Why choose to screen a natural product library?

The discovery of new small molecule leads for drug development has traditionally been achieved by four major approaches:

1) Identifying the active compound in an established traditional medicine
2) Screening a natural product library
3) Screening a synthetic compound library
4) Screening a compound structure database *in silico* using molecular docking software

There are inherent advantages and disadvantages to each of these approaches. In particular, there are two major differences between natural product extract libraries and synthetic compound libraries.

The first is that while a natural product library may contain hundreds or even thousands of different compounds per individual extract [1], a synthetic compound library will generally contain a relatively pure (typically >90%) preparation of just one compound in each vial.

The second key difference is that while the structure and means of synthesis of the man-made compound will be immediately available to the researcher as soon as a hit is identified, the active compound present in the natural product extract will require additional steps of activity-guided separation to purify it from the mixture, and further work will often be required to establish the structure of the novel molecule.

Largely for these reasons, most pharmaceutical companies refocused their efforts away from natural products in the 1990s towards the screening of very large libraries comprising millions of synthetic compounds. It was anticipated that bypassing the need for separation and structural elucidation of molecules from natural product extracts would accelerate the process of drug discovery and increase the rate of regulatory approvals. However, it is now widely accepted that the early promise of synthetic libraries has fallen short of expectation, and the rate of approval of new chemical entities has fallen considerably in recent years [2].

A number of factors are likely to explain the reduced success rate of synthetic compound library screening relative to natural products. First, synthetic compounds often demonstrate a less favourable toxicity profile when compared to compounds derived from living organisms [3]. Second, generally speaking, the 'hit rates' achieved by screens of synthetic compound libraries are far lower than those of libraries comprising natural products or their analogs [4-6]. This is likely because phytochemicals are constrained to exist only within the chemical space that is compatible with the basic machinery of eukaryotic cells, and interaction with conserved protein domains [4,7].

Phytochemicals also demonstrate far greater structural diversity and complexity than the relatively simple molecules that populate most synthetic compound libraries, and their structural features tend to be more drug-like

Caithness Biotechnologies Ltd., 72 Boston Road, Leicester, UK, LE4 1HB.
Tel: +44 (116) 326 3802| Company: 9395037 | VAT : 202 4512 64
contact@caithnessbiotechnologies.com | www.caithnessbiotechnologies.com

Phytotitre

Caithness BIOTECHNOLOGIES

Why choose to screen a natural product library?

than randomly synthesised compounds [8]. Molecules derived from natural sources also tend to exhibit more favourable absorption, distribution, metabolism and excretion (ADME) properties, than do randomly synthesised organic molecules, partly due to their common compatibility with cellular transporters [3].

Finally, the key advantage of natural product libraries with respect to 'hit rates' in biological assays stems from the fact that plants have faced millennia of intense selective pressure to evolve means of repelling infection by bacteria or fungi or grazing by insects and mammalian herbivores. As a result, many of the secondary metabolites synthesised by plants have evolved to target specific pathways regulating the activities of bacterial or animal cells [7]. Thus, plant extract libraries are profoundly enriched in molecules which have evolved to interact with and modify specific receptors and signalling pathways in animals, insects, bacteria and fungi, and many of these may have therapeutic potential.

Beyond molecular diversity and biocompatibility, a major practical consideration of smaller research groups is the size of the library that must be screened to generate hits. Synthetic libraries can contain millions of compounds, and hit rates can be as low as 0.001% [4]. This means that many thousands of plates may need to be screened to identify hits. Clearly, the costs of screening such assets in terms of labour and assay costs far exceed the means of most independent laboratories.

However, because natural product extracts contain hundreds to thousands of compounds per well, and typically yield a much higher hit rate, preliminary screens can be comfortably performed by smaller groups with much smaller libraries. In our view, the most cost-effective route to lead finding in the current setting is the screening of appropriately focused natural product extract libraries.

Finally, it should be noted that although most large pharmaceutical companies have shifted their focus to synthetic compound screening in recent years, natural product research has continued in the hands of academic research groups and smaller enterprises, and drugs are still being developed from natural leads. Indeed, of the 1,073 new chemical entities in the small molecule category approved between 1981 and 2010, more than half were based on a natural lead, and only 36% were fully synthetic [9]. Listed below are just a small number of the many highly successful drugs derived from or based on natural leads.

Notable successes of natural product-derived drugs:

- The statin family of cholesterol lowering agents (e.g. lovastatin, simvastatin)
- Most antibiotics (e.g. penicillin, tetracycline and erythromycin)
- The antiparasitic agent avermectin
- Antimalarial agents (e.g. quinine, artemisinin)
- Immunosuppressants (e.g. rapamycin, cyclosporine)
- Many commonly used anti-cancer drugs (e.g. taxol, doxorubicin)

Caithness Biotechnologies Ltd., 72 Boston Road, Leicester, UK, LE4 1HB.
Tel: +44 (116) 326 3802| Company: 9395037 | VAT : 202 4512 64
contact@caithnessbiotechnologies.com | www.caithnessbiotechnologies.com

Phytotitre

Caithness BIOTECHNOLOGIES

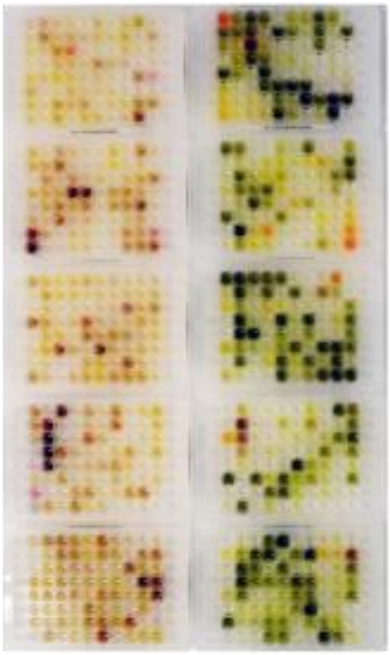
Why choose to screen a natural product library?

Ten reasons to choose a natural product library over a synthetic compound library:

1) Phytochemicals offer far greater structural diversity than synthetic alternatives
2) Phytochemicals occupy a more 'drug-like' chemical space than synthetic alternatives
3) Proven compatibility with conserved protein domains and eukaryotic machinery
4) Highly enriched in compounds targeting specific pathways in microbes and mammals
5) Phytochemicals tend to demonstrate a much higher 'hit rate' in biological assays
6) Plant-derived leads are typically of lower toxicity than purely synthetic compounds
7) Absorption, distribution, metabolism and excretion (ADME) profiles are often superior
8) Greatly reduced screening stage labour and assay costs (smaller number of plates)
9) Potential for the discovery of novel compounds (not possible with synthetic libraries)
10) Historically proven to be the most successful source of new drugs

tSummary of the pros and cons of natural and synthetic compound libraries

Property	Plant extract library	Synthetic compound library
Compounds per vial or well	Hundreds to thousands	One
Labour required for initial screening phase	Little (smaller number of plates)	Lots (larger number of plates)
Additional work required to separate active compound?	Yes	No
Additional work required to establish structure of active compound?	Often yes, sometimes no	No
Hit will be a known compound	Possibly	Definitely
Potential for the discovery of a novel compound	Yes	No
Diversity of molecular structures represented	Very high	Moderate
Proven compatibility with eukaryotic cell machinery	Yes	No
Highly enriched in molecules active on mammalian and bacterial signalling pathways through millennia of evolution	Yes	No
Typical hit rate in bioassays	Often good	Often poor
Typical absorption, distribution, metabolism and excretion (ADME) profile of lead molecules	Often good	Often poor
Typical toxicity of lead molecules	Often good	Often poor



References

[1] Hostettmann K. Strategy for the Biological and Chemical Evaluation of Plant Extracts. IUPAC (1999) [2] Scannell JW, *et al*. Diagnosing the decline in pharmaceutical R&D efficiency. Nat Rev Drug Discov 11:191-200 (2012) [3] Atanasov AG, *et al*. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv (2015) [4] Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science 325:161-5 (2009) [5] Weissman KJ, Leadlay PF. Combinatorial biosynthesis of reduced polyketides. Nat Rev Microbiol 3:925-36 (2005) [6] Wetzel S, Bon RS, Kumar K, Waldmann H. Biology-oriented synthesis. Angew Chem Int Ed 50:10800-10826 (2011) [7] Appendino G, Fontana G, Pollastro F. 3.08 - Natural products drug discovery. In: Liu H-W, Mander L. (Eds), Comprehensive Natural Products II. Elsevier, Oxford, pp. 205-236 (2010) [8] Henkel T, *et al*. Statistical investigation into the structural complementarity of natural products and synthetic compounds. Angew Chem Int Ed 38:643-647 (1999) [9] Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75:311-335 (2012)

Caithness Biotechnologies Ltd., 72 Boston Road, Leicester, UK, LE4 1HB.
Tel: +44 (116) 326 3802| Company: 9395037 | VAT : 202 4512 64
contact@caithnessbiotechnologies.com | www.caithnessbiotechnologies.com