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CHAPTER 3

Targeting Proteasomal Degradation of Soluble, Misfolded Proteins

3.1 UPS-MEDIATED DEGRADATION OF MISFOLDED PROTEINS

The elimination of misfolded protein copies is an appealing option to tackle neurodegenerative diseases (NDDs) and tauopathies. The ubiquitin–proteasome system (UPS) [1,2] is the most important cellular pathway to dispose of soluble cytosolic proteins.

UPS is responsible for most regulated proteolytic events, and depends on the 76-mer protein ubiquitin (UBQ) [3,4]. UBQ is a stable, compact protein with the six C-terminus amino acids (AAs) arranged as a flexible tail [4]. A first UBQ molecule is anchored to the ε-NH₂ group of a Lys residue in the substrate proteins through an isopeptide bond involving the C-terminus of UBQ. Mono-ubiquitination may target a specific Lys residue [5], or a domain [6] on the substrate protein. Multiple mono-ubiquitination on different Lys residues of the substrate protein (multimoно-убикутина́ция) is also observed [7]. Ubiquitinated proteins are usually tagged with polyUBQ chains. UBQ chain elongation implies the formation of an isopeptide bond between one of seven Lys residues (K6, K11, K27, K29, K33, K48, and K63), or between the Met1 residue of a substrate-anchored proximal UBQ molecule and the C-terminus of a free, distal UBQ protein. Different UBQ chain elongation enzymes bind to interaction surfaces on UBQ with diverse specificities, and promote UBQ elongation on a specific anchoring point.

Once a di-UBQ chain is formed, the two UBQ molecules assume an anchoring residue-dependent conformation. Five out of the eight UBQ
connections are structurally characterized by X-ray crystallography and/or nuclear magnetic resonance (NMR) [8]. Among them, K48-linked di-UBQ chains adopt a “closed,” compact conformation, where the two UBQ proteins interact with each other [9]. K63-linked di-UBQ chains adopt an “open” and more flexible conformation, where the two UBQ molecules do not interact beyond their isopeptide bond connection [10]. Highly flexible open conformations of di-UBQ chains provide even more alternative binding modes with their protein partners.

K48-linked polyUBQ chains are the most abundant homotypic poly-UBQ species [11], acting as labels on proteins to be degraded through the UPS [12]. Proteasome inhibition causes a fast increase of K48-polyUBQ proteins [13], and mutation of Lys residues in yeast UBQ show that K48 is the only essential residue among them [14].

There are eight Lys/Met anchoring points on UBQ, UBQ chain lengths varying between 1 (mono-UBQ) and >10 polyUBQs on each anchoring point, and >700 enzymes involved in a multi-step process (including UBQ activation, conjugation, transfer to a protein substrate, and chain trimming) [8]. The combinations of UBQ codes easily match the experimental observation of thousands of UBQ-labeled protein substrates on multiple sites, and ensure an exquisitely specific UBQ/UPS-dependent regulation of the functions reconducible to ubiquitinated proteins. But how can the UBQ machinery select the anchoring point, the UBQ chain length and nature, and the specific substrate to be ubiquitinated or deubiquitinated in a dynamic cellular environment?

UBQ is activated by two UBQ-activating (E1) enzymes through the formation of a high energy Cys–UBQ thioester bond [15,16]. E1 enzymes are relieved of their UBQ cargo by ≈40 UBQ-conjugating (E2) enzymes through a trans-thiolation reaction [17]. E2 enzymes contain a highly conserved 150–200 AA UBQ-conjugating (UBC) catalytic fold that acts as a scaffold for E1 enzymes, E3 UBQ ligases, and activated UBQ [18]. More than 600 E3 ligases receive UBQ from E2 enzymes and transfer it to substrates through three main mechanisms [19]. Really interesting new gene (RING) E3 ligases directly transfer UBQ from E2–UBQ complexes to RING E3-bound protein substrates [20]. Homologous to the E6AP carboxyl terminus (HECT) E3 ligases first bind UBQ onto a Cys residue of the HECT domain and release E2 enzymes, then bind protein substrates and transfer UBQ to them [21]. RING-in-between-RING (RBR) E3 ligases [22] act through a RING/HECT hybrid mechanism. Mono- and polyUBQ chains can be disassembled by a ≈100-membered class of isopeptide-specific deubiquitinating enzymes (DUBs) [23] that are essential to ensure proper processing of ubiquitinated proteins. Finally, the 26S proteasome complex is the protein degradation terminal for UBQ-tagged proteins in eukaryotes [24]. It is made by a 20S barrel-shaped catalytic core particle (CP) composed by 28 subunits, structurally arranged in four stacked seven membered rings [25], and by two
19S regulatory particles (RPs), composed each by 19 subunits (a 9 subunit lid, and a 10 subunit base structure [26]). The UBQ activation–conjugation–ligation-trimming cycle is extensively described in the biology-oriented companion book [27].

Any E1, E2, E3, and DUB enzyme may be considered a suitable target to restore the UPS activity in an impaired cellular environment. Proteasome activity may also be targeted for a direct effect on UPS. NDDs and tauopathies require the potentiation/restoring of cellular mechanisms leading to the elimination of misfolded/aggregated proteins. UPS inhibition may appear to lead in the opposite direction—decreasing UPS-mediated elimination of tau and other misfolded proteins. Certain enzymes in the UPS system, though, contribute to the elimination of proteins hindering the rescuing/refolding of misfolded proteins. Their inhibition, thus, should be beneficial.

In particular, the carboxy-terminus of Hsp70-interacting protein (CHIP) [28] is an E3 UBQ ligase, due to a U-box domain at its C-terminus [29], and a Hsp70/Hsp90 co-chaperone, due to its three tandem tetra tricopeptide (TPR) domains at the N-terminus [30]. CHIP is a key player in cellular management of misfolded proteins. Its role as an Hsp70-dependent, tau-ubiquitinating enzyme has been known for a decade [31,32]. The ubiquitin-specific protease USP14 is a UBQ-trimming, proteasome-bound DUB that frees and recycles UBQ before substrate protein degradation by the UPS [33]. The role of USP14 in physiological and pathological events of the CNS is well known [34,35]. The next two sections describe small molecule modulators acting on each selected target.

3.2 CHIP

The role of CHIP in cellular management of misfolded proteins is due to its three N-terminal TPR/chaperone-binding domain repeats, and to its C-terminal U-box/UBQ-binding domain. Modulation of the whole set of CHIP functions would likely impact on many physiological processes. Specificity may be targeted through chaperone-mediated CHIP-misfolded protein interactions (through the TPR domains), aiming to regulate the target/substrate protein clearance (i.e., to increase it in NDDs) [36]. Specificity may also be obtained by targeting CHIP–E2-conjugating enzyme interactions (through the U-box domain), aiming to regulate the clearance of misfolded proteins through the specific CHIP–E2 couple that ubiquitini nates a particular target/substrate protein [37].

NDDs often depend on pro-aggregation misfolded proteins. CHIP may contribute to their refolding, through complexation with Hsp90. When either neurotoxic protein oligomers exceed the refolding capacity of neurons, or the PQC machinery is impaired, CHIP may promote the aggregation of
protein oligomers into insoluble, less toxic aggregates to be cleared via autophagy (see autophagy and aggrephagy, Chapters 4 and 5 here and in the biology-oriented companion book [27]). Either Hsp90 inhibition or CHIP overexpression increase the amount of protein clearance-directing Hsp70–CHIP complexes. Hsp90 inhibitors are described in Chapter 2, while small molecules capable of promoting CHIP induction in cells are unknown.

Protein regulators of CHIP, capable of orienting the fate of misfolded proteins, are well known. Members of the Bcl-2-associated athanogene co-chaperone family (BAG) may mediate the docking of a CHIP–chaperone–target protein complex at the proteasome, facilitating proteasomal degradation (BAG-1 [38]). They may suppress CHIP-mediated ubiquitination and degradation of a target protein by abrogation of the CHIP–E2 interaction (BAG-2 [39]). They may promote autophagic clearance when complexed with Hsp70 and CHIP in aging and/or protein aggregate-rich tissues (BAG-3 [40]). Finally, they may inhibit CHIP-mediated ubiquitination and degradation of a target protein through unclarified molecular mechanisms (BAG-5) [41]. The Hsp70-binding protein 1 (HspBP1) co-chaperone causes a conformational change in CHIP–chaperone complexes and prevents the ubiquitination of Hsc70-bound client proteins [42]. The S100A2 and S100P proteins inhibit CHIP-mediated ubiquitination and proteasomal degradation in a Ca2+-dependent manner [43]. Stimulation of BAG-1- and BAG-3-promoted effects, and inhibition/prevention of CHIP negative regulation by BAG-2 and BAG-5 co-chaperones would be desirable in NDDs. Unfortunately, limited structural information on CHIP-containing chaperone complexes is available [44] to drive rational drug design projects.

Examples of selective modulation of CHIP–target protein complexes in the presence of Hsp chaperones are scarce [30]. Small molecules acting on TPR-mediated interactions of CHIP with chaperones, co-chaperones, and misfolded proteins are uncommon, and their selectivity is at best questionable. Thioflavin S (3.1a,b, Figure 3.1, see also 2.45a,b, Figure 2.9 and section 2.3.2) is a mixture of benzothiazolylammonium salts showing sub-μM potency in preventing the Hsc/Hsp70–BAG-1 interaction [45]. BAG-1–CHIP interactions could be modulated by 3.1a,b, although the cellular activity of thioflavin S could be due to Hsc/Hsp70–BAG-1-unrelated mechanisms.

The sulfonamide pifithrin-μ (PES, 3.2, see also 2.44, Figure 2.8 and section 2.3.1) shows multiple actions on the Hsp70 machinery [46]. Complexes between Hsp70 and CHIP, BAG-1 and other J-domain proteins are affected by PES. Its multiple and potent anticancer effects may be caused in part by the triple bond chemical reactivity, but probably are not entirely amenable to the Hsp70 chaperone network [46]. Naturally occurring gambogic acid (GA, 3.3) causes the UPS-dependent degradation of mutant p53 [47]. GA decreases the Hsp90–mutant p53 interaction and in parallel
increases the levels of the ternary, UPS-oriented Hsp70–CHIP–mutant p53 complex through molecular interactions that are not yet elucidated. UPS-mediated degradation of polyUBQ mutant p53 via selective CHIP ubiquitination may be a GA-driven effect shared by other misfolded proteins [47]. The tricyclic phenothiazine methylene blue (MB, 3.4), currently in clinical trials in AD patients as a tau aggregation inhibitor [48], negatively modulates polyQ protein degradation through Hsp70 binding and subsequent sub-μM inhibition of CHIP-mediated polyQ protein ubiquitination [49]. Its multi-targeted activity profile is discussed in detail in Chapter 6. Imidazole-based apoptozole (3.5, Figure 3.1) restores the defective cellular processing of ∆F508-CFTR (cystic fibrosis transmembrane conductance regulator), a mutant, misfolded protein involved in cystic fibrosis [50]. Its rescuing effect at sub-μM concentrations is likely due to the disruption of the tertiary Hsp70–CHIP–mutant protein complex, and to the prevention of CHIP-mediated ubiquitination and degradation of ∆F508-CFTR [50].

Small molecule modulators of chaperone-independent E3 ligase–target protein complexes are known [51]. Although they do not target CHIP-containing complexes, they indirectly prove the druggability of this protein–protein interaction (PPI) and must be mentioned. Two RING E3 ligases are targeted by candidates in clinical evaluation. The tetrasubstituted

![Figure 3.1](image-url)
imidazolidine nutlin-3 (3.6, Figure 3.2) [52] and indole-based serdemetan (3.7) [53] are clinically tested PPI inhibitors/anticancer agents targeting the p53-human double minute 2 (HDM2) PPI [54]. HDM2 is a RING E3 ligase that modulates UPS-mediated degradation of p53, and compounds 3.6 and 3.7 inhibit HDM2-mediated ubiquitination of p53 [55].

Bicyclic (AT-406, 3.8) [56], thiaiazole-, and thiazole-based monomeric (respectively GDC-0152 [57], 3.9 and LCL-161 [58], 3.10) and dimeric antagonists of inhibitor of apoptosis proteins (IAPs) (TL32711, 3.11, Figure 3.2) [59] are in clinical trials as anticancer agents. Their structure is inspired by the N-terminal AVPI sequence of the second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO), an endogenous ligand of IAPs [60]. They act as PPI inhibitors/Smac mimetics/IAP antagonists, preventing the interaction between IAP proteins and caspases [61]. They also bind to RING domain-containing cellular IAPs (cIAPs), activate their E3 ligase activity, and induce their auto-ubiquitination and rapid proteasomal degradation [62].
A few E3 ligase-containing complexes are targeted by small molecules at an earlier development stage. Such molecules are often discovered through high throughput screening (HTS) campaigns and assay formats that detect variations of E3 ligase activity [63]. The S-phase kinase-associated protein/Skp-cullin-F-box-containing (SCF) is the largest multi-protein RING E3 ligase family [64]. Diamino compound A (3.12, Figure 3.3) is identified through an HTS campaign targeted against inhibitors of the ubiquitination of p27Kip1 [65]. Enhanced UPS degradation of p27Kip1 is associated with poor prognosis in a variety of tumors, and SCF{Skp2} is the E3 ligase that degrades p27Kip1 [66]. Compound A moderately increases p27Kip1 levels in cells through the exclusion of Skp2 from the SCF{Skp2} E3 ligase complex, probably by inhibiting its binding with another complex member [65].

Inhibitors of SCF{Skp2} ligase activity stem from structure-based virtual screening of a 315K compound data set against an Skp2–Csk1–p27 ternary complex [67]. An alkylidene thiazolidine (compound C1, 3.13) reduces Skp2-mediated ubiquitination of p27 in vitro at low μM concentration, and increases p27 levels by decreasing its SCF{Skp2}-mediated degradation in melanoma cells [67]. The diacid SCF-I2 (3.14) is
identified as an inhibitor of SCF\textsuperscript{Cdc4} in an HTS campaign on a 50K member collection \cite{68}. SCF-I2 binds to the WP40 domain of the F-box protein cell division control protein 4 (Cdc4), as shown by the X-ray structure of the Cdc4–Skp1–SCF-12 complex. The conformational change induced in Cdc4 by SCF-I2 allosterically inhibits substrate recognition and ubiquitination by SCF\textsuperscript{Cdc4}. Although WP40 domains are shared by F-box proteins in several SCF RING E3 ligases, SCF-I2 appears to be selective against SCF\textsuperscript{Cdc4}. SCF-I2 is not active in cellular assays, as its two carboxylates prevent cellular permeability \cite{68}. LS-101 and LS-102 (respectively benzodiazepindione-based 3.15 and triazine-based 3.16) are identified from an =4M compound collection in an HTS campaign \cite{69} targeted against the auto-ubiquitination of synoviolin, a RING E3 ligase highly expressed in synoviocytes of patients suffering of rheumatoid arthritis \cite{70}. Both compounds show moderate \(\mu\)M potency against synoviolin auto-ubiquitination. LS-102 shows complete specificity \textit{vs}. three other RING E3 ligases, while LS-101 is non-selective. Both compounds show \textit{in vivo} activity in collagene-induced arthritis models \cite{69}. Tetra-cyclic SMER3 (3.17) is identified in a phenotype-based HTS looking for small molecule enhancers of the therapeutic effects of rapamycin (see also autophagy, Chapter 4 here and in the biology-oriented companion book \cite{27}) \cite{71}. SMER3 inhibits the RING E3 ligase SCF\textsuperscript{Met30}. It binds to the F-box motif in the Met30 protein, and it prevents its interaction with the SKC core protein Skp1. It is selective, as it is completely inactive against SCF\textsuperscript{Cdc4} \cite{71}. Finally, the bis-imide thalidomide (3.18, Figure 3.3) binds to cereblon (CRBN), a component of a RING E3 ligase complex, and inhibits its interaction with damaged DNA binding protein 1 (DDBP1) and Cullin 4 (Cul4) \cite{72}. It inhibits auto-ubiquitination in cells and \textit{in vivo}, both in zebrafish and chicken models. Teratogenicity of thalidomide is at least partially due to CRBN binding and E3 ligase inhibition \cite{72}.

Interactions between CHIP and E2 conjugating enzymes are extensively studied. The X-ray structures of murine CHIP complexed with the E2 enzymes Ubc13 \cite{73}, and of zebrafish CHIP and E2D1/UbcH5 \cite{74} are available. The conformational dynamics of the human CHIP–Ubc13 and CHIP–UbcH5 complexes, studied by amide hydrogen exchange mass spectrometry (HX-MS), highlight their differences and suggest that CHIP–E2 complexes in protein ubiquitination and chaperone interaction can be selectively modulated \cite{75}. A systematic study \cite{76} identifies a subset of E2 enzymes that bind CHIP through a common Ser-Pro-Ala motif, and promote target protein ubiquitination \textit{via} activation of E2–UBQ conjugates. Ubiquitination of target proteins depends on the E2 conjugating enzyme in terms of point of attachment (K48, K63, others) and UBQ chain length \cite{76}. For example, CHIP–UbcH5 preferentially catalyzes the mono-ubiquitination of target proteins through any Lys residue, while
CHIP–Ubc13 seems to be a polyUBQ-introducing complex with K63 specificity [77]. Finally, the tertiary E3:E2~UBQ complexes containing either breast cancer type 1 (BRCA1)/BRCA1–associated RING domain protein (BARD) or E4B/UFD2a as E3 ligases, and UbcH5c as E2 conjugating enzymes are studied by NMR [78]. The study provides valuable information on the role of RING/U-box E3 ligases such as CHIP in facilitating UBQ transfer and in promoting allosteric activation of E2~UBQ complexes [78]. The wealth of structural information is not yet translated into small molecules as regulators/modulators of CHIP:E2 binary, or CHIP:E2~UBQ tertiary complexes, and of their functions.

### 3.3 USP14

USP14 is a member of the largest USP/ubiquitin-specific protease subfamily, which contains ≈60 characterized family members [79,80]. Several USP enzymes are validated targets against various diseases [81], and CNS diseases in particular [34,35]. USP DUB inhibitors with varying degrees of inter-class selectivity are known [81–83]. UBQ analogs capable of specifically and irreversibly inactivating the thiol protease function of USPs include UBQ aldehyde [84] and UBQ vinyl sulfone [85]. They are useful probes to identify and characterize cysteine protease DUBs, but their peptidic nature and aspecificity hinder their use either as such, or as structural models for drug discovery efforts [81].

Aspecific USP inhibitors include electrophilic dienones, resulting from a computational pharmacophoric search on the National Cancer Institute (NCI) chemical database [86]. Curcumin (3.19, Figure 3.4), shikoccin (3.20), and ∆12-PGJ2 (3.21) are cytotoxic compounds whose cellular activity is at least partially due to DUB inhibition [86]. ∆12-PGJ2 is the most potent representative among electrophilic prostaglandins [87], whose activity against UCH DUBs is also reported [88]. Curcumin is covered in detail for its anti-aggregating properties on amyloidogenic peptides in Chapter 6 here and in the biology-oriented companion book [27].

The dienone NSC 632839 (3.22) shows similar, aspecific DUB inhibition [89]. Bis-isothiocyanate PR-619 (3.23) is an aspecific DUB inhibitor isolated from a pan-DUB-targeted HTS campaign [90]. It causes protein aggregation in neuronal cells and stabilizes microtubules (MTs), possibly with some effects on tau [91].

The tricyclic dinitrile HBX-41,108 (3.24a) results from structural optimization of hits from an HTS campaign targeted against the USP family member USP7 [92]. It stabilizes p53 and induces p53-dependent apoptosis in cancer cells through inhibition of the p53-deubiquitinating enzyme USP7 [92]. It inhibits at least five other USPs, and an UCH
DUB family member [93]. Compound 3.24b is reported as a selective USP8 inhibitor [82,94] but its structural similarity with 3.24a induces to suspect a limited selectivity against other DUBs. Gold complexes such as 3.25 potently inhibit DUBs and are endowed with cytotoxic activity [95].

The cyanoamide WP-1130 (3.26) is a member of a synthetic tyrphostin-like library [96]. It is active in a cell-based screen targeted towards the Janus-activated kinase (JAK)/signal transducer and activator of transcription (STAT) pathway [97]. WP-1130 is a partially selective, cell permeable USP inhibitor, active against USP5, USP9x, USP14, UCH-L1, and UCH37 [97]. It shows pro-apoptotic effects through up-regulation of p53 and down-regulation of myeloid cell leukemia sequence 1 (MCL-1) levels, and promotes aggresome formation in cancer cells [97]. The cellular effects of
WP-1130 are due to inhibition of the unknown DUB responsible for JAK-2 deubiquitination [98]. It also enhances bacterial killing via localization of inducible nitric oxide synthase (iNOS) to the macrophage phagosome [99], and shows antiviral activity through USP14-mediated induction of the unfolded protein response (UPR) [100].

The alkyliden-pyrazolidindione PYR-41 (3.27), originally reported as a selective and irreversible E1 UBQ-activating enzyme [101], inhibits several DUBs, and even unrelated Cys-containing enzymes, through displacement of their nitro function by Cys residues [102]. Betulinic acid (3.28, Figure 3.4) shows multiple DUB-inhibiting activities and cytotoxicity on proliferating cancer cells, while it does not have similar effects on normal cells [103]. This may be due to a general increase of DUB levels in proliferating vs. non-proliferating cells, or to partially selective inhibition by betulinic acid of a subset of DUBs that are highly overexpressed/much more active in cancer cells [103].

A chalcone-based library contains cytotoxic, UPS-inhibiting representatives [104]. Some of its members, such as RA-9 (3.29, Figure 3.5), are partially selective DUB inhibitors, inhibiting >50% overall DUB activity in cervical cancer HeLa cells at 10 µM [105]. UPS2, UPS5, UPS8, UCH-L1, and UCH-L3 are among the DUBs inhibited by RA-9, while USP7 is not affected. RA-9 and its analogs induce polyUBQ accumulation, deplete the free UBQ pool, and promote apoptosis in cancer cells, while being non-toxic to normal cells [105].

The anti-psychotic, phthalimide-based pimozide (3.30) is a selective, allosteric, low µM inhibitor of the DUB enzyme USP1–USP1 associated factor 1 (UAF1) complex [106]. Pimozide weakly inhibits USP7, and is selective against a wide set of cysteine proteases. It restores cisplatin sensitivity to cisplatin-resistant small lung cancer cells [106]. A phenotype screen aimed towards autophagy inhibitors identifies the specific and potent autophagy inhibitor-1 (spautin-1, 3.31) [107]. Spautin-1 is a selective nM inhibitor of USP10 and USP13. USP10 and USP13 are the DUBs acting on beclin-1, a key component of the autophagy-regulating kinase vacuolar protein sorting 34 (Vps34) complex (see also autophagy, Chapter 4 here and in the biology-oriented companion book [27]). Conversely, beclin-1 and the Vps34 complex regulate the levels of USP10 and USP13. Spautin-1-caused autophagy inhibition increases p53 levels and may represent a novel anticancer approach [107].

Thiophene-based P5091 (3.32) is a USP7–USP47-selective DUB inhibitor with low µM potency identified in a pan-DUB-targeted HTS campaign [90]. P5091 increases ubiquitinated HDM2, which is then degraded and leads to p53-mediated cytotoxicity in cancer cells [108]. P5091 restores sensitivity to bortezomib-resistant cancer cells, and shows synergistic effects when combined with proteasome and histone deacetylase (HDAC) inhibitors [108]. A structurally related analog (3.33) is more potent in cellular
assays, causing an increase in p53 levels and an induction of p21 [109]. The aminotetrahydroacridine HBX-19,818 (3.34) is a cell permeable, selective, moderately potent USP7 inhibitor identified through an HTS campaign [93]. HDM2-p53 regulation and subsequent cytotoxicity are observed in human colon carcinoma (HCT116) cancer cells [93].

The naphthylamide GRL0617 (3.35) is an nM, non-covalent inhibitor of the papain-like protease (PLpro) from the severe acute respiratory syndrome (SARS)-causing coronavirus [110]. PLpro acts as a DUB, and the antiviral properties of GRL0617 (the result of structural optimization on an HTS hit) stem from inhibition of the deubiquitinase activity of PLpro.
GRL0617 does not inhibit human DUBs, and its specificity is explained by
the X-ray structure of the PLpro–GRL0617 complex [110].

Two USP14-targeted small molecule inhibitors are known. The electrophilic
diene NSC687852/b-AP15 (3.36) is a pro-apoptotic compound
identified in an HTS campaign on an NCI chemical collection [111]. It
induces caspase-dependent apoptosis, and increases the levels of poly-
UBQ proteins through DUB inhibition [112]. b-AP15 selectively inhibits
with moderate potency two structurally unrelated, proteasomal DUBs,
USP14 and UCH37, probably because of their common association with
the proteasome [113]. It shows in vivo potency in animal models of leukemia,
colon, lung, and breast carcinoma [113,114]. Pyrrole-based IU1 (3.37,
Figure 3.5) is a selective, low μM inhibitor of USP14. It enhances in vitro
UBQ–transactive response/TAR DNA binding protein 43 (TDP-43), UBQ–
ataxin-3, and UBQ–tau levels, and increases their UPS-mediated proteolysis
in murine embryonic fibroblasts (MEFs) [115]. It shows antiviral prop-
erties against Dengue virus, most likely through UPS enhancement [116].
The tau- and ataxin-3-regulating role of USP14 (and consequently the pu-
tative therapeutic effect of IU1) is questionable [117], but a compensatory
increase of USP14 activity is observed in elderly cells [118].

Additional basic studies and potent, selective compounds are needed
to elucidate the potential of “clean” and “mixed” USP14 inhibitors against
neurodegeneration, as even small selectivity profile changes may induce
major alterations in cellular effects. For example, USP14–UCH37-targeted
bAP15 regulates caspase-1 activation and interleukin (IL)-1 release in an
inflammation model, while IU1, a “clean” USP14 inhibitor, is inactive in
the same model [119]. The existence of an additional, unknown DUB tar-
get for bAP15 cannot be ruled out, but the importance of cell-permeable
DUB inhibitors with finely tuned poly-DUB pharmacology is evident. The
available information regarding the structure of DUBs [79], and in partic-
ular the crystal structure of the 45 kDa catalytic domain of USP14 in isola-
tion and complexed with UBQ aldehyde [120], should assist the rational
design and synthesis of USP14 inhibitors with varying selectivity profiles.

3.4 RECAP

This chapter deals with small molecule modulators of neuropathologi-
cal alterations caused by protein misfolding and aggregation in general,
and by tau and/or tau-connected events in particular. A potential thera-
peutic mechanism was examined in detail in the biology-oriented com-
panion book [27], and two targets were arbitrarily chosen. Thirty-eight
scaffolds shown in Figures 3.1 to 3.5, acting on selected targets, are de-
scribed in detail in this chapter, and are briefly summarized in Table 3.1.
### TABLE 3.1 Compounds 3.1–3.37: Chemical Class, Target, Developing Organization, Development Status

| Number | Chemical cpd./class | Target | Organization | Dev. status |
|--------|---------------------|--------|--------------|-------------|
| 3.1a,b | Thioflavin S        | Hsp70–BAG-1 | Cancer Research, UK | DD          |
| 3.2    | Pifithrin-m, PES    | Hsp70, plus others | University of Pennsylvania | LO          |
| 3.3    | Gambogic acid, GA   | Hsp70–, Hsp90–CHIP regulation | Jangsu University, China | TM          |
| 3.4    | Methylene blue, MB  | Hsp70–CHIP regulation | TauRX Therapeutics | Ph III      |
| 3.5    | Apoptozole          | Hsp70, ATPase inhib. | Yonsei University, South Korea | LO          |
| 3.6    | Nutlin-3            | HDM2–p53 | Roche | Ph I         |
| 3.7    | Serdemetan          | HDM2–p53 | Johnson & Johnson | Ph I         |
| 3.8    | AT-406              | IAPs | Ascenta | Ph I         |
| 3.9    | GDC-0152            | IAPs | Genentech | Ph I         |
| 3.10   | LCL-161             | IAPs | Novartis | Ph II        |
| 3.11   | TL32711             | IAPs | Tetralogic | Ph II        |
| 3.12   | Diamines, compound A | Skp2 | University of North Carolina | DD          |
| 3.13   | Alkylidene thiazoli- | Skp2 | NY University | DD          |
|        | dines, compound C1  |        |              |             |
| 3.14   | Diacids, SKP-12     | Cdc4   | Mount Sinai Hosp., Toronto, Canada | DD          |
| 3.15   | Benzodiazepinediones, | Synoviolin | Tokyo Medical Univ. | LO          |
|        | LS-101              |        |              |             |
| 3.16   | Triazines, LS-102   | RING E3 ligases | Tokyo Medical Univ. | LO          |
| 3.17   | Tetracycles, SMER3  | Met30  | UCLA | LO          |
| 3.18   | Thalidomide         | Cereblon | Tokyo Institute of Technology | Ph III      |
| 3.19   | Curcumin            | Pan-DUB inhibition | University of Utah | TM          |
| 3.20   | Shikoccin           | Pan-DUB inhibition | University of Utah | DD          |
| 3.21   | Δ12-PGJ2            | Pan-DUB inhibition | Karolinska Institute | DD          |
| 3.22   | Dienones, NSC 632839 | Pan-DUB inhibition | Progenra | DD          |
| Number | Chemical cpd./class | Target | Organization | Dev. status |
|--------|--------------------|--------|--------------|-------------|
| 3.23   | Bis-isothiocyanate, PR-419 | Pan-DUB inhibition | Oldenburg University, Germany | DD |
| 3.24a,b | Tricyclic dinitriles, HBX-41,108 (3.24a) | USP DUBs | Hybrigenics | LO |
| 3.25   | Gold complexes | Pan-DUB inhibition | University of Hong Kong | DD |
| 3.26   | Tyrposthin-like WP-1130 | USP5, USP9x, USP14, UCH-L1, UCH37 | University of Michigan | LO |
| 3.27   | Alkyliden-pyrazolidindiones, PYR41 | Cys DUBs | University of Michigan | DD |
| 3.28   | Betulinic acid | Pan-DUB inhibition | University of Miami | PE |
| 3.29   | Chalcones, RA-9 | UPS2, UPS5, UPS8, UCH-L1, UCH-L3 | University of Minnesota | DD |
| 3.30   | Phthalimide based, pimozide | UAF1, USP7 | University of Delaware | DD |
| 3.31   | Spautin-1 | USP10, USP13 | Chinese Academy of Sciences | DD |
| 3.32   | Thiophene based, P5091 | USP7, USP47 | Harvard Med. School | LO |
| 3.33   | Thiophene based | USP7, USP47 | Progenra | LO |
| 3.34   | Aminotetrahydroacridines, HBX-19,818 | USP7 | Hybrigenics | Ph I |
| 3.35   | Naphthylamides, GRL0617 | PLpro | University of Illinois | LO |
| 3.36   | Electrophilic dienones, NSC687852/b-AP15 | USP14 | Karolinska Institute | LO |
| 3.37   | Pyrrole based, IU1 | USP14 | Harvard Med. School | LO |

Not progressed, NP; early discovery, DD; lead optimization, LO; preclinical evaluation, PE; clinical Phase I-II-III, Ph I–Ph III; marketed, MKTD; traditional medicine, TM. Please note that the most advanced status for NDD-targeted experiments is listed: for example, candidates in clinical trials for non-CNS indications with early in vitro characterization against proteinopathies/tauopathies are classified as DD.
The chemical core of each scaffold/compound is structurally defined; its molecular target is mentioned; the developing laboratory (either public or private) is listed; and the development status—according to publicly available information—is finally provided.

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