J. Microbiol. Biotechnol. (2006), **16**(5), 651–660

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Chemical Genomics with Natural Products

JUNG, HYE JIN AND HO JEONG KWON*

Chemical Genomics Laboratory, Department of Biotechnology, College of Engineering, Yonsei University, Seoul 120-749, Korea

Received: March 31, 2006
Accepted: May 2, 2006

Natural products are a rich source of biologically active small molecules and a fertile area for lead discovery of new drugs [10, 52]. For instance, 5% of the 1,031 new chemical entities approved as drugs by the US Food and Drug Administration (FDA) were natural products between 1981 and 2002, and another 23% were natural product-derived molecules [53]. These molecules have evolved through millions of years of natural selection to interact with biomolecules in the cells or organisms and offer unrivaled chemical and structural diversity [14, 37]. Nonetheless, a large percentage of nature remains unexplored, in particular, in the marine and microbial environments. Therefore, natural products are still major valuable sources of innovative therapeutic agents for human diseases.

However, even when a natural product is found to exhibit biological activity, the cellular target and mode of action of the compound are mostly mysterious. This is also true of many natural products that are currently under clinical trials or have already been approved as clinical drugs [11]. The lack of information on a definitive cellular target for a biologically active natural product prevents the rational design and development of more potent therapeutics. Therefore, there is a great need for new techniques to expedite the rapid identification and validation of cellular targets for biologically active natural products.

Chemical genomics is a new integrated research engine toward functional studies of genome and drug discovery [40, 69]. The identification and validation of cellular receptors of biologically active small molecules is one of the key goals of the discipline. This eventually facilitates subsequent rational drug design, and provides valuable information on the receptors in cellular processes. Indeed, several biologically crucial proteins have already been identified as targets for natural products using chemical genomics approach (Table 1). Herein, the representative case studies of chemical genomics using natural products derived from microbes, marine sources, and plants will be introduced.

Key words: Chemical genomics, natural products, target identification and validation

*Corresponding author
Phone: 82-2-2123-5883; Fax: 82-2-362-7265;
E-mail: kwonhj@yonsei.ac.kr

Chemical Genomics: An Integrated Research Engine for Target Identification and Validation of Natural Products

Chemical genomics is an inter- and multidisciplinary research engine, which utilizes small molecules to explore the function of genes and accelerate the drug discovery [40, 69]. Bioactive small molecules that are permeable to cellular membrane and bind to its cognate target protein can exert the phenotype changes of the cells or organisms. Thus, small molecules are temporally used to perturb biological phenotypes by binding to functional proteins, whereas genetic mutation permanently makes alteration of genetic composition and biological phenotypes in the conventional genetics. Generally, chemical genomics can be divided into two concepts, as with conventional genetics, depending on the starting tools used in the study, "forward" and "reverse" chemical genomics [39] (Fig. 1). Forward chemical genomics starts from the isolation of small molecules that exhibit specific activity toward certain phenotypes in cells and organisms, and then determines the protein targets of the chemicals by affinity-, genetics-, and genomics-based target identification [72]. Generally, affinity-based target identification of natural products have been mainly used for this aim. Figure 2 represents popular tags and linkers for construction of molecular probes of small molecules and target identification methods based on the affinity selection. Conversely, the newly identified targets from forward chemical genomics can be utilized to develop more potent and clinically druggable agents using

Table 1. Natural products and their target proteins identified by a chemical genomics approach.

Natural products	Target proteins	Natural products	Target proteins
Microbial natural products		Marine natural products	
Cyclosporin A	Cyclophilin/calcineurin [22]	Bengamide E	MetAP-2 [83]
Doxorubicin	hNopp140 [30]	Didemnin B	PPT [16]
Eponemycin	20S proteasome [50]	Halichondrin B	Tubulin [84]
FK506	FKBP/calcineurin [23]	Ilimaquinone	SAHase [61]
FR182877	CE-1 [2]	Pateamine A	eIF4A [46]
Fumagillin	MetAP-2 [77]		
Lactacystin	20S proteasome [17]	Plant natural products	
Leptomycin	Crm-1 [54]		
Myriocin	LCB1, 2 [12]	Colchicine	Tubulin [87]
Ovalicin	MetAP-2 [20]	Curcumin	APN [73]
Radicicol	Hsp90 [70]	HBC	Ca^{2+}/CaM [74]
Rapamycin	TOR [25]	Parthenolide	IKKβ [38]
Taxol	Tubulin [24]		
Trapoxin B	HDAC [80]		
Trichostatin A	HDAC [89]		

high throughput screening (HTS) of chemical libraries in reverse chemical genomics [49]. Structure-based drug design can also increase the efficiency and speed of the reverse chemical genomics process [27]. Consequently, these approaches of chemical genomics provide novel functions of protein targets as well as new lead compounds having therapeutic value. Biologically active natural products have proven to be invaluable resources in the advance of these system-wide approaches [59]. This review focuses on how chemical genomics has been applied to mode-of-

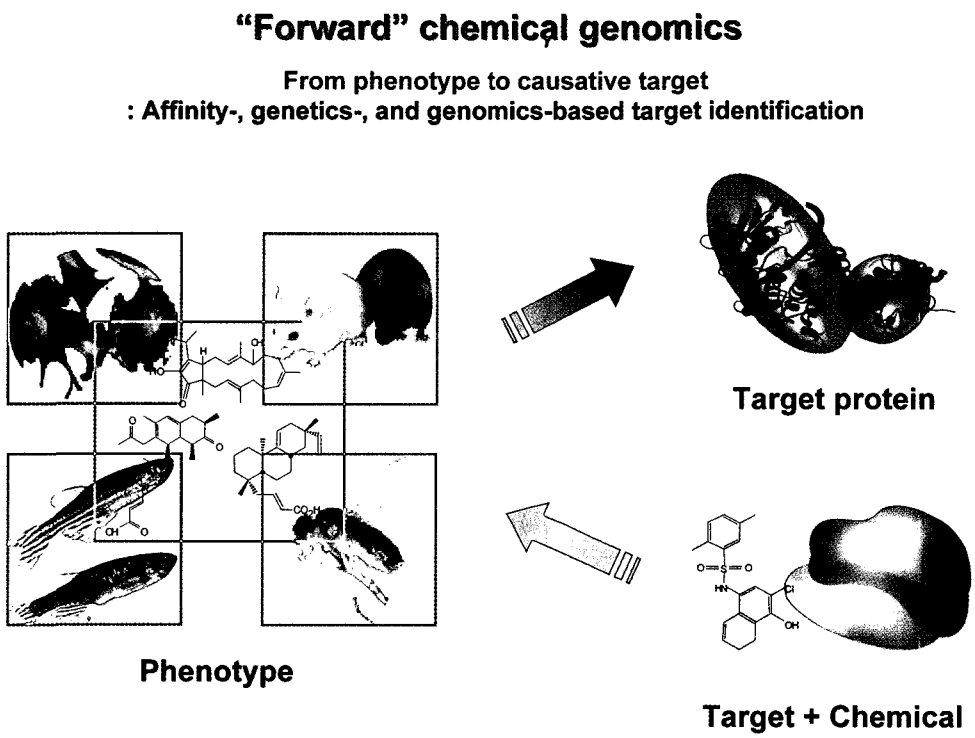

Fig. 1. Schematic illustration of chemical genomics.
Concepts of forward and reverse chemical genomics and their representative agenda.

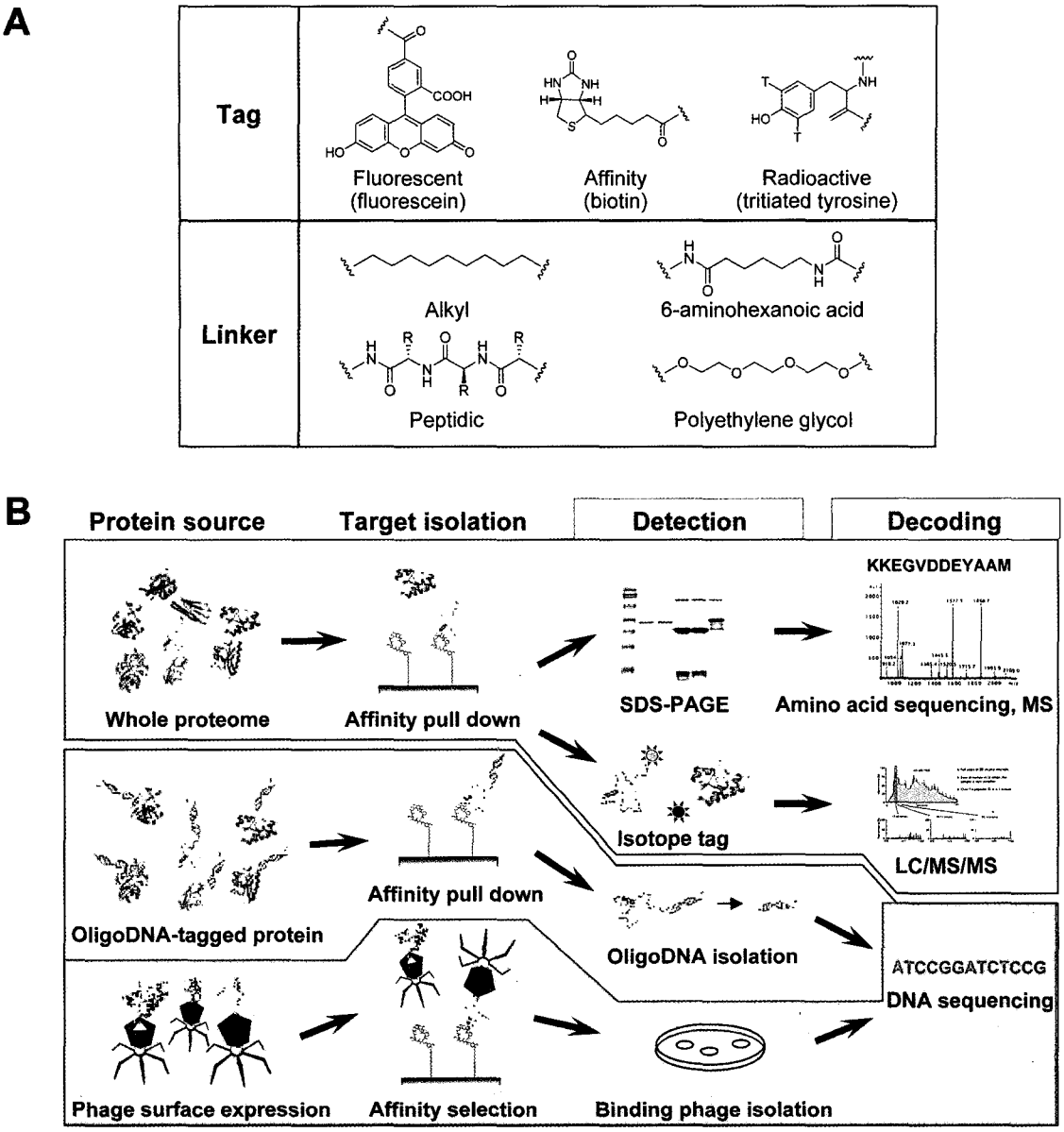

Fig. 2. Schematic representation of chemical genomics-based target identification for bioactive small molecules.
A. Representative tags and linkers for construction of molecular probes for small molecules; **B.** Several target identification methods based on the affinity selection.

action studies of natural products, and what have been the major benefits to modern biological, chemical, and medical sciences through this discipline (Fig. 3).

MICROBIAL NATURAL PRODUCTS: A RICH AND UNIQUE RESOURCE OF MOLECULAR PROBES FOR CHEMICAL GENOMICS

Hundreds of natural products from microbial metabolites have served as unique and novel resources to be specific biochemical agents as well as clinical drugs. As these compounds were generated from the natural evolution procedure, their biological receptors play key roles in a certain cellular circuit and signal pathway. Some of the representative studies exploring the mode of action of small molecules from microbes have provided new insights on the role of specific target proteins in the cells and led to the addition of new drug entities for therapeutic purposes.

Cyclosporin A and FK506

Cyclosporin A (CsA) is a cyclic undecapeptide isolated from the fungus *Tolypocladium inflatum* [65]. This small molecule is the principal immunosuppressant used in

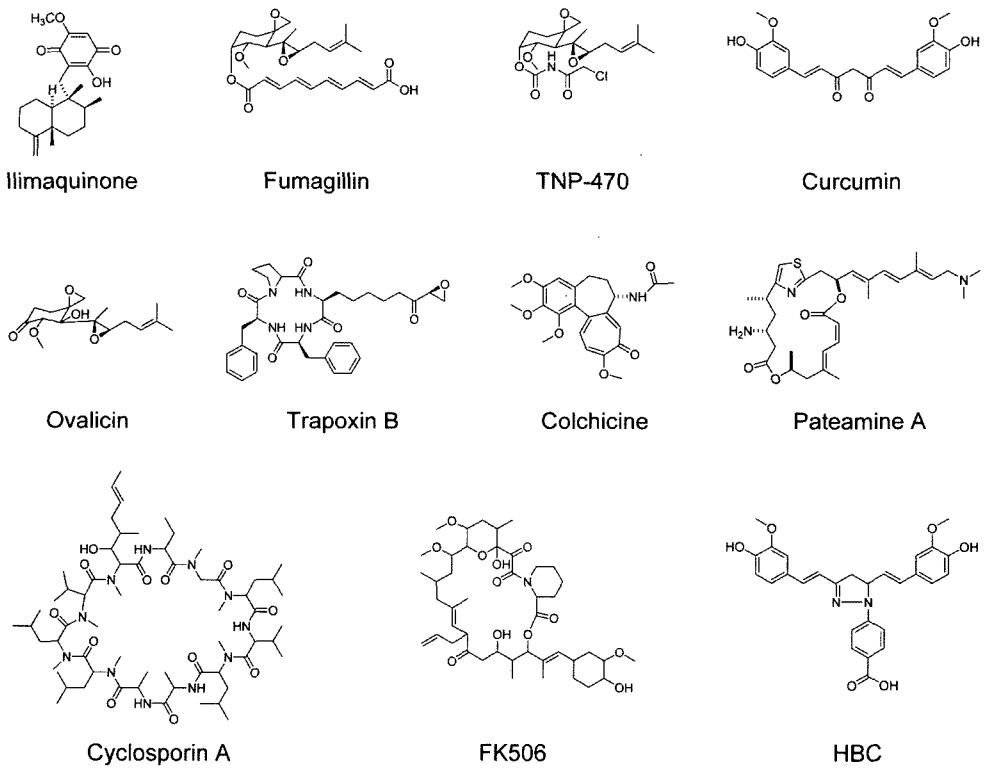

Fig. 3. Chemical structures of natural products introduced in this review.

organ transplantation and was approved by the US FDA in 1983 [7]. Although it was clear that CsA achieved its immunosuppression by inhibiting T lymphocyte activation, the exact cellular target and mode of action of the drug was unsolved. Handschumacher *et al.* [22] used a radioisotope labeled purification approach to identify the cellular target of CsA. A tritiated analog of CsA was incubated with homogenized bovine thymus, and gel electrophoresis of the final purified radioactive fractions revealed a protein band of 15 kDa. This protein could not be found in any protein database of the time, and it was named as "cyclophilin".

FK506, also known as tacrolimus, is a macrolide isolated from the actinomycete *Streptomyces tsukubaensis* [35]. FK506 also exhibits immunosuppression by inhibiting T lymphocyte activation, but at concentrations several hundred-fold lower than CsA [68]. Siekierka *et al.* [75] used a similar approach to that for the case of CsA to identify the cellular target of FK506. The cytosolic extract from homogenized human Jurkat T cells was incubated with a tritiated analog of FK506, and gel electrophoresis of the final purified material revealed a protein band of 12 kDa. Interestingly, however, the amino acid sequence of this protein showed no homology to cyclophilin, and this new protein was named as "FK506 binding protein (FKBP)". Harding *et al.* [23] also identified FKBP as a cellular target of FK506 by a different approach to the radioisotope labeling method, which was affinity chromatography using FK506-derivatized Affigel.

At the begining, it was thought that inhibition of the peptidyl-prolyl isomerase activities of cyclophilin and FKBP by CsA and FK506, respectively, might be responsible for the immunosuppressive effects of these drugs. Consequently, proteins bearing peptidyl-prolyl isomerase activities were named "immunophilins". However, further studies provided evidence that was inconsistent with a causative relationship between the enzyme activities and immunosuppression [6, 26]. These findings suggested that the CsA-cyclophilin and FK506-FKBP complexes may actually bind to another cellular target, and inhibition of this common target is responsible for immunosuppression. Liu *et al.* [43] once again used chemical genomics to identify the common cellular target of both CsA-cyclophilin and FK506-FKBP. A GST-FKBP fusion protein (GFK) was immobilized onto a glutathione-Sepharose resin, which was then preincubated with FK506. The FK506-charged GFK resin was incubated with homogenized bovine thymus, and the proteins retained by the affinity support were identified as calmodulin and subunits of calcineurin, a calcium- and calmodulin-dependent phosphatase. Similar results were obtained from a GST-cyclophilin fusion protein immobilized on a glutathione-Sepharose resin and preincubated with CsA.

The identification of calcineurin as a common cellular receptor for both FK506-FKBP and CsA-cyclophilin led to

the discovery that calcineurin is a key component in T cell activation [15]. It was found that calcineurin dephosphorylates nuclear factor NFAT, which is required for transcription of interleukin-2 and subsequent T cell activation. The study of CsA and FK506 clearly highlights the value of natural products, both as a source of new pharmaceuticals and as molecular probes, for understanding of cellular processes involved in the immune response.

Trapoxin B

Another popular example of natural products from microbes that was used as a molecular probe of chemical genomics could be trapoxin B. Trapoxin B is a cyclic tetrapeptide isolated from the fungus *Helicoma ambiens* [29]. The molecule is a potent anticancer agent that exhibits detransformation activity against *v-sis* oncogene-transformed NIH-3T3 cells. Biochemical studies revealed that trapoxin B is an irreversible inhibitor of histone deacetylation in cultured mammalian cells [33]. Despite that the importance of histone deacetylation in cell cycle progression had been known for over 30 years, the molecular entities responsible for these deacetylation reactions remained elusive. Taunton *et al.* [80] identified the putative histone deacetylase (HDAC) as the cellular target of trapoxin B. Homogenized bovine thymus was passed through a column of trapoxin B-derivatized Affigel, and amino acid sequencing of protein retained by the column revealed a new protein, named as "HD1". Subsequent experiments confirmed that HD1 has HDAC activity and this activity is inhibited by trapoxin B.

The discovery of HD1 has made it possible to study the role of histone deacetylation in gene transcription and cell cycle progression. It has also facilitated the design of new HDAC inhibitors as potential anticancer drugs [82]. Several other natural products, including apicidin, FR901228, depudecin, and FR235222 have proven to be inhibitors of HDAC [63]. In addition, a combinatorial library of 7,200 small molecules has been synthesized based on the structure of trapoxin B in an attempt to identify new and more potent HDAC inhibitors [78].

Fumagillin and Ovalicin

The final examples of microbial natural products as the molecular probe of chemical genomics are fumagillin and ovalicin. Fumagillin is an epoxide-containing natural product isolated from the fungus *Aspergillus fumigatus*. Recently, it was discovered that fumagillin is a potent inhibitor of angiogenesis, causing late G_1 phase endothelial cell cycle arrest [1]. However, the use of fumagillin as a therapeutic agent is limited by its bone marrow toxicity. TNP-470 is a semisynthetic analog of fumagillin that retains the activity of its parent compound, but has far lower bone marrow toxicity [28]. Despite the fact that TNP-470 had undergone numerous pharmacological studies and clinical trials, little was known about the cellular target and mode of action of fumagillin or TNP-470. Sin *et al.* [77] used an affinity matrix-based purification to identify the cellular target of fumagillin. A biotinylated analog of fumagillin was incubated with human umbilical venous endothelial cells, and biotinylated proteins were visualized using avidin-horseradish peroxidase. Amino acid sequencing identified this protein as human methionyl aminopeptidase (MetAP-2), a cobalt-dependent metalloprotease that cleaves methionine from the amino termini of peptides and proteins. Mass spectrometry and X-ray crystallography revealed that the imidazole nitrogen of histidine-231 at the active site of MetAP-2 forms a covalent bond with the carbon of the spirocyclic epoxide of fumagillin [44, 47].

Ovalicin is another epoxide-containing natural product isolated from the fungus *Pseudorotium ovalis* [76]. The compound, like fumagillin, is a potent inhibitor of angiogenesis. To identify the cellular target of ovalicin, Griffith *et al.* [20] incubated bovine aortic endothelial cell lysate with a biotinylated ovalicin probe and then added a streptavidin-agarose resin. The resin was separated from the mixture and the bound protein was identified as MetAP-2.

The identification of MetAP-2 as the cellular target of both fumagillin and ovalicin has led to better understanding of angiogenesis and cell cycle progression [67]. The findings have also provided the rational design and synthesis of a diverse range of new fumagillin analogs as angiogenesis inhibitors [62].

MARINE NATURAL PRODUCTS: ANOTHER UNIQUE SOURCE OF MOLECULAR PROBES FOR CHEMICAL GENOMICS

The marine environment is a unique pool for enriching the diverse complexity of life. In particular, several unique natural products from marine sponges as well as microbes have served as a valuable source of molecular probes for chemical genomics. Among the many examples, two representative studies will be demonstrated.

Pateamine A

Pateamine A (PatA) is a potent antiproliferative and proapoptotic marine natural product isolated from sponges of the *Mycale* species [55]. Very recently, Low *et al.* [46] identified the molecular target of PatA using affinity matrix-based purification. They generated the biotin-PatA in conjunction with streptavidin-Sepharose and affinity binding assays were subsequently performed with various cell lysates. Two putative PatA-binding proteins were detected in RKO cell lysates, with apparent molecular masses of 48 and 38 kDa. By using MALDI-TOF mass spectrometry, the 48 and 38 kDa bands were identified as

eIF4A, a translation initiation factor, and the serine/threonine kinase receptor associated protein (STRAP), respectively. The binding of eIF4A by biotin-PatA was reproducibly observed in HeLa cell lysate, wheat germ extract, and rabbit reticulocyte lysate. However, the binding of STRAP to biotin-PatA did not appear in all instances. The identity of the 48 kDa protein was confirmed by Western blot using eIF4A-specific antibodies after the pull-down assay. The specificity of eIF4A binding and its relation to biological activity was further confirmed with two biologically inactive analogs that were unable to compete with biotin-PatA for eIF4A binding and the active analog DMDA-PatA that was active in competition, consistent with their cellular activities. In addition, the overexpression of eIF4A in HeLa cells caused a 5-fold increase in IC_{50} for PatA, suggesting that eIF4A is likely the primary mediator of cell proliferation inhibition by PatA.

Translation initiation in eukaryotes is accomplished through the coordinated and orderly action of a large number of proteins, including the eIF4 initiation factors [19, 64]. PatA specifically targets eIF4A-dependent translation initiation in eukaryotes. As a consequence, it can cause either cell cycle arrest or apoptosis in transformed cells. Therefore, PatA could be a valuable molecular probe for future studies of eukaryotic translation initiation and a lead compound for the development of anticancer agents.

Ilimaquinone

Ilimaquinone is a sesquiterpene quinone isolated from the marine sponge *Hippospongia metachromia* [48]. This compound has been found to break down the Golgi apparatus into small vesicles, thereby blocking cellular secretion [79]. Radeke and Snapper [61] used a radioisotope and photoaffinity labeled derivative of the compound to identify cellular receptors for ilimaquinone. A tritiated azidobenzene moiety was attached to a synthetic chloroquinone analog of ilimaquinone to form a photoaffinity reagent. Bovine liver extract was incubated with the photoaffinity reagent in the presence of ultraviolet light, and amino acid sequencing of the purified 48 kDa protein revealed it to be the known enzyme *S*-adenosylhomocysteinase (SAHase). The binding of SAHase to ilimaquinone was reproducibly observed in a second experiment using an ilimaquinone-agarose affinity resin [60]. The following enzymatic assays revealed that ilimaquinone is a competitive inhibitor of SAHase.

SAHase plays a key role in cellular methylation chemistry by catalyzing the breakdown of *S*-adenosylhomocysteine (SAH) to homocysteine and adenosine [88]. The authors noted that SAHase inhibition would cause the intracellular accumulation of SAH, which is a potent feedback inhibitor of methyltransferases. These results support the assertion that methylation events play an important role in cellular secretory and vesicle-mediated processes.

Plant Natural Products: A Never-Ending Sources of Molecular Probes for Chemical Genomics

Many plants have traditionally been used as dietary and medicinal agents without having detail information on their modes of action. Accordingly, these pharmacological values of plants have attracted the attention to the target identification of active compounds as well as their role in pharmacological effect by chemical genomics.

Colchicine

Colchicine is a tropolone alkaloid isolated from meadow saffron (*Colchicum autumnale*) [9]. The compound was found to destabilize microtubules and thereby disrupt mitosis [42]. However, the exact composition of microtubules and the mode of action of colchicine were not known. Weisenberg *et al.* [8, 87] used a radioisotope labeling approach to explore the cellular receptor of colchicine and thereby determine the molecular composition of microtubules. Porcine brain extract was incubated with a tritiated analog of colchicine and the resulting mixture was fractionated by chromatography. The radioactive fractions

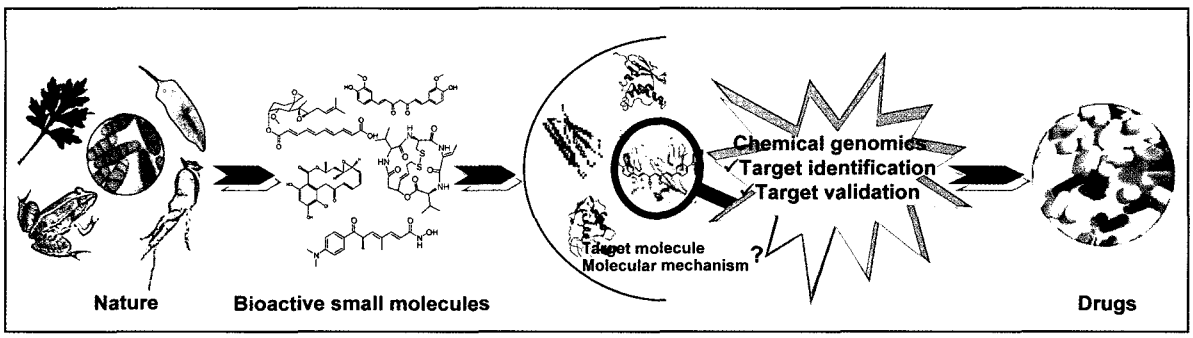

Fig. 4. Chemical genomics with natural products to study the function of target proteins and to develop therapeutic drugs.

were analyzed by gel electrophoresis, revealing a major protein band of 120 kDa. Treatment of this protein with guanidine hydrochloride yielded a single protein band of 57 kDa, suggesting that the colchicine-binding protein is a homodimer. Each dimer was found to bind two molecules of GTP, which stabilize the protein, and one molecule of colchicine. The authors concluded that this protein must be the subunit of microtubules. The amino acid sequence of the colchicine-binding protein was determined and the protein was named as "tubulin" [51].

The identification of tubulin as the cellular receptor for colchicine and the subunit of microtubules marked the beginning of a long history of using small molecules to probe the biology of the cytoskeleton. Since then, other natural products that interact with components of the cytoskeleton have been identified, including vinblastine, podophyllotoxin, cytochalasin B, paclitaxel (Taxol), latrunculin B, dolastatin-10, jasplakinolide, and epothilone A [24, 58]. Clearly, natural products are an extremely valuable source of structurally diverse compounds capable of interacting with components of the cytoskeleton. Indeed, the proteins of the cytoskeleton play a central role in cell division and hence are the target of an increasing number of anticancer drugs [3, 31].

Curcumin and HBC

Curcumin, a phenolic natural product isolated from the rhizome of *Curcuma longa*, is a potent chemopreventive agent, which has been entered into the Phase I clinical trials for colon cancer chemoprevention by the National Cancer Institute [32]. Recent evidences have shown that the potent chemopreventive activity of curcumin is, in part, through the direct inhibition of angiogenesis [4, 81]. However, the precise molecular mechanism of the angiogenesis inhibition by curcumin has not been fully understood.

Aminopeptidase N (APN) is a part of the M1 family of zinc-dependent metallopeptidase, which is mainly localized at the cellular membrane [45]. Accumulating evidence has shown that APN plays an important role in tumor metastasis via the stimulation of tumor cell invasion and angiogenesis [18, 66]. Our group has thus focused on the screening and development of new APN inhibitors from natural products and chemical libraries using a purified enzyme assay system. As a result, several candidates of APN inhibitors, including curcumin were isolated [73]. The interaction between curcumin and APN was confirmed by both *in vitro* surface plasmon resonance analysis and *in vivo* antibody competition assay. Interestingly, the invasiveness of APN+ cells was more sensitive against curcumin than that of APN- cells, implying that APN is a physiological target of curcumin for its antiangiogenic activity. These results demonstrate that APN is a promising target of angiogenesis-related diseases, and curcumin could be a new lead for the development of an APN inhibitor.

We thus developed several novel curcumin derivatives and evaluated their biological activities [71]. Among them, hydrazinobenzoylcurcumin (HBC), a synthetic benzoic acid derivative of curcumin, showed potent inhibitory activities against the proliferation of several human cancer cells. However, it turned out that HBC did not inhibit the activity of APN. We thus attempted to isolate the target protein of HBC using chemical genomics approaches [74]. Biotinyl-HBC was immobilized onto streptavidin-coated wells, and T7 phages encoding human cDNA libraries were used for affinity selection of the HBC-binding protein. As a result, Ca^{2+}/calmodulin (Ca^{2+}/CaM) was isolated as a direct binding protein of HBC.

Ca^{2+}/CaM is a calcium-binding protein that is implicated in a variety of cellular functions, including cell growth and proliferation, and has been recognized as a potential target for cancer chemotherapy [13, 21, 86]. In biological systems, HBC induces sustained phosphorylation of ERK1/2 and activates $p21^{WAF1}$ expression, resulting in the suppression of the cell cycle progression of HCT15 colon cancer cells. The biological activities of HBC are similar to those of other Ca^{2+}/CaM antagonists, suggesting that Ca^{2+}/CaM is a biologically relevant target of HBC [56, 85]. These results demonstrate that HBC is a new Ca^{2+}/CaM antagonist with a unique structure and offers a lead compound for the development of more potent Ca^{2+}/CaM antagonists.

CONCLUSION

Natural products and their derivatives have historically been invaluable as a source of therapeutic agents. However, in the past decade, research into natural products in the pharmaceutical industry has declined, owing to issues such as the lack of compatibility of traditional natural-product extract libraries with high-throughput screening. However, recent advances in separation technology and structure elucidation have revived the implication of natural products in drug discovery [57]. Natural products not only serve as drugs or templates for drugs directly, but also in many instances lead to the discovery of novel biology that provides a better understanding of targets and pathways involved in the disease process. To explore the great value of natural products, chemical genomics is a powerful approach as shown by the several cases in this review. Besides these several molecular probes from natural products described earlier, new bioactive small molecules with distinct structures have been continuously discovered from nature [5, 34, 36, 41]. An integrated approach using chemical genomics for target identification and validation of these new entities will certainly help to unblock hurdles in the process of drug discovery and development, and to decipher complicated circuits in the cells (Fig. 4).

Acknowledgments

This work was partly supported by grants from the Korea Research Foundation, Brain Korea 21 Project, and Yonsei University, Republic of Korea.

REFERENCES

1. Abe, J., W. Zhou, N. Takuwa, J. Taguchi, K. Kurokawa, M. Kumada, and Y. Takuwa. 1994. A fumagillin derivative angiogenesis inhibitor, AGM-1470, inhibits activation of cyclin-dependent kinases and phosphorylation of retinoblastoma gene product but not protein tyrosyl phosphorylation or protooncogene expression in vascular endothelial cells. *Cancer Res.* **54:** 3407–3412.
2. Adam, G. C., C. D. Vanderwal, E. J. Sorensen, and B. F. Cravatt. 2003. (–)-FR182877 is a potent and selective inhibitor of carboxylesterase-1. *Angew Chem. Int. Ed. Engl.* **42:** 5480–5484.
3. Altmann, K. H. 2001. Microtubule-stabilizing agents: A growing class of important anticancer drugs. *Curr. Opin. Chem. Biol.* **5:** 424–431.
4. Arbiser, J. L., N. Klauber, R. Rohan, R. van Leeuwen, M. T. Huang, C. Fisher, E. Flynn, and H. R. Byers. 1998. Curcumin is an *in vivo* inhibitor of angiogenesis. *Mol. Med.* **4:** 376–383.
5. Bae, M. A., K. Yamada, D. Uemura, J. H. Seu, and Y. H. Kim.1998. Aburatubolactam C, a novel apoptosis-inducing substance produced by marine *Streptomyces* sp. SCRC A-20. *J. Microbiol. Biotechnol.* **8:** 455–460.
6. Bierer, B. E., P. K. Somers, T. J. Wandless, S. J. Burakoff, and S. L. Schreiber. 1990. Probing immunosuppressant action with a nonnatural immunophilin ligand. *Science* **250:** 556 –559.
7. Borel, J. F., C. Feurer, C. Magnee, and H. Stahelin. 1977. Effects of the new anti-lymphocytic peptide cyclosporin A in animals. *Immunology* **32:** 1017–1025.
8. Borisy, G. G. and E. W. Taylor. 1967. The mechanism of action of colchicine. Binding of colchicine-3H to cellular protein. *J. Cell Biol.* **34:** 525–533.
9. Buchnicek, J. 1950. Colchicine in ripening seeds of the wild saffron (*Colchicum autumnale* L). *Pharm. Acta Helv.* **25:** 389–401.
10. Butler, M. S. 2004. The role of natural product chemistry in drug discovery. *J. Nat. Prod.* **67:** 2141–2153.
11. Butler, M. S. 2005. Natural products to drugs: Natural product derived compounds in clinical trials. *Nat. Prod. Rep.* **22:** 162–195.
12. Chen, J. K., W. S. Lane, and S. L. Schreiber. 1999. The identification of myriocin-binding proteins. *Chem. Biol.* **6:** 221–235.
13. Cheung, W. Y. 1980. Calmodulin plays a pivotal role in cellular regulation. *Science* **207:** 19–27.
14. Clardy, J. and C. Walsh. 2004. Lessons from natural molecules. *Nature* **432:** 829–837.
15. Clipstone, N. A. and G. R. Crabtree. 1992. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. *Nature* **357:** 695–697.
16. Crews, C. M., W. S. Lane, and S. L. Schreiber. 1996. Didemnin binds to the protein palmitoyl thioesterase responsible for infantile neuronal ceroid lipofuscinosis. *Proc. Natl. Acad. Sci. USA* **93:** 4316–4319.
17. Fenteany, G., R. F. Standaert, W. S. Lane, S. Choi, E. J. Corey, and S. L. Schreiber. 1995. Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. *Science* **5:** 726–731.
18. Fujii, H., M. Nakajima, I. Saiki, J. Yoneda, I. Azuma, and T. Tsuruo. 1995. Human melanoma invasion and metastasis enhancement by high expression of aminopeptidase N/ CD13. *Clin. Exp. Metastasis* **13:** 337–344.
19. Gingras, A. C., B. Raught, and N. Sonenberg. 1999. eIF4 initiation factors: Effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu. Rev. Biochem.* **68:** 913–963.
20. Griffith, E. C., Z. Su, B. E. Turk, S. Chen, Y. H. Chang, Z. Wu, K. Biemann, and J. O. Liu. 1997. Methionine aminopeptidase (type 2) is the common target for angiogenesis inhibitors AGM-1470 and ovalicin. *Chem. Biol.* **4:** 461–471.
21. Hait, W. N. 1987. Targeting calmodulin for the development of novel cancer chemotherapeutic agents. *Anticancer Drug Des.* **2:** 139–149.
22. Handschumacher, R. E., M. W. Harding, J. Rice, R. J. Drugge, and D. W. Speicher. 1984. Cyclophilin: A specific cytosolic binding protein for cyclosporin A. *Science* **226:** 544–547.
23. Harding, M. W., A. Galat, D. E. Uehling, and S. L. Schreiber. 1989. A receptor for the immunosuppressant FK506 is a *cis-trans* peptidyl-prolyl isomerase. *Nature* **341:** 758–760.
24. He, L., G. A. Orr, and S. B. Horwitz. 2001. Novel molecules that interact with microtubules and have functional activity similar to taxol. *Drug Discov. Today* **6:** 1153–1164.
25. Heitman, J., N. R. Movva, and M. N. Hall. 1991. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* **23:** 905–909.
26. Heitman, J., N. R. Movva, P. C. Hiestand, and M. N. Hall. 1991. FK 506-binding protein proline rotamase is a target for the immunosuppressive agent FK 506 in *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA* **88:** 1948–1952.
27. Hou, T. and X. Xu. 2004. Recent development and application of virtual screening in drug discovery: An overview. *Curr. Pharm. Des.* **10:** 1011–1033.
28. Ingber, D., T. Fujita, S. Kishimoto, K. Sudo, T. Kanamaru, H. Brem, and J. Folkman. 1990. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumor growth. *Nature* **348:** 555–557.
29. Itazaki, H., K. Nagashima, K. Sugita, H. Yoshida, Y. Kawamura, Y. Yasuda, K. Matsumoto, K. Ishii, N. Uotani, and H. Nakai. 1990. Isolation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation activities as antitumor agents. *J. Antibiot.* **43:** 1524–1532.

30. Jin, Y., J. Yu, and Y. G. Yu. 2002. Identification of hNopp140 as a binding partner for doxorubicin with a phage display cloning method. *Chem. Biol.* **9:** 157–162.
31. Jordan, M. A. and L. Wilson. 1998. Microtubules and actin filaments: Dynamic targets for cancer chemotherapy. *Curr. Opin. Cell Biol.* **10:** 123–130.
32. Kelloff, G. J., J. A. Crowell, E. T. Hawk, V. E. Steele, R. A. Lubet, C. W. Boone, J. M. Covey, L. A. Doody, G. S. Omenn, and P. Greenwald. 1996. Strategy and planning for chemopreventive drug development: Clinical development plans II. *J. Cell Biochem.* **26:** 54–71.
33. Kijima, M., M. Yoshida, K. Sugita, S. Horinouchi, and T. Beppu. 1993. Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. *J. Biol. Chem.* **268:** 22429–22435.
34. Kim, H. J., J. H. Kim, C. H. Lee, and H. J. Kwon. 2006. Gentisyl alcohol, an antioxidant from microbial metabolite, induces angiogenesis *in vitro*. *J. Microbiol. Biotechnol.* **16:** 475–479.
35. Kino, T., H. Hatanaka, S. Miyata, N. Inamura, M. Nishiyama, T. Yajima, T. Goto, M. Okuhara, M. Kohsaka, H. Aoki, and H. Imanaka. 1987. FK-506, a novel immunosuppressant isolated from a *Streptomyces*. *J. Antibiot.* **40:** 1256–1265.
36. Ko, H. R. 2002. PC-766B' and PC-766B, 16-membered macrolide angiogenesis inhibitors produced by *Nocardia* sp. RK97-56. *J. Microbiol. Biotechnol.* **12:** 829–833.
37. Koehn, F. E. and G. T. Carter. 2005. The evolving role of natural products in drug discovery. *Nat. Rev. Drug Discov.* **4:** 206–220.
38. Kwok, B. H., B. Koh, M. I. Ndubuisi, M. Elofsson, and C. M. Crews. 2001. The anti-inflammatory natural product parthenolide from the medicinal herb Feverfew directly binds to and inhibits IkappaB kinase. *Chem. Biol.* **8:** 759–766.
39. Kwon, H. J. 2003. Chemical genomics-based target identification and validation of anti-angiogenic agents. *Curr. Med. Chem.* **10:** 717–736.
40. Kwon, H. J. 2006. Discovery of new small molecules and targets towards angiogenesis via chemical genomics approach. *Curr. Drug Targets* **7:** 397–405.
41. Kwon, H. J., J. H. Kim, H. J. Jung, Y. G. Kwon, M. Y. Kim, J. R. Rho, and J. H. Shin. 2001. Anti-angiogenic activity of Acalycixenolide E, a novel marine natural product from *Acalycigorgia inermis*. *J. Microbiol. Biotechnol.* **11:** 656–662.
42. Levine, M. 1951. The action of colchicine on cell division in human cancer, animal, and plant tissues. *Ann. N. Y. Acad. Sci.* **51:** 1365–1408.
43. Liu, J., J. D. Jr. Farmer, W. S. Lane, J. Friedman, I. Weissman, and S. L. Schreiber. 1991. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* **66:** 807–815.
44. Liu, S., J. Widom, C. W. Kemp, C. M. Crews, and J. Clardy. 1998. Structure of human methionine aminopeptidase-2 complexed with fumagillin. *Science* **282:** 1324–1327.
45. Look, A. T., R. A. Ashmun, L. H. Shapiro, and S. C. Peiper. 1989. Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. *J. Clin. Invest.* **83:** 1299–1307.
46. Low, W. K., Y. Dang, T. Schneider-Poetsch, Z. Shi, N. S. Choi, W. C. Merrick, D. Romo, and J. O. Liu. 2005. Inhibition of eukaryotic translation initiation by the marine natural product pateamine A. *Mol. Cell* **20:** 709–722.
47. Lowther, W. T., D. A. McMillen, A. M. Orville, and B. W. Matthews. 1998. The anti-angiogenic agent fumagillin covalently modifies a conserved active-site histidine in the *Escherichia coli* methionine aminopeptidase. *Proc. Natl. Acad. Sci. USA* **95:** 12153–12157.
48. Luibrand, R. T., T. R. Erdman, J. J. Vollmer, P. J. Scheuer, J. Finer, and J. Clardy. 1979. Ilimaquinone, a sesquiterpenoid quinone from a marine sponge. *Tetrahedron* **35:** 609–612.
49. Macarron, R. 2006. Critical review of the role of HTS in drug discovery. *Drug Discov. Today* **11:** 277–279.
50. Meng, L., B. H. Kwok, N. Sin, and C. M. Crews. 1999. Eponemycin exerts its antitumor effect through the inhibition of proteasome function. *Cancer Res.* **59:** 2798–2801.
51. Mohri, H. 1968 Amino-acid composition of "Tubulin" constituting microtubules of sperm flagella. *Nature* **217:** 1053–1054.
52. Newman, D. J., G. M. Cragg, and K. M. Snader. 2000. The influence of natural products upon drug discovery. *Nat. Prod. Rep.* **17:** 215–234.
53. Newman, D. J., G. M. Cragg, and K. M. Snader. 2003. Natural products as sources of new drugs over the period 1981–2002. *J. Nat. Prod.* **66:** 1022–1037.
54. Nishi, K., M. Yoshida, D. Fujiwara, M. Nishikawa, S. Horinouchi, and T. Beppu. 1994. Leptomycin B targets a regulatory cascade of crm1, a fission yeast nuclear protein, involved in control of higher order chromosome structure and gene expression. *J. Biol. Chem.* **269:** 6320–6324.
55. Northcote, P. T., J. W. Blunt, and M. H. G. Munro. 1991. Pateamine: A potent cytotoxin from the New Zealand marine sponge, *Mycale* sp. *Tetrahedron Lett.* **32:** 6411–6414.
56. Osawa, M., M. B. Swindells, J. Tanikawa, T. Tanaka, T. Mase, T. Furuya, and M. Ikura. 1998. Solution structure of calmodulin-W-7 complex: The basis of diversity in molecular recognition. *J. Mol. Biol.* **276:** 165–176.
57. Paterson, I. and E. A. Anderson. 2005. The renaissance of natural products as drug candidates. *Science* **21:** 451–453.
58. Peterson, J. R. and T. J. Mitchison. 2002. Small molecules, big impact: A history of chemical inhibitors and the cytoskeleton. *Chem. Biol.* **9:** 1275–1285.
59. Piggott, A. M. and P. Karuso. 2004. Quality, not quantity: The role of natural products and chemical proteomics in modern drug discovery. *Comb. Chem. High Throughput Screen* **7:** 607–630.
60. Radeke, H. S., C. A. Digits, R. L. Casaubon, and M. L. Snapper. 1999. Interactions of (-)-ilimaquinone with methylation enzymes: Implications for vesicular-mediated secretion. *Chem. Biol.* **6:** 639–647.
61. Radeke, H. S. and M. L. Snapper. 1998. Photoaffinity study of the cellular interactions of ilimaquinone. *Bioorg. Med. Chem.* **6:** 1227–1232.
62. Rodeschini, V., J. G. Boiteau, P. Van de Weghe, C. Tarnus, and J. Eustache. 2004. MetAP-2 inhibitors based on the fumagillin structure. Side-chain modification and ring-substituted analogues. *J. Org. Chem.* **69:** 357–373.

63. Rosato, R. R. and S. Grant. 2004. Histone deacetylase inhibitors in clinical development. *Expert Opin. Investig. Drugs* **13:** 21–38.
64. Rozen, F., I. Edery, K. Meerovitch, T. E. Dever, W. C. Merrick, and N. Sonenberg. 1990. Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. *Mol. Cell Biol.* **10:** 1134–1144.
65. Ruegger, A., M. Kuhn, H. Lichti, H. R. Loosli, R. Huguenin, C. Quiquerez, and A. von Wartburg. 1976. Cyclosporin A, a peptide metabolite from *Trichoderma polysporum* Rifai, with a remarkable immunosuppressive activity. *Helv. Chim. Acta* **59:** 1075–1092.
66. Saiki, I., H. Fujii, J. Yoneda, F. Abe, M. Nakajima, T. Tsuruo, and I. Azuma. 1993. Role of aminopeptidase N (CD13) in tumor-cell invasion and extracellular matrix degradation. *Int. J. Cancer* **54:** 137–143.
67. Sato, Y. 2003. Aminopeptidases and angiogenesis. *Endothelium* **10:** 287–290.
68. Sawada, S., G. Suzuki, Y. Kawase, and F. Takaku. 1987. Novel immunosuppressive agent, FK506. *In vitro* effects on the cloned T cell activation. *J. Immunol.* **139:** 1797–1803.
69. Schreiber, S. L. 1998. Chemical genetics resulting from a passion for synthetic organic chemistry. *Bioorg. Med. Chem.* **6:** 1127–1152.
70. Sharma, S. V., T. Agatsuma, and H. Nakano. 1998. Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. *Oncogene* **16:** 2639–2645.
71. Shim, J. S., D. H. Kim, H. J. Jung, J. H. Kim, D. Lim, S. K. Lee, K. W. Kim, J. W. Ahn, J. S. Yoo, J. R. Rho, and H. J. Kwon. 2002. Hydrazinocurcumin, a novel synthetic curcumin derivative, is a potent inhibitor of endothelial cell proliferation. *Bioorg. Med. Chem.* **10:** 2987–2992.
72. Shim, J. S. and H. J. Kwon. 2004. Chemical genetics for therapeutic target mining. *Expert Opin. Ther. Targets* **8:** 653–661.
73. Shim, J. S., J. H. Kim, H. Y. Cho, Y. N. Yum, S. H. Kim, H. J. Park, B. S. Shim, S. H. Choi, and H. J. Kwon. 2003. Irreversible inhibition of CD13/aminopeptidase N by the antiangiogenic agent curcumin. *Chem. Biol.* **10:** 695–704.
74. Shim, J. S., J. Lee, H. J. Park, S. J. Park, and H. J. Kwon. 2004. A new curcumin derivative, HBC, interferes with the cell cycle progression of colon cancer cells via antagonization of the Ca2+/calmodulin function. *Chem. Biol.* **11:** 1455–1463.
75. Siekierka, J. J., S. H. Hung, M. Poe, C. S. Lin, and N. H. Sigal. 1989. A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* **341:** 755–757.
76. Sigg, H. P. and H. P. Weber. 1968. Isolation and structure elucidation of ovalicin. *Helv. Chim. Acta* **51:** 1395–1408.
77. Sin, N., L. Meng, M. Q. Wang, J. J. Wen, W. G. Bornmann, and C. M. Crews. 1997. The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. *Proc. Natl. Acad. Sci. USA* **94:** 6099–6103.
78. Sternson, S. M., J. C. Wong, C. M. Grozinger, and S. L. Schreiber. 2001. Synthesis of 7200 small molecules based on a substructural analysis of the histone deacetylase inhibitors trichostatin and trapoxin. *Org. Lett.* **3:** 4239–4242.
79. Takizawa, P. A., J. K. Yucel, B. Veit, D. J. Faulkner, T. Deerinck, G. Soto, M. Ellisman, and V. Malhotra. 1993. Complete vesiculation of Golgi membranes and inhibition of protein transport by a novel sea sponge metabolite, ilimaquinone. *Cell* **73:** 1079–1090.
80. Taunton, J., C. A. Hassig, and S. L. Schreiber. 1996. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* **272:** 408–411.
81. Thaloor, D., A. K. Singh, G. S. Sidhu, P. V. Prasad, H. K. Kleinman, and R. K. Maheshwari. 1998. Inhibition of angiogenic differentiation of human umbilical vein endothelial cells by curcumin. *Cell Growth Differ.* **9:** 305–312.
82. Tong, J. K., C. A. Hassig, G. R. Schnitzler, R. E. Kingston, and S. L. Schreiber. 1998. Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex.. *Nature* **395:** 917–921.
83. Towbin, H., K. W. Bair, J. A. DeCaprio, M. J. Eck, S. Kim, F. R. Kinder, A. Morollo, D. R. Mueller, P. Schindler, H. K. Song, J. van Oostrum, R. W. Versace, H. Voshol, J. Wood, S. Zabludoff, and P. E. Phillips. 2003. Proteomics-based target identification: Bengamides as a new class of methionine aminopeptidase inhibitors. *J. Biol. Chem.* **278:** 52964–52971.
84. Towle, M. J., K. A. Salvato, J. Budrow, B. F. Wels, G. Kuznetsov, K. K. Aalfs, S. Welsh, W. Zheng, B. M. Seletsk, M. H. Palme, G. J. Habgood, L. A. Singer, L. V. Dipietro, Y. Wang, J. J. Chen, D. A. Quincy, A. Davis, K. Yoshimatsu, Y. Kishi, M. J. Yu, and B. A. Littlefield. 2001. *In vitro* and *in vivo* anticancer activities of synthetic macrocyclic ketone analogues of halichondrin B. *Cancer Res.* **61:** 1013–1021.
85. Vandonselaar, M., R. A. Hickie, J. W. Quail, and L. T. Delbaere. 1994. Trifluoperazine-induced conformational change in Ca(2+)-calmodulin. *Nat. Struct. Biol.* **1:** 795–801.
86. Veigl, M. L., T. C. Vanaman, and W. D. Sedwick. 1984. Calcium and calmodulin in cell growth and transformation. *Biochim. Biophys. Acta* **738:** 21–48.
87. Weisenberg, R. C., G. G. Borisy, and E. W. Taylor. 1968. The colchicine-binding protein of mammalian brain and its relation to microtubules. *Biochemistry* **7:** 4466–4479.
88. Wong, D. L., R. J. Hayashi, and R. D. Ciaranello. 1985. Regulation of biogenic amine methyltransferases by glucocorticoids via *S*-adenosylmethionine and its metabolizing enzymes, methionine adenosyltransferase and *S*-adenosylhomocysteine hydrolase. *Brain Res.* **330:** 209–216.
89. Yoshida, M., M. Kijima, M. Akita, and T. Beppu. 1990. Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. *J. Biol. Chem.* **265:** 17174–17179.