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Natural product diversity and its role in chemical biology and drug discovery

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Abstract

Through the natural selection process, natural products possess a unique and vast chemical diversity and have been evolved for optimal interactions with biological macromolecules. Owing to their diversity, target affinity, and specificity, natural products have demonstrated enormous potential as modulators of biomolecular function, been an essential source for drug discovery, and provided design principles for combinatorial library development.

Introduction

Classical genetic techniques such as overexpression or conditional mutations have long played a crucial role in advancing the understanding of complex biological systems and provided key tools for exploring the intricacies of cellular pathways and elucidating the functions of proteins. While use of classical genetic methods has provided a wealth of information, its limitations include unwanted global side effects and difficulties in moving to higher order mammalian systems. As the need for more versatile tools grows, a new field has emerged that utilizes aspects of synthetic organic chemistry and biology to explore biological processes. This new approach is commonly referred to as chemical genetics/genomics [1,2].

In contrast to the irreversible deletion or mutagenesis strategies of classical genetics, chemical genetics/genomics uses small organic molecules to perturb living systems. These small molecules offer several advantages, including reversible, temporal, and dose-dependant control of gene products as well as greater versatility across model organisms. In addition, small molecules can be used to alter a single function of a protein whereas a deletion and/or a mutation result in a change of overall function. Considering these advantages, chemical genetic/genomic approaches are increasingly being utilized in drug discovery as well as in the study of important biological processes.

Natural product diversity

In chemical genetics/genomics, access to collections of structurally complex and diverse small molecules is extremely important. There are two major compound sources for chemical genetics/genomics: natural products (including natural product derivatives) and combinatorial chemistry libraries. With the advent of combinatorial chemistry, it is relatively easy to prepare a large number of small molecules. In addition, chemical

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modulators for important biological processes have been identified from screening small molecules originated from combinatorial chemistry libraries [3,4]. However, traditional combinatorial libraries through the ‘one-synthesis/one-scaffold’ approach generally show limited structural diversity.

On the contrary, natural products are known to possess a broader diversity in chemical space [5*,6*,7] and, as a result, have produced a profound impact on chemical biology and drug development (Figure 1). Most of the natural products produced by microorganisms or plants are not meant to bind to human proteins. However, for many years, microorganisms and plants have evolved to produce small ligands (or natural products) for their macromolecular targets within living organisms [8], and many human protein targets contain structural domains similar to the targets with which small ligands (or natural products) have coevolved [9**]. Through the natural selection process, natural products possess a unique and vast chemical diversity and have been evolved for optimal interactions with biological macromolecules. Therefore, natural products have proven to be by far the richest source of novel compound classes for biological studies and an essential source of new drug discovery.

Natural products as modulators of biomolecular function

Owing to their diversity, target affinity, and specificity, natural products have demonstrated enormous potential as modulators of biomolecular function [10**]. Numerous natural products, including brefeldin A, cyclosporine A, rapamycin, geldanamycin, TNP-470, trapoxin A, FTY720, and diazonamide A have been used for the study of important signaling pathways (Figure 2).

TNP-470

Fumagillin, a natural product of fungal origin [11], was discovered to act as a potent inhibitor of angiogenesis. A synthetic analog of fumagillin, *O*-(chloroacetylcarbamoyl) fumagillol (TNP-470, also known as AGM-1470), was found to be less toxic and a 50-fold more potent inhibitor of angiogenesis than fumagillin [12]. TNP-470 has been demonstrated to inhibit capillary endothelial cell growth induced by both basic fibroblast growth factor (bFGF) and vascular endothelial cell growth factor (VEGF), and preferentially inhibit endothelial cell growth in tumor vasculature. In an effort to decipher the molecular mechanism of angiogenesis inhibition by TNP-470, Liu and Crews identified a bifunctional protein, the type 2 methionine aminopeptidase (MetAPZ), as the molecular target of TNP-470 [13–15]. Using a combination of chemical modification of fumagillin and site-directed mutagenesis, they found that TNP-470 binds covalently to MetAP and inhibits its methionine aminopeptidase activity without affecting its inhibition of eIF-2 α phosphorylation. These studies showed that MetAP may play an important role in endothelial cell proliferation and angiogenesis and suggested that MetAP can be a potential therapeutic target for the treatment of cancer.

FTY720

The immunosuppressive agent FTY720 is a synthetic structural analogue of the fungal metabolite myriocin (ISP-1) [16], which was isolated from the ascomycete *Isaria sinclairii*. Unlike myriocin, FTY720 did not inhibit serine palmitoyl transferase, the first enzyme in sphingolipid biosynthesis. Subsequent studies showed that FTY720 did not reduce antigen-driven T-cell activation and proliferation at therapeutically relevant concentrations. But, FTY720 produced lymphopenia *in vivo* by sequestering lymphocytes from blood and spleen into lymph nodes and Peyer’s patches. These results prompted speculation that FTY720 may act by accelerating the chemokine-dependent homing of cells into the lymphoid organs.

However, subsequent studies showed that the sequestration occurred independent of the homing receptors CD62L, CCR7, and CXCR5, and the CCR7-ligand chemokines CCL19 and CCL21. In 2002, Rosen and Brinkmann demonstrated that FTY720 is rapidly phosphorylated *in vivo* by sphingosine kinase (SphK) and that the phosphate metabolite of FTY720 (FTY720-P) is the biologically active principle [17,18]. They also showed that the phosphorylated FTY720 metabolite acts *in vitro* as an agonist for all sphingosine 1-phosphate (S1P) receptors (except S1P₂) that regulate the lymphocyte recirculation pathway. The reverse pharmacological studies with FTY720 and its derivatives provided novel insights into the therapeutic relevance of the S1P pathway [19].

Diazonamide A

The marine natural product diazonamide A was originally isolated from the marine ascidian *Diazona angulata* [20]. Diazonamide A has remained of considerable interest, not only as a target for synthetic chemists, but also because of its potent *in vitro* cytotoxicity against human tumour cell lines [21]. At low-nanomolar concentrations, diazonamide A induces an M-phase growth arrest in a variety of cancer cell types. Data from the NCI's COMPARE screen implied a mechanistic similarity between diazonamide A and microtubule depolymerizing agents such as the vinca alkaloids and the taxanes. However, more detailed studies showed that diazonamide A did not compete with colchicine or vinblastine for tubulin binding, suggesting that either it had a unique binding site on tubulin or did not act by binding to tubulin at all. To address this issue, Harran, Wang, and co-workers used a biotinylated derivative of diazonamide A and a radiolabeled congener for direct binding measurements [22]. Neither interacted specifically with tubulin or microtubules *in vitro*, but both potently induced spindle abnormalities during mitosis indistinguishable from those caused by the natural product. They purified a mitochondrial matrix enzyme, ornithine delta-amino transferase (OAT), from HeLa cell and *Xenopus* egg extracts using a biotinylated derivative of diazonamide A. Diazonamide A did not inhibit the amino transferase activity of this enzyme, however, it disrupted the interaction of OAT with mitotic-spindle-promoting proteins. These studies suggested a unique mode of action involving OAT, revealed an unanticipated, paradoxical role for OAT in mitotic cell division, and identified the protein as a target for chemotherapeutic drug development.

Natural products as an essential source for new drug discovery

In addition to the utility of natural products as modulators of biomolecular functions, the enormous potential of natural products in drug development has increasingly been realized [23–25*]. For example, a total of 13 natural product and natural product-derived drugs were approved worldwide from 2005 to 2007 [25*]. Several natural product or natural product-derived drugs including ziconotide, exenatide, and ixabepilone are the first members of new human drug classes. It should be noted that 13 of 69 small molecule drug (19%) approved from 2005 to 2007 were natural products or derived from natural products, which further supports the importance of natural products in drug discovery [25*]. The great success of natural products in drug discovery comes from their structural diversity. General differences among biologically active natural products, combinatorial chemistry libraries, and existing synthetic drugs on the basis of structural and physicochemical properties have been extensively reviewed [6*].

In particular, drugs originated from natural products have been highly successful for protein–protein interactions, nucleic acid complexes, and antibacterial targets [26–33]. They are classically challenging targets for standard small-molecule drug discovery due to the involvement of large, flat interfaces and the macromolecular nature of their interactions. For example, macrocycles have demonstrated repeated success in modulating macromolecular

processes such as protein–protein interactions (Figure 2) [34**]. The macrocycle cyclosporine A has been extensively studied for its ability to enable the interaction of cyclophilin A with calcineurin [35]. Rapamycin similarly controls the mammalian target of rapamycin (mTOR) pathway through its interaction with FK506 binding protein 12 (FKBP12) to create a hybrid macrocycle–protein surface that facilitates the binding of FKBP12–rapamycin-associated protein (FRAP) [36]. Epothilone B, a macrocycle derived from myxobacteria, binds at the interface of the tubulin α - and β -subunits [37]. The macrolide antibiotic erythromycin functions by binding to the inner surface of the ribosomal tunnel and physically impedes the exit of nascent peptides from the ribosome by narrowing the passage [38]. These examples suggest that natural products are highly effective in modulating various types of macromolecular interactions.

Natural product-inspired small molecule libraries

Biologically active natural products can be regarded as chemical entities that were evolutionarily selected and validated for binding to particular protein domains. Many natural product scaffolds can be regarded as “privileged” scaffolds and could potentially address poorly populated, underexplored chemical space [9**]. Therefore, it is no surprise that the underlying structural complexities and architectures of natural products may provide powerful guiding principles for combinatorial library design. Over the last decade, efforts have been made to generate combinatorial chemistry libraries inspired by natural products [9**,26,39,40**].

Among the new techniques developed for combinatorial library design, diversity-oriented synthesis (DOS) has been increasingly popular as a choice of design principle for combinatorial library development (Figure 3) [41–43**]. Complex and diverse molecular architectures of natural products are displayed as three-dimensional surfaces of charges, polarities, and other specific bonding interactions. DOS aims to prepare collections of skeletally diverse small molecules to mimic the multitude of complexity and diversity of natural products.

In traditional target oriented synthesis (TOS), retrosynthetic analysis is used to plan a synthetic route from a complex product to structurally simple building blocks. TOS aims either to populate a distinctive point in chemical space (e.g. total synthesis) or to populate more densely a specific area of interest (e.g. focused library synthesis). In contrast to TOS, DOS requires the development of a forward synthetic analysis to enable the conversion of simple and similar starting materials into complex and diverse products. It aims to achieve a diverse and non-focused coverage of biologically active chemical space. However, as is the case with TOS, DOS also requires highly efficient and stereoselective reactions to be effective. By virtue of their structural diversity, collections of small molecules prepared by DOS interrogate larger areas of chemical space, compared with libraries produced using more traditional combinatorial chemistry. As a result, they possess a greater functional diversity than traditional combinatorial chemistry libraries. Over the past several years, structurally diverse collections of small molecules have been prepared by DOS and exploited successfully to identify modulators for biological systems [44,45].

Conclusions

As evidenced in this review, natural products offer a privileged starting point in the search for highly specific and potent modulators of biomolecular function as well as novel drugs. With the advent of high- throughput screening methods, genome mining, and novel heterologous expression systems to address the disadvantages with natural products such as difficulties in access and supply, and complexities of natural product chemistry, new natural

products possessing novel biological activities continue to be unveiled at an increasing rate. Owing to their diversity, target affinity, and specificity, it is no doubt that natural products will continue to make great impact on chemical biology and drug discovery.

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References and recommended reading

1. Schreiber SL. The small-molecule approach to biology. *Chem Eng News*. 2003; 81:51–61.
2. Stockwell BR. Exploring biology with small organic molecules. *Nature*. 2004; 432:846–854. [PubMed: 15602550]
3. Wurdak H, Zhu S, Min KH, Aimone L, Lairson LL, Watson J, Chopiuk G, Demas J, Charette B, Weerapana E, Cravatt BF, Cline HT, Peters EC, Zhang J, Walker JR, Wu C, Chang J, Tuntland T, Cho CY, Schultz PG. A small molecule accelerates neuronal differentiation in the adult rat. *Proc Natl Acad Sci USA*. 2010; 107:16542–16547. [PubMed: 20823227]
4. Bradner JE, Mak R, Tanguturi SK, Mazitschek R, Haggarty SJ, Ross K, Chang CY, Bosco J, West N, Morse E, Lin K, Shen JP, Kwiatkowski NP, Gheldof N, Dekker J, DeAngelo DJ, Carr SA, Schreiber SL, Golub TR, Ebert BL. Chemical genetic strategy identifies histone deacetylase 1 (HDAC1) and HDAC2 as therapeutic targets in sickle cell disease. *Proc Natl Acad Sci USA*. 2010; 107:12617–12622. [PubMed: 20616024]
- *5. Clardy J, Walsh C. Lessons from natural molecules. *Nature*. 2004; 432:829–837. A discussion of the rich structural diversity and complexity of natural products. [PubMed: 15602548]
- *6. Feher M, Schmidt JM. Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. *J Chem Inf Comput Sci*. 2003; 43:218–227. A discussion of the differences between three different compound classes: natural products, molecules from combinatorial synthesis, and drug molecules. [PubMed: 12546556]
7. Rosén J, Gottfries J, Muresan S, Backlund A, Oprea TI. Novel chemical space exploration via natural products. *J Med Chem*. 2009; 52:1953–1962. [PubMed: 19265440]
8. Firn RD, Jones CG. Natural products—a simple model to explain chemical diversity. *Nat Prod Rep*. 2003; 20:382–391. [PubMed: 12964834]
- **9. Bon RS, Waldmann H. Bioactivity-guided navigation of chemical space. *Acc Chem Res*. 2010; 43:1103–1114. A discussion of an approach termed biology-oriented synthesis (BIOS). [PubMed: 20481515]
- **10. Dixon N, Wong LS, Geerlings TH, Micklefield J. Cellular targets of natural products. *Nat Prod Rep*. 2007; 24:1288–1310. A review on the breadth of different cellular targets with which natural products interact. [PubMed: 18033580]
11. McCowen MC, Callender ME, Lawlis JF Jr. Fumagillin (H-3), a new antibiotic with amebicidal properties. *Science*. 1951; 113:202–203. [PubMed: 14809278]
12. Ingber D, Fujita T, Kishimoto S, Sudo K, Kanamaru T, Brem H, Folkman J. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. *Nature*. 1990; 348:555–557. [PubMed: 1701033]
13. Griffith EC, Su Z, Turk BE, Chen S, Chang YH, Wu Z, Biemann K, Liu JO. Methionine aminopeptidase (type 2) is the common target for angiogenesis inhibitors AGM-1470 and ovalicin. *Chem Biol*. 1997; 4:461–471. [PubMed: 9224570]
14. Sin N, Meng L, Wang MQW, Wen JJ, Bornmann WG, Crews CM. The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. *Proc Natl Acad Sci USA*. 1997; 94:6099–6103. [PubMed: 9177176]
15. Zhang Y, Griffith EC, Sage J, Jacks T, Liu JO. Cell cycle inhibition by the anti-angiogenic agent TNP-470 is mediated by p53 and p21WAF1/CIP1. *Proc Natl Acad Sci USA*. 2000; 97:6427–6432. [PubMed: 10841547]

16. Adachi K, Kohara T, Nakao N, Arita M, Chiba K, Mishina T, Sasaki S, Fujita T. Design, synthesis and structure-activity relationships of 2-substituted 2-amino-1,3-propanediols: Discovery of a novel immunosuppressant, FTY720. *Bioorg Med Chem Lett*. 1995; 5:853–856.
17. Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thornton R, Shei GJ, Card D, Keohane C, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science*. 2002; 296:346–349. [PubMed: 11923495]
18. Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J Biol Chem*. 2002; 277:21453–21457. [PubMed: 11967257]
19. Brinkmann V, Cyster JG, Hla T. FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. *Am J Transplant*. 2004; 4:1019–1025. [PubMed: 15196057]
20. Lindquist N, Fenical W, Van Duyne GD, Clardy J. Isolation and structure determination of diazonamides A and B, unusual cytotoxic metabolites from the marine ascidian *Diazona chinensis*. *J Am Chem Soc*. 1991; 113:2303–2304.
21. Lachia M, Moody CJ. The synthetic challenge of diazonamide A, a macrocyclic indole bis-oxazole marine natural product. *Nat Prod Rep*. 2008; 25:227–253. [PubMed: 18389137]
22. Wang G, Shang L, Burgett AW, Harran PG, Wang X. Diazonamide toxins reveal an unexpected function for ornithine delta-amino transferase in mitotic cell division. *Proc Natl Acad Sci USA*. 2007; 104:2068–2073. [PubMed: 17287350]
23. Harvey AL. Natural products in drug discovery. *Drug Discov Today*. 2008; 13:894–901. [PubMed: 18691670]
24. Ganesan A. The impact of natural products upon modern drug discovery. *Curr Opin Chem Biol*. 2008; 12:306–317. [PubMed: 18423384]
- *25. Butler MS. Natural products to drugs: natural product-derived compounds in clinical trials. *Nat Prod Rep*. 2008; 25:475–516. A review on natural product or natural product-derived drugs. [PubMed: 18497896]
26. Bauer RA, Wurst JM, Tan DS. Expanding the range of ‘druggable’ targets with natural product-based libraries: an academic perspective. *Curr Opin Chem Biol*. 2010; 14:308–314. [PubMed: 20202892]
27. Zhang MQ, Wilkinson B. Drug discovery beyond the ‘rule-of-five’. *Current Opin Biotechnol*. 2007; 18:478–488.
28. Reayi A, Arya P. Natural product-like chemical space: search for chemical dissectors of macromolecular interactions. *Curr Opin Chem Biol*. 2005; 9:240–247. [PubMed: 15939325]
29. Wells JA, McClendon CL. Reaching for high-hanging fruit in drug discovery at protein–protein interfaces. *Nature*. 2007; 450:1001–1009. [PubMed: 18075579]
30. Whitty A, Kumaravel G. Between a rock and a hard place? *Nat Chem Biol*. 2006; 2:112–118. [PubMed: 16484997]
31. Berg T. Inhibition of transcription factors with small organic molecules. *Curr Opin Chem Biol*. 2008; 12:464–471. [PubMed: 18706517]
32. Arndt HD. Small molecule modulators of transcription. *Angew Chem Int Ed*. 2006; 45:4552–4560.
33. Payne DJ, Gwynn MN, Holmes DJ, Pompilano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat Rev Drug Discov*. 2007; 6:29–40. [PubMed: 17159923]
- **34. Driggers EM, Hale SP, Lee J, Terrett NK. The exploration of macrocycles for drug discovery—an underexploited structural class. *Nat Rev Drug Discov*. 2008; 7:608–624. A review on macrocycles effective for challenging drug targets. [PubMed: 18591981]
35. Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506. *Immunol Today*. 1992; 13:136–142. [PubMed: 1374612]
36. Sehgal SN. Rapamune (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. *Clin Biochem*. 1998; 31:335–340. [PubMed: 9721431]
37. Goodin S, Kane MP, Rubin EH. Epothilones: mechanism of action and biologic activity. *J Clin Oncol*. 2004; 22:2015–2025. [PubMed: 15143095]

38. Schlünzen F, Zarivach R, Harms J, Bashan A, Tocilj A, Albrecht R, Yonath A, Franceschi F. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature*. 2001; 413:814–821. [PubMed: 11677599]
39. Kumar K, Waldmann H. Synthesis of natural product inspired compound collections. *Angew Chem Int Ed Engl*. 2009; 48:3224–3242. [PubMed: 19267376]
- **40. Nandy JP, Prakesch M, Khadem S, Reddy PT, Sharma U, Arya P. Advances in solution- and solid-phase synthesis toward the generation of natural product-like libraries. *Chem Rev*. 2009; 109:1999–2060. A review on the synthesis of natural product-like libraries. [PubMed: 19249849]
41. Burke MD, Schreiber SL. A planning strategy for diversity-oriented synthesis. *Angew Chem Int Ed*. 2004; 43:46–58.
42. Tan DS. Diversity-oriented synthesis: exploring the intersections between chemistry and biology. *Nat Chem Biol*. 2005; 1:74–84. [PubMed: 16408003]
- **43. Galloway WR, Isidro-Llobet A, Spring DR. Diversity-oriented synthesis as a tool for the discovery of novel biologically active small molecules. *Nat Commun*. 2010; 1:1–13. A review on diversity-oriented synthesis (DOS). [PubMed: 20975674]
44. Kuruvilla FG, Shamji AF, Sternson SM, Hergenrother PJ, Schreiber SL. Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays. *Nature*. 2002; 416:653–657. [PubMed: 11948353]
45. Koehler AN, Shamji AF, Schreiber SL. Discovery of an inhibitor of a transcription factor using small molecule microarrays and diversity-oriented synthesis. *J Am Chem Soc*. 2003; 125:8420–8421. [PubMed: 12848532]

Figure 1.
Natural product diversity

Figure 2.
Examples of natural products used in chemical biology and drug discovery

Target-Oriented Synthesis (TOS)

- Convergent Synthesis
- Retrosynthetic Analysis
simple $\longleftarrow$ single & complex target
- Aim to populate a discrete point in chemical space

Diversity-Oriented Synthesis (DOS)

- Divergent Synthesis
- Forward Synthetic Analysis
simple $\longrightarrow$ complex & diverse targets
- Aim to cover a diverse and non-focused coverage of chemical space

Figure 3.
Comparison of target-oriented synthesis (TOS) and diversity-oriented synthesis (DOS)