Target Identification of Bioactive Covalently Acting Natural Products

Daniel K. Nomura and Thomas J. Maimone

Contents

Abstract There are countless natural products that have been isolated from microbes, plants, and other living organisms that have been shown to possess therapeutic activities such as antimicrobial, anticancer, or anti-inflammatory effects. However, developing these bioactive natural products into drugs has remained challenging in part because of their difficulty in isolation, synthesis, mechanistic understanding, and off-target effects. Among the large pool of bioactive natural products lies classes of compounds that contain potential reactive electrophilic centers that can covalently react with nucleophilic amino acid hotspots on proteins and other biological molecules to modulate their biological action. Covalently

D. K. Nomura (✉) · T. J. Maimone (✉)
Department of Chemistry, University of California, 94720 Berkeley, CA, USA
e-mail: dnomura@berkeley.edu

T. J. Maimone
e-mail: maimone@berkeley.edu

Current Topics in Microbiology and Immunology
DOI 10.1007/82_2018_121

acting natural products are more amenable to rapid target identification and mapping of specific druggable hotspots within proteins using activity-based protein profiling (ABPP)-based chemoproteomic strategies. In addition, the granular biochemical insights afforded by knowing specific sites of protein modifications of covalently acting natural products enable the pharmacological interrogation of these sites with more synthetically tractable covalently acting small molecules whose structures are more easily tuned. Both discovering binding pockets and targets hit by natural products and exploiting druggable modalities targeted by natural products with simpler molecules may overcome some of the challenges faced with translating natural products into drugs.

1 Introduction

Natural products from microbes, plants, and other living organisms are a rich source of medicinal compounds that have been utilized by humankind for thousands of years. Their further characterization and exploitation of important biological pathways have led to many different types of modern medicines to treat pathologies such as bacterial and fungal infections, inflammation, diabetes, neurodegenerative diseases, and cancer (Koehn and Carter 2005; Kingston 2011; Harvey et al. 2015; Laraia et al. 2018). Between 25 and 50% of marketed drugs are thought to be derived from natural products, and while such compounds populate many therapeutic areas, they have been particularly important to the areas of oncology and infectious diseases (Kingston 2011). However, while many natural products have shown therapeutic potential, developing natural products into drugs has remained challenging and most US pharmaceutical companies have reduced or eliminated their natural product groups. This is in part due to the difficulty of synthesis and execution of global structure–activity relationship (SAR) studies, challenges in large-scale isolation, slower discovery, and development compared to target-based high-throughput screening campaigns involving simpler, often "flat" molecules, hurdles in understanding mode of action or direct targets, and complex polypharmacology (Beutler 2009; Romo and Liu 2016). Despite these challenges, natural products have not only yielded classical active-site inhibitors against druggable targets, but have also been able to uniquely access druggable modalities that would be considered undruggable or difficult to tackle with traditional drug discovery efforts. These include, for example, FK506 or tacrolimus, which reduces peptidylprolyl isomerase activity by binding to FKBP12, creating a new complex FKBP12–FK506 complex, leading to the interaction with and inhibition of calcineurin resulting in inhibition of T cell signaling (Liu et al. 1991). Another example comes from rapamycin or sirolimus, which also binds to FKBP12 to form a FKBP12–rapamycin complex which then interacts with and inhibits mTORC1 signaling (Saxton and Sabatini 2017). Even in the twenty-first century, these molecular functions are difficult to "design" from first principles. Thus, target identification of natural products remains a critically important endeavor not just for

translational development of natural products into drugs, but also to gain insight into new modalities that can be targeted for drug discovery and to gain insight into natural strategies for target engagement. Upon identifying particular binding pockets within proteins targeted by natural products, synthetically simpler libraries of small molecules can potentially be developed against these sites to overcome the synthetic and isolation challenges faced with natural products. In this review, we will highlight activity-based protein profiling (ABPP) chemoproteomic strategies for mapping targets of covalently acting natural products and covalent ligand discovery approaches that can be utilized to potentially develop more synthetically tractable compounds that mimic the actions of the more complex natural products. We will also discuss diverse examples of electrophilic natural product scaffolds and their targets.

2 Covalently Acting Natural Products

Hundreds of natural products have been shown, or proposed, to engage a wide array of biological targets in covalent bond formation including proteins, nucleic acids, and even small biological molecules themselves. Equally diverse are the chemical structures of the "reactive warhead" embedded into the parent natural product scaffold–motifs which in some cases would often be difficult to design from first principles (Potashman and Duggan 2009; Baillie 2016; Lagoutte et al. 2017), yet are continually influencing modern covalent drug design (Lagoutte and Winssinger 2017). Several excellent reviews have been written on covalently acting natural products, particularly with respect to their protein targeting abilities (Drahl et al. 2005; Leslie and Hergenrother 2008; Gersch et al. 2012; Wright and Sieber 2016; Pan et al. 2016; Jackson et al. 2017). Here, we briefly highlight various classes of electrophilic species presented by natural products that have been implicated in their mechanism of action.

2.1 Strained Ring-Containing Electrophiles

Nature extensively exploits ring strain in the creation of covalent warheads. Reactive carbonyl groups embedded in strained rings, as typified by the β-lactam and β-lactone functional group, are some of the oldest known and most intensely studied covalent pharmacophores found in natural products (Fig. 1a) (Böttcher and Sieber 2012). Indeed, the β-lactam antibiotics, typified by penicillin V (**1**), are perhaps the greatest success story in the use of covalently acting natural products to treat human disease. Their primary target (an active-site serine of bacterial transpeptidases) and other modes of action are discussed in Sect. 3. In the past three decades, β-lactones have garnished significant attention due to their protein targeting ability (Böttcher and Sieber 2012). Naturally occurring lipstatin (**2**) inspired

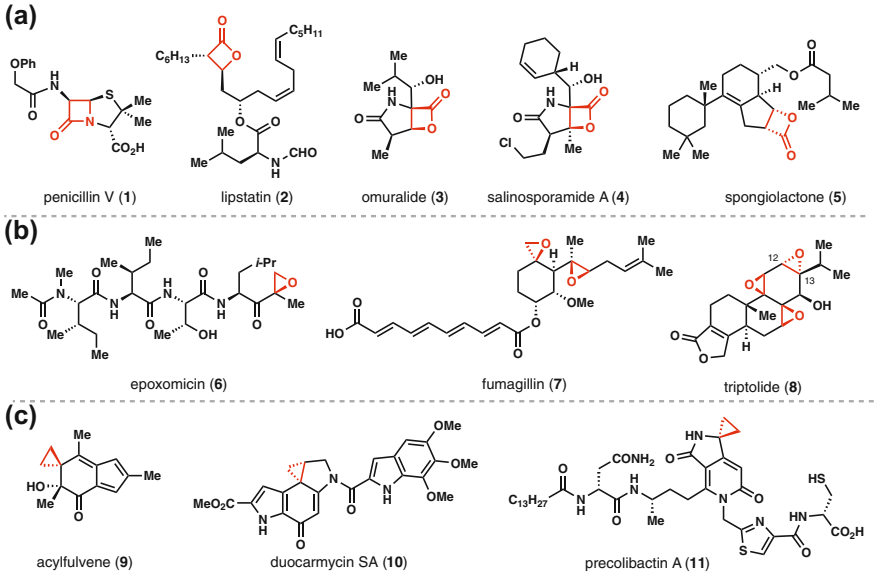


Fig. 1 Covalently acting natural products exploiting strained ring electrophiles. **a** β-lactams and β-lactones, **b** reactive epoxides, and **c** electrophilic cyclopropanes

the antiobesity drug and lipase inhibitor orlistat (Bialecka-Florjanczyk et al. 2018). Omuralide (**3**) and salinosporamide A (**4**) are both potent cytotoxic agents which covalently modify an active-site threonine of the 20S proteasome subunit (Gulder and Moore 2010). This family of natural products is involved in various clinical trials for the treatment of myriad cancers (Potts et al. 2011). Despite the target specificity for **1**–**4**, proteomic profiling studies of spongiolactone (**5**) revealed a complex portrait of targets and thus likely polypharmacological effects dictating its antiproliferative effects (Wright et al. 2017).

The epoxide functional group is another major class of reactive electrophile frequently exploited by natural products (Fig. 1b). The intrinsic affinity of the parent molecule to the target, prior to covalent engagement, is a common theme for promoting reactivity of otherwise unreactive substrates (Swinney 2009; Bauer 2015; Strelow 2017). Thus, many complex epoxide-containing molecules studied are not promiscuous, and in the case of multiple epoxidized molecules, only a single epoxide often reacts as discussed below. The bacterial-derived natural product epoxomicin (**6**) is a potent covalent proteasome inhibitor whose understanding of mechanism of action led to the discovery of the anticancer drug carfilzomib (Kim and Crews 2013). Fumagillin (**7**) targets the methionine aminopeptidase MetAP-2 wherein only the spiroepoxide is essential. Target identification for both **6** and **7** is discussed in Sect. 3. The diterpene triepoxide triptolide (**8**) has long been used in traditional Chinese medicine to treat a host of diseases including inflammation and cancer, and derivatives of **8** have entered clinical trials for the treatment of cancer. Triptolide induces apoptosis and cell growth arrest and is a potent inhibitor of

NF-κB- and NF-AT-mediated transcription (Meng et al. 2014). Many additional targets of **8** (both covalent and non-covalent) have been proposed (Leuenroth and Crews 2005). Triptolide covalently modifies a reactive cysteine on the XPB subunit of the transcription factor TFIIH (Titov et al. 2011). Notably, the covalent locus of reactivity is believed to be the 12, 13-epoxide (He et al. 2015).

Cyclopropanes adjacent to electron-deficient π-systems (i.e., homo-Michael acceptors) have been frequently implicated in the alkylation of biological nucleophiles, particularly DNA bases (Fig. 1c). Not surprisingly, many natural products containing such motifs are potent cytotoxins including both acylfulvene (**9**) and duocarmycin SA (**10**), derivatives of which are being investigated for the treatment of cancer (Tanasova and Sturla 2012). DNA alkylation by **9** impairs transcription by RNA Pol II (Malvezzi et al. 2017), while **10** is a highly sequence-selective covalent alkylator of adenine-N^3 in AT-rich sequences (Boger et al. 1994). The human gut microbe-derived family of colibactins, as typified by precolibactin A (**10**), alkylate and cross-link DNA and have been implicated in human colorectal cancers (Vizcaino and Crawford 2015).

2.2 Unsaturated Ketones, Esters, and Amides

By far, the largest class of covalently acting natural product is those susceptible to hetero-Michael addition reactions–often by the nucleophilic amino acid side chains of proteins (Fig. 2) (Jackson et al. 2017). These addition reactions can be both reversible and irreversible. Notably, hetero-Michael acceptors are prevalent across much of natural product space (peptides, alkaloids, polyketides, and terpenes) and serve as the basis for much of modern covalent drug design (De Cesco et al. 2017). Nature employs both α,β-unsaturated amides (see **12**), esters (see **13**), and ketones (see **14**) to achieve covalent target modification (Fig. 2a). Particularly important from a natural product's perspective is the large class of exocyclic methylene lactones of which over 5000 members are believed to exist (see **15**–**18**, Fig. 2b) (Kitson et al. 2009). Many of these compounds are reactive to thiols, both cysteine side chains and the pool of free intracellular thiols. The anti-inflammatory and anticancer sesquiterpene parthenolide (**15**) has been used for proteomics-based target identification as discussed in Sect. 3. The anticancer pseudoguaianolide helenalin (**16**) is also thiol-reactive (Widen et al. 2017). Nature also constructs various dimeric and higher-order structures featuring multiple electrophilic sites as typified by ainsliadimer A (**17**) and ainsliatrimer A (**18**). Mode of action studies by Lei et al. has found these compounds target IKKα/β and PPARγ (Li et al. 2014; Dong et al. 2015).

An important but less frequently encountered class of hetero-Michael acceptors is the family of quinone methides, which aromatize the following conjugate addition and thus providing an additional driving force for conjugation (Fig. 2c) (Bai et al. 2014). Celastrol (**19**), which possesses a myriad of biological activities including anticancer and anti-inflammatory effects, is cysteine-reactive (Klaić et al.

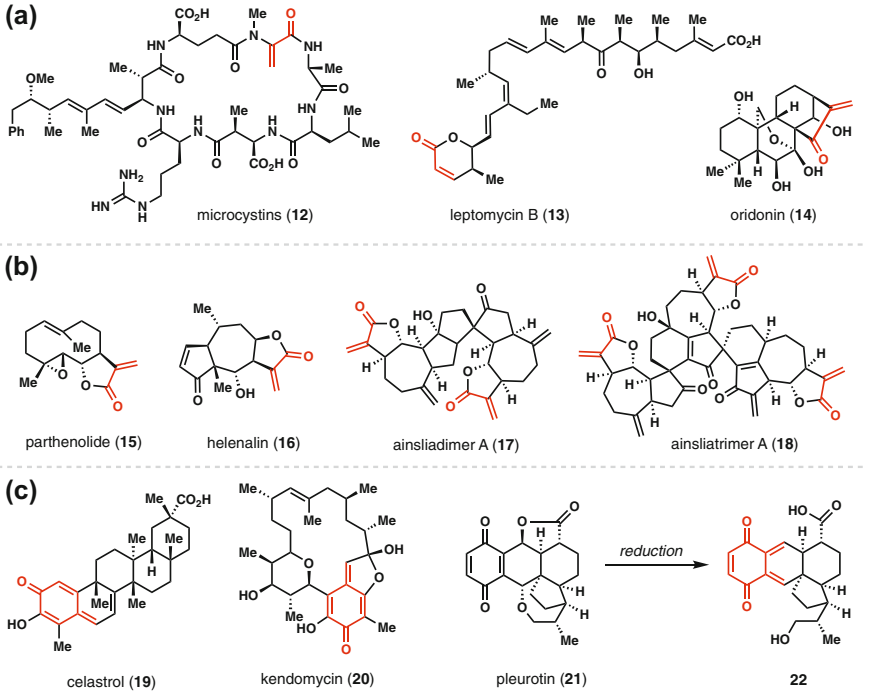


Fig. 2 Natural products susceptible to hetero-Michael addition. **a** α,β-unsaturated amides, esters, and ketones. **b** selected exocyclic methylene lactone **c** electrophilic quinone methides

2012; Zhou et al. 2016a). The antibacterial and cytotoxic natural product kendomycin (**20**) reacts with a proteasome histidine residue (Beck et al. 2014). Finally, it should be noted that few "stable" quinone methides exist; in many cases, the reactive electrophile is generated in situ (Bai et al. 2014). Pleurotin (**21**), for example, has been hypothesized to undergo *in situ* reduction to generate the reactive quinone methide **22** (Moore 1977).

2.3 *Aldehydes, Hemiacetals, and Aminals*

Nature also utilizes natural products containing electrophilic carbonyl groups at the aldehyde oxidation state, including free aldehydes, aminals, and hemiacetals, for covalent target engagement. The phytotoxic sesterterpene ophiobolin A (**23**) has been shown to condense with a lysine side chain of the calcium-binding protein calmodulin, presumably forming an extended enamine intermediate (Leung et al. 1988). Of note, 1, 4-dicarbonyl-containing natural products, including **23**, have also been implicated in the formation of pyrroles via a Paal–Knorr-type process with intracellular amines (Chidley et al. 2016; Kornienko and La Clair 2017).

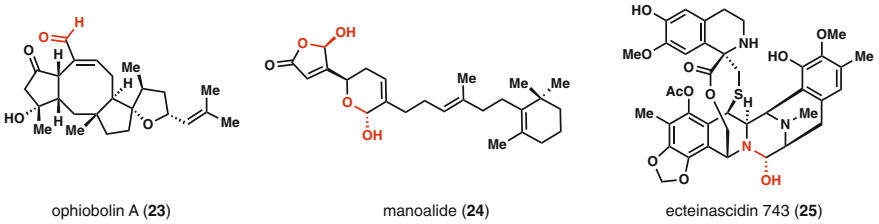


Fig. 3 Natural products containing reactive functional groups capable of condensation reactions

The *bis*-hemiacetal marine natural product manoalide (**24**) has also been proposed to react with lysines (Pettinger et al. 2017). Ecteinascidin 743 (**25**), an aminal-containing anticancer agent and approved drug (trabectedin), generates an iminium ion which covalently bonds to DNA bases (Aune et al. 2002) (Fig. 3).

In summary, nature has evolved a myriad of chemical warheads to covalently modify a range of biological nucleophiles and many historically successful natural product-based drugs have exploited these features. Given the enormous number of naturally occurring epoxide and α,β-unsaturated carbonyl-containing metabolites, one can assume that we have only scratched the surface with respect to detailed target annotation as well as understanding the complex polypharmacology of bioactive natural products. Moreover, nature continues to impress the organic and medicinal chemist with its complex arsenal and sometimes bizarre mechanisms for generating reactive warheads (Fig. 4) (Kwan and Luesch 2010). One cannot help but stand in awe at processes such as the thiol-mediated conversion of leinamycin

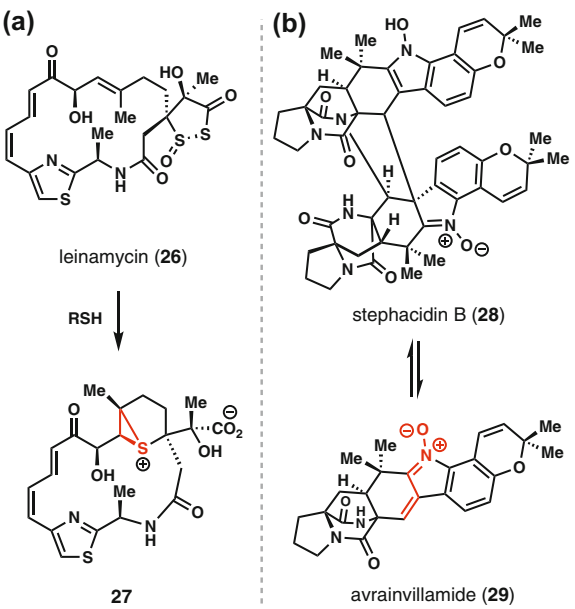


Fig. 4 Natural products containing exotic warheads: Unsaturated nitrones and episulfonium ions

(**26**) to DNA-reactive episulfonium ion **27** or the retro-dimerization of the alkaloid stephacidin B (**28**) into protein-reactive, α,β-unsaturated nitrone-containing avrainvillamide (**29**) (Asai et al. 1996; Myers and Herzon 2003; Wulff et al. 2007). By studying these processes, searching for new ones, and distilling the essential features needed for efficient bond formation, new chemistry concepts relevant to small molecule drug discovery will no doubt be unearthed (Crane and Gademann 2016).

3 Chemoproteomic Approaches to Target Identification of Covalently Acting Natural Products

3.1 Reversible Versus Irreversible Natural Products and Methods for Target Identification

Many strategies have arisen to tackle the challenge of identifying direct protein targets of natural products. These include classical approaches wherein natural products can be immobilized onto solid supports for affinity-based isolation of protein targets (Wright and Sieber 2016). Approaches that enable target identification without chemical modification have also arisen, including cellular thermal shift assay (CETSA) or thermal proteome profiling (TPP) (Chang et al. 2016). Among various approaches, chemoproteomic strategies have been thus far been the dominant approach for natural product target identification, which relies on: (1) direct derivatization of natural products to incorporate photoaffinity cross-linkers (e.g., diazirines, benzophenones), biorthogonal handles (e.g., alkynes or azides), and/or biotin enrichment handles to enable covalent capture and enrichment; or (2) activity-based protein profiling (ABPP)-based competitive chemoproteomic profiling approaches pioneered by Benjamin Cravatt, wherein the parent natural product, usually covalently acting, is competed against a broadly reactive probe—for subsequent identification of the natural product protein targets by mass-spectrometry-based proteomics (Wright and Sieber 2016; Kanoh 2016; Pan et al. 2016). For reversibly acting natural products, these target identification approaches still remain quite challenging and laborious due to low abundance of the targets, high degree of non-specific binding, or synthetic difficulty in natural product derivatization without losing bioactivity. Furthermore, while these types of experiments can provide direct protein targets, identifying the specific site of binding within the protein target often requires additional structural elucidation.

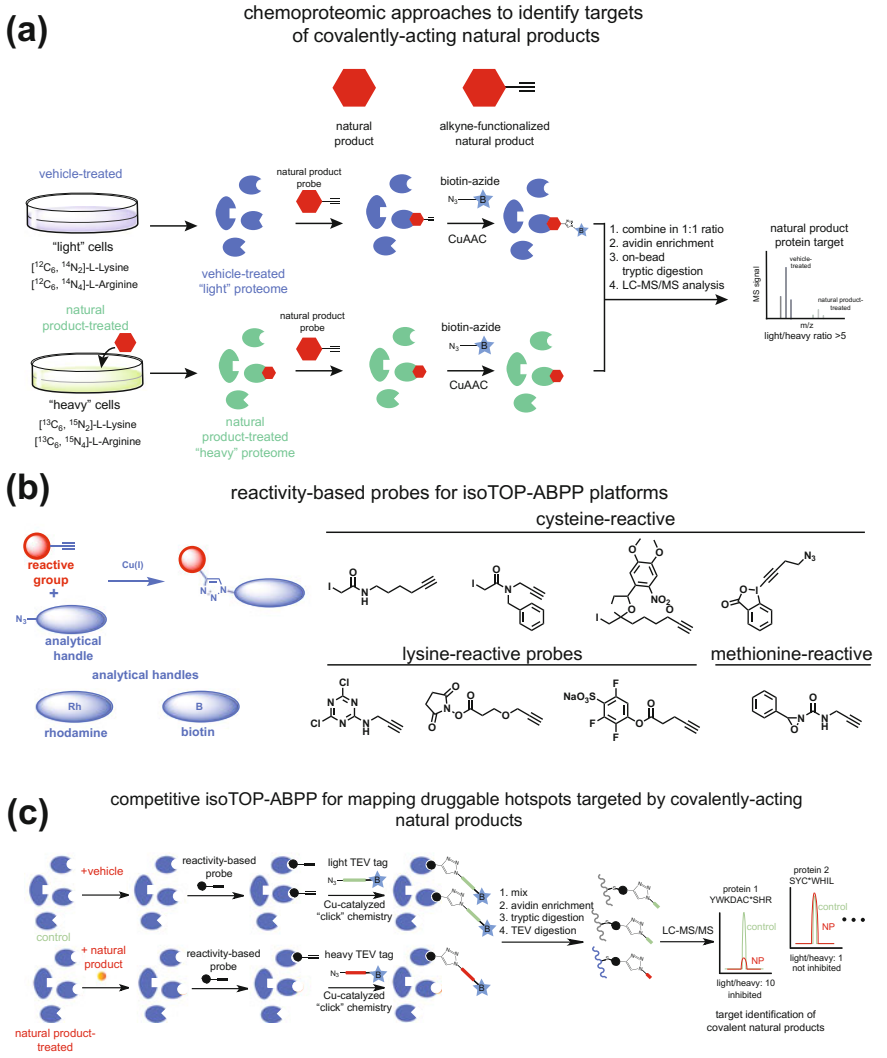
As mentioned, many natural products possess electrophilic fragments capable of covalently reacting with nucleophilic amino acids within proteins to disrupt their biological functions (Drahl et al. 2005; Gersch et al. 2012). As seen in Sect. 2, β-lactones/lactams, exocyclic methylene lactones, spiroepoxides, enones, α,β-unsaturated amides and esters, quinone methides, and aldehydes all take part in protein modification. These reactive groups have been shown to covalently modify

mostly serine, cysteine, threonine, and lysine, but also other amino acids such as histidine, arginine, and aspartic and glutamic acid on particular protein targets (Drahl et al. 2005; Gersch et al. 2012). The advantage of covalently acting natural products is their relative ease in target identification compared to reversibly acting molecules. This is in part because direct covalent attachment by the natural product itself enables harsher washing steps of the protein during the enrichment processes to reduce non-specific binding. It also abrogates non-specific reactivity issues that arise from photoaffinity tags and photo-crosslinking. It is also easier to identify the specific site of covalent modification or the druggable hotspots targeted by reactive natural products compared to reversibly acting natural products using competitive ABPP methods subsequently described. As previously mentioned, understanding the specific amino acid targeted by the natural product within a specific protein directly from complex proteomes or living systems provides significant benefit over just the identification of the protein target; the druggable hotspot(s) may represent a unique and functional modality that can be exploited for drug discovery without the laborious and sometimes impossible efforts of having to solve the three-dimensional structure of the natural product bound to the protein by either crystallographic or spectroscopic means. Furthermore, these sites of covalent natural product modification now represent ligandable hotspots that can be interrogated with far simpler and more synthetically tractable covalently acting small-molecule libraries using high-throughput competitive ABPP screening approaches to be subsequently discussed. We will discuss the two primary chemoproteomic methods for target identification of covalently acting natural products.

3.2 Target Identification Using Natural Product-Based Probes

The chemoproteomics approach that has most commonly been used for covalently acting natural products is to derivatize or synthesize the natural product analog incorporating either a biotin enrichment handle or an alkyne to which a fluorophore-azide or biotin-azide handle can be appended by copper-catalyzed azide–alkyne cycloaddition reactions (CuAAC) (Fig. 5a) (Tornøe et al. 2002; Rostovtsev et al. 2002; Heeres and Hergenrother 2011; Gersch et al. 2012; Ursu and Waldmann 2015; Wright and Sieber 2016; Pan et al. 2016). This general strategy can also be coupled with competition studies with the parent natural product and quantitative proteomic approaches with stable isotopic labeling of cells (SILAC) or isotopic tagging of peptides (e.g., TMT labeling) to identify the direct targets of these natural products (Ong et al. 2002; Thompson et al. 2003; McAlister et al. 2014).

With natural product probes bearing the full linker and biotin handles, the utility of these probes is often limited to in vitro experiments since the larger nature of the probe precludes cell penetrance. Nonetheless, this approach has been used repeatedly to identify the targets of many covalently acting natural products. Craig



Crews' group has used this approach to gain invaluable insights into the mechanisms of action of multiple covalently acting natural products (Sin et al. 1997; Meng et al. 1999; Kwok et al. 2001). His group synthesized a biotinylated parthenolide analog and showed that this natural product derived from the feverfew plant exerts anti-inflammatory activity through targeting IKKβ and showed through mutagenesis studies that it reacts with cysteine C179 (Kwok et al. 2001). His team also discovered that epoxomicin (**6**), isolated from *Actinomyces,* covalently reacts with the LMP7, X, MECL1, and Z catalytic subunits of the proteasome using a biotinylated epoxomicin derivative (Sin et al. 1999; Meng et al. 1999). Subsequent studies by the Crews Laboratory showed that epoxomicin formed a morpholino ring

◂**Fig. 5** Chemoproteomic approaches to target identification of covalently acting natural products. **a** Natural products can be derivatized with functional biorthogonal alkyne handles to enable chemoproteomic target identification. Cells subjected to SILAC can be treated with vehicle or natural product. Cell proteomes can then be treated with the alkyne-functionalized natural product probe and subjected to CuAAC to append a biotin-azide handle after which probe-labeled isotopically light or heavy proteins can be combined in a 1:1 ratio, avidin-enriched, subjected to on-bead tryptic digestion, and subsequent tryptic peptides can be analyzed by LC-MS/MS. Those proteins showing high light-to-heavy SILAC ratios are targets of the covalently acting natural product probe which are competed by the parent natural product. **b** isoTOP-ABPP uses reactivity-based probes to profile proteome-wide reactive and ligandable hotspots in complex proteomes. Reactivity-based probes consist of a broadly reactive warhead and an alkyne or azide handle which can be appended onto an analytical handle (e.g., fluorophore, biotin, or cleavable biotin handles) by CuAAC. Shown are examples of cysteine, lysine, and methionine reactive probes. (C) In an isoTOP-ABPP experiment, proteomes are treated with vehicle or covalently acting natural product and subsequently labeled with reactivity-based probes. Probe-labeled proteins are then appended to a TEV protease-cleavable biotin-azide tag bearing isotopically light or heavy handles for control or treated proteomes, respectively. Control and treated proteomes are subsequently mixed in a 1:1 ratio, probe-labeled proteins are avidin-enriched, tryptically digested, and probe-modified tryptic peptides are eluted by TEV protease and analyzed by LC-MS/MS. Those probe-modified peptides that show high light-to-heavy ratios indicate the druggable hotspot targeted by the covalently acting natural product

between the amino-terminal threonine and the epoxomicin pharmacophore (Groll et al. 2000) and epoxomicin analogs were eventually developed into the multiple myeloma cancer drug carfilzomib or kyprolis (Kim and Crews 2013). Similarly, his group discovered that the antiangiogenic natural product fumagillin (**7**) was found to covalently react with methionine aminopeptidase, MetAP-2, using a biotinylated derivative of **7** (Sin et al. 1997). Through site-directed mutagenesis studies using a fluorescein-labeled fumagillin analog, Jun Liu's group subsequently identified the site of covalent modification as His231 in MetAP-2 (Griffith et al. 1998). The anticancer natural product withaferin A, a steroidal lactone from the withanolide class of natural products found in *Withania somnifera* and other plant species, was shown to react with C328 of the intermediate filament protein vimentin using a biotinylated derivative of withaferin A (Bargagna-Mohan et al. 2007). Herbert Waldmann's group also synthesized a biotinylated derivative of the tetramic acid natural product melophlin A and found that it targeted dynamin to modulate RAS signaling (Knoth et al. 2009).

Natural product probes bearing a biorthogonal alkyne handle can be used in complex proteomes, live cells, or in live animals, after which fluorescent or enrichment handles can be appended onto probe-labeled proteins for visualization or enrichment and identification of natural product targets. Cravatt et al., who have pioneered these types of chemoproteomic approaches, have utilized this approach successfully to map the on and off targets of countless pharmaceutical drugs, drug candidates, and tool compounds (Ahn et al. 2009, 2011; Bachovchin et al. 2011a; Lanning et al. 2014; Whitby et al. 2017; Niessen et al. 2017). In relation to natural products, Cravatt et al. synthesized a diverse alkyne-functionalized library of spiroepoxides, inspired by the many covalently acting natural products that bear

this scaffold including fumagillin and lumanicin D. They then screened this library for antiproliferative effects in breast cancer cells and identified a particular spiroepoxide MJE3 that covalently modified and inhibited the glycolytic enzyme PGAM1 (Evans et al. 2005). Edward Tate's group has also made seminal contributions to chemoproteomic discoveries in natural product target identification. Their group showed, for example, that the reactive cyclic sesquiterpene zerumbone that has been shown to possess anti-inflammatory and anticancer activity reacts with numerous targets in cells involved in cell death, metabolism, cell cycle, and cytoskeletal pathways (Kalesh et al. 2015). Stephen Sieber's group has also repeatedly used these approaches to successfully identify protein targets of covalently acting antibacterial natural products (Staub and Sieber 2008; Böttcher and Sieber 2010; Zeiler et al. 2011; Wirth et al. 2012; Nodwell et al. 2012; Battenberg et al. 2013; Wright and Sieber 2016). To highlight a few examples, the Sieber group functionalized multiple β-lactam antibiotics with alkyne handles for labeling diverse penicillin-binding proteins and subsequently introduced a series of new β-lactam probes that covalently labeled and inhibited a selection of additional bacterial protein targets, including the virulence-associated enzyme ClpP and resistance-associated β-lactamase (Staub and Sieber 2009). In another study, they functionalized a potent nucleoside antibiotic showdomycin with an alkyne handle and showed that it covalently binds to cysteines and inhibits multiple bacterial pathogenesis-associated enzymes, including MurA1 and MurA2 enzymes required for cell wall biosynthesis (Böttcher and Sieber 2010). They also defined multiple antibacterial targets of the dihydro-α-pyrone natural product rugulactone that bears two potentially cysteine-reactive Michael acceptors using an alkyne-functionalized rugulactone derivative, including covalent inhibition of 4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate kinase (ThiD), involved in thiamine biosynthesis (Nodwell et al. 2012).

3.3 Activity-Based Protein Profiling (ABPP) for Identifying Proteome-Wide Druggable Hotspots Targeted by Covalently Acting Natural Products

The aforementioned approaches have been very successful at identifying the targets of covalently acting natural products. However, many natural products are incredibly complex to synthesize or may not necessarily have appropriate sites for derivatization. Furthermore, identifying the specific site that has been covalently modified within the target can be challenging and laborious and requires considerable mutagenesis experiments upfront to nail down the specific site of modification. Identifying the specific sites of modification of large reactive natural products by mass spectrometry may also be challenging if the specific modified adduct on the protein is unstable, semi-reversible, or does not ionize well for mass spectrometry detection.

A chemoproteomic platform pioneered by the Cravatt group that greatly facilitates the identification of specific druggable hotspots targeted by covalently acting natural products is ABPP, which uses reactivity-based chemical probes to profile proteome-wide reactive, functional, and ligandable hotspots directly in complex proteomes (Fig. 5b, c). ABPP utilizes active-site-directed probes to profile the activities of entire enzyme classes. ABPP has now evolved to utilize broader reactivity-based probes to map proteome-wide ligandable amino acid hotspots. Pioneered by Benjamin Cravatt, Eranthie Weerapana, and Chu Wang, this modern version of ABPP termed isotopic tandem orthogonal proteolysis–ABPP (isoTOP-ABPP) enables the specific and quantitative mapping of proteome-wide sites of probe modifications (Weerapana et al. 2010). Reactivity-based probes consist of three features which enable this approach: (1) chemical probes that consist of an electrophilic warhead that can react with nucleophilic hotspots on proteins; (2) an alkyne handle for CuAAC conjugation of an enrichment handle for probe-labeled proteins and peptides; and (3) an azide-functionalized TEV protease recognition peptide linker bearing an isotopically light or heavy valine and a biotin group which can be appended onto probe-labeled proteins for subsequent avidin enrichment of probe-labeled proteins, digestion, isolation of probe-labeled tryptic peptides, and TEV release of probe-labeled peptides for subsequent quantitative proteomic analyses comparing isotopically light-to-heavy peptide ratios of probe-modified tryptic peptides. These probes react not only with catalytic sites within enzymes, but also solvent-accessible binding pockets, post-translational modification sites, cysteine oxidation sites, protein–protein interaction sites, and other types of regulatory or functional domains across the proteome. When coupled with isoTOP-ABPP to map reactivity of specific sites of probe modification, this overall approach enables a global method for mapping protein functionality and, more importantly, facilitates the identification of ligandable hotspots within protein targets that may have previously been undruggable (Weerapana et al. 2010).

When used in a competitive manner (competitive isoTOP-ABPP) (Fig. 5c), covalently acting small molecules, such as electrophilic natural products, can be competed against the binding of their corresponding reactivity-based probes to rapidly identify not only the targets, but also the specific sites of modifications, of these molecules (Weerapana et al. 2008; Wang et al. 2014; Backus et al. 2016; Hacker et al. 2017a). This isoTOP-ABPP approach has also evolved to employ chemically or UV-cleavable isotopic linkers, SILAC, multiplexed isotopic labeling strategies, isotopic incorporation into reactivity-based probes, and caged reactivity-based probes for in-cell use (Adibekian et al. 2011; Qian et al. 2013; Abo and Weerapana 2015; Abegg et al. 2015; Abo et al. 2017; Tian et al. 2017). There are multiple advantages of this method compared to the direct incorporation of analytical handles, conjugation to solid supports, or thermal stability assays. First, because the method involves not just the enrichment of proteins labeled with the broad reactivity-based probes, but also further enrichment of the specific probe-modified peptides, the proteome-wide coverage of probe-labeled sites is substantial. Second, this method allows for the use of the parent covalent natural product directly in vitro, in situ, or in vivo and does not require synthesis of

derivatives which may result in altered biological activity. Third and most importantly, this approach enables the identification of not only the direct target of the reactive compound, but also the specific amino acid druggable hotspot that is targeted by the natural product. There are also drawbacks to this approach though. First, this method is most amenable for covalently acting compounds as these entities are competed against other covalently acting reactivity-based probes. Second, this approach does not encompass all types of natural product reactivities. The chemical biology field has developed versatile reactivity-based probes that are compatible with proteomic profiling of probe-modified peptides for many amino acids, such as cysteines, lysines, serines, and methionines (Liu et al. 1999; Weerapana et al. 2008, 2010; Adibekian et al. 2011; Shannon et al. 2014; Lin et al. 2017; Ward et al. 2017; Hacker et al. 2017a). While there are many chemoselective bioconjugation reactions that have been reported and are being discovered for other amino acids, it is unclear whether these probes will be compatible with ABPP methods (Shannon and Weerapana 2015; Mix et al. 2016; deGruyter et al. 2017; Martín-Gago et al. 2017). Thus, if the covalently acting natural product (e.g., epoxomicin, fumagillin) reacts outside of the cysteine, lysine, serine, and methionine functional space, this approach currently does not work. Also, currently existing reactivity-based probes do not likely functionalize all possible ligandable sites within the amino acid scope of the probe and thus may still miss natural product targets. Third, there is no direct enrichment of natural product targets, so low abundance targets of natural products may be missed.

Nonetheless, ABPP platforms have been successfully used to identify targets and druggable hotspots targeted by many covalently acting natural products. The isoTOP-ABPP platform (pioneered by the Cravatt, Weerapana, and Wang groups) has been used to map druggable hotspots targeted by a multitude of reactive metabolites, drugs, drug candidates, and tool compounds (Weerapana et al. 2010; Banerjee et al. 2013; Wang et al. 2014; Shannon et al. 2014; Lewallen et al. 2015; Blewett et al. 2016; Backus et al. 2016; Zhou et al. 2016b; Chen et al. 2017, 2018; Hacker et al. 2017a; Bar-Peled et al. 2017). Focusing on natural products, the Cravatt group has also used ABPP platforms and the serine hydrolase-directed fluorophosphonate activity-based probe to profile the targets of β-lactone natural product tetrahydrolipstatin (THL) in mouse brain proteomes and showed that this compound inhibits multiple serine hydrolase enzymes, including Bat5, Abhd12, Pla2g7, and Tpp2 (Hoover et al. 2008). In another study, Porco et al. used ABPP platforms to discover that a novel rocaglate-derived β-lactone inhibits serine hydrolases ABHD10, ACOT1/2, CTSA, and SCPEP1 in prostate cancer cells (Lajkiewicz et al. 2014). Adibekian et al. adapted isoTOP-ABPP platforms using a cysteine-reactive alkynyl benziodoxolone probe and a UV-cleavable linker bearing a biotin enrichment handle to discover the proteome-wide targets of curcumin, an anticancer diarylheptanoid natural product and showed that curcumin covalently modifies several key players of cell signaling and metabolism, including casein kinase I gamma (Abegg et al. 2015). Yang et al. used a similar multiplexed iTRAQ-ABPP strategy to profile the disparate proteome-wide cysteine reactivity of seven exocyclic methylene lactone-containing natural products (Tian et al. 2017).

A recent elegant collaborative study led by Michael Fischbach's group showed that a family of non-ribosomal peptide synthetase gene clusters in gut bacteria encode for pyrazinone and dihydropyrazinone natural products. They showed in collaboration with the Cravatt group using isoTOP-ABPP platforms that the active form of these molecules is the initially released peptide aldehyde, which bears potent protease inhibitory activity and selectively targets the catalytic cysteines of a subset of human cathepsins (Guo et al. 2017). In another study, Nomura and colleagues used isoTOP-ABPP to map the proteome-wide cysteine reactivity of licochalcone A and found that this natural product impairs breast cancer cell viability through targeting C239 of prostaglandin reductase (Roberts et al. 2017).

3.4 Covalent Ligand Discovery Against Druggable Hotspots Targeted by Natural Products

The advantage of identifying the direct targets and druggable hotspots targeted by covalently acting anticancer natural products is that these targets can then be further pharmacologically interrogated with more synthetically accessible covalently acting chemical scaffolds for drug discovery efforts. Upon identifying the protein and druggable hotspot targeted by the covalently acting natural product that is responsible for its biological action, ABPP-based covalent ligand screening can potentially be used to pharmacologically interrogate this site in a target-based manner using moderate- to high-throughput screening approaches utilized by traditional target-based drug discovery efforts (Fig. 6a, b) (Bachovchin et al. 2009, 2010, 2011b). This method contrasts with having to perform medicinal chemistry efforts on natural product scaffolds that can be synthetically challenging, with readouts based on their bioactivity rather than affinity to specific protein targets. Cravatt and Backus showed the power of chemoproteomics-enabled covalent ligand discovery approaches in pharmacologically targeting classically undruggable or therapeutically intractable ligandable hotspots with cysteine-reactive covalent ligands.[47] This work has been followed up by Cravatt, Weerapana, Nomura, and many others showing that cysteine- and lysine-reactive covalent ligands can be used to target classically intractable targets for potential therapy (Shannon et al. 2014; Bateman et al. 2017; Hacker et al. 2017a, b; Anderson et al. 2017; Grossman et al. 2017).

In Cravatt and Backus's study, they identified covalent ligands against >700 cysteines found in both druggable and more intractable proteins, including transcription factors, adaptor/scaffolding proteins, and uncharacterized proteins. Among the many validated examples of covalent ligands targeting characterized and previously unrecognized functional cysteines, one particular unique example they demonstrated was a cysteine-reactive covalent ligand that targeted the catalytic cysteine C360 of caspase 8, only in its inactive zymogen form, but not its active form, wherein they used these compounds to distinguish extrinsic apoptosis pathways in human cell lines versus primary human T cells (Backus et al. 2016). In another study, Nomura and Bateman performed a phenotypic screen with

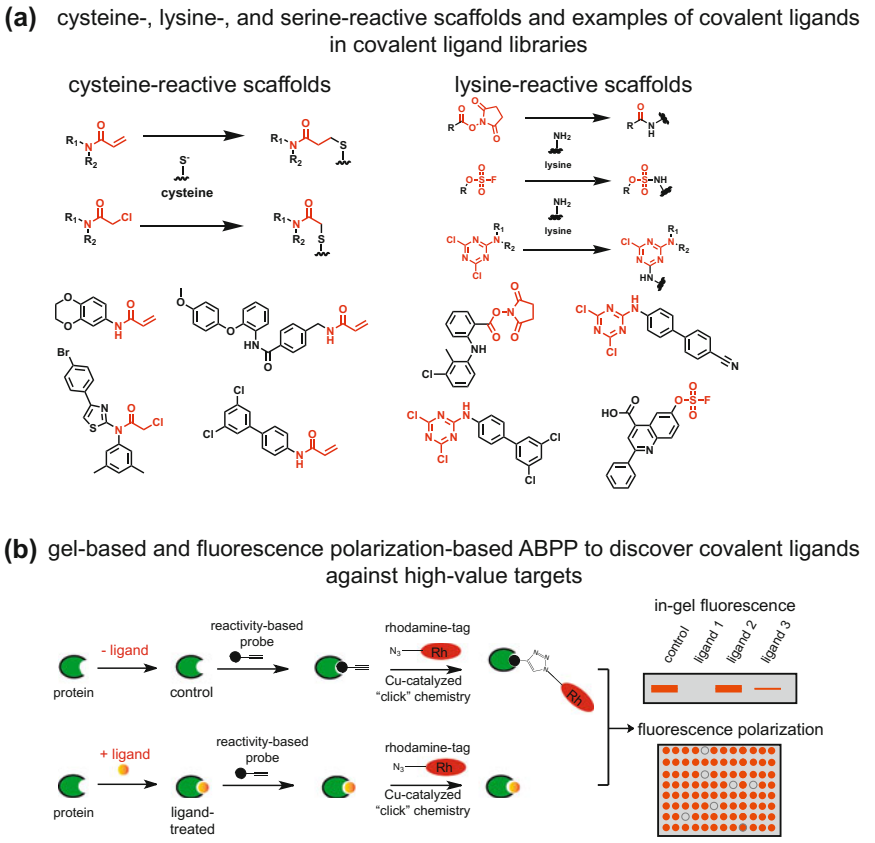


Fig. 6 Covalent ligand discovery against druggable hotspots targeted by covalently acting natural products. Upon identifying druggable hotspots targeted by covalently acting natural products using chemoproteomic platforms, such as isoTOP-ABPP, more synthetically tractable covalent ligand libraries can be competed against the natural product target using higher throughput ABPP approaches. **a** Shown are reactive scaffolds for covalent ligand libraries for cysteines and lysines. **b** Covalent ligands can be screened against targets using gel-based or fluorescence polarization-based ABPP assays in a target-based screening paradigm

cysteine-reactive covalent ligands to identify hits that impaired colorectal cancer cell viability. From this screen, they identified a cysteine-reactive covalent ligand that targeted C1101 of reticulon 4 (RTN4), a protein involved in endoplasmic reticulum (ER) membrane curvature and tubule formation. The authors showed that targeting this C1101 of RTN4 impaired ER tubular formation and nuclear morphology during cell division leading to impaired colorectal cancer pathogenicity (Bateman et al. 2017).

The feasibility of simpler covalent ligands that can react with druggable hotspots targeted by covalently acting natural products was shown by Nomura, Grossman, and Ward recently (Grossman et al. 2017). The authors used isoTOP-ABPP

platforms to map the proteome-wide cysteine reactivity of withaferin A and found that the primary target in breast cancer cells was an activating C377 on the protein phosphatase 2A (PP2A) regulatory subunit PPP2R1A, which led to the activation of PP2A activity, dephosphorylation and inactivation of AKT signaling, and impaired breast cancer pathogenicity (Grossman et al. 2017). They performed a parallel phenotypic screen with a simpler cysteine-reactive library and identified a simpler chloroacetamide that targeted this same C377 on PPP2R1A to recapitulate many of the properties observed with withaferin A. Further medicinal chemistry efforts led to the generation of JNS 1-40, a chloroacetamide that was in vitro, in situ, and in vivo selective for C377 on PPP2R1A, which activated PP2A activity, inactivated AKT signaling, and impaired breast cancer tumorigenesis in vivo (Grossman et al. 2017). While the simper covalent ligand identified in this study was fortuitously identified to modify the same site as withaferin A, this study shows that gel-based or fluorescence polarization-based moderate- to high-throughput competitive ABPP assays can likely be performed against natural product targets to rapidly screen for simpler ligands that bind to the same sites and recapitulate the actions of their more complex natural product counterparts (Fig. 6b).

4 Conclusions

In conclusion, there are countless reactive natural products that likely target unique druggable hotspots across a diversity of therapeutic targets. Moreover, many more are likely yet to be discovered, some of which may exploit completely novel reaction chemistry and mechanisms of action. The translational potential of these natural products into drugs has been hampered in part by difficulty of synthesis, isolation, mechanistic understanding, and speed of translation compared to target-based drug discovery efforts. At least with covalently acting natural products, chemoproteomic platforms are becoming increasingly powerful and robust to enable target identification in a reasonable time frame. Covalent ligand discovery against druggable hotspots targeted by reactive natural products using target-based ABPP screening strategies may help to overcome some of the other challenges faced with natural product translation by developing more synthetically tractable small molecule leads that are inspired, not necessarily by the natural product scaffold itself, but rather the target and druggable hotspot.

While this review focused on covalently acting natural products and covalent ligand discovery, these strategies can also be applied to reversibly acting natural products and chemoproteomics-enabled fragment screening using photoaffinity-labeling strategies. Recent studies by the Cravatt group showed how proteome-wide ligandable hotspots can be massively expanded through the use of quantitative chemoproteomic platforms and “fully functionalized” fragment probes bearing a variable small-molecule fragment conjugated to a constant tag bearing an alkyne and photoactivable diazirine group (Parker et al. 2017). These types of strategies may be coupled with traditional photoaffinity-labeling-based natural product target

identification approaches to discover more synthetically tractable compounds that target druggable sites targeted by reversibly acting natural products. The future of this research area will no doubt greatly benefit small-molecule drug discovery.

References

Abegg D, Frei R, Cerato L et al (2015) Proteome-Wide Profiling of Targets of Cysteine reactive Small Molecules by using Ethynyl Benziodoxolone Reagents. Angew Chem Int Ed Engl 54:10852–10857. https://doi.org/10.1002/anie.201505641

Abo M, Bak DW, Weerapana E (2017) Optimization of caged electrophiles for improved monitoring of cysteine reactivity in living cells. ChemBioChem 18:81–84. https://doi.org/10.1002/cbic.201600524

Abo M, Weerapana E (2015) A Caged Electrophilic Probe for Global Analysis of Cysteine Reactivity in Living Cells. J Am Chem Soc 137:7087–7090. https://doi.org/10.1021/jacs.5b04350

Adibekian A, Martin BR, Wang C et al (2011) Click-generated triazole ureas as ultrapotent in vivo-active serine hydrolase inhibitors. Nat Chem Biol 7:469–478. https://doi.org/10.1038/nchembio.579

Ahn K, Johnson DS, Mileni M et al (2009) Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. Chem Biol 16:411–420. https://doi.org/10.1016/j.chembiol.2009.02.013

Ahn K, Smith SE, Liimatta MB et al (2011) Mechanistic and pharmacological characterization of PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces inflammatory and noninflammatory pain. J Pharmacol Exp Ther 338:114–124. https://doi.org/10.1124/jpet.111.180257

Anderson KE, To M, Olzmann JA, Nomura DK (2017) Chemoproteomics-Enabled Covalent Ligand Screening Reveals a Thioredoxin-Caspase 3 Interaction Disruptor That Impairs Breast Cancer Pathogenicity. ACS Chem Biol 12:2522–2528. https://doi.org/10.1021/acschembio.7b00711

Asai A, Hara M, Kakita S et al (1996) Thiol-Mediated DNA Alkylation by the Novel Antitumor Antibiotic Leinamycin. J Am Chem Soc 118:6802–6803. https://doi.org/10.1021/ja960892w

Aune GJ, Furuta T, Pommier Y (2002) Ecteinascidin 743: a novel anticancer drug with a unique mechanism of action. Anticancer Drugs 13:545–555

Bachovchin DA, Brown SJ, Rosen H, Cravatt BF (2009) Identification of selective inhibitors of uncharacterized enzymes by high-throughput screening with fluorescent activity-based probes. Nat Biotechnol 27:387–394. https://doi.org/10.1038/nbt.1531

Bachovchin DA, Ji T, Li W et al (2010) Superfamily-wide portrait of serine hydrolase inhibition achieved by library-versus-library screening. Proc Natl Acad Sci U S A 107:20941–20946. https://doi.org/10.1073/pnas.1011663107

Bachovchin DA, Mohr JT, Speers AE et al (2011a) Academic cross-fertilization by public screening yields a remarkable class of protein phosphatase methylesterase-1 inhibitors. Proc Natl Acad Sci U S A 108:6811–6816. https://doi.org/10.1073/pnas.1015248108

Bachovchin DA, Mohr JT, Speers AE et al (2011b) Academic cross-fertilization by public screening yields a remarkable class of protein phosphatase methylesterase-1 inhibitors. Proc Natl Acad Sci U S A 108:6811–6816. https://doi.org/10.1073/pnas.1015248108

Backus KM, Correia BE, Lum KM et al (2016) Proteome-wide covalent ligand discovery in native biological systems. Nature 534:570–574. https://doi.org/10.1038/nature18002

Bai W-J, David JG, Feng Z-G et al (2014) The domestication of ortho-quinone methides. Acc Chem Res 47:3655–3664. https://doi.org/10.1021/ar500330x

Baillie TA (2016) Targeted Covalent Inhibitors for Drug Design. Angew Chem Int Ed Engl 55:13408–13421. https://doi.org/10.1002/anie.201601091

Banerjee R, Pace NJ, Brown DR, Weerapana E (2013) 1, 3, 5-Triazine as a modular scaffold for covalent inhibitors with streamlined target identification. J Am Chem Soc 135:2497–2500. https://doi.org/10.1021/ja400427e

Bargagna-Mohan P, Hamza A, Kim Y et al (2007) The tumor inhibitor and antiangiogenic agent withaferin A targets the intermediate filament protein vimentin. Chem Biol 14:623–634. https://doi.org/10.1016/j.chembiol.2007.04.010

Bar-Peled L, Kemper EK, Suciu RM et al (2017) Chemical Proteomics Identifies Druggable Vulnerabilities in a Genetically Defined Cancer. Cell 171(696–709):e23. https://doi.org/10.1016/j.cell.2017.08.051

Bateman LA, Nguyen TB, Roberts AM et al (2017) Chemoproteomics-enabled covalent ligand screen reveals a cysteine hotspot in reticulon 4 that impairs ER morphology and cancer pathogenicity. Chem Commun Camb Engl 53:7234–7237. https://doi.org/10.1039/c7cc01480e

Battenberg OA, Yang Y, Verhelst SHL, Sieber SA (2013) Target profiling of 4-hydroxyderricin in S. aureus reveals seryl-tRNA synthetase binding and inhibition by covalent modification. Mol BioSyst 9:343–351. https://doi.org/10.1039/c2mb25446h

Bauer RA (2015) Covalent inhibitors in drug discovery: from accidental discoveries to avoided liabilities and designed therapies. Drug Discov Today 20:1061–1073. https://doi.org/10.1016/j.drudis.2015.05.005

Beck P, Heinemeyer W, Späth A-L et al (2014) Interactions of the natural product kendomycin and the 20S proteasome. J Mol Biol 426:3108–3117. https://doi.org/10.1016/j.jmb.2014.06.019

Beutler JA (2009) Natural Products as a Foundation for Drug Discovery. Curr Protoc Pharmacol Editor Board SJ Enna Ed–Chief Al 46:9.11.1–9.11.21. https://doi.org/10.1002/0471141755.ph0911s46

Bialecka-Florjanczyk E, Fabiszewska AU, Krzyczkowska J, Kurylowicz A (2018) Synthetic and Natural Lipase Inhibitors. Mini Rev Med Chem 18:672–683. https://doi.org/10.2174/1389557516666160630123356

Blewett MM, Xie J, Zaro BW et al (2016) Chemical proteomic map of dimethyl fumarate-sensitive cysteines in primary human T cells. Sci Signal 9:rs10. https://doi.org/10.1126/scisignal.aaf7694

Boger DL, Johnson DS, Yun W, Tarby CM (1994) Molecular basis for sequence selective DNA alkylation by (+)- and ent-(-)-CC-1065 and related agents: alkylation site models that accommodate the offset AT-rich adenine N3 alkylation selectivity. Bioorg Med Chem 2:115–135

Böttcher T, Sieber SA (2010) Showdomycin as a versatile chemical tool for the detection of pathogenesis-associated enzymes in bacteria. J Am Chem Soc 132:6964–6972. https://doi.org/10.1021/ja909150y

Böttcher T, Sieber SA (2012) β-Lactams and β-lactones as activity-based probes in chemical biology. MedChemComm 3:408–417. https://doi.org/10.1039/c2md00275b

Chang J, Kim Y, Kwon HJ (2016) Advances in identification and validation of protein targets of natural products without chemical modification. Nat Prod Rep 33:719–730. https://doi.org/10.1039/C5NP00107B

Chen Y-C, Backus KM, Merkulova M et al (2017) Covalent Modulators of the Vacuolar ATPase. J Am Chem Soc 139:639–642. https://doi.org/10.1021/jacs.6b12511

Chen Y, Liu Y, Lan T et al (2018) Quantitative Profiling of Protein Carbonylations in Ferroptosis by an Aniline-Derived Probe. J Am Chem Soc 140:4712–4720. https://doi.org/10.1021/jacs.8b01462

Chidley C, Trauger SA, Birsoy K, O'Shea EK (2016) The anticancer natural product ophiobolin A induces cytotoxicity by covalent modification of phosphatidylethanolamine. eLife 5. https://doi.org/10.7554/elife.14601

Crane EA, Gademann K (2016) Capturing Biological Activity in Natural Product Fragments by Chemical Synthesis. Angew Chem Int Ed Engl 55:3882–3902. https://doi.org/10.1002/anie.201505863

De Cesco S, Kurian J, Dufresne C et al (2017) Covalent inhibitors design and discovery. Eur J Med Chem 138:96–114. https://doi.org/10.1016/j.ejmech.2017.06.019

deGruyter JN, Malins LR, Baran PS (2017) Residue-Specific Peptide Modification: a chemist's guide. Biochemistry (Mosc) 56:3863–3873. https://doi.org/10.1021/acs.biochem.7b00536

Dong T, Li C, Wang X et al (2015) Ainsliadimer A selectively inhibits IKKα/β by covalently binding a conserved cysteine. Nat Commun 6:6522. https://doi.org/10.1038/ncomms7522

Drahl C, Cravatt BF, Sorensen EJ (2005) Protein-reactive natural products. Angew Chem Int Ed Engl 44:5788–5809. https://doi.org/10.1002/anie.200500900

Evans MJ, Saghatelian A, Sorensen EJ, Cravatt BF (2005) Target discovery in small-molecule cell-based screens by in situ proteome reactivity profiling. Nat Biotechnol 23:1303–1307. https://doi.org/10.1038/nbt1149

Gersch M, Kreuzer J, Sieber SA (2012) Electrophilic natural products and their biological targets. Nat Prod Rep 29:659–682. https://doi.org/10.1039/c2np20012k

Griffith EC, Su Z, Niwayama S et al (1998) Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2. Proc Natl Acad Sci U S A 95:15183–15188

Groll M, Kim KB, Kairies N et al (2000) Crystal Structure of Epoxomicin:20S Proteasome Reveals a Molecular Basis for Selectivity of α′, β′-Epoxyketone Proteasome Inhibitors. J Am Chem Soc 122:1237–1238. https://doi.org/10.1021/ja993588m

Grossman EA, Ward CC, Spradlin JN et al (2017) Covalent Ligand Discovery against Druggable Hotspots Targeted by Anti-cancer Natural Products. Cell Chem Biol 24(1368–1376):e4. https://doi.org/10.1016/j.chembiol.2017.08.013

Gulder TAM, Moore BS (2010) Salinosporamide natural products: Potent 20 S proteasome inhibitors as promising cancer chemotherapeutics. Angew Chem Int Ed Engl 49:9346–9367. https://doi.org/10.1002/anie.201000728

Guo C-J, Chang F-Y, Wyche TP et al (2017) Discovery of Reactive Microbiota-Derived Metabolites that Inhibit Host Proteases. Cell 168(517–526):e18. https://doi.org/10.1016/j.cell.2016.12.021

Hacker SM, Backus KM, Lazear MR et al (2017a) Global profiling of lysine reactivity and ligandability in the human proteome. Nat Chem advance online publication. https://doi.org/10.1038/nchem.2826

Hacker SM, Backus KM, Lazear MR et al (2017b) Global profiling of lysine reactivity and ligandability in the human proteome. Nat Chem 9:1181–1190. https://doi.org/10.1038/nchem.2826

Harvey AL, Edrada-Ebel R, Quinn RJ (2015) The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 14:111–129. https://doi.org/10.1038/nrd4510

He Q-L, Titov DV, Li J et al (2015) Covalent modification of a cysteine residue in the XPB subunit of the general transcription factor TFIIH through single epoxide cleavage of the transcription inhibitor triptolide. Angew Chem Int Ed Engl 54:1859–1863. https://doi.org/10.1002/anie.201408817

Heeres JT, Hergenrother PJ (2011) High-throughput screening for modulators of protein-protein interactions: use of photonic crystal biosensors and complementary technologies. Chem Soc Rev 40:4398–4410. https://doi.org/10.1039/b923660k

Hoover HS, Blankman JL, Niessen S, Cravatt BF (2008) Selectivity of inhibitors of endocannabinoid biosynthesis evaluated by activity-based protein profiling. Bioorg Med Chem Lett 18:5838–5841. https://doi.org/10.1016/j.bmcl.2008.06.091

Jackson PA, Widen JC, Harki DA, Brummond KM (2017) Covalent Modifiers: A Chemical Perspective on the Reactivity of α,β-Unsaturated Carbonyls with Thiols via Hetero-Michael Addition Reactions. J Med Chem 60:839–885. https://doi.org/10.1021/acs.jmedchem.6b00788

Kalesh KA, Clulow JA, Tate EW (2015) Target profiling of zerumbone using a novel cell-permeable clickable probe and quantitative chemical proteomics. Chem Commun Camb Engl 51:5497–5500. https://doi.org/10.1039/c4cc09527h

Kanoh N (2016) Photo-cross-linked small-molecule affinity matrix as a tool for target identification of bioactive small molecules. Nat Prod Rep 33:709–718. https://doi.org/10.1039/C5NP00117J

Kim KB, Crews CM (2013) From epoxomicin to carfilzomib: chemistry, biology, and medical outcomes. Nat Prod Rep 30:600–604. https://doi.org/10.1039/c3np20126k

Kingston DGI (2011) Modern Natural Products Drug Discovery and its Relevance to Biodiversity Conservation. J Nat Prod 74:496–511. https://doi.org/10.1021/np100550t

Kitson RRA, Millemaggi A, Taylor RJK (2009) The renaissance of alpha-methylene-gamma-butyrolactones: new synthetic approaches. Angew Chem Int Ed Engl 48:9426–9451. https://doi.org/10.1002/anie.200903108

Klaić L, Morimoto RI, Silverman RB (2012) Celastrol analogues as inducers of the heat shock response. Design and synthesis of affinity probes for the identification of protein targets. ACS Chem Biol 7:928–937. https://doi.org/10.1021/cb200539u

Knoth T, Warburg K, Katzka C et al (2009) The Ras Pathway Modulator Melophlin A Targets Dynamins. Angew Chem Int Ed 48:7240–7245. https://doi.org/10.1002/anie.200902023

Koehn FE, Carter GT (2005) The evolving role of natural products in drug discovery. Nat Rev Drug Discov 4:206–220. https://doi.org/10.1038/nrd1657

Kornienko A, La Clair JJ (2017) Covalent modification of biological targets with natural products through Paal-Knorr pyrrole formation. Nat Prod Rep 34:1051–1060. https://doi.org/10.1039/c7np00024c

Kwan JC, Luesch H (2010) Weapons in disguise–activating mechanisms and protecting group chemistry in nature. Chem Weinh Bergstr Ger 16:13020–13029. https://doi.org/10.1002/chem.201001562

Kwok BH, Koh B, Ndubuisi MI et al (2001) The anti-inflammatory natural product parthenolide from the medicinal herb Feverfew directly binds to and inhibits IkappaB kinase. Chem Biol 8:759–766

Lagoutte R, Patouret R, Winssinger N (2017) Covalent inhibitors: an opportunity for rational target selectivity. Curr Opin Chem Biol 39:54–63. https://doi.org/10.1016/j.cbpa.2017.05.008

Lagoutte R, Winssinger N (2017) Following the Lead from Nature with Covalent Inhibitors. Chimia 71:703–711. https://doi.org/10.2533/chimia.2017.703

Lajkiewicz NJ, Cognetta AB, Niphakis MJ et al (2014) Remodeling natural products: chemistry and serine hydrolase activity of a rocaglate-derived β-lactone. J Am Chem Soc 136:2659–2664. https://doi.org/10.1021/ja412431g

Lanning BR, Whitby LR, Dix MM et al (2014) A road map to evaluate the proteome-wide selectivity of covalent kinase inhibitors. Nat Chem Biol 10:760–767. https://doi.org/10.1038/nchembio.1582

Laraia L, Robke L, Waldmann H (2018) Bioactive compound collections: from design to target identification. Chem 4:705–730. https://doi.org/10.1016/j.chempr.2018.01.012

Leslie BJ, Hergenrother PJ (2008) Identification of the cellular targets of bioactive small organic molecules using affinity reagents. Chem Soc Rev 37:1347–1360. https://doi.org/10.1039/b702942j

Leuenroth SJ, Crews CM (2005) Studies on calcium dependence reveal multiple modes of action for triptolide. Chem Biol 12:1259–1268. https://doi.org/10.1016/j.chembiol.2005.09.009

Leung PC, Graves LM, Tipton CL (1988) Characterization of the interaction of ophiobolin A and calmodulin. Int J Biochem 20:1351–1359

Lewallen DM, Bicker KL, Subramanian V et al (2015) Chemical proteomic platform to identify citrullinated proteins. ACS Chem Biol 10:2520–2528. https://doi.org/10.1021/acschembio.5b00438

Li C, Dong T, Li Q, Lei X (2014) Probing the anticancer mechanism of (-)-ainsliatrimer A through diverted total synthesis and bioorthogonal ligation. Angew Chem Int Ed Engl 53:12111–12115. https://doi.org/10.1002/anie.201407225

Lin S, Yang X, Jia S et al (2017) Redox-based reagents for chemoselective methionine bioconjugation. Science 355:597–602. https://doi.org/10.1126/science.aal3316

Liu J, Farmer JD, Lane WS et al (1991) Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell 66:807–815

Liu Y, Patricelli MP, Cravatt BF (1999) Activity-based protein profiling: the serine hydrolases. Proc Natl Acad Sci U S A 96:14694–14699

Malvezzi S, Farnung L, Aloisi CMN et al (2017) Mechanism of RNA polymerase II stalling by DNA alkylation. Proc Natl Acad Sci U S A 114:12172–12177. https://doi.org/10.1073/pnas.1706592114

Martín-Gago P, Fansa EK, Winzker M et al (2017) Covalent Protein Labeling at Glutamic Acids. Cell Chem Biol 24(589–597):e5. https://doi.org/10.1016/j.chembiol.2017.03.015

McAlister GC, Nusinow DP, Jedrychowski MP et al (2014) MultiNotch MS3 enables accurate, sensitive, and multiplexed detection of differential expression across cancer cell line proteomes. Anal Chem 86:7150–7158. https://doi.org/10.1021/ac502040v

Meng L, Mohan R, Kwok BHB et al (1999) Epoxomicin, a potent and selective proteasome inhibitor, exhibits in vivo antiinflammatory activity. Proc Natl Acad Sci 96:10403–10408. https://doi.org/10.1073/pnas.96.18.10403

Meng C, Zhu H, Song H et al (2014) Targets and molecular mechanisms of triptolide in cancer therapy. Chin J Cancer Res 26:622–626. https://doi.org/10.3978/j.issn.1000-9604.2014.09.01

Mix KA, Aronoff MR, Raines RT (2016) Diazo compounds: versatile tools for chemical biology. ACS Chem Biol 11:3233–3244. https://doi.org/10.1021/acschembio.6b00810

Moore HW (1977) Bioactivation as a model for drug design bioreductive alkylation. Science 197:527–532

Myers AG, Herzon SB (2003) Identification of a novel Michael acceptor group for the reversible addition of oxygen- and sulfur-based nucleophiles. Synthesis and reactivity of the 3-alkylidene-3H-indole 1-oxide function of avrainvillamide. J Am Chem Soc 125:12080–12081. https://doi.org/10.1021/ja0372006

Niessen S, Dix MM, Barbas S et al (2017) Proteome-wide Map of Targets of T790M-EGFR-Directed Covalent Inhibitors. Cell Chem Biol 24(1388–1400):e7. https://doi.org/10.1016/j.chembiol.2017.08.017

Nodwell MB, Menz H, Kirsch SF, Sieber SA (2012) Rugulactone and its analogues exert antibacterial effects through multiple mechanisms including inhibition of thiamine biosynthesis. Chembiochem Eur J Chem Biol 13:1439–1446. https://doi.org/10.1002/cbic.201200265

Ong S-E, Blagoev B, Kratchmarova I et al (2002) Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics MCP 1:376–386

Pan S, Zhang H, Wang C et al (2016) Target identification of natural products and bioactive compounds using affinity-based probes. Nat Prod Rep 33:612–620. https://doi.org/10.1039/C5NP00101C

Parker CG, Galmozzi A, Wang Y et al (2017) Ligand and target discovery by fragment-based screening in human cells. Cell 168(527–541):e29. https://doi.org/10.1016/j.cell.2016.12.029

Pettinger J, Jones K, Cheeseman MD (2017) Lysine-targeting covalent inhibitors. Angew Chem Int Ed Engl 56:15200–15209. https://doi.org/10.1002/anie.201707630

Potashman MH, Duggan ME (2009) Covalent modifiers: an orthogonal approach to drug design. J Med Chem 52:1231–1246. https://doi.org/10.1021/jm8008597

Potts BC, Albitar MX, Anderson KC et al (2011) Marizomib, a proteasome inhibitor for all seasons: preclinical profile and a framework for clinical trials. Curr Cancer Drug Targets 11:254–284

Qian Y, Martell J, Pace NJ et al (2013) An isotopically tagged azobenzene-based cleavable linker for quantitative proteomics. Chembiochem Eur J Chem Biol 14:1410–1414. https://doi.org/10.1002/cbic.201300396

Roberts LS, Yan P, Bateman LA, Nomura DK (2017) Mapping novel metabolic nodes targeted by anti-cancer drugs that impair triple-negative breast cancer pathogenicity. ACS Chem Biol 12:1133–1140. https://doi.org/10.1021/acschembio.6b01159

Romo D, Liu JO (2016) Editorial: Strategies for cellular target identification of natural products. Nat Prod Rep 33:592–594. https://doi.org/10.1039/C6NP90016J

Rostovtsev VV, Green LG, Fokin VV, Sharpless KB (2002) A stepwise huisgen cycloaddition process: copper(I)-catalyzed regioselective “ligation” of azides and terminal alkynes. Angew

Chem Int Ed Engl 41:2596–2599. https://doi.org/10.1002/1521-3773(20020715)41:14%3c2596:AID-ANIE2596%3e3.0.CO;2-4
Saxton RA, Sabatini DM (2017) mTOR Signaling in Growth, Metabolism, and Disease. Cell 168:960–976. https://doi.org/10.1016/j.cell.2017.02.004
Shannon DA, Banerjee R, Webster ER et al (2014) Investigating the proteome reactivity and selectivity of aryl halides. J Am Chem Soc 136:3330–3333. https://doi.org/10.1021/ja4116204
Shannon DA, Weerapana E (2015) Covalent protein modification: the current landscape of residue-specific electrophiles. Curr Opin Chem Biol 24:18–26. https://doi.org/10.1016/j.cbpa.2014.10.021
Sin N, Kim KB, Elofsson M et al (1999) Total synthesis of the potent proteasome inhibitor epoxomicin: a useful tool for understanding proteasome biology. Bioorg Med Chem Lett 9:2283–2288
Sin N, Meng L, Wang MQW et al (1997) The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. Proc Natl Acad Sci U S A 94:6099–6103
Staub I, Sieber SA (2008) Beta-lactams as selective chemical probes for the in vivo labeling of bacterial enzymes involved in cell wall biosynthesis, antibiotic resistance, and virulence. J Am Chem Soc 130:13400–13409. https://doi.org/10.1021/ja803349j
Staub I, Sieber SA (2009) Beta-lactam probes as selective chemical-proteomic tools for the identification and functional characterization of resistance associated enzymes in MRSA. J Am Chem Soc 131:6271–6276. https://doi.org/10.1021/ja901304n
Strelow JM (2017) A Perspective on the Kinetics of Covalent and Irreversible Inhibition. SLAS Discov Adv Life Sci R D 22:3–20. https://doi.org/10.1177/1087057116671509
Swinney DC (2009) The role of binding kinetics in therapeutically useful drug action. Curr Opin Drug Discov Devel 12:31–39
Tanasova M, Sturla SJ (2012) Chemistry and biology of acylfulvenes: sesquiterpene-derived antitumor agents. Chem Rev 112:3578–3610. https://doi.org/10.1021/cr2001367
Thompson A, Schäfer J, Kuhn K et al (2003) Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. Anal Chem 75:1895–1904
Tian C, Sun R, Liu K et al (2017) Multiplexed Thiol Reactivity Profiling for Target Discovery of Electrophilic Natural Products. Cell Chem Biol 24(1416–1427):e5. https://doi.org/10.1016/j.chembiol.2017.08.022
Titov DV, Gilman B, He Q-L et al (2011) XPB, a subunit of TFIIH, is a target of the natural product triptolide. Nat Chem Biol 7:182–188. https://doi.org/10.1038/nchembio.522
Tornøe CW, Christensen C, Meldal M (2002) Peptidotriazoles on solid phase: [1, 2, 3]-triazoles by regiospecific copper(i)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. J Org Chem 67:3057–3064
Ursu A, Waldmann H (2015) Hide and seek: Identification and confirmation of small molecule protein targets. Bioorg Med Chem Lett 25:3079–3086. https://doi.org/10.1016/j.bmcl.2015.06.023
Vizcaino MI, Crawford JM (2015) The colibactin warhead crosslinks DNA. Nat Chem 7:411–417. https://doi.org/10.1038/nchem.2221
Wang C, Weerapana E, Blewett MM, Cravatt BF (2014) A chemoproteomic platform to quantitatively map targets of lipid-derived electrophiles. Nat Methods 11:79–85. https://doi.org/10.1038/nmeth.2759
Ward CC, Kleinman JI, Nomura DK (2017) NHS-Esters as Versatile Reactivity-Based Probes for Mapping Proteome-Wide Ligandable Hotspots. ACS Chem Biol 12:1478–1483. https://doi.org/10.1021/acschembio.7b00125
Weerapana E, Simon GM, Cravatt BF (2008) Disparate proteome reactivity profiles of carbon electrophiles. Nat Chem Biol 4:405–407. https://doi.org/10.1038/nchembio.91
Weerapana E, Wang C, Simon GM et al (2010) Quantitative reactivity profiling predicts functional cysteines in proteomes. Nature 468:790–795. https://doi.org/10.1038/nature09472

Whitby LR, Obach RS, Simon GM et al (2017) Quantitative Chemical Proteomic Profiling of the in Vivo Targets of Reactive Drug Metabolites. ACS Chem Biol 12:2040–2050. https://doi.org/10.1021/acschembio.7b00346

Widen JC, Kempema AM, Villalta PW, Harki DA (2017) Targeting NF-κB p65 with a Helenalin Inspired Bis-electrophile. ACS Chem Biol 12:102–113. https://doi.org/10.1021/acschembio.6b00751

Wirth T, Schmuck K, Tietze LF, Sieber SA (2012) Duocarmycin analogues target aldehyde dehydrogenase 1 in lung cancer cells. Angew Chem Int Ed Engl 51:2874–2877. https://doi.org/10.1002/anie.201106334

Wright MH, Sieber SA (2016) Chemical proteomics approaches for identifying the cellular targets of natural products. Nat Prod Rep 33:681–708. https://doi.org/10.1039/c6np00001k

Wright MH, Tao Y, Drechsel J et al (2017) Quantitative chemoproteomic profiling reveals multiple target interactions of spongiolactone derivatives in leukemia cells. Chem Commun Camb Engl 53:12818–12821. https://doi.org/10.1039/c7cc04990k

Wulff JE, Siegrist R, Myers AG (2007) The natural product avrainvillamide binds to the oncoprotein nucleophosmin. J Am Chem Soc 129:14444–14451. https://doi.org/10.1021/ja075327f

Zeiler E, Braun N, Böttcher T et al (2011) Vibralactone as a tool to study the activity and structure of the ClpP1P2 complex from Listeria monocytogenes. Angew Chem Int Ed Engl 50:11001–11004. https://doi.org/10.1002/anie.201104391

Zhou Y, Li W, Wang M et al (2016a) Competitive profiling of celastrol targets in human cervical cancer HeLa cells via quantitative chemical proteomics. Mol BioSyst 13:83–91. https://doi.org/10.1039/c6mb00691d

Zhou Y, Wynia-Smith SL, Couvertier SM et al (2016b) Chemoproteomic Strategy to Quantitatively Monitor Transnitrosation Uncovers Functionally Relevant S-Nitrosation Sites on Cathepsin D and HADH2. Cell Chem Biol 23:727–737. https://doi.org/10.1016/j.chembiol.2016.05.008