The foregoing illustrates a major paradox in the application of the enormously complex ubiquitin proteasome pathway to cancer therapeutics, which can be articulated in the following question: why has ubiquitin-based therapy heretofore been more readily achieved by using a nonselective approach (carpet bombing with proteasome inhibitors) than by employing a selective approach (surgical strike with highly selective E3 ligase or DUB inhibitors)? A trivial answer may be that the biochemistry of certain malignancies (proteasome inhibitors have not yet demonstrated utility over a broad range of tumor types) makes them hypersensitive to proteasome inhibitor-induced apoptosis, hence a therapeutic window. An interesting alternative explanation is that sparing a large number of proteins from degradation by inhibiting the proteasome ensures that multiple pro-apoptotic, anti-survival activities are allowed to proceed, while blocking a single oncogenic DUB or E3 ligase with an exquisitely selective inhibitor has a limited effect owing to circumvention by functional redundancy or compensation by other enzymes. It is possible that inhibitors of limited, rather than absolute selectivity against DUBs or ligases will exhibit improved efficacy, as in the case of recently approved dual kinase inhibitors [44]. Ongoing work with new inhibitors of the proteasome and of ubiquitin pathway enzymes will help to answer these questions.

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