Drug Discovery, Natural Substances & Pharmaceutical Industry

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Abstract

The purpose of this lecture is to discuss the advantages and disadvantages of natural product based libraries, the specific challenges faced by natural products chemistry: supply according to the strict respect of biodiversity conventions and laws, compatibility with HTS, strategic choices, isolation and structural elucidation, patent issues, pharmaceutical development, re-supply…

The Research and Development process, in the pharmaceutical industry, is one of the strictest amongst industrial processes. In fact tens of thousands of compounds must be examined before enabling registration of a new drug in order to reach the market. This low productivity process is long and very expensive (approx. $0.5 billion to 1 billion).

In order to save the therapeutic innovation, three key technologies have been introduced:

- **High Throughput Screening (HTS)**
  HTS enables thousands of biological experiments per day by using one robot in a standardized way.
- **Genomics**
  Genomics and Proteomics are to bring thousands of new targets from the knowledge of human genome (around 25'000 genes) and functional proteome (around 100'000 functional proteins = potential targets for the drug discovery)
- **Combinatorial Chemistry**
  CombiChem allows the build-up of very large libraries, in a standardized format, with little or no problem of re-supply, and the possibility of patenting. The proof of concept has still to be validated.

The research process is presented in Figure 1 (from Prof A. TARTAR).

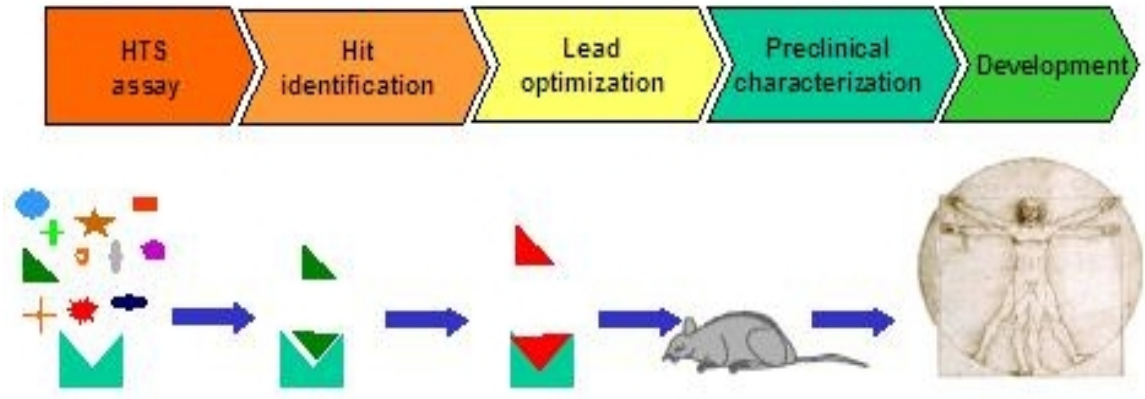


Figure 1

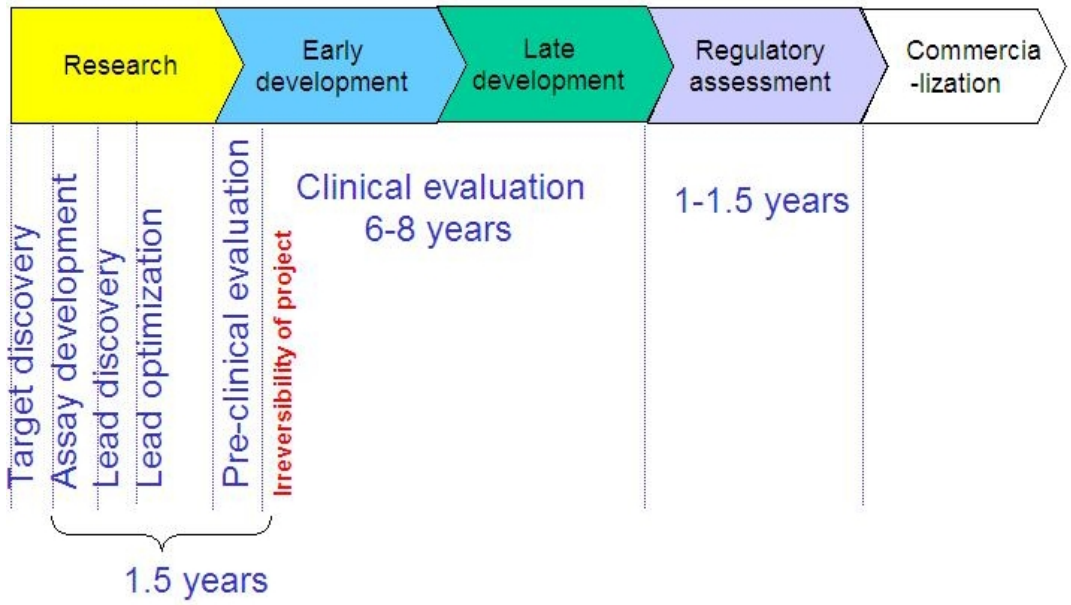


Figure 2

The duration of the different steps in research and development is presented in Figure 2 (from Prof A. TARTAR).

Compared with aeronautics the steps are quite the opposite.
In aeronautics:
Industrials start selling a pre-project to customers. They continue improving the project until the airplanes are produced and delivered to customers.
In pharmacy:
Scientists start with millions of projects (chemical entities) followed by drastic attrition until one molecule enters in development. The project is then fixed for about 10 years during the development before the expected registration, commercialization and delivery to patients.

In this context, natural products present some important advantages:

In theory, they are in very large numbers with an excellent chemical diversity; the proof of concept has been documented for many years, (cf. conferences of Prof. Kinston and G. Gragg). Natural products are « naturally bioactive ». They come from life organisms and have been tailored to play a biological role (crossing of membrane, interactions more or less with enzymes and proteins).

Nevertheless, they present some drawbacks for the pharmaceutical industry:

- Difficult strategic choices have to be made between crude extracts, fractions and pure compounds for the pharmacological screening.

- Concentration of active compounds in a fraction or in an extract is unknown. So we have to keep kissing thousands of frogs in order to find the ones which, after a long kissing process, will finally turn into charming princes.

- Many biological interferences occur between NP and enzymatic based screening tests.

- NP are often chemically complex for medicinal chemists. They possess many chiral centres

- The access of biodiversity is considered to be complex, too expensive, with uncertain and difficult re-supply issues.

- The convention on biodiversity recognises access of biodiversity to everybody. The retribution to the source country should be fair and follow local laws. But in practice, it is difficult to find the right office (or administrative centre) which has the legal mandate to deal with these issues.

- The rights attached to natural products are sensible and complex.

- NP are not easy to patent.

- Dereplications and isolation steps are quite long.

- When we isolate an active product, hemisynthetic or synthetic derivatives of this compound have to be made to improve activity and to get quantitative structure-activity-relationship information.

This chemical work is often uneasy because natural products are complex and difficult to handle.

This situation has forced many companies to reduce or terminate their natural product programs.
Out or almost out:
BMS, Abbott, Pfizer, GSK, Lilly, MSD, Monsanto, Novo Nordisk, Roche…
Still in:
Sanofi Aventis, Ajinomoto, Bayer, B. Ingelheim, Novartis, Pierre Fabre, Schering Plough, Servier, Takeda, Wieth… Research on NP have often been externalized by big Pharmas to small sized spin-off companies e.g., GSK to Merlion in Singapore.

When researching bioactive natural products many choices are possible:

- To work on crude extracts (an ethanolic extract for example): Preparation of a crude extract is very simple, fast and does not need much effort, the chemiodiversity is present. Nevertheless, the quality of the biological data obtained is poor (molecular soup effect) and isolation of the active compound is fastidious because no isolation process has started.

- Preparation of isolated crude compounds before a screening is a heavy job because one needs to isolate and identify the compounds one by one in order to avoid duplication. This work is colossal and delicate since minor compounds escape the attention of the chemist who has to obtain hundreds of compounds from hundreds of vegetal sources. The advantage is the excellent quality of the biological data since

compounds are tested as an isolated pure molecule. Identification of the hits is instantaneous if the huge identification work has already been performed.

- Working on semi-purified compounds is a good compromise between the quantity of preliminary work to be done and the quality of the biological results obtained. This is the choice we have made in our team.

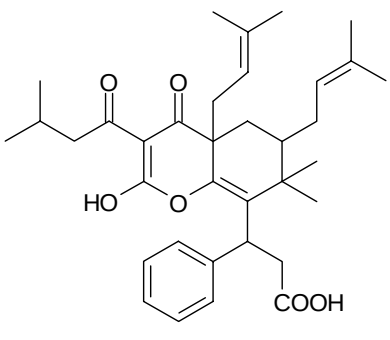
The process begins with the botanical collection in the fields. The samples are collected in accordance with the different laws and decrees on the biological diversity exchanges. Of course endangered or rare plants are excluded from the collection. The choice of the plants is important since the botanical diversity is the source of the chemical diversity. Collections are carried out by different national institutions having mandate to deal with the botanical diversity in their countries applying absolute respects of the laws on biological diversity.

Botanical identification is very important in order to avoid collection in duplicate. Moreover, it allows an efficient dereplication with data bases which indicate what compound is known (or identified) from a definite botanical species. Queries can be made on the data base with the MS data and the botanical name. Our library is rich of more than 12'000 botanical samples and to optimise the work the storage was normalised. In the lab the process begins with extraction of dry ground samples. Extracts are fractionated by chromatography and the dry fractions are dissolved in DMSO and transferred in 96' well mother plates (deep well plate) for HTS biological evaluations. Our library is periodically screened with new targets. After each campaign, chemists have only a few weeks to isolate by bio-guided fractionation the active compounds. Structural identification is performed by mass spectrometry and nuclear magnetic resonance using homo and hetero nuclear 1D and 2D experiments. Actives identified molecules are then evaluated on complementary tests.

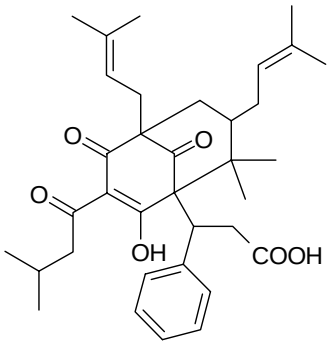
Choice of the target for HTS is a key issue. What enzyme or protein is to be inhibited?
This choice is important because the targets must be relevant for disease, specific…, original…, miniaturisable, robotisable.
The downstream experiments must be planned.

Example of results

For example, we have carried out research and found inhibitors of DNA polymerase β, an enzyme involved in reparation of DNA and therefore of interest in oncology.



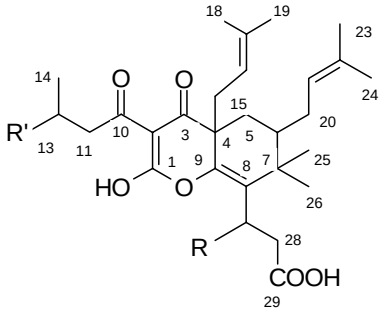
5 new cpds: Mahureone A-E



Laxifloranone

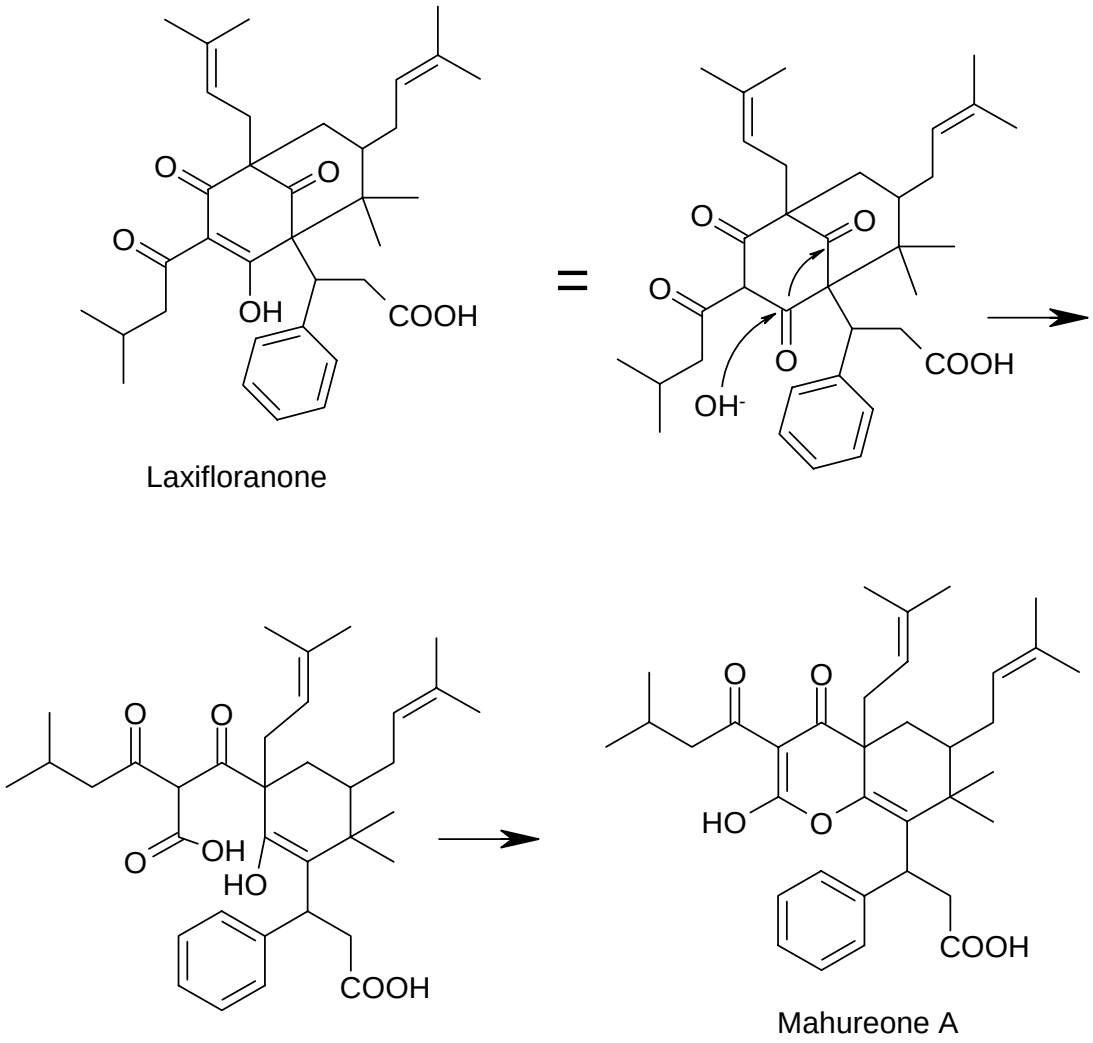
DNA polymerase β (Pol β) is indeed an essential enzyme involved in the DNA synthesis step of the base excision repair and single-stranded break repair processes. Because of the lack of

associated proof reading activity, it is distinguishable from replicative DNA polymerases (Pol δ and Pol ε) by high infidelity in replicating DNA. DNA polymerase β belongs to a newly-identified family of mutagenic DNA polymerases whose cellular functions are to allow cells to adapt to genotoxic stress by promoting DNA repair or to bypass DNA adducts or to participate in the immune response by promoting somatic hypermutation. High levels of Pol β have been found in prostate, breast, and colon cancer tissues, and the possible involvement of Pol β in some tumorigenesis processes and resistance to bifunctional alkylating agents has been suggested because of its ability both to perturb the accurate replicative machinery and to replicate across derived DNA adducts. Inhibitors of Pol β have been sought actively among natural products in the hope of finding use as adjunct therapy with, for example, cis-platinum or camptothecin derivatives. During the course of a large-scale screening of extracts to find new inhibitors of Pol β, we have discovered an active non-polar extract from the leaves of a Guttiferae (Clusiaceae) collected in French Guyana *Mahurea palustris*. Bioassay-guided fractionation of this extract led to a series of new acylphloroglucinol derivatives which were named mahurenones A-E. The more interesting compounds present an IC50 around 10 µM for inhibition of the polymerase.



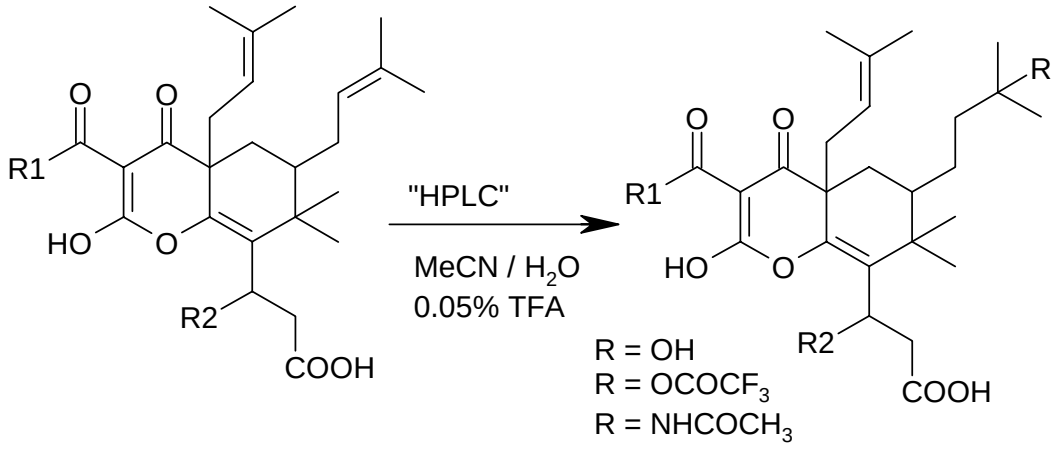
R = Ph R' = $(CH_3)_2CHCH_2$-	Mahureone A IC_{50}= 28 µM
R = Ph R' = $CH_3CH_2CH(CH_3)$-	Mahureone B IC_{50}= 73 µM
R = Ph R' = $(CH_3)_2CH$-	Mahureone C I inactive
R = $CH_3CH_2CH_2$- R' = $(CH_3)_2CHCH_2$-	Mahureone D IC_{50}= 12 µM
R = $CH_3CH_2CH_2$- R' = $CH_3CH_2CH(CH_3)$-	Mahureone E IC_{50}= 340µM

From a biosynthetic standpoint, mahureone A **1** most probably arises from laxifloranone **2** through ring opening of the strained non-enolizable 1,3 diketone ring in basic medium or under nucleophile attack followed by ring closure of the intermediate enol (Scheme 1).



Scheme 1

During HPLC bio-guided fractionation on reversed phase (gradient H_2O, MeCN, 0.05% TFA), we observed apparition of artifacts. The dimethylallyl appendage is prone to protonation, which results in the direct formation of tertiary alcohol **1** and of its trifluoroacetate **2**, along with a Ritter reaction product **3**, formed by the addition of acetonitrile to the double bond (Scheme 2).



Scheme 2

Therefore when the HPLC solvents used to separate this class of compound include traces of acids, care must be taken during the final isolation step. Worthy of note is the selectivity of these reactions, since the other highly similar isoprene unit does not behave similarly, and the absence of neighboring group participation due to the distance between the potentially reactive groups.

News trends in field of Natural Product Drug Discovery

New advances have modified the field of natural products drug discovery.

- Automatic isolation is nowadays possible with devices like the SepBox® from Sepiatec Company. This apparatus is able to prepare pure compounds from a crude extract by iterative HPLC.
- NMR has moved forward with an impressive boost in sensitivity with high fields (900 Mhz), capillary-NMR, cryogenic probes, LC-NMR. According to Bruker Company, it is possible, in theory, to screen in a NMR tube proteins ligand interactions. Therefore, one–should have access to information such as:–what is the active molecule in the crude mixture? what is the binding site on the protein...?
- On-line multi pharmacological detections are today possible after HPLC separation with Kiadis Company device, which allow parallel flow bioassay lines for biological activity, selectivity analyses and spectrometric data in order to obtain structural information.
- Progress in metabolomics will soon permit to predict the chemical composition of a plant extract through the genome, transcriptome and proteome (enzymes) data. This has already been described with the study of a *Saccharomyces* species (Bentley 2002).

Conclusion

Natural products have always played a major role in human therapy and represent a huge reservoir of bioactive chemical diversity. They are privileged sources for lead compounds for the pharmaceutical industry. It is important to preserve this impressive chemical and biological patrimony which is being burned down at the dramatic rate of world deforestation. Protection of this fantastic animal and vegetal treasure should be a tool for preservation and durable development in biodiversity rich countries.

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