Will natural products remain an important source of drug research for the future?

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In the highly competitive environment of contemporary pharmaceutical research, natural products provide a unique element of molecular diversity and biological functionality which is indispensable for drug discovery. The emergence of strategies to deliver drug leads from natural products within the same time frame as synthetic chemical screening has eliminated a major limitation of the past. At a more functional level, the application of molecular genetics techniques has permitted the manipulation of biosynthetic pathways for the generation of novel chemical species as well as rendering hitherto uncultivatable microorganisms accessible for secondary metabolite generation. These developments augur well for an industry confronted with the challenge of finding lead compounds directed at the plethora of new targets arising from genomics projects. The exploitation of structural chemical databases comprising a wide variety of chemotypes, in conjunction with databases on target genes and proteins, will facilitate the creation of new chemical entities through computational molecular modelling for pharmacological evaluation.

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Introduction

The study of secondary metabolites that organisms such as microbes and plants have evolved, largely for the purpose of their own survival, has historically proved of immense benefit in drug discovery, providing a rich source of structurally novel bioactive molecules, many of which have become life-saving drugs. Natural product successes have included drugs for a variety of therapeutic indications, exemplified by cyclosporin (immunosuppression), mevinolin (hypercholesterolaemia), avermectin (parasitic disease), the vinca alkaloids and taxol (cancer). Additionally, natural products have made a contribution to fundamental biological science. The field of intracellular signal transduction, for example, has been developed, in large part, through the study of cyclosporin, FK506 and rapamycin, which also exemplify the principle of small molecule-induced modulation of complex protein interactions.

Today, the discovery and development of drugs derived from natural products has to be viewed against the background of the recent developments in automated synthetic chemistry which offer significant complementary routes to drug leads. Small molecule libraries, with molecular weights principally in the range 250–600, originally conceived for lead optimisation purposes and constructed around an ever-increasing number of building blocks or scaffolds are becoming available on an unprecedented scale for *de novo* drug discovery, relative to the synthetic libraries of the past, which were centred, for the most part, upon a particular pharmacological profile. Typically generated in tens of thousands of compounds in a single experiment, compounds synthesised by combinatorial chemistry are immediately available for screening, with the added advantage that a 'hit' meeting appropriate criteria is, by definition, also rapidly accessible for further evaluation and scale-up synthesis.

By contrast, microorganisms and plants have the capability to create unusual chemical structures in a myriad of different and unpredictable ways. The genes and pathways by which this is achieved are increasingly well understood and just beginning to be rationally exploited in drug discovery. At one level, the biosynthesis of secondary metabolites in microbes and plants represents combinatorial chemistry at its most expansive and versatile, in as much as natural product biosynthesis is driven enzymatically from a vast gene pool that has evolved over billions of years.

From the perspective of drug lead finding, two factors critically distinguish natural products from synthetic chemicals, namely, molecular diversity and biological functionality. It is universally recognised that molecular diversity among natural products far outweighs that of todays combinatorial libraries based on scaffolds, which, despite considerable advances, are still relatively limited in scope. Furthermore, as the products of organisms which are known to have many homologies with mammalian systems, natural chemicals sometimes exhibit properties other than, or in addition to, the anti-microbial activity with which they are traditionally linked.

One of the issues hitherto associated with natural products as a source of new drug leads has been that the screening materials are complex. Uncharacterised biological mixtures often contain interfering substances and so require extensive, costly and time-consuming deconvolution and iterative bioassay-guided chemical fractionation to identify the active principle. Such approaches are becoming obsolete and are being superseded by innovative technologies and process engineering that render natural products as amenable to screening as synthetic libraries in terms of cost-effectiveness and time to lead finding, as well as providing an indispensable, complementary source of

molecular diversity for the numerous new disease targets emerging from genomics research.

A second issue associated with natural products is that, as lead compounds, they are not uncommonly refractory to immediate synthesis. It is clear that where the lead compound has proved to be a 'home run' (i.e. requiring minimal or no chemical modification) structural complexity has not prevented its progress to the market. Even in the case of a structure as complex as Taxol (discussed below) the generation of more active compounds by derivatisaton of the original structure has been feasible. The new paradigm of using natural products in drug discovery discussed in this article anticipates that structural information will be used in new ways made possible by advances in molecular modelling and chemoinformatics.

This article will review the progress of some well-known natural products through development in different therapeutic fields, illustrate how certain natural products are being modified to generate compounds with superior therapeutic properties, exemplify natural products with new mechanisms of action and with unprecedented activities against new targets, and summarise the technological innovations and process efficiencies which are revolutionising the approach to using natural products in drug discovery.

An array of newly launched and pipeline products

In cancer, for example, there has been burgeoning interest in the anti-tumour taxanes as a class. Taxol, (Paclitaxel) and its derivatives, prevent cell division by accelerating the polymerisation of tubulin and stabilising microtubules [1–3]. The development of taxol has been well reviewed [1]. Taxotere (Docetaxel) is a taxoid derivative synthesised during the search for a semi-synthetic production route to taxol from 10-deacetylbaccatin III, a precursor extracted from the needles of the European Yew, *Taxus baccata* [2,3]. Docetaxel has been launched for the treatment of ovarian, breast and non-small cell lung cancers and is being investigated for wider clinical application [4]. Further synthetic modification of taxol is an active research area and the synthesis and structure activity relationships of a second generation of anti-tumour taxoids was recently reported [5]. These new taxoids show improved cytotoxic potency compared to taxol and taxotere against a range of cell lines, including drug-resistant lines, and are postulated to exhibit reduced binding to the membrane efflux pump P-glycoprotein, which makes them less susceptible to efflux by this energy-dependent pump. A new family of natural products, the epothilones, from the myxobacterium *Sorangium cellulosum*, have recently been reported to prevent cells from proliferating by the same mechanism as taxol and have potential advantages in terms of activity against drug-resistant cell lines, water-solubility and ease of production [6]. A second class of semi-synthetic antineoplastic agents from plants consists of the camptothecin derivatives irinotecan, topotecan and 9-aminocamptothecin. These compounds were developed after identification of the mechanism of action of camptothecin, an alkaloid from the Chinese tree *Camptotheca acuminata*, as topoisomerase 1 inhibitors [7,8].

Compounds with the ability to modulate multi-drug resistance P-glycoprotein function *in vitro* are not uncommon, and include several natural products, notably cyclosporin and its non-immunosuppressive analogue, PSC 833. The appropriate pharmacological profile for drug development, however, has hitherto been difficult to achieve, as PSC 833 causes unwanted side-effects in humans. Screening of a *Streptomyces* sp yielded a new class of lead compound [9,10] from which drug development candidates have been optimised and entered into clinical trials.

Marine organisms are also proving to be an abundant source of potentially useful antineoplastic substances [11], several of which are at various stages of preclinical and clinical evaluation [11,12•]. Evaluation is most advanced for bryostatin 1, a metabolite of the colonial bryozoan, *Bugula neritina*. Bryostatin 1 is a protein kinase C activator which exhibits potent and selective activity against leukaemia cell lines and excellent *in vivo* activity [12]. The compound is also reported to inhibit the cyclin-dependent kinases within tumour cells, probably by stimulation of the p53 target p21 [13]. As is the case for other promising marine organism metabolites, material supply is a concern, not only in respect of enabling efficient and timely evaluation but also from the point of view of marine habitat conservation. Large scale collections of *B. neritina* have been processed to provide sufficient bryostatin 1 for completion of the phase II clinical trials currently in progress.

In the area of immunosuppression, a number of microbial products have been introduced or are being evaluated, and this area has also been recently reviewed [14]. FK506 (Tacrolimus) has been introduced for transplant rejection [15] but, although more potent *in vitro*, it appears to have a lower therapeutic efficacy than cyclosporin A [14]. Rapamycin is currently in development for use in combination with cyclosporin A [14]. Rapamycin and cyclosporin act by different mechanisms which, theoretically, should achieve an effect that is at least additive and may be synergistic. Mycophenolate mofetil is the morphylinoethyol ester of the *Penicillium* sp. metabolite mycophenolic acid. It acts as a prodrug for mycophenolic acid and has been introduced for the prevention of acute kidney transplant rejection in conjunction with other immunosuppressive therapy and for treatment of refractory acute kidney graft rejection [4,14].

In the cardiovascular area, microbial screening has been widely used. In the search for low molecular weight inhibitors of plasminogen activator inhibitor (PAI)-1, two

diketopiperazines, isolated from *Streptomyces* sp., were found to inhibit PAI-1 activity in an amidolytic assay for tissue plasminogen activator-mediated plasmin generation. These compounds enhance fibrinolysis *ex vivo* and protect against thrombus formation in rats [16,17]. Medicinal chemistry has generated derivatives with improved properties, principally *in vitro* potency and *in vivo* efficacy, [18], establishing this series as the first of a potentially new therapeutic class. The interaction of PAI-1 with a small natural molecule further exemplifies the potential of natural products to provide small molecules that modulate critical protein functions, particularly where no small molecule ligand pre-exists, in a therapeutically beneficial way.

New technologies in natural products drug discovery

As discussed above, natural product screening programmes have traditionally involved the testing of crude extracts of microbial fermentation broths, plants or marine organisms. More recently, short discovery project timeline expectations have driven such natural product screening operations to apply process re-engineering methods to maximise efficiency in order to compete with other chemical library approaches [19•]. For example, the preparation of microbial samples by resin adsorption and elution methods to reduce assay interference from proteins; dereplication and prioritisation of active samples by chemical fingerprinting, spectral database-matching and biological profiling to facilitate selection of the most active organisms for structural elucidation of the biologically active principle.

A completely different approach is provided by a chemical screening approach to the search for novel metabolites. Physico-chemical screening of natural product extracts with photodiode array and mass spectrometric detection enables sample component characterisation by UV-visible and mass spectra and known compound identification by automated spectral library searching [20]. This screening method provides the basis for establishing a library of purified natural products selected for potential novelty or rarity and avoiding known, non-selective or nuisance compounds [20]. Compounds are purified on a multi-milligram scale to permit both extensive screening at defined assay concentrations and full structure elucidation as soon as an interesting biological activity is discovered. Screening pure compounds facilitates both qualitative and quantitative interrogation of actives in secondary assays, while elimination of a refermentation/resupply step provides the structures of active compounds in the shortest time frame. This approach combines the breadth of natural chemical diversity with the convenience of chemical library screening [20].

Recently, we have also witnessed the first application of combinatorial chemistry approaches to natural products. Based on the technology for the combinatorial synthesis of peptide libraries, a highly efficient solid-phase strategy for synthesizing cyclic peptide-DNA intercalator (actinomycin D) conjugates has been devised [21•]. The number of natural product templates amenable to this type of structural exploitation can be expected to increase significantly in future in line with advances in combinatorial chemistry technologies. This development will not only greatly facilitate the lead optimisation of natural products but also provide the basis for sub-library generation for primary screening.

A further approach is the application of combinatorial biosynthesis. Elucidation of the biosynthetic pathways involved in the production of major families of bioactive natural products, such as the polyketides, is providing the means to build on natural chemical diversity using protein/genetic engineering strategies [22••]. Several different classes of drug are polyketide in nature and are attracting considerable interest because the genes encoding the synthases that catalyse the formation of several classes of these compounds have been isolated, sequenced and functionally analysed. Polyketides can be designed rationally by recombinant assembly of enzyme subunits to produce 'unnatural' natural products. Two new aromatic polyketides were prepared using *Streptomyces* strains engineered to express combinations of appropriate enzymatic subunits from naturally occurring polyketide synthases [23]. The same general approach is being studied more widely for natural products other than polyketides [24]. DNA technology is also being investigated for the production of interesting natural products from organisms that are difficult to cultivate, such as marine organisms [24]. This approach endeavours to by-pass the isolation of microbes from the environment and to isolate genes instead. Collections of DNA representing partial or complete biosynthetic pathways are thereby employed in combination with surrogate host organisms to produce the secondary metabolites of non-cultivatable microbes.

New potential for natural products

Two examples of natural products as drug candidates or agrochemical agents which have generated much productive recent research are the enediyne and β-methoxyacrylate classes of antibiotics. The enediyne antitumour antibiotics include members of diverse structural origin, including the calicheamicins from *Micromonospora echinospora* ssp *calichensis* [25] and the dynemicins from *Micromonospora chersina* [26]. They all possess a 1,5-diyn-3-ene or similar moiety which is responsible for their potent double stranded DNA-cleaving ability via a reactive 1,4-aryl diyl intermediate [25]. In addition to total syntheses of calicheamicin 1 and dynemicin A, a number of synthetic mimics of the 1,5-diyn-3-ene portions of these molecules have been reported [27•,28]. The calicheamicins also possess an aryltetrasaccharide domain which is responsible for their binding to specific DNA sequences. The aryltetrasaccharide of calicheamicin 1 inhibits sequence-specific DNA–protein interactions

and transcription *in vivo*. This has been proposed to form the basis of a development strategy for novel transcriptional antagonists [29]. The β-methoxyacrylate class of antifungal antibiotics includes the strobilurin and oudemansin families of basidiomycete metabolites [30]. Strobilurin A was used as a topical antifungal agent even before its structure was known. The strobilurins and oudemansins are potent inhibitors of mitochondrial respiration, binding specifically to cytochrome b, at least in part by virtue of their β-methoxyacrylate moieties. The strobilurins have been adopted as lead templates for the development of agricultural fungicides, leading to an extensive number of patents and the launch of fungicidal products containing synthetic strobilurin derivatives [30,31].

Natural products research in the area of immune-inflammation has highlighted the potential of a number of compounds to inhibit cytokine production. Several of these have been identified in cell-based assays and the molecular mechanism, where amenable, was subsequently elucidated. A notable example is the radicicol series of compounds, which interfere with cytokine production by inducing mRNA instability [32]. These particular compounds may not have the necessary properties for progression into drugs, for example, lack of potency, pharmaceutical inefficiency and adverse pharmacology, but they comprise the first of a new family of agents with which to interrogate the mechanism of mRNA degradation of cytokines and proto-oncogenes. More recent discoveries indicate that compounds with other, putatively more specific, modes of action on cytokine production can be found by cell-based natural product screening.

Conclusions

This review has demonstrated that natural products continue to sustain their reputation as a viable and fertile area for drug discovery and development. Without them there would be a significant therapeutic deficit in several important clinical areas, such as, neurodegenerative disease, cardiovascular disease, most solid tumours and immune-inflammatory diseases such as rheumatoid arthritis. Most encouraging is the continuing emergence of new natural product chemotypes with interesting structures and biological activities and the potential for sub-library generation for targeted screening.

Most currently marketed natural product-based drugs were identified before the introduction of high-throughput screening and bio- and chemo-infomatics. Since the introduction of high-throughput screening in many companies, natural products have become gradually, but inexorably, deprioritised in favour of combinatorial synthetic libraries. This fact may explain, at least in part, the dearth of high quality lead compounds generally recognised throughout the industry. The number of potential new drug targets emerging from the Human Genome Project and microbial genomics is now set to increase dramatically and the high-throughput screening technologies are already in place to accommodate them. Molecular diversity thus becomes the rate-limiting factor in determining how productive these new screens will be. Driven by these considerations the prospects for natural products to deliver drug leads are probably greater than in the recent past. Increasingly available as pure compounds, natural products are highly amenable to the much broader screening opportunities presented by the plethora of new targets. While the rapid identification of a lead compound, or lead compound series, remains the primary objective of all high-throughput screening regardless of chemical library input, natural products are uniquely well placed to provide structural information from which virtual compounds can be created by computational chemistry and allied technologies. Part of the future direction for natural products, therefore, also lies in using structural chemical databases in conjunction with those of target proteins and genes, and contributing to the generation of new chemical entities through computational molecular modelling. With their structural versatility, natural products can be expected to play a major role in the ongoing transition from empirical drug screening to rational drug design.

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