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Drug Design

Small-Molecule Control of Intracellular Protein Levels through Modulation of the Ubiquitin Proteasome System

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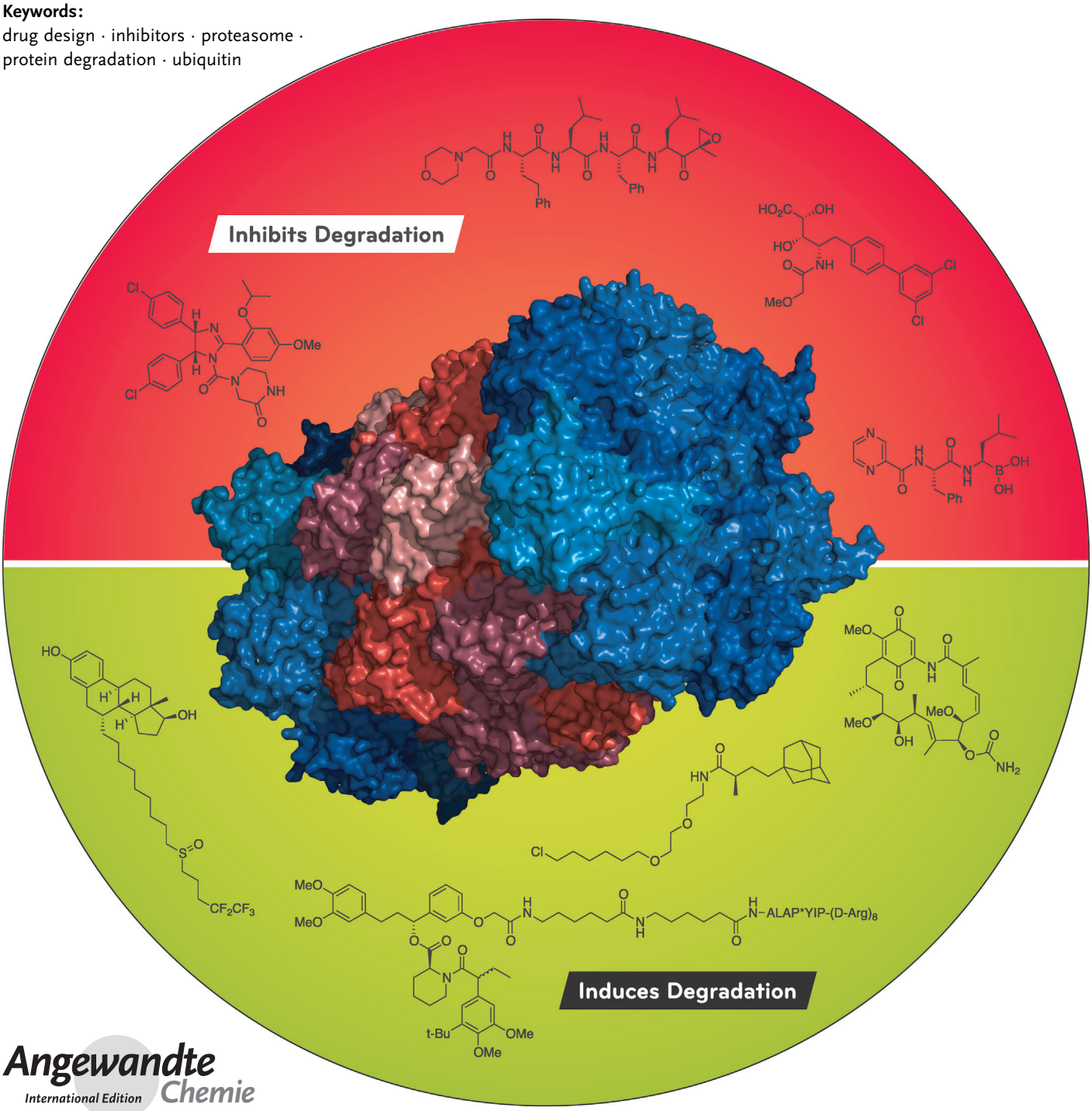

Traditionally, biological probes and drugs have targeted the activities of proteins (such as enzymes and receptors) that can be readily controlled by small molecules. The remaining majority of the proteome has been deemed "undruggable". By using small-molecule modulators of the ubiquitin proteasome, protein levels, rather than protein activity, can be targeted instead, thus increasing the number of druggable targets. Whereas targeting of the proteasome itself can lead to a global increase in protein levels, the targeting of other components of the UPS (e.g., the E3 ubiquitin ligases) can lead to an increase in protein levels in a more targeted fashion. Alternatively, multiple strategies for inducing protein degradation with small-molecule probes are emerging. With the ability to induce and inhibit the degradation of targeted proteins, small-molecule modulators of the UPS have the potential to significantly expand the druggable portion of the proteome beyond traditional targets, such as enzymes and receptors.

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1. Introduction

Current techniques in molecular biology and genetics enable virtually any gene to be knocked down or overexpressed in cell culture and in animal models. However, pharmacological control of genes is desirable both for the development of therapeutics and for biological studies owing to the improved temporal control and reversibility.[1] Unfortunately, currently available small-molecule probes and drugs are much more limited in their possible targets than genetic methods.[2] Traditionally, pharmacological probes have been limited to the targeting of enzymes and receptors that contain well-defined pockets that tightly bind small molecules. Thus, many proteins, including those that act through protein–protein interactions as scaffolds, were deemed "undruggable".[3] Finally, although small molecules that activate, rather than inhibit, protein activity are known (such as receptor agonists), they act on an even smaller subset of the proteome than inhibitors.

An alternative to the small-molecule inhibition and activation of protein activity that would more closely mimic the results of molecular-biological techniques would be the control of protein levels, which is possible at the post-translational level through the modulation of the ubiquitin proteasome system (UPS). Small-molecule control of protein levels at the level of transcription, another exciting area of research with its own set of challenges, is beyond the scope of this Review. Small-molecule inhibitors of the ubiquitination cascade or of the proteasome offer the possibility of stabilizing proteins targeted by the UPS with varying levels of specificity depending on the target. Inhibitors of deubiquitinases and chaperones that rescue proteins from proteasomal degradation offer the ability to decrease the levels of specific proteins. Additionally, multiple strategies are being developed that seek to highjack the UPS to induce the degradation of proteins not already targeted by the UPS.

1.1. Overview of the Ubiquitin Proteasome System

The ubiquitin proteasome system operates by a complex, coordinated process involving the covalent coupling of the 76 amino acid protein ubiquitin to targeted proteins, leading to their subsequent recognition and degradation by the 26S proteasome.[4] The process begins with the E1 ubiquitin-activating enzyme (UAE or Uba1), which first adenylates the C terminus of ubiquitin (consuming adenosine triphosphate (ATP) in the process) to form a reactive thioester bond with a surface cysteine residue. E1 then transfers the activated ubiquitin to an E2 ubiquitin-conjugating enzyme to form a new thioester. One of the over 600 E3 ligases then acts as an adaptor (except in the case of the HECT family of E3 ligases, which promote the formation of a third thioester intermediate) to bring the E2–ubiquitin complex into proximity with the target protein and thus facilitate the transfer of ubiquitin to a surface lysine residue to form an isopeptide bond (Figure 1). The addition of ubiquitin is also reversible through the action of various deubiquitinating enzymes (DUBs).[4a,6]

The process can then be repeated, by the transfer additional ubiquitin units to the N terminus or one of the seven lysine residues of ubiquitin. The formation of a chain consisting of at least four ubiquitin moieties linked through Lys^{48} is recognized as being sufficient to target the target protein for degradation by the 26S proteasome.[5] This process involves the removal of the ubiquitin units (which are recycled) and subsequent processive degradation of the target protein into short peptide fragments (Figure 1).[4a] However, alternative linkages of ubiquitin chains are also possible and can lead to a variety of biological consequences besides proteasome-mediated degradation. One possibility is the formation of Lys^{63} chains, which have been shown to be

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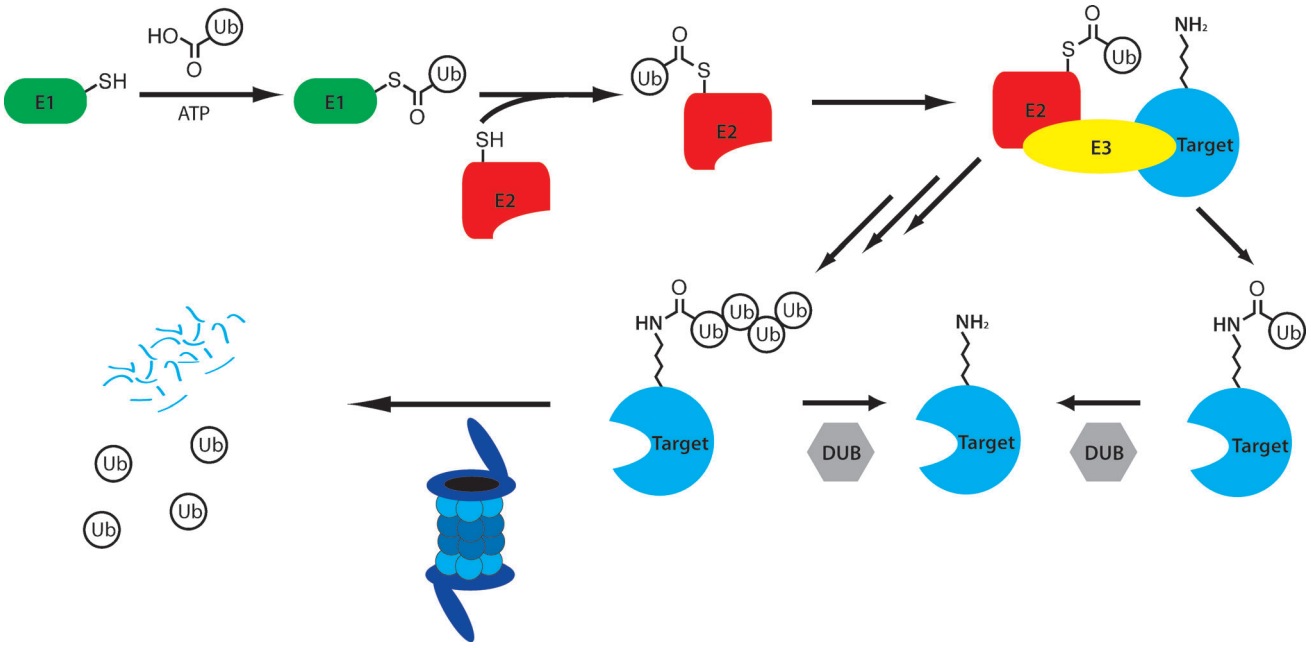


Figure 1. Summary of the ubiquitin proteasome system. Ubiquitin is activated by the E1 ubiquitin-activating enzyme and transferred to an E2 ubiquitin-conjugating enzyme. The E2 enzyme then transfers the ubiquitin to a target protein with the assistance of an E3 ubiquitin ligase, which recognizes the target protein. The process may then be repeated to form polyubiquitin chains, which bind to the regulatory particle of the 26S proteasome, thus leading to the degradation of the target protein and the recycling of the ubiquitin units.[4a,5]

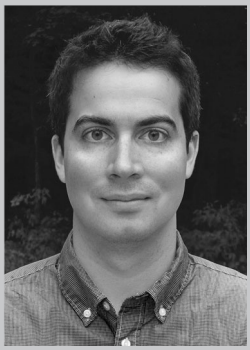
involved in the regulation of endocytosis. Monoubiquitination of proteins can have diverse biological effects, sometimes through the induction of a conformational change in the target. Additionally, numerous ubiquitin-like proteins (UBLs) have been identified, such as SUMO and NEDD8, which are ligated to other proteins through an analogous system but involving distinct enzymes. These UBLs can also have numerous effects, often through the induction of conformational changes in the target proteins.[6]

2. Small-Molecule-Mediated Protein Stabilization: Inhibitors of Protein Degradation

Owing to the multiple effects of ubiquitination on diverse biological processes (including cell-cycle progression, apoptosis, oncogenesis, protein-quality control, and angiogenesis),[7] the inhibition of proteasome-dependent degradation presents numerous attractive targets for the development of therapeutic treatment for many diseases. Each class of enzymes, ranging from the E1 and roughly 40 E2 enzymes to the over 600 E3 ligases and the multiple subunits of both the constitutive and immunoproteasomes, has both advantages and disadvantages as drug targets in terms of their biological role, specificity, and druggability.[6] Other processes (e.g., autophagy) can also control protein degradation; the small-molecule control of these systems is beyond the scope of this Review but has been recently reviewed elsewhere.[8]

2.1. Proteasome Inhibitors

Currently, there are only two FDA-approved drugs that target the UPS: bortezomib/Velcade and carfilzomib/Kyprolis, which both directly inhibit the proteasome.[6b,9] Numerous classes of proteasome inhibitors have been described,[7,9,10] including peptide aldehydes, peptidyl boronates, epoxyketones, vinyl sulfones,[11] β-lactones,[12] hydroxy-



Dennis Buckley completed his undergraduate studies at the State University of New York at Geneseo in 2008. He then joined the research group of Craig Crews at Yale University, where he recently obtained his PhD in chemistry with a focus on the development of ligands for the E3 ligase VHL and their use as biological probes.

Craig Crews studied chemistry at the University of Virginia and received his doctorate from Harvard University under the supervision of R. L. Erikson. Following a postdoctoral fellowship with S. L. Schreiber, he joined the Yale faculty in 1995. His research is focused on the small-molecule control of intracellular protein levels and led to the development of carfilzomib/Kyprolis, an FDA-approved next-generation proteasome inhibitor based on the lead compound YU101, which was discovered in his laboratory.

ureas,[13] and α-ketoaldehydes.[14] Many contain an electrophile that targets the key nucleophilic Thr[1] residues in the catalytic β1, β3, and β5 subunits of the proteasome.[15] These electrophiles are often attached to a linear or cyclic peptide chain that mimics the substrate protein. Additionally, noncovalent inhibitors have been reported, such as TMC-95A[16] and the noncompetitive imidazoline class.[17]

The first class of proteasome inhibitors identified were the peptide aldehydes,[18] of which MG132 (or Z-LLL, developed by the company Myogenics) is the most studied (Figure 2).[19]

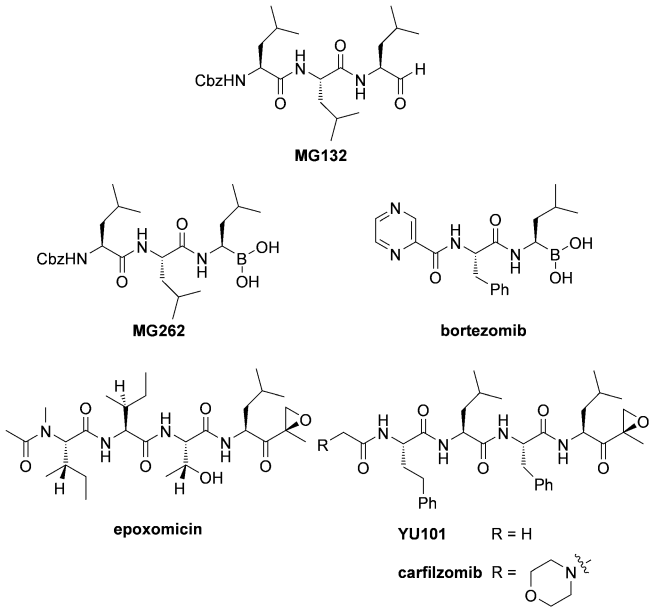


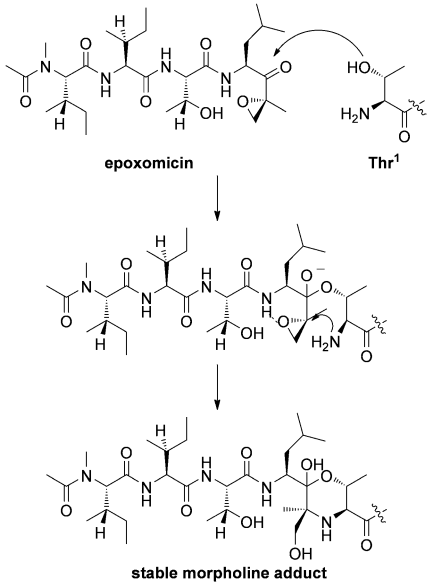
Figure 2. Proteasome inhibitors, including the widely used probe compound MG132, as well as bortezomib/Velcade and carfilzomib/Kyprolis, FDA-approved drugs for the treatment of multiple myeloma.

These compounds are potent ($K_i = 4$ nM)[19] covalent inhibitors of the chymotryptic-like activity of the β5 subunit. However, issues related to lack of selectivity towards other proteases (such as calpains and cathepsins), rapid oxidation, and the rapid reversibility of the formation of a hemiketal with Thr[1] prevented these compounds from being useful therapeutically. Nonetheless, the combination of potency and wide availability (at relatively low cost) has led to the establishment of MG132 as one of the most used proteasome inhibitors in biological studies.[7] Besides synthetic peptide aldehydes, natural products, such as fellutamide B, have also been shown to inhibit the proteasome.[20]

Studies on peptide aldehydes led directly to the discovery of the peptidyl boronates; it was found that the conversion of the aldehyde group of MG132 into a boronic acid (MG262 or Z-LLL-boronate) led to a vast improvement in potency ($K_i =$ 18 pM).[7] This increased potency enabled the development of smaller, dipeptidyl boronic acids, such as bortezomib (developed by Myogenics/ProScript). This class of compounds had the added benefit of increased specificity as compared to peptide aldehydes, as the boronic acid moiety is far less capable of reacting with cysteine proteases, such as the cathepsins and calpains.[21] Bortezomib reversibly forms a tetrahedral borate with Thr[1], as demonstrated by X-ray crystallography.[22] After being purchased by Millennium Pharmaceuticals (now owned by Takeda), bortezomib entered clinical trials in 1997 and was approved by the FDA in 2003 for use in multiple myeloma. Marketed as Velcade, it is administered intravenously or subcutaneously and is currently under investigation for the treatment of other cancers, such as non-Hodgkin lymphoma.[6b,21,23]

Another class of proteasome inhibitors are the epoxyketones. The first epoxyketones described were eponemycin[24] and epoxomicin (Figure 2),[25] natural products isolated by Bristol-Myers Squibb in the early 1990s in view of their activity against melanoma cell lines. However, the mechanism of action of these compounds remained unknown until our research group completed the total syntheses of eponemycin,[26] epoxomicin,[27] and their respective biotinylated affinity reagents, which were used to show that both compounds were potent inhibitors of the proteasome.[28] We also demonstrated that the epoxyketones reacted with Thr[1] of the proteasome to first form a hemiketal, and that the subsequent attack of the terminal amine on the epoxide led to a stable morpholine ring.[29] This two-step nucleophilic attack on the epoxyketone not only leads to irreversible inhibition of the proteasome (as opposed to the slowly reversible inhibition by peptidyl boronates, such as bortezomib), but also leads to specificity, owing to the uniqueness of the N-terminal threonine catalytic residue amongst proteases (Scheme 1).

After identifying the proteasome as the target of epoxomicin, we sought to optimize the selectivity of epoxyketones for the chymotrypsin-like activity of the proteasome over the other two primary activities, the trypsin-like activity and the caspase-like activity. A more selective inhibitor was desirable



Scheme 1. Proposed mechanism for the inactivation of the proteasome by epoxomicin. Initial hemiketal formation is followed by epoxide opening by the terminal amine to form a stable morpholine adduct, which has been observed by X-ray crystallography.[29]

as an improved chemical probe to help tease out the inhibitory effects of each of the catalytic subunits. Additionally, most proteasome inhibitors (including bortezomib and epoxomicin) already showed significant selectivity for the chymotrypsin-like activity of the β5 subunit, thus suggesting that the therapeutic effects of these inhibitors were primarily due to the inhibition of this activity. The result of this optimization was YU101,[30] which exhibited increased selectivity for the chymotrypsin-like activity over the trypsin-like activity and nearly 8000-fold selectivity for the chymotrypsin-like activity over the caspase-like activity (Figure 2). We then were able to correlate the K_{obs}/[I] ratio for the chymotrypsin-like activity with the inhibition of cellular proliferation.[31]

These results were then licensed to the company Proteolix, which modified the compound by adding a morpholine moiety to increase the solubility of the molecule. The resulting compound, carfilzomib,[32] then entered clinical trials for multiple myeloma in patients resistant to bortezomib and either thalidomide or lenalidomide. In patients with refractory multiple myeloma, carfilzomib (administered intravenously) had an overall response rate of 23.7%, with a median duration of response of 7.8 months.[33] On the basis of this phase II study and other data showing a generally comparable safety profile (to that of bortezomib) as well as significantly improved rates of peripheral neuropathy, carfilzomib was approved by the FDA in 2012, and is currently being marketed by Onyx Pharmaceuticals (which purchased Proteolix in 2009) as Kyprolis.[34]

In addition to the two FDA-approved proteasome inhibitors, there remains a strong interest in the development of improved proteasome inhibitors for clinical use. One avenue of active research is to improve the bioavailability of proteasome inhibitors; currently there are multiple orally bioavailable proteasome inhibitors in clinical trials, such as ixazomib citrate (MLN9708) from Millennium Pharmaceuticals and oprozomib (ONX 912) from the company Onyx.[35] Another active area of research is the development of inhibitors that are selective for the immunoproteasome, which is expressed in lymphocytes. Inhibition of the immunoproteasome has been suggested to be a possible strategy for the treatment of autoimmune and neurodegenerative disorders. Inhibitors such as ONX 914 show selectivity for the immunoproteasome over the constitutive proteasome. Crystallographic studies have suggested that selectivity can be attributed to the larger P1 pocket of the immunoproteasome.[36]

One concern about the use of proteasome inhibitors is that the inhibition of protein degradation leads to the formation of protein aggregates. Protein aggregation is linked to neurodegenerative disorders, such as Alzheimer's disease. However, bortezomib and carfilzomib do not readily cross the blood–brain barrier, and their effect on the central nervous system should therefore be limited,[32b,37] although, as noted above, peripheral neuropathy is a serious side effect of bortezomib treatment.

2.2. Inhibitors of the E1 Ubiquitin-Activating Enzyme and Related E1 Proteins

The E1 ubiquitin-activating enzyme (UAE) carries out its function in two discrete steps. It first adenylates the C terminus of ubiquitin and then forms a covalent thioester bond with the active-site cysteine residue. Inhibition at the E1 level would prevent ubiquitination and thus globally disrupt the UPS. It may therefore have similar effects to proteasome inhibition (as opposed to the targeting of E2, E3, or DUB enzymes, which may have more specific effects). There has been significant interest in developing E1 inhibitors, and the topic has recently been reviewed.[38]

The first cell-permeable inhibitor was the pyrazone PYR-41 (Figure 3), which was found to covalently inhibit UAE in cells and thus prevent the ubiquitination and degradation by

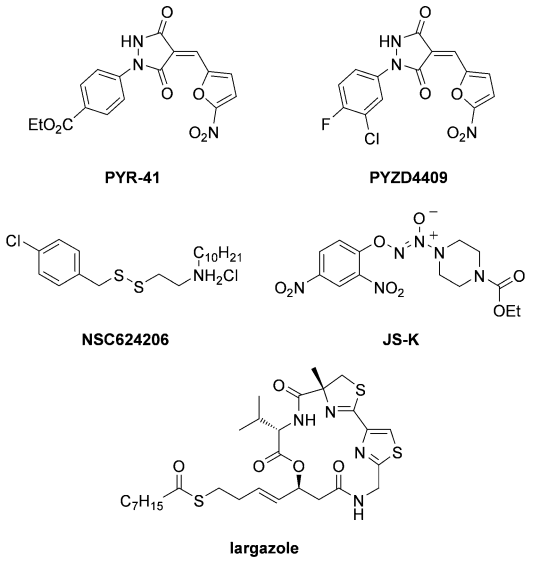
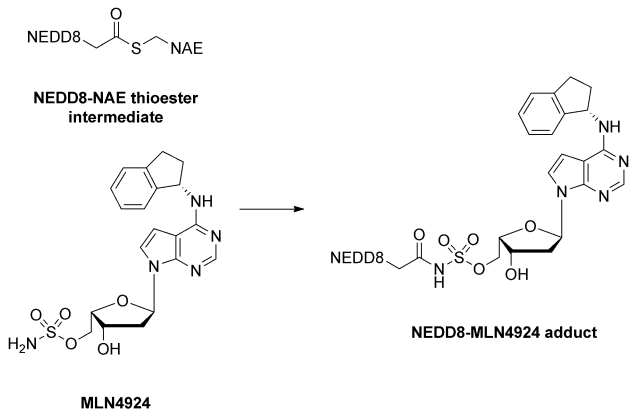


Figure 3. Small-molecule inhibitors of the E1 ubiquitin-activating enzyme.

the proteasome of target proteins, such as p53.[39] A similar inhibitor, PYZD4409, was also shown to induce cell death in malignant cells and have activity in a mouse model of leukemia.[40] Besides the pyrazone UAE inhibitors, reported inhibitors include the nitric oxide prodrug JS-K[41] and the disulfide NSC624206,[42] both of which act by modification of the catalytic cysteine residue. The natural product largazole, better known for its effects on histone deacetylases, has also been shown to inhibit UAE by blocking ubiquitin adenylation (but not thioester formation).[43] Although numerous UAE inhibitors have been developed, it is likely that new classes of inhibitors with increased specificity and druglike properties will need to be developed before they are of use therapeutically.[38]

Besides the ubiquitin-activating enzyme (UAE), there are seven other E1 proteins that activate other ubiquitin-like proteins (UBLs). Millennium Pharmaceuticals has developed a small-molecule inhibitor of the NEDD8-activating enzyme (NAE), MLN4924, which acts through an interesting mech-

anism termed substrate-assisted inhibition. MLN4924 is an adenine mimic which, in the presence of NAE, forms a covalent bond with NEDD8 through nucleophilic attack by the sulfamate group on the intermediary thioester (Scheme 2). The NEDD8–MLN4924 adduct is then able to



Scheme 2. MLN4924 is able to react with the activated NEDD8–NAE intermediate to form a non-hydrolyzable covalent bond with NEDD8. The MLN4924–NEDD8 adduct then acts as a mimic of the adenylated-NEDD8 substrate to competitively inhibit NAE.[44]

act as a potent inhibitor of NAE, as a nonhydrolyzable mimic of the adenylated-NEDD8 intermediate.[44] As the NEDDylation of cullin is important to the activity of cullin–RING ligases (CRLs), a family of E3 ligases, the inhibition of NAE has therapeutic potential.[6a,44b] As a result, MLN4924 has entered phase I and I/II trials as a potential treatment for both hematologic and nonhematologic malignancies.[45]

2.3. Inhibition of E2 Ubiquitin-Conjugating Enzymes

The approximately 40 E2 proteins are responsible for accepting ubiquitin or UBL units from the E1 enzyme and transferring them to the target protein in conjunction with the E3 ligase (or for the transfer of ubiquitin to HECT E3 ligases). Despite the existence of a catalytic cysteine residue, the development of E2 inhibitors has lagged behind that of E1 inhibitors, and only two E2 inhibitors have been reported. The first inhibitor of an E2 enzyme was described by Ceccarelli et al. It was discovered in a high-throughput screen of inhibitors of $p27^{Kip1}$ ubiquitination, which is important to cell-cycle progression and a possible target for cancer therapy. The cell-free assay reconstituted all the components of the ubiquitinylation machinery: biotinylated ubiquitin, the E1 enzyme Uba1, the E2 protein hCdc34, the E3 complex SCF^{Skp2} (as well as Cks1), and the phosphorylated substrate, $p27^{Kip1}$. CC0651 (Figure 4) was found to inhibit $p27^{Kip1}$ ubiquitination. The activity of CC0651 was found to be due to allosteric inhibition of hCdc34, as confirmed by X-ray crystallography. CC0651 was shown to inhibit the proliferation of cells and cause the accumulation of $p27^{Kip1}$.[46]

The inhibition of a distinct E2 protein, Rad6 (essential for postreplication DNA repair), was recently reported. The

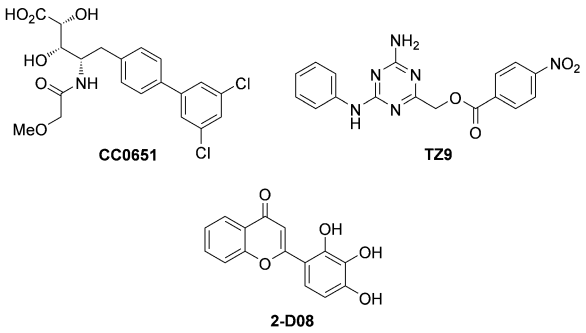


Figure 4. Small-molecule inhibitors of E2 ligases. CC0651 inhibits hCdc34;[46a] TZ9 inhibits Rad6;[47] 2-D08 inhibits the SUMO E2 UBc-9.[48]

compound TZ9 was developed by the use of a virtual screen and designed to bind to the catalytic site of Rad6. TZ9 was successful at inhibiting histone ubiquitination in vitro and inhibited cell proliferation. Unlike CC0651, which acts through an allosteric mechanism, TZ9 is predicted to block thioester formation; this mechanism of action would make TZ9 the first competitive E2-ligase inhibitor.[47] The SUMO E2 protein, Ubc-9, has also been targeted for inhibition. Schneekloth and co-workers recently reported the identification of the flavonoid 2-D08, which inhibited the transfer of SUMO from Ubc-9 to a model substrate and inhibited SUMOylation of topoisomerase-1 in a cellular assay.[48]

2.4. Small-Molecule Inhibitors of E3 Ligases

E3 ligases catalyze the addition of ubiquitin or UBLs to their target proteins. There are over 600 E3 ligases,[6b] which can be divided into four families: HECT-domains E3s, U-box E3s, monomeric RING E3s, and multisubunit RING E3s.[6a] The substrate specificity of the UPS is derived primarily from the selectivity of the E3 ligases for their targets; the E3 ligases are therefore attractive targets for the development of therapeutics. Unfortunately, most E3 proteins lack any enzymatic activity. Instead, they act by bringing ubiquitin-loaded E2 proteins into proximity with target proteins (with the exception of the HECT E3s, which form a thioester bond with ubiquitin before transferring it to their substrates). Therefore, the inhibition of E3 ligases has generally required the targeting of protein–protein interactions, which are notoriously difficult to modulate with small-molecule agents.[3]

The first E3 ligase successfully targeted was MDM2, which ubiquitinates the tumor suppressor p53. The company Roche reported the discovery of Nutlins, *cis*-imidazoline inhibitors of the MDM2–p53 interaction that stabilize p53 in cells and inhibit the growth of tumor xenographs in nude mice.[49] Since then, an orally administered Nutlin derivative, RG7112 (Figure 5),[50] has advanced to phase I clinical trials for the treatment of solid and hematological tumors.[51] An additional MDM2 inhibitor, the tryptamine JNJ-26854165, is also orally administered and in phase I clinical trials but appears to act instead by blocking the interaction of the p53–

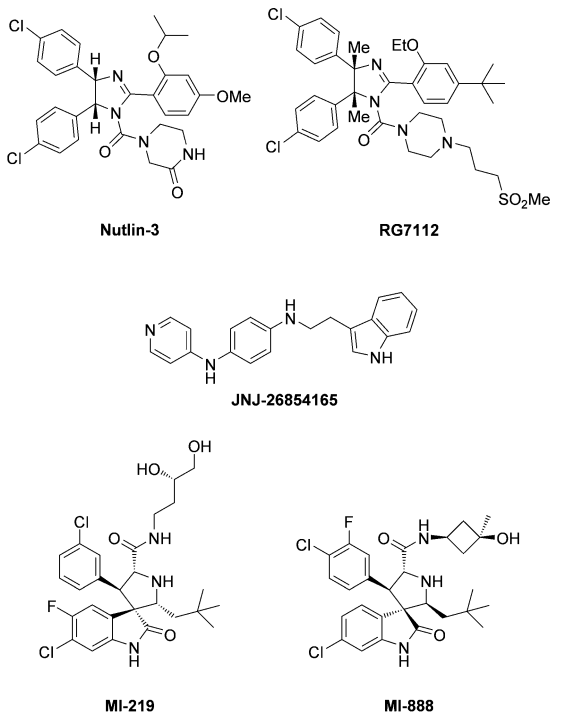


Figure 5. Nutlin, the first MDM2 inhibitor, and other selected MDM2 inhibitors.

MDM2 complex with the proteasome.[51,52] Numerous other classes of MDM2 inhibitors have been developed,[53] including the spirooxindoles, which were discovered through structure-based design[54] and include MI-219[55] and MI-888.[56] However, although p53 is an important tumor suppressor, attempts to stabilize it will have little effect on the large percentage of cancers with mutated p53.[57]

The inhibitor of apoptosis protein (IAP) family of E3 ligases has also been extensively targeted, usually with largely peptidic or peptidomimetic inhibitors inspired by the natural protein inhibitor of the IAPs, SMAC/DIABLO. The IAPs regulate apoptosis through numerous pathways, including the regulation of caspase 3, 7, and 9. Inhibitors of IAPs have recently been reviewed extensively[58] and include numerous compounds in clinical trials (Figure 6).[6b] SMAC mimics bind to the BIR3 domain of IAPs, which is distinct from the RING domain. Pioneering studies by Abbott led to the optimized peptidic ligand compound 11 (Figure 6).[59] The research group of Shaomeng Wang at the University of Michigan optimized this core structure by replacing the cyclohexylglycine–proline motif with a bicyclic structure, such as that present in SM-122.[60] An additional peptidic IAP antagonist is MV1, which was developed by the company Genentech.[61]

The SCF (SKP1, CUL1, F-box protein) E3 ligases are a subfamily of the multisubunit cullin–RING ligases (CRLs). They contain various F-box proteins, which confer substrate specificity, and often recognize post-translational modifications on their targets, such as phosphorylation (Figure 7).[64] Numerous SCF members have been targeted with small-molecule inhibitors. SCF^{Skp2} ubiquitinates numerous proteins involved in cell-cycle control, such as $p27^{Kip1}$. SCF^{Skp2}

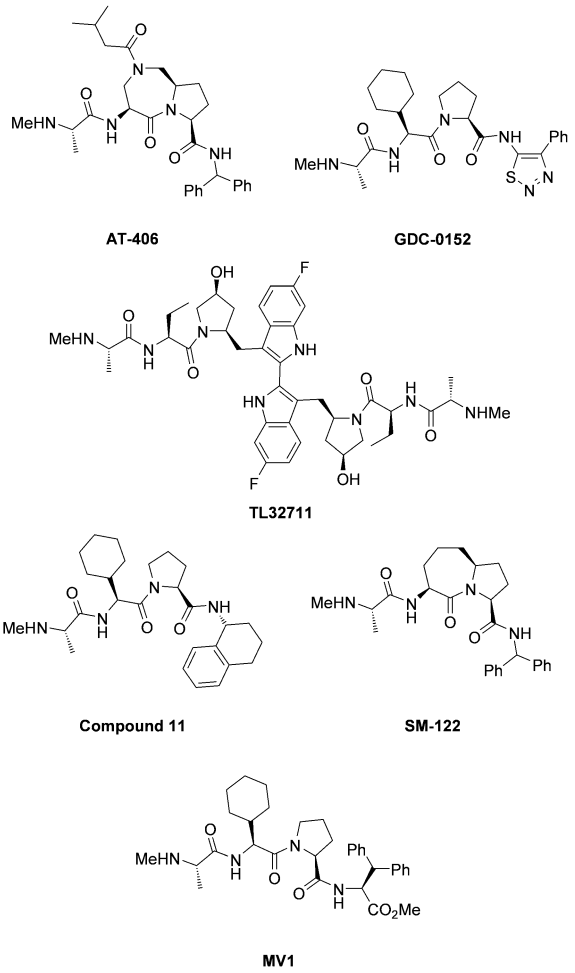


Figure 6. IAP inhibitors, including AT-406 (developed by Ascenta Therapeutics and the University of Michigan),[62] which is administered orally in phase I clinical trials for solid tumors and lymphoma, GDC-0152 (developed by Genentech/Roche), which is administered intravenously and is in phase I clinical trials for metastatic malignancies,[6b,63] and the bivalent compound TL32711 (developed by Tetralogics Pharma), which is administered intravenously.[6b,58] LCL161 (Novartis), AEG35156 and AEG40826 (Aegera), and YM155 (Astellas Pharma) are also in clinical trials but are not shown.[6b,58] Selected SMAC mimics, such as SM-122 and MV1, are also shown but are not in clinical trials.

inhibitors were developed through virtual screening to target the SCF^{Skp2}–p27 interface. Both the rhodanine C1 and the pyrrolinone C2 inhibited the ubiquitination of p27 in vitro. This inhibition led to p27 accumulation in cells and thus induced G1/S arrest in cells.[65] Recently, a class of chromones was also reported to inhibit SCF^{Skp2} by preventing the binding of Skp2 to the remainder of the SCF complex. The lead, compound 25, was found to have antitumor activity in animal models.[66] SMER3 was discovered through the use of a chemical-genetics screen of enhancers of rapamycin and found to inhibit Met4 ubiquitination by SCF^{Met30} through blockage of the interaction between Met30 and the core of the SCF complex.[67] Racemic SCFI2 was reported to inhibit the interaction between SCF^{Cdc4} and its target, phosphorylated

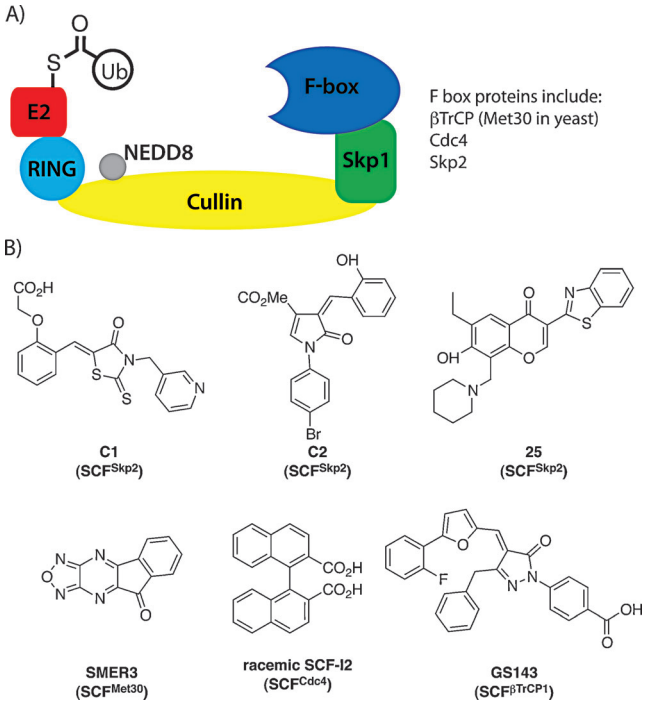


Figure 7. A) Depiction of the complex of SCF E3 ligases. The complex contains a cullin (which is NEDDylated when active), which binds a RING domain as well as the adaptor Skp1. Skp1 also binds various F-box proteins (such as βTrCP, Cdc4, and Skp2), which function as the substrate interaction motif and bind target proteins that are ubiquitinated. B) Inhibitors of SCF E3 ligases. The specific E3 inhibited is shown in parentheses.

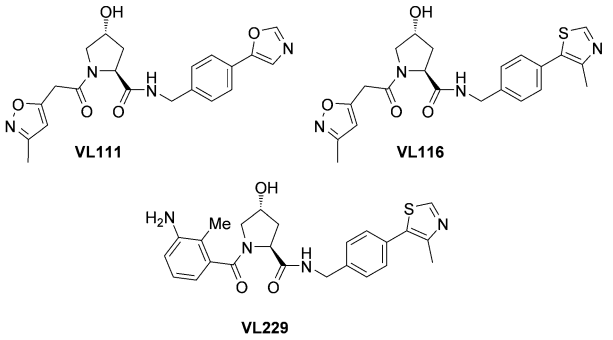


Figure 8. Structures of hydroxyproline-based molecules capable of inhibiting the interaction between VHL and a peptide derived from HIF in vitro.

Sic1. However, crystallographic studies showed that SCFI2 binds the WD40 propeller domain 25 Å away from the substrate-binding site and inhibits SCF^{Cdc4} allosterically.[67b,68] GS143 (Figure 7) was reported as a putative $SCF^{\beta TrCP1}$ inhibitor. GS143 stabilizes IκBα in cells. The authors hypothesized that it inhibits the $SCF^{\beta TrCP1}$–IκBα interaction after excluding other mechanisms for the stabilization; however, no evidence for the direct binding of GS143 to either $SCF^{\beta TrCP1}$ or IκBα was reported.[69]

Recently, we reported the design of small molecules that can competitively bind to the primary HIF-binding site on the VHL protein, the substrate-recognition subunit of a CRL. Drawing inspiration from the key hydroxyproline residue of HIF, we developed VL111 (Figure 8).[70] Guided by the X-ray crystal structures of VL111 and other early derivatives bound to VHL,[71] we optimized the VHL ligand and obtained VL116 and VL229.[72] However, although these compounds were able to inhibit the interaction between VHL and fluorescent peptides derived from HIF in vitro, we have been unable to demonstrate ligand-induced HIF-stabilization activity in cell-based assays.

Thalidomide was originally developed for its sedative properties before it was infamously discovered to be a potent teratogen that causes serious birth defects, such as phocomelia (limb defects) and amelia (the lack of one or more limbs). Although its use as a sedative was discontinued, it is still in use today for the treatment of serious disorders, such as leprosy and multiple myeloma, despite its serious side effects. Recently, a study found that thalidomide binds to and inactivates the E3 ligase cereblon (CRBN), a component of a CRL important to limb development. This finding strongly indicates a mechanism for the side effects of thalidomide, and could possibly enable the development of thalidomide derivatives that do not target CRBN.[6b,73]

2.5. Direct Small-Molecule Stabilization of Destabilized Proteins and Shield-1

Direct ligand binding can also increase the stability of a protein, through a number of mechanisms. Ligand binding commonly increases the thermal stability of a protein and thus enables the use of differential scanning fluorimetry in high-throughput screening.[75] It can also reduce the protease susceptibility of the target protein, which forms the basis of DARTS, a technique used to identify the cellular targets of small molecules.[76] However, the most striking stabilization in cells results from the use of ligand-responsive degrons.[77]

Building on their earlier use of rapamycin derivatives to stabilize mutated Frb*,[78] Wandless and co-workers mutated an FKBP12–YFP fusion protein by using error-prone PCR and subsequently analyzed fluorescence in the presence and then in the absence of an FKBP12 ligand to screen for destabilized mutants of FKBP12 that are rescued upon addition of the ligand. The L106P mutant was then analyzed further by the use of a related ligand, Shld1 (Figure 9), and was found to rescue the degradation of numerous fusion proteins in cell culture[74] and in mice.[79] This technology was then modified to enable cleavage of the degron to yield the native protein in the presence of the stabilizing ligand.[80] An alternative use of Shld1 has the opposite effect and stabilizes proteins fused with mutated FKBP and a cryptic degron. This approach involves the tethering of FKBP to a degron sequence as well as a proline-rich sequence designed to bind to the active site of FKBP. In the absence of Shld1, the degron is masked, but upon its addition, the degron becomes exposed, which leads to the degradation of the fusion protein.[81] Although these systems require genetic manipulation, they have been widely used and enable the time-

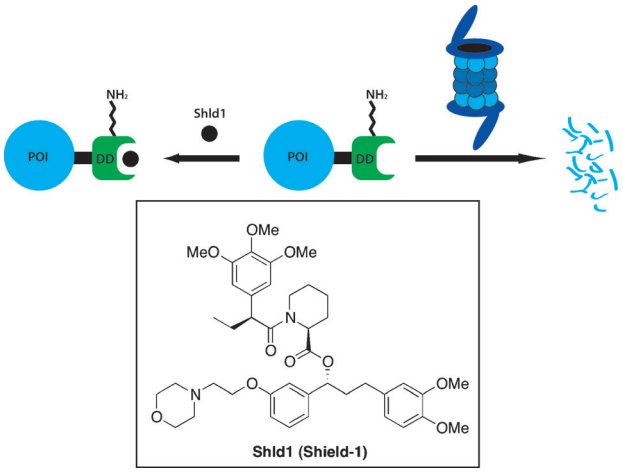


Figure 9. Shld1 is used to stabilize mutant FKBP proteins (as well as FKBP fusion proteins).[74] DD = destabilization domain, POI = protein of interest.

sensitive control of dendritic proteins[82] and transcription factors.[83]

3. Inducers of Protein Degradation

The induction of protein degradation is also a possible avenue for the development of therapeutics. Whereas enzyme inhibitors and antagonists are limited to specific subsets of proteins (i.e. enzymes and receptors), theoretically, any protein can be targeted for degradation. Proteins such as transcription factors or scaffolds, which act through protein–protein interactions (PPIs) or protein–nucleic acid interactions, have long been considered "undruggable".[3] The induction of their degradation, for example, by the use of compounds known as PROTACs, requires only a ligand capable of binding to these targets, rather than a ligand that inhibits interactions that occur across large surface areas. Additionally, whereas inhibitors and antagonists control only a specific activity of their targets, degradation of the protein would lead to a loss of function of other activities (such as scaffolding functions).[2] Outside of the field of therapeutics, the use of small-molecule probes that induce degradation is in many cases preferable to genetic knockdown with techniques such as RNAi owing to the better temporal control and reversibility.[1b]

Induced degradation can also be useful in cases in which resistance to therapeutics emerges. Mechanisms of resistance, such as protein overexpression, could theoretically be overcome by inducing protein degradation. In the case of the androgen receptor, resistance can emerge to antagonists, such as flutamide, not by prevention of the binding of the antagonist, but by mutations that convert the antagonists into agonists.[84] Small-molecule degraders avoid this issue, as the target protein would be degraded, thus preventing mutations that increase the activity of the drug from generating resistance.

3.1. PROTACs: Heterobifunctional Molecules That Recruit Specific E3 Ligases to Targeted Proteins

Proteolysis targeting chimeras, or PROTACs, are heterobifunctional molecules[85] that contain a ligand for an E3 ligase, a linker, and a ligand for a protein that is to be targeted for degradation. The molecule can then bind to both the E3 ligase and the target and thus induce the formation of a ternary complex. This hijacking of the E3 ligase can lead to the polyubiquitination of the target protein, followed by its degradation by the proteasome (Figure 10).[1]

The first PROTAC described (PROTAC-1) contained a phosphopeptide derived from IκBα, which binds to the E3 ligase $SCF^{\beta TrCP}$, as well as a moiety derived from ovalicin, which covalently binds MetAP-2 (Figure 11). PROTAC-1 was able to specifically and covalently bind to MetAP-2, which was then recruited to $SCF^{\beta TrCP}$ and ubiquitinated in vitro; however, PROTAC-1 lacked cell permeability.[86] Similar PROTACs synthesized with the same IκBα phosphopeptide targeted both the androgen receptor (AR) and the estrogen receptor (ER), but also lacked cell permeability.[87]

The first cell-permeable PROTACs (PROTAC-4 and PROTAC-5) were developed by the incorporation of a peptide derived from HIF (ALAPYIP) that binds to VHL (after hydroxylation by PHD enzymes in situ), the substrate-recognition portion of a CRL E3 ligase. A poly-D-arginine chain was also added to aid cell permeability (Figure 11). It was linked to a ligand for FKBP12 (F36V)[88] to give PROTAC-4, which was able to degrade GFP–FKBP12 (F36V) in cells efficiently (at 25 μM). PROTAC-5 contained a DHT moiety and was used to degrade an androgen receptor/GFP fusion protein (at 25 μM) in cells.[89] The same HIF peptide and related sequences have been used in numerous PROTACs to target the estrogen receptor[90] and the aryl hydrocarbon receptor.[91] Often degradation occurred in cells without the need for the poly-D-arginine chain.

Recently, we reported two peptidic PROTACs with activity dependent upon the cellular response to external stimuli. In response to stimulus by growth factors, receptor tyrosine kinases dimerize and undergo transautophosphorylation of specific Tyr residues. This phosphorylation event leads to the recruitment of effectors/substrates containing PTP and SH2 domains. We designed $^{TrkA}PP_{FRS2\alpha}$ by combining the peptidic phosphorylation sequence from the NGF receptor, TrkA; the VHL-binding fragment from previous PROTACs; and the octa-D-Arg sequence to enable cell permeability. Treatment of NGF-stimulated PC12 cells with $^{TrkA}PP_{FRS2\alpha}$ (60 μM) led to roughly 90% knockdown of the effector/substrate FRS2α. No degradation was observed in cells that were not treated with NGF, or in cells that were treated with the control $^{TrkA}NP_{FRS2\alpha}$.[92]

On the basis of these results, a second phosphoPROTAC, $^{ErbB2}PP_{PI3K}$, was designed. $^{ErbB2}PP_{PI3K}$ contained the peptidic sequence ErbB3, which is phosphorylated by ErbB2 in response to neuregulin, thus leading to the binding of PI3K. $^{ErbB2}PP_{PI3K}$ caused neuregulin-dependent degradation of PI3K and decreased the activation of its downstream effector Akt. This activity led to dose-dependent toxicity of $^{ErbB2}PP_{PI3K}$ in MCF-7 cells, whereas the control, $^{ErbB2}NP_{PI3K}$, had negligible

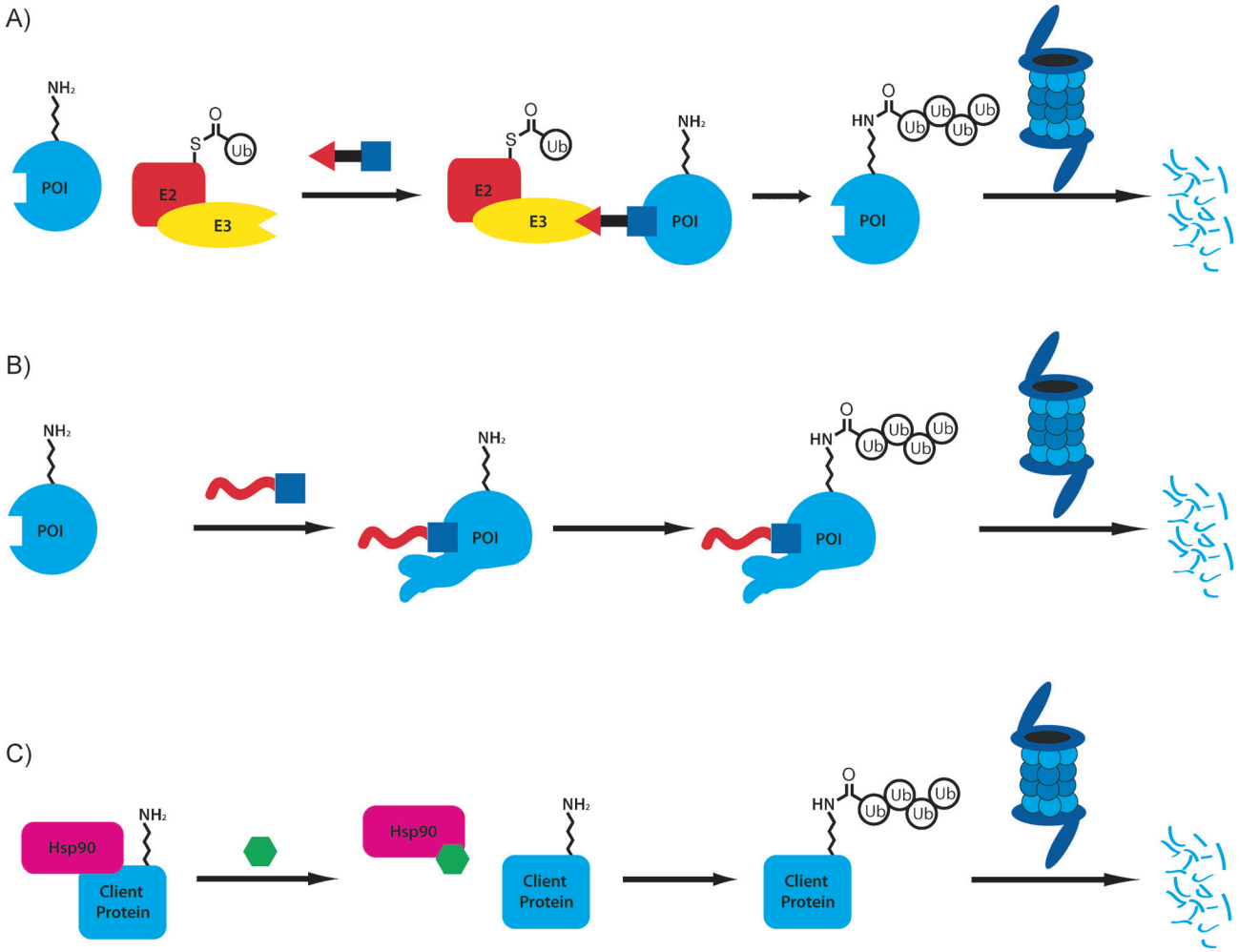


Figure 10. Strategies for induced protein degradation include A) direct recruitment of an E3 ligase with PROTACs, B) induced protein misfolding (or the mimicking of misfolding) with hydrophobic tags (or ligand-mediated degradation), and C) the inhibition of chaperones, such as Hsp90.

toxicity. In a mouse xenograft model, daily treatment with $^{ErbB2}PP_{PI3K}$ (10 mg kg^{-1}, intraperitoneal) led to a 40% reduction in tumor size as compared to the control (treatment with $^{ErbB2}NP_{PI3K}$). This study was the first demonstration of PROTAC activity in a mouse model.[92]

Although peptidic PROTACs have shown efficacy in mice, there is a strong desire to design nonpeptidic PROTACs by the use of small-molecule ligands for E3 ligases, such as MDM2[93] and cIAP1 (Figure 11).[94] Such PROTACs would theoretically be more druglike and would lead to more stable probe compounds or could possibly be used as future therapeutics. We found that the use of a Nutlin-based moiety to hijack the E3 ligase MDM2 could lead to the degradation of the androgen receptor (AR),[93] albeit much less effectively than in our previous study with peptidic VHL ligands.[89]

Hashimoto and co-workers have exploited bestatin esters, which recruit the cellular inhibitor of apoptosis protein 1 (cIAP1), to promote the degradation of cellular retinoic acid binding proteins (CRABPs)[94] as well as nuclear receptors, such as the AR, the estrogen receptor, and the retinoic acid receptor.[95] This subclass of PROTACs, termed SNIPERs (specific and nongenetic IAPs-dependent protein erasers), have been fairly successful but have numerous off-target effects owing to the lack of specificity of bestatin (which had been developed as an aminopeptidase inhibitor before its binding to cIAP1 was discovered).[96] These compounds, like bestatin itself (as well as other IAP inhibitors),[62] also lead to the destabilization and degradation of cIAP1. Additionally, the reliance on hydrolytically unstable ester and oxime[97] linkages raises issues with regard to stability. Furthermore, both PROTACs (peptidic and small-molecule) and SNIPERs suffer from low potency: They often require concentrations up to 25 μM to induce sufficient degradation. These issues highlight the need for improved E3-ligase-targeting moieties to enable the development of more potent and druglike PROTACs.

3.2. Hydrophobic Tagging and Ligand-Mediated Degradation

Besides PROTACs, which cause the degradation of a target protein through the use of a direct E3-ligase ligand, there are a number of examples of molecules that cause

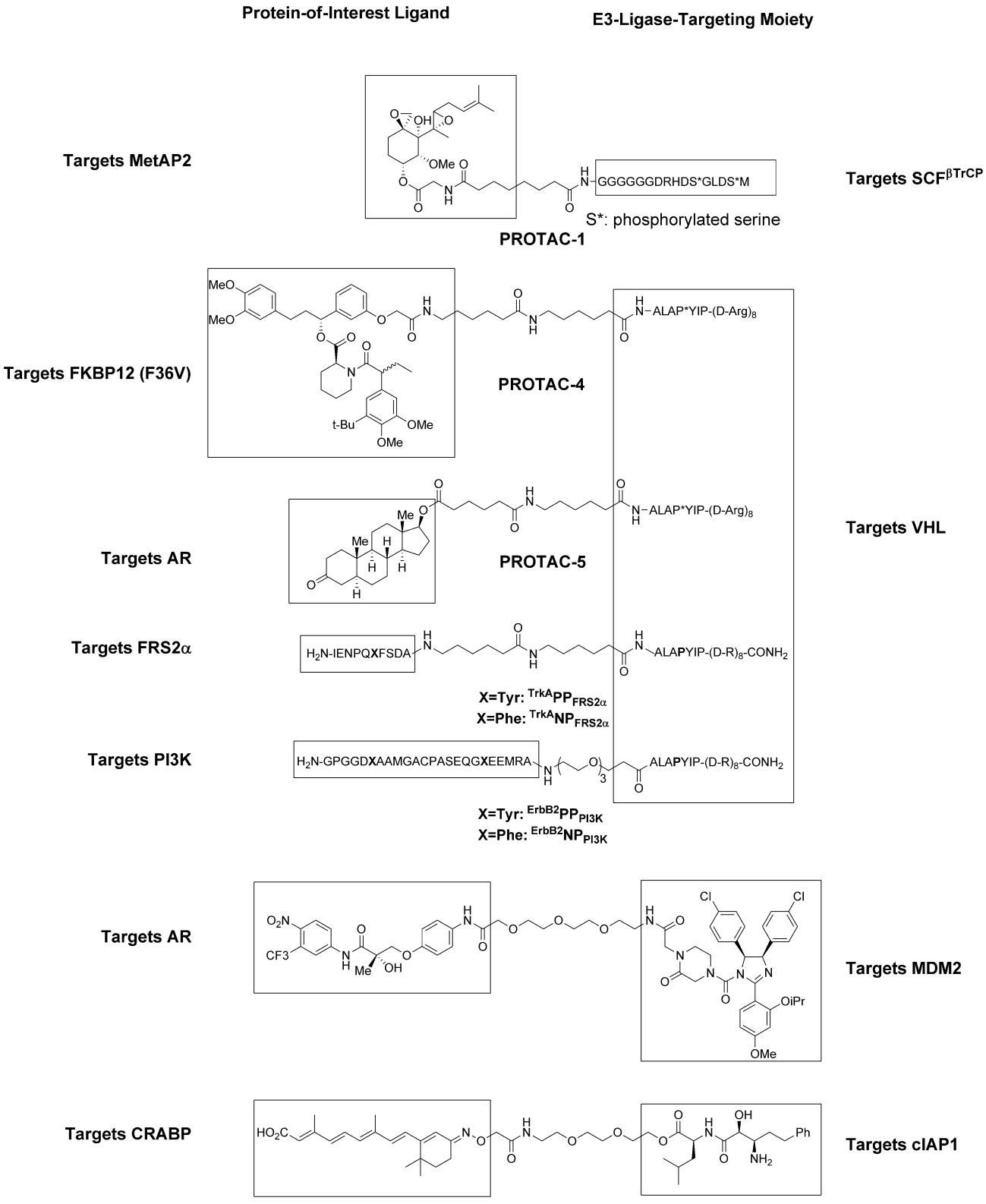


Figure 11. PROTACs are heterobifunctional molecules that combine an E3-ligase ligand (shown on the right) with ligands for various proteins of interest (shown on the left). The E3 ligase is recruited in this way to the protein of interest, thus leading to ubiquitination and degradation. Peptidic ligands have been used to target the E3 ligases $SCF^{\beta TrCP}$ and VHL; small-molecule ligands have been used to target MDM2 and cIAP1.

ligand-mediated degradation although they lack the ability to directly bind E3 ligases. One such compound was discovered in our laboratory after the observation that the use of ligands tagged with hexyl chloride for HaloTag2[98] fusion proteins led to their degradation. We then optimized the chloroalkane ligands for their ability to cause degradation of the HaloTag2 fusion proteins and found that the addition of more hydrophobic ligands led to more potent degradation. This trend led us to term this approach "hydrophobic tagging". We found the adamantaneacetamide HyT13 to be the most successful ligand at inducing this degradation, as it led to roughly 75% degradation of numerous proteins (GFP, luciferase, Hras1^{G12V}, Ror2, etc.) fused with HaloTag2 (Figure 12).[99] Furthermore,

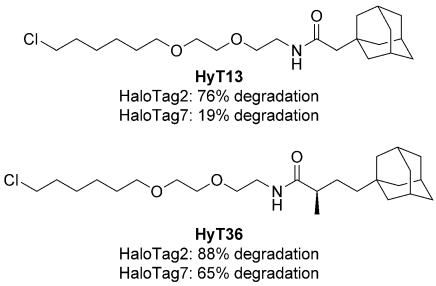


Figure 12. Structures of HyT13 and HyT36 and their ability to degrade HaloTag–GFP fusion proteins at 10 μM.[101]

HyT13 was shown to be effective in degrading HaloTag2 fusion proteins in vivo, with the knockdown of HaloTag–Smad5 in zebrafish and of HaloTag–Hras1^{G12V} in mice, thus leading to a reduction in tumor size in a xenograft model.[99] During the course of a small-molecule screen, a compound (named HALTS) was discovered that stabilized HaloTag2 fusion proteins (in the absence of HyT13) through direct binding to the active site (as determined by crystallography). This stabilization, reminiscent of the Shield system described above, enables the simultaneous small-molecule-induced degradation and stabilization of the same system.[100]

Owing in large part to stability issues associated with HaloTag2, the company Promega has continued to optimize the HaloTag system to increase the stability and decrease the propensity for aggregation of the fusion proteins. Their result was the HaloTag7 protein,[102] which contains 22 point mutations with respect to HaloTag2. We found that HyT13 was much less efficacious in inducing the degradation of HaloTag7 fusion proteins: Less than 20% degradation of HaloTag7–GFP was observed. After much optimization, we found that related HyT36 (Figure 12) was able to degrade more than 50% of HaloTag7–GFP.[101]

A similar system involving the attachment of a Boc_3Arg group to covalent inhibitors of glutathione S-transferase (GST) and a noncovalent inhibitor of eDHFR was recently reported by Hedstrom and co-workers (Figure 13). Treatment with EA–Boc_3Arg led to the efficient degradation of roughly 80% of GST in lysates and whole cells. The noncovalent TMP–Boc_3Arg was less effective: It led to 60% degradation

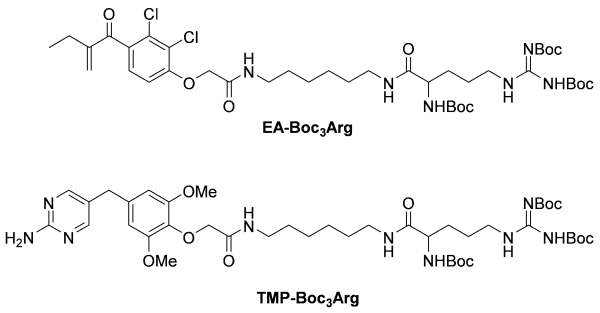


Figure 13. Structures of Boc_3Arg-containing degraders of GST and eDHFR.

of eDHFR in lysates but only 30% degradation in whole cells.[103]

In addition to these methods that have been designed for the degradation of carefully constructed systems and fusion proteins, similar ligand-mediated degradation has been observed serendipitously in the course of traditional medicinal-chemistry programs. The most notable example is the compound fulvestrant (Figure 14), an FDA-approved estro-

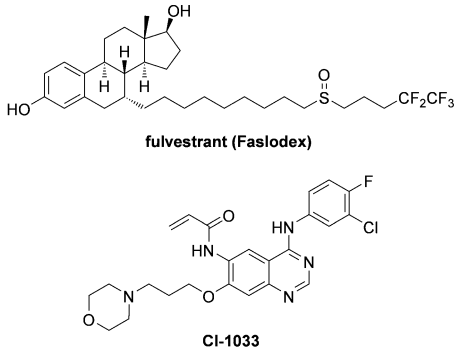


Figure 14. Although designed as traditional antagonists or inhibitors, fulvestrant and CI-1033 were discovered to induce the degradation of the ER and ErbB2, respectively.

gen-receptor (ER) antagonist that functions by inhibiting ER dimerization and nuclear localization.[104] However, the binding of fulvestrant leads to a conformational change in the ER and the formation of a less stable complex, leading to its downregulation.[105] Another example is CI-1033, a covalent inhibitor of ErbB2 that induces its degradation by the proteasome.[106] It was proposed that the covalent modification of the ATP-binding pocket alters the site and leads to its ubiquitination (and subsequent degradation) by a chaperone-mediated destructive system.[106]

Although these examples of ligand-mediated degradation lack the clear degradation signals of PROTACs (i.e. ligands to directly recruit E3 ligases), they share similar features and may operate by related, albeit distinct mechanisms. A common motif found in many of these degraders is a hydrophobic patch that is attached through a linker to the ligand. The ligand often covalently binds to the target. These ligands

then can induce a conformational change or disrupt binding to other members of a multiprotein complex. Recognition of these non-native states of the target protein by the cell machinery may lead to the degradation and ubiquitination of the misfolded proteins. However, the nature of this proposed mechanism, which is heavily reliant upon the ability of the target to adopt a non-native state, may prevent the rational application of this approach to novel targets.

3.3. Related Systems Requiring Genetic Manipulation

Although the above systems have often targeted genetically modified targets, they have all been shown to operate on endogenous proteins as well. A variety of related methods have proved successful for the targeted degradation of proteins, but require genetic manipulation to operate. A notable example involved the fusion of the proteasomal subunit Rpn10 with Fpr1 in yeast.[107] The addition of rapamycin led to chemically induced dimerization with Tor1 fusion proteins, which led to their degradation. The success of this approach demonstrated that direct localization to the proteasome was sufficient for the degradation of some proteins in the absence of polyubiquitination. A related method that lacks small-molecule control but is highly analogous to the concept of PROTACs involves the development of chimeric E3 ligases.[108] Zhou and co-workers were able to generate chimeric F-box proteins based upon Cdc4p and βTrCP that contained the N-terminal domain of a viral protein, E7, which binds to the retinoblastoma protein (pRB). Expression of these complexes in yeast and mammalian cells, respectively, led to the knockdown of pRB.[109] This system was also used to generate a βTrCP-E–cadherin chimera that binds to and degrades mutant β-catenin that had developed resistance to its normal degradation pathway involving the APC protein.[110]

3.4. Geldanamycin Derivatives and Other Hsp90 Inhibitors

Geldanamycin (Figure 15), an ansamycin antibiotic, is a natural product identified on the basis of its potent growth-inhibitory effects on cells transformed with the tyrosine kinase v-Src. Although it was originally believed to directly inhibit Src, its effects were found in 1994 to be due to its inhibition of Hsp90, a molecular chaperone that assists in the refolding of damaged proteins.[111] The inhibition of Hsp90 thus leads to the degradation of Src.[112] Further investigation showed that geldanamycin treatment led to the degradation of numerous other Hsp90 client proteins, such as the androgen receptor (AR), the estrogen receptor (ER), and kinases such as Raf[113] and HER. As many of these proteins are oncogenic, Hsp90 inhibitors have been investigated as chemotherapeutics. The development of these inhibitors has been reviewed extensively[114] and is therefore only briefly summarized herein.

Whereas geldanamycin itself was too toxic for clinical studies, a number of derivatives with varying substitution of the quinone, such as 17-AAG and 17-DMAG, have been

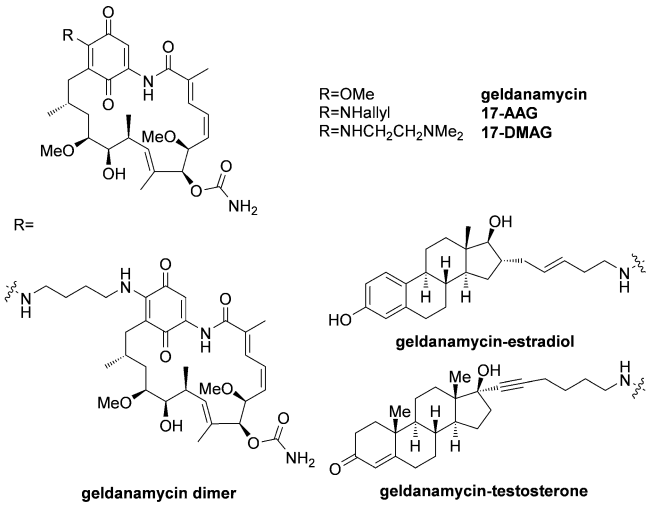


Figure 15. Geldanamycin and analogues.

studied in prostate and breast cancer models for their effects resulting from degradation of the AR and HER2. 17-AAG progressed through phase I and phase II trials before it was discontinued.[114e] As the quinone moiety was largely responsible for the toxicity and metabolic instability of geldanamycin and its derivatives, purely synthetic Hsp90 inhibitors were developed. An early example was the purine PU3,[115] which inspired the development of the clinical candidate PU-H71 (administered intravenously; Figure 16).[116] Other examples

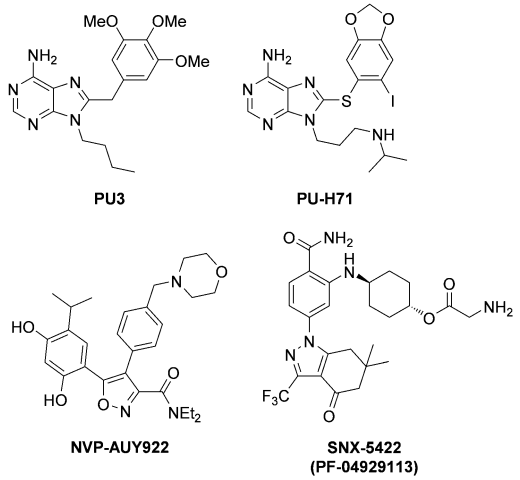


Figure 16. Selected synthetic Hsp90 inhibitors: PU3,[115] PU-H71,[116] NVP-AUY922,[117] and SNX-5422.[118]

of synthetic Hsp90 inhibitors in clinical trials include the isoxazole NVP-AUY922[117] and the prodrug SNX-5422 (PF-04929113).[118]

As geldanamycin and other Hsp90 inhibitors lack the ability to selectively degrade specific client proteins, an effort was made to develop geldanamycin hybrids that increased the specificity of the protein knockdown. The creation of a geldanamycin–estradiol hybrid was found to maintain the

level of ER knockdown of geldanamycin, while reducing the knockdown of Her2, Raf-1, and IGF1R.[119] In a similar manner, in comparison to geldanamycin, a geldanamycin–testosterone hybrid led to sustained inhibition of cell growth in cells dependent upon the AR but to significantly lower growth inhibition of other cell lines that were AR-independent.[120] As HER kinases are particularly sensitive to Hsp90 inhibition and require dimerization to become activated, it was hypothesized that a geldanamycin dimer would have increased selectivity for HER2 degradation. In fact, a dimer with a four-carbon-atom linker was found to increase selectivity for HER2 degradation over that of Raf and IGFR.[121]

3.5. Small-Molecule Inhibitors of Deubiquitinases

Ubiquitin and UBL units are linked to their protein targets (or to other units to form chains) through stable peptide and isopeptide bonds; however, the formation of these linkages is readily reversible through the action of the roughly 80 human deubiquitinases (DUBs). DUBs are subdivided into five families, JAMMs, which are zinc metalloproteases, as well as ubiquitin-specific proteases (USPs), UCHs, OTUs, and Josephins, which are all cysteine proteases. The purposes of these enzymes vary and include the maturation of ubiquitin and other UBLs, the removal of ubiquitin chains to prevent degradation and to reverse nondegradation UBL signals, and the recycling of ubiquitin chains during degradation by the proteasome. The specificity of DUBs varies and can involve the recognition of specific target proteins or specific types of ubiquitin linkages. Owing to their wide-reaching effects within the UPS as well as their relatively druggable enzymatic activity, many DUBs may prove to be attractive therapeutic targets.[122]

USP7 (ubiquitin-specific protease 7) is a deubiquitinase that has activity on numerous targets, including the E3 ligase MDM2. As USP7 stabilizes MDM2 and thus downregulates anti-oncogenic p53, USP7 presents an attractive target for the development of therapeutics for the treatment of cancer. The first small-molecule inhibitor of USP7 described was HBX 41,108 (Figure 17);[123] however, this compound was later determined to be a nonspecific DUB inhibitor.[124] HBX 19,818 was found to specifically inhibit USP7 by covalently modifying the active Cys^{223} residue (it lacked activity against a panel of other USPs and other DUBs).[124] Additionally, P005091 and P0050429 were recently reported to have dual activity against USP7 and USP47 in the low micromolar range and caused inhibition in cells.[125]

Various other DUB inhibitors have been reported. LS1 was discovered to inhibit UCH-L3 through the screening of a library of 1000 compounds in a FRET assay by using fluorescently labeled ubiquitin attached to model substrates with fluorescent quenchers.[126] PR-619 was found to inhibit a broad range of DUBs and had good selectivity over other classes of cysteine proteases.[127] LDN91946 was reported to inhibit UCH-L1, which is potentially involved in Parkinson's disease.[128]

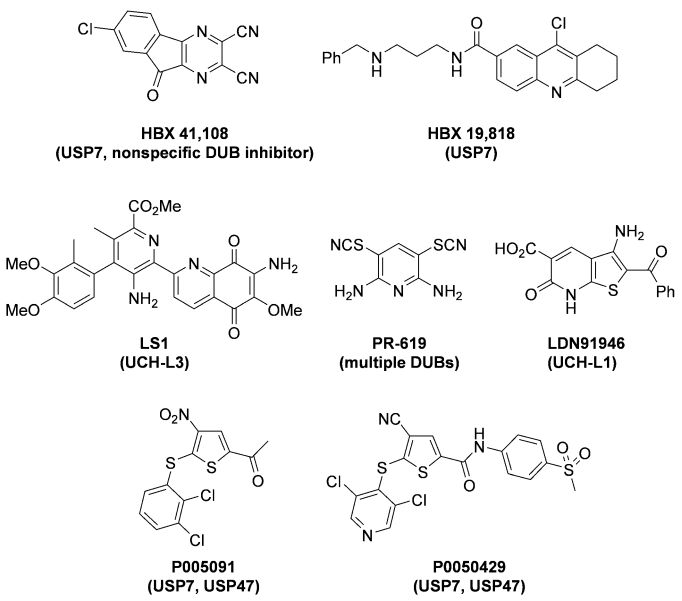


Figure 17. Inhibitors of DUBs. The specific DUB inhibited is shown in parentheses.

Although inhibitors of DUB enzymes generally lead to increased degradation of their substrates, this activity is not always observed. The 26S proteasome has deubiquitinase activity, which is localized to subunits in the 19S regulatory unit. These deubiquitinases remove the polyubiquitin chains of proteins targeted for degradation by the proteasome, and their removal of ubiquitin is necessary for the degradation of the target protein. Therefore, inhibition of the DUB activity in the 19S regulatory particle should have similar effects to those of the proteasome inhibitors described above. The small molecule b-AP15 was reported to inhibit the 19S-associated DUBs UCH-L5 (ubiquitin C-terminal hydrolase 5) and USP14, thus leading to accumulation of polyubiquitin. By the use of a mouse model, b-AP15 was then shown to inhibit tumor growth in vivo, which validated 19S-associated DUBs as a target for cancer treatment (Figure 18).[129] However, selective inhibition of USP14 addition with IU1 was reported to have the opposite effect and to lead to an increase in proteasome activity.[130] These results suggest either opposing effects of USP14 and UCH-L5, or a possible redundancy, whereby UCHL5 can compensate for inhibited USP14.

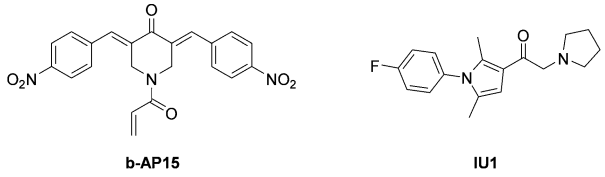


Figure 18. Inhibitors of DUBs associated with the 19S regulatory particle. IU1 inhibits USP14, whereas b-AP15 inhibits USP14 and UCH-L5.

4. Summary and Outlook

In the 10 years since the approval of bortezomib/Velcade, the first marketed drug to target the proteasome, great strides have been made in small-molecule inhibitors of the UPS; however, these results have been slow to translate to the clinic. In 2012, the second proteasome inhibitor, carfilzomib/Kyprolis, was approved for the treatment of relapsed and refractory multiple myeloma.[34] Currently, additional next-generation proteasome inhibitors are being developed. These endeavors include efforts to make an orally bioavailable drug and to selectively target the immunoproteasome.[35b]

The development of inhibitors of targets in the UPS other than the proteasome has also made great strides in the past decade, although no compounds have made it to the market yet. The targeting of E3 ligases is likely to allow for more specificity in the control of protein stability than that observed for proteasome inhibitors but is also more difficult owing to their lack of defined catalytic residues. In 2004, the first E3-ligase inhibitors were reported; these compounds targeted MDM2[49] as well as XIAP.[59] Since then, inhibitors of MDM2 and related IAPs have advanced to clinical trials, and these E3 ligases remain targets for various forms of cancer. The past five years have also seen reports of inhibitors of numerous other E3 ligases, including various SCF complexes. Despite the current lack of clinically approved drugs that target E3 ligases, the successes over the past decade should put to rest the once popular idea[57] that E3 ligases could not be targeted by small molecules.

Another approach that is more specific than direct inhibition of the proteasome is the inhibition of the E1 protein of the UBL NEDD8 (NAE). As NEDDylation of cullins is important for the activity of the CRL class of E3 ligases, NAE inhibitors, such as MLN4924 (which is currently in phase I/II trials), can regulate a whole class of E3 ligases at once.[44b,c]

Inducers of protein degradation remain an attractive possibility for the development of therapeutics owing to their ability to target all of the functions of a protein (such as scaffolding), as opposed to inhibitors, which only target enzymatic activity.[2] Hsp90 inhibitors are capable of inducing the degradation of a large swath of client proteins relevant to cancer. Owing to the large number of Hsp90 inhibitors that have advanced to clinical trials, they remain a promising class of possible therapeutics.[114d]

More research is required for the development of general methods to induce targeted degradation. Although fulvestrant is capable of inducing degradation of the estrogen receptor, it remains unclear how much of its efficacy is due to its activity as an estrogen-receptor antagonist and how much to its activity as an inducer of degradation.[104a] While there are other reports of ligand-mediated degradation,[99] it is unclear which proteins make good targets for such methods and what functionality is required to induce the process. Systems such as PROTACs help solve this problem through the use of bifunctional molecules that can recruit specific E3 ligases to a target protein; however, they suffer from a lack of druglikeness and still require a potent ligand for the target protein.[86,89,94]

Abbreviations

AR	androgen receptor
Boc_3Arg	an arginine residue containing three *tert*-butoxycarbonyl (Boc) protecting groups
CRABP	cellular retinoic acid binding protein
CRL	cullin–RING ligases, a multisubunit class of E3 ligases
DARTS	drug affinity responsive target stability
DHT	dihydrotestosterone
DUB	deubiquitinase
eDHFR	*Escherichia coli* dihydrofolate reductase
ER	estrogen receptor
FKBP	FK506-binding protein
Frb	FKBP12–rapamycin-binding domain
GFP	green fluorescent protein
HECT	homologous to the E6-AP carboxyl terminus, a class of E3 ligases
HIF	hypoxia-inducible factor, a transcription factor that is ubiquitinated by VHL
IAP	inhibitor of apoptosis protein, a family of E3 ligases
MDM2	mouse double minute 2 homolog, an E3 ligase
NAE	NEDD8-activating enzyme
NEDD8	neural precursor cell expressed, developmentally down-regulated 8, a UBL
PPI	protein–protein interaction
PROTAC	proteolysis targeting chimera, a heterobifunctional molecule that hijacks E3 ligases to degrade proteins
RING	really interesting new gene, a domain found in many E3 ligases
SCF	Skp, cullin, F-box protein, a subfamily of the CRL E3 ligases that use multiple F-box proteins as adaptors for their substrates
SMAC	second mitochondria-derived activator of caspases, a protein that inhibits the activity of IAP E3 ligases
SNIPER	specific and nongenetic IAPs-dependent protein eraser, a subclass of PROTACs that hijacks an IAP E3 ligase
SUMO	small ubiquitin-like modifier, a UBL
TMP	trimethoprim, an antibiotic that binds DHFR
UAE	ubiquitin-activating enzyme
Ub	ubiquitin
UBL	ubiquitin-like protein
UPS	ubiquitin proteasome system
VHL	Von Hippel–Lindau tumor suppressor, the substrate-recognition subunit of an E3 ligase
YFP	yellow fluorescent protein

We thank Dr. John Hines for helpful input on portions of the manuscript and Eric Buckley for the design of the frontispiece. The frontispiece depicts the crystal structure of the proteasome in complex with fellutamide B (PDB 3D29).[20a]

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[1] a) K. Raina, C. M. Crews, *J. Biol. Chem.* **2010**, *285*, 11057 – 11060; b) J. S. Schneekloth, Jr., C. M. Crews, *ChemBioChem* **2005**, *6*, 40 – 46.
[2] C. M. Crews, *Chem. Biol.* **2010**, *17*, 551 – 555.
[3] a) M. R. Arkin, J. A. Wells, *Nat. Rev. Drug Discovery* **2004**, *3*, 301 – 317; b) J. A. Wells, C. L. McClendon, *Nature* **2007**, *450*, 1001 – 1009.
[4] a) A. Hershko, *Angew. Chem.* **2005**, *117*, 6082 – 6094; *Angew. Chem. Int. Ed.* **2005**, *44*, 5932 – 5943; b) A. Hershko, A. Ciechanover, *Annu. Rev. Biochem.* **1992**, *61*, 761 – 807.
[5] Y. Kravtsova-Ivantsiv, T. Sommer, A. Ciechanover, *Angew. Chem.* **2013**, *125*, 202 – 209; *Angew. Chem. Int. Ed.* **2013**, *52*, 192 – 198.
[6] a) L. Bedford, J. Lowe, L. R. Dick, R. J. Mayer, J. E. Brownell, *Nat. Rev. Drug Discovery* **2011**, *10*, 29 – 46; b) P. Cohen, M. Tcherpakov, *Cell* **2010**, *143*, 686 – 693.
[7] A. F. Kisselev, A. L. Goldberg, *Chem. Biol.* **2001**, *8*, 739 – 758.
[8] M. Zhou, R. Wang, *ChemMedChem* **2013**, *8*, 694 – 707.
[9] K.-B. Kim, C. M. Crews, *J. Med. Chem.* **2008**, *51*, 2600 – 2605.
[10] A. Rentsch, D. Landsberg, T. Brodmann, L. Bülow, A.-K. Girbig, M. Kalesse, *Angew. Chem.* **2013**, *125*, 5560 – 5599; *Angew. Chem. Int. Ed.* **2013**, *52*, 5450 – 5488.
[11] a) M. Bogyo, J. S. McMaster, M. Gaczynska, D. Tortorella, A. L. Goldberg, H. L. Ploegh, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6629 – 6634; b) M. Bogyo, S. Shin, J. S. McMaster, H. L. Ploegh, *Chem. Biol.* **1998**, *5*, 307 – 320; c) P. P. Geurink, W. A. van der Linden, A. C. Mirabella, N. Gallastegui, G. de Bruin, A. E. M. Blom, M. J. Voges, E. D. Mock, B. I. Florea, G. A. van der Marel, C. Driessen, M. van der Stelt, M. Groll, H. S. Overkleeft, A. F. Kisselev, *J. Med. Chem.* **2013**, *56*, 1262 – 1275.
[12] a) E. J. Corey, W. D. Li, *Chem. Pharm. Bull.* **1999**, *47*, 1 – 10; b) L. R. Dick, A. A. Cruikshank, L. Grenier, F. D. Melandri, S. L. Nunes, R. L. Stein, *J. Biol. Chem.* **1996**, *271*, 7273 – 7276; c) R. H. Feling, G. O. Buchanan, T. J. Mincer, C. A. Kauffman, P. R. Jensen, W. Fenical, *Angew. Chem.* **2003**, *115*, 369 – 371; *Angew. Chem. Int. Ed.* **2003**, *42*, 355 – 357; d) G. Fenteany, R. F. Standaert, W. S. Lane, S. Choi, E. J. Corey, S. L. Schreiber, *Science* **1995**, *268*, 726 – 731; e) T. A. M. Gulder, B. S. Moore, *Angew. Chem.* **2010**, *122*, 9534 – 9556; *Angew. Chem. Int. Ed.* **2010**, *49*, 9346 – 9367.
[13] N. Gallastegui, P. Beck, M. Arciniega, R. Huber, S. Hillebrand, M. Groll, *Angew. Chem.* **2012**, *124*, 251 – 254; *Angew. Chem. Int. Ed.* **2012**, *51*, 247 – 249.
[14] M. A. Gräwert, N. Gallastegui, M. Stein, B. Schmidt, P.-M. Kloetzel, R. Huber, M. Groll, *Angew. Chem.* **2011**, *123*, 563 – 566; *Angew. Chem. Int. Ed.* **2011**, *50*, 542 – 544.
[15] a) J. Löwe, D. Stock, B. Jap, P. Zwickl, W. Baumeister, R. Huber, *Science* **1995**, *268*, 533 – 539; b) A. J. Marques, R. Palanimurugan, A. C. Matias, P. C. Ramos, R. J. Dohmen, *Chem. Rev.* **2009**, *109*, 1509 – 1536.
[16] M. Groll, Y. Koguchi, R. Huber, J. Kohno, *J. Mol. Biol.* **2001**, *311*, 543 – 548.
[17] T. A. Lansdell, M. A. Hurchla, J. Xiang, S. Hovde, K. N. Weilbaecher, R. W. Henry, J. J. Tepe, *ACS Chem. Biol.* **2013**, *8*, 578 – 587.
[18] A. Vinitsky, C. Michaud, J. C. Powers, M. Orlowski, *Biochemistry* **1992**, *31*, 9421 – 9428.
[19] V. J. Palombella, O. J. Rando, A. L. Goldberg, T. Maniatis, *Cell* **1994**, *78*, 773 – 785.
[20] a) J. Hines, M. Groll, M. Fahnestock, C. M. Crews, *Chem. Biol.* **2008**, *15*, 501 – 512; b) J. S. Schneekloth, Jr., J. L. Sanders, J. Hines, C. M. Crews, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3855 – 3858.
[21] a) J. Adams, *Oncologist* **2002**, *7*, 9 – 16; b) J. Adams, M. Behnke, S. Chen, A. A. Cruickshank, L. R. Dick, L. Grenier, J. M. Klunder, Y.-T. Ma, L. Plamondon, R. L. Stein, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 333.
[22] M. Groll, C. R. Berkers, H. L. Ploegh, H. Ovaa, *Structure* **2006**, *14*, 451 – 456.
[23] J. Adams, *Curr. Opin. Chem. Biol.* **2002**, *6*, 493 – 500.
[24] K. Sugawara, M. Hatori, Y. Nishiyama, K. Tomita, H. Kamei, M. Konishi, T. Oki, *J. Antibiot.* **1990**, *43*, 8 – 18.
[25] M. Hanada, K. Sugawara, K. Kaneta, S. Toda, Y. Nishiyama, K. Tomita, H. Yamamoto, M. Konishi, T. Oki, *J. Antibiot.* **1992**, *45*, 1746 – 1752.
[26] N. Sin, L. Meng, H. Auth, C. M. Crews, *Bioorg. Med. Chem.* **1998**, *6*, 1209 – 1217.
[27] N. Sin, K.-B. Kim, M. Elofsson, L. Meng, H. Auth, B. H. Kwok, C. M. Crews, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2283 – 2288.
[28] a) K.-B. Kim, J. Myung, N. Sin, C. M. Crews, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3335 – 3340; b) L. Meng, B. H. Kwok, N. Sin, C. M. Crews, *Cancer Res.* **1999**, *59*, 2798 – 2801; c) L. Meng, R. Mohan, B. H. Kwok, M. Elofsson, N. Sin, C. M. Crews, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10403 – 10408.
[29] M. Groll, K.-B. Kim, N. Kairies, R. Huber, C. M. Crews, *J. Am. Chem. Soc.* **2000**, *122*, 1237 – 1238.
[30] K. B. Kim, C. M. Crews, *Nat. Prod. Rep.* **2013**, *30*, 600.
[31] M. Elofsson, U. Splittgerber, J. Myung, R. Mohan, C. M. Crews, *Chem. Biol.* **1999**, *6*, 811 – 822.
[32] a) H.-J. Zhou, M. A. Aujay, M. K. Bennett, M. Dajee, S. D. Demo, Y. Fang, M. N. Ho, J. Jiang, C. J. Kirk, G. J. Laidig, E. R. Lewis, Y. Lu, T. Muchamuel, F. Parlati, E. R. Ring, K. D. Shenk, J. Shields, P. J. Shwonek, T. Stanton, C. M. Sun, C. Sylvain, T. M. Woo, J. Yang, *J. Med. Chem.* **2009**, *52*, 3028 – 3038; b) S. D. Demo, C. J. Kirk, M. A. Aujay, T. J. Buchholz, M. Dajee, M. N. Ho, J. Jiang, G. J. Laidig, E. R. Lewis, F. Parlati, K. D. Shenk, M. S. Smyth, C. M. Sun, M. K. Vallone, T. M. Woo, C. J. Molineaux, M. K. Bennett, *Cancer Res.* **2007**, *67*, 6383 – 6391; c) D. Kuhn, Q. Chen, P. Voorhees, J. Strader, K. D. Shenk, C. M. Sun, S. D. Demo, M. K. Bennett, F. W. B. van Leeuwen, A. Chanan-Khan, *Blood* **2007**, *110*, 3281.
[33] D. S. Siegel, T. Martin, M. Wang, R. Vij, A. J. Jakubowiak, S. Lonial, S. Trudel, V. Kukreti, N. Bahlis, M. Alsina, A. Chanan-Khan, F. Buadi, F. J. Reu, G. Somlo, J. Zonder, K. Song, A. K. Stewart, E. Stadtmauer, L. Kunkel, S. Wear, A. F. Wong, R. Z. Orlowski, S. Jagannath, *Blood* **2012**, *120*, 2817 – 2825.
[34] J. L. Thompson, *Ann. Pharmacother.* **2013**, *47*, 56 – 62.
[35] a) http://www.clinicaltrials.gov/; b) E. M. Huber, M. Groll, *Angew. Chem.* **2012**, *124*, 8838 – 8850; *Angew. Chem. Int. Ed.* **2012**, *51*, 8708 – 8720.
[36] E. M. Huber, M. Basler, R. Schwab, W. Heinemeyer, C. J. Kirk, M. Groettrup, M. Groll, *Cell* **2012**, *148*, 727 – 738.
[37] A. Gilardini, P. Marmiroli, G. Cavaletti, *Curr. Med. Chem.* **2008**, *15*, 3025 – 3035.
[38] S. R. da Silva, S.-L. Paiva, J. L. Lukkarila, P. T. Gunning, *J. Med. Chem.* **2013**, *56*, 2165 – 2177.
[39] Y. Yang, J. Kitagaki, R.-M. Dai, Y. C. Tsai, K. L. Lorick, R. L. Ludwig, S. A. Pierre, J. P. Jensen, I. V. Davydov, P. Oberoi, C.-C. H. Li, J. H. Kenten, J. A. Beutler, K. H. Vousden, A. M. Weissman, *Cancer Res.* **2007**, *67*, 9472 – 9481.
[40] G. W. Xu, M. Ali, T. E. Wood, D. Wong, N. Maclean, X. Wang, M. Gronda, M. Skrtic, X. Li, R. Hurren, X. Mao, M. Venkatesan, R. B. Zavareh, T. Ketela, J. C. Reed, D. Rose, J. Moffat, R. A. Batey, S. Dhe-Paganon, A. D. Schimmer, *Blood* **2010**, *115*, 2251 – 2259.
[41] J. Kitagaki, Y. Yang, J. E. Saavedra, N. H. Colburn, L. K. Keefer, A. O. Perantoni, *Oncogene* **2009**, *28*, 619 – 624.
[42] D. Ungermannova, S. J. Parker, C. G. Nasveschuk, D. A. Chapnick, A. J. Phillips, R. D. Kuchta, X. Liu, *J. Biomol. Screening* **2012**, *17*, 421 – 434.

[43] D. Ungermannova, S. J. Parker, C. G. Nasveschuk, W. Wang, B. Quade, G. Zhang, R. D. Kuchta, A. J. Phillips, X. Liu, *PLoS ONE* **2012**, *7*, e29208.

[44] a) J. E. Brownell, M. D. Sintchak, J. M. Gavin, H. Liao, F. J. Bruzzese, N. J. Bump, T. A. Soucy, M. A. Milhollen, X. Yang, A. L. Burkhardt, J. Ma, H.-K. Loke, T. Lingaraj, D. Wu, K. B. Hamman, J. J. Spelman, C. A. Cullis, S. P. Langston, S. Vyskocil, T. B. Sells, W. D. Mallender, I. Visiers, P. Li, C. F. Claiborne, M. Rolfe, J. B. Bolen, L. R. Dick, *Mol. Cell* **2010**, *37*, 102 – 111; b) T. A. Soucy, P. G. Smith, M. A. Milhollen, A. J. Berger, J. M. Gavin, S. Adhikari, J. E. Brownell, K. E. Burke, D. P. Cardin, S. Critchley, C. A. Cullis, A. Doucette, J. J. Garnsey, J. L. Gaulin, R. E. Gershman, A. R. Lublinsky, A. Mcdonald, H. Mizutani, U. Narayanan, E. J. Olhava, S. Peluso, M. Rezaei, M. D. Sintchak, T. Talreja, M. P. Thomas, T. Traore, S. Vyskocil, G. S. Weatherhead, J. Yu, J. Zhang, L. R. Dick, C. F. Claiborne, M. Rolfe, J. B. Bolen, S. P. Langston, *Nature* **2009**, *458*, 732 – 736; c) T. A. Soucy, P. G. Smith, M. Rolfe, *Clin. Cancer Res.* **2009**, *15*, 3912 – 3916.

[45] J. I. Toth, L. Yang, R. Dahl, M. D. Petroski, *Cell Reports* **2012**, *1*, 309 – 316.

[46] a) D. F. Ceccarelli, X. Tang, B. Pelletier, S. Orlicky, W. Xie, V. Plantevin, D. Neculai, Y.-C. Chou, A. Ogunjimi, A. Al-Hakim, X. Varelas, J. Koszela, G. A. Wasney, M. Vedadi, S. Dhe-Paganon, S. Cox, S. Xu, A. Lopez-Girona, F. Mercurio, J. Wrana, D. Durocher, S. Meloche, D. R. Webb, M. Tyers, F. Sicheri, *Cell* **2011**, *145*, 1075 – 1087; b) J. W. Harper, R. W. King, *Cell* **2011**, *145*, 1007 – 1009.

[47] M. A. Sanders, G. Brahemi, P. Nangia-Makker, V. Balan, M. Morelli, H. Kothayer, A. D. Westwell, M. P. Shekhar, *Mol. Cancer Ther.* **2013**, *12*, 373 – 383.

[48] Y. S. Kim, K. Nagy, S. Keyser, J. S. Schneekloth, *Chem. Biol.* **2013**, *20*, 604 – 613.

[49] L. T. Vassilev, B. T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi, E. A. Liu, *Science* **2004**, *303*, 844 – 848.

[50] F. Gonzalez-Lopez de Turiso, D. Sun, Y. Rew, M. D. Bartberger, H. P. Beck, J. Canon, A. Chen, D. Chow, T. L. Correll, X. Huang, L. D. Julian, F. Kayser, M.-C. Lo, A. M. Long, D. McMinn, J. D. Oliner, T. Osgood, J. P. Powers, A. Y. Saiki, S. Schneider, P. Shaffer, S.-H. Xiao, P. Yakowec, X. Yan, Q. Ye, D. Yu, X. Zhao, J. Zhou, J. C. Medina, S. H. Olson, *J. Med. Chem.* **2013**, *56*, 4053 – 4070.

[51] Y. Yuan, Y.-M. Liao, C.-T. Hsueh, H. R. Mirshahidi, *J. Hematol. Oncol.* **2011**, *4*, 16.

[52] K. Kojima, J. K. Burks, J. Arts, M. Andreeff, *Mol. Cancer Ther.* **2010**, *9*, 2545 – 2557.

[53] a) L. T. Vassilev, *Trends Mol. Med.* **2007**, *13*, 23 – 31; b) Y. Yang, J. Kitagaki, H. Wang, D.-X. Hou, A. O. Perantoni, *Cancer Sci.* **2009**, *100*, 24 – 28.

[54] K. Ding, Y. Lu, Z. Nikolovska-Coleska, S. Qiu, Y. Ding, W. Gao, J. Stuckey, K. Krajewski, P. P. Roller, Y. Tomita, D. A. Parrish, J. R. Deschamps, S. Wang, *J. Am. Chem. Soc.* **2005**, *127*, 10130 – 10131.

[55] A. S. Azmi, P. A. Philip, F. W. J. Beck, Z. Wang, S. Banerjee, S. Wang, D. Yang, F. H. Sarkar, R. M. Mohammad, *Oncogene* **2011**, *30*, 117 – 126.

[56] Y. Zhao, L. Liu, W. Sun, J. Lu, D. McEachern, X. Li, S. Yu, D. Bernard, P. Ochsenbein, V. Ferey, J.-C. Carry, J. R. Deschamps, D. Sun, S. Wang, *J. Am. Chem. Soc.* **2013**, *135*, 7223 – 7234.

[57] K. Garber, *J. Natl. Cancer Inst.* **2005**, *97*, 166 – 167.

[58] S. Fulda, D. Vucic, *Nat. Rev. Drug Discovery* **2012**, *11*, 109 – 124.

[59] T. K. Oost, C. Sun, R. C. Armstrong, A.-S. Al-Assaad, S. F. Betz, T. L. Deckwerth, H. Ding, S. W. Elmore, R. P. Meadows, E. T. Olejniczak, A. Oleksijew, T. Oltersdorf, S. H. Rosenberg, A. R. Shoemaker, K. J. Tomaselli, H. Zou, S. W. Fesik, *J. Med. Chem.* **2004**, *47*, 4417 – 4426.

[60] a) J. Lu, L. Bai, H. Sun, Z. Nikolovska-Coleska, D. McEachern, S. Qiu, R. S. Miller, H. Yi, S. Shangary, Y. Sun, J. L. Meagher, J. A. Stuckey, S. Wang, *Cancer Res.* **2008**, *68*, 9384 – 9393; b) H. Sun, Z. Nikolovska-Coleska, J. Lu, J. L. Meagher, C.-Y. Yang, S. Qiu, Y. Tomita, Y. Ueda, S. Jiang, K. Krajewski, P. P. Roller, J. A. Stuckey, S. Wang, *J. Am. Chem. Soc.* **2007**, *129*, 15279 – 15294; c) H. Sun, Z. Nikolovska-Coleska, C.-Y. Yang, D. Qian, J. Lu, S. Qiu, L. Bai, Y. Peng, Q. Cai, S. Wang, *Acc. Chem. Res.* **2008**, *41*, 1264 – 1277; d) H. Sun, Z. Nikolovska-Coleska, C.-Y. Yang, L. Xu, M. Liu, Y. Tomita, H. Pan, Y. Yoshioka, K. Krajewski, P. P. Roller, S. Wang, *J. Am. Chem. Soc.* **2004**, *126*, 16686 – 16687.

[61] E. Varfolomeev, J. W. Blankenship, S. M. Wayson, A. V. Fedorova, N. Kayagaki, P. Garg, K. Zobel, J. N. Dynek, L. O. Elliott, H. J. A. Wallweber, J. A. Flygare, W. J. Fairbrother, K. Deshayes, V. M. Dixit, D. Vucic, *Cell* **2007**, *131*, 669 – 681.

[62] Q. Cai, H. Sun, Y. Peng, J. Lu, Z. Nikolovska-Coleska, D. McEachern, L. Liu, S. Qiu, C.-Y. Yang, R. Miller, H. Yi, T. Zhang, D. Sun, S. Kang, M. Guo, L. Leopold, D. Yang, S. Wang, *J. Med. Chem.* **2011**, *54*, 2714 – 2726.

[63] S. Fulda, *J. Med. Chem.* **2012**, *55*, 4099 – 4100.

[64] a) M. D. Petroski, R. J. Deshaies, *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 9 – 20; b) T. Cardozo, M. Pagano, *BMC Biochem.* **2007**, *8*, S9; c) T. J. Cardozo, M. Pagano, *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 739 – 751.

[65] a) E. Rico-Bautista, D. A. Wolf, *Chem. Biol.* **2012**, *19*, 1497 – 1498; b) L. Wu, A. V. Grigoryan, Y. Li, B. Hao, M. Pagano, T. J. Cardozo, *Chem. Biol.* **2012**, *19*, 1515 – 1524.

[66] C.-H. Chan, J. K. Morrow, C.-F. Li, Y. Gao, G. Jin, A. Moten, L. J. Stagg, J. E. Ladbury, Z. Cai, D. Xu, C. J. Logothetis, M.-C. Hung, S. Zhang, H.-K. Lin, *Cell* **2013**, *154*, 556 – 568.

[67] a) M. Aghajan, N. Jonai, K. Flick, F. Fu, M. Luo, X. Cai, I. Ouni, N. Pierce, X. Tang, B. Lomenick, R. Damoiseaux, R. Hao, P. M. Del Moral, R. Verma, Y. Li, C. Li, K. N. Houk, M. E. Jung, N. Zheng, L. Huang, R. J. Deshaies, P. Kaiser, J. Huang, *Nat. Biotechnol.* **2010**, *28*, 738 – 742; b) J. R. Lydeard, J. W. Harper, *Nat. Biotechnol.* **2010**, *28*, 682 – 684.

[68] S. Orlicky, X. Tang, V. Neduva, N. Elowe, E. D. Brown, F. Sicheri, M. Tyers, *Nat. Biotechnol.* **2010**, *28*, 733 – 737.

[69] H. Nakajima, H. Fujiwara, Y. Furuichi, K. Tanaka, N. Shimbara, *Biochem. Biophys. Res. Commun.* **2008**, *368*, 1007 – 1013.

[70] D. L. Buckley, I. Van Molle, P. C. Gareiss, H. S. Tae, J. Michel, D. J. Noblin, W. L. Jorgensen, A. Ciulli, C. M. Crews, *J. Am. Chem. Soc.* **2012**, *134*, 4465 – 4468.

[71] I. Van Molle, A. Thomann, D. L. Buckley, E. C. So, S. Lang, C. M. Crews, A. Ciulli, *Chem. Biol.* **2012**, *19*, 1300 – 1312.

[72] a) D. L. Buckley, J. L. Gustafson, I. Van Molle, A. G. Roth, H. S. Tae, P. C. Gareiss, W. L. Jorgensen, A. Ciulli, C. M. Crews, *Angew. Chem.* **2012**, *124*, 11630 – 11634; *Angew. Chem. Int. Ed.* **2012**, *51*, 11463 – 11467.

[73] T. Ito, H. Ando, T. Suzuki, T. Ogura, K. Hotta, Y. Imamura, Y. Yamaguchi, H. Handa, *Science* **2010**, *327*, 1345 – 1350.

[74] L. A. Banaszynski, L.-C. Chen, L. A. Maynard-Smith, A. G. L. Ooi, T. J. Wandless, *Cell* **2006**, *126*, 995 – 1004.

[75] F. H. Niesen, H. Berglund, M. Vedadi, *Nat. Protoc.* **2007**, *2*, 2212 – 2221.

[76] B. Lomenick, R. Hao, N. Jonai, R. M. Chin, M. Aghajan, S. Warburton, J. Wang, R. P. Wu, F. Gomez, J. A. Loo, J. A. Wohlschlegel, T. M. Vondriska, J. Pelletier, H. R. Herschman, J. Clardy, C. F. Clarke, J. Huang, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21984 – 21989.

[77] L. A. Banaszynski, T. J. Wandless, *Chem. Biol.* **2006**, *13*, 11 – 21.

[78] a) J. H. Bayle, J. S. Grimley, K. Stankunas, J. E. Gestwicki, T. J. Wandless, G. R. Crabtree, *Chem. Biol.* **2006**, *13*, 99 – 107; b) K. Stankunas, J. H. Bayle, J. E. Gestwicki, Y.-M. Lin, T. J. Wandless, G. R. Crabtree, *Mol. Cell* **2003**, *12*, 1615 – 1624.

[79] L. A. Banaszynski, M. A. Sellmyer, C. H. Contag, T. J. Wandless, S. H. Thorne, *Nat. Med.* **2008**, *14*, 1123 – 1127.
[80] a) H. D. Lau, J. Yaegashi, B. W. Zaro, M. R. Pratt, *Angew. Chem.* **2010**, *122*, 8636 – 8639; *Angew. Chem. Int. Ed.* **2010**, *49*, 8458 – 8461; b) M. R. Pratt, E. C. Schwartz, T. W. Muir, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11209 – 11214.
[81] K. M. Bonger, L.-C. Chen, C. W. Liu, T. J. Wandless, *Nat. Chem. Biol.* **2011**, *7*, 531 – 537.
[82] S. Al-Bassam, M. Xu, T. J. Wandless, D. B. Arnold, *Cell Reports* **2012**, *2*, 89 – 100.
[83] M. D. Shoulders, L. M. Ryno, C. B. Cooley, J. W. Kelly, R. L. Wiseman, *J. Am. Chem. Soc.* **2013**, *135*, 8129 – 8132.
[84] B. J. Feldman, D. Feldman, *Nat. Rev. Cancer* **2001**, *1*, 34 – 45.
[85] T. W. Corson, N. S. Aberle, C. M. Crews, *ACS Chem. Biol.* **2008**, *3*, 677 – 692.
[86] K. Sakamoto, K.-B. Kim, A. Kumagai, F. Mercurio, C. M. Crews, R. J. Deshaies, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 8554 – 8559.
[87] K. Sakamoto, K.-B. Kim, R. Verma, A. Ransick, B. Stein, C. M. Crews, R. J. Deshaies, *Mol. Cell. Proteomics* **2003**, *2*, 1350 – 1358.
[88] W. Yang, L. W. Rozamus, S. Narula, C. T. Rollins, R. Yuan, L. J. Andrade, M. K. Ram, T. B. Phillips, M. R. van Schravendijk, D. Dalgarno, T. Clackson, D. A. Holt, *J. Med. Chem.* **2000**, *43*, 1135 – 1142.
[89] J. S. Schneekloth, Jr., F. N. Fonseca, M. Koldobskiy, A. K. Mandal, R. J. Deshaies, K. Sakamoto, C. M. Crews, *J. Am. Chem. Soc.* **2004**, *126*, 3748 – 3754.
[90] P. Bargagna-Mohan, S.-H. Baek, H. Lee, K.-B. Kim, R. Mohan, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2724 – 2727.
[91] a) H. Lee, D. Puppala, E.-Y. Choi, H. Swanson, K.-B. Kim, *ChemBioChem* **2007**, *8*, 2058 – 2062; b) D. Puppala, H. Lee, K. B. Kim, H. I. Swanson, *Mol. Pharmacol.* **2008**, *73*, 1064 – 1071.
[92] J. Hines, J. D. Gough, T. W. Corson, C. M. Crews, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 8942 – 8947.
[93] A. R. Schneekloth, M. Pucheault, H. S. Tae, C. M. Crews, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5904 – 5908.
[94] Y. Itoh, M. Ishikawa, M. Naito, Y. Hashimoto, *J. Am. Chem. Soc.* **2010**, *132*, 5820 – 5826.
[95] Y. Itoh, R. Kitaguchi, M. Ishikawa, M. Naito, Y. Hashimoto, *Bioorg. Med. Chem.* **2011**, *19*, 6768 – 6778.
[96] a) S. Sato, M. Tetsuhashi, K. Sekine, H. Miyachi, M. Naito, Y. Hashimoto, H. Aoyama, *Bioorg. Med. Chem.* **2008**, *16*, 4685 – 4698; b) K. Sekine, K. Takubo, R. Kikuchi, M. Nishimoto, M. Kitagawa, F. Abe, K. Nishikawa, T. Tsuruo, M. Naito, *J. Biol. Chem.* **2008**, *283*, 8961 – 8968.
[97] J. Kalia, R. T. Raines, *Angew. Chem.* **2008**, *120*, 7633 – 7636; *Angew. Chem. Int. Ed.* **2008**, *47*, 7523 – 7526.
[98] G. V. Los, L. P. Encell, M. G. McDougall, D. D. Hartzell, N. Karassina, C. Zimprich, M. G. Wood, R. Learish, R. F. Ohana, M. Urh, D. Simpson, J. Mendez, K. Zimmerman, P. Otto, G. Vidugiris, J. Zhu, A. Darzins, D. H. Klaubert, R. F. Bulleit, K. V. Wood, *ACS Chem. Biol.* **2008**, *3*, 373 – 382.
[99] T. K. Neklesa, H. S. Tae, A. R. Schneekloth, M. J. Stulberg, T. W. Corson, T. B. Sundberg, K. Raina, S. A. Holley, C. M. Crews, *Nat. Chem. Biol.* **2011**, *7*, 538 – 543.
[100] T. K. Neklesa, D. J. Noblin, A. Kuzin, S. Lew, J. Seetharaman, T. B. Acton, G. Kornhaber, R. Xiao, G. T. Montelione, L. Tong, C. M. Crews, *ACS Chem. Biol.* **2013**, *8*, 2293 – 2300.
[101] H. S. Tae, T. B. Sundberg, T. K. Neklesa, D. J. Noblin, J. L. Gustafson, A. G. Roth, K. Raina, C. M. Crews, *ChemBioChem* **2012**, *13*, 538 – 541.
[102] R. F. Ohana, L. P. Encell, K. Zhao, D. Simpson, M. R. Slater, M. Urh, K. V. Wood, *Protein Expression Purif.* **2009**, *68*, 110 – 120.
[103] M. J. C. Long, D. R. Gollapalli, L. Hedstrom, *Chem. Biol.* **2012**, *19*, 629 – 637.
[104] a) A. Howell, *Crit. Rev. Oncol. Hematol.* **2006**, *57*, 265 – 273; b) C. K. Osborne, A. Wakeling, R. I. Nicholson, *Br. J. Cancer* **2004**, *90*, S2 – S6.
[105] a) A. L. Wijayaratne, D. P. McDonnell, *J. Biol. Chem.* **2001**, *276*, 35684 – 35692; b) B. M. Wittmann, A. Sherk, D. P. McDonnell, *Cancer Res.* **2007**, *67*, 9549 – 9560.
[106] A. Citri, I. Alroy, S. Lavi, C. Rubin, W. Xu, N. Grammatikakis, C. Patterson, L. Neckers, D. W. Fry, Y. Yarden, *EMBO J.* **2002**, *21*, 2407 – 2417.
[107] D. M. Janse, B. Crosas, D. Finley, G. M. Church, *J. Biol. Chem.* **2004**, *279*, 21415 – 21420.
[108] P. Zhou, *Curr. Opin. Chem. Biol.* **2005**, *9*, 51 – 55.
[109] a) J. Zhang, N. Zheng, P. Zhou, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14127 – 14132; b) P. Zhou, R. Bogacki, L. McReynolds, P. M. Howley, *Mol. Cell* **2000**, *6*, 751 – 756.
[110] F. Cong, J. Zhang, W. Pao, P. Zhou, H. Varmus, *BMC Mol. Biol.* **2003**, *4*, 10.
[111] C. Schneider, L. Sepp-Lorenzino, E. Nimmesgern, O. Ouerfelli, S. Danishefsky, N. Rosen, F. U. Hartl, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 14536 – 14541.
[112] L. Whitesell, E. G. Mimnaugh, B. De Costa, C. E. Myers, L. M. Neckers, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 8324 – 8328.
[113] L. F. Stancato, A. M. Silverstein, J. K. Owens-Grillo, Y. Chow, R. Jove, W. B. Pratt, *J. Biol. Chem.* **1997**, *272*, 4013 – 4020.
[114] a) T. Taldone, A. Gozman, R. Maharaj, G. Chiosis, *Curr. Opin. Pharmacol.* **2008**, *8*, 370 – 374; b) M. Sgobba, G. Rastelli, *ChemMedChem* **2009**, *4*, 1399 – 1409; c) T. Taldone, W. Sun, G. Chiosis, *Bioorg. Med. Chem.* **2009**, *17*, 2225 – 2235; d) M. A. Biamonte, R. Van de Water, J. W. Arndt, R. H. Scannevin, D. Perret, W.-C. Lee, *J. Med. Chem.* **2010**, *53*, 3 – 17; e) L. Neckers, P. Workman, *Clin. Cancer Res.* **2012**, *18*, 64 – 76.
[115] G. Chiosis, M. N. Timaul, B. Lucas, P. N. Munster, F. F. Zheng, L. Sepp-Lorenzino, N. Rosen, *Chem. Biol.* **2001**, *8*, 289 – 299.
[116] a) E. Caldas-Lopes, L. Cerchietti, J. H. Ahn, C. C. Clement, A. I. Robles, A. Rodina, K. Moulick, T. Taldone, A. Gozman, Y. Guo, N. Wu, E. de Stanchina, J. White, S. S. Gross, Y. Ma, L. Varticovski, A. Melnick, G. Chiosis, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 8368 – 8373; b) R. M. Immormino, Y. Kang, G. Chiosis, D. T. Gewirth, *J. Med. Chem.* **2006**, *49*, 4953 – 4960.
[117] S. A. Eccles, A. Massey, F. I. Raynaud, S. Y. Sharp, G. Box, M. Valenti, L. Patterson, A. de Haven Brandon, S. Gowan, F. Boxall, W. Aherne, M. Rowlands, A. Hayes, V. Martins, F. Urban, K. Boxall, C. Prodromou, L. Pearl, K. James, T. P. Matthews, K.-M. Cheung, A. Kalusa, K. Jones, E. McDonald, X. Barril, P. A. Brough, J. E. Cansfield, B. Dymock, M. J. Drysdale, H. Finch, R. Howes, R. E. Hubbard, A. Surgenor, P. Webb, M. Wood, L. Wright, P. Workman, *Cancer Res.* **2008**, *68*, 2850 – 2860.
[118] P. Fadden, K. H. Huang, J. M. Veal, P. M. Steed, A. F. Barabasz, B. Foley, M. Hu, J. M. Partridge, J. Rice, A. Scott, L. G. Dubois, T. A. Freed, M. A. R. Silinski, T. E. Barta, P. F. Hughes, A. Ommen, W. Ma, E. D. Smith, A. W. Spangenberg, J. Eaves, G. J. Hanson, L. Hinkley, M. Jenks, M. Lewis, J. Otto, G. J. Pronk, K. Verleysen, T. A. Haystead, S. E. Hall, *Chem. Biol.* **2010**, *17*, 686 – 694.
[119] S. D. Kuduk, F. F. Zheng, L. Sepp-Lorenzino, N. Rosen, S. J. Danishefsky, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1233 – 1238.
[120] S. D. Kuduk, C. R. Harris, F. F. Zheng, L. Sepp-Lorenzino, Q. Ouerfelli, N. Rosen, S. J. Danishefsky, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1303 – 1306.
[121] F. F. Zheng, S. D. Kuduk, G. Chiosis, P. N. Münster, L. Sepp-Lorenzino, S. J. Danishefsky, N. Rosen, *Cancer Res.* **2000**, *60*, 2090 – 2094.
[122] D. Komander, M. J. Clague, S. Urbé, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 550 – 563.
[123] F. Colland, E. Formstecher, X. Jacq, C. Reverdy, C. Planquette, S. Conrath, V. Trouplin, J. Bianchi, V. N. Aushev, J. Camonis, A.

Calabrese, C. Borg-Capra, W. Sippl, V. Collura, G. Boissy, J.-C. Rain, P. Guedat, R. Delansorne, L. Daviet, *Mol. Cancer Ther.* **2009**, *8*, 2286 – 2295.

[124] C. Reverdy, S. Conrath, R. Lopez, C. Planquette, C. Atmanene, V. Collura, J. Harpon, V. Battaglia, V. Vivat, W. Sippl, F. Colland, *Chem. Biol.* **2012**, *19*, 467 – 477.

[125] J. Weinstock, J. Wu, P. Cao, W. D. Kingsbury, J. L. McDermott, M. P. Kodrasov, D. M. McKelvey, K. G. S. Kumar, S. J. Goldenberg, M. R. Mattern, B. Nicholson, *ACS Med. Chem. Lett.* **2012**, *3*, 789 – 792.

[126] S. Ohayon, L. Spasser, A. Aharoni, A. Brik, *J. Am. Chem. Soc.* **2012**, *134*, 3281 – 3289.

[127] M. Altun, H. B. Kramer, L. I. Willems, J. L. McDermott, C. A. Leach, S. J. Goldenberg, K. G. S. Kumar, R. Konietzny, R. Fischer, E. Kogan, M. M. Mackeen, J. McGouran, S. V. Khoronenkova, J. L. Parsons, G. L. Dianov, B. Nicholson, B. M. Kessler, *Chem. Biol.* **2011**, *18*, 1401 – 1412.

[128] A. H. Mermerian, A. Case, R. L. Stein, G. D. Cuny, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3729 – 3732.

[129] P. D'Arcy, S. Brnjic, M. H. Olofsson, M. Fryknäs, K. Lindsten, M. De Cesare, P. Perego, B. Sadeghi, M. Hassan, R. Larsson, S. Linder, *Nat. Med.* **2011**, *17*, 1636 – 1640.

[130] B.-H. Lee, M. J. Lee, S. Park, D.-C. Oh, S. Elsasser, P.-C. Chen, C. Gartner, N. Dimova, J. Hanna, S. P. Gygi, S. M. Wilson, R. W. King, D. Finley, *Nature* **2010**, *467*, 179 – 184.