The fall and rise of natural products screening for drug discovery

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Of the 520 new drugs approved between 1983 and 1994, 39% were natural products or derived from natural products. Nine of the 20 best-selling non-protein drugs in 1999 were either derived from or developed as the result of leads generated from natural products, and had annual sales of US$16B. Forty percent of the chemical scaffolds found in a published database of natural products are absent from synthetic chemistry. Despite these impressive figures, the enthusiasm for screening natural product extracts has followed peaks and troughs over a number of years. In recent years, the availability of large libraries of compounds produced by combinatorial chemistry and the pressure to shorten lead discovery timelines signalled another decline of interest in natural product extracts due to the "difficulty" of working with these complex mixtures of compounds. A number of approaches however, have been adopted by companies to improve the speed of, and more importantly, the effectiveness of screening natural products. As a result the use of natural products in industrial drug discovery programmes is currently undergoing a renaissance as some of the difficulties that were traditionally associated with using natural products in high throughput screening programmes are overcome. In addition, the dynamics of the drug screening business has been affected by the consolidation of large pharmaceutical companies, and the impact of gene sequencing and the increase in screen targets and technologies. Some of these changes and their effects upon natural products screening are also outlined.

Key words: ***

Introduction

Natural products screening for drug discovery has been undertaken for many decades, but the popularity of this approach to drug discovery is cyclical, with peaks and troughs occurring over time. It's fair to say that natural products screening has not been the "favoured son" for some time, with many pharmaceutical companies closing or downsizing their natural products groups. In this essay, I will explore the reasons for this and outline a number of strategies that are being undertaken by biotechnology companies to remedy

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this. Some changes to the business of natural products screening as a result of its most recent fall from favour also will be outlined.

The historical success of natural products screening

From a number of perspectives, natural products have been the most consistently successful source of drug leads.

From a medical perspective, microbial and plant secondary metabolites have doubled our lifespan during the twentieth century (Demain, 2002). Of the 520 new drugs approved between 1983-1994, 39% were natural products or derived from natural products. In addition, 60% of new antibacterial or anticancer drugs approved between 1983-1994 were derived from natural products (Cragg *et al*., 1997).

From a chemistry perspective, 40% of the chemical scaffolds found in a published database of natural products are absent in synthetic chemistry. Until 1995, drug development had used only 244 prototypic molecular scaffolds, 83% of which came from natural sources and only 17% from noticing unexpected biological effects of compounds or from chemical synthesis (Harvey, 2001).

From the revenue perspective of a drug company, of the 20 best-selling non-protein drugs in 1999, nine were either derived from or developed as a result of leads generated from natural products. These nine compounds had combined sales of US$16B in that year (Harvey, 2001).

Yet major pharma companies have terminated or are winding down their natural product activities in some form or another. For example, Abbott and Pfizer have terminated their natural product activities, Aventis has abandoned natural product extract screening and undertake pure compound screening only in-house (Bindseil *et al.*, 2001), and GlaxoSmithKline have moved away from natural product screening and have sold-off their sample collections.

What has gone wrong and put an end to the dominance of natural products in drug discovery?

Challenges to natural products screening

The following are some of the reasons for the lack of enthusiasm for screening natural product extracts.

Perceptions

"Antibiotics are 'appropriate' natural products because there's a battle in nature between fungi and bacteria, the microorganisms in the soil. They use

antibiotics to fight with each other. So we adapt those antibiotics for our own use in fighting organisms, modifying them chemically in ways we deem appropriate …….. No battle is being waged in the soil around mammalian viruses. So there's almost no natural product chemistry that's likely to be helpful against viruses, or against cancer, or against a multitude of targets" (David Baltimore, CalTech President and Nobel Prize winner, 1999 as noted in Longman, 2001).

Negative comments such as this concerning the value of natural products in drug discovery are barriers to their continued use.

Timelines

The development of roboticised high throughput screening has increased the pace of the drug discovery process from assay to drug development. Many screening assays now run for periods of months rather than years and the cycle times of lead generation through the traditional activity-guided natural product isolation are often considered too long as they may take the equivalent of the lifetime of a high throughput screen.

Increasingly sophisticated assays

Screening assays have become increasingly more sophisticated and have moved from simple cell-based killing assays to enzyme inhibition assays, receptor-based assays, protein-protein interactions and other pharmacologically and biochemically relevant assay systems (Strohl, 2000).

It is widely accepted that testing chemical entities in living cells is a most powerful tool for discovering and analysing their activity. Cell-based screening methods offer the opportunity to evaluate chemical entities under physiological conditions, and at the same time allow the discovery of molecules with novel mechanisms of action. Most biological extracts, however, are complex mixtures containing an abundance of compounds that may interfere in a non-specific manner with living cells, thus not allowing the differentiation between specific inhibition of the targeted mechanism and general cytotoxicity.

Thus, natural product extracts are often considered to be too "dirty" or "difficult" to assay in these systems. There is a belief that screening natural products may give rise to large numbers of artefacts and that synergistic actions between different components in an extract may be a common occurrence (Harvey, 2001).

Chemistry of natural products

Many "easy to find" antibacterial, antifungal and antitumour natural products have been found and, as a result, known structures are rediscovered frequently during screening programmes. In addition, no information on structure-activity relationships is generated in the first round of high throughput screening as structures are not known and only active compounds are isolated.

The molecules isolated in natural product screening campaigns may be chemically unattractive (albeit to medicinal chemists) due to features such as structural complexity, multiple hydroxyl groups, ketones and chiral centres (Strohl, 2000). In addition synthetic chemists may be reluctant to work with natural products.

New strategies for natural products screening

Having outlined some of the perceived and real problems of working with natural products, what are some of the measures that are being undertaken to improve their success rates in screening programmes?

It is evident that no single strategy will be successful. A combination of strategies is required to improve the:

1. Speed (reducing the time frame of screen to lead to weeks or a few months)
2. Quality (extracts that have reduced false positives and interference)
3. Diversity (effectively accessing structural diversity)

There is little doubt that there are new and wondrous molecules remaining to be discovered in nature, but the challenge is to find more effective strategies to discover them amongst the already known and less interesting molecules that are also there in abundance.

Some of these strategies will be outlined below.

Fractionation

Fractionation of crude extracts can be undertaken to maximise access to chemical diversity through the separation of toxicity from activity. Some compound classes may be lost through using this approach but new strategies can also be developed for the isolation of bioactive compound classes.

The value of this approach to enhance screening effectiveness has been demonstrated through the identification of hits in many cell-based and molecular screens, from which active molecules have subsequently been

isolated and characterized. These could not otherwise have been detected due to overt cytotoxicity or overwhelming non-specific activity displayed by parent extracts. For example, at Cerylid Biosciences, more than 90% of all sub-samples of a number of crude plant extracts showed little or no cytotoxic activity (30% or less) when tested against a human cell line. By comparison, when 60 unfractionated plant tissue extracts were tested in the same cytotoxicity assay at equivalent concentrations, approximately 95% showed marked cytotoxicity.

Thus, testing of the sub-samples yields significant improvements in the effectiveness of cell-based screens by unmasking biological activities which would otherwise remain obscured by an overwhelming background of cytotoxicity. This approach will lead to an increase in the level of chemical diversity which can be accessed effectively from crude extracts.

Data processing to select samples for dereplication

It is common for 100-200K samples to be tested in a typical screen. With typical hit rates of between 0.1-1% this may result in between 100–2,000 hits. Many of these hits are of no interest as they may be due to commonly-occurring compounds, they may represent the combined effects of a number of compounds of low potency in an extract, or they may be due to compounds of high potency but which have undesirable properties.

As it is not practicable to progress all of these hits for chemical isolation, the application of clustering and other algorithms based on patterns of bioactivity and the polarity distribution of fractionated extracts can be helpful in selecting the most varied samples for progression to dereplication.

Dereplication technologies

The aim of dereplication is to provide as much information as possible on the active constituents at an early stage to help decide the necessarily small number of hits that should be progressed into the final phase of compound isolation and characterisation. A streamlined process using short tailored HPLC gradients to provide high resolution over appropriate polarity ranges is one approach that has been applied. In addition, automation of this process may also be undertaken to increase throughput and, at Cerylid Biosciences for example, automation processes currently allow the throughput of over 100 hits per day.

At the dereplication stage, further selectivity/cytotoxicity assays may be applied to the active HPLC fractions, which frequently contain no more than 1-

3 compounds. Data profiles can then be generated for screen hits using physicochemical (chromatographic) and bioactivity (screening) data, leading to more informed selection of extracts that are most likely to yield lead compounds.

Known compounds can frequently be identified at this stage by their UV-characteristics, their MS data, and polarity by comparison with data compiled in the established natural products databases. This ensures that samples which progress to the final stage of chemical isolation and structural elucidation are those which are most likely to contain interesting and novel compounds.

Pre-screened sub-libraries

Another approach to address speed and quality issues is the use of pre-screened sub-libraries of extracts which are enriched for extracts with particular bioactivities, for example, the creation of subsets of extracts demonstrating antibacterial, antifungal, or antitumour activity.

The use of appropriate sample sets can lead to improved hit rates on screening and in the number of bioactive compounds identified from a screen. There are advantages, for example, in testing extracts that have shown activity in whole cell assays against subsequent specific molecular targets, as the odds of finding a hit against a mechanistic target that translates back into a compound affecting whole cell activity are greatly improved.

This approach has been used successfully at Cerylid Biosciences, where several specialised extract libraries have been constructed for antibacterial, antifungal and oncology screening. These focussed libraries represent a movement away from the concept of libraries simply being collections of compounds in search of targets.

Information systems and data mining

Commercially available natural product databases such as the Chapman & Hall Dictionary of Natural Products, Antibase and Berdy are often used from the dereplication stage onwards. As noted previously, known compounds can frequently be identified at this stage by their chemical characteristics by comparison with data compiled in these natural products databases. This ensures that samples which progress to the final stage of chemical isolation and structural elucidation are those which are most likely to contain interesting and novel compounds. Rapid structure elucidation helps to determine compound novelty and to shorten the time frame of screen to lead compound.

Data mining of past screening data can be useful for identifying consistently bioactive extracts and trends or relationships between extracts and to identify and categorise relationships between compound classes and activities. Information systems and data mining are smart strategies for selecting extracts for testing in order to more effectively access structural diversity.

Pure or peak compound libraries

Combinatorial chemistry has become integrated into drug discovery organisations engaged in high throughput screening activities as its processes have the ability to provide very large libraries of compounds for testing.

Pure natural product compound libraries have been created to overcome the speed (of testing), quality of samples (single compounds can be tested) and diversity (structures of compounds are known) issues noted earlier. It can be argued that pure natural compound libraries, despite perhaps having fewer compounds in them than combinatorial chemistry libraries, are of greater value. This is due to their greater chemical diversity (less variations on a common theme) and surprising chemical structures that are not constrained by chemists' thinking.

Peak libraries are an intermediate solution between extract libraries and pure compound libraries. Their production through the fractionation of extracts is less costly than that of pure compound libraries. Peak libraries will include the minor compounds that might be absent from pure compound libraries. Redundancy and dereplication issues however remain, resulting in higher costs for assays and follow-up work (Bindseil *et al.*, 2001). The compounds in the peak library however, may be at least partially characterised, particularly with respect to the amount (weight) and molecular mass. There are a number of technologies now available for determining these parameters in a relatively high throughput manner.

Combinatorial biosynthesis and combinatorial biology

Combinatorial biosynthesis techniques have been used to generate chemical diversity within a microorganism that may be producing compounds of interest through modifications of its genetic make-up. The genes required for the production of secondary metabolites are often found in clusters, and the deletion, shuffling and blocking of gene domains has enabled the production of novel chemical structures. Combinatorial biosynthesis may provide access to a

greater portion of a species' genetic and chemical diversity than is currently possible using traditional approaches.

As only a small percentage of microorganisms in the environment have been isolated and cultivated in the laboratory, an enormous wealth of bioactive compounds remain to be discovered and tested for their medicinal properties. Indeed, as many microbes will be difficult to cultivate at all, combinatorial biological approaches have been developed to isolate genetic material from unknown microbes that are difficult to cultivate and to clone this DNA into cultivable strains. These surrogate hosts can then be grown in the laboratory to allow for the cloning, screening and production of enzymes and biosynthetic pathways leading to novel small molecule natural products. The application of combinatorial biology approaches to drug discovery is, however, at an early stage at present.

A further step in the exploitation of the potential of biosynthetic genes in nature will be the identification of rapid and reliable ways to overproduce natural products using combinatorial biosynthesis and biology methods (see Newman *et al*., 2000 for references to a number of combinatorial biosynthesis and biology papers).

Changes to the natural products screening business

Figure 1 is a simple diagrammatic representation of the types of interactions between a large pharmaceutical company and smaller biotechnology companies that existed in the past. It also includes the more recent situation of the emergence of very large pharma companies, pharma split-ups and the creation of biotech alliances. In the past (Figure 1A), the pharma company often received a service (samples, screen targets or screening technologies) from a biotech company and in return paid in cash or in some other way (e.g., a royalty on eventual sale of products) for that service.

At noted previously, major pharmaceutical companies have terminated or are winding down their natural product activities. In fact, Bindseil *et al*. (2001) observed that there was a general consensus that, in the absence of new approaches, natural product discovery programmes were likely to be marginalised in the 21st century. In response to this change in attitude by the larger pharma companies and the new strategies and technologies I have outlined for natural products screening, there are now a greater number of smaller players on the scene with many covering a limited aspect of the compound discovery process.

As a result, there are now greater interactions within the smaller companies and between the smaller players and larger companies in order to take the drug

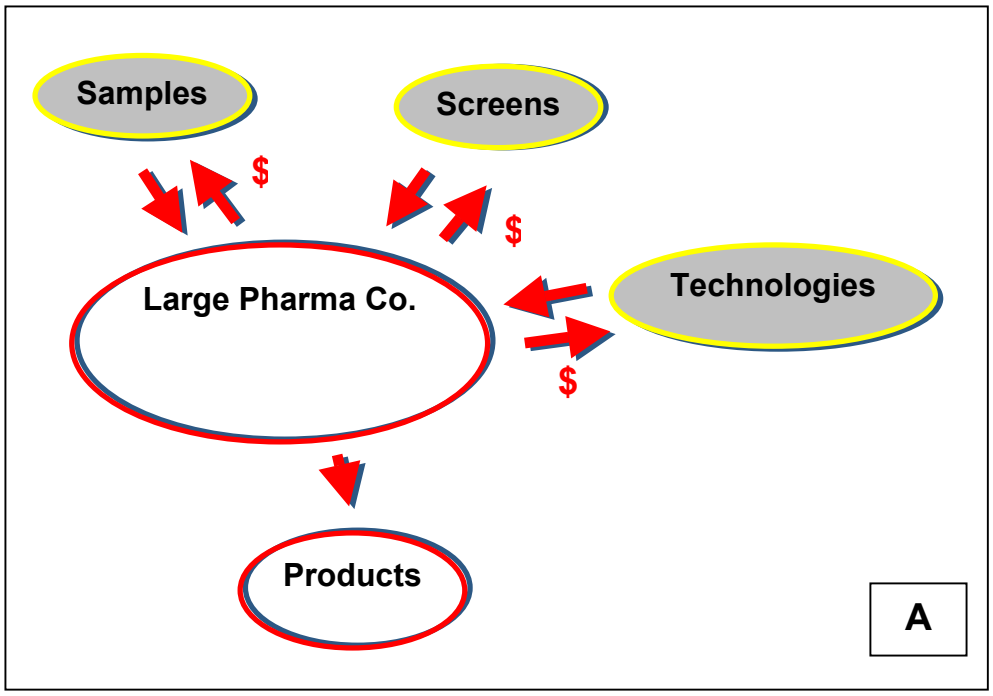
discovery process through to a lead compound. There have also been large pharma mergers, large pharma split-ups, pharma/biotech acquisitions, pharma/biotech strategic alliances, pharma/biotech spin-offs and biotech/biotech alliances. In the period 1993-2001 there was a greater than 500% increase in the number of new biotech/large pharma collaborations (Burrill, 2002). Perhaps more importantly, the number of biotech/biotech deals has increased by almost 70% since 1999 and now exceeds the number of pharma/biotech deals (Berg *et al*., 2002).

Figure 1B is a representation of the more recent complex situation with the emergence of very large pharma companies, pharma split-ups and the creation of biotech alliances. Biotech companies are often working at the front end of the drug development value chain and the pharma companies lend their strengths at the tail end in clinical development, manufacturing and marketing (Berg *et al*., 2002). The biotech interactions and alliances (dotted circle) represent interesting areas as they involve the sometimes necessary interactions of smaller players who may be asset-rich (samples, screen targets, IP on technologies) but cash-poor. New and innovative ways of interacting are required in order to get a lead compound to the stage where it might be taken up as a candidate drug by a large pharma company or pharma spin-off for further development.

Having said that, rather than licensing their compounds to pharma companies that have established development and marketing skills, there are now more biotech companies and alliances developing and marketing drugs on their own in order to establish the full value chain within themselves (Figure 1B). From 1991-94, the US FDA approved the launch of 20 drugs by biotech companies, with that number growing to 50 between 1996-99 (Jern and Bohlin, 2001).

Conclusions

Natural products screening is alive and well, but has changed address from large pharma to smaller biotech companies. New and exciting scientific strategies continue to be devised for the discovery of lead compounds from natural products. There is now a greater interaction between smaller biotech companies in the lead compound discovery process. New and cleverer business strategies are required and are being devised by small companies to co-discover, develop and possibly market lead compounds using the more limited financial resources that may be available.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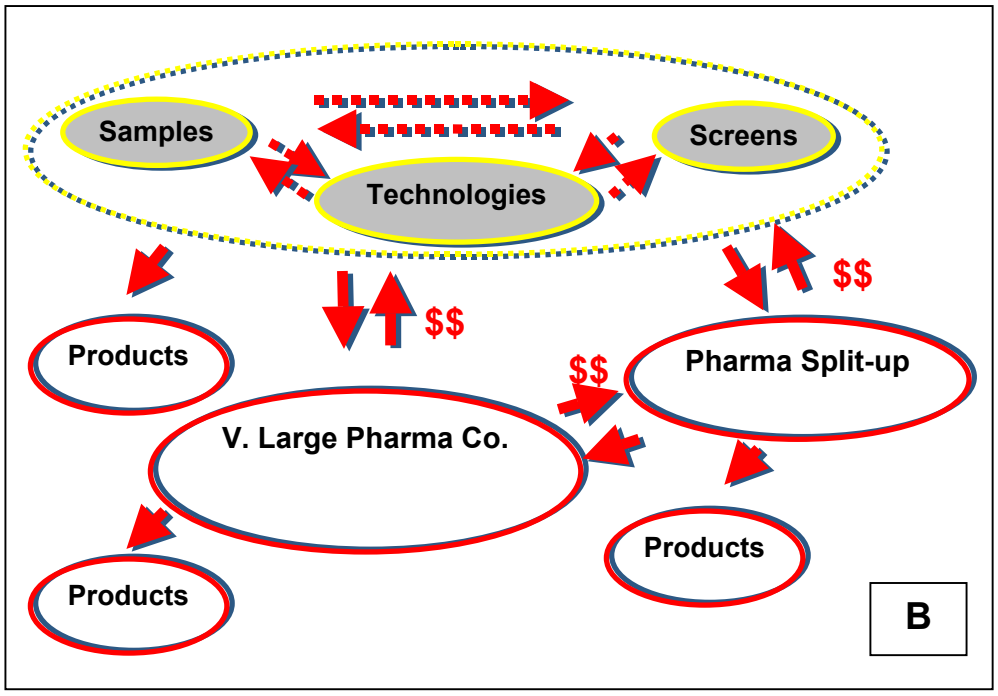
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Fig. 1. Diagrammatic representations of **(A)** the types of interactions between a large pharmaceutical company and smaller biotechnology companies that existed in the past and **(B)** the more recent situation with the emergence of very large pharma companies, pharma split-ups and the creation of biotech alliances. Arrows represent the flow of services leading to a product and the rewards for those services.

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