Chinese plants for modern drug discovery campaigns

Process innovation and new approaches in natural product drug discovery

ABSTRACT

China is getting into focus for building up and conducting pharmaceutical R&D and outsourcing activities. The reason for turning towards China is mostly politically or economically motivated. Scientific aspects often do not play an important role, but China with its huge plant biodiversity is an ideal site for drug discovery and development starting with natural compounds.

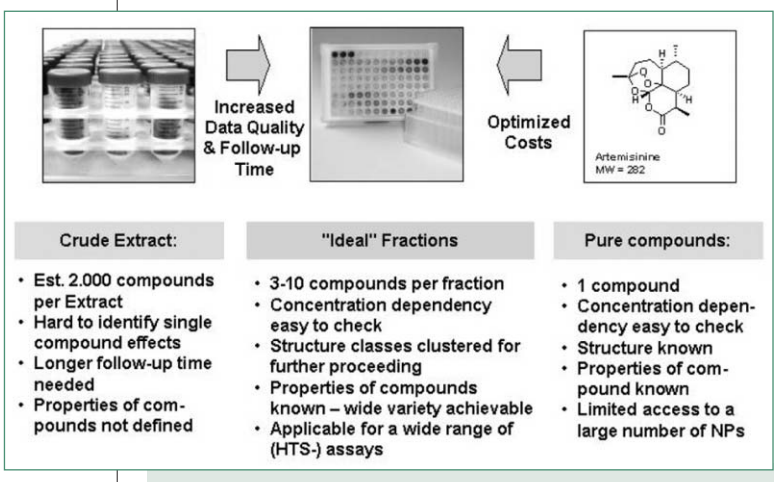
This article gives an overview about advantages and disadvantages of natural product strategies in drug discovery. The authors describe how to deal with small molecules from plants as valuable source for successful drug discovery campaigns and how to meet the demands and standards of Western pharmaceutical industries.

INTRODUCTION/BACKGROUND

Most recently China is getting into focus for building up and conducting pharmaceutical R&D and outsourcing activities (1, 2). Political reasons might be one aspect to gain access to the emerging Chinese pharmaceutical market. Another motivation for pharmaceutical companies to step into China is the cost advantage and access to Chinese talents (3, 4).

Over these economical and financial motives, scientific aspects have partly taken a back seat: China with its huge plant biodiversity, its history in plant derived medication, and its reasonable research infrastructure is an ideal site for drug discovery and development from natural sources.

Drug discovery using secondary metabolites from plants itself became out of fashion in Western industry with the establishment of novel highly automated technologies for drug discovery. These technologies all built on the statistical approach that high numbers of compounds in R&D can count out high quality small molecules. An example for one up so far not-successful-as-expected approach is combinatorial chemistry in the small molecule sector (5). A recently published overview over the last 25 years of drug development could only identify one out of the 1184 new chemical entities (NCEs), which was a *de novo* combinatorial compound that finally got approved (6). Despite these emerging approaches, natural products (NPs) and its derivatives are up to date still the most successful source for drug discovery campaigns. The natural product field is yet producing or involved in 50% of all small molecules as NCEs approvals in the years 2000-2006 (6). To feed the growing demand for reasonable NCEs, it is time to dig into natural resources again. This article shows how to overcome hurdles in the drug development process based on small molecules from (Chinese) plants.



Scheme 1. Pros & Cons in Screening Approaches with NPs

APPROACHES IN DRUG DISCOVERY FROM NATURE

Chinese plants are well-known as treatment tools for Traditional Chinese Medicine (TCM) in various forms. But TCM is 1) an individualized therapy based on 2) mostly complex mixtures of active agents. Hence TCM-"indications" can hardly be translated into Western drugs with its 1) statistical therapeutic concept (same treatment for a group of patients) and 2) mono-agent/single compound drugs. There are rare exceptions documented, where TCM indications and Western medical requirements are overlapping. Such an exception is artemisinine (Qinghaosu), a natural compound from a Chinese plant, traditionally used as fever lowering agent since 1600 years (7). In Western medicine it is used more specifically as anti-Malaria treatment.

Nevertheless choosing Chinese plants for Western drug discovery cannot be first of all a matter of their use in TCM but of being the most creative donors of new chemical structures (plant secondary metabolites) during their millions of year's ongoing race of survival of the fittest.

Main reasons why natural products – in spite of their proven success – have been less and less considered for drug discovery campaigns are lying in two long applied strategies for finding active secondary metabolites from plants and their drawbacks in time, efforts, and costs. On each end of the strategies you will find:

1) Testing of extracts and activity guided isolation. Advantage is that plant extracts are well available, and relatively cheap; but disadvantages are severe. Because of their complexity (+1,000 different molecules), extracts often give (false) positive test results in screening and have to be seen as non-compatible with high throughput screening systems. Further purification/fractionation and re-test often cause a lot of frustration, because of lost activity, or an activity caused by combination effects, or the fact that activity is caused by highly active but minor compounds, which cannot be followed up during repeated isolation/re-testing circles. Last but not least time frame needed starting from extracts to leads can be considered as not acceptable within modern drug discovery campaigns.

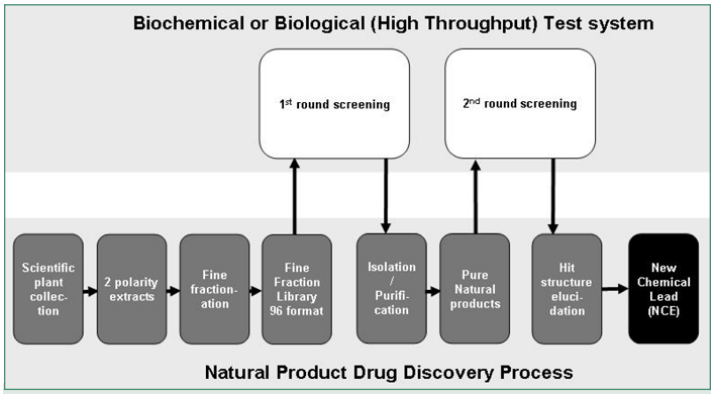
2) Testing of pure natural products with known structure. Advantage is that natural compounds in this approach can be used like other, synthetic compound libraries. Disadvantages are that the number of overall structure

isolated natural products is very low (< 1% of all known compounds) (8) in comparison to synthetic compounds. Often only main compounds from plant species are characterized. In addition the question remains how to gain access to all these compounds, when structure elucidation of interesting natural products can easily reach budgets of several thousand Euros. From already existing libraries with seldom more than several thousand different natural products, it is hard to get NCEs. On the other side it is very cost intensive to achieve new pure natural product libraries at all. Isolation and structure elucidation are costly and cannot compete at least in number with automated synthesis.

THE THIRD WAY

Thus we turned our attention to a third way balancing costs and quality: technologies and process innovation that can bring back small molecules as leads for drug discovery and development. Here in the described case they are derived from endemic Chinese plants from the tropical region of South China.
This approach is the **testing of fine fractions**. These are fractionated extracts according to a robust, innovative separation system with a proprietary separation phase. It allows high separation capacity and reproducibility and therefore reflects to the optimised approach with "ideal" fractions as tools to identify activity related to the plethora of compounds found in plants.
One plant organ is extracted in two extracts, a non-polar and a more polar extract and subsequent fractionated into 180 fine fractions altogether, sorted roughly according to their physical shown logP value.
One of these fine fractions is containing around 3-10 compounds. Advantage of fine fractions is their reduced complexity compared with extracts that turns them ready to use in (high-throughput) screening assays. Within only two rounds of screening, active agents can be identified and then structure elucidated. The development time can be cut significantly. Unlike common pure natural product libraries, that concentrate on (often known) main compounds, it is now possible to build up libraries with also minor compounds and their novel chemical structures, that before were hard to identify from extracts. In comparison to other pure natural product libraries, costly structure elucidation is only done for active, interesting and promising compounds.
Besides the general concept this innovative process is accomplished by:

- Scientific plant collection and characterisation including herbarium voucher, digital pictures and GPS positioning to guarantee source of NCE in close collaboration with renowned Chinese taxonomists.
- Normalizing of fine fractions: every fine fraction prepared for testing has the same weight. This allows the identification of dose dependent activities within primary screening and sort out of false positive activities early on.
- Tailor made profile sub-libraries for special indications based on logP (e.g. higher probability to cross blood-brain barrier)
- The activity guided two round screening processes for fast access to active small molecules.
- Compatibility with a wide range of test systems, ranging from biochemical screenings (e.g. with optical read-out), over whole cell tests to *in-vivo* test systems.
- Follow-up development can reach from medicinal chemistry over cell based secondary metabolites fermentation process to plantations to gain access to larger amounts of material.



Scheme 2: Two-Round Screening Process

CONCLUSION

A Chinese site enables to directly collaborate with local botanists in identifying plant species, laying a solid base for an innovative process combined with state-of-the-art R&D infrastructure, like one can find in Shanghai. The above described technologies and processes bring drug discovery from natural products back into a strong position also in comparison to other small molecule sources in terms of time and costs and are without a doubt superior in terms of novelty/patentability of structure and drug-likeness. A set-up which is drastically needed to increase the number of NCEs for modern drug research.

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