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International collaboration in drug discovery and development from natural sources*

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Abstract: Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, particularly plants, with many based on their use in traditional medicine. The past century, however, has seen an increasing role played by microorganisms in the production of the antibiotics and other drugs for the treatment of serious diseases, and more recently, marine organisms have proved to be a rich source of novel bioactive agents. Natural products will continue to play a crucial role in meeting this demand through the expanded investigation of the world's biodiversity, much of which remains unexplored. By using medicinal chemistry, and combinatorial chemical and biosynthetic technology, novel natural product leads will be optimized on the basis of their biological activities to yield effective chemotherapeutic and other bioactive agents. With much of the biological diversity found in tropical and subtropical regions, the investigation of these resources requires multidisciplinary international collaboration in the discovery and development process. Such collaboration can result in substantial short-term benefits accruing to source countries, with the potential for the generation of significant longer-term benefits in the select cases of those agents that proceed into advanced development, and possible commercialization.

Keywords: Biodiversity; drug discovery; drug development/costs and timeframes; multidisciplinary collaboration; international collaboration; benefit-sharing.

BIODIVERSITY: MEDICINAL USES

Recorded history

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2600 BC, while the best-known Egyptian pharmaceutical record is the Ebers Papyrus dating from 1500 BC; this documents some 700 drugs (mostly plants), and includes formulas, such as gargles, snuffs, poultices, infusions, pills, and ointments, with beer, milk, wine, and honey being commonly used as vehicles. The Chinese Materia Medica has been extensively documented over the centuries, with the first record dating from about 1100 BC (Wu Shi Er Bing Fang, containing 52 prescriptions), followed by works such as the Shennong Herbal (~100 BC; 365 drugs), and the Tang Herbal (659 AD; 850 drugs). Likewise, documentation of the Indian Ayurvedic system dates from about 1000 BC (Susruta and Charaka).

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In the ancient Western world, the Greeks contributed substantially to the rational development of the use of herbal drugs. The philosopher and natural scientist, Theophrastus (~300 BC), in his *History of Plants*, dealt with the medicinal qualities of herbs, and noted the ability to change their characteristics through cultivation. Dioscorides, a Greek physician (100 AD), during his travels with Roman armies, recorded the collection, storage, and use of medicinal herbs, and Galen (130–200 AD), who practiced and taught pharmacy and medicine in Rome, published no less than 30 books on these subjects, and is well known for his complex prescriptions and formulas used in compounding drugs, sometimes containing dozens of ingredients ("galenicals").

During the Dark and Middle Ages (5^{th} to 12^{th} centuries), the monasteries in countries such as England, Ireland, France, and Germany, preserved the remnants of this Western knowledge, but it was the Arabs who were responsible for the preservation of much of the Greco-Roman expertise, and for expanding it to include the use of their own resources, together with Chinese and Indian herbs unknown to the Greco-Roman world. The Persian pharmacist, physician, philosopher, and poet, Avicenna, contributed much to the sciences of pharmacy and medicine through works such as *Canon Medicinae*, regarded as "the final codification of all Greco-Roman medicine". Information on this and other Arabic works may be found on the Web site of the National Library of Medicine (NLM), U.S. National Institutes of Health (NIH) at <www.nlm.nih.gov/hmd/medieval/arabic.html>. A comprehensive review of the history of medicine may be found on the NLM History of Medicine homepage at <www.nlm.nih.gov/hmd/hmd.html>.

Plant sources

Traditional medicine and drug discovery

As mentioned above, plants have formed the basis for traditional medicine systems, which have been used for thousands of years in countries such as China [1] and India [2]. The use of plants in the traditional medicine systems of many other cultures has been extensively documented [3–5]. These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization (WHO) that approximately 80 % of the world's inhabitants rely mainly on traditional medicines for their primary health care [6]. Plant products also play an important role in the health care systems of the remaining 20 % of the population, mainly residing in developed countries, and at least 119 chemical substances, derived from 90 plant species, can be considered as important drugs currently in use in one or more countries [6]. Of these 119 drugs, 74 % were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine.

An important example is the antimalarial drug, quinine, which formed the basis for the synthesis of the commonly used antimalarial drugs, chloroquine and mefloquine (Fig. 1). It was originally isolated in 1820 by French pharmacists Caventou and Pelletier from the bark of *Cinchona* species (e.g., *C. officinalis*) which had long been used by indigenous groups in the Amazon region for the treatment of fevers, and was first introduced into Europe in the early 1600s for the treatment of malaria. With the emergence of resistance to these drugs in many tropical regions, another plant, *Artemisia annua* (Quinhaosu), long used in the treatment of fevers in traditional Chinese medicine, has yielded the agents artemisinin and its derivatives, artemether and arteether (Fig. 1), effective against resistant strains [7]. In addition, the use of so-called complementary or alternative herbal products, many of which are derived from medicinal plants, has expanded in recent decades [8].

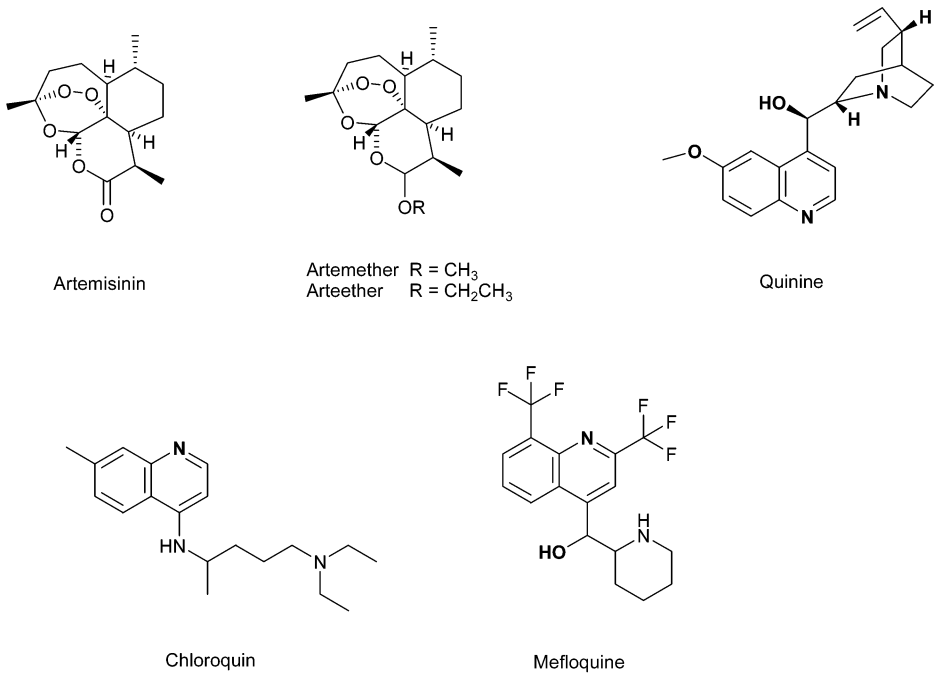


Fig. 1 Plant-derived antimalarial agents.

Plant-derived anticancer agents

Plants have a long history of use in the treatment of cancer. Hartwell [9], in his review of plants used against cancer, lists more than 3000 plant species that have reportedly been used in the treatment of cancer. In many instances, however, the "cancer" is undefined, or reference is made to conditions such as "hard swellings", abscesses, calluses, corns, warts, polyps, or tumors, to name a few. Many of the claims for efficacy in the treatment of cancer, however, should be viewed with some skepticism because cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine [10]. This is in contrast to other plant-based therapies used in traditional medicine for the treatment of afflictions such as malaria and pain, which are more easily defined, and where the diseases are often prevalent in the regions where traditional medicine systems are extensively used.

Of the plant-derived anticancer drugs in clinical use, among the best known are the so-called vinca alkaloids, vinblastine (VLB) and vincristine (VCR) (Fig. 2), isolated from the Madagascar periwinkle, *Catharanthus roseus*. *C. roseus* was used by various cultures for the treatment of diabetes, and VLB and VCR were first discovered during an investigation of the plant as a source of potential oral hypoglycemic agents. Their discovery, therefore, may be indirectly attributed to the observation of an unrelated medicinal use of the source plant. It is interesting to note that though the plant was originally endemic to Madagascar, the samples used in the discovery of VLB and VCR were collected in Jamaica and the Philippines. More recent semisynthetic analogs of these agents are vinorelbine (VRLB) and vindesine (VDS). These agents are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers.

The two clinically active agents, etoposide and teniposide, which are semisynthetic derivatives of the natural product, epipodophyllotoxin, may be considered being more closely linked to a plant originally used for the treatment of cancer. Epipodophyllotoxin is an isomer of podophyllotoxin, which was isolated as the active antitumor agent from the roots of various species of the genus *Podophyllum*. These plants possess a long history of medicinal use by early American and Asian cultures, including the treatment of skin cancers and warts [9].

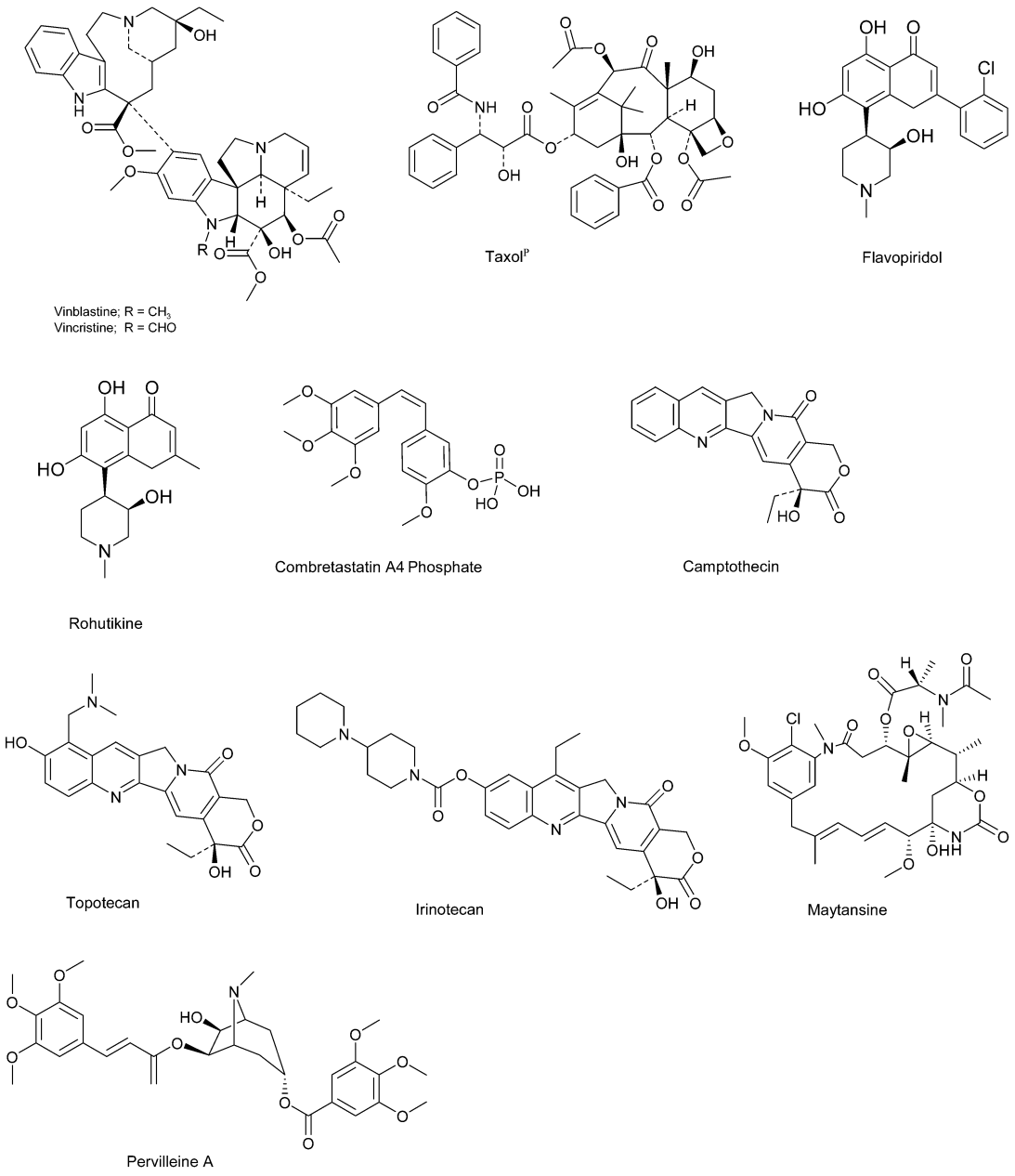


Fig. 2 Plant-derived anticancer agents.

More recent additions to the armamentarium of plant-derived chemotherapeutic agents are the taxanes and camptothecins. Paclitaxel (Taxol®; Fig. 2) initially was isolated from the bark of *Taxus brevifolia*, collected in Washington state as part of a random collection program by the U.S. Department of Agriculture (USDA) for the U.S. National Cancer Institute (NCI) [11]. The use of various parts of *T. brevifolia* and other *Taxus* species (e.g., *canadensis, baccata*) by several Native American tribes for the treatment of some noncancerous conditions has been reported [12], while the leaves of *T. baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system [2], with one reported use in the treatment of cancer [9]. Paclitaxel, along with several key precursors (the baccatins), occurs in the

leaves of various *Taxus* species, and the ready semisynthetic conversion of the relatively abundant baccatins to paclitaxel, and active paclitaxel analogs, such as docetaxel [13], have provided a major, renewable natural source of this important class of drugs. An economically feasible process for production of paclitaxel by plant cell culture fermentation has also recently been announced (see <http://www.epa.gov/greenchemistry/aspa04.html>).

Likewise, the clinically active agents, topotecan (hycamptamine) and irinotecan (CPT-11) are semisynthetically derived from camptothecin (Fig. 2), isolated from the Chinese ornamental tree, *Camptotheca acuminata* [14]. Camptothecin (as its sodium salt) was in clinical trials at NCI in the 1970s, but was dropped because of severe bladder toxicity. Flavopiridol (Fig. 2), was made by the Indian subsidiary of Hoechst (now Aventis) following the isolation and synthesis of the plant-derived natural product, rohitukine (Fig. 2), and is currently in phase III clinical trials both as a single agent and in combination with other agents, particularly paclitaxel and *cis*-platinum [15].

African plant-derived anticancer agents: Recent developments

Combretastatins: Models for combinatorial chemistry

The combretastatins were isolated from the South African "bush willow", *Combretum caffrum* (Eckl. & Zeyh.) Kuntze, collected in Southern Africa in the 1970s for the NCI by the USDA, working in collaboration with the Botanical Research Institute of South Africa. These collections were part of a random collection program aimed at the discovery of novel anticancer agents. Species of the *Combretum* and *Terminalia* genera, both of which belong to the Combretaceae family, are used in African and Indian traditional medicine for the treatment of a variety of diseases, including hepatitis and malaria. Several *Terminalia* species have reportedly been used in the treatment of "cancer". The combretastatins are a family of stilbenes that act as anti-angiogenic agents, causing vascular shutdown in tumors and resulting in tumor necrosis [16]. A water-soluble analog, combretastatin A-4 phosphate (CA4) (Fig. 2), has shown promise in early clinical trials. Of interest is the number of combretastatin (CA4) mimics being developed [17]. Three are in clinical trials, while 11 are in preclinical development. This chemical class has served as a model for the synthesis of a host of analogs containing the essential trimethoxy aryl moiety (Fig. 2) linked to substituted aromatic moieties through a variety of two or three atom bridges, including heterocyclic rings and sulfonamides. This is an impressive display of the power of a relatively simple natural product structure to spawn a prolific output of medicinal and combinatorial chemistry.

Maytansine: Targeting toxic natural products

A recurring liability of natural products, at least in the area of cancer chemotherapy, is that, although many are generally very potent, they have limited solubility in aqueous solvents and exhibit considerable toxicity, often reflected in narrow therapeutic indices. These factors have resulted in the demise of a number of pure natural products, such as the plant-derived agents, bruceantin and maytansine, as promising leads. An alternative approach to utilizing such agents is to investigate their potential as "warheads" attached to monoclonal antibodies specifically targeted to epitopes on tumors of interest [18].

A promising case is that of maytansine [19]. Maytansine (Fig. 2) was isolated in the early 1970s from the Ethiopian plant, *Maytenus serrata* (Hochst. Ex A. Rich.) Wilczek collected for the NCI as part of a random collection program performed through a collaboration with the USDA. The yields were very low (2×10^{-5} % based on plant dry weight), but its extreme potency in testing against cancer cell lines permitted the production of sufficient limited quantities for pursuit of preclinical and clinical development. Despite very promising activity observed in preclinical animal testing, no significant efficacy was observed in clinical trials, and it was dropped from further study in the early 1980s. Related compounds, the ansamitocins, were subsequently isolated from a microbial source, the Actinomycete, *Actinosynnema pretiosum*, and this posed the question as to whether the maytansines are actually plant products, or are produced through an association between a microbial symbiont and the plant; this is a

topic of continuing study. The microbial source of closely related compounds allows for easier production of larger quantities of this class of compounds, and this factor, together with their extreme potency, has stimulated continued interest in pursuing their development. A derivative of maytansine, DM1, conjugated with a monoclonal antibody (mAb) targeting small-cell lung cancer cells, is being developed as huN901-DM1 by the U.S. company, ImmunoGen, Inc. and British Biotech for the treatment of small-cell lung cancer. Another conjugate, known as SB408075 or huC242-DM1 (also known as Cantuzumab Mertansine), produced by the coupling of DM1 to huC242, a mAb directed against the *muc*1 epitope expressed in a range of cancers, including pancreatric, biliary, colorectal, and gastric cancers, was being developed by Glaxo-SmithKline, and is currently in phase I clinical trials in the United States. DM1 has also been conjugated to J591, a mAb targeting the prostate specific membrane antigen (PSMA), and is in clinical trials against prostate cancer.

Pervilleines: Potential multidrug resistance inhibitors

The resistance developed by many cancer patients to treatment with standard anticancer agents is a serious problem encountered in cancer chemotherapy [20]. Resistance to a drug may develop in a cell population through repeated exposure to treatment with that particular drug, and this cell population may subsequently show broad cross-resistance to other anticancer agents even though it has never been exposed those agents. This phenomenon is called multidrug resistance (MDR), and may be related to the presence of an MDR1gene encoding a protein (Pgp; P-glycoprotein), which effectively pumps the drugs out of the cell, thereby precluding their antitumor actions. A good number of compounds which reverse this effect in vitro in cell line studies (so-called MDR inhibitors) have been discovered, but their effectiveness in the clinic has been disappointing in many cases. Thus, there is a continuing search for more effective MDR inhibitors. The pervilleines, isolated from the Madagascar plant, *Erythroxylum pervillei* Baillon, have shown promising MDR activity both in vitro and in vivo, and pervilleine A (Fig. 2) is currently in preclinical development through a collaboration between an NCI-supported National Cooperative Drug Discovery Group (NCDDG) and the Institut Malgache de Recherches Appliquées [21,22].

Marine sources

While marine organisms do not have a significant history of use in traditional medicine, the ancient Phoenicians employed a chemical secretion from marine mollusks to produce purple dyes for woolen cloth, and seaweeds have long been used to fertilize the soil. The world's oceans, covering more than 70 % of the earth's surface, are home to an enormous diversity of organisms, with all but 2 of the 28 major animal phyla being represented and 8 being exclusively aquatic, mainly marine [23]. With the development of reliable scuba diving techniques some 40 years ago, the collection of marine organisms in depths from approximately 3–35 m became routinely attainable, and the marine environment has proved to be a rich source of bioactive compounds, many of which belong to totally novel chemical classes not found in terrestrial sources [24].

Even though no compound isolated from a marine source has, as yet, advanced to commercial use as a chemotherapeutic agent, several are in various phases of clinical development as potential anticancer agents. The most prominent of these is bryostatin 1 (Fig. 3), isolated from the bryozoan, *Bugula neritina* [25]. To date, bryostatin 1 has been in more than 80 human clinical trials, with more than 20 being completed at both the phase I and phase II levels [26]. The sea hare, *Dolabella auricularia* from the Indian Ocean, is the source of more than 15 cytotoxic cyclic and linear peptides, the dolastatins. Dolastatin 10, a linear depsipeptide that was shown to be a tubulin interactive agent, entered phase I clinical trials in the 1990s, and progressed through to phase II trials as a single agent, but has been dropped due to lack of significant activity. As a result of the synthetic processes, many derivatives of the dolastatins have been synthesized with TZT-1027 (Auristatin PE or Soblidotin) now in phase II clinical trials in Europe, Japan, and the United States [24].

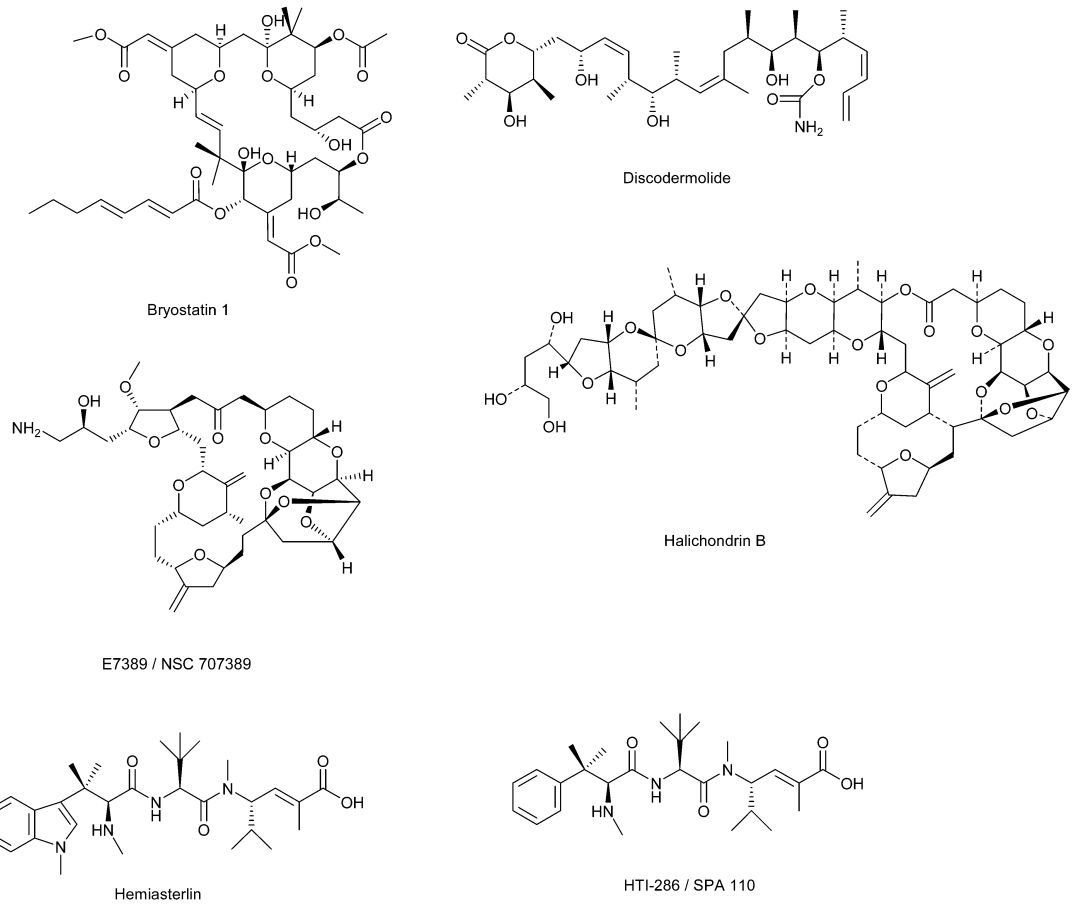


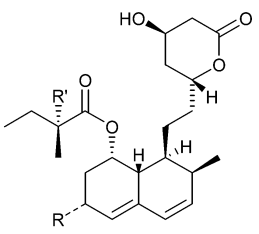
Fig. 3 Marine-derived anticancer drugs.

Sponges are a rich source of bioactive compounds in a variety of pharmacological screens [23], and a number of sponge-derived agents are in clinical development as potential anticancer agents [24]. These include the polyhydroxylated lactone, discodermolide (Fig. 3), isolated from the Caribbean sponge, *Discodermia dissoluta* [27]; HTI-286, a synthetic analog of hemiasterlin (Fig. 3) [28] originally isolated from a South African sponge, *Hemiasterella minor* [29], and soon thereafter from a Papua New Guinea sponge from the genus *Cymbastela*. [30]; and a synthetic analog of halichondrin B (Fig. 3) [31] which was originally isolated in 1985 from the Japanese sponge, *Halichondria okadai*, and subsequently from *Axinella* sp. from the Western Pacific, *Phakellia carteri* from the Eastern Indian Ocean, and from *Lissodendoryx* sp. off the East Coast of South Island, New Zealand [24]. Other marine-derived compounds currently in clinical trials against cancer include ecteinascidin 743, isolated from the Caribbean ascidian, *Ecteinascidia turbinata* [32], aplidine, the dehydro analog of didemnin B, isolated from the Caribbean tunicate, *Tridemnum solidum* [33], and kahalalide F, isolated from the Hawaian mollusk, *Elysia rufescens* [34,35].

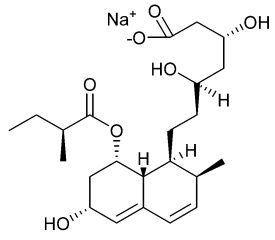
Microbial sources

The serendipitous discovery of penicillin from the filamentous fungus, *Penicillium notatum*, by Fleming in 1929, and the observation of the broad therapeutic use of this agent in the 1940s, promoted the intensive investigation of nature as a source of novel bioactive agents. Microorganisms have proved to be

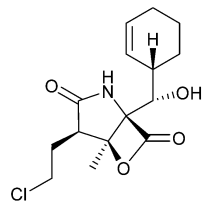
a prolific source of structurally diverse bioactive metabolites, which have yielded some of the most important products of the pharmaceutical industry. These include antibacterial agents [the penicillins (from *Penicillium* species), cephalosporins (from *Cephalosporium cryptosporium*), aminoglycosides, tetracyclines, and other polyketides of many structural types (from the *Actinomycetales*)]; immunosuppressive agents [the fungal metabolites, the cyclosporins, and rapamycin (from *Streptomyces* species)]; cholesterol-lowering agents {mevastatin (compactin) and pravastatin (from *Penicillium* species) (Fig. 4) [7]}; and antitumor antibiotics which are among the most important of the cancer chemotherapeutic agents, which include members of the anthracycline, bleomycin, actinomycin, mitomycin, and aureolic acid families [36].



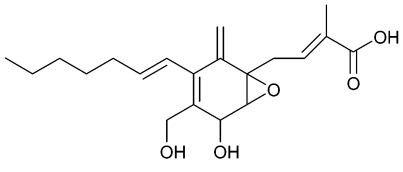
Compactin; R, R' = H
Mevinolin; R = CH_3, R' = H
Simvastin; R, R' = CH_3



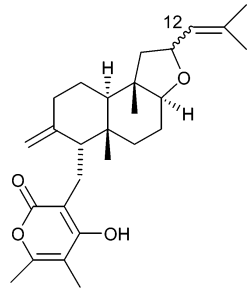
Pravastatin



Salinosporamide A



Ambuic Acid



Subglutinol A; 12 = *S*
Subglutinol B; 12 = *R*

Fig. 4 Microbial-derived drugs.

Despite the tremendous contribution of microbes to the discovery and development of highly efficacious drugs, such as the antibiotics, which ushered in a new age of medicine, microbial diversity has been barely explored. It has been estimated that "less than 1 % of bacterial species and less than 5 % on fungal species are currently known", and recent evidence indicates that millions of microbial species remain undiscovered [37]. Improved culturing procedures [38] and the extraction and manipulation of the nucleic acids of thousands of uncultured microbes (the so-called metagenome) from environmental samples, such as soil and marine animals [39], hold promise for expanding the drug discovery potential of as yet uncultured microbes.

Endophytic microbes

As discussed above, plants are a prolific source of bioactive metabolites; however, the endophytic microbes that reside in the tissues between living plant cells have received scant attention. The promise of this area of research has recently been reviewed [40], and amongst the new bioactive molecules dis-

cussed are ambuic acid (Fig. 4), an antifungal agent, which has been recently described from several isolates of *Pestalotiopsis microspora*, found in many of the world's rainforests, and subglutinols A and B, immunosuppressive compounds produced by *Fusarium subglutinans*, an endophyte of *T. wilfordii*.

New microbes from marine sediments

Recent research has shown that deep ocean sediments are a valuable source of new actinomycete bacteria that are unique to the marine environment. The first truly marine actinomycete genus named *Salinospora* has been cultured using appropriate selective isolation techniques, and a very potent cytotoxin and proteasome inhibitor, salinosporamide A (Fig. 4), has been isolated [41]. Members of this genus are ubiquitous, and are found in sediments on tropical ocean bottoms and in more shallow waters, and also appear on the surfaces of numerous marine plants and animals [42].

MULTIDISCIPLINARY COLLABORATION AND THE GENERATION OF MOLECULAR DIVERSITY

From the foregoing discussion, it is clear that natural products have made, and continue to make, an indispensable contribution to the discovery and development of effective drugs for the treatment of many of the diseases afflicting humankind. A recent analysis of the new drugs marketed during the period between 1981 and 2002 shows that some 50 % owe their origin in one way or another to natural sources, and in some disease areas well over 60 % are derived from natural products; thus, 79 % of the antibacterial, 62 % of the anticancer, and 74 % of the antihypertensive drugs are natural product related [43]. In addition, natural products are an invaluable source of molecular probes in the study of pathways influencing cell cycle progression [44; <http://www.sb-roscoff.fr/CyCell/Frames80.htm>].

While natural products are a proven source of novel, bioactive molecules, the actual compound isolated from the natural source often is not suitable for development into an effective drug product. It may, however, be regarded as a lead molecule which can form the basis for further chemical of biochemical modification. The discovery of promising bioactive molecules always involves close collaboration with biologists in the provision of suitable disease-oriented screens, while the refinement of the lead molecule often requires significant input from medicinal and synthetic chemists. The preclinical development of an agent always requires close collaboration with pharmacologists and toxicologists in the determination of the optimal pharmacodynamic and toxicological parameters suitable for advancement of the agent into clinical trials with human patients.

Combinatorial chemistry and natural products

The analysis of the human genome, as well as those of pathogenic microbes and parasites [45], is resulting in the identification of many proteins associated with disease processes. Combinatorial chemistry is a technique originally developed for the synthesis of large chemical libraries for high-throughput screening against the large number of new molecular targets being developed from such proteins [46]. The *de novo* combinatorial chemistry approach "has been less productive than anticipated ten years ago" [47], and has led to advocacy for "small, focused libraries with truly more diverse structures", with natural products playing an important role [47]. This approach is embodied in the concept of "privileged structures" advanced by Nicolaou et al. [48–50], and is exemplified by the use of benzopyran moieties (Fig. 5). Using solid-phase synthetic methodology has led to the identification and subsequent optimization of benzopyrans bearing a cyanostilbene substitution that are effective against vancomycin-resistance bacteria (Fig. 5) [51]. The combretastatins discussed above provide another impressive example of a relatively simple natural product structure generating a wealth of productive medicinal and combinatorial chemistry.

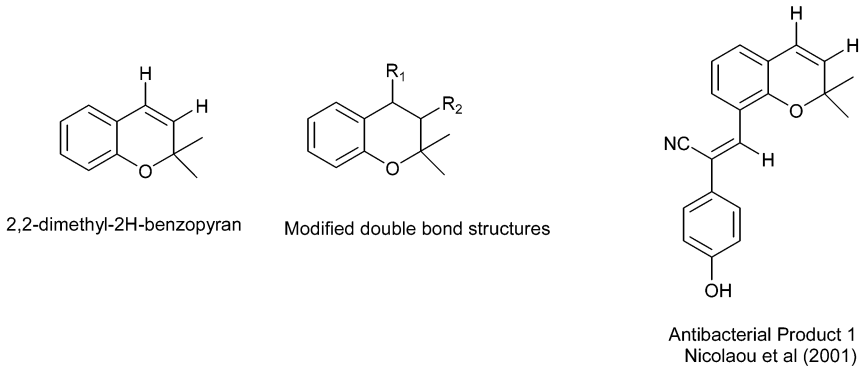


Fig. 5 Privileged structures.

Total synthesis of natural products

The total synthesis of complex natural products has long presented a challenge to the top synthetic chemistry groups worldwide, and has led to significant advances in synthetic methodology [52]. En route to the total synthesis of the natural product, it may be possible to identify a simpler precursor or analog containing the essential features of the molecule necessary for activity (the pharmacophore), and having similar or better activity. A significant example is the synthesis of the marine-derived antitumor agent, halichondrin B (Fig. 3) reported [53], which permitted the synthesis of a large number of variants, particularly smaller molecules that maintained the biological activity, but were intrinsically more chemically stable, due to the substitution of a ketone for the ester linkage in the macrolide ring. One of the compounds, (NSC 707389/E7389) (Fig. 3), is now in phase I clinical trials [31].

CURRENT STATUS OF DRUG DISCOVERY

As indicated in the earlier discussion, natural products play a pivotal role in the discovery of novel lead compounds for drug development. In recent years, there has been a steady decline in the output of the R&D programs of the pharmaceutical industry, and the number of new active substances, also known as new chemical entities, hit a 20-year low of 37 in 2001 [54]. Further evidence of this drop in productivity is evident from the report that only 16 new drug applications had been received by the U.S. Food and Drug Administration (FDA) in 2001, down from 24 the previous year [54]. This downturn has been attributed in part to disruption of laboratory activities by the surge in company mergers and acquisitions, the mounting costs of drug development, and the FDA over-caution in the drug approval process [54]; no mention was made, however, of a contributing factor being the de-emphasis by many companies of the "tried and true" exploration of nature as the source of novel leads for drug development.

Recently, there have been reports of a rekindling of interest in "rediscovering natural products" [55]. As stated by one authority, "We would not have the top-selling drug class today, the statins; the whole field of angiotensin antagonists and angiotensin-converting-enzyme inhibitors; the whole area of immunosuppressives; nor most of the anticancer and antibacterial drugs. Imagine all of those drugs not being available to physicians or patients today." It is clear that nature has played, and will continue to play, a vital role in the drug discovery process.

Despite the intensive investigation of terrestrial flora, it is estimated that only 5–15 % of the approximately 250 000 species of higher plants have been systematically investigated, chemically and pharmacologically [56]. The potential of large areas of tropical rainforests remains virtually untapped, and many source country organizations (SCOs) and scientists are well placed to take a leadership role in this area. The marine environment as a source of novel drugs has already been discussed, but the potential of this area remains virtually unexplored, and, as mentioned above, it has been estimated that

"less than 1 % of bacterial species and less than 5 % on fungal species are currently known", and recent evidence indicates that millions of microbial species remain undiscovered [37].

INTERNATIONAL COLLABORATION AND NATURAL PRODUCT DRUG DISCOVERY AND DEVELOPMENT

As discussed above, effective discovery and development of novel drugs requires a multidisciplinary collaboration. In the area of natural product drug discovery, close international collaboration is an additional, important requirement. Much of the world's biological diversity resides in countries located in tropical and subtropical regions, many of which are developing. Productive interaction between these biodiversity-rich source countries and more developed countries located mainly in the temperate north, and involved in advanced drug discovery and development, must follow terms of fair and equitable collaboration and benefit-sharing as spelled out in the United Nations Convention on Biological Diversity (CBD; <http://www.biodiv.org/convention/ articles.asp>). Consideration may also be given to establishing regional collaborative research programs between qualified source country institutions. Examples of such regional collaborations are Programa Iberoamericano de Ciencia Y Tecnologia Para el Desarrolo (CYTED; <http://www.cyted.org>), comprising over 20 Central and South American countries and Portugal and Spain, and AFASSA (<http://www.afassa.org/>), a program promoting so-called South-South collaboration, and its component networks in Africa, Asia, and South America (<http://www.afassa.org/member_networks.htm>).

NCI Experience

The NCI has been involved in drug discovery and development for close to 50 years, and has made significant contributions to the development of many of the anticancer drugs currently used clinically. The success of much of this effort has depended on close collaboration with organizations worldwide, and international collaboration continues to be an important feature of the NCI programs.

Achievements: 1955–1982

The NCI (<http://www.nci.nih.gov>) was established in 1937, its mission being "to provide for, foster and aid in coordinating research related to cancer." In 1955, NCI set up the Cancer Chemotherapy National Service Centre (CCNSC) to coordinate a national voluntary cooperative cancer chemotherapy program, involving the procurement of drugs, screening, preclinical studies, and clinical evaluation of new agents. The responsibility for drug discovery and preclinical development at NCI now rests with the Developmental Therapeutics Program (DTP; <http://dtp.nci.nih.gov>), a major component of the Division of Cancer Treatment and Diagnosis (DCTD). Thus, NCI has for the past 50 years provided a resource for the preclinical screening of compounds and materials submitted by scientists and institutions, public and private, worldwide, and has played a major role in the discovery and/or development of many of the available commercial and investigational anticancer agents. During this period, more than 500 000 chemicals, both synthetic and natural, have been screened for antitumor activity.

Initially, most of the materials screened were pure compounds of synthetic origin, but the program also recognized that natural products were an excellent source of complex chemical structures with a wide variety of biological activities. The original plant collections from 1960 to 1982 were performed by the USDA through an interagency agreement with NCI, and involved the random collection of over 35 000 plant samples, mainly from temperate regions. These collections led to the discovery of paclitaxel (Taxol) and camptothecin, which formed the basis for the semisynthesis of several clinically effective drugs. Marine invertebrates were generally collected by academic investigators, mainly funded through grants from the NCI, while microbial samples were obtained from pharmaceutical companies and research institutes, such as the Institute of Microbial Chemistry in Japan, some of which were funded through contracts with the NCI. From 1960 to 1982, over 180 000 microbial-derived, some

16 000 marine organism-derived, and over 114 000 plant-derived extracts were screened for antitumor activity, mainly by the NCI, and, as mentioned above, a number of clinically effective chemotherapeutic agents have been developed [36].

Contract collections: 1986–present, the NCI Letter of Collection

The systematic collection of marine invertebrates and terrestrial plants was initiated in 1986, and is coordinated by the DTP Natural Products Branch (NPB; <http://dtp.nci.nih.gov/branches/npb/index.html>). Marine organism collections originally focused in the Caribbean and Australasia, but, in 1992, were expanded to the Central and Southern Pacific and to the Indian Ocean (off East and Southern Africa) through a contract with the Coral Reef Research Foundation, which is based in Palau in Micronesia. With the renewal of the contract in 2002, collections are now performed worldwide. Terrestrial plant collections have been carried out in over 25 countries in tropical and subtropical regions worldwide through contracts with the Missouri Botanical Garden (Africa and Madagascar), the New York Botanical Garden (1986–1996; Central and South America), the University of Illinois at Chicago (Southeast Asia), the Morton Arboretum, and World Botanical Associates (U.S. mainland and territories).

The NCI collection contractors are required to obtain all the necessary permits, including visas, collecting, shipping and export permits, from the appropriate source country agencies or departments. The NCI provides the contractors with the NCI Letter of Collection (LOC; <http://ttb.nci.nih.gov/nploc.html>) [57] for transmission to the appropriate source country authorities and scientific organizations. The LOC states NCI's willingness to collaborate with local scientists and/or authorities in the discovery and development of novel drugs from organisms (plants, marine invertebrates, microbes) collected in their countries and/or territorial waters, and, if requested, the NCI will enter into formal agreements based on the LOC with the relevant source country government agency or organization. Fourteen such agreements have been signed; there are also 25 countries which have not, as yet, signed formal agreements with the NCI. These countries, however, are fully aware of the terms of the LOC, and granted the necessary permits for NCI contractor activities without requiring a formal agreement. In this respect, the NCI is totally committed to the terms of the LOC irrespective of whether or not a formal agreement has been signed [58]. This absence of formal agreements has not been due to lack of effort on the part of the NCI contractors and/or NCI staff to solicit formal agreements from the source countries involved. Indeed, NPB staff have interacted with source country government representatives and scientists, both in their countries, or more frequently during NCI-sponsored visits to NCI and contractor U.S.-based home facilities. The purpose of these visits is to provide opportunities for source country officials and scientists to observe the NCI drug discovery facilities and the processes to which their raw materials are subjected, and to discuss collaboration in the drug discovery process. During the past 15 years, over 65 source country officials and scientists have visited NCI, either to discuss participation in NCI contract collections or direct collaboration in the drug discovery process.

Source country collaboration

In carrying out collections, the NCI contractors work closely with qualified organizations in each of the source countries. Botanists and marine biologists from SCOs collaborate in field collection activities and taxonomic identifications, and their knowledge of local species and conditions is indispensable to the success of the NCI collection operations. SCOs provide facilities for the preparation, packaging, and shipment of the samples to the NCI's Natural Products Repository (NPR) in Frederick, Maryland. The collaboration between the SCOs and the NCI collection contractors, in turn, provides support for expanded research activities by source country biologists, and the deposition of a voucher specimen of each species collected in the national herbarium or repository is expanding source country holdings of their biota. NCI contractors also provide training opportunities for local personnel through conducting workshops and presentation of lectures, both in-country and at the contractor's U.S. facilities.

In addition, through its LOC and agreements based upon it, the NCI: (1) sponsors visits by scientists nominated by SCOs to its facilities, and/or equivalent facilities in other approved U.S. or local organizations, for 1–12 months to participate in collaborative natural products research involving disciplines pertaining to drug discovery, such as the screening and bioassay-directed fractionation of extracts (over 20 such visits have been sponsored); and (2) dictates arrangements for benefit-sharing and use of source country resources in the event of the licensing and development of a promising drug candidate. Successful licensees of patented agents discovered from NCI contract collections are required to negotiate agreements with the relevant source country government agencies or organizations dictating terms of collaboration and compensation. The terms apply irrespective of whether the potential drug is the actual natural isolate or a product structurally based upon the isolate, a synthetic material for which the natural product material provided a key development lead, or a method of synthesis or use of any aforementioned isolate, product, or material. The terms (e.g., percentage of royalties) negotiated as payment, however, might vary depending upon the relationship of the marketed drug to the originally isolated product.

The formulation of the NCI policies for collaboration and compensation embodied in the LOC predated the drafting of the CBD (<http://www.biodiv.org/convention/articles.asp>) in Rio de Janeiro by some four years. It must be stressed that the NCI abides by the terms of the LOC even if the collaborating source country has not signed a formal agreement. This is in line with the U.S. Government's policy to follow the principles articulated in the CBD (<www.State.gov/g/oes/rls/or/25962.htm>), which calls for sharing in a fair and equitable way the results of research and development, and the benefits arising from the commercial and other utilization of genetic resources, with the source country providing such resources (UN CBD; Article 15.7).

Direct collaboration with source country organizations: The NCI Memorandum of Understanding

As discussed above, the collections of plants and marine organisms have been carried out in over 25 countries through contracts with qualified botanical and marine biological organizations working in close collaboration with qualified SCOs, and all collections are performed subject to the terms of the NCI LOC. Particularly in the area of plant-related studies, source country scientists and governments are becoming increasingly committed to performing more of the drug discovery operations in-country, as opposed to the export of raw materials. The NCI has recognized this fact for several years, and contract collections of plants are now being de-emphasized in favor of establishing direct collaborations with qualified organizations in the source countries where the necessary expertise and infrastructure exist.

The NCI has established collaborative agreements [Memoranda of Understanding (MOUs); <http://dtp.nci.nih.gov/branches/npb/agreements.html>] with over 20 SCOs suitably qualified to perform in-country processing. In establishing these agreements, the NCI undertakes to abide by the same policies of collaboration and compensation as specified in the LOC. NCI also assists the SCOs establish their own drug discovery programs through training in techniques of antitumor screening and natural product isolation. NCI has sponsored long-term visitors from 18 countries for purposes of such collaboration and training. Where suitable infrastructure is available at source country institutions, the NCI will provide human cancer cell lines, as well as the appropriate cell line and virus (genetically modified to be noninfectious) for a cell-based anti-HIV screen, to those institutions to enable them to set up screens for their own in-house drug discovery programs.

It is anticipated that the discovery of novel anticancer drugs will be performed by the SCO at its own expense, with assistance from the NCI in terms of free secondary in vitro and in vivo testing. All results from such secondary testing are considered the sole intellectual property of the SCO (the NCI regards such testing as a routine service to the scientific community), and can be used by the SCO in the application for patents covering sufficiently promising inventions. The NCI will devote its resources to collaborating with the SCO in the preclinical and clinical development of any SCO-discovered drug

which meets the NCI selection criteria, and will make a sincere effort to transfer any knowledge, expertise, and technology developed during such collaboration to the SCO, subject to the provision of mutually acceptable guarantees for the protection of intellectual property associated with any patented technology.

Through this mechanism, collaborations have been established with organizations in Australia, Bangladesh, Brazil (5 SCOs), China (3 SCOs), Costa Rica, Fiji, Iceland, South Korea, Mexico, New Zealand, Nicaragua, Pakistan, Panama, Papua New Guinea, South Africa (2 SCOs), and Zimbabwe.

Cooperative drug discovery group programs

Substantial support for source country operations is also provided through NCI-funded U.S. grantee programs such as NCDDGs programs, where the grantees may have collaborations with appropriate SCOs [59]. Since grantee research is regarded as independent, collaborating institutions in source countries may receive support from the grantee in the form of equipment and materials, in addition to training. Similar opportunities for collaboration exist through the International Cooperative Biodiversity Group (ICBG) program coordinated by the Fogarty International Center of the U.S. NIH (<http://www.fic.nih.gov/programs/icbg.html>).

NCI screening agreement

As mentioned above, the NCI has played a major role in the discovery and development of many of the available commercial and investigational anticancer agents. Organizations or individuals wishing to have pure compounds tested in the NCI drug-screening program, such as pharmaceutical and chemical companies or academic research groups worldwide, may submit their compounds for free testing through an online submission process (<http://dtp.nci.nih.gov/docs/misc/common_files /submit_compounds.html>). A screening agreement may be signed with the NCI DCTD, which includes terms stipulating confidentiality and levels of collaboration in the drug development process (see <http://dtp.nci.nih.gov/docs/misc/common_files/canagr.html>). Should a compound show promising anticancer activity in the routine screening operations, the NCI may propose the establishment of a more formal collaboration for further development, such as a Cooperative Research and Development Agreement (CRADA) or a Clinical Trial Agreement (CTA) (<http://ttb.nci.nih.gov/forms.html>).

Case studies: Anti-HIV agents in development with NCI collaboration

From 1987 to 1996, the NCI tested over 60 000 extracts of natural origin in an in vitro cell-based anti-HIV screen which determined the degree of HIV-1 replication in treated infected lymphoblastic cells versus that in untreated infected control cells. Several plant-derived natural products have shown in vitro activity (<http://www.niaid.nih.gov/daids/dtpdb/natprod.htm>), and the development of two of them is discussed below.

Michellamine B: A potential anti-HIV agent from the Cameroon liana, Ancistrocladus korupensis

Michellamine B (Fig. 6) was isolated as the main in vitro active anti-HIV agent from the leaves of the liana, *Ancistrocladus korupensis*, collected in the Korup region of southwest Cameroon through an NCI contract with Missouri Botanical Garden (MBG) [60]. This new species [61] is found only in and around the Korup National Park, and vine densities are very low, on the order of one large vine per hectare. While fallen leaves do contain michellamine B, and their collection provided sufficient biomass for the isolation of enough drug to complete preclinical development, it was clear that extensive collections of fresh leaves could pose a possible threat to the limited and sparse wild population.

Thus far, no other *Ancistrocladus* species has been found to contain michellamine B, and investigation of the feasibility of cultivation of the plant as a reliable biomass source was initiated in 1993 through a contract with the Center for New Crops and Plant Products of Purdue University working in close collaboration with the University of Yaounde 1, the World Wide Fund for Nature Korup Project,

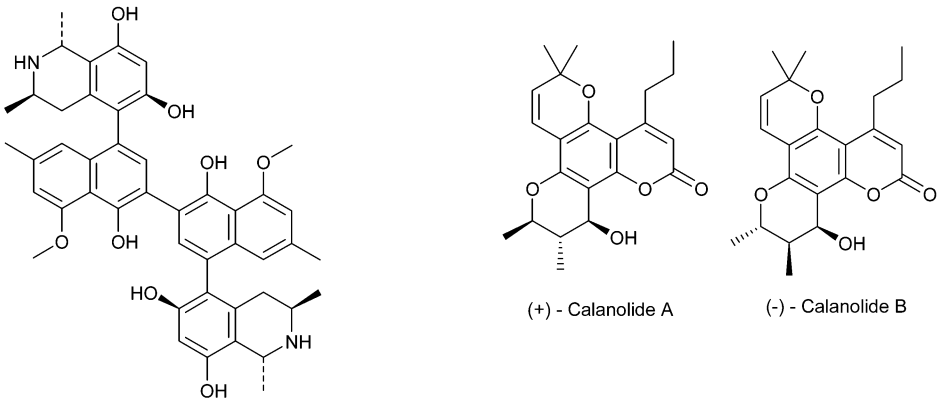


Fig. 6 Plant-derived anti-HIV agents.

MBG, Oregon State University, and the NCI-Frederick contractor, Science Applications International Corporation (SAIC). An extensive botanical survey was undertaken, and the range and distribution of the species were mapped, and dried leaves were analyzed for michellamine B content. Promising plants were re-sampled for confirmatory analysis, and those showing reproducible high concentrations were targeted for vegetative propagation. A medicinal plant nursery was established for the *A. korupensis* collection near Korup Park Headquarters in Mundemba, and through selection of promising plants from the wild and their subsequent propagation and growth in the nursery, it was demonstrated that michellamine content well above the wild average could be produced routinely. In keeping with the NCI policies of collaboration with source countries, all the cultivation studies were performed in Cameroon and involved the local population, particularly those in the Korup region where the plant was originally discovered.

Based on the observed activity and the efficient formulation of the diacetate salt, the NCI committed michellamine B to advanced preclinical development, but continuous infusion studies in dogs indicated that in vivo effective anti-HIV concentrations could only be achieved close to neurotoxic dose levels. Thus, despite in vitro activity against an impressive range of HIV-1 and HIV-2 strains, the difference between the toxic dose level and the anticipated level required for effective antiviral activity (the therapeutic index) was small, and NCI decided to discontinue further studies aimed at clinical development. However, the discovery of novel antimalarial agents, the korupensamines, from the same species [62], adds further promise for this species.

Calanolides: Potential anti-HIV agents from Calophyllum species, Sarawak, Malaysia

An extract of the leaves and twigs of the tree, *Calophyllum lanigerum*, collected in Sarawak, Malaysia in 1987, yielded (+)-calanolide A (Fig. 6), which showed significant anti-HIV activity [63]. Efforts to relocate the original tree failed, and collections of other specimens of the same species gave only trace amounts of calanolide A. A detailed survey of *C. lanigerum* and related species showed that latex of *Calophyllum teysmanii* yielded extracts with significant anti-HIV activity. The active constituent was found to be (–)-calanolide B (Fig. 6), which was isolated in yields of 20 to 30 %. While (–)-calanolide B is slightly less active than (+)-calanolide A, it has the advantage of being readily available from the latex, which is tapped in a sustainable manner by making small slash wounds in the bark of mature trees without causing any harm to the trees. The calanolides were licensed by NCI/NIH to Medichem Research, Inc., which, as required by the NCI LOC, negotiated an agreement with the Sarawak State Government. The drugs are being developed by Sarawak Medichem Pharmaceuticals, a joint venture company formed between the Sarawak State Government and Medichem Research, Inc. (+)-Calanolide A (which was synthesized by Medichem chemists) is currently in phase II clinical trials, while

(–)-calanolide B is in preclinical development. The development of the calanolides has been reviewed as a "Benefit-Sharing Case Study" for the Executive Secretary of the Convention on Biological Diversity (Ten Kate and Wells, <http://www.biodiv.org/programmes/socio-eco/benefit/case-studies.asp.>; case study number 19).

DRUG DEVELOPMENT: THE REALITIES

Commercial drug: A rarity!

The signing of the treaty at the Convention on Biological Diversity was accompanied by a rash of optimistic expectations that the rich biodiversity located in many source countries, particularly in tropical and subtropical regions, would yield a host of new miracle drugs, rapidly generating considerable wealth for many source (mainly developing) countries (so-called green gold). Unfortunately, the realities of natural product drug discovery and development are in direct contradiction to such expectations. An oft quoted estimate is that 1 in 10 000 biologically active leads will result in a commercial drug, but with natural products this ignores the initial phase of isolating and identifying the active lead compound from the original active crude extract.

In their chapter on natural products and the pharmaceutical industry, Laird and Ten Kate [64] estimate that the primary screening of 5 million "compounds", presumably crude extracts, will yield 1000 "hits" (a "hit rate" of 1/5000), which, after bioassay-guided fractionation and purification, dereplication [65], structural elucidation, and secondary screening, will yield 10 novel leads. Subsequent optimization [yield improvement, analog synthesis, formulation and bioavailability studies, enhancement of the activity versus toxicity ratio (the therapeutic index)] narrows the field to five drug candidates that enter advanced preclinical development, and, after approval by the regulatory authorities, enter clinical trials. After phase III clinical trials, one of these candidates is finally approved for registration and marketing. These estimates probably apply to the discovery of new antibiotics from microbial broths produced by the fermentation of many microbial cultures under multiple different fermentation conditions, but they dramatically illustrate the extremely low probability of discovering a novel commercial drug.

Possibly a more relevant example is the NCI experience in the early years of its natural product drug discovery and development program. From 1960 to 1982, some 35 000 plant samples (representing about 12 000 to 13 000 species) were processed to yield 114 000 extracts. Though a significant number of interesting active chemotypes were discovered from these extracts, only two compounds advanced to the stage of development into commercial products. These were taxol (e.g., paclitaxel and its analog, docetaxel) and camptothecin, which, though it proved to be too toxic in clinical trials to become a commercial drug, has yielded commercial analogs, such as topotecan (Hycamptine®) and irinotecan (Camptosar®). One other product, homoharringtonine, has advanced through clinical trials for the treatment of refractory leukemias. Thus, 114 000 extracts derived from approximately 12 000 species, thus far, have given only two compounds currently yielding products of commercial value (further derivatives and analogs of taxol and camptothecin are being developed which will probably become commercial products), with homoharrintonine being another a likely product. As mentioned in earlier discussions, new developments aimed at the targeting of toxic products, such as maytansine, to tumors through conjugation to suitable carrier molecules, such as monoclonal antibodies, may well improve the success rate.

Irrespective of which scenario is used, a clear message is that the chances of discovering a product that will eventually become a commercial drug are extremely small! Having made this observation, it is also clear that the chances of discovering a useful commercial drug are enhanced by exposing the initial natural product extracts to as many screens as possible, and source countries can optimize the potential value of their resources through expanding their collaborations with suitable screening organizations, subject to the negotiation of agreements protecting the rights of all the parties.

Development costs and timeframe

Numerous estimates of costs and times of drug development have been proposed. In 1991, the estimated cost of "bringing a new medicine to the market" was USD $231 million [66]; current estimates range from over USD $800 million to $1.7 billion [67]. In considering costs, allowance must be made for the considerable costs of research and development devoted to the many potential leads and candidates which eventually fail to become commercial products, as well as the substantial costs of "borrowing money" to finance these very expensive enterprises.

The timeframes for drug discovery and development also vary considerably. Laird and Ten Kate provide estimates ranging from 7 to 18 years. In the case of taxol, the time elapsed from initial collection of the source plant material (1962), to final approval of paclitaxel (1992) was 30 years. The discovery and development of the cholesterol-lowering drug, lovastatin, provides another valuable case study [66], with some 30 years elapsing between the discovery of HMG-CoA as the major rate-limiting step in cholesterol biosynthesis to the final approval of lovastatin as a drug.

With the streamlining of the discovery and development processes, the timeframe for "bringing a new medicine to the market" should be greatly reduced, but, nevertheless, it remains an extremely costly and time-consuming operation.

Optimizing the benefits for source countries

Given the timeframes and costs of drug discovery and development, and the extremely low probability of eventually developing a commercial product, it would seem to make good sense to take advantage of "short-term" benefits, rather than banking on the remote possibility of reaping a monetary reward in terms of royalties from the sale of a commercial product.

Short-term benefits can be substantial in terms of training, technology transfer, and infrastructure building, which contribute to the capacity of source country scientists to perform the discovery of promising lead compounds in-country. Such in-country discovery permits the application for patent coverage of the discovery by the source country scientists, either as sole inventors, or, at least, as co-inventors with colleagues in collaborating organizations. This will ensure that the source country derives direct benefit from the discovery, and has a key role in subsequent licensing negotiations. Also, in licensing negotiations, attention may be given to milestone payments and alternative benefits, such as provision by the licensee of free supplies of the drug or another drug of value to the source country, rather than concentrating on the payment of percentage royalties, which in all likelihood will never materialize!

Thus, we would argue that the drug discovery and development process may best be considered as occurring in two phases, as is illustrated by the terms of the NCI LOC and MOU:

- The first phase (phase I) involving terms A1-6 in the LOC can be regarded as basic research, in which many thousands of extracts are screened, and active extracts are subjected to bioassay-guided fractionation in an effort to identify lead compounds for development as potential drug candidates. Applications for patent coverage may be filed for those leads exhibiting sufficient promise. This first phase, in which 1/1000 extracts may yield a promising drug lead candidate at best, should be regarded as truly basic research, and should be subject to application for a basic research agreement, as opposed to a commercial research agreement. In such instances, a basic research agreement must include mention of the absolute requirement for negotiation of a new agreement to cover the development of any promising drug candidate lead (phase II).
- The second phase (phase II) involves the preclinical development of the identified drug candidate, which, if successful, permits the advancement of the drug to clinical trials after approval by the FDA or an equivalent regulatory body in the source country. It is at this second phase that a compound may be considered to have possible commercial potential, even though commercialization is still fairly remote, and may take many (5–10) years to achieve. In the LOC, entry into this second phase triggers a new agreement between the licensee and appropriate source country government agency and/or organization (LOC; Terms A8-11), which will determine appropriate

terms of collaboration in the development process, sustainable and environmentally sound use of source country resources in the production of the drug, and equitable sharing of benefits (e.g., milestone payments, eventual royalty payments if the drug ever reaches the commercialization stage).

CONCLUSION

Nature continues to be a major source of molecular diversity, which, through the pursuit of multidisciplinary, international, collaborative research, can result in the discovery of promising lead compounds, some of which may be developed into commercial drugs. Source countries can derive substantial benefits, both short-term in terms of training, technology transfer, and capacity building, and longer-term in select cases, in terms of milestone payments, royalties, and/or free or low-cost supplies of drugs of importance to the health of their communities. Importantly, such international collaborative research provides significant benefit to the global patient population. The continuing threat to biodiversity through the destruction of terrestrial and marine ecosystems, however, lends urgency to the need to expand the collaborative exploration of these resources as a source of novel bioactive agents.

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