REVIEW

ACS chemical biology

Natural Products as Chemical Probes

Erin E. Carlson*

Departments of Chemistry and Molecular and Cellular Biochemistry, Indiana University, 212 S. Hawthorne Drive, Bloomington, Indiana 47405

ABSTRACT Natural products have evolved to encompass a broad spectrum of chemical and functional diversity. It is this diversity, along with their structural complexity, that enables nature's small molecules to target a nearly limitless number of biological macromolecules and to often do so in a highly selective fashion. Because of these characteristics, natural products have seen great success as therapeutic agents. However, this vast pool of compounds holds much promise beyond the development of future drugs. These features also make them ideal tools for the study of biological systems. Recent examples of the use of natural products and their derivatives as chemical probes to explore biological phenomena and assemble biochemical pathways are presented here.

Since the dawn of medicine, compounds derived from animals, plants, and microbes have been used as therapeutic agents. Prior to the 1800s, the active constituents of most medicines, which were generally plant-based, were unknown. Isolation of the well-known drug, morphine, from the opium poppy occurred in 1817 (*1, 2*). Commercialization of the antibiotic penicillin in the 1940s was arguably the most significant milestone in drug discovery research, as it spurred an enormous amount of effort in this field. As a result, about 40% of today's therapeutic agents are derived from biological sources (*3–5*). These compounds can be derived directly, by use of semisynthetic natural product analogs, or indirectly, through the use of synthetic compounds based upon natural product pharmacophores. Interest in natural product-based drug discovery experienced a decline in the 1980s and 1990s due in large part to the advent of combinatorial chemistry coupled with high-throughput screening methods. The accompanying marked decline in the number of new drug candidates, however, has rekindled interest in expanding natural product-based efforts (*6*).

Natural products have long been recognized as privileged scaffolds because they have evolved specifically to interact with biological macromolecules, especially proteins (*7, 8*). As evidence of this fact, natural product-based screening libraries routinely yield higher hit rates than synthetically produced small molecule libraries (*9*). The exquisite selectivity of many natural product–protein binding events often results from a polyvalent network of noncovalent interactions (*7*). A proportionally smaller, but equally important group of natural products also bind to their targets through a covalent interaction (*7, 10*). Namely, they utilize a highly reactive functional group, such as an epoxide or a lactone/lactam, to covalently modify their target protein(s), the most famous example being the antibiotic penicillin.

***Corresponding author, carlsone@indiana.edu.**

Received for review April 21, 2010 and accepted May 28, 2010.

Published online May 28, 2010

10.1021/cb100105c

Although the therapeutic value of natural products is clear, use of these structurally complex compounds presents a number of challenges (*7*). First, accessing sufficient quantities of a given natural product using either isolation strategies or (bio)synthetic methods is often difficult. Thus, full characterization of both the biological and chemical properties of lead compounds can be problematic. However, this complexity, in combination with the success of natural products in the development of therapeutic agents, has long attracted the attention of synthetic chemists. Accordingly, many elegant strategies to generate a growing number of natural products have been reported. In addition, biosynthetic and semisynthesis techniques play a critical role in providing an adequate supply of many natural products. A comprehensive review of these areas cannot be presented here, but several recent reviews are provided (*11–14*).

As with most drug candidates, deconvolution of the molecular target of a natural product is one of the greatest challenges. The process is often time-consuming and labor intensive. Additionally, natural product–protein interactions that occur with low affinity and/or specificity can complicate interpretation of experimental results (*15, 16*). Recent advances in proteomics, three-hybrid systems, phage and mRNA display technologies, chemical synthesis, and affinity chromatography-based methods have greatly accelerated target identification (*16–20*). Most of these methods require derivatization of the lead compound to facilitate either covalent interactions between the natural product and the target biomolecule(s) or conjugation of the natural product to a read-out tag, such as biotin or a fluorophore. Ideally, a natural product would be functionalized at a discrete position to produce a derivative with an activity profile that is comparable to the parent structure. In reality, this process often requires either total synthesis or semisynthesis of lead structures to enable installation of a chemical handle. In addition, the functionalized natural products can suffer from a dramatic decrease in the desired bioactivity. Researchers are working to address this barrier by development of chemo- and/or regioselective functionalizations (*21–23*). Such transformations facilitate the direct functionalization of natural products and eliminate the need for development of lengthy syntheses to access each analog of interest.

Largely due to the aforementioned challenges, the exploration of natural products as therapeutics has seen variable interest. However, many believe that compounds devised by nature are often far superior to even the best synthetic moieties in terms of diversity, specificity, binding efficiency, and propensity to interact with biological targets (*7, 8*). It is these same characteristics that have piqued the interest of many researchers to the possibility of utilizing natural products in studies that go beyond the identification of potential therapeutic agents. That is, to use them as chemical probes: small molecules that facilitate exploration of a biological system (*24*).

A BRIEF HISTORY OF NATURAL PRODUCTS AS CHEMICAL PROBES

One of the first studies to utilize a natural product-based chemical probe was published in 1996 by Schreiber and co-workers (*25, 26*). At the outset of their work, it was known that trapoxin (Figure 1, panel a), a cyclotetrapeptide isolated from the fungus *Helicoma ambiens* (*27*), caused cell cycle arrest in mammalian cells and inhibited histone deacetylation. It was not clear, however, what the molecular target of this natural product was or how the state of acetylation of the histones might be related to the observed phenotype. The authors hypothesized that the electrophilic epoxyketone moiety may be important for inhibition as it could participate in a covalent interaction with the protein target, particularly given the similarity of this fragment to the endogenous substrate, *N*-acetyl lysine (Figure 1, panel a). They took advantage of this electrophilic center by developing a trapoxin-based affinity resin, known as K-trap, to facilitate the enrichment and identification of the protein target (*25*). Using this resin, they detected a protein possessing histone deacetylase activity that was 60% identical to Rpd3, a transcriptional repressor found in yeast (*26*). Rpd3 was not known to have histone deacetylase activity. Thus, these studies brought to light the relationship between histone deacetylases (HDACs) and transcriptional regulation and cell cycle progression. Ultimately, this knowledge led to the development of HDAC inhibitors for cancer treatment.

Another example of a natural product that has changed our understanding of biology is rapamycin, a macrolide produced by *Streptomyces hygroscopicus*. Rapamycin and its derivatives have played important roles in the clarification of several cellular processes including cell growth, proliferation, and survival as well as protein synthesis and transcription (*28, 29*). Rapamy-

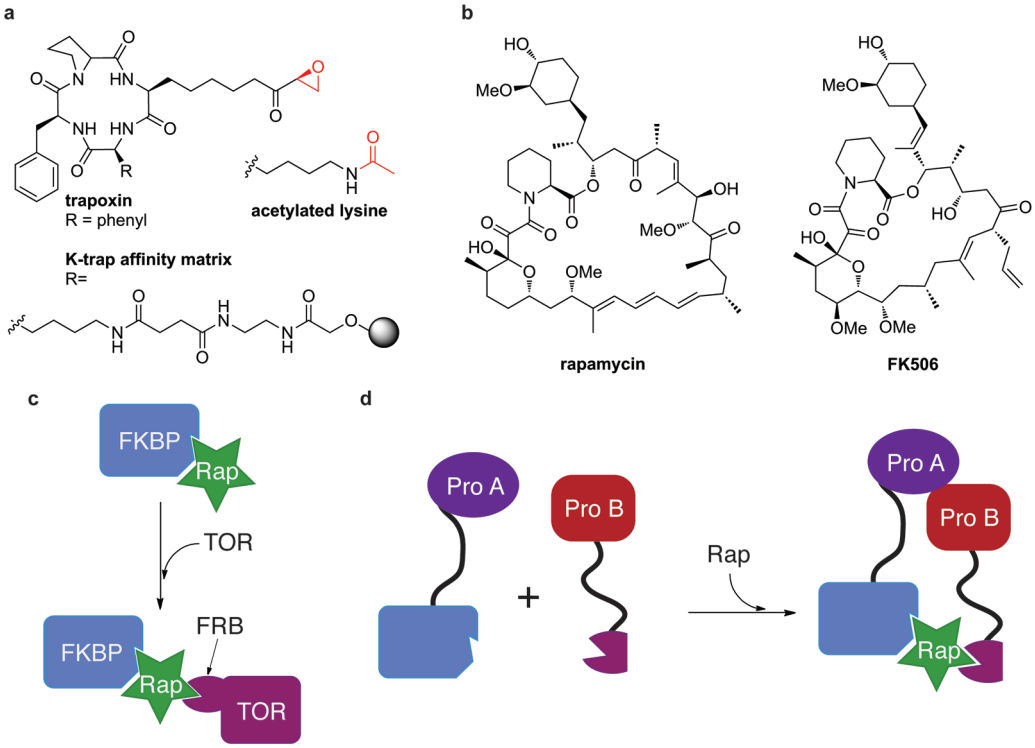

Figure 1. Early examples of natural products that were utilized as chemical probes. a) A derivative of trapoxin was used in studies to identify the first HDAC. Trapoxin was immobilized on a matrix, resulting in the conjugate called K-trap, to enable enrichment of its binding partners. The electrophilic epoxyketone in trapoxin mimics the acetylated lysine substrates of these enzymes and promotes covalent modification of this natural product's protein targets. b) Rapamycin and FK506 have similar structures but influence T-cell signal transduction by distinct mechanisms. c) Rapamycin binds to FKBP forming a complex that subsequently interacts with TOR through the FKBP–rapamycin-binding (FRB) domain. d) Rapamycin can be used to dimerize two proteins by conjugation of the target proteins to FKBP and FRB. Treatment with rapamycin enables temporal control over association of the target proteins or promotes interactions between two proteins that do not ordinarily associate.

cin was first identified as an antibiotic/antifungal agent (Figure 1, panel b) (*30*, *31*); however, this compound has become most well-known for its potent immunosuppressive and antiproliferative properties. These characteristics have led to the current use and study of rapamycin and several analogs ("rapalogs") as drugs and in clinical trials (*32*).

The activity of rapamycin is governed by interaction with a peptidyl–prolyl *cis*/*trans* isomerase, FKBP (FK506 binding protein). FKBP had previously been identified as a target of the immunosuppressive drug, FK506 (*33*, *34*). Despite the similarity in structure of rapamycin and FK506 (Figure 1, panel b), the FKBP–FK506 complex inhibits calcineurin and reduces T-cell signal transduction and interleukin-2 (IL-2) production, whereas the FKBP–rapamycin complex targets mTOR (mammalian target of rapamycin; Figure 1, panel c) inhibiting response to IL-2 (*35*, *36*). mTOR is currently being targeted for treatment of transplant rejection (*37*) and is under investigation for cancer therapy (*38*). Thus, the two natural product–protein complexes, FK506–FKBP and rapamycin–FKBP, influence discrete T-cell signal transduction pathways (*34*).

Although proteins were once thought to act as discrete entities, performing their functions without significant crosstalk with other macromolecules, today it is widely recognized that proteins function through a complex network of biomolecule interactions, the so-called interactome (*39*, *40*). Accordingly, development of tools either to study natural protein–protein interactions or

to bring together two proteins that are not known to be associated to create unique functions has been an area of intense interest (*41, 42*). In the case of rapamycin, its innate ability to promote protein–protein interactions or to act as a "chemical inducer of dimerization" has been exploited in many of these studies (Figure 1, panel d) (*41–43*). Additionally, investigations involving two other natural products, FK506 and brefeldin, have been instrumental in highlighting the utility of natural products, not only to uncover new therapeutic targets but also to bring to light fundamentally new strategies for drug development and to facilitate study of complex biological processes.

KEYWORDS

Activity-based protein profiling: Chemical proteomics strategy that utilizes small molecule probes to tag active enzymes but not their inactive precursors or inhibitor bound forms. This technology provides information about the functional state of an enzyme.

Affinity tag: A moiety, often biotin, which is added to a natural product to facilitate its immobilization onto a solid support by interaction with avidin. The resulting functionalized resin is used in mechanism of action studies to identify the binding partners of the natural product of interest.

Chemical probe: A small molecule utilized in the exploration of biological phenomena and/or assembly of biochemical pathways. These compounds can either be natural products or produced synthetically.

LC/LC-MS/MS: Liquid chromatography (LC) and mass spectrometry (MS). LC-MS analysis is commonly utilized for the identification of proteins and peptides in proteomics studies. LC/LC refers to tandem chromatography steps that enable better resolution of the sample contents. MS/MS refers to analysis of the parent species followed by fragmentation of the ion to enable peptide sequencing.

Mechanism of action: Studies to determine the biological target(s), typically a protein, of a natural product that elicits an effect on an organism or pathway.

Natural product: A secondary metabolite produced by a living organism ranging from plants and animals to bacteria and fungi. Absence of these compounds does not result in the immediate death of an organism unlike the loss of primary metabolites.

Natural product biosynthesis: Synthesis of secondary metabolites by an assembly line of enzymes. Some natural products, including polyketides and nonribosomal peptides, are produced by a series of modular enzymes.

The preceding examples highlight several influential studies involving natural product-based chemical probes. In the remainder of this review, instead of providing an exhaustive discussion of myriad roles that natural products can play, the focus will be placed on recent examples of creative and significant utilization of natural product-based probes. These studies can be categorized into three general themes: use of natural product probes to map biochemical pathways, utilization of natural product-based tools to study poorly characterized biological processes, and development of natural product tools for proteomic profiling and biomarker identification.

NATURAL PRODUCT PROBES TO MAP BIOCHEMICAL PATHWAYS

Natural product discovery efforts are largely focused on the utilization of bioactivity-guided isolation. Once samples have been procured from a natural source, the material is screened for bioactivity. Cell-based assays are often used and identify compounds that, for example, inhibit growth or prompt apoptosis. The active component of the crude natural product mixture is then purified and structurally characterized. In most cases, however, little is known about the exact mechanism by which these natural products produce the observed bioactivity. Thus, mode of action studies are required to reveal the protein target(s) of each natural product. Nature continually presents compounds that do not function through known mechanisms/pathways. This section will highlight examples of natural products that elicit bioactivity in a novel fashion. These studies serve to illustrate the role that natural products have and will continue to play in mapping important biochemical networks and identifying novel therapeutic strategies.

New Function for an Old Enzyme. Much of what is currently known about the structure and organization of the mitotic spindle was determined by the use of small molecules. Generally, these compounds are tubulin-binding agents (*e.g.*, taxol), and in some cases, they have been developed into successful cancer therapeutic agents. However, given the importance of mitosis in all cells that are undergoing division, it is not surprising that treatments that interfere globally with microtubule formation are also associated with serious side effects, such as weight loss and a dramatic decrease in the level of neutrophils (*i.e.*, neutropenia) (*44*). Thus, exploration of compounds that selectively target spindle assembly or components other than the tubulins may provide highly efficacious drugs with fewer side effects than the currently utilized treatments.

Recent research has shown that diazonamide A, a natural product isolated from the marine ascidian *Diazona angulata* (*45*), may possess these desired features (Figure 2, panel a). Initial studies of its bioactivity revealed that, like tubulin poisons, diazonamide A inhibits the growth of several cancer cell lines. Unlike these agents, however, no evidence that diazonamide A directly interacts with tubulin or the microtubules *in vitro* was observed (*46*). In addition, treatment of nude mice with therapeutically relevant doses of this compound revealed that it does not cause the side effects so commonly observed with tubulin-interacting antimitotics (*47*). These data suggest that diazonamide A may function by a previously unknown mechanism and prompted further study of its mode of action. Harran, Wang, and co-workers utilized a biotinylated diazonamide A ana-

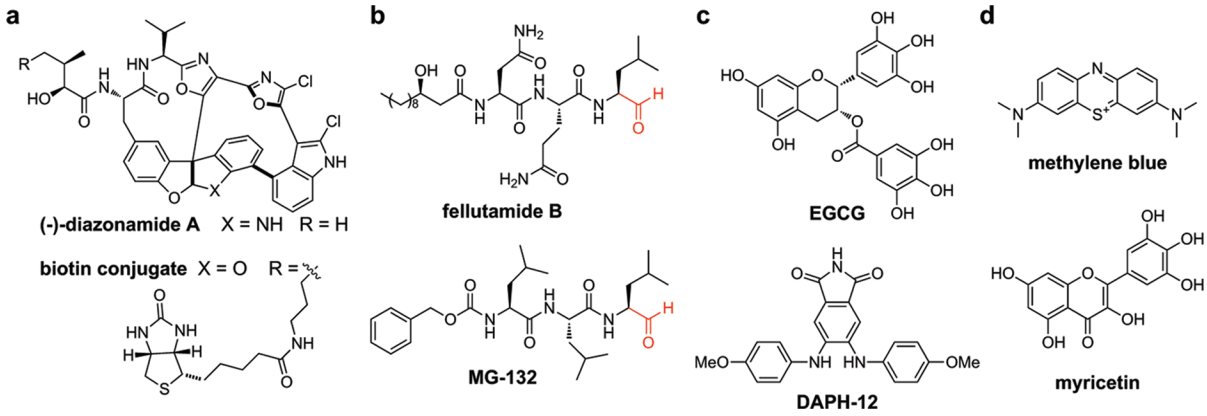

Figure 2. A diversity of natural products have been used as chemical probes to understand processes ranging from mitotic spindle assembly to amyloid formation. a) Identification of the target of diazonamide A revealed the identity of an enzyme not previously known to be involved in mitosis, ornithine δ-amino transferase. b) The role of fellutamide B in nerve growth factor secretion was elucidated following the recognition that this natural product resembled MG-132, a known proteasome inhibitor. The electrophilic aldehyde in these compounds is involved in their inhibitory activity. c) A combination of epigallocatechin-3-gallate (EGCG) and 4,5-bis-(4-methoxyanilio)phthalimide (DAPH-12) targets myriad mechanisms of prionogenesis. d) Methylene blue and myricetin both inhibit Hsp70 ATPase activity resulting in τ degradation, demonstrating that this protein may be a viable target for the treatment of neurodegenerative diseases.

log to identify the binding partner of this natural product (Figure 2, panel a) (*48*). Unexpectedly, they found that it targets an enzyme not known to be involved in mitosis. This receptor, ornithine δ-amino transferase (OAT), was previously characterized as a mitochondrial enzyme essential in ornithine catabolism (*49*). OAT is not required for survival (nonlethal in knockout mice or in humans with mutant inactive forms), suggesting that it is not essential for normal cell development (*50, 51*). OAT knockdown in several cancer lines inhibited cell division, which suggests that this enzyme may offer a selective way to target rapidly dividing cells, such as cancer cells, in preference to normal cells (*48*).

Further studies will be required to determine the exact role of OAT in cancer cell mitotic function. However, these thought-provoking data and a subsequently published study examining a diazonamide A analog suggest that these compounds may be cancer-selective agents (*47*). In addition, these studies highlight the important roles that chemical probes can play in understanding biological processes. Here, use of diazonamide A enabled identification of a potentially important player in cancer therapeutic development that was unlikely to have been otherwise discovered, given that OAT function was not known to be associated with mitosis.

Novel Strategy for Development of Neurotrophic Agents. Interest in the marine natural product, fellutamide B (*52*), produced by the fungus *Penicillium fellutanum* (Figure 2, panel b), was spurred by the discovery that this compound induces nerve growth factor (NGF) secretion in fibroblasts and glial-derived cells (*53*). NGF and other neurotrophins provide neuroprotective effects that may be important in the development of treatments for neuronal injuries or neurodegeneration caused by stroke or central nervous system diseases, such as Alzheimer's and Parkinson's disease (*54*). However, the mechanism of action of this fungal metabolite was not understood. Crews and co-workers recently reported evidence that the neuroprotective effect of this natural product stems from an unanticipated action, inhibition of the proteasome, a function not previously associated with NGF secretion (*55*).

The investigations that lead to the discovery of this seemingly disparate role for fellutamide B were inspired by the observation that this natural product possesses structural similarity to a known proteasome inhibitor, MG132 (Figure 2, panel b). Like MG132, fellutamide B targets the active site threonine in the chymotrypsin-like site through covalent modification of its aldehyde warhead. Fellutamide B was also found to inhibit the trypsin- and caspase-like sites, although with lower efficacy. In light of this result, the authors confirmed that

other proteasome inhibitors also promote NGF secretion. These data suggest that the neurotrophic activity of fellutamide B stems from inhibition of the proteasome, which the authors hypothesize regulates NGF gene expression by an unidentified transcriptional factor. Although additional studies will be required to characterize the relationship between proteasome inhibition and NGF secretion fully, these results strongly suggest that proteasome inhibitors, many of which have been identified for other therapeutic applications, will play an important role in the development of neurotrophic agents.

NATURAL PRODUCTS TOOLS TO STUDY POORLY CHARACTERIZED BIOLOGICAL PROCESSES

The above examples highlight studies that were prompted by the identification of natural products that elicit a desired phenotype in a cell-based assay (*e.g.*, cell death). This section will present examples of research that stemmed from an interest in the understanding of a particular biological process, such as the function of a specific enzyme. In these targeted studies, natural products were identified, usually through the use of a high-throughput screen, for their activity against a discrete binding partner. Following identification, these natural product probes were utilized to further the understanding of the biological question of interest.

Natural Product Probes of Amyloid Formation. Many neurodegenerative diseases, such as Alzheimer's disease (AD) and Creutzfeldt–Jakob disease (CJD), are associated with the formation of proteinaceous plaques, known as amyloid, in the central nervous system (*56*). Although significant progress has been made toward the development of treatments, in all cases, neurodegenerative diseases are ultimately fatal. Amyloid structures, typically high in β-sheet content, can be formed by a variety of normal, endogenous proteins (*e.g.*, prion protein in CJD and τ and amyloid β (Aβ) in AD), giving rise to the various symptomologies of the different diseases. The plaques that result from the aggregation of these distinct protein types are equally dissimilar, and this conformational diversity complicates efforts to understand and prevent amyloidogenesis. Thus, continued exploration of small molecule probes to further our understanding of protein oligomerization and plaque formation is of paramount importance. Here, two recent examples of natural product-based probes that facilitated substantial steps forward in the comprehension of neurodegenerative disease progression and potential treatment avenues are highlighted.

Prions represent one of the classes of proteins that can undergo a dramatic conformational change leading to amyloidogenesis. Prion-based plaques are associated with disorders such as CJD and mad cow disease. Prion protein occurs naturally throughout the body and in its normal, soluble form is called PrP^{c} for "cellular" or "common". Prion proteins may also be present in an infectious form called PrP^{Sc}, which can populate many different structural conformations and are the forms involved in amyloid formation. Identification of a small molecule that can effectively disrupt the highly stabilized protein–protein interfaces in amyloids and address these many isoforms is a daunting challenge.

Recently, Shorter, Duennwald, and co-workers examined the effects of epigallocatechin-3-gallate (EGCG), an antioxidant found in tea (*57*), and 4,5-bis-(4-methoxyanilio)phthalimide (DAPH-12; Figure 2, panel c) on amyloidogenesis of different strains of the well-studied yeast prion protein, Sup35 (*58*). Discrete infectious structures or "strains" of pure Sup35 can be readily generated, providing an ideal platform for study. The research team found that, although neither compound alone could address all of the prion strains, treatment with both EGCG and DAPH-12, each of which antagonizes prionogenesis by a distinct mechanism, inhibited and reversed amyloidogenesis in a range of prion strains. These studies provide the first example of synergistic inhibition and reversal of prion formation with two mechanistically distinct small molecules and show promise for the development of small molecule treatments for amyloid-associated diseases.

The most well-known of all neurodegenerative diseases is AD. AD is characterized by the formation of amyloid plaques composed of Aβ peptide and intracellular "tangles" of protein τ (*59*). Current evidence suggests that generation of therapeutic strategies to facilitate the removal of abnormal τ may be efficacious. However, the ideal target for this goal is not known, making full characterization of τ processing mechanisms essential. To achieve this goal, identification of selective inhibitors of the potential players will be required. In a recent study, Dickey, Gestwicki, and coworkers focused their efforts on characterization of Hsp70, a heat shock protein that is associated with protein folding (*60*). This molecular chaperone is expressed in response to stress and is emerging as a po-

tentially important target in not only therapeutic development for neurodegenerative diseases but also cancer, infectious disease, and immunity (*61, 62*). In particular, the authors sought to examine the role that Hsp70s ATPase activity might play in the stability of τ.

Recent development of a high-throughput screen for Hsp70 (*63*) facilitated the examination of a 2800-member library of known bioactive compounds. Several inhibitors and activators of Hsp70 were identified, including the antioxidant dye, methylene blue, and the flavonoid, myricetin (*64*) (both are inhibitors; Figure 2, panel d). Interestingly, inhibitors of Hsp70 ATPase activity promoted τ degradation, while activators elevated the level of τ. These modulating agents were shown to affect the rate of ATP consumption of this enzyme, a process that is intimately associated with the release or the sequestration of the protein client, in this case τ. This suggests a model in which Hsp70 inhibitors hold the enzyme in a conformation that accelerates the releasing and targeting of τ to the proteasome for degradation. Although the authors had originally anticipated that activation of Hsp70 would increase τ degradation, they found exactly the opposite. This finding strongly suggests that inhibition of the ATPase activity of Hsp70 may provide a viable therapeutic strategy.

In subsequent studies, the identified compounds have been utilized in research aimed at understanding the relationship between Hsp70 enzymatic activity and clearance of another protein, the cancer-associated kinase, Akt (*65*), and between Hsp70 and polyglutamine protein degradation (*66*).

Characterization of Unannotated Enzymes. The era of proteomics has made possible the identification and study of thousands of proteins. One of the major challenges remaining in this field is the astounding number of proteins, estimated to be 30–50% of the human proteome (*67*), whose functions have yet to be annotated. All too often, proteins are identified to be important in a particular cellular process or disease state, but the manner in which these proteins contribute is poorly understood. Unannotated enzymes are generally challenging to study since identification of selective chemical probes is difficult without the use of high-throughput substrate-based assays. Recently, Cravatt, Rosen, and co-workers reported a novel strategy for the identification of small molecules that can be applied to the study of uncharacterized enzymes (*68*). To accomplish this goal, the authors take advantage of probes developed for the chemical proteomics method, activity-based protein profiling (ABPP). This technology utilizes chemical probes that have been designed to target large classes of mechanistically related enzymes. Probes label proteins in an activity-dependent fashion by covalent interaction with an active site residue (*69*).

To enable the identification of selective small molecule inhibitors of a given enzyme, compound libraries can be screened in competition with ABPP probes to identify molecules that prevent the proteomics probes from labeling the enzyme (Figure 3, panel a). This method, termed competitive ABPP, has been used to identify selective inhibitors of, for example, unannotated serine hydrolases (*70*). However, this approach is incompatible with high-throughput screening (HTS), as it requires the use of one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels. To address this challenge, Cravatt and Rosen adapted competitive ABPP to a fluorescence polarization (FP)-based assay, where binding of the reactive probe to the enzyme results in a lower depolarization signal, while competition with a screening compound, which results in a high concentration of free fluorescent probe in solution, yields an elevated depolarization signal (Figure 3, panel a).

To identify a small molecule useful for characterization of the cancer-associated serine hydrolase RBBP9, the authors screened a library of ~19 000 compounds. One selective inhibitor, the alkaloid emetine (*71*), which is produced by the ipecac root was identified (Figure 3, panel b). Emetine is known to be a highly cytotoxic agent, but the mechanism of action of this natural product has not been elucidated. Further examination of ~75 structurally related compounds demonstrated that RBBP9 displays a high degree of binding specificity even against closely related analogs, such as dehydroemetine (Figure 3, panel b). Thus, emetine will likely serve as a useful tool for characterization of the biological functions of this enzyme. Although there is still much to be learned about the relationship between emetine and RBBP9, this work suggests that the described high-throughput competitive ABPP platform will see future application to the study of unannotated enzymes.

Probe To Advance Understanding of Translation Initiation. The mechanisms that regulate the translation of mRNA into proteins are highly complex and, to date, only partially understood. Translation initiation has been recognized as an important cancer therapeutic tar-

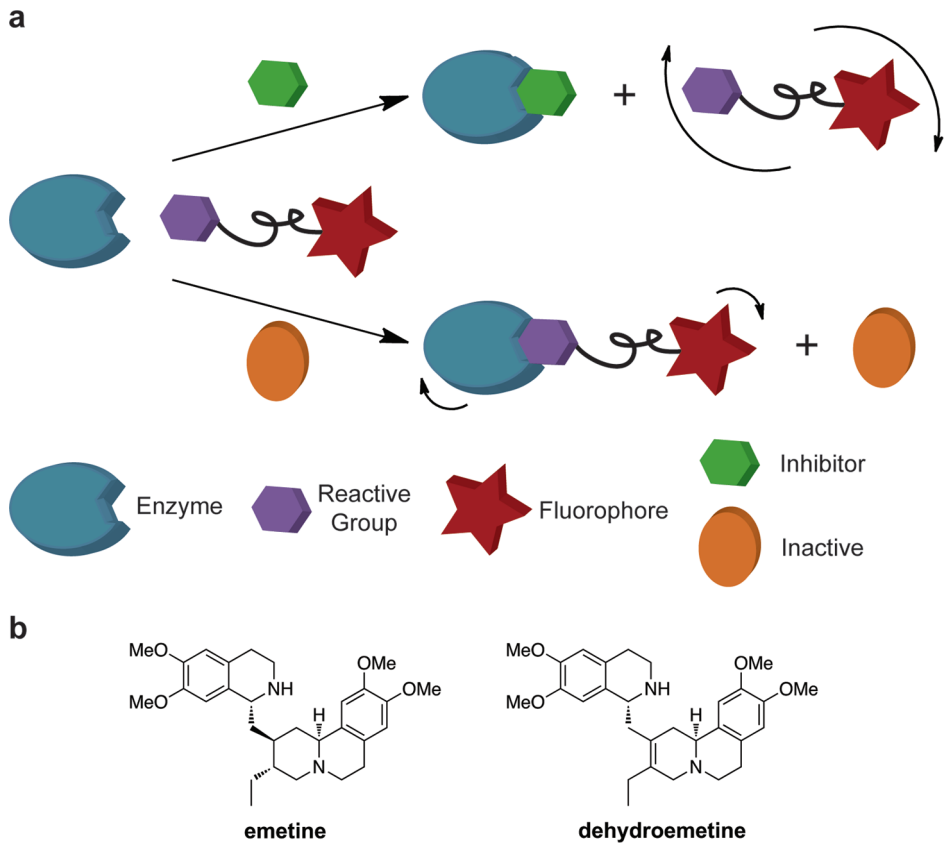

Figure 3. Development of a high-throughput screen enabled the functional annotation of a cancer-associated protein. a) Enzyme inhibitors were identified using a fluorescence polarization (FP) assay. Each compound was incubated with RBBP9 followed by addition of a reactive probe that specifically labels the active site of the serine hydrolase. Active compounds prevented the probe from tagging the enzyme, leaving it free in solution where it tumbles quickly, causing light depolarization (top). Inactive compounds do not bind the enzyme leaving it susceptible to probe labeling. The bound probe tumbles more slowly than the free compound yielding a lower depolarization response (bottom). b) Using the devised FP assay, emetine was identified as a selective inhibitor of RBBP9. Examination of ~75 structurally related compounds showed that this binding interaction is highly specific, even the dehydroemetine analog, which differs by only one unit of unsaturation, was not bound by RBBP9.

get due to the success seen with small molecules, such as rapamycin, that are known to affect this process (*72*). Development of additional tools to tease apart how translation is regulated is of critical importance.

The identification of the natural product pateamine A, from the sponge *Mycale* sp. has provided such a chemical probe (Figure 4, panel a). Pateamine A was first noted for its selective cytotoxicity profile against rapidly growing cells, such as P388 leukemia cells (*73*). In 2005, it was demonstrated that this cytotoxicity is due to inhibition of protein translation by interaction with eIF4A, a component of the translation initiation complex, eIF4F. This result was reported by two independent research groups, one working to examine the mechanism of action of pateamine A, and the other that sought to identify inhibitors of eukaryotic protein synthesis by performing a high-throughput screen (*74*, *75*). Importantly, unlike other known inhibitors of protein translation, pateamine A showed a high level of selectivity for the eIF4A-based activity and did not appear to affect other steps of translation, making it an ideal biological probe.

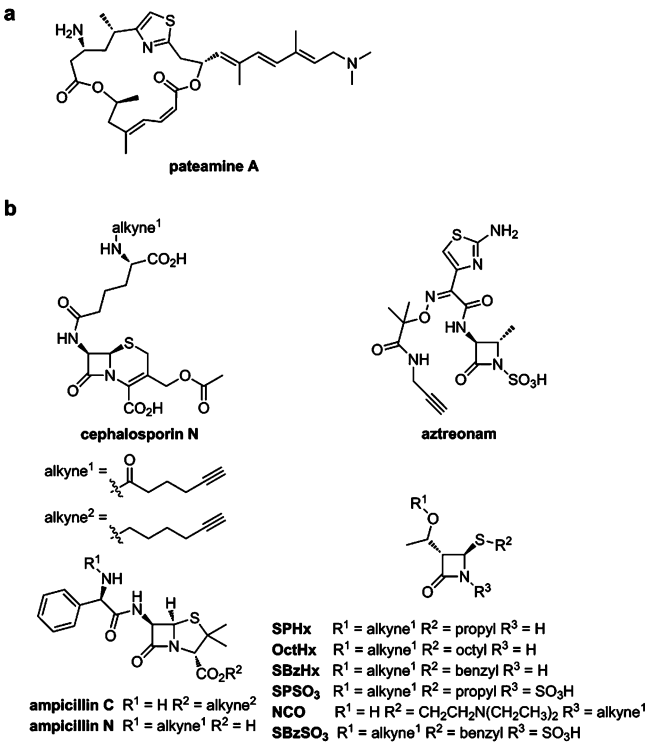

Figure 4. Natural product-based compounds often yield highly selective chemical probes. a) Pateamine A targets eIF4A-based translation activity but does not affect other steps in translation. b) A suite of natural product-inspired probes was developed for profiling of penicillin binding protein (PBP) activity *in vivo*. The alkyne-functionalized natural product analogs are depicted here. Together, these probes targeted both the anticipated PBPs and a pool of virulence- and resistance-associated enzymes.

Pateamine A increases the ATPase and helicase activities of eIF4A (*74, 75*). Recent studies have shed light on the relationship between the enzymatic activity and the complex formation, indicating that binding of pateamine A induces a conformational change in eIF4A that increases its enzymatic activity and thus, increases its residence time on its mRNA substrates (*74*). When bound to mRNA, eIF4A cannot participate in the protein–protein interactions required for formation of the eIF4F initiation complex. Accordingly, pateamine A inhibits protein translation by sequestering eIF4A and preventing formation of the initiation complex (*76, 77*). This unique mechanism of action has prompted great interest in both the continued study of the role of eIF4A in translation initiation and cancer and the potential therapeutic utility of pateamine A and related analogs (*78, 79*).

NATURAL PRODUCTS AS TOOLS FOR PROTEOMIC PROFILING

In the above examples, natural products were utilized as probes of a particular biological system. In general, these compounds were highly specific for the protein target of interest and promoted a deeper understanding of the role of that biological target in a disease state or cellular process. This section focuses on research aimed at the development of natural product-based probes to facilitate the global analysis of a target system. Such chemical proteomics methods seek to identify new therapeutic targets and/or assist in determination of the activity, function, and regulation of a large number of proteins. The final example presented in this review combines chemical proteomics with the area of natural products discovery by development of natural product-inspired probes to map the activities of the machinery involved in natural product biosynthesis.

Probes To Target Antibiotic Resistance- and Virulence-Associated Enzymes. Infectious diseases are the second-leading cause of death worldwide and the third-leading cause of death in economically advanced countries. With the advent of the "age of antibiotics" in the 1940s, many believed that these dangerous microbes had been conquered. Despite early optimism, it quickly became apparent that the ability of bacteria to evolve resistance had been sorely underestimated. In fact, penicillin-resistant strains were detected only months after clinical introduction of this drug (*80*). Generally, resistant bacteria are observed within four years of the introduction of a new antibiotic. Accordingly, a dire need to identify new therapeutic targets exists, particularly for the treatment of resistant organisms.

β-Lactam-containing compounds, such as the natural product penicillin, are one of the most commonly utilized classes of antibiotics. These agents inhibit bacterial growth by covalently modifying penicillin binding proteins (PBPs), a class of enzymes that is required for

bacterial cell wall biosynthesis. Although it is widely appreciated that PBPs are targeted by β-lactam antibiotics, little is known about how these enzymes are regulated *in vivo*.

To address this challenge, Sieber and Staub generated a library of antibiotic-inspired chemical probes featuring analogs of cephalosporin, isolated from *Acremonium* (*81*), and aztreonam and ampicillin, synthetic β-lactam-containing antibiotics (Figure 4, panel b) (*82*). In addition, they generated six structurally simplified β-lactam probes to potentially expand the scope of the enzymes addressed in these studies. These β-lactam-containing compounds will only label catalytically active enzymes, making them ideal activity-based probes. Each probe was functionalized with a bioorthogonal alkyne moiety to enable conjugation of labeled proteins to a read-out tag, such as rhodamine, following proteome labeling. This small suite of probes was applied to the exploration of the *Pseudomonas putida*, *Listeria welshimeri*, and *Bacillus licheniformis* proteomes. The four antibiotic-based probes labeled a diverse set of PBPs as anticipated. Intriguingly, the synthetic β-lactams did not label any PBPs but instead a pool of unrelated enzymes, including the virulence- and resistance-associated enzymes ClpP and a β-lactamase (β-Lact), respectively.

In a subsequent study, this probe library was applied to the comparative analysis of antibiotic-sensitive *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) to identify resistance-associated enzymes (*83*). Several known resistance-conferring enzymes were identified. In addition, three enzymes of unknown function, a dipeptidase (Dipep), an esterase (E28), and a serine protease (SPD_0), were detected only in the MRSA proteomes. Preliminary characterization of the Dipep and E28 proteins revealed that they display β-lactamase activity with preference for the hydrolysis of antibiotics in the penicillin family. Additionally, it was determined that the dipeptidase is likely a metallo-β-lactamase, a class of enzymes that are insensitive to the current repertoire of lactamase inhibitors, making them crucial for bacterial resistance (*84*). These results highlight the potential power of β-lactam-containing probes for the identification of other unknown resistance-conferring enzymes in pathogenic bacteria and suggest that development of additional natural product-based proteomics probes will also yield efficacious compounds for functional proteomic studies (*85*).

Proteomic Profiling of Natural Product Biosynthesis. Since the advent of the genomic era, more than 1000 bacterial genomes have been sequenced, enabling estimation of the natural product biosynthetic pathways available in a particular species (*86, 87*). These efforts have revealed that there are many more biosynthetic gene clusters in a given organism than there are known secondary metabolites (*88, 89*). Thus, the biosynthetic potential of microorganisms has been greatly underexplored. Researchers are making great strides in the characterization and manipulation of many classes of biosynthetic enzymes (*90–93*). However, these efforts generally examine one or a small number of enzymes at a time making global exploration of the biosynthetic machinery of an organism difficult. Recently, several strategies for the proteomic profiling of natural product biosynthesis have been reported (*94–96*).

One of these methods utilizes substrate analogs to map biosynthetic pathways for two classes of natural products: polyketides and nonribosomal peptides (*96*). Together, these natural product families encompass a significant percentage of known natural product-based therapeutic agents, making continued study of their biosynthesis of great importance. The modular enzymes responsible for synthesis of these natural products (polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS)) can be "mixed and matched" to enable an organism to produce a disparate collection of compounds. Thus, it is very difficult to predict the structures of natural products that may be produced by a given species *a priori*.

Many PKS and NRPS enzymes have been characterized using a combination of biochemical, genetic, and structural-based techniques as well as small molecule probes (*97–99*). However, a method for the global analysis of the PKS and NRPS repertoire of a given organism is still required. Such a tool would enable comparative analysis of the functional state of PKS and NRPS enzymes in various organisms and assessment of the dynamics of biosynthetic enzymes. In addition, a global profiling method would facilitate discovery of novel compounds by enabling the optimization of natural product production. As noted above, sequencing of a number of bacterial genomes revealed that there are many more biosynthetic gene clusters in a given organism than there are known secondary metabolites, making development of tools to facilitate biosynthetic optimization an important objective (*88, 89*).

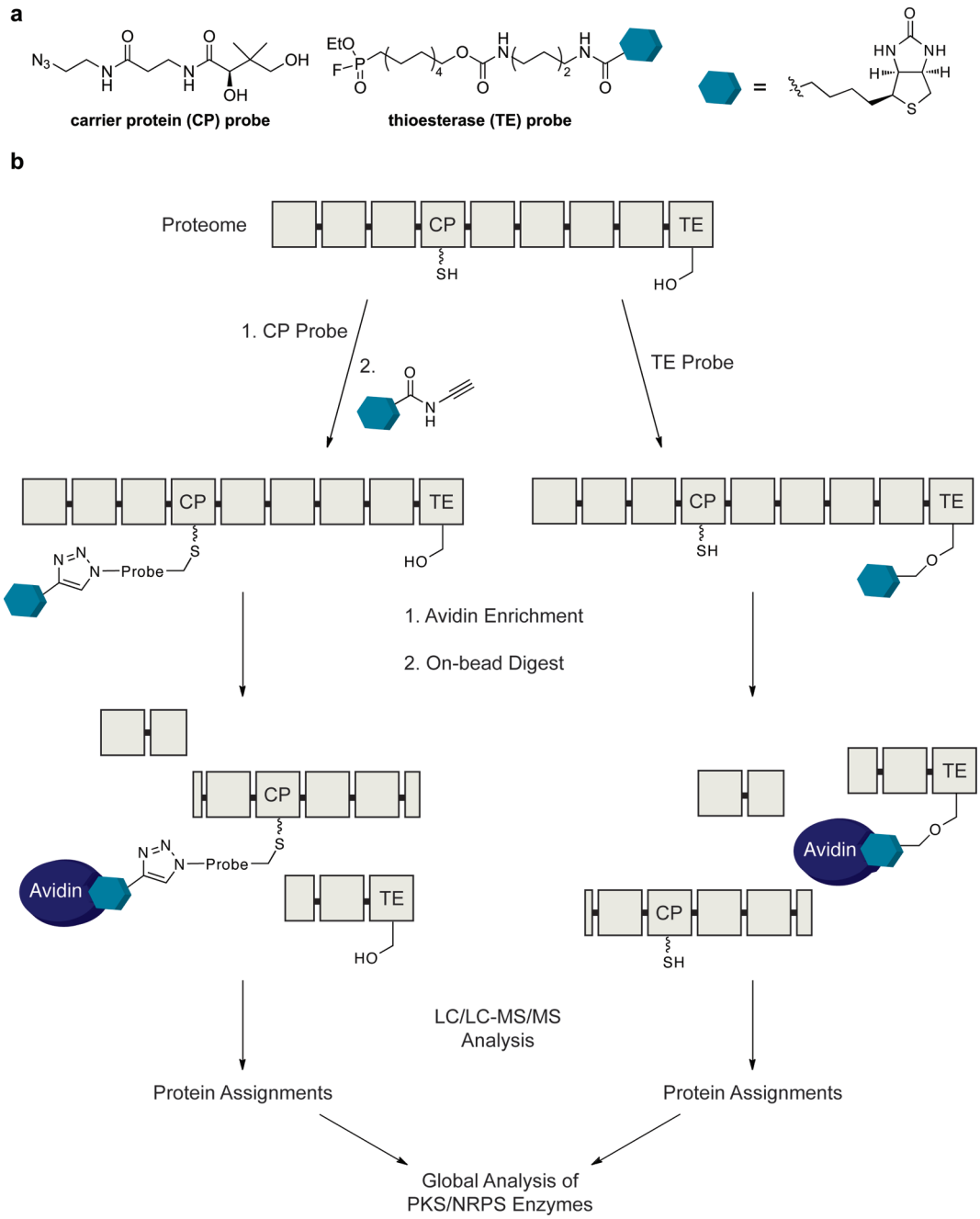

Figure 5. Chemical probes were developed to facilitate profiling of PKS and NRPS enzymes. a) Carrier proteins were targeted with a coenzyme A precursor analog (CP probe), while thioesterase domains were tagged with a fluorophosphonate probe (TE probe) known to be specific for serine hydrolases. b) The OASIS method. Isolated proteomes were tagged with either the CP or TE probe. Proteomes were conjugated to the biotin tag using copper-catalyzed click chemistry. The biotin-labeled proteins were enriched on avidin beads and subjected to on-bead trypsin digestion. The cleaved peptides were analyzed by liquid chromatography-mass spectrometry (LC/LC-MS/MS) to yield protein identifications. Utilization of these two probes enabled global analysis of PKS and NRPS enzymes. Note that some peptides were common between the two probes, while many differed.

With this goal in mind, Burkart, Cravatt, and coworkers sought to develop an active site-directed strategy for identification and analysis of PKS and NRPS enzymes called Orthogonal Active Site Identification System (OASIS) (*96*). This technique utilizes a combination of substrate analogs and small molecule inhibitors to label the carrier protein (CP) and thioesterase (TE) domains of active enzymes. Probes were designed to take advantage of the catalytic mechanisms of these domains. Accordingly, CPs were targeted with a coenzyme A (CoA) precursor (CP probe), while TEs were labeled with a fluorophosphonate-containing probe (TE probe) known to tag serine nucleophiles (Figure 5) (*100*). Labeled enzymes were conjugated to the enrichment tag biotin, using the Cu(I)-catalyzed [3 + 2] cycloaddition reaction ("click chemistry") (*101*) and identified by means of the liquid chromatography-mass spectrometry (LC/LC-MS/MS)-based method multidimensional protein identification (MudPIT) (*102*). Using this platform, the authors examined the proteome of a model organism, *Bacillus subtilis*.

OASIS analysis with the CP probe facilitated the detection of enzymes from all four known PKS and NRPS gene clusters, identifying 12 of the 16 CP-containing enzymes found in the wild-type *B. subtilis* strain 6051. In addition, comparative analysis of the activity of TE domains in *B. subtilis* stains 6051 and 168, an organism containing a deletion that affects synthase activation, revealed an upregulation of the NRPS activities of four enzymes, SrfAC, SrfAD, PpsE, and DhbF in strain 168. This result is consistent with what is known about strain 168. For example, levels of SrfAC expression were previously found to be higher in the wild-type strain 168 in gel-based proteomic experiments; however, follow-up studies will be required to understand the biological significance of this variation. One PKS, PksR, which is involved in the biosynthesis of bacillaene showed upregulated activity in strain 6051.

These proof-of-principle experiments suggest that OASIS will represent a powerful new technology for comparative profiling of the biosynthetic capabilities of various organisms, exploration of the dynamics of natural product synthesis, optimization of natural product biosynthesis, and discovery of new compounds. Certainly, work remains to develop OASIS into a global profiling strategy and to further validate the ability of this method to provide the quantitative data required for comparative analysis. However, these preliminary studies suggest that biosynthetic precursors will likely play an important role in future chemical proteomics studies.

SUMMARY AND FUTURE DIRECTIONS IN NATURAL PRODUCT-BASED PROBE DEVELOPMENT

Although about 200 000 natural compounds are currently known (*103*), it is likely that this group encompasses only a small percentage of the biologically produced small molecule universe. The continued exploration of the small molecule repertoire of many organisms, such as plants and microbes, is bound to prove fruitful for the discovery of novel bioactive compounds. Chemical probes are an essential component of today's research arsenal and are utilized to dissect complex biological processes, to wield temporal control over biochemical pathways, and ultimately, to identify novel therapeutic targets. Natural products have and will continue to play critical roles as biological probes given their innate ability to interact with high affinity and selectivity with biological targets. The complexity of natural products has decreased interest in these compounds in the pharmaceutical realm. However, researchers are constantly working to provide new tools to make identification, functional annotation, and synthesis more straightforward. The power of nature's small molecules far outweighs their challenges, and it has become clear that renewed interest in natural products will be essential to the future of both biological studies and drug development.

Acknowledgment: Thanks to R. Carlson, K. Garber, C. Salisbury, and M. Schmitt for critical reading of the manuscript. This work was supported by NIH R00GM82983.

REFERENCES

1. Sertuerner, F. (1817) Ueber das morphium, eine neue salzfähige grundlage, und die mekonsäure, als hauptbestandtheile des opiums, *Ann. Phys. 55*, 56–89.
2. Newman, D. J., and Cragg, G. M. (2010) Natural products as drugs and leads to drugs: the historical perspective, in *RSC Biomolecular Sciences No. 18; Natural Product Chemistry for Drug Discovery* (Buss, A. D., Butler, M. S., Eds.), pp 3–27, Royal Society of Chemistry, Cambridge, U.K.
3. Li, J. W.-H., and Vederas, J. C. (2009) Drug discovery and natural products: end of an era or an endless frontier? *Science 325*, 161–165.
4. Newman, D. J., Cragg, G. M., and Snader, K. M. (2003) Natural products as sources of new drugs over the period 1981–2002, *J. Nat. Prod. 66*, 1022–1037.

5. Harvey, A. (2000) Strategies for discovering drugs from previously unexplored natural products, *Drug Discovery Today 5*, 294–300.
6. Koehn, F. E., and Carter, G. T. (2005) The evolving role of natural products in drug discovery, *Nat. Rev. Drug Discovery 4*, 206–220.
7. Clardy, J., and Walsh, C. (2004) Lesson from natural molecules, *Nature 432*, 829–837.
8. Piggott, A. M., and Karuso, P. (2004) Quality, not quantity: The role of natural products and chemical proteomics in modern drug discovery, *Comb. Chem. High Throughput Screening 7*, 607–630.
9. Koch, M. A., Schuffenhauer, A., Scheck, M., Wetzel, S., Casaulta, S., Odermatt, A., Ertl, P., and Waldmann, H. (2005) Charting biologically relevant chemical space: A structural classification of natural products (SCONP), *Proc. Natl. Acad. Sci. U.S.A. 102*, 17272–17277.
10. Drahl, C., Cravatt, B. F., and Sorensen, E. J. (2005) Protein-reactive natural products, *Angew. Chem., Int. Ed. Engl. 44*, 5788–5809.
11. Kirschning, A., Taft, F., and Knobloch, T. (2007) Total synthesis approaches to natural product derivatives based on the combination of chemical synthesis and metabolic engineering, *Org. Biomol. Chem. 5*, 3245–3259.
12. Maimone, T. J., and Baran, P. S. (2007) Modern synthetic efforts toward biologically active terpenes, *Nat. Chem. Biol. 3*, 396–407.
13. McCoy, E., and O'Connor, S. E. (2008) Natural products from plant cells cultures, *Prog. Drug Res. 65*, 331–370.
14. Morris, J. C., Nicholas, G. M., and Phillips, A. J. (2007) Marine natural products: Synthetic aspects, *Nat. Prod. Rep. 24*, 87–108.
15. Burdine, L., and Kodadek, T. (2004) Target identification in chemical genomics: The (often) missing link, *Chem. Biol. 11*, 593–597.
16. Terstappen, G. C., Schlüpen, C., Raggiaschi, R., and Gaviraghi, G. (2007) Target deconvolusion strategies in drug discovery, *Nat. Rev. Drug Discovery 6*, 891–903.
17. Böttcher, T., Pitscheider, M., and Sieber, S. A. (2010) Natural products and their biological targets: Proteomic and metabolomic labeling strategies, *Angew. Chem., Int. Ed. 49*, 2680–2699.
18. Cheng, K.-W., Wong, C.-C., Wang, M., He, Q.-Y., and Chen, F. (2010) Identification and characterization of molecular targets of natural products by mass spectrometry, *Mass Spectrom. Rev. 29*, 126–155.
19. Rodríguez, A. D., Lear, M. J., and La Clair, J. J. (2008) Identification of the binding of sceptrin to MreB via a bidirectional affinity protocol, *J. Am. Chem. Soc. 130*, 7256–7258.
20. Hughes, C. C., Yang, Y.-L., Liu, W.-T., Dorrestein, P. C., La Clair, J. J., and Fenical, W. (2009) Marinopyrrole A target elucidation by acyl dye transfer, *J. Am. Chem. Soc. 131*, 12094–12096.
21. Peddibhotla, S., Dang, Y., Liu, J. O., and Romo, D. (2007) Simultaneous arming and structure/activity studies of natural products employing O-H insertions: An expedient and versatile strategy for natural products-based chemical genetics, *J. Am. Chem. Soc. 129*, 12222–12231.
22. Shenvi, R. A., O'Malley, D. P., and Baran, P. S. (2009) Chemoselectivity: The mother of invention in total synthesis, *Acc. Chem. Res. 42*, 530–541.
23. Lewis, C. A., Longcore, K. E., Miller, S. J., and Wender, P. A. (2009) An approach to the site-selective diversification of apoptolidin A with peptide-based catalysts, *J. Nat. Prod. 72*, 1864–1869.
24. Pucheault, M. (2008) Natural products: chemical instruments to apprehend biological symphony, *Org. Biomol. Chem. 6*, 424–432.
25. Taunton, J., Collins, J. L., and Schreiber, S. L. (1996) Synthesis of natural and modified trapoxins, useful reagents for exploring histone deacetylase function, *J. Am. Chem. Soc. 118*, 10412–10422.
26. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p, *Science 272*, 408–411.
27. Itazaki, H., Nagashima, G., Sugita, K., Yoshida, H., Kawamura, Y., Yasuda, Y., Matsumoto, K., Ishii, K., Uotani, N., Nakai, H., Terui, A., Yoshimatsu, S., Ikenishi, Y., and Nakagawa, V. (1990) Solation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation activities as antitumor agents, *J.Antibiot. 43*, 1524–1532.
28. Abraham, R. T. (1998) Mammalian target of rapamycin: Immunosuppressive drugs uncover a novel pathway of cytokine receptor signaling, *Curr. Opin. Immun. 10*, 330–336.
29. Crespo, J. L., and Hall, M. N. (2002) Elucidating TOR signaling and rapamycin action: Lessons from Sacchromyces cerevisiae, *Microbiol. Mol. Biol. Rev. 66*, 579–591.
30. Véniza, C., Kudelski, A., and Sehgal, S. N. (1975) Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle, *J.Antibiot. (Tokyo) 28*, 721–726.
31. Sehgal, S. N., Baker, H., and Vézina, C. (1975) Rapamycin (AY-22, 989), a new antifungal antibiotic. II. Fermentation, isolation and characterization, *J.Antibiot. (Tokyo) 28*, 727–732.
32. Dancey, J. (2010) mTOR signaling and drug development in cancer, *Nat. Rev. Clin. Oncol. 7*, 209–219.
33. Harding, M. W., Galat, A., Uehling, D. E., and Schreiber, S. L. (1989) A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase, *Nature 341*, 758–760.
34. Bierer, B. E., Mattila, P. S., Standaert, R. F., Herzenberg, L. A., Burakoff, S. J., Crabtree, G., and Schreiber, S. L. (1990) Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK506 or rapamycin, *Proc. Natl. Acad. Sci. U.S.A. 87*, 9231–9235.
35. Heitman, J., Movva, N. R., and Hall, M. N. (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast, *Science 253*, 905–909.
36. Fingar, D. C., and Blenis, J. (2004) Target of rapamycin (TOR): An integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression, *Oncogene 23*, 3151–3171.
37. Abraham, R. T., and Wiederrecht, G. J. (1996) Immunopharmacology of rapamycin, *Annu. Rev. Immunol. 1996*, 483–510.
38. Guertin, D. A., and Sabatini, D. M. (2007) Defining the role of mTOR in cancer, *Cancer Cell 12*, 9–22.
39. Cusick, M. E., Klitgord, N., Vidal, M., and Hill, D. E. (2005) Interactome: Gateway into systems biology, *Hum. Mol. Genet. 14*, R171–R181.
40. Gordo, S., and Giralt, E. (2009) Knitting and untying the protein network: Modulation of protein ensembles as a therapeutic strategy, *Protein Sci. 18*, 481–493.
41. Gestwicki, J. E., and Marinee, P. S. (2007) Chemical control over protein-protein interactions: Beyond inhibitors, *Comb. Chem. High Throughput Screening 10*, 667–675.
42. Corson, T. W., Aberle, N., and Crews, C. M. (2008) Design and applications of bifunctional small molecules: Why two heads are better than one, *ACS Chem. Bio. 3*, 677–692.
43. Crabtree, G. R., and Schreiber, S. L. (1996) Three-part inventions: Intracellular signaling and induced proximity, *Trends Biochem. Sci. 21*, 418–422.
44. Wood, K. W., Cornwell, W. D., and Jackson, J. R. (2001) Past and future of the mitotic spindle as an oncology target, *Curr. Opin. Pharmacol. 1*, 370–377.
45. Lindquist, N., Fenical, W., Van Duyne, G. D., and Clardy, J. (1991) Isolation and structure determination of diazonamides A and B, unusual cytotoxic metabolites from the marine ascidian Diazona chinensis, *J. Am. Chem. Soc. 113*, 2303–2304.
46. Cruz-Monserrate, Z., Vervoort, H. C., Bai, R., Newman, D. J., Howell, S. B., Los, G., Mullaney, J. T., Williams, M. D., Pettit, G. R., Fenical, W., and Hamel, E. (2003) Diazonamide A and a synthetic structural analog: disruptive effects on mitosis and cellular microtubules and analysis of their interactions with tubulin, *Mol. Pharmacol. 63*, 1273–1280.

47. Williams, N. S., Burgett, A. W. G., Atkins, A. S., Wang, X., Harran, P. G., and McKnight, S. L. (2007) Therapeutic anticancer efficacy of a synthetic diazonamide analog in the absence of overt toxicity, *Proc. Natl. Acad. Sci. U.S.A. 104*, 2074–2079.
48. Wang, G., Shang, L., Burgett, A. W. G., Harran, P. G., and Wang, X. (2007) Diazonamide toxins reveal an unexpected function for ornithine ä-amino transferase in mitotic cell division, *Proc. Natl. Acad. Sci. U.S.A. 104*, 2068–2073.
49. Seiler, N. (2000) Ornithine aminotransferase, a potential target for the treatment of hyperammonemias, *Curr. Drug Targets 1*, 119–153.
50. Brody, L. C., Mitchell, G. A., Obie, C., Michaud, J., Steel, G., Fontaine, G., Robert, M. F., Sipila, I., Kaiser-Kupfer, M., and Valle, D. (1992) Ornithine delta-aminotransferase mutations in gyrate atrophy. Allelic heterogeneity and functional consequences, *J. Biol. Chem. 15*, 3302–3307.
51. Wang, T., Lawler, A. M., Steel, G., Sipila, I., Milam, A. H., and Valle, D. (1995) Mice lacking ornithine-delta-aminotransferase have paradoxical neonatal hypoornithinaemia and retinal degeneration, *Nat. Genet. 11*, 185–190.
52. Shigemori, H., Wakuri, S., Yazawa, K., Nakamura, T., Sasaki, T., and Kobayashi, J. I. (1991) Fellutamides A and B, cytotoxic peptides from a marine fish-possessing fungus Penicillium fellutanum, *Tetrahedron 47*, 8529–8534.
53. Yamaguchi, K., Tsuji, T., Wakuri, S., Yazawa, K., Kondo, K., Shigemori, H., and Kobayashi, J. I. (1993) Stimulation of nerve growth factor synthesis and secretion by fellutamide A *in vitro*, *Biosci., Biotechnol., Biochem. 57*, 195–199.
54. Castellanos-Ortega, M. R., Cruz-Aguado, R., and Martinez-Matrí, L. (1999) Nerve growth factor: possibilities and limitations of its clinical applications, *Rev. Neurol. 29*, 439–447.
55. Hines, J., Groll, M., Fahnestock, M., and Crews, C. M. (2008) Proteasome inhibition by Fellutamide B induces nerve growth factor synthesis, *Chem. Biol. 15*, 501–512.
56. Skovronsky, D. M., Lee, V. M.-Y., and Trojanowski, J. Q. (2006) Neurodegenerative diseases: New concepts of pathogenesis and their therapeutic implications, *Annu. Rev. Pathol.: Mech. Dis. 1*, 151–170.
57. Butt, M. S., and Sultan, M. T.20069Green tea: Nature's defense against malignancies, *Crit. Rev. Food Sci. Nutr. 49*, 463–473.
58. Roberts, B. E., Duennwald, M. L., Wang, H., Chung, C., Lopreitiato, N. P., Sweeny, E. A., Knight, M. N., and Shorter, J. (2009) A synergistic small-molecule combination directly eradicates diverse prion strain structures, *Nat. Chem. Biol. 5*, 936–946.
59. Oddo, S., Caccamo, A., Shepherd, J. D., Murphy, M. P., Golde, T. E., Kayed, R., Metherate, R., Mattson, M. P., Akbari, Y., and LaFerla, F. M. (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: Intracellular Abeta and synaptic dysfunction, *Neuron 39*, 409–421.
60. Jinwal, U. K., Miyata, Y., Koren, J., III, Jones, J. R., Trotter, J. H., Chang, L., O'Leary, J., Morgan, D., Lee, D. C., Shults, C. L., Rousaki, A., Weeber, E. J., Zuiderweg, E. R. P., Gestwicki, J. E., and Dickey, C. A. (2009) Chemical manipulation of Hsp90 ATPase activity regulates tau stability, *J. Neurosci. 29*, 12079–12088.
61. Patury, S., Miyata, Y., and Gestwicki, J. E. (2009) Pharmacological targeting of the Hsp70 chaperone, *Curr. Top. Med. Chem. 9*, 1337–1351.
62. Evans, C. G., Chang , L., and Gestwicki, J. E.Heat shock protein 70 (Hsp70) as an emerging drug target *J. Med. Chem.*; DOI: 2010. 1021/jm100054f.
63. Chang, L., Bertelsen, E. B., Wisén, S., Larsen, E. M., Zuiderweg, E. R., and Gestwicki, J. E. (2008) High-throughput screen for small molecules that modulate the ATPase activity of the molecular chaperone DnaK, *Anal. Biochem. 372*, 167–176.
64. Ross, J. A., and Kasum, C. M. (2002) Dietary flavonoids: Bioavailability, metabolic effects, and safety, *Ann. Rev. Nutr. 22*, 19–34.
65. Koren, J., III, Jinwal, U. K., Jin, Y., O'Leary, J., Jones, J. R., Johnson, A. G., Blair, L. J., Abisambra, J. F., Chang, L., Miyata, Y., Cheng, A. M., Guo, J., Cheng, J. Q., Gestwicki, J. E., and Dickey, C. A. (2009) Facilitating Akt clearance via manipulation of Hsp70 activity and levels, *J. Biol. Chem. 285*, 2498–2505.
66. Wang, A. M., Morishima, Y., Clapp, K. M., Peng, H.-M., Pratt, W. B., Gestwicki, J. E., Osawa, Y., and Lieberman, A. P. (2010) Inhibition of Hsp70 by methylene blue affects signaling protein function and ubiquitination and modulates polyglutamine protein degradation, *J. Biol. Chem. 285*, 15714–15723.
67. Galperin, M. Y., and Koonin, E. V. (2004) 'Conserved hypothetical' proteins: Prioritization of targets for experimental study, *Nucleic Acids Res. 32*, 5452–5463.
68. Bachovchin, D. A., Brown, S. J., Rosen, H., and Cravatt, B. F. (2009) Identification of selective inhibitors of uncharacterized enzymes by high-throughput screening with fluorescent activity-based probes, *Nat. Biotechnol. 27*, 387–394.
69. Cravatt, B. F., Wright, A. T., and Kozarich, J. W. (2008) Activity-based protein profiling: From enzyme chemistry to proteomic chemistry, *Annu. Rev. Biochem. 77*, 383–414.
70. Li, W., Blankman, J. L., and Cravatt, B. F. (2007) A functional proteomic strategy to discovery inhibitors for uncharacterized hydrolases, *J. Am. Chem. Soc. 129*, 9594–9595.
71. Paul, B. H., and Cownley, A. J. (1894) Chemistry of ipecacuanha, *Pharm. J. 25*, 111–115373–374, 690–692.
72. Abraham, R. T., and Eng, C. H. (2008) Mammalian target of rapamycin as a therapeutic target in oncology, *Expert Opin. Ther. Targets 12*, 209–222.
73. Northcote, P., Blunt, J. W., and Munro, M. H. G. (1991) Pateamine: a potent cytotoxin from the New Zealand marine sponge Mycale sp, *Tetrahedron Lett.* 32.
74. Low, W.-K., Dang, Y., Schneider-Poetsch, T., Shi, Z., Choi, N. S., Merrick, W. C., Romo, D., and Liu, J. O. (2005) Inhibition of eukaryotic translation initiation by the marine natural product pateamine A, *Mol. Cell 20*, 709–722.
75. Brodeleau, M.-E., Matthews, J., Wojnar, J. M., Lindquist, L., Novac, O., Jankowsky, E., Sonenberg, N., Northcote, P., Teesdale-Spittle, P., and Pelletier, J. (2005) Stimulation of mammalian translation initiation factor eIF4A by a small molecule inhibitor of eukaryotic translation, *Proc. Natl. Acad. Sci. U.S.A. 102*, 10460–10465.
76. Low, W.-K., Dang, Y., Bhat, S., Romo, D., and Liu, J. O. (2007) Substrate-dependent targeting of eukaryotic translation initiation factor 4A by pateamine A: Negation of domain-linker regulation of activity, *Chem. Biol. 14*, 715–727.
77. Brodeleau, M.-E., Cencic, R., Lindquist, L., Oberer, M., Northcote, P., Wagner, G., and Pelletier, J. (2006) RNA-mediated sequestration of the RNA helicase eIF4A by pateamine A inhibits translation initiation, *Chem. Biol. 13*, 1287–1295.
78. Dang, Y., Low, W.-K., Xu, J., Gehring, N. H., Dietz, H. C., Romo, D., and Liu, J. O. (2009) Inhibition of nonsense-mediated mRNA decay by the natural product pateamine A through eukaryotic initiation factor 4AIII, *J. Biol. Chem. 284*, 23613–23621.
79. Kuznetsov, G., Xu, Q., Rudolph-Owen, L., TenDyke, K., Liu, J., Towle, M., Zhao, N., Marsh, J., Agoulnik, S., Twine, N., Parent, L., Chen, Z., Shie, J.-L., Jiang, Y., Zhang, H., Du, H., Boivin, R., Wang, Y., Romo, D., and Littlefield, B. A. (2009) Potent *in vitro* and *in vivo* anticancer activities of des-methyl, des-amino pateamine A, a synthetic analogue of marine natural product pateamine A, *Mol. Cancer Ther. 8*, 1250–1260.
80. Walsh, C. (2003) *Antibiotics: Actions, Origins, Resistance*, ASM Press, Washington, D.C.
81. Newton, G. C., and Abraham, E. P. (1956) Isolation of cephalosporin C, a penicillin-like antibiotic containing D-alpha-aminoadipic acid, *Biochem. J. 62*, 651–658.

82. Staub, I., and Sieber, S. A. (2008) β-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.
83. Staub, I., and Sieber, S. A. (2009) β-Lactam probes as selective chemical-proteomic tools for the identification and functional characerization of resistance associated enzymes in MRSA, *J. Am. Chem. Soc. 131*, 6271–6276.
84. Bebrone, C. (2007) Metallo-beta-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily, *Biochem. Pharmacol. 74*, 1688–1701.
85. Evans, M. J., Saghatelian, A., Sorensen, E. J., and Cravatt, B. F. (2005) Target discovery in small-molecule cell-based screens by in situ proteome reactivity profiling, *Nat. Biotechnol. 23*, 1303–1307.
86. Bode, H. B., and Muller, R. (2005) The impact of bacterial genomics on natural product research, *Angew. Chem., Int. Ed. Engl. 44*, 6828–6846.
87. Nett, M., Ikeda, H., and Moore, B. S. (2009) Genomic basis for natural product biosynthetic diversity in the actinomycetes, *Nat. Prod. Rep. 26*, 1362–1384.
88. Jenke-Kodama, H., Sandmann, A., Muller, R., and Dittmann, E. (2005) Evolutionary implications of bacterial polyletide synthases, *Mol. Biol. Evol. 22*, 2027–2039.
89. Van Lanen, S. G., and Shen, B. (2006) Microbial genomics for the improvement of natural product discovery, *Curr. Opin. Microbiol. 9*, 252–260.
90. Usera, A. R., and O'Connor, S. E. (2009) Mechanistic advances in plant natural product enzymes, *Curr. Opin. Chem. Biol. 13*, 492–498.
91. Das, A., and Khosla, C. (2009) Biosynthesis of aromatic polyketides in bacteria, *Acc. Chem. Res. 42*, 631–639.
92. Neumann, C. S., Fujimori, D. G., and Walsh, C. T. (2008) Halogenation strategies in natural product biosynthesis, *Chem. Biol. 15*, 99–109.
93. Jones, A. C., Gu, L. C., Sorrels, C. M., Sherman, D. H., and Gerwick, W. H. (2009) New tricks from ancient algae: natural product biosynthesis in marine cyanobacteria, *Curr. Opin. Chem. Biol. 13*, 216–223.
94. Bumpus, S. B., Evans, B. S., Thomas, P. M., Ntai, I., and Kelleher, N. L. (2009) A proteomics approach to discovering natural products and their biosynthetic pathways, *Nat. Biotechnol. 27*, 951–956.
95. Meier, J. L., Mercer, A. C., and Burkart, M. D. (2008) Fluorescent profiling of modular biosynthetic enzymes by complementary metabolic and activity based probes, *J. Am. Chem. Soc. 130*, 5443–5445.
96. Meier, J. L., Niessen, S., Hoover, H. S., Foley, T. L., Cravatt, B. F., and Burkart, M. D. (2009) An orthogonal active site identification system (OASIS) for proteomic profiling of natural product biosynthesis, *ACS Chem. Bio. 4*, 948–957.
97. Fischerbach, M. A., and Walsh, C. T. (2006) Assembly-line enzymology for polyketide and nonribosomal peptide antibiotics: Logic, machinery, and mechanisms, *Chem. Rev. 106*, 3468–3496.
98. Sieber, S. A., and Marahiel, M. A. (2005) Molecular mechanisms underlying nonribosomal peptide synthesis: Approaches to new antibiotics, *Chem. Rev. 105*, 715–738.
99. Meier, J. L., and Burkart, M. D. (2009) The chemical biology of modular biosynthetic enzymes, *Chem. Soc. Rev. 38*, 2012–2045.
100. Liu, Y., Patricelli, M. P., and Cravatt, B. F. (1999) Activity-based protein profiling: the serine hydrolases, *Proc. Natl. Acad. Sci. U.S.A. 96*, 14694–14699.
101. Speers, A. E., and Cravatt, B. F. (2004) Profiling enzyme activities in vivo using click chemistry methods, *Chem. Biol. 11*, 535–546.
102. Washburn, M. P., Wolters, D., and Yates, J. R. (2001) Large-scale analysis of the yeast proteome by multidimensional protein identification technology, *Nat. Biotechnol. 19*, 242–247.
103. Tulp, M., and Bohlin, L. (2005) Rediscovery of known natural products: nuisance or goldmine? *Bioorg. Med. Chem. 13*, 5274–5282.