Modern methods to produce natural-product libraries

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Natural sources offer a wealth of chemically diverse compounds that have been evolutionary preselected to modulate biochemical pathways. Several industrial and academic groups are accessing this source using advanced technology platforms. Methods have been reported to generate large and diverse natural-product libraries optimised for high-throughput screening and for a fast discovery process. In addition to prefractionated and pure natural-product libraries, parallel synthesis gives access to synthetic, semi-synthetic and natural-product-like libraries. Natural-product chemistry and organic synthesis are powerful tools for optimising natural leads and for generating new diversity from natural scaffolds. The amalgamation of both may be expected to become an important strategy in future drug design.

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Abbreviations

BMBF	Bundesministerium für Bildung und Forschung
DYMONS	diversity-modified natural scaffold
GBF	Gesellschaft für Biotechnologische Forschung
HTS	high-throughput screening
SAR	structure–activity relationship

Introduction

The major research focus of the pharmaceutical and agrochemical industries is the fast and efficient detection of active compounds ('leads') with a pronounced developmental and market potential. During the past decade, remarkable efforts have been made to accelerate the lead discovery process (Figure 1). Enabling technologies (e.g. robotics, high-throughput screening [HTS] assay technologies as well as genomics and proteomics) were put into place throughout the industry to generate the necessary high-profile clinical and developmental candidates. After 20 years of molecular modelling with relatively moderate success in *ab initio* lead discovery and the short and stormy period of combinatorial peptide libraries, a whole industry was waiting for the seemingly unlimited scopes of combinatorial chemistry [1,2].

From a mathematical point of view, the virtual generation of chemical diversity seemed to be unlimited, as long as one has not the responsibility to synthesize it in the laboratory. Now, after 10 years, lots of good news has been provided to the chemical community, with developments in small-scale and high-throughput synthesis on solid support and in solution, automation and cheminformatics for the generation of focused libraries. The bad news is that the total output of new chemical entities could not be increased at all. Top management as well as medicinal chemists had to realize that there is still no technology at hand to generate large and diverse chemical libraries to fulfil the needs of HTS. On the other hand, there exists a huge pool of compounds with a seemingly unlimited chemical and functional diversity: natural compounds from various biological sources with a pronounced secondary metabolism [3•,4]. Even though major therapeutic breakthroughs are intrinsically connected to natural compounds (e.g. antibiotics, cyclosporin, statins, taxol acarbose and many more), many pharmaceutical companies lost touch with their 'good old friend'. But this is going to change rapidly. The seemingly unlimited genetic diversity of soil microbes (more than 99% of species are still uncharacterized), in connection with the generation of very diverse compound libraries that fit the needs of HTS as well as rapid isolation and structure elucidation open natural compounds as a new and very innovative avenue for clinical development.

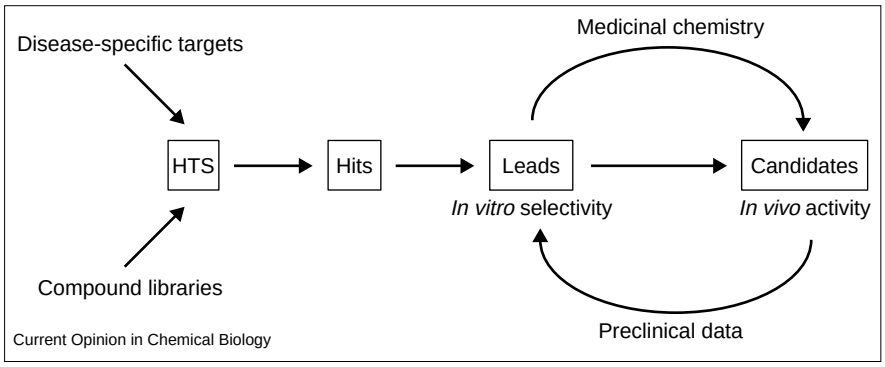
In this article, we describe the generation of high-quality libraries that reflect to a large degree the biosynthetic potential of the respective microbes. Several studies have proven that these compounds provide very few false-positive results but generate leads for extensive developmental studies. In addition, we describe the DYMONS™ (diversity-modified natural scaffolds) concept, a combination of natural compounds and parallel synthesis.

Prefractionated natural-product libraries

Several companies try to access biological diversity with prefractionated extracts of natural origin. bioLeads generates libraries of pre-purified natural products of unknown structure, so called subfractions [5]. These subfractions are derived from cultures of microorganisms (fungi and bacteria) that are filtered first. The mycelium (the mass of branched, tubular filaments [hyphae] of fungi and actinomycetes) of fungi is extracted separately with organic solvents to give a non-polar subfraction. The filtrate is adsorbed onto two different columns and fractionated into nine subfractions with defined weights. This semi-automatic process using the Isoleader System and ContiWeigher System [6,7] allows the generation of about 8000 enriched subfractions per month, which contain the biological diversity of 800 different microorganisms (Figure 2).

Several customers are provided with aliquots of the subfractions for their screening systems. After hit notification, the repository of the subfraction is time fractionated with a semi-preparative HPLC system. The tenth part of these fractions is retested. The repository of the active fraction is analysed by LC-MS and eventually NMR. In most cases, this information is sufficient to perform dereplication of

Figure 1



The current lead-discovery process.

the structure with the advantage of an extremely shortened timeline from hit to structure. The second advantage of this process is the low cost because only structures from the active fractions are elucidated and only the strains with non-replicable substances have to be recultivated for structure elucidation.

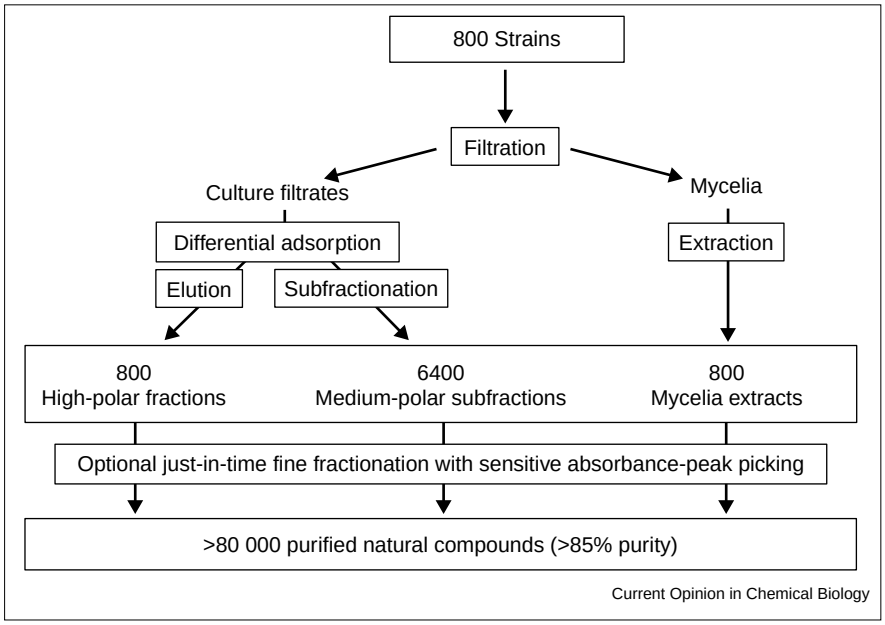
Comparable to this is the approach of Molecular Nature Limited, a company based in the UK, which provides natural compounds from plants. Before preparative fractionation, a chemical fingerprinting of the plant extract is taken to minimize the risk of compound duplicates. The fractions derived from extract are composed of one to four main components.

Pure natural-product libraries

A natural-substance library with defined structures would overcome several problems connected with compounds derived from nature. When a 'hit' is identified in the first screening run, it can immediately be evaluated with respect to its potential drug viability and accessibility by synthetic means.

In collaboration, AnalytiCon Discovery and Aventis Pharma tried to evaluate a system to generate a library of semi-characterized pure natural compounds with a purity of greater than 80% and an amount of more than 5 mg [8]. Within 18 months, it was possible to get 4000 non-redundant substances from plants, bacteria and fungi, 400 of which were randomly selected and classified by their main structure element. The major problem to solve was the high number of redundant and ubiquitous substances. Furthermore, a comparison between this library, the historical Rhone–Poulenc Rorer compound collection (> 50 000 single compounds) and combinatorial libraries (> 50 000 single compounds) was made. A combination of

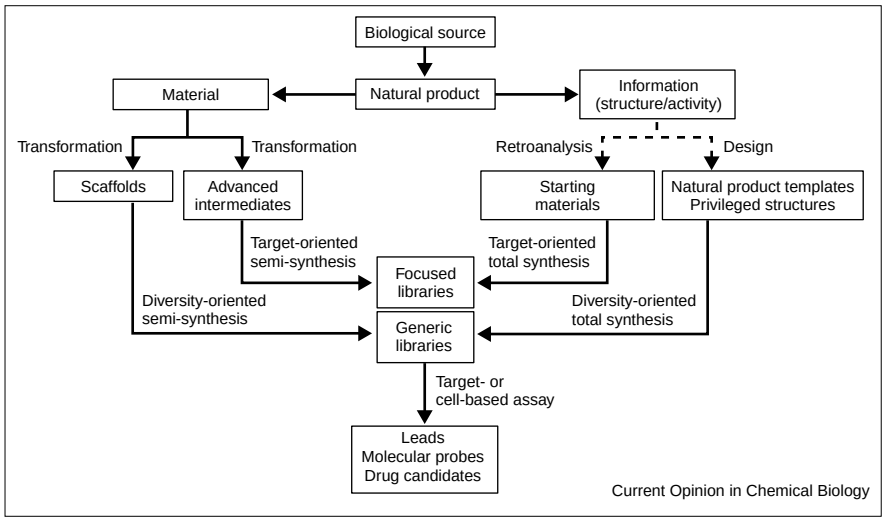
Figure 2



The approach of bioLeads to generate prefractionated and purified natural compound libraries.

Figure 3

Approaches towards natural-product-derived libraries depending on the accessibility of starting materials and library focus.



libraries derived from synthesis and natural sources was found to be ideal for modern drug-discovery process because the distribution of pharmacophores in natural products and synthetic compounds are complementary to one another: natural products contain more oxygen and synthetic substances more nitrogen pharmacophoric groups. On the other hand, the generation of such a library is expensive and relatively slow.

A less time- and money-consuming concept is provided by bioLeads. The subfractions derived from the Isoleader process are further fractionated by peak-based semi-preparative HPLC to generate libraries of purified natural products without structure information but with an average purity of 85%. Within one month, it is possible to produce a library of more than 80 000 substances (Figure 2).

Another approach to build up a diverse compound library is to recruit pure compounds from different sources (e.g. academia, research establishments, small companies and professional providers of natural products such as the Dutch SPECS and BioSPECS BV and Interbioscreen in Moscow). Most of these sources acquire their pure substances from scientists in academia, to sell them profitably to the pharmaceutical industry or agro chemistry. Usually, providers of natural compound libraries have only small quantities (1 mg to 2 g) of each substance in stock. Requirements for additional quantities of a compound of interest for further evaluation (e.g. biological testing) often result in difficulties.

One of the first systematic approaches in the direction of pure compound screening, which ensured direct access to the supplier, was made by the Hans-Knoell-Institut in Jena, Germany. A collection of several thousand natural products and derivatives (Natural Product Pool) was established under the leadership of the Hans-Knoell-Institut in cooperation with partners in academia, the scientific institute GBF and AnalytiCon Discovery within about five years [9–11]. In December 2001, the Natural Product Pool comprised more than 6000 compounds derived from more than 70 laboratories, primarily from Germany. Beyond the BMBF-supported initiation period, the Natural Product pool is exclusively funded by eight industrial partners in Germany and Switzerland since April 1999 (Asta Medica, Aventis Crop Science, Boehringer Ingelheim Pharma, E Merck, Hoffmann-LaRoche, Oncotest, Schering and AnalytiCon Discovery). Approximately 800 to 1000 pure substances in quantities of 1 mg per compound are delivered to the industrial partners per year. For a period of 12 months after delivery, the industrial partners can acquire options or licenses on Natural Product Pool substances. Contract regulations and guidelines enable the industrial partners a secured access to subsequent deliveries of a substance as well as possible patent applications with consideration of the inventive proportions of the Natural Product Pool suppliers. Until now, the industrial partners have requested about 120 compounds for further evaluation.

bioLeads is in the process of generating a combined pure compound library from natural sources and synthesis with detailed structure information. This natural compound library is being completed with partners in academia all over the world. The structures and stereochemistry are confirmed by various physicochemical analytical methods, including MS and NMR spectroscopy. The purity of the compounds is 90–98%. Our libraries provide access to a wide chemical diversity of fungi, terrestrial and marine actinomycetes, as well as plants.

Synthetic and semi-synthetic natural-product libraries

The isolation of natural products from organisms yields large and diverse compound libraries covering huge areas

Table 1

Total synthesis and semi-synthesis of natural product and natural-product-like libraries.

Library	Technique	Author
(*S*)-zearalenone [28]	Total synthesis on solid support	Nicolaou *et al.*
Carpanone [29]	Total synthesis on solid support	Shair *et al.*
Vitamin D3 [30]	Total synthesis on solid support	Takahashi *et al.*
Balanol [31]	Total synthesis on solid support	Nielsen *et al.*
Cycloserin derivatives [32]	Oligomer synthesis on solid support	Gordeev *et al.*
Dysidiolid [33˙]	Total synthesis on solid support	Waldmann *et al.*
Epothilone A [34,35]	Total synthesis on solid support	Nicolaou *et al.*
Fumiquinazoline F [36]	Solid-phase total synthesis	Ganesan *et al.*
Flavones [37]	Solution-phase total synthesis	Marder *et al.*
Indolactam V [38]	Solid-phase total synthesis	Waldmann *et al.*
Fumitremorgin C [39]	Solution-phase total synthesis	Van Loevenzijn *et al.*
Kramerixin [40]	Solution-phase total synthesis	Fecik *et al.*
Mappicine [41]	Solution-phase total synthesis using solid-phase reagents	Curran *et al.*
Muscone [42]	Solid-phase total synthesis	Nicolaou *et al.*
Olomoucine [43]	Solid-phase semi-synthesis based on purine scaffold	Schultz *et al.*
Prostaglandins [44–45]	Total synthesis on solid support	Ellman *et al.*; Janda *et al.*
Rauwolfia alkaloids [46]	Solid-phase synthesis based on yohimbine and rauwolscine	Atuegbu *et al.*
Sarcodictyn [47]	Solution- and solid-phase synthesis from a synthetic precursor	Nicolaou *et al.*
Steroids [48,49]	Solution- and solid-phase synthesis based on natural scaffold	Poirier *et al.*
Tambjamine [50]	Solution-phase semi-synthesis from natural scaffold	Davis *et al.*
Taxoids [51]	Solid-phase semi-synthesis from baccatin III	Xiao *et al.*
Vancomycin [52–57]	Solid-phase semi-synthesis based on synthetic precursor/natural scaffold	Nicolaou *et al.*; Ellman *et al.*

in diversity space [12••,13]. However, only in some cases are sets of structurally related natural compounds isolated that are suitable for the analysis of structure–activity relationships (SARs). Synthesizing analogues that possess appropriate structural variations can elude this obstacle. The new paradigm of creating small-molecule libraries by combinatorial and parallel synthesis [14] has been applied to natural-product synthesis, enabling chemists to rapidly build vast arrays of derivatives based on a common scaffold [15•,16,17•]. Using solid-support resins has led to the manufacture of natural-product libraries by the split-and-pool protocol [18]. Alternatively, solid-phase-supported reagents and scavengers [19] offer a particularly facile parallelisation of already known synthesis pathways.

Focused Libraries

The size and design of a library depends on the knowledge about the addressed biological target. If a natural product proves to be a valid lead for a known target, relatively small libraries of derivatives are usually synthesized to explore the neighbouring diversity space. SAR data can be resubmitted into an iterative process of redesign and follow-up-synthesis aimed at the optimisation of pharmacological and physicochemical properties. If the starting material for library synthesis cannot be obtained in sufficient quantities from natural sources or if substituents need to be varied on positions not accessible by semi-synthesis, a total synthesis strategy is applied (Figure 3). The advent in multistep solid-phase organic synthesis has lead to several libraries of complex natural-product derivatives such as epothilones and prostaglandins (Table 1). A semi-synthesis starting from isolated natural products or semi-synthetic advanced intermediates can be superior to a total synthesis in many respects but a sufficient supply of material from organisms is always prerequisite.

Generic libraries

In contrast to focused or biased libraries resulting from target-directed design, generic libraries consist of highly diverse compounds covering larger areas in diversity space. They are thought to be especially useful for screening in cell-based and chemical-genetic assays eventually leading to the discovery of new drug leads, previously unidentified targets and biochemical pathways. In particular, highly functionalised and rigid polycyclic natural products can be regarded as scaffolds that orient attached substituents in defined spatial directions (exit vectors), comparable to the function of small-molecule peptide mimics [20]. Such natural-product templates can be easily 'decorated' by combinatorial permutation of substituents attached using chemoselective reactions or appropriate protective-group strategies.

Stuart Schreiber's group presented a chemical genetic approach to generate and screen natural-product-like libraries [21•,22]. Starting from synthetic precursors, complexity-generating reactions and diversity-oriented forward synthesis yields large and diverse compound libraries. In a series of papers, Nicolaou *et al.* [23–25] describe the synthesis of generic libraries based on privileged structures (e.g. core motifs, such as benzopyrans, that show especially high occurrence in known bioactive natural compounds).

Diversity-modified natural scaffolds (DYMONS)

We recently launched a program for the development and production of biased, natural-product-derived compound libraries. Natural-product scaffolds are selected from commercial (*Dictionary of Natural Products*; Chapman and Hall) and in-house databases by their lead-likeness [26], as judged by molecular weight, number of rings, number of

rotatable bonds, functional groups, elemantal distribution and calculated logP, as well as by their biological activity. Another important yet often-underestimated criterion is the biological availability of natural products, for instance the production yield from a fermentation process or the seasonal availability of fungal fruiting bodies. Upon isolation, the natural scaffolds are chemically transformed to be orthogonally protected or to undergo chemoselective reactions. In the final step, diverse substituents bearing pharmacophoric groups [27•] are introduced in a combinatorial way yielding libraries of what we named diversity-modified natural scaffolds (DYMONS). The integration of microbiology, separation technology, analytics and high-throughput synthesis proved to be a premise for such an undertaking.

Conclusions

Although natural-compound screening has been an old and very successful technology, we are far from knowing what the real and quantitative content of the biosynthetic genes of secondary metabolites is and making conclusions in respect to the vast and unknown microbial diversity is very premature. Under the aspect of the upcoming huge numbers of disease-related targets and the need for large and diverse compound libraries, it seems more than helpful to combine natural compounds with parallel synthesis and medicinal chemistry for the benefit of patients as well as the innovation-driven pharmaceutical industry.

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