Chapter 6

Natural Product Inhibitors of Ubiquitin Conjugation and Deconjugation

Wilfried Hess*, Mukram M. Mackeen§, Holger B. Kramer*,[1]
*University of Oxford, Oxford, United Kingdom; §Universiti Kebangsaan Malaysia, Bangi, Malaysia
[1]Corresponding author: E-mail: holger.kramer@dpag.ox.ac.uk

Chapter Outline

INTRODUCTION

Cellular ubiquitination is a complex posttranslational protein modification. It involves the attachment of the highly conserved 76-amino acid polypeptide ubiquitin to lysine residues of a substrate protein. This attachment is performed

through formation of an isopeptide bond between the *C*-terminus of ubiquitin and the ε-amino group of the lysine side chain. The most well-known function of protein ubiquitination is the intracellular degradation of poly-ubiquitinated proteins, in which a chain of ubiquitin molecules is attached to the substrate, by the 26S proteasome. In addition to that ubiquitination is also intimately involved in DNA damage response, protein trafficking, NF-κB signaling, and many other cellular processes. The inhibition of proteasomal degradation by natural products and synthetic inhibitors has been extensively described and reviewed previously [1–6]. Our understanding of the molecular mechanisms determining proteasomal degradation of cellular proteins has advanced significantly. To a large extent this was enabled by extensive biochemical, mechanistic, and structural studies of natural product inhibitors of the proteasome, such as epoxomicin [7]. These developments culminated in the successful development of bortezomib as an FDA-approved proteasome inhibitor, which is used clinically in the treatment of multiple myeloma, a malignancy of antibody-producing plasma cells. It is hoped that similar investigations into the many facets of the ubiquitin–proteasome system (UPS) will allow further successful translation into clinically useful compounds.

In comparison to proteasomal degradation, inhibitors of the enzyme systems responsible for ubiquitin conjugation and deconjugation have attracted less attention. Attachment of ubiquitin to substrate proteins is mediated by an enzyme cascade of the ubiquitin-activating enzyme E1, a ubiquitin-conjugating enzyme E2, and a ubiquitin ligase E3 (Fig. 6.1). The activation of ubiquitin involves *C*-terminal adenylation of ubiquitin and formation of a covalent thioester-linked intermediate with the E1 enzyme. A transfer of ubiquitin then occurs from E1 to the E2 conjugating enzyme, resulting in another thioester-linked conjugate. The ubiquitin ligase E3 brings together the E2-ubiquitin conjugate
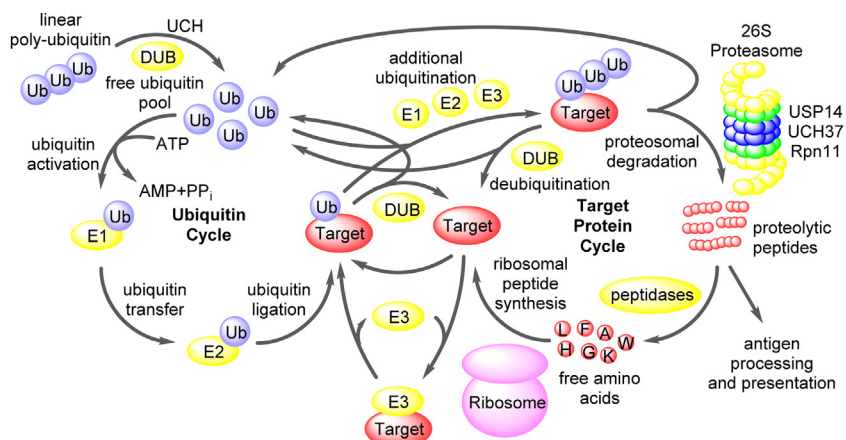

FIGURE 6.1 Schematic overview of the ubiquitin–proteasome system (UPS).

with the substrate protein destined for ubiquitination. Selectivity for ubiquitination of specific substrate proteins is conferred through the E3 enzymes in this process and to date several hundred members are known in this protein family.

Ubiquitin deconjugation is regulated by a family of proteases termed deubiquitinating enzymes or deubiquitinases (DUBs) [8–10]. This enzyme family consists mainly of cysteine proteases and a smaller subfamily of metalloproteases. DUBs form an integral part of the ubiquitin system and in addition to their role of counteracting the function of the ubiquitin conjugation cascade, they are involved in the generation of free monoubiquitin from linear poly-ubiquitin precursors, the remodeling of different types of ubiquitin linkages on modified substrate proteins and the removal of ubiquitin from substrates of proteasomal degradation directly prior to proteolysis.

The ubiquitin conjugation system and the DUB family of proteases comprise a large number of structurally and functionally complex proteins. In this system there are several hundred recognized E3 ubiquitin ligases and almost 100 DUB proteases [11]. This complexity is further increased when taking into account the multitude of protein subunits, which form part of ubiquitin E3 ligase complexes and the regulation of many of these proteins by posttranslational modifications [12,13]. This degree of complexity and molecular diversity may at least in part explain the relative lack of detailed structural and mechanistic characterization when compared to the more conserved proteasome. There is also a significant number of protein–protein interaction (PPI) inhibitors in the group of natural products targeting ubiquitin conjugation by inhibition of E3 ligase complexes. These usually act through binding to protein surfaces involved in PPIs rather than through binding to an enzyme active site.

In addition to the cellular ubiquitin system several related modification systems of ubiquitin-like (Ubl) proteins have been characterized [14]. These proteins share many features including isopeptide bond formation between the *C*-terminus of the Ubl modifier and lysine residues on the substrate, conjugation by E1, E2, and E3 enzyme systems, and deconjugation by specialized proteases. The biological functions of Ubl modifiers differ from those of ubiquitin itself and include immune signaling and interferon response (ISG15), transcriptional regulation and nuclear-cytosolic transport (SUMO) and regulation of cullin components of E3 ligase complexes and thereby of cell cycle progression (NEDD8).

The most well-characterized Ubl modifier is the small ubiquitin-related modifier (SUMO) and a large number of targets of protein SUMOylation have been identified to date [15,16]. SUMO is involved in various cellular processes, including cell cycle regulation, transcription, nuclear-cytoplasmic transport, DNA replication and repair, chromosome dynamics, apoptosis, and ribosome biogenesis [17].

After protein translation the SUMO precursor is cleaved by Ubl protein-specific proteases (Ulp) in yeast or sentrin-specific proteases (SENP) in mammalian cells to obtain a C-terminal diglycine motif (Fig. 6.2). Then the
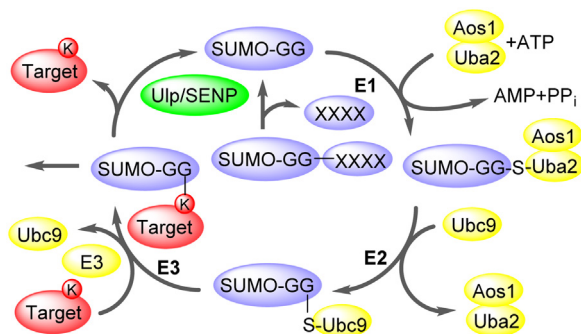
FIGURE 6.2 Schematic overview of the SUMOylation pathway.

E1 heterodimer, consisting of activator of SUMO 1 (Aos1) and Ubl modifier-activating enzyme 2 (Uba2), forms a thioester bond with the SUMO protein in an ATP-dependent process. The modifier is subsequently transferred to the E2 enzyme Ubc9. Finally, SUMO is transferred with the help of specific E3 ligases onto lysine residues on the target protein. Ligase-independent SUMOylation has also been observed with Ubc9 alone [17]. The process is reversible by the action of SUMO-specific proteases [18,19], which cleave SUMO from modified target protein (deSUMOylases) [20].

NEDD8, the product of the neural precursor cell-expressed, developmentally downregulated gene 8, shows the greatest sequence similarity with ubiquitin (58%) among the Ubl proteins. NEDDylation has been demonstrated predominantly for members of the cullin protein family, which are important scaffold proteins for the assembly of multicomponent RING E3 ligase complexes in the ubiquitin pathway [21].

Full-length NEDD8 protein is hydrolyzed by the proteases UCHL3 and SENP8 to reveal the free *C*-terminal diglycine motif (Fig. 6.3). The E1 enzyme complex is assembled, consisting of a dimer of the regulatory amyloid precursor protein-binding protein 1 (APPBP1) and Ubl modifier-activating enzyme 3 (Uba3) in order to activate NEDD8 by formation of a thioester bond in an ATP-dependent process [22]. Therefore the activation of NEDD8 occurs, similarly to SUMO, through the action of a heterodimeric E1 enzyme system. This is in contrast to the activation of ubiquitin and other Ubl proteins, which are generally activated by monomeric E1 enzymes. Subsequently the thioester-linked NEDD8 is transferred onto the cognate E2 enzyme, Ubc12. In collaboration with specific E3 ligases Ubc12 then transfers NEDD8 onto lysine residues of target proteins. Analogous to ubiquitin and SUMO, NEDDylation is a reversible process through the action of DUBs with cross-reactivity for cleavage of NEDD8 conjugates.
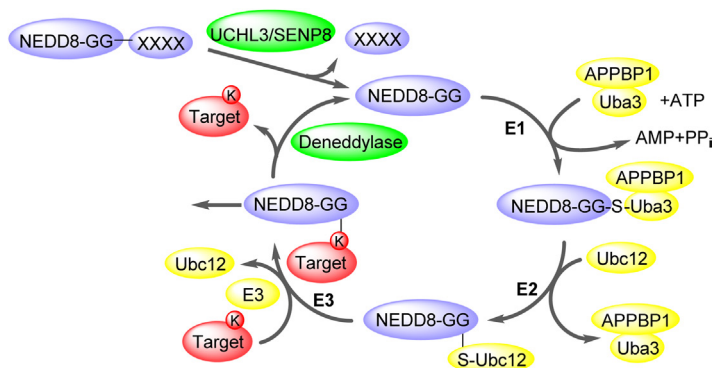
FIGURE 6.3 Schematic overview of the NEDDylation pathway.

The identification and development of compounds capable of interfering with the complex systems of ubiquitin and Ubl protein conjugation and deconjugation in a selective manner represents a significant challenge. Natural products allow us to address this challenge due to a number of attractive features: There is precedence of a number of identified natural product inhibitors and presumably much larger numbers of to date unknown compounds exist. Natural products have previously developed our understanding of complex cellular systems and have been a source of highly active and sometimes selective compounds. Many natural products are structurally very complex and access to such structures when extracted from natural sources is not limited by ease of preparation using synthetic organic chemistry techniques. Finally, the wide distribution of the ubiquitin system in eukaryotic organisms, including plants, animals, and fungi, suggests that secondary metabolites could have evolved, which are specialized at interfering with particular steps in this system.

In the following sections, natural product inhibitors and their semisynthetic derivatives and structurally related compounds are discussed. We begin with compounds acting on proteins involved in the ubiquitin conjugation machinery, followed by inhibitors of DUBs responsible for disassembly of Ub conjugates. This is followed by a treatment of inhibitors of enzymes involved in Ubl protein conjugation and deconjugation, which includes discussions of the Ubl modifiers SUMO, NEDD8, and ISG15.

UBIQUITIN-ACTIVATING ENZYME E1 INHIBITORS

The first natural product with activity against the ubiquitin-activating enzyme (E1) was described in 2002. (+)-Panepophenanthrin (**1**) was isolated from the fermentation broth of the basidiomycete mushroom *Panus rudis* Fr. IFO 8994 (Fig. 6.4) [23]. It represents a highly functionalized natural product with a
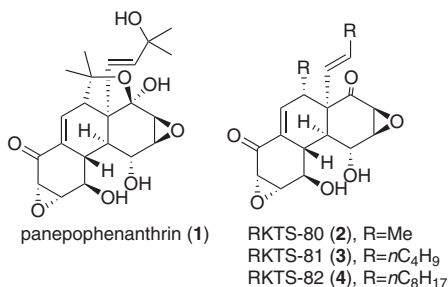
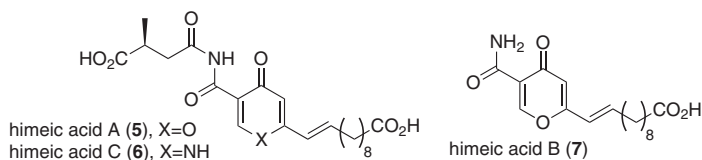**FIGURE 6.4**   Structures of panepophenanthrin and synthetic analogs.

FIGURE 6.5   Structures of himeic acid A, B and C.

tetracyclic core, the biosynthesis of which occurs by a Diels–Alder dimerization of epoxyquinol monomers. **1** has been shown to inhibit the formation of the E1-ubiquitin complex in a dose-dependent manner. The IC_{50} value was determined in immunoblotting experiments as 17.0 µg/mL. Interestingly, no significant inhibitory effect was observed in intact cells up to a concentration of 50 µg/mL, which was attributed to poor membrane permeability. **1** possesses a rather complex structure making it a challenging synthetic target. Despite these challenges, several successful syntheses have been published in the years following its initial discovery, including the preparation of some modified derivatives [24–29]. The introduction of hydrophobic side chains into the structure allowed the generation of cell-permeable E1 inhibitors, such as RKTS-80, -81, and -82 (**2–4**). The IC_{50} values of **2–4** were determined by immunoblotting experiments as 9.4, 3.5, and 90 µM, respectively. These natural product derivatives also blocked cell growth in a dose-dependent manner with IC_{50} values of 5.4, 1.0, and 3.6 µM, respectively [30].

The second natural product, which was shown to have inhibitory activity toward ubiquitin-activating enzyme (E1), was himeic acid A (**5**) (Fig. 6.5). This compound was isolated in 2005 from the culture of a marine-derived fungus, *Aspergillus sp.* isolated from the mussel, *Mytilus edulis*, collected in Toyama Bay [31]. **5** inhibits the formation of the E1-ubiquitin intermediate by 65% at a concentration of 50 µM as determined by immunoblotting analysis. On the other hand, the two related compounds, himeic acids B and C (**7** and **6**), showed no significant E1 inhibitory activity.

Largazole (**8**) was isolated from marine cyanobacteria of the *Symploca* genus and its structure was characterized and reported in 2008 (Fig. 6.6) [32]. It showed significant cytotoxicity against cancer cells and antiproliferative activity in transformed mammary epithelial cells. Its ability to preferentially
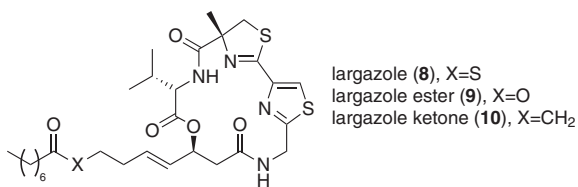
largazole (**8**), X=S
largazole ester (**9**), X=O
largazole ketone (**10**), X=CH$_2$

FIGURE 6.6 Structure of largazole and largazole derivatives.

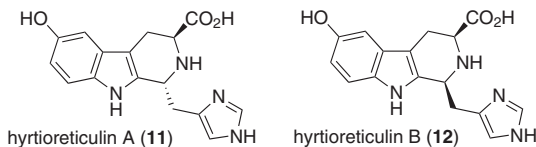hyrtioreticulin A (**11**)   hyrtioreticulin B (**12**)

**FIGURE 6.7** Structures of hyrtioreticulin A and B.

target tumor cells over nonmalignant cells spurred further interest into more detailed investigations of this compound. Largazole (**8**) consists of a 16-membered macrocycle containing a substituted 4-methylthiazoline linearly fused to a thiazole and an unusual octanoic thioester side chain. A total synthesis of **8** and a series of derivatives in a rapid and convergent eight-step synthesis was published in 2008 [33] This study confirmed the original finding of selective growth inhibition of cancer cells versus a nontransformed epithelial cell line. Furthermore, it demonstrated the requirement for the thioester functional group and supported the notion that histone deacetylase (HDAC) inhibition is the basis of its antiproliferative (chemopreventing) activity [33]. Numerous analogs of **8** have been generated in efforts to understand the structure–activity relationship toward inhibition of HDACs [33–43]. Largazole (**8**) and its oxoester analog (**9**) inhibit E1 with IC$_{50}$ values of approximately 29 and 25 μM. The ketone derivative **10** was also found to be active, but the free thiol form of **8** failed to inhibit the E1 activating enzyme, suggesting that the carbonyl group is important for inhibition [44]. Largazole (**8**) and its derivatives (**9** and **10**) inhibit the ubiquitin adenylation step of the E1 enzyme reaction while having no effect on subsequent thioester formation. The inhibition shows selectivity toward ubiquitin-activating enzyme E1 and does not significantly inhibit the Ubl SUMO E1 activating enzyme.

Hyrtioreticulins A and B (**11** and **12**), which are tetrahydro-β-carboline alkaloids, were isolated from the marine sponge *Hyrtios reticulates* (Fig. 6.7).

They inhibited E1 enzyme activity with IC$_{50}$ values of 0.75 and 11 μg/mL (2.4 and 35 μM), respectively, as determined by an immunoblotting-based assay [45]. The results indicate that hyrtioreticulins A and B are unable to inhibit the binding of ubiquitin to E1, in contrast to himeic acid A (**5**), which suggests a different mode of action.

Ginsenosides or panaxosides are steroid glycosides, which are found in the plant genus *Panax* (ginseng). Six different ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 (**13a-f**) were found to alter E1-activating enzyme activity and were

investigated in quantitative inhibition assays (Fig. 6.8). It was shown in immunoblotting experiments that ginsenosides Re (**13e**) and Rg1 (**13f**) inhibited E1 activity with IC_{50} values of 62.8 and 3.5 μM. In contrast, ginsenosides Rb1, Rb2, Rc, and Rd (**13a-d**) increased E1 enzymatic activity by a factor of 1.74–2.2 at 50 μM [46]. Additionally, ginsenoside Rg1 (**13f**) was found to inhibit the chymotrypsin-like activity of the 26S proteasome.
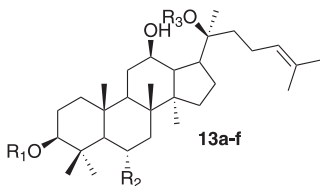
Ginsenoside	Rb1 (13a)	Rb2 (13b)	Rc (13c)	Rd (13d)	Re (13e)	Rg1 (13f)
R_1	Glc2- Glc	Glc2- Glc	Glc2- Glc	Glc2- Glc	H	H
R_2	H	H	H	H	O- Glc2-Rha	O Glc
R_3	Glc6- Glc	Glc6-Ara(p)	Glc6-Ara(f)	Glc	Glc	Glc

FIGURE 6.8 Structures of various ginsenosides.

UBIQUITIN-CONJUGATING ENZYME E2 INHIBITORS

Leucettamol A (**14**) is an antimicrobial compound, which was originally isolated in 1993 from the marine sponge *Leucetta microrhaphis* collected in Bermuda (Fig. 6.9) [47]. **14** was the first Ubc13-Uev1A interaction inhibitor, which was established in an ELISA test, revealing an IC_{50} value of 50 μg/mL (106 μM). The fully hydrogenated derivative of leucettamol A (**15**) was also investigated and was a more potent inhibitor with an IC_{50} value of 4 μg/mL (8 μM), while its tetraacetate derivative was inactive, indicating that the hydroxy and/or amino groups are required for inhibition [48].
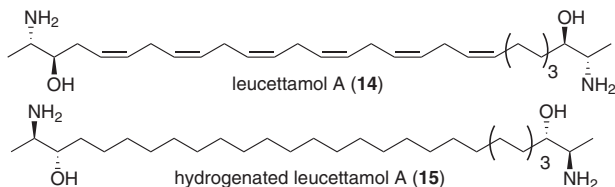
FIGURE 6.9 Structures of leucettamol A and hydrogenated leucettamol.

In 2012 the same group reported the isolation of two new dimeric sterols, manadosterols A (**16**) and B (**17**), from the marine sponge *Lissodendryx fibrosa* collected in Indonesia (Fig. 6.10). Both compounds contain two sulfonated sterol cores, which are linked via their side chains. Manadosterols A (**16**) and B (**17**) inhibited the Ubc13–Uev1A interaction measured by an ELISA assay with
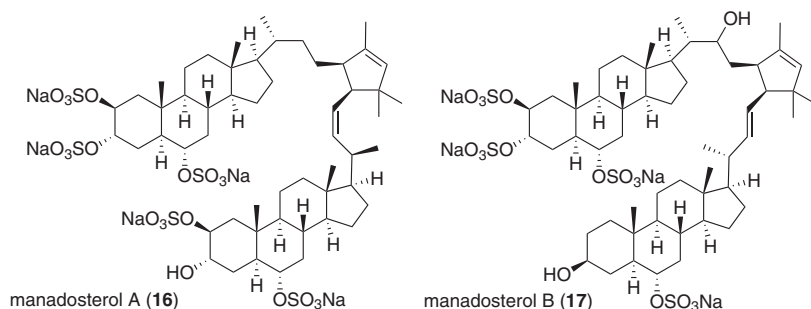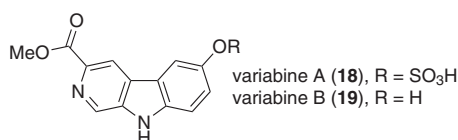
FIGURE 6.10 Structures of manadosterol A and B.

FIGURE 6.11 Structure of variabine A and B.

IC$_{50}$ values of 0.09 and 0.13 μM, respectively making them significantly more potent than leucettamol A (**14**) [49].

Recently, Tsukamoto and coworkers reported the isolation of two new β-carboline alkaloids, variabines A (**18**) and B (**19**), from the Indonesian marine sponge *Luffariella variabilis* (Fig. 6.11). Structure elucidation revealed that **18** was the sulfonated derivative of **19**. The latter inhibited the chymo-trypsin-like activity of the proteasome and the Ubc13–Uev1A interaction as determined by ELISA with IC$_{50}$ values of 4 and 5 μg/mL (16 and 20 μM) but showed no inhibition of E1 activity or the p53–HDM2 (E3) interaction at 5 μg/mL (20 μM) [50]. Additionally the sulfonated compound **18** showed no detectable inhibitory effect.

UBIQUITIN LIGASE E3 INHIBITORS

Inhibition of E3 ligases is an attractive prospect for the development of novel therapeutic strategies. Their role of targeting specific protein sub-strates for ubiquitination and potentially proteasomal degradation means that it may be possible to selectively stabilize selected protein substrates. The challenge lies in the identification of sufficiently potent and selective inhibitors of E3 ligases. A number of natural products have been identified which possess some of the desired characteristics and many of these were shown to be PPI inhibitors. The most closely studied interaction between an E3 ligase and its substrate protein is that of mouse double minute 2 (MDM2) homologue or its respective human form (HDM2) with its substrate p53. The central importance of the p53 pathway in many cancers and its promise

as a molecular target explain the effort, which has gone into the investigation of inhibitors of this interaction.

p53–HDM2 (MDM2) Interaction Inhibitors

Chlorofusin (**20**) was first isolated from the fermentation broth of *Microdochium caespitosum*, a plant pathogenic fungus, in 2001 and was characterized as the first natural product p53–HDM2 (MDM2) interaction inhibitor (Fig. 6.12) [51,52]. **20** consists of a densely functionalized chromophore linked through the terminal amine of an ornithine residue to a 27-membered cyclic peptide.

Subsequently, **20** was also isolated from the filamentous fungus *Fusarium* sp. 22026. Chlorofusin (**20**) was found to be the most abundant inhibitory compound in the fermentation extract with an IC_{50} value of 4.6 μM measured by DELFIA-modified ELISA [51]. The compound antagonizes p53–HDM2 interactions by binding to the *N*-terminal domain of HDM2 [53]. The unfunctionalized cyclic peptide was synthesized in 2004 but showed no inhibitory activity [54,55]. This unusual disruption of a PPI makes chlorofusin (**20**) an attractive lead structure for anticancer drug development. The compound does not show any cytotoxic effect at 4 μM against Hep G2 cells or antimicrobial activity against various test strains. The proposed structure of chlorofusin (**20**) was later revised and a total synthesis was published [56].

(*R*)-(−)-3-Hexylitaconic acid (**21a**) was isolated from a culture of *Arthrinium sp.*, a marine-derived fungus (Fig. 6.13). **21a** inhibits the p53–HDM2
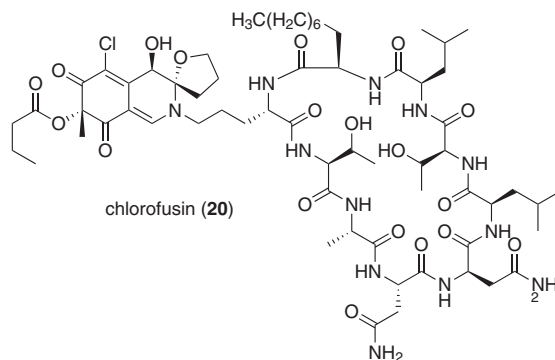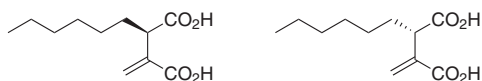

FIGURE 6.12   Structure of chlorofusin.

(*R*)-(-)-3-hexylitaconic acid (**21a**)   (*S*)-(+)-3-hexylitaconic acid (**21b**)

FIGURE 6.13   Structures of (*R*)-(−)-hexylitaconic acid and (*S*)-(+)-hexylitaconic.
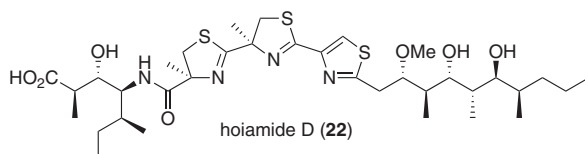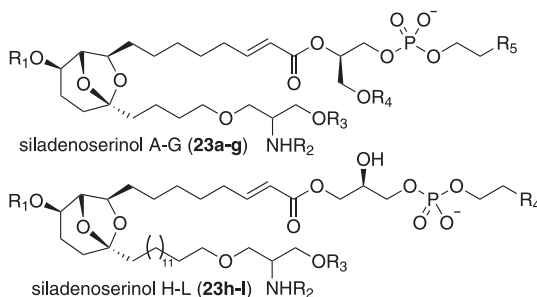
FIGURE 6.14 Structure of hoiamide D.

entry	23	R$_1$	R$_2$	R$_3$	R$_4$	R$_5$	entry	23	R$_1$	R$_2$	R$_3$	R$_4$
1	a	Ac	SO$_3$H	H	Ac	NMe$_3^+$	8	h	Ac	SO$_3$H	H	NMe$_3^+$
2	b	Ac	SO$_3$H	H	Ac	NH$_3^+$	9	i	Ac	H	SO$_3$H	NMe$_3^+$
3	c	H	H	SO$_3$H	Ac	NH$_3^+$	10	j	H	H	SO$_3$H	NH$_3^+$
4	d	Ac	SO$_3$H	H	H	NMe$_3^+$	11	k	H	SO$_3$H	H	NMe$_3^+$
5	e	H	H	SO$_3$H	Ac	NMe$_3^+$	12	l	H	H	SO$_3$H	NMe$_3^+$
6	f	H	SO$_3$H	H	Ac	NMe$_3^+$						
7	g	H	H	SO$_3$H	Ac	NMe$_3^+$						

FIGURE 6.15 Structures of siladenoserinols A-L.

interaction with an IC$_{50}$ value of 50 μg/mL [57]. The enantiomer (+)-hexyli-taconic acid (**21b**), which was first isolated as a plant growth regulator from *Aspergillus niger* cultivated from field soil, shows an equal inhibition of the p53–HDM2 interaction [58].

Hoiamide D (**22**) was isolated via bioassay-guided fractionation from the cyanobacterium *Symploca sp.* collected near Cape Point, Papua New Guinea in 2006 (Fig. 6.14). Compound **22** is a polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS)-derived natural product that features two consecutive thiazolines and a thiazole, as well as a modified isoleucine residue. Hoiamide D (**22**) displayed interesting inhibitory activity against the p53–MDM2 interaction (EC$_{50}$ = 4.5 μM), which was measured in a high-throughput Förster resonance energy transfer (FRET)-based competition assay [59].

The marine natural products siladenoserinol A-L (**23a-l**) were isolated from a tunicate and were characterized as inhibitors of the p53–HDM2 interaction (Fig. 6.15). These compounds are sulfonated serinol derivatives, which con-tain a 6,8-dioxabicyclo[3.2.1]octane unit and either a glycerophosphocholine or
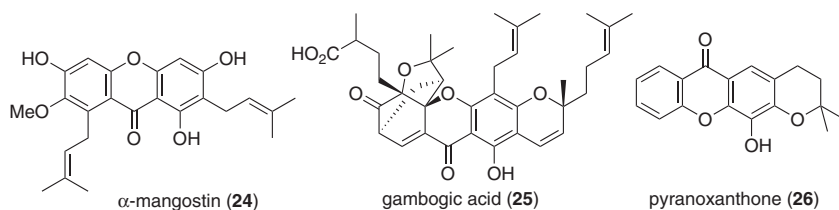
FIGURE 6.16 Structures of α-mangostin, gambogic acid, and pyranoxanthone.

glycerophosphoethanolamine unit [60]. They exhibit IC_{50} values in the range of 2.0–55 μM with siladenoserinol A and B (**23a, b**) being the most potent inhibitors with IC_{50} values of 2.0 μM.

α-Mangostin (**24**) and gambogic acid (**25**) are prenylated xanthones, which can be obtained from the mangosteen fruit of *Garcinia mangostana* L. (Clusiaceae) and from the resin of *Garcinia hanburyi* Hook.f. (Fig. 6.16). Both compounds show cytotoxic activity toward human tumor cell lines and in animal models [61–64]. Drug-like molecules with a xanthone scaffold were previously shown to be inhibitors of the p53−MDM2 interaction [65]. Using a yeast-based assay it was established that pyranoxanthone (3,4-dihydro-12-hydroxy-2,2-dimethyl-2*H*,6*H*-pyrano- [3,2-b]xanthen-6-one) (**26**) is an inhibitor of p53–MDM2 interaction after virtual screening of a library of xanthones. Computational studies indicated that **26** binds to the p53-binding site of MDM2. Therefore, it was envisaged that α-mangostin (**24**) and gambogic acid (**25**) could also be inhibitors. The same yeast-based assay was used to establish that both xanthones are potential inhibitors of the p53−MDM2 interaction. This was confirmed by a gene reporter assay in a human tumor cell line as well as computational docking studies [66]. A concentration of 10 μM was the lowest concentration at which a significant effect on MDM2 was observed. α-Mangostin has also been made by total synthesis and some derivatives were prepared and their anticancer properties were investigated [67–69].

HDM2 inhibitors stabilize p53 to subsequently inhibit cell proliferation and induce apoptosis, which is a promising approach in the development of anticancer therapies. The identification of HDM2 inhibitors by high-throughput screening was used to discover three new alkaloids, isolissoclinotoxin B (**27**), diplamine B (**30**), and lissoclinidine B (**31**) from the marine invertebrate *Lissoclinum* cf. *badium* (Fig. 6.17) [70]. Diplamine B (**30**) and lissoclinidine B (**31**) stabilize HDM2 and p53 in cells. Compound **31** selectively kills transformed cells expressing wild-type p53. All three compounds show HDM2 inhibitory activity in the range of 58–101 μM in a cell-free assay with **30** and **31** displaying enhanced activity in cells (10 μM). Varacin (**28**) and *N,N*-dimethyl-5-methylvaracin (**29**), which have similar structures, were also isolated but were found to have very little activity.

In 2008 O'Keefe and coworkers presented a new electrochemiluminescence assay system for the screening of natural product extracts for MDM2 inhibition activity. By screening more than 144,000 natural product extracts, sempervirine

isolissoclinotoxin B (**27**)   varacin (**28**)   *N,N*-dimethyl-5-methylvaracin (**29**)

diplamine B (**30**)   lissoclinidine B (**31**)
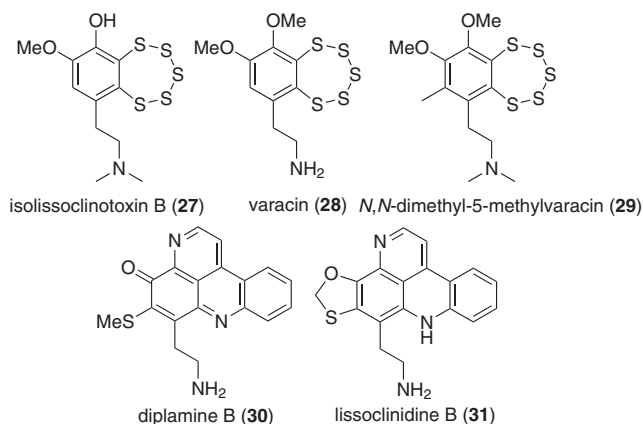
entry	Compound	Name	IC$_{50}$ (µM) HDM2
1	**27**	isolissoclinotoxin B	58.6 ± 4
2	**28**	varacin	>295
3	**29**	*N,N*-dimethyl-5-methylvaracin	120.8 ± 9
4	**30**	diplamine B	101.3 ± 4
5	**31**	lissoclinidine B	98.1 ± 6

FIGURE 6.17   Structures of alkaloid HDM2 inhibitors.
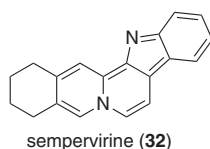
sempervirine (**32**)

FIGURE 6.18   Structure of sempervirine.

(**32**) was identified, which inhibits MDM2 autoubiquitination (IC$_{50}$ value of 8 µg/mL), MDM2-mediated p53 degradation, and led to accumulation of p53 in cells (Fig. 6.18) [71]. Sempervirine (**32**) preferentially induced apoptosis in transformed cells expressing wild-type p53, suggesting that it could be a potential lead for anticancer therapeutics. Over several decades, multiple synthetic routes to sempervirine (**32**) have been established and published in the literature [72–76].

MDM4–p53 Interaction Inhibitors

In silico screening for inhibitors of the E3-ligase MDM4 led to the generation of a compound library from which lithocholic acid (**33**) was identified as an endogenous inhibitor of both MDM4 and MDM2 (Fig. 6.19). **33** was identified

FIGURE 6.19   Structure of lithocholic acid.

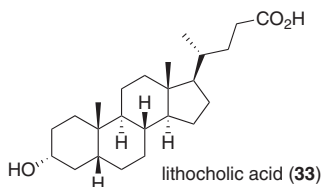after screening of 295 carefully selected candidate compounds in a fluorescence anisotropy-based assay measuring the displacement of a labeled p53 peptide from the N-terminus of MDM4 [77].

33 is a secondary bile acid generated by bacteria in the gut from chenodeoxycholic acid (CDCA) and is variously reported in the literature to have carcinogenic or apoptotic properties [78,79]. The dissociation constants of **33** were determined in a competition assay to be 15.4 ± 0.6 μM and 66.0 ± 3.3 μM for the MDM4 and MDM2 complexes, respectively [77]. **33** is a dual inhibitor of MDM4/MDM2 with a moderate preference for MDM4. More than 50 derivatives and related steroid compounds were tested with **33** showing the highest affinity, leading to the hypothesis that **33** might be a natural ligand for MDM2/MDM4 and that the MDM family of proteins may be able to act as sensors for specific steroids.

β-TrCP1-Pdcd4 Interaction Inhibitors

A luciferase reporter system for the assay of proteasomal degradation of the tumor suppressor Pdcd4 was used in a high-throughput screen format to identify active components from natural product extracts. The protein is a substrate of an SCF ubiquitin ligase complex, which contains the F-box protein β-TrCP1 as the substrate recognition subunit [80,81]. Ubiquitination and proteasomal degradation of Pdcd4 is induced by mitogenic stimuli after p70[S6K1]-dependent protein phosphorylation. This high-throughput assay revealed that an extract from the wooly sunflower *Eriophyllum lanatum* led to stabilization of Pdcd4 from 12-*O*-tetradecanoylphorbol-1,3-acetate (TPA)-induced degradation. As a result, erioflorin (**34**) was isolated and characterized as the active component, which was effective at low micromolar concentrations (Fig. 6.20) [82]. It was also shown that this compound selectively inhibited the β-TrCP1-Pdcd4 interaction in vitro and in cell culture but did not disrupt other known E3 ligase–substrate interactions. This inhibition leads to decreased ubiquitination of Pdcd4 and in turn results in
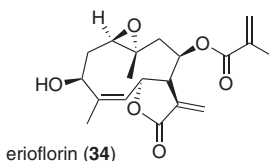

erioflorin (**34**)

FIGURE 6.20   Structure of erioflorin.

its stabilization from proteasomal degradation. Erioflorin (**34**) is a sesquiterpene lactone possessing a tricyclic germacranolide skeleton. The natural product was first isolated in 1981 from another sunflower species, *Helianthus tuberosus* [83].

von Hippel–Lindau–Hypoxia-Inducible Factor-1α Interaction Inhibitors

Bavachinin (**35**) is a flavonate from the seeds of the medicinal plant *Psoralea corylifolia* L., native to eastern Asia, and has been traditionally used in the treatment of various diseases (Fig. 6.21) [84].
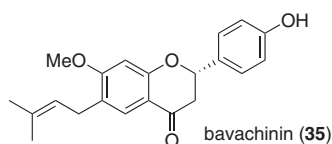

bavachinin (**35**)

FIGURE 6.21   Structure of bavachinin.

In 2012 Soh and coworkers studied the anti-angiogenic activity of bavachinin (**35**) via hypoxia-inducible factor-1 (HIF-1) in vitro and in vivo and reported that the natural product binds HIF-1, which consists of two subunits, HIF-1α and HIF-1β, the former interacting with von Hippel–Lindau (VHL) protein, the substrate recognition subunit of a cullin E3 ubiquitin ligase complex. Treatment of KB cells with 1 μM of bavachinin increased VHL binding to endogenous HIF-1α by 2.5-fold showing that this natural product is one of the rare cases of an activator of binding of a E3 ligase–substrate protein pair [85].

DEUBIQUITINASE INHIBITORS

The deconjugation of ubiquitin from substrate proteins by ubiquitin isopeptidases, which have become known as deubiquitinating enzymes (DUBs), is an important feature of the UPS. This group of approximately 100 enzymes in the human genome consists predominantly of cysteine proteases. The functions of DUBs are wide ranging and include negative regulation of the ubiquitin conjugation machinery, remodeling of ubiquitinated protein substrates, and deconjugation of linear ubiquitin polyproteins to monoubiquitin. Studies of natural products as inhibitors of the DUB family of enzymes will be discussed later.

Fitzpatrick and coworkers have published several studies on natural product inhibitors of DUBs. The first one appeared in 2001 regarding prostaglandins of the J series [86]. It was reported that $\Delta 12$-PGJ_2 (**36**), which contains a doubly unsaturated ketone with one exocyclic alkene (Fig. 6.22) is able to inactivate wild-type tumor suppressor p53 in a manner analogous to prostaglandins of the A series [87]. The compound also induces apoptosis despite p53 inactivation and p21 stabilization by inhibiting ubiquitin isopeptidase activity in the range
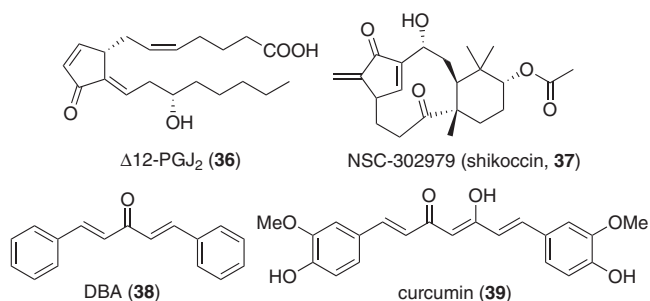
FIGURE 6.22 Structures of Δ12-PGJ₂, Shikoccin, Dibenzylideneacetone, and curcumin.

of 100 μM, which was measured by the Z-LRGG-AMC cleavage assay. This fluorogenic peptide was used as a general substrate of ubiquitin isopeptidase activity, but the identity of the specific DUBs involved was not elucidated at the time [86]. Based on these results, a pharmacophore model for inhibitors of ubiquitin isopeptidases was envisaged, containing a double unsaturated ketone in which both electrophilic β-carbons are easily accessible.

After searching the National Cancer Institute's Developmental Therapeutics Database with a cross-conjugated unsaturated dienone as a pharmacophore, three compounds were identified: shikoccin (NSC-302,979, **37**), dibenzylidene-acetone (DBA, **38**), and curcumin (**39**) (Fig. 6.22) [88]. The ability of **37**, **38,** and **39** to inhibit DUBs was verified by cell-based assays using either Z-LRGG-AMC or a ubiquitin substrate with a 18-amino acid C-terminal extension (Ub-PEST) as isopeptidase substrates. In 2004, Figueiredo-Pereira and coworkers investigated the mechanism of action of Δ12-PGJ₂ (**36**). The ubiquitin hydrolases, UCH-L1 ($K_i \sim 3.5$ μM) and UCH-L3 ($K_i \sim 8.1$ μM), were identified as targets of **36** with no direct inhibition of the cellular 26S proteasome [89].

In the same year, Fitzpatrick and Ireland identified punaglandins (**40a-e**), chlorinated prostaglandin derivatives, which contain a halogenated enone motif as inhibitors of DUBs (Fig. 6.23). The punaglandins were isolated from the soft coral *Telesto riisei* collected in Hawaii [90].

In vitro isopeptidase activity assays showed that these marine natural products were stronger DUB inhibitors than the previously investigated A and J series prostaglandins. The cross-conjugated punaglandins (**40b-d**) showed the most potent-inhibiting isopeptidase activity at concentrations of around 20–60 μM [90]. The proposed mechanism for the DUB inhibition is the nucleophilic trapping of a thiol at the enzyme active site to the electrophilic *exo*-alkene moiety.

In 2006, Ireland and coworkers prepared halogenated prostaglandin-A2 derivatives (**42–44**) by semisynthesis from (15S)-prostaglandin-A2 (**41**) isolated from the sea whip *Plexaura homomalla* (Fig. 6.24). These derivatives were investigated as inhibitors of ubiquitin-specific isopeptidase activity [91]. Potency in this series was reported to decrease in the order I > Br ≥ Cl with the iodo compound being more active than Δ12-PGJ₂ (**36**) but less potent than the punaglandins.
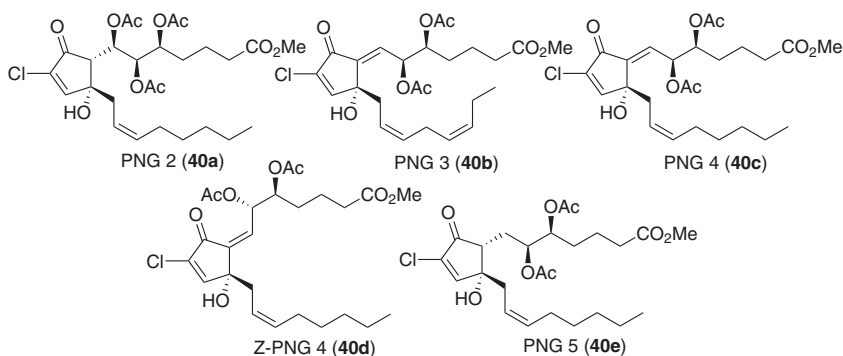
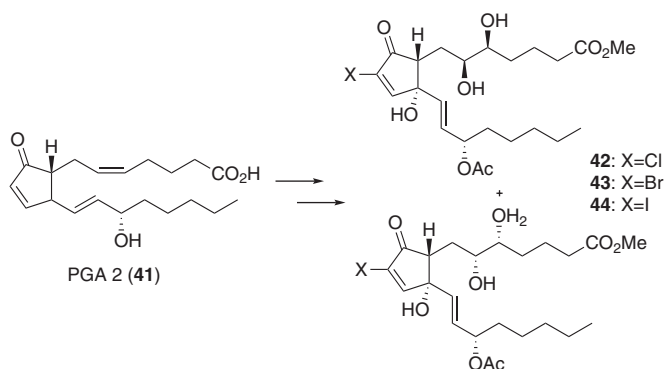**FIGURE 6.23** Structures of punaglandins.

FIGURE 6.24 Synthesis of halogenated prostaglandin-A2 derivatives.

A natural product library composed of marine invertebrate extracts led to the first USP7 inhibitor isolated from the marine sponge *Stylissa massa* [92]. The screen was performed with recombinant full-length USP7 in combination with ubiquitin-Rh110 as a quenched, fluorescent substrate in in vitro deubiquitination assays [93]. Three potent compounds spongiacidin C (**45**) debromohymenialdisine (**46**), and hymenialdisine (**47**) were isolated and characterized (Fig. 6.25). **45** was the most potent inhibitor with an IC_{50} value of 3.8 μM and the other two derivatives (**46** and **47**) exhibited 20% inhibition at 20 μM. This suggests that the hydantoin motif is crucial for potent inhibitory activity. Spongiacidin C (**45**) possesses some selectivity for USP7 showing moderate inhibition of USP21 but is inactive against USP2, USP8, or SENP1.

Vialinin A (**48**), isolated from the Chinese mushroom *Thelephora vialis* was identified as an inhibitor of ubiquitin-specific peptidase 5/isopeptidase T (USP5/IsoT) by a beads-probe method (Fig. 6.26) [94].

The IC_{50} of vialinin A (**48**) was found to be 5.9 μM, measured by the in vitro ubiquitin-7-amido-4-methylcoumarin (Ub-AMC) hydrolysis assay. **48** shows
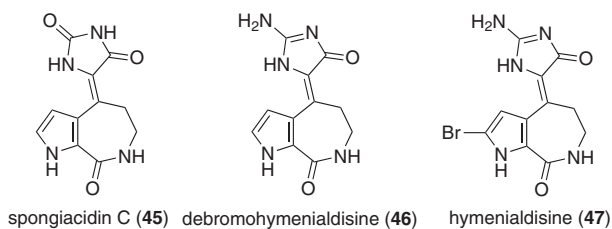
spongiacidin C (**45**)   debromohymenialdisine (**46**)   hymenialdisine (**47**)

FIGURE 6.25 Structures of spongiacidin C, debromohymenialdisine, and hymenialdisine.

vialinin A (**48**)   DMT (**49**)
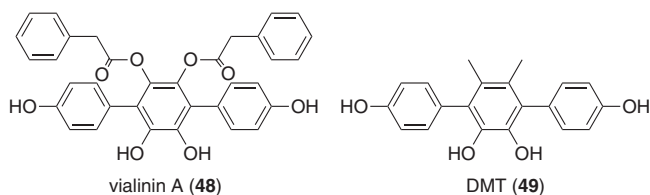
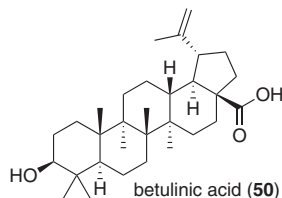**FIGURE 6.26** Structures of vialinin A and simplified synthetic analog DMT.

betulinic acid (**50**)

FIGURE 6.27 Structure of betulinic acid.

some selectivity, but USP4 (IC_{50} = 1.5 μM) and UCH-L1 (IC_{50} = 22.3 μM) are also inhibited while no inhibition was observed for UCH-L3, USP2, and USP8. A simplified synthetic analog of vialinin A, DMT (**49**) was also investigated showing less inhibitory activity with IC_{50} values of approximately 20 μM against USP4, USP5, and UCH-L1. Vialinin A (**48**) was also shown to be an inhibitor of TNFα production and in another study it was demonstrated that USP5 siRNA knockdown in rat basophilic leukemia cells (RBL-2H3) led to decreased production of TNFα, which suggest that USP5 is one of the essential regulators of production of TNFα [95].

Betulinic acid (**50**) is found in several plant species, the white birch (*Betula pubescens*) being one of them (Fig. 6.27). It can increase apoptosis specifically in cancer but not in normal cells, making it potentially an attractive anticancer agent. **50** inhibited multiple DUBs in prostate cancer cells at a concentration of 10 μM, which results in the accumulation of poly-ubiquitinated proteins, decreased levels of oncoproteins, and increased apoptotic cell death [96]. The authors reported that deubiquitination was inhibited in transgenic adenocarcinoma of the mouse prostate (TRAMP) prostate cancer cells but not normal murine fibroblasts. Their data suggest that degradation of multiple cell cycle
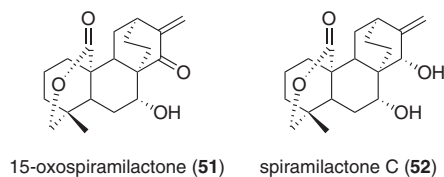
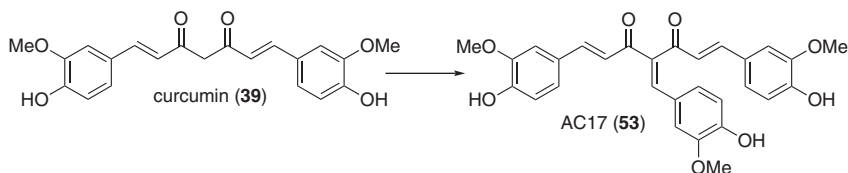**FIGURE 6.28** Structure of 15-oxospiramilactone.

FIGURE 6.29 Synthesis of curcumin analog AC17 via Knoevenagel condensation.

and prosurvival proteins is involved. It was suggested that **50** specifically inhibits USP7, 9x, and 10 in PC3 cells, but the corresponding primary data to support this hypothesis were not shown.

Chen and coworkers identified 15-oxospiramilactone (**51**), a semisynthetic diterpenoid derivative of spiramilactone C (**52**) from *Spiraea japonica* (Rosaceae), as an inducer of mitochondrial fusion through inhibition of the mitochondrion-localized DUB USP30 (Fig. 6.28) [98].

It was shown that **51** is an irreversible inhibitor with the enone reacting with the cysteine residue in the active site of the enzyme. The study thereby identified ubiquitination of mitofusins-1 and -2 (Mfn1 and Mfn2) as a regulatory modification, which promotes mitochondrial fusion.

In a 2013 study, a 4-arylidene curcumin analog, AC17 (**53**), was investigated as an inhibitor of the DUB activity of the 19S regulatory subunit of the proteasome (Fig. 6.29) [99].

AC17 (**53**) is an irreversible inhibitor with an IC$_{50}$ value of 4.23 ± 0.010 μM, measured in an Ub-AMC protease assay, resulting in inhibition of the NF-κB pathway and reactivation of the proapoptotic protein p53. AC17 (**53**) shows improved oral bioavailability, metabolic stability, and moderately potent anticancer activities against several different cancer cell lines. It was not demonstrated by the authors whether the inhibition targets USP14, UCH37, Rpn11 (POH1), or a combination of these proteasome-associated DUBs. Addition of a thiol to one of the enones was proposed as the inhibitory mechanism, which is in agreement with the observed irreversibility of inhibition and the loss of inhibitory activity after treatment with glutathione.

Curcusone D (**54**), a diterpene isolated from *Jatropha curcas* (barbados nut), was identified to be a UPS inhibitor using the ubiquitin G76V yellow fluorescent protein fusion, Ub-G76V-YFP reporter assay (Fig. 6.30) [100]. Tumor inhibition and chemotoxicity of a leaf extract of *J. curcas* were reported previously [101].
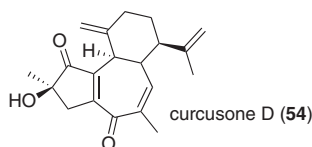
FIGURE 6.30 Structure of curcusone D.

Investigation of the mechanism of UPS inhibition revealed that curcusone D (**54**) did not inhibit the proteasome's chymotrypsin-like, trypsin-like, or caspase-like activities, but it reduced the activity of cellular DUBs. It was suggested that curcusone D (**54**) induces reactive oxygen species (ROS), which may correlate with its inhibition of DUBs and the UPS to induce cellular growth inhibition and apoptosis [100]. In a covalent-labeling assay with the activity-based probe HAUbVS [102], a significant reduction in the labeling of the DUBs, USP5, USP7, USP8, USP15, USP14, and USP22 was observed in cells treated with curcusone D (**54**) at 10 μM for 24 h, suggesting that this compound possesses some degree of selectivity.

INHIBITORS OF THE UPS WITH UNKNOWN MOLECULAR TARGETS

Girolline (**55**), a 2-aminoimidazole derivative, was originally isolated in 1988 from the marine sponge *Peudaxinyssa cantharella* collected in New Caledonia [103] and later from the marine sponge *Axinella brevistyla* (Fig. 6.31) [104]. The compound was prepared synthetically in enantiomerically pure form in 1991 [105].
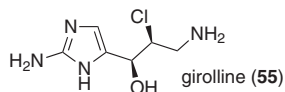
FIGURE 6.31 Structure of girolline.

Girolline (**55**) induces G2/M cell cycle arrest in several tumor cell lines. It has been suggested that this can be explained by the lack of recruitment of poly-ubiquitinated p53 to the proteasome [106]. Nevertheless, the precise molecular targets explaining this effect have not been identified. Toxicological studies in animals showed little toxic effect. However, in a phase I clinical study, severe side effects with no antitumor activity were observed [107]. More recently there have been conflicting reports regarding the ability of **55** to inhibit cellular protein translation [108,109].

Piperlongumine (**56**) was originally isolated from the fruit of the long pepper (*Piper longum*), which is found in Southeast Asia (Fig. 6.32) [110].

Piperlongumine (**56**) inhibits platelet aggregation [111] and it was shown to possess selective cytotoxicity toward tumor cells and has antitumor effects in animal models [112]. The cellular action of **56** was investigated in cell-based

FIGURE 6.32 Structure of piperlongumine.

FIGURE 6.33 Structures of physalin B and C.

assays and by mass spectrometry-based proteomics identifying increases in levels of ROS as well as inhibition of the UPS. Nevertheless the compound displays no inhibition of the 20S proteolytic activity or the 19S deubiquitinating activity of the proteasome at concentrations inducing cytotoxicity. Additionally, no inhibition of cellular DUBs in cell lysates was observed indicating that **56** inhibits 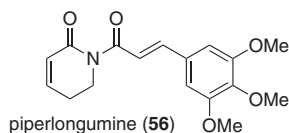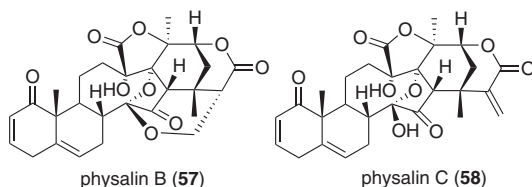the UPS at another preproteasomal step [113]. No specific molecular target of piperlongumine (**56**) in the UPS has been unambiguously identified yet. Despite this it was proposed that UPS inhibition is the primary effect of **56,** which correlates with a strong activation of ROS consistent with previous reports [112].

In 2007 Ausseil et al. developed a cell-based bioluminescent assay using a linear tetraubiquitin–luciferase (4Ub-Luc) reporter protein to screen for UPS inhibitors [114]. In this study, 21 molecules and 66 plant extracts from a total of 15,744 natural extracts and fractions inhibited the UPS. One plant extract hit, *Physalis angulata* L. (Solanaceae), was investigated further due to its previously reported immunomodulatory, antimicrobial, and antitumor biological activities.

Using a bioassay-guided approach, two UPS inhibiting secosteroids, physalin B (**57**) and C (**58**), were isolated from the extract of *Physalis angulata* L. (Fig. 6.33). The accumulation of poly-ubiquitinated proteins was confirmed by immunoblotting with EC_{50} values of 3.8 and 4.4 μM for physalin B (**57**) and C (**58**), respectively. The design of the assay only allows for the identification of inhibitors acting on proteasomal recruitment, proteasomal deubiquitination, or proteolytic processing by the proteasome. Physalin B (**57**) inhibited the CTL and PGPH proteolytic processing of the proteasome in the range of 20 and 40 μM, respectively, while 19-fold increase of bioluminescence in the luciferase assay was observed at 5 μM. This suggests that direct proteasome inhibition does not fully explain its (**57**) activity profile [115].

Ausseil and coworkers utilized the same assay for the further investigation of natural product extracts and pure compounds leading to the isolation of neoboutomellerones 1 (**59**) and 2 (**60**) together with 30 other cycloartane

derivatives from the leaves of the *Neoboutonia melleri* (Euphorbiaceae) (Fig. 6.34). At 1 μM induction factors (IF, increase in luciferase signal) of 48 and 45 were measured in the 4Ub-Luc-DLD-1 cell assay for cycloartanes **59** and **60**, respectively.

In further investigations a small library of analogs consisting of natural products isolated from *Neoboutonia melleri* and of semisynthetic derivatives was investigated with the goal to improve activity and solubility [115]. The compounds were tested for activity in the same cell-based 4Ub-luc DLD-1 assay (best results: IFs at 0.5 μM of 76, 66, and 23). In addition inhibition of the CTL or PGPH activities of the proteasome was observed but no straightforward correlation between the two was obtained. All compounds showed IC_{50} values higher than 10 μM in the proteasome inhibition assays. This suggests that the inhibitory activity of the neoboutomellerone compounds is not or is only partially explained by proteasome inhibition and interference with other components of the UPS is more significant for its activity.
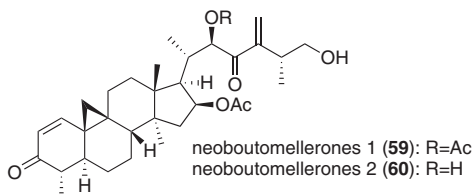
FIGURE 6.34   Structure of neoboutomellerones 1 and 2.

INHIBITORS OF UBIQUITIN-LIKE PROTEIN CONJUGATION

SUMO-Activating Enzyme E1 Inhibitors

Yoshida and coworkers identified ginkgolic acid (**61**) and anacardic acid (**62**) as inhibitors of protein SUMOylation [116] with an in situ cell-based screening assay of plant extracts [117] (Fig. 6.35).
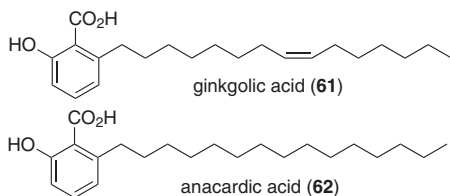
FIGURE 6.35   Structures of ginkgolic acid and anacardic acid.

Both compounds inhibit protein SUMOylation in vitro and in vivo without affecting ubiquitination. IC_{50} values of **61** and **62** against the SUMOylation of RanGAP1-C2 are 3.0 μM and 2.2 μM, respectively [116]. Ginkgolic acid (**61**) binds to E1 blocking the formation of an E1-SUMO thioester complex. For both

compounds, in vivo activities have been reported but currently it is not clear if SUMOylation inhibition is responsible for these effects.

The same group isolated kerriamycin B (**63**) from an unidentified strain of *Actinomycetes* [118] with an in situ cell-based SUMOylation assay (Fig. 6.36) [117].
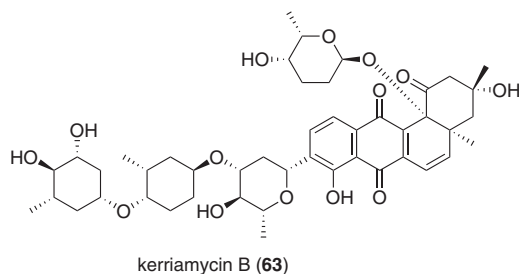
kerriamycin B (**63**)

FIGURE 6.36 Structure of kerriamycin B.
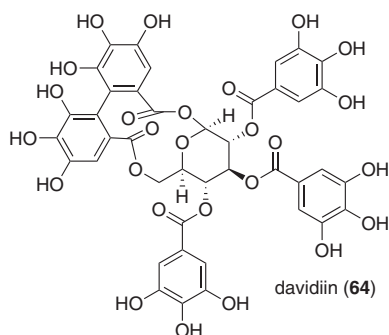
davidiin (**64**)

FIGURE 6.37 Structure of davidiin.

Samples from microbial culture broths were screened for inhibitors of protein SUMOylation and **63** inhibited the formation of the E1-SUMO intermediate with an IC_{50} value of 11.7 μM against the SUMOylation of RanGAP1-C2 [118]. Again no inhibition of ubiquitination was observed in in vitro assays. *In vivo* studies showed that kerriamycin B (**63**) reduced the amount of high-molecular weight SUMO conjugates at 100 μM in 293T cells expressing epitope-tagged Flag-SUMO.

Recently, the same group identified another natural product by the same screening methodology. A leaf extract of the deciduous tree *Davidia involucrata* (Nyssaceae) inhibited in vitro SUMOylation and davidiin (**64**) was subsequently purified and characterized (Fig. 6.37) [119].

In the SUMOylation assay, dose-dependent inhibition was observed in vitro with an IC_{50} value of 0.15 μM [119]. Additionally, in vivo inhibition was observed reducing the level of SUMOylation of p53 in a dose-dependent manner without influencing the cellular levels of ubiquitinated

proteins. Mechanistic investigations revealed the tannin derivative **64** inhibits protein SUMOylation by inhibiting the formation of the E1-SUMO intermediate.

SUMO-Conjugating Enzyme E2 Inhibitors

Yoshida and coworkers utilized a compound library in combination with an in situ cell-based assay to identify spectomycin B1 (**65**) from *Streptomyces spectabilis* as a SUMOylation inhibitor (Fig. 6.38) [120].
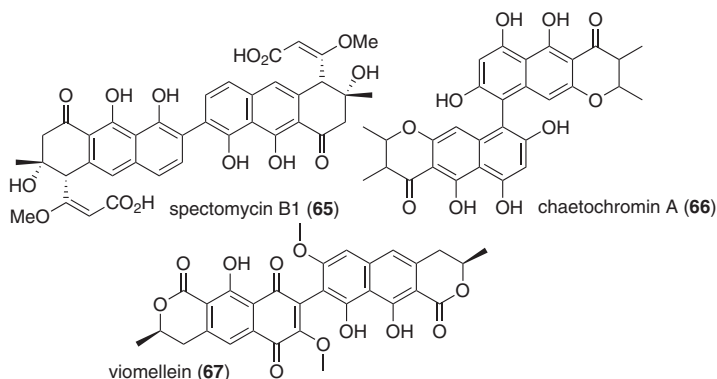
FIGURE 6.38 Structures of spectomycin B1, chaetochromin A, and viomellein.

The microbial natural product inhibits in vitro SUMOylation in a dose-dependent manner with an IC_{50} value of 4.4 μM. The structurally related compounds chaetochromin A (**66**) and viomellein (**67**) were also investigated and showed IC_{50} values of 3.7 μM and 10.2 μM, respectively (Fig. 6.38). Spectomycin B1 (**65**) directly binds to the SUMO-conjugating enzyme E2 (Ubc9) and selectively blocks the formation of the E2-SUMO intermediate without affecting protein ubiquitination in cells.

Using a novel medium throughput microfluidic electrophoretic mobility shift assay to monitor substrate SUMOylation in vitro, Schneekloth and coworkers investigated 500 pure compounds as potential inhibitors [121].

Eight of the ten most active compounds identified were flavone derivatives including the synthetic derivative 2-D08 (**68**) and quercetin (**69**) with **68** being the most active with an IC_{50} value of 6.0 ± 1.3 μM (Fig. 6.39).

Structure–activity studies were performed and mechanistic investigations revealed that 2-D08 (**68**) inhibited SUMOylation by preventing transfer of SUMO from the Ubc9–SUMO thioester to the substrate and without global inhibition of ubiquitination. Subsequently the same group also published the synthesis of 2-D08 (**68**) [122].
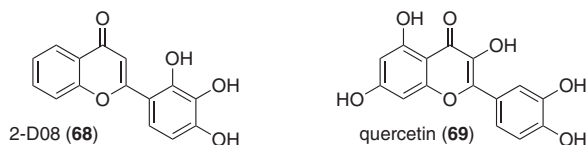
FIGURE 6.39 Structures of 2-D08 and quercetin.

NEDD8-Activating Enzyme Inhibitors

The first natural product derivative inhibitor of NEDD8-activating enzyme (NAE) was identified by an in silico screening approach and then confirmed experimentally. 6,6″-Biapigenin (**70**) has not been found in nature but was synthesized from the hexaacetate, which in turn was obtained from succedaneaflavanone (6,6″-binaringenin) (**71**) from the plant species *Rhus succedanea* (Anacardiaceae) (Fig. 6.40) [123].

70 is a competitive and in vitro inhibitor of NAE with an IC_{50} value of ~20 μM. Activity in human epithelial colorectal adenocarcinoma cells (Caco-2 cells) was also determined with an IC_{50} value of ~20 μM [124].

Virtual screening of the ZINC database of natural products for NAE inhibitors led to the identification of the dipeptide-conjugated deoxyvasicinone derivative (**72**) (Fig. 6.41) [125]. Molecular modeling results suggested that **72** may be a noncovalent competitive inhibitor of NAE by blocking the ATP-binding domain. **74** also shows activity in both cell-free ($IC_{50} = 0.8$ μM) and cell-based assays (Caco-2, $IC_{50} = 6$ μM) with selectivity over analogous ubiquitin and SUMO E1 enzymes. **72** also shows moderate cytotoxicity against Caco-2 cells ($IC_{50} = 10$ μM), which could be attributed, at least in part, to the inhibition of NAE activity.
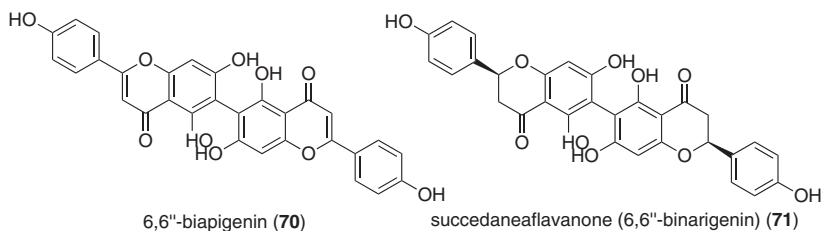
FIGURE 6.40 Structures of 6,6″-biapigenin and succedaneaflavanone.
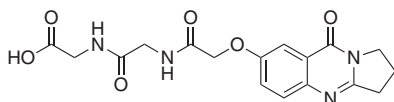
FIGURE 6.41 Structure of dipeptide-conjugated deoxyvasicinone derivative.
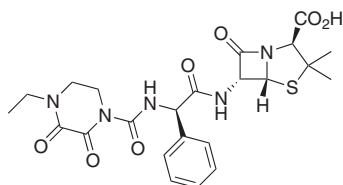
piperacillin (**73**)

FIGURE 6.42 Structure of piperacillin.
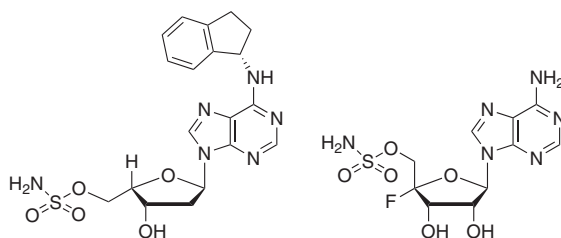
MLN4924 (**74**)   nucleocidin (**75**)

FIGURE 6.43 Structures of MLN4924 and nucleocidin.

The same group used an analogous approach toward the identification of further NAE inhibitors and found and confirmed piperacillin (**73**) as an inhibitor of this enzyme (Fig. 6.42) [126].

Antibiotic (**73**) represents a semisynthetic beta-lactam, which was first developed in the 1970s. The compound is an FDA-approved drug and showed an IC$_{50}$ value of 1 μM for NAE inhibition in vitro. Additionally, it also led to a significant reduction of Ubc12–NEDD8 conjugate levels in Caco-2 cells at 13.5 μM. At the same time the activity of SUMO E1-activating enzyme was not affected at these concentrations.

MLN4924 (**74**) is structurally related to the natural product nucleocidin (**75**) and was reported as a potent and selective inhibitor of NAE (IC$_{50}$ = 5.7 ± 1.5 nM) (Fig. 6.43). (**75**), a naturally occurring nucleoside, represents one of the few natural products to contain a fluorine atom. It was originally isolated from *Streptomyces calvus* and anti-trypanosomal antibiotic activity was reported [127,128]. Derivative (**74**) acts as an AMP analog and forms a covalent NEDD8–MLN4924 adduct, which cannot be processed further and thereby blocks the active site of the enzyme [129]. The NAE inhibition leads to apoptotic death in human tumor cells and suppresses growth of a murine human tumor xenograft model [130].

INHIBITORS OF UBIQUITIN-LIKE PROTEIN DECONJUGATION

DeISGylase Inhibitors

The viral papain-like protease (PLpro), which is encoded by the severe acute respiratory syndrome coronavirus (SARS-CoV), has received a considerable
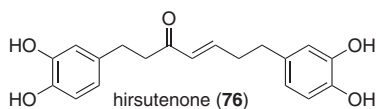
FIGURE 6.44 Structure of hirsutenone.

cryptotanshinone (**77**)
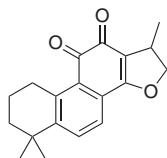
FIGURE 6.45 Structure of cryptotashinone.

attention from both natural product chemists and medicinal chemists in efforts to develop suitable inhibitors. The protease is capable of cleaving the viral replicase polyprotein as well as Ub and ISG15 conjugates with preference for the latter. This enzymatic activity is important for the viral infection process making it a target of interest in the development of anti-infective therapies.

Park et al. described the isolation of nine diarylheptanoids from the bark of *Alnus japonica*, a tree in the Betulaceae family and tested the compounds as inhibitors of SARS-CoV PLpro [131]. Inhibitory activity of these compounds, together with two synthetic curcumin analogues, against PLpro was measured in a fluorogenic assay and hirsutenone (**76**) was found to be the most potent inhibitor with an IC_{50} of 4.1 ± 0.3 μM (Fig. 6.44). Structure–activity studies demonstrated that the enone and catechol moieties were important for successful inhibition of SARS-CoV cysteine protease.

In the same year, Park et al. reported the isolation and testing of tanshinone compounds from *Salvia miltiorrhiza*, a perennial Lamiaceae, which is used in traditional Chinese medicine. Seven tanshinones were found to be selective inhibitors for the SARS-CoV 3CLpro and PLpro viral cysteine proteases with IC_{50} values from 0.8 to 30.0 μM. Inhibitory activity against PLpro was most potent for cryptotanshinone (**77**) (Fig. 6.45) [132].

Cho et al. isolated and identified 12 flavonoids from the fruits of *Paulownia tomentosa*, a deciduous tree in the Paulowniaceae family, as SARS-CoV PLpro inhibitors by activity-guided fractionation. Five new compounds, tomentin A (**78a**), tomentin B (**78b**), tomentin C (**78c**), tomentin D (**78d**), and tomentin E (**78e**) contain an unusual 3,4-dihydro-2H-pyran moiety derived from cyclization of a geranyl group with a phenol functionality (Fig. 6.46). Compounds **78a–78l** inhibit PLpro, as determined by a fluorogenic assay, with IC_{50} values ranging from 5.0 to 14.4 μM with compound **78e** being the most potent. All newly identified natural products showed better inhibition than their noncyclized parent compounds [133].

In a recent study to identify inhibitors of PLpro, six cinnamic amides (**79a–f**) and ferulic acid (**80**) were isolated from the fruits of the annual plant *Tribulus*

FIGURE 6.46 Structures of tomentins and related geranylated flavonoids.
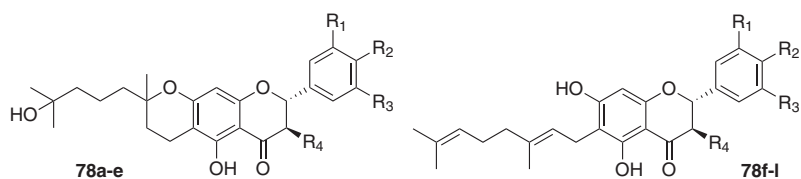
entry	78	R_1	R_2	R_3	R_4	entry	78	R_1	R_2	R_3	R_4
1	a	OH	OH	H	H	6	f	OMe	OH	H	OH
2	b	OH	OMe	H	H	7	g	OH	OMe	H	OH
3	c	OMe	OMe	OH	H	8	h	OMe	OH	H	H
4	d	OMe	OH	OMe	H	9	i	OH	OMe	H	H
5	e	OMe	OH	H	OH	10	j	H	OH	H	H
						11	k	OH	OH	H	H
						12	l	OMe	OH	OMe	H

79a: R_1=OH, R_2=H, Y=H_2
79b: R_1=H, R_2=H, Y=H_2
79c: R_1=OMe, R_2=H, Y=H_2
79d: R_1=OMe, R_2=H, Y=O
79e: R_1=OMe, R_2=H, Y=H, OH
79f: R_1=OMe, R_2=OH, Y=H_2
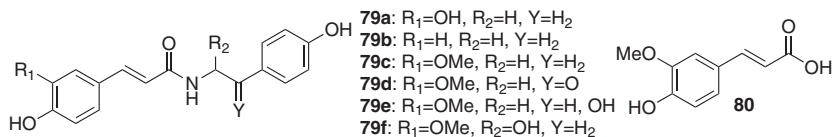
FIGURE 6.47 Structures of cinnamic amides and ferulic acid.

terrestris, a member of the Zygophyllaceae family, which is used in traditional Chinese medicine (Fig. 6.47) [97].

Compounds **79a–f** and **80** are present in the native fruits in high quantities and displayed significant inhibitory activity (Fig. 6.47). Inhibitory activities were determined in in vitro assays with recombinant PLpro enzyme using the peptide Z-RLRGG-AMC as a fluorogenic substrate. The IC_{50} values were found to be in the range of 15.8–70.1 μM and terrestrimin **79f** was the most potent inhibitor with an IC_{50} value of 15.8 μM while ferulic acid **80** showed no detectable activity [97].

Inhibitors Awaiting Experimental Confirmation

In silico methods gain increasing importance for the identification of small molecules as potential drug targets. A data set of 40 compounds previously isolated from fungi were virtually screened to investigate their potential as p53–MDM2 interaction inhibitors using the docking algorithm AutoDock4 with known crystal structures [134].

The steroids ganoderic acids X (**81b**) (predicted K_i = 44 nM), Y (**81d**) (predicted K_i = 47 nM) and F (**81e**) (predicted K_i = 59 nM) showed potential as
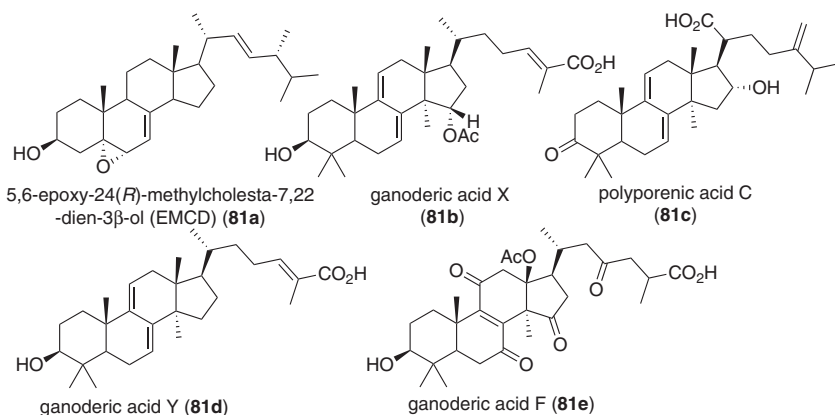
FIGURE 6.48 Structure of ganoderic acids and 5,6-epoxy-24(*R*)-methylcholesta-7,22-dien-3*β*-ol (EMCD).

MDM2 inhibitors (Fig. 6.48). These ganoderic acids were originally isolated and identified from the medicinal mushroom *Ganoderma lucidum* and show cytotoxicity against tumor cell lines [135]. Another potential inhibitor was EMCD (5,6-epoxy-24(*R*)-methylcholesta-7,22-dien-3*β*-ol) (**81a**) (predicted K_i = 106 nM), which was originally isolated from the entomoparasitic fungus *Cordyceps sinensis* and is known to inhibit growth of several tumor cell lines [136]. A further compound of interest polyporenic acid C (**81c**) (predicted K_i = 158 nM) was isolated from the polyporus bracket fungi *Piptoporus betulinus* and *Daedalea dickinsii*. The proposed mechanism for the observed antiproliferative activities of the top ranked compounds was the disruption of the p53–MDM2 interaction and subsequent increase in active p53 levels. These computational results still await confirmation by experimental studies to validate molecular docking of known natural products with protein crystal structure data as a predictive approach. We hope that follow-up studies will be conducted in order to confirm or discount these findings.

CONCLUSIONS

The discovery of natural product inhibitors with activities in the conjugation/deconjugation systems of ubiquitin and Ubl proteins has been enabled by suitably designed assays. This includes in vitro as well as cell-based assays and their design is critical toward throughput, specificity, and robustness of the method. We expect that the wider availability of powerful assays for the analysis of enzymatic activities in the ubiquitin system will lead to the identification of further natural product inhibitors of ubiquitination and deubiquitination. It is hoped that this will improve our understanding of biological function in the ubiquitin system and of Ubl modifiers. In order to realize this potential, it is of fundamental importance that the molecular mechanism and targets of the identified compounds are fully elucidated. We expect that collaborative research efforts and the use of modern

technologies in proteome analysis (such as chemical proteomics and tandem mass spectrometry) will enable further progress in this area.

ABBREVIATIONS

293T	human embryonic kidney 293 cells
4Ub-Luc	linear tetraubiquitin-luciferase
Ac	acetate
AMC	7-amido-4-methylcoumarin
AMP	adenosine monophosphate
APPBP1	amyloid precursor protein-binding protein 1
Aos1	activator of SUMO 1
Ara	arabinose
ATP	adenosine triphosphate
Caco-2	human epithelial colorectal adenocarcinoma
CDCA	chenodeoxycholic acid
CTL	chymotrypsin-like
DBA	dibenzylideneacetone
DELFIA	dissociation-enhanced lanthanide fluorescent immunoassay
DHFR	dihydrofolate reductase
DLD-1	colorectal adenocarcinoma cell line (by D.L. Dexter)
DUB	deubiquitinating enzyme
DMT	dimethyl terphenyl
ELISA	enzyme-linked immunosorbent assay
EMCD	5,6-epoxy-24(R)-methylcholesta-7,22-dien-3β-ol
FDA	Food and Drug Administration
Flag	polypeptide protein tag
FRET	Förster resonance energy transfer
Glc	glucose
HAUbVS	HA-tagged ubiquitin vinyl sulfone
Hep G2	human liver carcinoma cell line
HDAC	histone deacetylase
HDM2	human double minute 2 homologue
HeLa	cervical cancer cell line taken from Henrietta Lacks
HIF-1	hypoxia-inducible factor-1
IC$_{50}$	half maximal inhibitory concentration
IF	induction factor
IsoT	isopeptidase T
KB	keratin-forming tumor cell line
MCF-7	breast cancer cell line
MDM	mouse double minute homologue
Me	methyl
MfnX	mitofusin-X
NAE	NEDD8-activating enzyme
NEDD8	neural precursor cell expressed developmentally downregulated protein 8
NF-κB	nuclear factor kappa-light chain enhancer of activated B cells
NRPS	nonribosomal peptide synthetase
p21	cyclin-dependent kinase inhibitor 1

p53	tumor protein p53
PC3	human prostate cancer cell line
Pdcd4	programmed cell death protein 4
PEST	proline (P)-, glutamic acid (E)-, serine (S)-, and threonine (T)-rich peptide sequence
PGPH	peptidylglutamyl-peptide hydrolyzing
PI3 Kinase	phosphatidylinositol-4,5-bisphosphate 3-kinase
PKS	polyketide synthase
PLpro	papain-like protease
POH1	pad one homolog-1
PP_i	pyrophosphate
RanGAP1-C2	Ran GTPase-activating protein 1 C-terminal region
RBL-2H3	rat basophilic leukemia cells-2H3
Rha	rhamnose
Rh110	rhodamine-110
RING	really interesting new gene
ROS	reactive oxygen species
Rpn11	Regulatory Particle Non-ATPase 11
SARS-CoV	severe acute respiratory syndrome coronavirus
SENP1	sentrin-specific peptidase 1
siRNA	small interfering RNA
SUMO	small ubiquitin-like modifier
TNFα	tumor necrosis factor alpha
TPA	12-O-tetradecanoylphorbol-13-acetate
TRAMP	transgenic adenocarcinoma of the mouse prostate
β-TrCP1	beta-transducin repeat-containing protein 1
Ub	ubiquitin
Ubl	ubiquitin-like
UbcX	ubiquitin-conjugating enzyme X
UCH-LX	ubiquitin carboxyterminal hydrolase LX
Uev1A	ubiquitin-conjugating enzyme variant
Ulp	ubiquitin-like protein-specific protease
UPS	ubiquitin–proteasome system
USPX	ubiquitin-specific peptidase X
VHL	von Hippel–Lindau
YFP	yellow fluorescent protein
ZINC	ZINC is not commercial database

ACKNOWLEDGMENTS

REFERENCES

[1] M. Bogyo, E.W. Wang, in: Curr. Top. Microbiol. Immunol., vol. 268, 2002, p. 185.

[2] A.F. Kisselev, W.A. Van Der Linden, H.S. Overkleeft, Chem. Biol. 19 (2012) 99.

[3] J. Myung, K.B. Kim, C.M. Crews, Med. Res. Rev. 21 (2001) 245.

[4] J.S. Schneekloth Jr., C.M. Crews, Curr. Drug Targets 12 (2011) 1581.

[5] S. Tsukamoto, H. Yokosawa, Planta Med. 76 (2010) 1064.

[6] S. Tsukamoto, H. Yokosawa, Curr. Med. Chem. 13 (2006) 745.

[7] M. Groll, R. Huber, Biochim. Biophys. Acta – Mol. Cell Res. 1695 (2004) 33.

[8] K.R. Love, A. Catic, C. Schlieker, H.L. Ploegh, Nat. Chem. Biol. 3 (2007) 697.

[9] Z.M. Eletr, K.D. Wilkinson, Biochim. Biophys. Acta – Mol. Cell Res. 1843 (2014) 114.

[10] F.E. Reyes-Turcu, K.H. Ventii, K.D. Wilkinson, in: Annu. Rev. Biochem., vol. 78, 2009, p. 363.

[11] S.M.B. Nijman, M.P.A. Luna-Vargas, A. Velds, T.R. Brummelkamp, A.M.G. Dirac, T.K. Sixma, R. Bernards, Cell 123 (2005) 773.

[12] B.M. Kessler, M.J. Edelmann, Cell Biochem. Biophys. 60 (2011) 21.

[13] C.M. Pickart, M.J. Eddins, Biochim. Biophys. Acta – Mol. Cell Res. 1695 (2004) 55.

[14] O. Kerscher, R. Felberbaum, M. Hochstrasser, in: Annu. Rev. Cell Dev. Biol., vol. 22, 2006, p. 159.

[15] R.T. Hay, Mol. Cell. 18 (2005) 1.

[16] S. Jentsch, I. Psakhye, in: Annu. Rev. Genet., vol. 47, 2013, p. 167.

[17] Y. Wang, M. Dasso, J. Cell Sci. 122 (2009) 4249.

[18] S.J. Li, M. Hochstrasser, Nature 398 (1999) 246.

[19] T. Suzuki, A. Ichiyama, H. Saitoh, T. Kawakami, M. Omata, C.H. Chung, M. Kimura, N. Shimbara, K. Tanaka, J. Biol. Chem. 274 (1999) 31131.

[20] J. Zhao, Cell. Mol. Life Sci. 64 (2007) 3017.

[21] G. Rabut, M. Peter, EMBO Rep. 9 (2008) 969.

[22] B.A. Schulman, J. Wade Harper, Nat. Rev. Mol. Cell Biol. 10 (2009) 319.

[23] R. Sekizawa, S. Ikeno, H. Nakamura, H. Naganawa, S. Matsui, H. Iinuma, T. Takeuchi, J. Nat. Prod. 65 (2002) 1491.

[24] L. Comméiras, J.E. Moses, R.M. Adlington, J.E. Baldwin, A.R. Cowley, C.M. Baker, B. Albrecht, G.H. Grant, Tetrahedron 62 (2006) 9892.

[25] J. Li, D. Lee, Chem. Asian J. 5 (2010) 1298.

[26] X. Lei, R.P. Johnson, J.A. Porco Jr., Angew. Chem. Int. Ed. Engl. 42 (2003) 3913.

[27] G. Mehta, K. Islam, Tetrahedron Lett. 45 (2004) 7683.

[28] G. Mehta, S.S. Ramesh, Tetrahedron Lett. 45 (2004) 1985.

[29] J.E. Moses, L. Commeiras, J.E. Baldwin, R.M. Adlington, Org. Lett. 5 (2003) 2987.

[30] M. Matsuzawa, H. Kakeya, J. Yamaguchi, M. Shoji, R. Onose, H. Osada, Y. Hayashi, Chem. Asian J. 1 (2006) 845.

[31] S. Tsukamoto, H. Hirota, M. Imachi, M. Fujimuro, H. Onuki, T. Ohta, H. Yokosawa, Bioorg. Med. Chem. Lett. 15 (2005) 191.

[32] K. Taori, V.J. Paul, H. Luesch, J. Am. Chem. Soc. 130 (2008) 1806.

[33] C.G. Nasveschuk, D. Ungermannova, X. Liu, A.J. Phillips, Org. Lett. 10 (2008) 3595.

[34] A.A. Bowers, T.J. Greshook, N. West, G. Estiu, S.L. Schreiber, O. Wiest, R.M. Williams, J.E. Bradner, J. Am. Chem. Soc. 131 (2009) 2900.

[35] A.A. Bowers, N. West, T.L. Newkirk, A.E. Troutman-Youngman, S.L. Schreiber, O. Wiest, J.E. Bradner, R.M. Williams, Org. Lett. 11 (2009) 1301.

[36] A.K. Ghosh, S. Kulkarni, Org. Lett. 10 (2008) 3907.

[37] X. Li, Z. Tu, H. Li, C. Liu, Z. Li, Q. Sun, Y. Yao, J. Liu, S. Jiang, ACS Med. Chem. Lett. 4 (2013) 132.

[38] T. Seiser, F. Kamena, N. Cramer, Angew. Chem. Int. Ed. Engl. 47 (2008) 6483.

[39] J.A. Souto, E. Vaz, I. Lepore, A.C. Pöppler, G. Franci, R. Álvarez, L. Altucci, A.R. De Lera, J. Med. Chem. 53 (2010) 4654.

[40] J. Su, Y. Qiu, K. Ma, Y. Yao, Z. Wang, X. Li, D. Zhang, Z. Tu, S. Jiang, Tetrahedron 70 (2014) 7763.

[41] Q. Xiao, L.P. Wang, X.Z. Jiao, X.Y. Liu, Q. Wu, P. Xie, J. Asian Nat. Prod. Res. 12 (2010) 940.

[42] Y. Ying, Y. Liu, S.R. Byeon, H. Kim, H. Luesch, J. Hong, Org. Lett. 10 (2008) 4021.

[43] X. Zeng, B. Yin, Z. Hu, C. Liao, J. Liu, S. Li, Z. Li, M.C. Nicklaus, G. Zhou, S. Jiang, Org. Lett. 12 (2010) 1368.

[44] D. Ungermannova, S.J. Parker, C.G. Nasveschuk, W. Wang, B. Quade, G. Zhang, R.D. Kuchta, A.J. Phillips, X. Liu, PLoS One 7 (2012).

[45] R. Yamanokuchi, K. Imada, M. Miyazaki, H. Kato, T. Watanabe, M. Fujimuro, Y. Saeki, S. Yoshinaga, H. Terasawa, N. Iwasaki, H. Rotinsulu, F. Losung, R.E.P. Mangindaan, M. Namikoshi, N.J. De Voogd, H. Yokosawa, S. Tsukamoto, Bioorg. Med. Chem. 20 (2012) 4437.

[46] T.L. Chang, Y.H. Huang, Y.D. Ou, J. Funct. Foods 7 (2014) 462.

[47] F. Kong, D.J. Faulkner, J. Org. Chem. 58 (1993) 970.

[48] S. Tsukamoto, T. Takeuchi, H. Rotinsulu, R.E.P. Mangindaan, R.W.M. van Soest, K. Ukai, H. Kobayashi, M. Namikoshi, T. Ohta, H. Yokosawa, Bioorg. Med. Chem. Lett. 18 (2008) 6319.

[49] S. Ushiyama, H. Umaoka, H. Kato, Y. Suwa, H. Morioka, H. Rotinsulu, F. Losung, R.E.P. Mangindaan, N.J. De Voogd, H. Yokosawa, S. Tsukamoto, J. Nat. Prod. 75 (2012) 1495.

[50] E. Sakai, H. Kato, H. Rotinsulu, F. Losung, R.E.P. Mangindaan, N.J. De Voogd, H. Yokosawa, S. Tsukamoto, J. Nat. Med. 68 (2014) 215.

[51] S.J. Duncan, S. Grüschow, D.H. Williams, C. McNicholas, R. Purewal, M. Hajek, M. Gerlitz, S. Martin, S.K. Wrigley, M. Moore, J. Am. Chem. Soc. 123 (2001) 554.

[52] S.J. Duncan, D.H. Williams, M. Ainsworth, S. Martin, R. Ford, S.K. Wrigley, Tetrahedron Lett. 43 (2002) 1075.

[53] S.J. Duncan, M.A. Cooper, D.H. Williams, Chem. Commun. (Cambridge, U.K.) 9 (2003) 316.

[54] P. Desai, S.S. Pfeiffer, D.L. Boger, Org. Lett. 5 (2003) 5047.

[55] J.P. Malkinson, M. Zloh, M. Kadom, R. Errington, P.J. Smith, M. Searcey, Org. Lett. 5 (2003) 5051.

[56] W.J. Qian, W.G. Wei, Y.X. Zhang, Z.J. Yao, J. Am. Chem. Soc. 129 (2007) 6400.

[57] S. Tsukamoto, T. Yoshida, H. Hosono, T. Ohta, H. Yokosawa, Bioorg. Med. Chem. Lett. 16 (2006) 69.

[58] A. Nakahashi, N. Miura, K. Monde, S. Tsukamoto, Bioorg. Med. Chem. Lett. 19 (2009) 3027.

[59] K.L. Malloy, H. Choi, C. Fiorilla, F.A. Valeriote, T. Matainaho, W.H. Gerwick, Bioorg. Med. Chem. Lett. 22 (2012) 683.

[60] Y. Nakamura, H. Kato, T. Nishikawa, N. Iwasaki, Y. Suwa, H. Rotinsulu, F. Losung, W. Maarisit, R.E.P. Mangindaan, H. Morioka, H. Yokosawa, S. Tsukamoto, Org. Lett. 15 (2013) 322.

[61] O. Chantarasriwong, A. Batova, W. Chavasiri, E.A. Theodorakis, Chem. Eur. J. 16 (2010) 9944.

[62] C. Chitchumroonchokchai, J.M. Thomas-Ahner, J. Li, K.M. Riedl, J. Nontakham, S. Suksumrarn, S.K. Clinton, A.D. Kinghorn, M.L. Failla, Mol. Nutr. Food Res. 57 (2013) 203.

[63] J. Pedraza-Chaverri, N. Cárdenas-Rodríguez, M. Orozco-Ibarra, J.M. Pérez-Rojas, Food Chem. Toxicol. 46 (2008) 3227.

[64] J. Zhao, Q. Qi, Y. Yang, H.Y. Gu, N. Lu, W. Liu, W. Wang, L. Qiang, L.B. Zhang, Q.D. You, Q.L. Guo, Eur. J. Pharmacol. 589 (2008) 127.

[65] M. Leão, C. Pereira, A. Bisio, Y. Ciribilli, A.M. Paiva, N. Machado, A. Palmeira, M.X. Fernandes, E. Sousa, M. Pinto, A. Inga, L. Saraiva, Biochem. Pharmacol. 85 (2013) 1234.

[66] M. Leão, S. Gomes, J. Pedraza-Chaverri, N. MacHado, E. Sousa, M. Pinto, A. Inga, C. Pereira, L. Saraiva, J. Nat. Prod. 76 (2013) 774.

[67] X. Fei, M. Jo, B. Lee, S.B. Han, K. Lee, J.K. Jung, S.Y. Seo, Y.S. Kwak, Bioorg. Med. Chem. Lett. 24 (2014) 2062.

[68] K. Iikubo, Y. Ishikawa, N. Ando, K. Umezawa, S. Nishiyama, Tetrahedron Lett. 43 (2002) 291.

[69] D. Xu, Y. Nie, X. Liang, L. Ji, S. Hu, Q. You, F. Wang, H. Yea, J. Wang, Nat. Prod. Commun. 8 (2013) 1101.

[70] J.A. Clement, J. Kitagaki, Y. Yang, C.J. Saucedo, B.R. O'Keefe, A.M. Weissman, T.C. McKee, J.B. McMahon, Bioorg. Med. Chem. 16 (2008) 10022.

[71] C.A. Sasiela, D.H. Stewart, J. Kitagaki, Y.J. Safiran, Y. Yang, A.M. Weissman, P. Oberoi, I.V. Davydov, E. Goncharova, J.A. Beutler, J.B. McMahon, B.R. O'Keefe, J. Biomol. Screen. 13 (2008) 229.

[72] A. Chatterjee, A. Sahu, M. Saha, J. Banerji, Monatsh. Chem. 127 (1996) 1259.

[73] T.S. Chinta Rao, S. Saha, G.B. Raolji, B. Patro, P. Risbood, M.J. Difilippantonio, J.E. Tomaszewski, S.V. Malhotra, Tetrahedron Lett. 54 (2013) 487.

[74] G.W. Gribble, T.C. Barden, D.A. Johnson, Tetrahedron 44 (1988) 3195.

[75] T.M. Lipińska, Tetrahedron 62 (2006) 5736.

[76] K.T. Potts, G.S. Mattingly, J. Org. Chem. 33 (1968) 3985.

[77] S.M. Vogel, M.R. Bauer, A.C. Joerger, R. Wilcken, T. Brandt, D.B. Veprintsev, T.J. Rutherford, A.R. Fersht, F.M. Boeckler, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 16906.

[78] A.F. Hofmann, Drug Metab. Rev. 36 (2004) 703.

[79] B.W. Katona, S. Anant, D.F. Covey, W.F. Stenson, J. Biol. Chem. 284 (2009) 3354.

[80] H. Suzuki, T. Chiba, M. Kobayashi, M. Takeuchi, T. Suzuki, A. Ichiyama, T. Ikenoue, M. Omata, K. Furuichi, K. Tanaka, Biochem. Biophys. Res. Commun. 256 (1999) 127.

[81] H. Suzuki, T. Chiba, T. Suzuki, T. Fujita, T. Ikenoue, M. Omata, K. Furuichi, H. Shikama, K. Tanaka, J. Biol. Chem. 275 (2000) 2877.

[82] J.S. Blees, H.R. Bokesch, D. Rübsamen, K. Schulz, L. Milke, M.M. Bajer, K.R. Gustafson, C.J. Henrich, J.B. McMahon, N.H. Colburn, T. Schmid, B. Brüne, PLoS One 7 (2012).

[83] H. Morimoto, H. Oshio, J. Nat. Prod. 44 (1981) 748.

[84] K.K. Anand, M.L. Sharma, B. Singh, B.J. Ghatak, Indian J. Exp. Biol. 16 (1978) 1216.

[85] M. Nepal, H. Jung Choi, B.Y. Choi, S. Lim Kim, J.H. Ryu, D. Hee Kim, Y.H. Lee, Y. Soh, Eur. J. Pharmacol. 691 (2012) 28.

[86] J.E. Mullally, P.J. Moos, K. Edes, F.A. Fitzpatrick, J. Biol. Chem. 276 (2001) 30366.

[87] P.J. Moos, K. Edes, F.A. Fitzpatrick, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 9215.

[88] J.E. Mullally, F.A. Fitzpatrick, Mol. Pharmacol. 62 (2002) 351.

[89] Z. Li, F. Melandri, I. Berdo, M. Jansen, L. Hunter, S. Wright, D. Valbrun, M.E. Figueiredo-Pereira, Biochem. Biophys. Res. Commun. 319 (2004) 1171.

[90] S.M. Verbitski, J.E. Mullally, F.A. Fitzpatrick, C.M. Ireland, J. Med. Chem. 47 (2004) 2062.

[91] A.S. Ratnayake, T.S. Bugni, C.A. Veltri, J.J. Skalicky, C.M. Ireland, Org. Lett. 8 (2006) 2171.

[92] M. Yamaguchi, M. Miyazaki, M.P. Kodrasov, H. Rotinsulu, F. Losung, R.E.P. Mangindaan, N.J. De Voogd, H. Yokosawa, B. Nicholson, S. Tsukamoto, Bioorg. Med. Chem. Lett. 23 (2013) 3884.

[93] B. Nicholson, C.A. Leach, S.J. Goldenberg, D.M. Francis, M.P. Kodrasov, X. Tian, J. Shanks, D.E. Sterner, A. Bernal, M.R. Mattern, K.D. Wilkinson, T.R. Butt, Protein Sci. 17 (2008) 1035.

[94] K. Okada, Y.Q. Ye, K. Taniguchi, A. Yoshida, T. Akiyama, Y. Yoshioka, J.I. Onose, H. Koshino, S. Takahashi, A. Yajima, N. Abe, S. Yajima, Bioorg. Med. Chem. Lett. 23 (2013) 4328.

[95] Y. Yoshioka, Y.Q. Ye, K. Okada, K. Taniguchi, A. Yoshida, K. Sugaya, J.I. Onose, H. Koshino, S. Takahashi, A. Yajima, S. Yajima, N. Abe, PLoS One 8 (2013).

[96] T. Reiner, R. Parrondo, A. de las Pozas, D. Palenzuela, C. Perez-Stable, PLoS One 8 (2013).

[97] Y.H. Song, D.W. Kim, M.J. Curtis-Long, H.J. Yuk, Y. Wang, N. Zhuang, K.H. Lee, K.S. Jeon, K.H. Park, Biol. Pharm. Bull. 37 (2014) 1021.

[98] W. Yue, Z. Chen, H. Liu, C. Yan, M. Chen, D. Feng, H. Wu, L. Du, Y. Wang, J. Liu, X. Huang, L. Xia, L. Liu, X. Wang, H. Jin, J. Wang, Z. Song, X. Hao, Q. Chen, Cell Res. 24 (2014) 482.

[99] B. Zhou, Y. Zuo, B. Li, H. Wang, H. Liu, X. Wang, X. Qiu, Y. Hu, S. Wen, J. Du, X. Bu, Mol. Cancer Ther. 12 (2013) 1381.

[100] M.N. Cao, Y.B. Zhou, A.H. Gao, J.Y. Cao, L.X. Gao, L. Sheng, L. Xu, M.B. Su, X.C. Cao, M.M. Han, M.K. Wang, J. Li, Biochim. Biophys. Acta – Gen. Subjects 1840 (2014) 2004.

[101] M.P. Gupta, A. Monge, G.A. Karikas, A. Lopez De Cerain, P.N. Solis, E. De Leon, M. Trujillo, O. Suarez, F. Wilson, G. Montenegro, Y. Noriega, A.I. Santana, M. Correa, C. Sanchez, Pharm. Biol. 34 (1996) 19.

[102] A. Borodovsky, H. Ovaa, N. Kolli, T. Gan-Erdene, K.D. Wilkinson, H.L. Ploegh, B.M. Kessler, Chem. Biol. 9 (2002) 1149.

[103] A. Ahond, M.B. Zurita, M. Colin, C. Fizames, P. Laboute, F. Lavelle, D. Laurent, C. Poupat, J. Pusset, et al., C. R. Acad. Sci. Ser. 2 307 (1988) 145.

[104] S. Tsukamoto, K. Tane, T. Ohta, S. Matsunaga, N. Fusetani, R.W.M. Van Soest, J. Nat. Prod. 64 (2001) 1576.

[105] A. Commercon, J.M. Paris, Tetrahedron Lett. 32 (1991) 4905.

[106] S. Tsukamoto, K. Yamashita, K. Tane, R. Kizu, T. Ohta, S. Matsunaga, N. Fusetani, H. Kawahara, H. Yokosawa, Biol. Pharm. Bull. 27 (2004) 699.

[107] G. Catimel, R. Coquard, J.P. Guastalla, Y. Merrouche, N. Le Bail, M.K. Alakl, A. Dumortier, M. Foy, M. Clavel, Cancer Chemother. Pharmacol. 35 (1995) 246.

[108] D. Diop, C. Chauvin, S. Salhi, C. Poupat, A. Ahond, O. Jean-Jean, Comptes Rendus – Biologies 330 (2007) 855.

[109] S.Y. Fung, V. Sofiyev, J. Schneiderman, A.F. Hirschfeld, R.E. Victor, K. Woods, J.S. Piotrowski, R. Deshpande, S.C. Li, N.J. De Voogd, C.L. Myers, C. Boone, R.J. Andersen, S.E. Turvey, ACS Chem. Biol. 9 (2014) 247.

[110] A. Chatterjee, C.P. Dutta, Tetrahedron 23 (1967) 1769.

[111] M. Iwashita, N. Oka, S. Ohkubo, M. Saito, N. Nakahata, Eur. J. Pharmacol. 570 (2007) 38.

[112] L. Raj, T. Ide, A.U. Gurkar, M. Foley, M. Schenone, X. Li, N.J. Tolliday, T.R. Golub, S.A. Carr, A.F. Shamji, A.M. Stern, A. Mandinova, S.L. Schreiber, S.W. Lee, Nature 475 (2011) 231.

[113] M. Jarvius, M. Fryknäs, P. D'Arcy, C. Sun, L. Rickardson, J. Gullbo, C. Haglund, P. Nygren, S. Linder, R. Larsson, Biochem. Biophys. Res. Commun. 431 (2013) 117.

[114] F. Ausseil, A. Samson, Y. Aussagues, I. Vandenberghe, L. Creancier, I. Pouny, A. Kruczynski, G. Massiot, C. Bailly, J. Biomol. Screen. 12 (2007) 106.

[115] J. Beck, Y. Guminski, C. Long, L. Marcourt, F. Derguini, F. Plisson, A. Grondin, I. Vandenberghe, S. Vispé, V. Brel, Y. Aussagues, F. Ausseil, P.B. Arimondo, G. Massiot, F. Sautel, F. Cantagrel, Bioorg. Med. Chem. 20 (2012) 819.

[116] I. Fukuda, A. Ito, G. Hirai, S. Nishimura, H. Kawasaki, H. Saitoh, K.i. Kimura, M. Sodeoka, M. Yoshida, Chem. Biol. 16 (2009) 133.

[117] N. Saitoh, Y. Uchimura, T. Tachibana, S. Sugahara, H. Saitoh, M. Nakao, Exp. Cell Res. 312 (2006) 1418.

[118] I. Fukuda, A. Ito, M. Uramoto, H. Saitoh, H. Kawasaki, H. Osada, M. Yoshida, J. Antibiot. 62 (2009) 221.

[119] M. Takemoto, Y. Kawamura, M. Hirohama, Y. Yamaguchi, H. Handa, H. Saitoh, Y. Nakao, M. Kawada, K. Khalid, H. Koshino, K.I. Kimura, A. Ito, M. Yoshida, J. Antibiot. 67 (2014) 335.

[120] M. Hirohama, A. Kumar, I. Fukuda, S. Matsuoka, Y. Igarashi, H. Saitoh, M. Takagi, K. Shin-Ya, K. Honda, Y. Kondoh, T. Saito, Y. Nakao, H. Osada, K.Y.J. Zhang, M. Yoshida, A. Ito, ACS Chem. Biol. 8 (2013) 2635.

[121] Y.S. Kim, K. Nagy, S. Keyser, J.S. Schneekloth Jr., Chem. Biol. 20 (2013) 604.

[122] Y.S. Kim, S.G.L. Keyser, J.S. Schneekloth Jr., Bioorg. Med. Chem. Lett. 24 (2014) 1094.

[123] F.C. Chen, Y.M. Lin, Phytochemistry 14 (1975) 1644.

[124] C.H. Leung, D.S.H. Chan, H. Yang, R. Abagyan, S.M.Y. Lee, G.Y. Zhu, W.F. Fong, D.L. Ma, Chem. Commun. (Cambridge, U.K.) 47 (2011) 2511.

[125] H.J. Zhong, V. Pui-Yan Ma, Z. Cheng, D. Shiu-Hin Chan, H.Z. He, K.H. Leung, D.L. Ma, C.H. Leung, Biochimie 94 (2012) 2457.

[126] H.J. Zhong, L.J. Liu, D.S.H. Chan, H.M. Wang, P.W.H. Chan, D.L. Ma, C.H. Leung, Biochimie 102 (2014) 211.

[127] J.J. Jaffe, J.J. McCormack, E. Meymerian, Exp. Parasitol. 28 (1970) 535.

[128] G.O. Morton, J.E. Lancaster, G.E. Van Lear, W. Fulmor, W.E. Meyer, J. Am. Chem. Soc. 91 (1969) 1535.

[129] 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 37 (2010) 102.

[130] 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 458 (2009) 732.

[131] J.Y. Park, H.J. Jeong, J.H. Kim, Y.M. Kim, S.J. Park, D. Kim, K.H. Park, W.S. Lee, Y.B. Ryu, Biol. Pharm. Bull. 35 (2012) 2036.

[132] J.Y. Park, J.H. Kim, Y.M. Kim, H.J. Jeong, D.W. Kim, K.H. Park, H.J. Kwon, S.J. Park, W.S. Lee, Y.B. Ryu, Bioorg. Med. Chem. 20 (2012) 5928.

[133] J.K. Cho, M.J. Curtis-Long, K.H. Lee, D.W. Kim, H.W. Ryu, H.J. Yuk, K.H. Park, Bioorg. Med. Chem. 21 (2013) 3051.

[134] H.J.C. Froufe, R.M.V. Abreu, I.C.F.R. Ferreira, J. Enzym. Inhib. Med. Chem. 28 (2013) 569.

[135] J. Jiang, B. Grieb, A. Thyagarajan, D. Sliva, Int. J. Mol. Med. 21 (2008) 577.

[136] J.W. Bok, L. Lermer, J. Chilton, H.G. Klingeman, G.H.N. Towers, Phytochemistry 51 (1999) 891.