

## Successful Drug Discovery from Natural Products: Methods and Results

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### Abstract

The approach to new drugs through natural products has proved to be the single most successful strategy for the discovery of new drugs, but in recent years its use has been deemphasized by many pharmaceutical companies in favor of approaches based on combinatorial chemistry and genomics, among others.

This article reviews some of the past successes of the natural products approach, with an emphasis on natural products with anticancer activity. It then explores some of the reasons why it has fallen out of favor among major pharmaceutical companies in the USA and Europe. Some newer approaches to drug discovery from natural products will also be discussed.

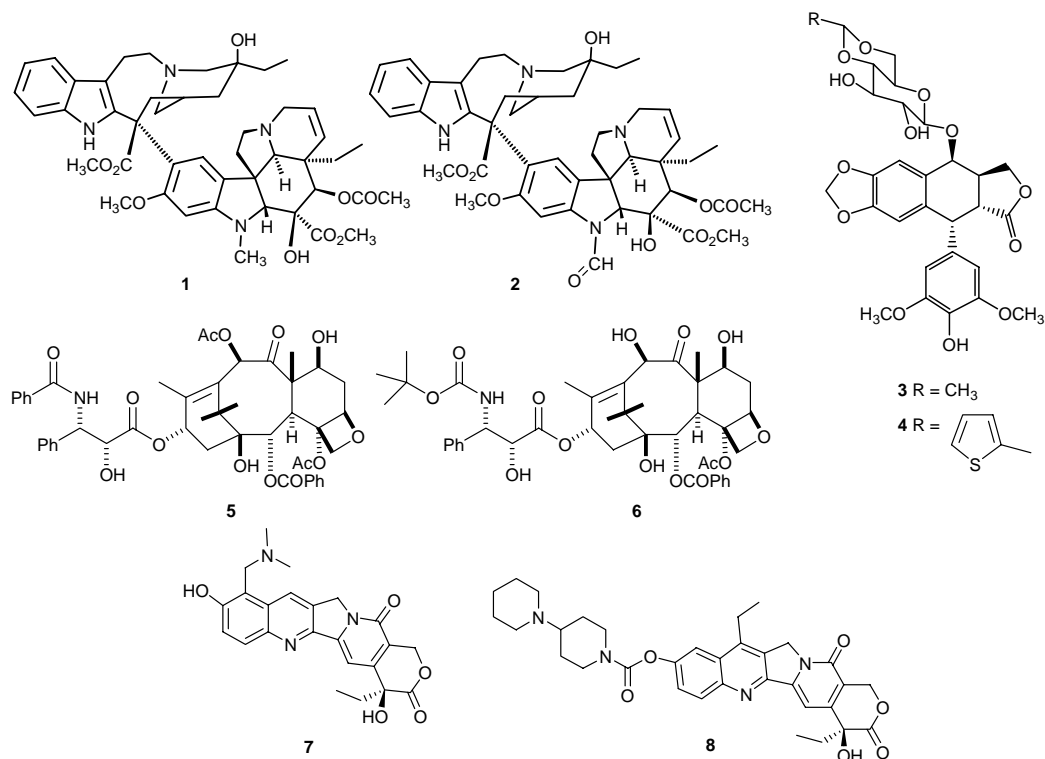
The article concludes with a discussion of International Cooperative Biodiversity Group (ICBG) Program at the National Institutes of Health, USA. We are involved in a collaborative program to discover potential pharmaceuticals in the rainforests of Suriname and Madagascar. This collaboration involves participants from all three countries and from academic, industrial, and non-profit organizations. The benefits of this general approach to biodiversity conservation, economic development, and drug discovery are explained.

**Keywords.** Biodiversity; anticancer activity; drug discovery; bioassays.

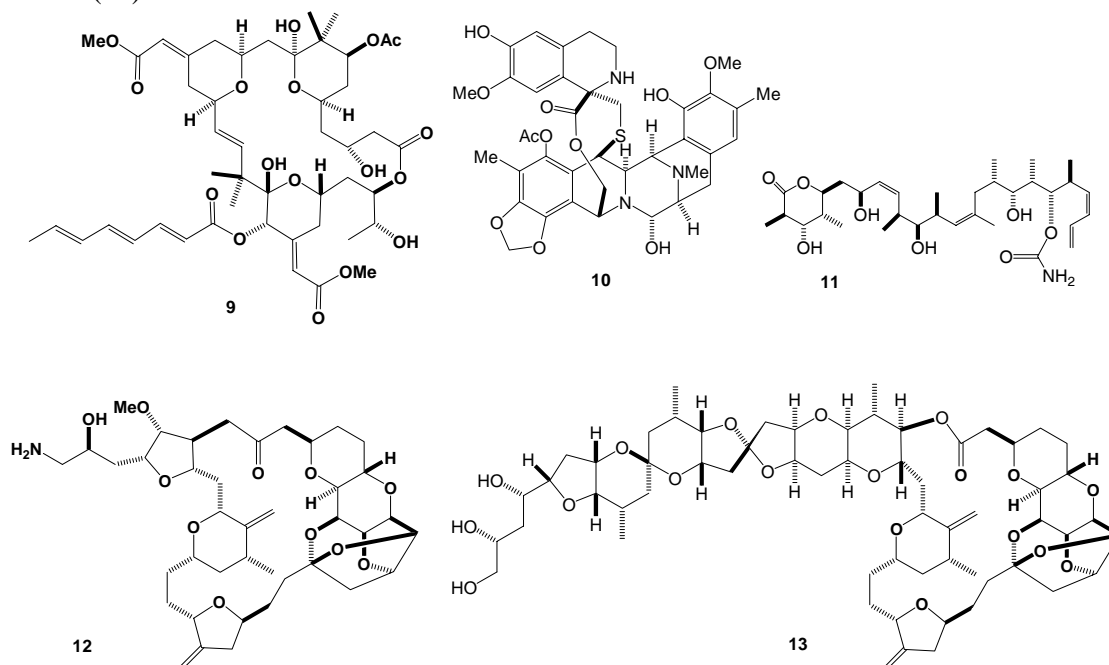
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### The Significance of Natural Products as Pharmaceuticals

The study of natural products, or “Nature’s Combinatorial Library” has a long history as a source of drugs, and especially anticancer drugs. Thus half of all prescriptions dispensed in the U.S.A. contain substances of natural origin, one quarter of all prescriptions contain a plant-derived active principle, and over \$8 billion of U.S. prescription drugs in 1980 were estimated to be plant-based <sup>(i)</sup>. Using more recent data, a recent authoritative review concluded that 61% of all the new drugs introduced worldwide during 1981-2002 can be traced to or were inspired by natural products <sup>(ii)</sup>. Examples of plant-derived clinically used anticancer agents include vinblastine (1) and vincristine (2), etoposide (3) and teniposide (4), taxol (5) and docetaxel (6), and topotecan (7) and irinotecan (8).



In addition to the plant-derived anticancer agents mentioned above, several marine natural products and related compounds are in clinical and advanced preclinical trials as anticancer agents (<sup>iii</sup>); these include bryostatin 1 (**9**), ecteinascidin 743 (**10**), discodermolide (**11**), and E7389 (**12**).



It is instructive to ask why it is that natural products, including plant, marine, and microbial products, have proved such a prolific source of bioactive agents. There are several reasons. In the first place, plants and other organisms produce many biologically active substances for defense and other purposes (<sup>iv</sup>). These substances are often so complex that they would never be prepared synthetically as drug candidates, so isolation from natural sources is the only

feasible way to access them. Furthermore, most natural products have built-in chirality, whereas most synthetic compounds are achiral. Natural products are thus more “druglike” than most synthetic compounds.

This “druglike” nature of natural products has been demonstrated both by statistical analyses (<sup>v</sup>, <sup>vi</sup>) and by various analyses of the importance of natural products as pharmaceuticals. A recent review (<sup>vii</sup>) makes a comparison between natural products and drugs, and shows that there is more similarity between natural products and drugs in several areas (LogP, number of chiral centers, number of nitrogen atoms, number of oxygen atoms, percent of aromatic rings) than between synthetic compounds and drugs.

Natural products were significant from the perspective of a major pharmaceutical company in 1998 (<sup>viii</sup>), and are still significant for some companies. Thus Butler, writing from the perspective of a small pharmaceutical company that is heavily invested in natural products, writes “Another misconception has been that NP research has failed to deliver many new compounds that have undergone clinical evaluation over the last few years. However, in reality, 15 NP-derived drugs have been launched in the key markets of the United States, Europe, and Japan over the last three years, and an additional 15 NP-derived compounds were in Phase III clinical trials at the end of 2003.” (<sup>ix</sup>).

In addition to the use of natural products as drugs, natural products can also lead to new analogs with greater synthetic accessibility or improved activity. A nice example of this is shown by the synthesis of E7389 (**12**) as a synthetically accessible active analog of halichondrin B (**13**). Compound **12** has been prepared in large quantities in an impressive synthesis, and is currently in clinical trials (<sup>x</sup>). The many examples of taxol analogs in clinical trials (<sup>xi</sup>) also demonstrate the value of synthetic modifications of natural products as anticancer agents, as does the exciting activity of 26-trifluoro-(*E*)-9,10-dehydro-12,13-desoxyepothilone B as an improved epothilone analog (<sup>xii</sup>).

### **The Decline in Interest in Natural Products as Pharmaceuticals**

In spite of the obvious successes of the natural products approach to drug discovery, in recent years it has lost some favor, particularly within the pharmaceutical industry. The reasons for this are complex, but can be summarized as being due to a combination of factors, including the incompatibility of crude extracts with the high throughput assays used in the pharmaceutical industry, the cost of sample collection, problems with the lack of reproducibility and the presence of artifacts in some extracts, the difficulty in isolating active compounds, the long resupply times for active extracts, problems with large scale supply if a drug should emerge, the difficulty of complying with the Rio Treaty on Biodiversity, and last but not least, the diversion of resources to combinatorial chemical approaches to drug discovery (<sup>xiii</sup>). However, there is evidence that some people now realize that the move to discontinue natural products research in favor of combinatorial chemistry may have been a mistake. The authors of the review previously cited (<sup>vii</sup>) conclude with these trenchant observations: “The early years of combinatorial chemistry suffered from an excess of hype, and a major victim was natural-product screening. Many organizations went through an irreversible shift in policy, and prematurely discontinued their efforts in this area. We are now seeing the backlash from this knee-jerk reaction. The early combinatorial strategies were flawed and unproven, and have yet to deliver any blockbuster drugs. Meanwhile, we have lost the uniqueness of screening natural-product space as a complement to synthetic compounds. If past indicators are any guide, there are undoubtedly many more unique and potent biologically active natural products waiting to be discovered.”

### **Some Ways to Address the Perceived Problems with Natural Products as Pharmaceuticals**

Although as natural products researchers we may decry the apparent decline in interest in our field by the pharmaceutical industry, we must do more than simply lament this decline. The situation is not in fact as bleak as it might appear at first sight, since several small companies have been formed to take up the challenge of drug discovery from natural products. These include Galileo Pharmaceuticals and SelectX Pharmaceuticals in the USA, Ecopia BioSciences in Canada, and MerLion Pharma in Singapore. It is likely that one or more of these and other similar companies will develop an important new drug from a natural product, which will in turn increase the overall level of interest in natural products.

Having said this, what can be done now to stimulate drug discovery from natural products? The following suggestions range from those that can be implemented by an individual academic researcher to those that require the resources of a major company or the cooperation of national governments.

The incompatibility of crude extracts with high throughput assays can be addressed by some degree of prefractionation of extracts. This can range from simple polyamide filtration to remove polyphenolics which interfere with enzyme and receptor-based assays to the generation of “peak libraries” of partially purified compounds by automated HPLC of crude extracts. A second approach to this problem is to develop and use “smart” assays that are compatible with natural product extracts. These assays can usefully be cell-based, since the cell wall will limit “hits” to compounds that can pass through it, excluding many nuisance compounds such as tannins. As examples, Roberge and his group have developed cell-based assays that have proven effective for discovering novel inhibitors of mesenchymal tumor cell invasion and migration (<sup>xiv</sup>) and for antimitotic agents (<sup>xv</sup>). The use of simple yeast-based assays has also proven effective in discovering DNA-damaging agents in our own work (<sup>xvi</sup>). The development of more assays of this type will certainly be beneficial to the academic researcher in the search for bioactive natural products.

The difficulty in isolating and characterizing complex natural products is becoming less of a problem as new methods for isolation and structure elucidation of natural products continue to be developed. Thus it is likely that HPLC-MS and HPLC-NMR will become routine techniques, assisting with the rapid characterization of complex mixtures. In addition, microprobe NMR will lessen the sample size requirements for structure elucidation; a recent illustration of the power of this technique is the isolation and characterization of thirteen steroids from fifty fireflies (<sup>xvii</sup>).

Problems with resupply and large scale production can be minimized by careful collection work, using GPS to return to the exact location of the original collection. Large scale production can sometimes be carried out by semisynthesis (as in the case of taxol) or by total synthesis (as in the case of E7389). In the case of taxol, the natural product was obtained in only 0.04% yield from yew bark, and there was little initial interest from the pharmaceutical industry, in part because of supply problems. The supply problem was initially solved by semisynthesis from the more abundant 10-deacetylbaocatin III, and taxol is now also available by plant tissue culture (xi).

The geopolitical issues associated with bioprospecting can be approached in various ways. In the first place, it goes without saying that natural product scientists must be careful to collect

biomaterial in approved and legal ways, with preservation of the rights of all parties (including those of indigenous peoples). To enable this to be possible with the minimum amount of bureaucracy, it is desirable that the process for obtaining collection permits should be open and transparent. Although this is true in many countries, it is not universally the case, and this is an area where active collaboration with and (in some cases) education of government authorities is necessary.

Another approach to the use of natural products is as herbal medicines. It is estimated that 80% of the world's population use herbal preparations as their main source of medication, and so there is a great need to standardize and validate these preparations. The National Center for Complementary and Alternative Medicine (NCCAM) in the USA actively supports such work; recent awards by NCCAM include grants on phytoestrogens and aging, on the effect of Chinese herbal medicine on food allergy, on estrogen receptor-selective herbs for menopause symptoms, and on natural product therapeutics in Alzheimer's disease.

### **The Madagascar-Suriname ICBG Project: Development and Biodiversity Conservation**

Our own work has been carried out with support from the International Cooperative Biodiversity Group (ICBG) program at the National Institutes of Health, USA. This approach has several important features. Thus, consistent with the Rio Treaty, bioprospecting is done with the full informed consent of all parties, and bioprospecting is combined with economic development activities, since much deforestation is caused directly or indirectly by poverty. The work is done in partnership with a pharmaceutical company (and in our case with an agrochemical company too), so that there is a natural pathway to drug development when a lead compound is discovered. A part of the agreement between the parties involved is a commitment to return any royalty payments in part to the host country in compensation for the use of its biodiversity. Our work was originally based in Suriname, and is now based in Madagascar.

The structure of our group and the activities undertaken by each partner are as follows:

The Missouri Botanical Garden, under Dr. Jim Miller, carries out botanical collections and makes biodiversity surveys. Conservation International-Madagascar is involved with economic development, biodiversity conservation, and benefit sharing activities. The Centre National d'Application des Recherches Pharmaceutiques prepares the plant extracts, and also does screening for antimalarial activity and isolation of compounds with antimalarial activity. The Centre National de L'Environnement makes collections of marine organisms, and will also carry out some microbial isolations. Eisai Research Institute has an independent program of bioassay drug discovery and development, with an emphasis on the anticancer and immunological areas. Dow Agrosiences does bioassay and agrochemical discovery and development, while our own program at VPI&SU (Virginia Tech) is focused on bioassay and anticancer drug discovery.

Although our work in Suriname is now over, there has been one significant legacy of our time there, the Central Suriname Nature Reserve. In the early 1990's Suriname had three major National Parks in the center of the country. In the mid 1990's some lumber companies from southeast Asia approached the government of Suriname and offered to pay a significant sum of money for the rights to carry out logging operations in the center of the country. These proposed logging concessions would have logged much of central Suriname, would have completely isolated one National Park, and would have destroyed the forest close to many of the villages, thus denying the indigenous peoples the ability to use this key resource.

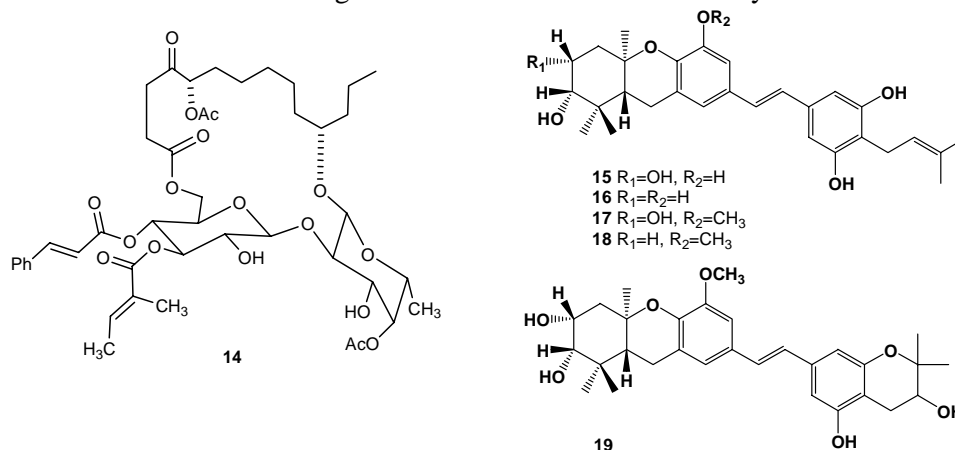
Our ICBG partner Conservation International argued against this potential catastrophe, and used the ICBG program as an important factor in arguments against logging. Using funds from private donors, Conservation International offered to lease a large section of central Suriname, thus replacing the income that would have been generated by logging, but at no cost to the environment. Conservation International and the government of Suriname then jointly established the Central Suriname Nature Reserve, linking the three major parks into one contiguous unit, and providing a major forest resource for future bioprospecting and biodiversity conservation.

Our conservation and development work in Madagascar has not been on this scale, but it has included several small-scale projects for the villages in the area of our plant collections, namely the Zahamena Park area. These projects were selected by the villagers themselves, and the funds for the work came from upfront compensation funds provided by our industrial partners. Examples of the projects are the construction of an agricultural warehouse in Antanandava, renovation of a primary school in Manakambahiny, and construction of a river bridge to enable villagers to reach a clinic during the rainy season. Other projects have focused on developing ecotourism and farming self-sufficiency.

A further important result of our work in Madagascar has been the assistance our program, and especially the teams from the Missouri Botanical Garden, Conservation International-Madagascar, and the Centre National d'Application des Recherches Pharmaceutiques, have been able to give to the government of Madagascar as part of its ambitious program to double the nation's protected areas. The botanical survey work carried out by these partners as part of the overall project has provided a sound scientific basis for the selection of future protected areas.

### The Madagascar-Suriname ICBG Project: Drug Discovery

Much of our work in the drug discovery area has been reported previously (<sup>xviii</sup>, <sup>xix</sup>), and so it will not be reproduced here. Two results, one each from Madagascar and Suriname, are selected for discussion. Work in Suriname led to the discovery that the leaves of *Ipomoea squamosa* yielded a moderately cytotoxic extract. Fractionation of this extract by flash chromatography on a C18 column followed by HPLC on C18 and then phenyl columns yielded five cytotoxic compounds designated ipomoeassins A-E (<sup>xx</sup>). These compounds turned out to have similar structures, of which **14** is representative. Interestingly, **14** was two orders of magnitude more cytotoxic to the A2780 cell line than some of its close congeners, so there are some interesting SAR effects which we do not as yet understand.



Our work in Madagascar has led, among other findings, to the isolation of compounds **15** – **19**. These potent cytotoxins are related to the schweinfurthins (<sup>xxi</sup>), and are of possible interest for drug development. The major stumbling block to further work is the lack of any mechanistic rationale for their activity, but ongoing studies at the National Cancer Institute may resolve this issue and open the way for potential development of these interesting compounds.

### Conclusion

The ICBG program represents an ambitious attempt to integrate the areas of biodiversity conservation, economic development, and drug discovery into a coherent whole. The approach that we have taken in Suriname and Madagascar has yielded modest but tangible benefits in the economic development area, and has contributed significantly to biodiversity conservation. This is especially the case in Suriname, where the Central Suriname Nature Reserve stands as a testament to the foresight and diligence of our partners at Conservation International and to the effectiveness of this integrated approach. In the drug discovery area we have isolated over 380 bioactive compounds, over 180 of which are new to science, and several of which have promising bioactivities. At this point none of the isolated compounds are clear drug candidates, but the full story on all of them has not yet been worked out, and so it is still possible that this area of work could eventually be as successful as the other two areas.

### Acknowledgements

The work described above has been very much a group effort, with participation from many individuals and organizations. The chemical work at Virginia Polytechnic Institute and State University was carried out most recently by Dr. Shugeng Cao, Dr. Maged Abdel-Kader, Dr. Prakash Chaturvedula, Mr. John Berger, Mr. Eba Adou, Mr. Brent Yoder, Mr. Russell Williams, and Mr. Brian Murphy, with able technical assistance from Ms. Jennifer Schilling and Mr. Andrew Norris. Work at our partner organizations was led by Dr. Jim Miller (MBG), Josette Rahantamalala (CI), Drs. Rabodo Adriantsiferana and Etienne Rakotobe (CNARP), and Dr. Jean Maharavo, (CNRE/CNRO). Financial support was provided by the Fogarty International Center, the National Cancer Institute, the National Science Foundation, the National Heart Lung and Blood Institute, the National Institute of Mental Health, the National Center for Complementary and Alternative Medicine, and the Office of Dietary Supplements, Office of the Director of NIH, under Cooperative Agreement U01 TW000313 with the International Cooperative Biodiversity Groups, and this support is gratefully acknowledged. I also acknowledge the US government scientific coordinators of the ICBG project, Drs. Joshua Rosenthal and Flora Katz, and Dr. Yali Hallock of the National Cancer Institute. I also thank Drs. Gordon Cragg and David Newman of the Natural Products Branch, National Cancer Institute, for providing access to extracts of Madagascar plant collections from the NCI Natural Products Repository.

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