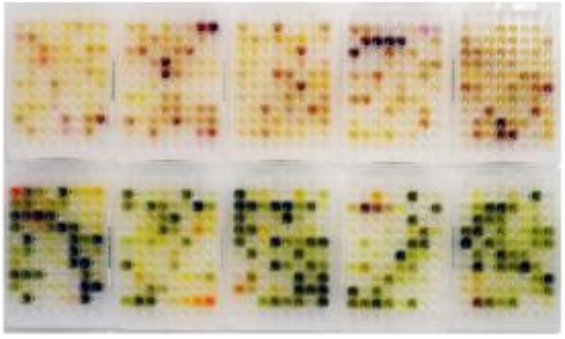
Refinements of the *Phytotitre* natural extract library

Big pharma's recent shift to synthetic library screening was brought about in large part by the view that the throughput of natural product library screening was too slow, typically because of delays introduced by a number of commonly observed difficulties. In particular, these issues included the viscosity of some crude extracts that impeded the accurate pipetting of small volumes, the colour and fluorescence of samples impairing commonly used assay readouts, frequent difficulties and delays in the recollection of rare plants and issues regarding environmental impacts and sovereignty of the discovery when hits were identified in resources from low-income countries or ecologically sensitive regions [1]. In addition to these challenges, smaller research groups seeking to develop new drugs from natural products face a number of other practical considerations. The *Phytotitre* library addresses these concerns in the following ways:

1) Balancing diversity with library size

Most natural product libraries have been developed with the aim of capturing the greatest possible geographical and biochemical diversity and, as a result, are very large (thousands of extracts and hundreds of plates). While this approach provides a good chance of identifying dozens of hits, the costs of screening such libraries, particularly in terms of labour and reagents, are far beyond the means of most small research groups. Fortunately, it is now recognised that many phytochemicals are expressed widely across species of the same genus, and it has been shown that a broad sampling of biodiversity may not be essential for successful natural product-based drug discovery [2].

Indeed, a recent analysis of all alkaloids in medical use today showed that 93% of these molecules occurred more than 50 times in the Global Biodiversity Information Facility (GBIF) database, and only two had less than 10 occurences [3]. These observations indicate that an acceptable hit-rate may be obtained from modestly sized natural product libraries. The *Phytotitre* library has been carefully sized to optimally balance high molecular diversity and potential for lead finding with appropriate workflow by independent research groups.



2) Focused selection of plant species to maximise hit rate

The *Phytotitre* library furthermore seeks to maximise the potential for hit-finding against diverse molecular targets by focusing only on plants with a history of use as traditional medicines, or association with reduced risk of disease in human dietary and epidemiological studies. Notably, the power of using ethnomedicinal information to guide discovery in this way was recently highlighted by a review of 122 approved drugs derived from plant compounds, which found that 80% were prescribed to treat the same condition treated by the parent plant in ethnomedicinal use [4].

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Natural product extract library PT800

3) Recollection and the Nagoya protocol

The Nagoya protocol is a 2010 supplementary agreement to the 1992 Convention on Biological Diversity (CBD). It provides a transparent legal framework to support the fair and equitable sharing of benefits arising from the utilization of genetic resources, thereby contributing to the conservation and sustainable use of biodiversity. The development of molecules from rare or endangered plants may therefore be hindered not only by issues of difficulties in resupply and potential damage to fragile ecosystems, but also with respect to potential legal considerations and the sovereignty of the discovery. To address these concerns, the *Phytotitre* library comprises only plants which are commercially available, so negating any potential issues with recollection, sustainability or national genetic resources. Rapid resupply of raw materials is possible, either through Caithness Biotechnologies, or from other suppliers.

4) Viscosity and precipitation

Insoluble or viscous residues have been removed from all extracts after resuspension, allowing easy pipetting of samples with micro-tips.

5) Appropriate pre-fractionation

A balance needs to be struck between the number of fractions of each extract used to prepare a natural product library prior to screening, and the costs of the subsequent screening efforts. Pre-fractionation can reduce interference between compounds within samples, so increasing the hit-rate, and simplifies subsequent activity-guided separation [5]. However, increasing the number of pre-fractions also increases the number of plates that must be screened, and accordingly the labour, time and cost of the screening component of the work. *Phytotitre* provides two pre-fractions per sample, one polar and one lipophilic, since this balances optimum library size with ease of subsequent separation techniques.

6) Accessibility to smaller research groups

Most libraries are sized and priced beyond the means and throughput of smaller laboratories. *Phytotitre* has been carefully sized and selected to optimally balance excellent structural diversity with accessibility and workflow for smaller research groups.

7) Hygroscopic nature of DMSO

DMSO is highly hygroscopic, and readily absorbs moisture from the atmosphere, particularly when cold and when plates are poorly sealed. Such introduction of water into extracts can cause the precipitation of compounds, and accelerates the degradation of biological activities of extracts in storage [6]. All *Phytotitre* plates are provided with DMSO-resistant re-sealable cap-mats that maximise sample integrity, and are compatible with storage at temperatures as low as -80°C.

8) Separation and compound identification

Following the identification of hits with the primary screen, and triage against appropriate counter-screens, identification of the active compound(s) will be required. This can be achieved either through collaboration with your academic partners, or we can assist you with this process. Please contact us for further details of how we may help you with fractionation of lead extracts and activity-guided separation of your hits.

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Some further tips to help reduce interference in your assays:

- All hits from primary screens should be triaged against an appropriate counterscreen, which is most often an assay based on a related but non-relevant receptor or pathway, or an alternative assay readout
- Cytotoxicity assays will help determine whether the observed effects of hits are related to non-specific effects on cell viability
- If using a fluorescence assay, give preference to red shifted tags, such as rhodamine or Texas Red, and avoid the use of ultraviolet range and green tags
- Because plant secondary metabolites often interfere with light absorbance, time-resolved or background-corrected readouts are often superior to single timepoint measurements
- Consider subfractionation of 'near-miss' hits using low-resolution silica gel chromatography before re-assay

References

[1] Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov 4:206-220 (2005)
[2] Tulp M, Bohlin L. Functional versus chemical diversity: is biodiversity important for drug discovery? Trends Pharmacol Sci 5:225-231 (2002)
[3] Amirkia V, Heinrich M. Alkaloids as drug leads - a predictive structural and biodiversity-based analysis. Phytochem Lett 10:xlviii-liii (2014)
[4] Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 109(S1):69-75 (2001)
[5] Potterat O, Hamburger M. Combined use of extract libraries and HPLC-based activity profiling for lead discovery: potential, challenges, and practical considerations. Planta Med 80:1171-81 (2014)
[6] Zitha-Bovens E, et al. COMDECOM: Predicting the lifetime of screening compounds in DMSO solution. J Biomol Screen 14:557-565 (2009)

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