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HIGHLIGHT

Natural products and Pharma 2011: Strategic changes spur new opportunities

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Although natural products have been marginalized by major pharmaceutical companies over the last 20–30 years, the changing landscape of drug discovery now favors a greatly enhanced role for Nature's privileged structures. Screening for drug leads in phenotypic screens provides the best opportunity to realize the value of natural products. Advances in total synthesis, especially function-oriented syntheses and biosynthetic technologies offer new avenues for the medicinal chemical optimization of biologically active secondary metabolites. Genomic research has given new insights into biosynthetic processes as well as providing evidence that a wealth of unrealized biosynthetic potential remains to be explored. As Pharma strives to develop innovative and highly effective new drugs, natural products will be increasingly valued as sources of novel leads whose further development will be expedited by emerging technologies.

In the 50+ years since the celebrated "Golden Age of Antibiotic Discovery", natural products have gradually been marginalized by major pharmaceutical research companies (Pharma) as sources for new drugs. Today, however, there is a renewed opportunity to integrate natural products into the mainstream of Pharma R&D – it is clearly time for a change in strategy. The accepted paradigm for drug discovery in the last 25 years has failed to provide sufficient numbers of innovative and effective new drugs to continue to fuel the R&D engine. High throughput screening (HTS) of hundreds of thousands of small molecules against single targets, followed by medicinal chemical engineering of lead compounds as the "magic bullet" has led to a much higher rate of failure than predicted by the established attrition model. Drug approvals by the US Food and Drug Administration have continued to fall from the levels of the 1990's, when the overall success rate of compounds entering Phase 1 was 11%.[1] The industry also faces continual threats to revenue through expensive product recalls, aggressive generic competition and the looming patent cliff. The patent expiry situation will certainly have a major financial impact on Big Pharma this year.[2] Owing to these factors, the industry has been forced to downsize R&D operations and refocus efforts on therapies that are most likely to yield sustainable products. A critical part of this refocusing includes the re-evaluation of drug discovery processes. This is where the value of natural products as sources of drug leads is once again gaining traction.

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Natural products and evolving screening platforms for drug discovery

For some time scientists involved in drug discovery have been questioning the wisdom of the reductionist philosophy of hitting single targets as the mechanism to ameliorate complex disease states.[3] Furthermore, it has become increasingly clear that many effective drugs act by interactions with multiple receptors and/or enzymes, although they may have been designed to be highly selective. Engineering compounds to have affinities for several targets by *de novo* synthetic chemistry is a highly complex undertaking. Nonetheless, the concept of designing such compounds, known as "Designed Multiple Ligands" (DML), has entered the lexicon of Pharma.[4] One of the main limitations with the DML approach is the ability to define the set of targets that is causative for a particular disease state. Designing compounds that will hit the key targets with a desirable ratio of potencies is certainly a daunting challenge. A viable alternative to DML that embraces the multi-target nature of disease control is to employ multi-parametric and/or phenotypic screening.

In response to this conundrum there has been a trend toward screening platforms that enable interrogation of a broader range of biological phenomena. Greater emphasis has been given to disrupting protein–protein interactions,[5] for example in signaling cascades. Inhibition of such interactions requires larger and/or more complex compounds than would typically be designed for inhibition of an enzyme or blockage of a receptor. Regrettably, such multifaceted compounds have been systematically purged from the screening libraries in Pharma owing to their non-compliance to Lipinski's "rule of five" criteria for acceptable ADME properties. According to Wells and McClendon, the typical screening library may not be effective in these assays since the compounds were designed to hit traditional targets. They

conclude: "New classes of target often require new chemotypes."[6]

Peptides have been promoted as having the properties needed for such new chemotypes in screens targeting protein–protein interactions.[7] Obviously peptides are most closely related chemically to the binding surfaces of interest and, additionally, offer the advantages of being readily synthesized and modified. Structurally constrained peptides that lock productive binding conformations in place and protect the peptide from hydrolysis are particularly desirable as leads. Cyclic peptides offer these advantages and are widely distributed in nature. Recently, the case has been made that macrocyclic compounds in general are ideal modulators of biomolecular interactions since they exhibit multiple binding domains.[8] In addition to peptides, other natural product classes offer multifaceted binding properties; perhaps the most attractive are polyketide-derived secondary metabolites (PKs). Furthermore, cyclic PKs arguably represent the most structurally varied macrocycles known, in terms of ring size, functional groups, substitution patterns and stereochemistry, and thus are exquisite new chemotypes for new targets. Despite their obvious advantages, natural cyclic peptides and PKs have not been widely investigated in biomolecular screening. The main reason that they have not been screened to a greater extent is because they are not readily available as pre-packaged libraries of pure compounds. Neither class is readily available through chemical synthesis, rather they must be isolated from natural sources. Despite this perceived limitation, the two classes of natural macrocycles share the advantage of a modular templated biosynthetic origin that has been well studied and engineered in bacteria. Although Pharma may have some historical collections of these compounds, there is no doubt that a screening collection containing a broad range of chemically diverse NRPS-derived peptides and PKs would be of great interest.

In a major shift from the single target paradigm, the industry has returned to screening in complex biological systems. Phenotypic screening has re-emerged as a means to uncover new mechanisms for disease intervention. Whether using high content screening with fast readouts, or monitoring the behavior and developmental stages of zebrafish or any intermediate level of complexity – this approach has once again become credible. In a particularly enlightened strategy, Eli Lilly has offered to screen novel collections of synthetic and natural compounds from academic and biotech labs in order to access novel chemotypes in a series of well validated phenotypic assays.[9] A key tactic in this phenotypic renaissance, as exemplified by Lilly, is the use of unconventional screening libraries. Owing to the biological complexity underpinning a desirable phenotypic effect, it is critical that compounds in the screening set represent well developed pharmacophores rather than the simplified scaffolds used for typical single-target HTS. Currently, Pharma companies maintain collections of advanced leads and/or near-miss clinical candidates as well as standard collections of drug substances for this purpose, however, these are of limited scope. Natural products represent Nature's fully developed small molecule effectors and thus are ideally suited for phenotypic screening.

As the trend of screening in multi-parametric systems continues, the demand for natural products libraries will grow. Companies with highly diverse collections of natural products will gain a competitive advantage over others without access to these privileged structures. Ideally, natural product screening libraries would consist of pure compounds in order to obviate confounding responses derived from mixtures, and avoid the lag time required to deconvolute a mixture in order to identify the active component. Assembling a highly diverse library of pure natural products for screening is a major endeavor. The commercial availability of pure natural products remains quite limited. Hence, the real question becomes: How does one access significant chemical (and therefore functional) diversity for a natural products screening resource? The pragmatic solution resides in compromising the principle of pure compound screening to allow mixtures to be evaluated. Pre-fractionated natural products libraries couple the beneficial effects of incorporating much greater chemical diversity with reasonably consistent biological readouts.[10] Simplified mixtures resulting from pre-fractionation expedite the downstream processes of dereplication and compound purification and therefore reduce the lag time. It is only through the incorporation of such mixtures that the broadest range of natural chemical diversity can be screened in the foreseeable future. Of utmost importance is that pre-fractionation schemes aim to include the bulk of constituents, rather than only including major components or "peaks", so the opportunity to discover novel compounds is preserved.

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Guy Carter obtained a bachelors degree in Chemistry at Lehigh University and a Ph.D. in Biochemistry from the University of Wisconsin. He then pursued marine natural products with an NIH-sponsored post-doctoral fellowship in the laboratory of the late Kenneth Rinehart at the University of Illinois, before beginning a career in the pharmaceutical industry in 1978. Most of these years were spent in Pearl River, NY – first with American Cyanamid's Lederle Laboratories, and continuing with Wyeth Research. During this period he gradually moved out of the lab into managerial roles but maintained responsibility for the natural products discovery program. In 2010 he began a new career as a private consultant to advance and promote natural products R & D.

Natural products and lead optimization in drug discovery

The logical progression of research following the finding of a natural product "active" in a phenotypic screen is to understand its mechanism of action. Chemical biology experiments employing the natural product as an affinity tag, can reveal molecular targets that guide the development of drug candidates. The design and synthesis of FKBP ligands that mimic FK506's binding represent the fruitful application of this approach. The dual binding modes of FK506 were revealed in pioneering research from the Schreiber laboratory.[11] FK506 was shown to

exert its immunosuppressive activity by first binding with FKBP-12 and then this pre-organized complex recruits and inhibits the action of calcineurin. The FKBP-binding region of the compound consists of the pipecolate residue flanked by the "tricarbonyl" region, as shown in Fig. 1. When it was subsequently discovered that non-immunosuppressive analogs of FK506 (**1**) had neurotrophic properties, several groups were inspired to create simplified mimetic analogs of the FKBP-binding region.[12] One of the most advanced benchmark compounds derived from this strategy is GPI-1046 (**2**). Compounds of this general structural class have shown promising neuroprotective and neuroregenerative activity in a variety of animal models and some derivatives have progressed into clinical studies.[13] This "dissective" approach to creating a simplified drug-like compound is an attractive strategy for capturing the pharmaceutical potential of a complex natural product.

Natural products that are discovered as leads in screening may well serve as the starting point for medicinal chemistry programs aimed at enhancing their biological profiles. The decision to pursue a natural product-based medicinal chemistry program will be predicated on adequate compound supply and therefore renewable, highly productive sources are greatly favored. In most instances the starting material has been derived from microbial fermentations or isolation from abundant plant species, however as will be discussed shortly, de novo synthetic processes can also be effective. Often relatively straightforward structural modifications result in product profiles that meet criteria for further development. The new generation of rapamycin analogs recently approved for clinical use, temsirolimus (**3**), everolimus (**4**), zotarolimus (**5**) and deferolimus (**6**),[14] (Fig. 2) are simple examples of this approach. The compounds were designed in part for improved aqueous solubility to facilitate IV administration, as well as for enhancements in other properties. The C40 position on the pendant cyclohexyl moiety has been recognized as a viable point for chemical modification. These rapalogs are the result of focused semi-synthetic medicinal chemistry that relies on pre-existing structural features (*e.g.* an unhindered hydroxyl function) as an entry point for modifications. Owing to developments in genetic engineering, it is now feasible to practice *biosynthetic* medicinal chemistry for lead enhancement. The targets of biosynthetic medicinal chemistry are not restricted by

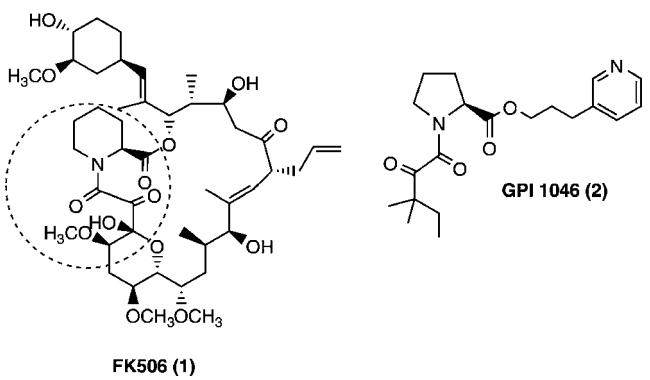


Fig. 1 Structures of the FKBP ligands FK506 (**1**) and its synthetic mimic GPI-1046 (**2**). The dashed circle roughly indicates the FKBP-binding region of FK506.

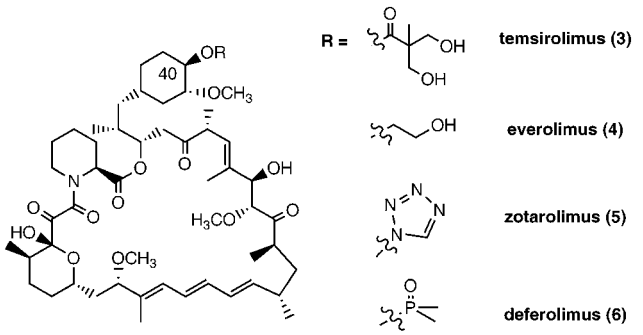


Fig. 2 Structures of the recently approved derivatives of rapamycin: temsirolimus (**3**), everolimus (**4**), zotarolimus (**5**), and deferolimus (**6**).

pre-existing functional group "handles" on a scaffold, and thus are often complimentary to the products of semi-synthesis.

An incisive example of biosynthetic medicinal chemistry is illustrated in Fig. 3 for the Hsp90 inhibitor macbecin (**7**). As with other ansamycins, such as geldanamycin, the quinone moiety was implicated in causing off-target effects leading to toxicity. With a firm knowledge of the biosynthetic sequence in hand, Martin and coworkers at Biotica Technologies genetically engineered a strain of *Actinosynnema pretiosum* in which the gene responsible for oxidation at C21 was deleted.[15] Eliminating this oxygenation step prevented the formation of the quinone and resulted in the exclusive production of non-quinone products **8** and **9**. Evidently blocking quinone formation also inhibited the downstream tailoring processes that are responsible for the C4,5 desaturation, C15 hydroxylation and C11,15 *O*-methylation. Compound **8** was the most promising of these analogs, retaining the cellular potency of macbecin, while its toxicity was dramatically reduced. In order to enhance the overall production of **8**, the downstream tailoring gene for C15 hydroxylation was also deleted, resulting in a productive strain that yielded essentially the single component **8** at 200 mg L^{-1}. In those cases where biosynthetic pathways are available for manipulation, combining biosynthetic medicinal chemistry with semi-synthetic processes can provide access to a much wider range of scaffolds and analogs.

Structurally unique natural products that demonstrate tantalizing biological properties will continue to provide the inspiration for the total synthesis of these compounds as potential drug candidates. The most relevant total syntheses from a pharmaceutical perspective are those that truly resolve the "supply issue"

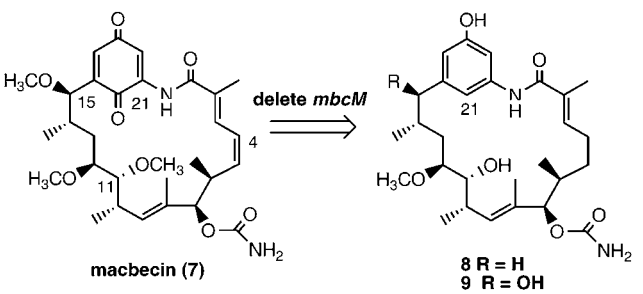


Fig. 3 Biosynthetic medicinal chemistry of macbecin (**7**): Deletion of the C21 oxidase gene *mbcM* results in non-quinone products **8** and **9**.

and provide routes for the creation of improved analogs. Paul Wender's program on the synthesis and simplification of bryostatin (**10**) analogs for cancer chemotherapy demonstrates the power of modern synthetic chemistry guided by structural insights of the mechanism of action. The initial investment of research toward understanding the structural requirements for bryostatin's biological activity enabled the design of simplified analogs *via* function-oriented synthesis.[16] As illustrated in Fig. 4, the upper portion of the metabolite acts as scaffolding to properly orient the key oxygen atoms in the lower half, and therefore this portion of the molecule could be simplified without loss of function. The simplified bryolog (**11**) requires less than half of the synthetic steps initially required to make the parent compound and has enhanced potency against tumor cells. Given this exquisite potency (an 8–12 week dosing regimen requires only 1–2 mg) this approach may be cost effective.

With few exceptions, the dogma in Pharma has been that total syntheses of complex natural products, such as bryostatin, are purely academic exercises that would never be commercially feasible. It has now become apparent that under the right direction the knowledge obtained from total synthesis can successfully transcend the academic laboratory. The synthesis of halichondrin B analogs as anticancer agents is a superb example of translational research that has reached fruition. Following the elegant total synthesis of halichondrin B (**12**) by Kishi and coworkers in 1992, the Eisai Research Institute directed its resources to realizing the potential of this highly complex marine metabolite originally discovered by Uemura and Hirata.[17] Once it became clear that the tubulin-inhibitory activity of the parent structure could be replicated by simpler analogs containing the macrocycle, a synthetic strategy could be developed.[18] By adopting the convergent strategy of Kishi, the Eisai team was able to prepare a series of highly potent analogs resulting in the selection of E7389 (**13**) for further development (see Fig. 5).[19] In November 2010, E7389 (now eribulin mesylate or Halaven), was approved by the US Food and Drug Administration for the treatment of metastatic breast cancer.[20]

The development of antibody–drug conjugates (ADC), particularly for cancer therapy, represents a growing area of natural products research in industry. The focus of ADC research has been to selectively deliver potent cytotoxic agents to tumor cells, and thus avoid collateral damage to surrounding

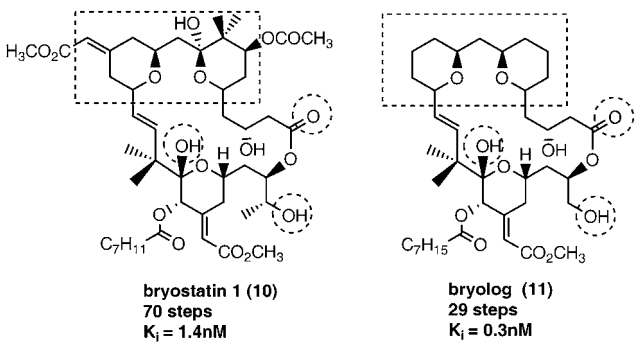


Fig. 4 Structures of bryostatin **1** and simplified bryolog **11**. The dashed rectangle depicts the portions of the structures that act as scaffolding to orient the three key oxygen atoms (circled).

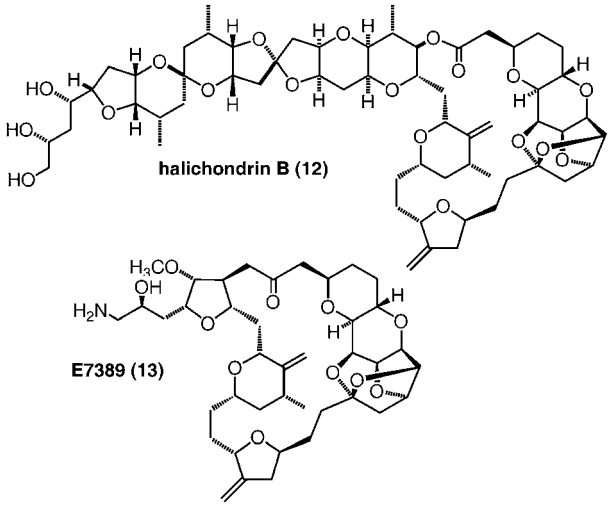


Fig. 5 Structures of halichondrin B (**12**) and the simplified synthetic analog E7389 (**13**).

normal tissues.[21] Lead optimization in this context involves not only the conjugation chemistry for coupling the natural product to the antibody, but also the design of linkers and selection of antibodies. The first ADC approved for clinical use was Mylotarg®, which delivers a calicheamicin derivative to cells bearing the CD33 antigen.[22] Other compounds that have sufficiently high potency for these applications include the maytansinoids and auristatins; the latter are synthetic analogs of dolastatin. ADC's bearing each of these classes of natural product derivatives are currently in various stages of clinical evaluation.[23] Many other natural cytotoxins have been evaluated for the ADC route to cancer chemotherapy, and several of these (*e.g.* taxanes, ansamycins, duocarmycins) are being actively pursued in Pharma. As the technologies for production of antibody conjugates continues to be developed, the ADC approach will figure prominently in the optimization of new classes of potent cytotoxic metabolites.

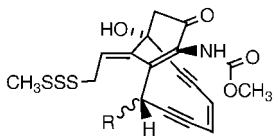
Successful lead optimization will typically require multiple iterations of structure modification and biological evaluation to understand the structure–activity and structure–property relationships necessary to transform the lead into a drug candidate. Both compound supply and an effective structural modification strategy are critical determinants for the success of natural product-based medicinal chemistry programs.

The impact of genomics on natural products drug discovery & development

Recent developments in our understanding of the genetics of biosynthesis and the insights provided by the sequencing and annotation of genomes, particularly of highly productive Actinomycetes, has led to new and greater expectations from natural products research.[24] While much of this promise remains to be realized, there are a number of areas in which genomics has enabled natural products research applied to drug discovery and development.

The impact of biosynthetic medicinal chemistry in drug discovery has already been mentioned – and this avenue for lead enhancement will expand and thrive in concert with the

development of new tools for genetic manipulation. The use of genomic information to discover selected pathway-specific biosynthetic genes has become a powerful means to access a desired series of compounds. An early application of genomics, which did not require whole genome sequencing, was described by workers at Ecopia Biosciences.[25] In their genome scanning approach, DNA fragments derived from bacterial genomes are compared to a database of known biosynthetic gene sequences to identify related pathways. In this particular example, the unique arrangement of five genes responsible for the biosynthesis of the reactive "warhead" in enediyne antibiotics (**14**) was used to probe naïve actinomycetes for related pathways. Fully 15% of the bacterial strains tested showed strong evidence for the existence of enediyne biosynthetic pathways. These were the first experiments that demonstrated the widespread occurrence of this potent family of DNA-damaging compounds in Nature. Given the current state of knowledge regarding the genetics of enediyne biosynthesis, the pathways revealed in this genome-scanning approach could be cloned and further assessed for novelty, or the strains could be grown for subsequent chemical analysis.[26]



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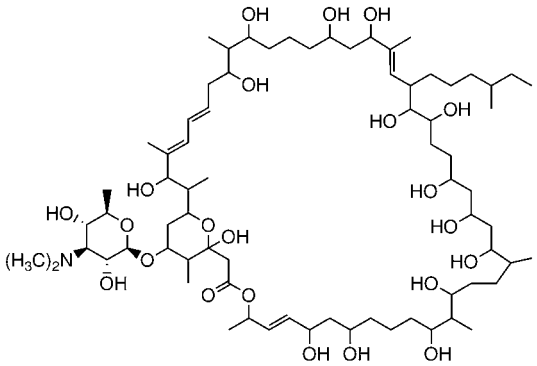
The rapidly advancing technologies for genome sequencing and annotation have already greatly expedited the processes for searching for biosynthetic pathways. With the ready availability of sequence data, *in silico* genome mining has now become a standard approach for the identification of biosynthetic clusters. Specific strategies are devised for discovering broad classes of products, *e.g.* ribosomally synthesized natural products,[27] or specific structural classes, *e.g.* γ-lactam-β-lactone derivatives.[28] Polyketide pathways are particularly widespread in bacteria and these are readily identified in sequencing data. Recently, a unique PK pathway was recognized by genome mining of the partial sequence of *Streptomyces ambofaciens* that illustrates the application of genomic methods for the discovery of novel compounds.[29] The intriguing feature of the pathway was its large size of 150 kb, which would place it among the largest PK's known from bacteria. Although *S. ambofaciens* was known to produce spiramycin and congocidine, no large PK product had previously been observed. Transcriptional analysis indicated that the pathway was not expressed under ordinary growth conditions. Detailed *in silico* analysis of the novel gene cluster led to the identification of a putative regulatory gene that, when overexpressed successfully, induced production. Thus with the aid of genomics a series of novel 51-membered macrolides, now known as the stambomycins, was isolated. The stambomycins (*e.g.* stambomycin A **15**) have some activity in antimicrobial assays and show moderately potent cytotoxic activity against certain cancer cell lines. The stambomycin story clearly illustrates the power of genome mining for the discovery of natural products. As the wealth of genomic information continues to grow these technologies will revolutionize natural product research and development.



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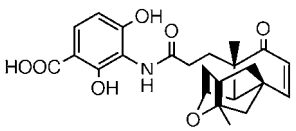
In the downstream environment of pharmaceutical development, genetic advances will play an increasing role in making natural product-derived drugs a practical reality. Cost-effective production of microbial metabolites as active ingredients or crucial intermediates requires levels of production generally in the range of 1–10 g L^{-1}. Such heroic titers have been achieved by intensive cycles of fermentation medium improvement and strain improvement. The latter was traditionally accomplished by random mutagenesis and screening for mutant strains with derepressed levels of production. These processes have had a remarkable rate of success but are exceptionally resource-intensive and lengthy.[30] The unfolding knowledge of the genetic regulation of the expression of secondary metabolites has enabled substantial progress in rational strain improvement.[31] The results reported by Ben Shen and coworkers on strain improvement of Merck's platensimycin (**16**) producer *Streptomyces platensis* illustrate the power of the strategy.[32] Bioinformatic analysis of the platensimycin gene cluster revealed the presence of a gene (*ptmR1*) that was identified as a negative regulator of pathway expression. Targeted knock out of this gene resulted in a strain with 100-fold enhancement in production titers of the antibiotic, reminiscent of the huge gains in production obtained through random mutagenesis.



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Product profiles, or the spectrum of metabolites expressed in microbial fermentations, are of major concern to biopharmaceutical development scientists. Complex profiles introduce the need for expensive, high-resolution refining steps, such as chromatography, in order to obtain a single purified product. Accordingly, one goal of fermentation development efforts is the creation of strains and fermentation conditions that yield the single component of interest. Tailoring the product profile requires some insight into the biosynthetic process, and ideally the identification of a step in the biosynthetic sequence that is responsible for the generation of unwanted components. Efforts can then be devoted to minimizing the impact of that divergent step by adjusting fermentation conditions or through genetic modification. In the production of the avermectin analog doramectin (**17**), *aveC* is a gene of unknown function that affects the

ratio of desirable to undesirable congeners in fermentations of *Streptomyces avermitilis.* The desired product doramectin contains an olefinic bond at C22–23, whereas the coproduced congener CHC-B2 (**18**) lacks the double bond and has a hydroxyl group at C23, as shown in Fig. 6. Knock out of *aveC* results in abolition of avermectin production, and overexpression has no effect, therefore more subtle changes were needed to alter the product ratios. In a series of experiments using error-prone PCR and semi-synthetic DNA shuffling, the *aveC* gene was evolved in a stepwise fashion.[33] In order to facilitate the screening of clones derived from these experiments miniaturized solid phase fermentations were conducted in 96-well plates. Product ratios were analyzed by a high-throughput MS/MS procedure. Following four iterations of this process, improved strains were obtained in which the ratio of doramectin to the undesired CHC-B2 metabolite were improved more than 20-fold. This work highlights the independent value of genomics as a tool. Although the exact biochemical mechanism of AveC remained unknown, it was possible to alter the product profiles by an understanding of sequence–function relationships alone.

Conclusions

The strategic shift of the major pharmaceutical R & D companies away from engineering drugs that bind to single targets back to the discovery of agents that induce desirable phenotypic effects, greatly enhances the potential of natural products in drug discovery. Polyketide-derived natural products represent a major class of secondary metabolites whose unrivaled chemical diversity and structural plasticity have led to the discovery and development of a wealth of biomedical products. The maturation of the science of genetic engineering of PKs, coupled with the development of innovative biologically complex screening systems presents a great opportunity to reinvest in these compounds. The two major obstacles to advancing a natural product lead into drug development are compound supply and/or adequate structural diversification strategies. One must not underestimate how much material may be needed. Even the most straightforward courses of pre-clinical studies require hundreds of grams of highly consistent well-characterized product, which represents a major hurdle for natural products derived from non-renewable sources. Functional synthesis and applications of biosynthetic technologies in microbial systems offer the means to successfully translate the beneficial properties of scarce natural resources into pharmaceutical realities.

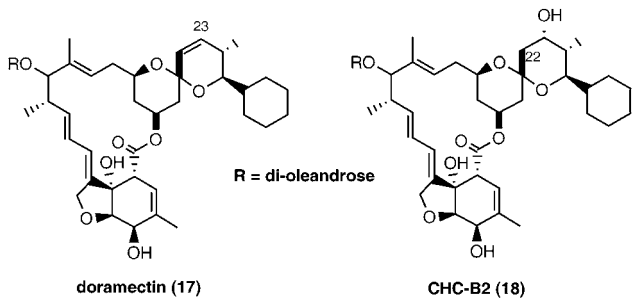

Fig. 6 Antiparasitic agent doramectin (**17**) and the undesired co-produced metabolite CHC-B2 (**18**).

Products of microbial biosynthesis, particularly those derived from actinomycetes, continue to provide viable platforms for drug development. Genomics applied to biosynthetic pathways is providing crucial insights for the regulation of production in bacterial systems that promises to shortcut the construction of highly productive strains and thus expedite product development. Perhaps the most promising finding from the study of the genomes of actinomycetes is the revelation that numerous cryptic biosynthetic pathways are present in even the most thoroughly studied species. The products of these pathways are likely to represent the starting points for the next generation of drug discovery.

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