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Biodiversity: A continuing source of novel drug leads*

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Abstract: Nature has been a source of medicinal agents for thousands of years and continues to be an abundant source of novel chemotypes and pharmacophores. With only 5 to 15 % of the approximately 250 000 species of higher plants systematically investigated, and the potential of the marine environment barely tapped, these areas will remain a rich source of novel bioactive compounds. Less than 1 % of bacterial and 5 % of fungal species are currently known, and the potential of novel microbial sources, particularly those found in extreme environments, seems unbounded. To these natural sources can be added the potential to investigate the rational design of novel structure types within certain classes of microbial metabolites through genetic engineering. It is apparent that Nature can provide the novel chemical scaffolds for elaboration by combinatorial approaches (chemical and biochemical), thus leading to agents that have been optimized on the basis of their pharmacological activities. The proven natural product drug discovery track record, coupled with the continuing threat to biodiversity through the destruction of terrestrial and marine ecosystems and the current low number of new chemical entities in pharmaceutical industry pipelines, provides a compelling argument in favor of expanded multidisciplinary and international collaboration in the exploration of Nature as a source of novel leads for the development of drugs and other valuable bioactive agents.

BIODIVERSITY: MEDICINALS FOR THE MILLENNIA

Recorded history

Throughout the ages, humans have relied on Nature for their basic needs for the production of foodstuffs, shelters, clothing, means of transportation, fertilizers, flavors and fragrances, and, not least, medicines. 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 B.C.; among the substances they used were oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh), and *Papaver somniferum* (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. Egyptian medicine dates from about 2900 B.C., but the best known Egyptian pharmaceutical record is the Ebers Papyrus dating from 1500 B.C.; 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

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the centuries, with the first record dating from about 1100 B.C. (Wu Shi Er Bing Fang, containing 52 prescriptions), followed by works such as the Shennong Herbal (~100 B.C.; 365 drugs), and the Tang Herbal (659 A.D.; 850 drugs). Likewise, documentation of the Indian Ayurvedic system dates from about 1000 B.C. (Susruta and Charaka), and this system formed the basis for the primary text of Tibetan Medicine, Gyu-zhi (Four Tantras) translated from Sanskrit during the eighth century A.D. [1].

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 B.C.), 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 A.D.), during his travels with Roman armies, recorded the collection, storage, and use of medicinal herbs, and Galen (130–200 A.D.), 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 (fifth to twelfth centuries A.D.), 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 Arabs were the first to establish privately owned drug stores in the eighth century, and 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 [2] and India [3]. The use of plants in the traditional medicine systems of many other cultures has been extensively documented [4–9]. These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization that approximately 80 % of the world's inhabitants rely mainly on traditional medicines for their primary health care [10]. Plant products also play an important role in the health care systems of the remaining 20 % of the population, mainly residing in developed countries. Analysis of data on prescriptions dispensed from community pharmacies in the United States from 1959 to 1980 indicates that about 25 % contained plant extracts or active principles derived from higher plants, 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 [10]. 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. In addition, the use of so-called complementary or alternative herbal products has expanded in recent decades [11].

The isolation of the antimalarial drug, quinine (Fig. 1), from the bark of *Cinchona* species (e.g., *C. officinalis*), was reported in 1820 by the French pharmacists, Caventou and Pelletier. The bark 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. Quinine formed the basis for the synthesis of the commonly used antimalarial drugs, chloroquine and mefloquine (Fig. 1). With the emergence of resistance to these drugs in many tropical regions, another plant long used in the treatment of fevers in traditional Chinese medicine, *Artemisia annua* (Quinhaosu), has yielded the agents, artemisinin and its derivatives, artemether and arteether (Fig. 1), effective against resistant strains [12].

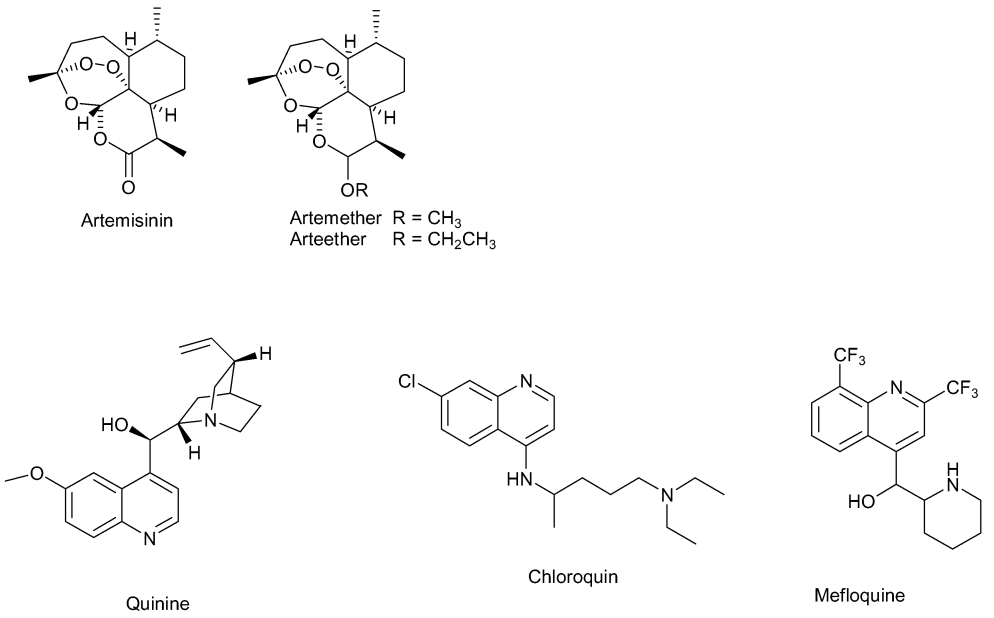


Fig. 1 Plant-derived antimalarial agents.

The analgesic, morphine, isolated in 1816 by the German pharmacist, Serturner, from the opium poppy, *Papaver somniferum,* used in ancient Mesopotamia (vide infra), laid the basis for alkaloid chemistry and the development of a range of highly effective analgesic agents [12].

Other significant drugs developed from traditional medicinal plants include: the antihypertensive agent, reserpine, isolated from *Rauwolfia serpentina* used in Ayurvedic medicine for the treatment of snakebite and other ailments [3]; ephedrine, first isolated in 1887 from *Ephedra sinica* (Ma Huang), a plant long used in traditional Chinese medicine, and the basis for the synthesis of the anti-asthma agents (beta agonists), salbutamol and salmetrol; and the muscle relaxant, tubocurarine, isolated from *Chondrodendron* and *Curarea* species used by indigenous groups in the Amazon as the basis for the arrow poison, curare [12].

Plants have a long history of use in the treatment of cancer [13], though many of the claims for the efficacy of such treatment 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 [14]. Of the plant-derived anticancer drugs in clinical use, among the best known are the so-called vinca alkaloids, vinblastine and vincristine (Fig. 2), isolated from the Madagascar periwinkle, *Catharanthus roseus. C. roseus* was used by various cultures for the treatment of diabetes, and vinblastine and vincristine 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. 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 [13].

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 for the U.S. National Cancer Institute (NCI) [15]. The use of various parts of *T. brevifo-*

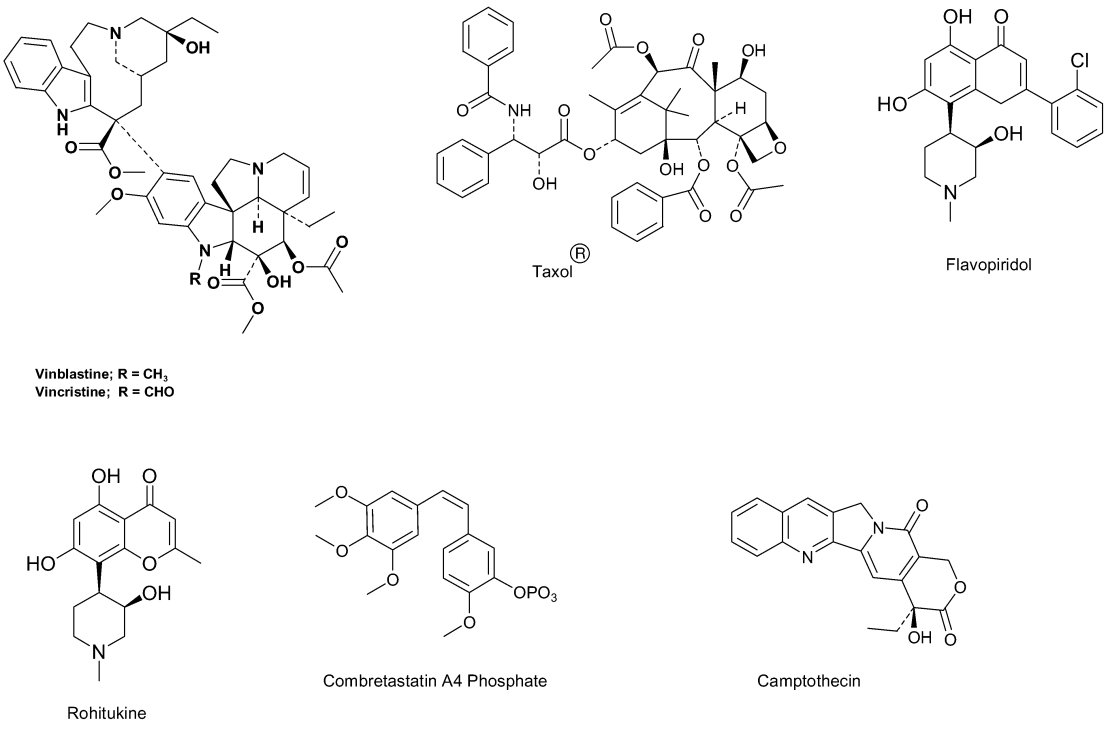


Fig. 2 Plant-derived anticancer agents.

lia and other *Taxus* species (e.g., *canadensis*, *baccata*) by several Native American tribes for the treatment of some noncancerous conditions has been reported [16], while the leaves of *T. baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system [3], with one reported use in the treatment of cancer [13]. 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 [17], has provided a major, renewable natural source of this important class of drugs. Likewise, the clinically active agents, topotecan (hycamptamine) and irinotecan (CPT-11), are semisynthetically derived from camptothecin, isolated from the Chinese ornamental tree, *Camptotheca acuminata* [18] Camptothecin (as its sodium salt) was in clinical trials at NCI in the 1970s, but was dropped because of severe bladder toxicity.

A significant number of plant-sourced agents are still in clinical trials for the treatment of cancer. Some are being investigated as direct cytotoxins, whereas others are being studied from the aspect of their potential role as inhibitors of particular cell cycle enzymes, proteins, or pathways [19]. Homoharringtonine, from the Chinese tree *Cephalotaxus harringtonia* var. *drupacea* (Sieb and Zucc), is still in clinical trials against various leukemias in the West, but is reported as being used in China as an anticancer agent [20]. 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 [21]. The combretastatins, derived from *Combretum caffrum,* are a family of stilbenes which act as anti-angiogenic agents, causing vascular shutdown in tumors and resulting in tumor necrosis, and a water-soluble analog, combretastatin A-4 phosphate (Fig. 2), has shown promise in early clinical trials and is currently in Phase II [19,22]. A significant number of compounds based upon the combretastatin skeleton have been synthesized in the search for more effective anticancer agents [23].

With the emergence of the AIDS pandemic in the 1980s, the NCI, along with other organizations, has been involved in the exploration of nature as a source of potential agents for the treatment of AIDS and associated opportunistic infections. Over 60 000 extracts of marine and plant origin were tested in vitro against lymphoblastic cells infected with HIV-1. Several plant extracts showed significant activity, and the active compounds were isolated. Of these, the calanolides, isolated from *Calophyllum* species [24, see also Convention on Biological Diversity, <http://www.biodiv.org/programmes/socio-eco/benefit/case-studies.asp>, Case Study 19], and prostratin, isolated from *Homalanthus nutans* [25,26] have progressed into clinical and preclinical development, respectively (Fig. 3).

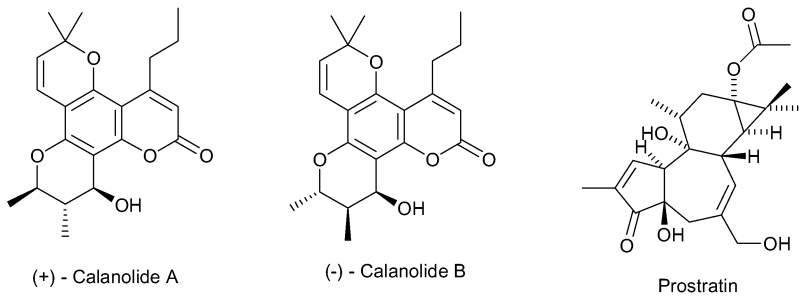


Fig. 3 Plant-derived anti-HIV agents.

Microbial sources and the Golden Age of Antibiotics

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, ushered in a new era in medicine, the so-called Golden Age of Antibiotics [27]. This discovery promoted the intensive investigation of nature as a source of novel bioactive agents, and 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, such as the penicillins (from *Penicillium* species), cephalosporins (from *Cephalosporium cryptosporium*), aminoglycosides, tetracyclines, and other polyketides of many structural types (from the *Actinomycetales*); immunosuppressive agents, such as the fungal metabolites, the cyclosporins, and rapamycin (from *Streptomyces* species); cholesterol-lowering agents, such as mevastatin (compactin) and lovastatin (from *Penicillium* species) (Fig. 4); and anthelmintics and antiparasitic drugs, such as the ivermectins (from *Streptomyces* species) [12].

Antitumor antibiotics are among the most important of the cancer chemotherapeutic agents, which include members of the anthracycline, bleomycin, actinomycin, mitomycin, and aureolic acid families [19]. Clinically useful agents from these families are the daunomycin-related agents, daunomycin itself, doxorubicin, idarubicin, and epirubicin; the glycopeptidic bleomycins A_2 and B_2 (Blenoxane®); the peptolides exemplified by d-actinomycin; the mitosanes such as mitomycin C; and the glycosylated anthracenone, mithramycin. All were isolated from various *Streptomyces* species, as were two other clinically active agents, streptozocin and deoxycoformycin [28]. Calicheamicin (Fig. 4), possibly the most potent antitumor compound to be approved for clinical use, languished for a number of years as it was just too toxic to pursue, in spite of its exquisite subpicomolar level activity [29,30]. The compound has been linked to a specific monoclonal antibody directed against chronic myologenous leukemia, and recently has been approved for clinical use as Mylotarg®. Thus, the compound is carried to the site where needed and released in situ, thereby reducing the systemic toxicity, but not its innate activity [31].

The epothilones (Fig. 4), isolated from the *Myxomycetales* (gliding bacteria) are in clinical development, and are of great interest as potential antitumor agents due to their mechanism of action being the same as that of paclitaxel (vide infra) though having at first glimpse, quite a different topology [32].

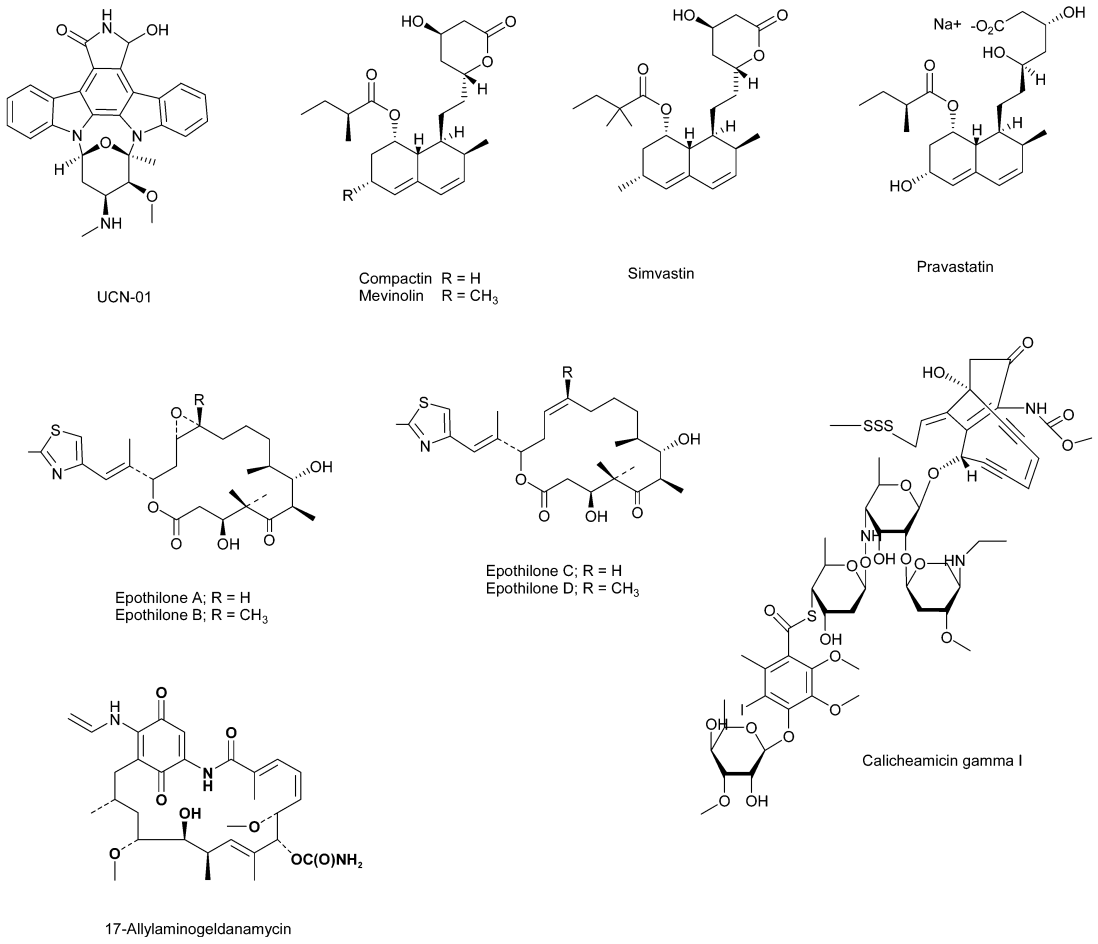


Fig. 4 Microbial-derived drugs.

Other interesting microbially derived chemotypes include the indolocarboxazoles, represented by staurosporine and its simple derivative UCN-01 (Fig. 4), which is in Phase I clinical trials [21,33], and the geldanamycin derivatives, represented by 17-allylamino-geldanamycin (17-AAG) (Fig. 4) which is also in Phase I trials [34,35].

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, represent an enormous resource for the discovery of potential chemotherapeutic agents. All but two of the 28 major animal phyla are represented in aquatic environments, with eight being exclusively aquatic, mainly marine [36]. Prior to the development of reliable scuba diving techniques some 40 years ago, the collection of marine organisms was limited to those obtainable by skin diving. Subsequently, depths from approximately 3 to 35 meters became routinely attainable, and the marine environment has been increasingly explored as a source of novel bioactive agents. Deep water collections can be made by dredging or trawling, but these methods suffer from disadvantages, such as environmental damage and nonselective sampling. These disadvantages can be partially over-

come by use of manned submersibles or remotely operated vehicles (ROVs); however, the high cost of these forms of collecting precludes their extensive use in routine collections.

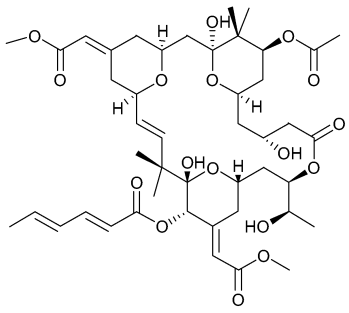
The first notable discovery of biologically active compounds from marine sources was the serendipitous isolation of the C-nucleosides, spongouridine, and spongothymidine, from the Caribbean sponge, *Cryptotheca crypta*, in the early 1950s. These compounds were found to possess antiviral activity, and synthetic analog studies eventually led to the development of cytosine arabinoside (Ara-C) as a clinically useful anticancer agent approximately 15 years later [36], together with Ara-A as an antiviral agent. The systematic investigation of marine environments as sources of novel biologically active agents only began in earnest in the mid-1970s. These studies have clearly demonstrated that the marine environment is a rich source of bioactive compounds, many of which belong to totally novel chemical classes not found in terrestrial sources [37].

As yet, no compound isolated from a marine source has advanced to commercial use as a chemotherapeutic agent, though several are in various phases of clinical development as potential anticancer agents. The most prominent of these is bryostatin 1 (Fig. 5), isolated from the bryozoan, *Bugula neritina* [38]. 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 [39]. However, administration as a single agent is probably not the optimal usage for this agent, and when combined with cytotoxic drugs such as the vinca alkaloids, paclitaxel, fludarabine, cisplatin, etc., responses in Phase I trials have been reported [38]. The sea hare, *Dolabella auricularia* from the Indian Ocean, is the source of more than 15 cytotoxic cyclic and linear peptides, the dolastatins. Due to its potency and mechanism of action, dolastatin 10, a linear depsipeptide which 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 [19].

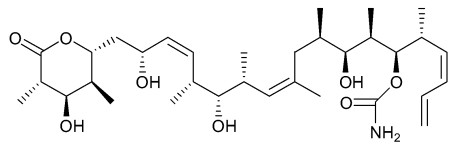
Sponges are traditionally a rich source of bioactive compounds in a variety of pharmacological screens [36], and a number of sponge-derived agents are in clinical development as potential anticancer agents [19]. These include the polyhydroxylated lactone, discodermolide (Fig. 5), isolated from the Caribbean sponge, *Discodermia dissoluta* [40]; HTI-286, a synthetic analog of hemiasterlin (Fig. 5) [41] originally isolated from a South African sponge, *Hemiasterella minor* [42], and soon thereafter from a Papua New Guinea sponge from the genus *Cymbastela.* [43]; and a synthetic analog (E7389) of halichondrin B (Fig. 5) [44], 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 [19]. Girolline isolated from *Pseudaxinyssa cantharella*, and LAF-389, a synthetic analog of bengamide A, isolated from *Jaspis* cf. *coraciae*, advanced into clinical trials, but were dropped due to lack of efficacy [19].

Other marine-derived compounds currently in clinical trials against cancer include ecteinascidin 743, isolated from the Caribbean ascidian, *Ecteinascidia turbinata* [45]; aplidine, the dehydro analog of didemnin B, isolated from the Caribbean tunicate, *Tridemnum solidum* [46]; kahalalide F, isolated from the Hawaian mollusk, *Elysia rufescens* [47,48]; spisulosine, isolated from the marine clam, *Spisula polynyma* [49]; squalamine, isolated from the common dogfish shark, *Squalus acanthias*, collected off the New England coast [50]. The cryptophycins are metabolites isolated from a terrestrial cyanophyte (*Nostoc* sp.) and an Okinawan sponge, *Dysidea arenaria,* and a semisynthetic derivative, cryptophycin 52 (LY355703), progressed to Phase II clinical trials, but was withdrawn in 2002 [51].

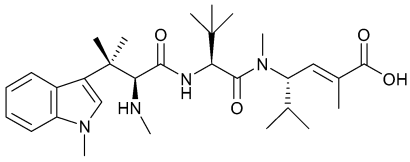
The extremely potent venoms (conatoxins) of predatory cone snails (*Conus* species) have yielded complex mixtures of small peptides (6–40 amino acids), which have provided models for the synthesis of novel painkillers (e.g., Ziconotide®) which currently is in a pivotal Phase III trial [52].



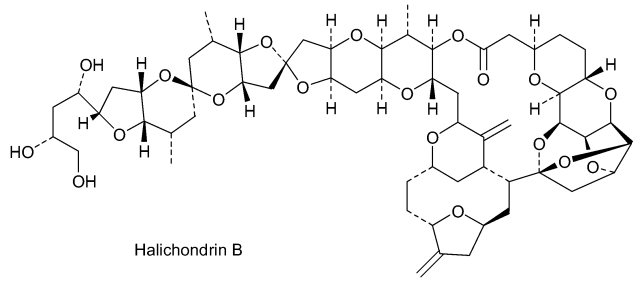
Bryostatin 1



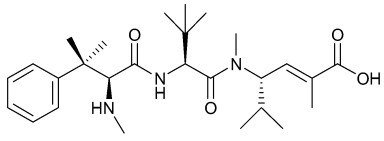
Discodermolide



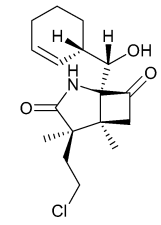
Hemiasterlin



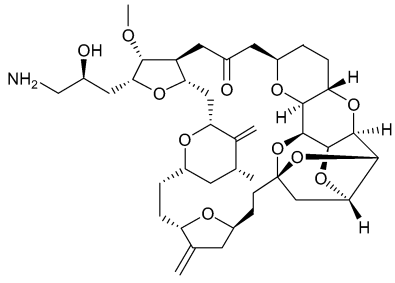
Halichondrin B



HTI-286 / SPA 110



Salinosporamide A



E7389 / NSC 707389

Fig. 5 Marine-derived anticancer drugs.

Other sources

Teprotide, isolated from the venom of the pit viper, *Bothrops jaracaca*, led to the design and synthesis of the ACE inhibitors, captopril and enalapril [12], used in the treatment of cardiovascular disease, while epibatidine, isolated from the skin of the poisonous frog, *Epipedobates tricolor*, has led to the development of a novel class of painkillers [53].

CURRENT STATUS OF DRUG DISCOVERY

The interest in nature as a source of potential chemotherapeutic agents continues [54]. An analysis of natural products as sources of new drugs over the period 1981–2002 indicates that 67 % of the 877

small molecule, new chemical entities (NCEs) are formally synthetic, but 16.4 % correspond to synthetic molecules containing pharmacophores derived directly from natural products [55]. Furthermore, 12 % are actually modeled on a natural product inhibitor of the molecular target of interest, or mimic (i.e., competitively inhibit) the endogenous substrate of the active site, such as ATP. Thus, only 39 % of the 877 NCEs can be classified as truly synthetic in origin [55]. In the area of anti-infectives (anti-bacterial, -fungal, -parasitic, and -viral), close to 70 % are naturally derived or inspired, while in the cancer treatment area 67 % are in this category.

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 [56]. 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 [56]. 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 [56]; 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" [57]. 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.

BIODIVERSITY AND THE CONTINUED GENERATION OF MOLECULAR DIVERSITY

Expanded exploration of classical environments

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 [58]. The potential of large areas of tropical rainforests remains virtually untapped, and many source country organizations 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. Another vast untapped resource is that of the insect world, and organizations, such as the Instituto Nacional de Biodiversidad (INBio, <http://www.inbio.org>) in Costa Rica, are investigating the potential of this and other natural resources, in collaboration with academia and industry (<http://www.inbio.ac.cr/papers/insectoscr/Insectcr.html>).

Unexplored potential of microbial diversity

Until recently, microbiologists were greatly limited in their study of natural microbial ecosystems due to an inability to cultivate most naturally occurring microorganisms. In a report released by the American Academy of Microbiology entitled "The Microbial World: Foundation of the Biosphere", it is 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 [59].

Improved culturing procedures

Recent developments of procedures for cultivating and identifying microorganisms will aid microbiologists in their assessment of the earth's full range of microbial diversity. Application of a technique for the massive parallel cultivation of gel-encapsulated single cells (gel micro-droplets, GMDs) derived from microbes separated from environmental samples (sea water and soil) has recently been published [60]. Use of "nutrient-sparse" media under conditions simulating the original natural environment,

"permits the simultaneous and relatively non-competitive growth of both slow- and fastgrowing microorganisms", thereby preventing overgrowth by the fast-growing "microbial weeds". This has resulted in the identification of previously undetected species (using 16S rRNA gene sequencing), and the culturing and scale-up cultivation of previously uncultivated microbes.

Extraction of environmental samples

In addition, procedures based on the extraction of nucleic acids (the metagenome) from environmental samples permit the identification of uncultured microorganisms through the isolation and sequencing of ribosomal RNA or rDNA (genes encoding for rRNA); samples from soils are currently being investigated, and the methods may be applied to other habitats, such as the microflora of insects and marine animals [61]. Valuable products and information are certain to result from the cloning and understanding of the novel genes that will be discovered through these processes. Heterologous expression of gene clusters encoding the enzymes involved in biosynthetic pathways in viable host organisms, such as *Escherichia coli,* should permit the production of novel metabolites produced from as yet uncultured microbes. A recent example of heterologous expression of genomic DNA is the production of the antibiotic, pantocin A (Fig. 6), from the bacterium, *Pantoea agglomerans* [62]. Low titers and the complexity of the mixture of metabolites produced, made production of pantocin A by the microbe grown in liquid culture impractical; however, expression of a genomic DNA library from *P. agglomerans* in *E. coli* provided access to reasonable quantities of the small molecule antibiotics of interest.

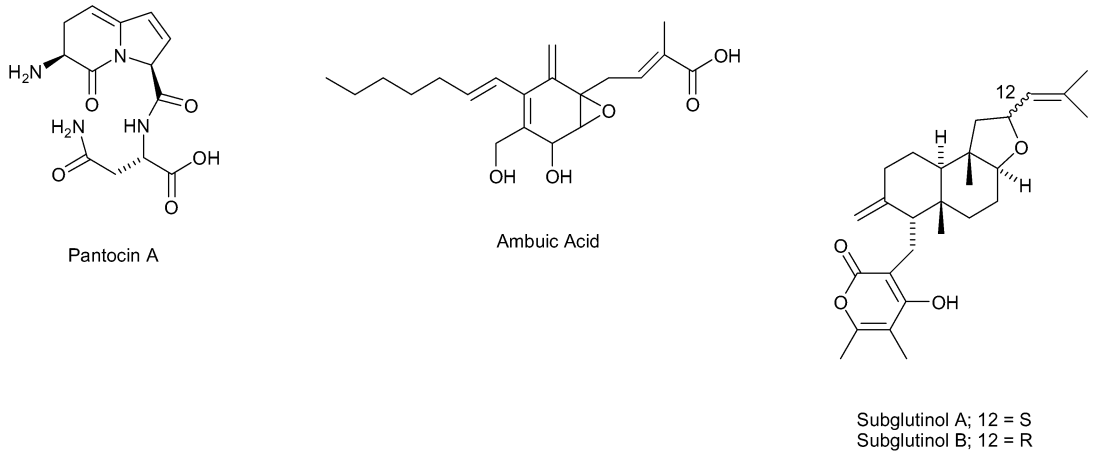


Fig. 6 Drugs derived from endophytic fungi.

Extremophiles

Extreme habitats harbor a host of extremophilic microbes (extremophiles), such as acidophiles (acidic sulfurous hot springs), alkalophiles (alkaline lakes), halophiles (salt lakes), piezo (baro)- and thermophiles (deep-sea vents) [63], and psychrophiles (Arctic and Antarctic waters, alpine lakes) [64]. While investigations thus far have focused on the isolation of thermophilic and hyperthermophilic enzymes [65], there are reports of useful enzymes being isolated from other extreme habitats (<www.diversa.com>). These extreme environments will also undoubtedly yield novel bioactive chemotypes.

Endophytes

While plants have received extensive study as sources of bioactive metabolites, the endophytic microbes that reside in the tissues between living plant cells have received scant attention. The relationship established between the endophytes and their host plants may vary from symbiotic to pathogenic, and limited studies have revealed an interesting realm of novel chemistry [66]. Among the new bioactive molecules discovered are: novel wide-spectrum antibiotics, kakadumycins, isolated from the endophytic

streptomycete associated with the fern-leafed grevillea (*Grevillea pteridifolia*) from the Northern Territory of Australia [67]; ambuic acid (Fig. 6), an antifungal agent which has been recently described from several isolates of *Pestalotiopsis microspora,* found in many of the world's rainforests [68]; and subglutinols A and B, immunosuppressive compounds produced by *Fusarium subglutinans*, an endophyte of *T. wilfordii* [69].

Marine sediments

Recent research has revealed that deep ocean sediments are a valuable source of new actinomycete bacteria that are unique to the marine environment. Based on combined culture and phylogenetic approaches, the first truly marine actinomycete genus named *Salinospora* has been described [70]. Members of the genus are ubiquitous and are found in sediments on tropical ocean bottoms and in more shallow waters, often reaching concentrations up to 10^4 per cc of sediment. They also appear on the surfaces of numerous marine plants and animals. They can be cultured using the appropriate selective isolation techniques, and significant antibiotic and cytotoxic activity has been observed, leading to the isolation of a very potent cytotoxin, salinosporamide A (Fig. 5), a very potent proteasome inhibitor (IC_{50} = 1.3 nM) [71].

As Dr. Rita Colwell, Director of the U.S. National Science Foundation, commenting on the importance of exploration and conservation of microbial diversity, has stated: "Hiding within the as-yet undiscovered microorganisms are cures for diseases, means to clean polluted environments, new food sources, and better ways to manufacture products used daily in modern society" [72].

Combinatorial biosynthesis

Advances in the understanding of bacterial aromatic polyketide biosynthesis have led to the identification of multifunctional polyketide synthase enzymes (PKSs) responsible for the construction of polyketide backbones of defined chain lengths, the degree and regiospecificity of ketoreduction, and the regiospecificity of cyclizations and aromatizations, together with the genes encoding for the enzymes [73–75]. Since polyketides constitute a large number of structurally diverse natural products exhibiting a broad range of biological activities (e.g., tetracyclines, doxorubicin, and avermectin), the potential for generating novel molecules with enhanced known bioactivities, or even novel bioactivities, appears to be high [76].

A recent example of the power of this technique when applied to natural products is the development of an efficient method for scale-up production of epothilone D (Fig. 4), currently undergoing clinical trials as a potential anticancer agent. Epothilone D is the most active of the epothilone series isolated from the myxobacterium, *Sorangium cellulosum*, and is the des-epoxy precursor of epothilone B (Fig. 4). The isolation and sequencing of the polyketide gene cluster producing epothilone B from two *S. cellulosum* strains has been reported [77,78], and the role of the last gene in the cluster, epoK, encoding cytochrome P450, in the epoxidation of epothilone D to epothilone B has been demonstrated. Heterologous expression of the gene cluster minus the epoK into *Myxococcus xanthus* resulted in large-scale production of crystalline epothilone D [79,80].

Total synthesis of natural products

The total synthesis of complex natural products has long posed challenges to the top synthetic chemistry groups worldwide, and has led to the discovery of many novel reactions, and to developments in chiral catalytic reactions [81]. More recently, the efforts of some groups have been focused on the synthesis and modification of drugs that are difficult to isolate in sufficient quantities for development. In the process of total synthesis, it is often possible to determine the essential features of the molecule necessary for activity (the pharmacophore), and, in some instances, this has led to the synthesis of simpler analogs having similar or better activity. A notable example is that of the marine-derived antitumor

agent, halichondrin B (Fig. 5) mentioned earlier. In 1992, the synthesis of both halichondrin B and norhalichondrin B was reported [82], and the synthetic schemes were utilized to synthesize a large number of variants of halichondrin B, 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. Two of these agents were subsequently evaluated by NCI in conjunction with the Eisai Research Institute in the United States, and one of the compounds, (NSC 707389/E7389) (Fig. 5), is now in Phase I clinical trials [83,84].

The synthesis of the epothilones (Fig. 4) by several groups has permitted the preparation of a large number of designed analogs and detailed structure–activity studies, which have been reviewed [85]. These studies have identified desirable modifications, which might eventually lead to more suitable candidates for drug development, but thus far none of the analogs has been reported to surpass epothilone B in its potency against tumor cells.

Combinatorial chemistry and natural products

The analysis of the human genome, as well as advances in the description of the genomes of pathogenic microbes and parasites [86], is permitting the determination of the structures of many of the proteins associated with disease processes. With the development of these new molecular targets, there is an increasing demand for novel molecular diversity for screening. Combinatorial chemistry is a technique originally developed for the synthesis of large chemical libraries for high-throughput screening against such targets [87]. This has led to the development of robotic systems and tools, such as solid-phase synthesis and new immobilization strategies involving novel resins, reagents, and linkers, which have permitted high-throughput parallel approaches to the synthesis of very large libraries of millions of compounds. While there are claims that new leads are being found [87], the declining numbers of new NCEs [56] indicate that the use of de novo combinatorial chemistry approaches to drug discovery over the past decade have been disappointing, with some of the earlier libraries being described as "poorly designed, impractically large, and structurally simplistic" [87]. As stated in the recent article [87], "an initial emphasis on creating mixtures of very large numbers of compounds has largely given way in industry to a more measured approach based on arrays of fewer, well-characterized compounds" with "a particularly strong move toward the synthesis of complex natural-product-like compounds—molecules that bear a close structural resemblance to approved natural-product-based drugs".

The synthesis of natural product-like libraries is exemplified by the work of the Schreiber group who have combined the simultaneous reaction of maximal combinations of sets of natural product-like core structures ("latent intermediates") with peripheral groups ("skeletal information elements") in the synthesis of libraries of over 1000 compounds bearing significant structural and chiral diversity [88a,b].

Over the last few years, detailed analyses of active natural product skeletons have led to the identification of relatively simple key precursor molecules which form the building blocks for use in combinatorial synthetic schemes that have produced numbers of potent molecules, thereby enabling structure–activity relationships to be probed. Thus, in the study of the structure–activity relationships of the epothilones (Fig. 4), solid-phase synthesis of combinatorial libraries was used to probe regions of the molecule important to retention or improvement of activity [85]. The combinatorial approach, using an active natural product as the central scaffold, can also be applied to the generation of large numbers of analogs for structure–activity studies, the so-called parallel synthetic approach [89].

The importance of natural products as leads for combinatorial synthetic approaches is embodied in the concept of "privileged structures" advanced by Nicolaou et al. [90–92], and stated as follows: "We were particularly intrigued by the possibility that using scaffolds of natural origin, which presumably have undergone evolutionary selection over time, might confer favorable bioactivities and bioavailabilities to library members". A search of the literature yielded nearly 4000 2,2-dimethyl-2H-benzopyran moieties (Fig. 7), with another 8000 structures identified through the inclusion of a slight modification of the search (see Fig. 2 in ref. [90]). Nicolaou's group then proceeded to develop the nec-

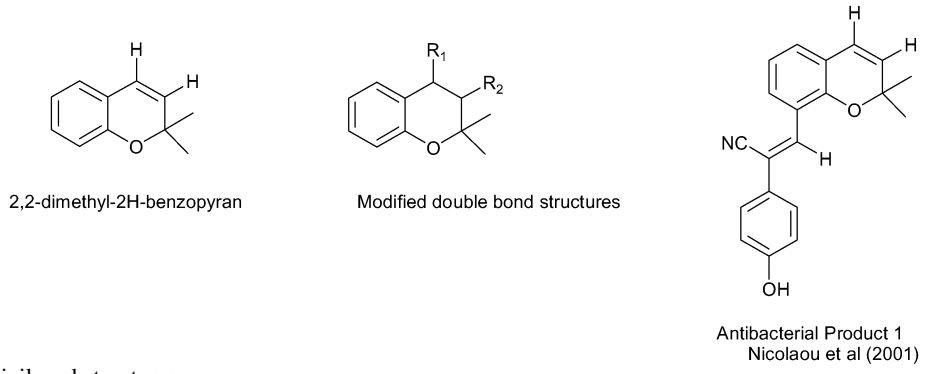


Fig. 7 Privileged structures.

essary solid-phase synthetic methods by modifying a reagent that they had reported in the literature a couple of years earlier, a polystyrene-based selenenyl bromide resin [93]. Application of this methodology has led to the identification and subsequent optimization of benzopyrans with a cyanostilbene substitution that are effective against vancomycin-resistance bacteria (Fig. 7) [94].

The approach of probing complex biological processes by altering the function of proteins through binding with small molecules has been called chemical genetics [95]. This technique has been applied to the modification of the natural product galanthamine (Fig. 8) [96] using combinatorial techniques, and then assaying the products using a novel screen that looked for inhibition of protein trafficking in the Golgi apparatus. They identified a modified nucleus derived from galanthamine, which they named secramine (Fig. 8), that inhibited this process. The only other known agents to do this were based on the microbial product, brefeldin, an entirely different structure in a formal sense.

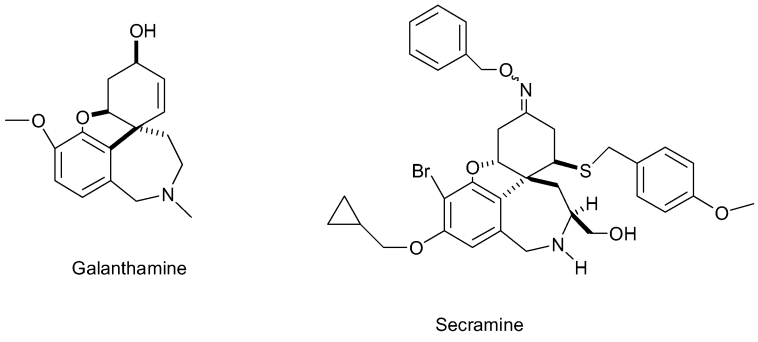


Fig. 8 Application of chemical genetics.

Targeting 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 narrow therapeutic indices. These factors have resulted in the demise of a number of pure natural products, such as 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 [97]. The first FDA-approved, natural product-based example (Mylotarg®), using the microbial metabolite, calicheamicin, as the warhead was mentioned earlier (vide infra) [31]. Another conjugate, huN901-DM1, produced by the coupling of DM1, a cytotoxic agent derived from maytansine, with a monoclonal antibody targeting small-cell lung cancer cells, is being developed for the treatment of small-cell lung cancer [98]. The same maytansinoid derivative linked to a different anti-

body directed against the *muc*1 epitope in gastric cancers, known as SB408075, is currently in Phase I clinical trials in the United States [99].

Another novel strategy for delivery of anticancer drugs to the tumor site involves the coupling of cytotoxins to water-soluble copolymers. Coupling of doxorubicin to an *N*-(2-hydroxypropyl)-methacrylamide (HPMA) copolymer produces the construct known as PK1 which is currently in Phase II trials [100]. Addition of a sugar to the polymer enables specific targeting for the hepatocyte, and this construct PK2 is currently in clinical trials [101].

Another strategy of interest is the use of antibodies as vectors for enzymes capable of activating a nontoxic drug precursor (prodrug) to a potent cytotoxic moiety. After injection and localization of an antibody-enzyme conjugate at the tumor, a nontoxic prodrug is administered, and while remaining innocuous to the normal tissues, it is converted to the cytotoxin by the enzyme localized at the tumor site. This approach, called "antibody-directed enzyme prodrug therapy" (ADEPT), provides further potential for the application of potent natural products to cancer treatment [102].

COLLABORATION: AN ESSENTIAL FACTOR IN DRUG DISCOVERY AND DEVELOPMENT

The effective discovery and development of novel drugs requires close international and multidisciplinary collaboration. This involves disciplines ranging from botany, marine biology, and microbiology, through cell and molecular biology and chemistry, to pharmacology, toxicology, and clinical trials. Consideration may be given to establishing collaborative programs between qualified institutions on a regional basis. An excellent example of such regional collaboration is the Programa Iberoamericano de Ciencia Y Tecnologia Para el Desarrolo (CYTED, <http://www.cyted.org>), an organization comprising over 20 Central and South American countries and Portugal and Spain, having the goal of promoting international collaboration in scientific research.

In addition, opportunities exist for establishing collaborations with academia and industry in industrialized countries, such as through the International Cooperative Biodiversity Group (ICBG) program coordinated by the U.S. National Institutes of Health (NIH, <http://www.fic.nih.gov/programs/icbg.html>). Current programs involve collaboration between U.S. academic groups; source country organizations in Costa Rica, Jamaica, Jordan, Laos, Madagascar, Panama, Papua New Guinea, Philippines, Samoa, Uzbekistan, and Vietnam; U.S. government agencies involved in conservation, drug discovery, and development and economic development; nongovernment organizations (NGOs) such as Conservation International; and pharmaceutical companies (<http://www.nih.gov/news/pr/dec2003/fic-16.htm>). A similar program is supported by the NCI through its National Cooperative Drug Discovery Group (NCDDG) program [103].

The continuing threat to biodiversity through the destruction of terrestrial and marine ecosystems lends urgency to the need to expand the collaborative exploration of these resources as a source of novel bioactive agents.

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