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NATURAL PRODUCTS IN DRUG DISCOVERY: RECENT ADVANCES

GORDON M. CRAGG, PAUL G. GROTHAUS, AND DAVID J. NEWMAN

1.1 INTRODUCTION

Throughout the ages, Nature has provided humans with the means to cater for their basic needs, not the least of which are medicines for the treatment of a wide spectrum of diseases. Plants, in particular, played a significant role forming the basis of sophisticated traditional medicine systems. Records dating from around 2600 BCE document the uses of approximately 1000 plant-derived substances in Mesopotamia. These included oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh), and *Papaver somniferum* (poppy juice), all of which continue to be used today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation [1]. Although Egyptian medicine dates from about 2900 BCE, the best-known record is the "Ebers Papyrus," which dates from 1500 BCE and documents over 700 drugs, mostly of plant origin [1]. The Chinese *Materia Medica* has been extensively documented over the centuries [2]; the first record dates from about 1100 BCE (Wu Shi Er Bing Fang, containing 52 prescriptions), and is followed by works such as the Shennong Herbal (∼100 BCE; 365 drugs) and the Tang Herbal (659 CE; 850 drugs). Likewise, documentation of the Indian Ayurvedic system dates from before 1000 BCE (Charaka; Sushruta and Samhitas with 341 and 516 drugs, respectively) [3,4].

The Greeks and Romans made substantial contributions to the rational development of the use of herbal drugs in the ancient "Western" world. The Greek physician,

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Dioscorides (100 CE), accurately documented the collection, storage, and use of medicinal herbs while traveling with Roman armies throughout the then "known world," while Galen (130–200 CE), a practitioner and teacher of pharmacy and medicine in Rome, is well known for his complex prescriptions and formulae used in compounding drugs. It was, however, the Arabs who preserved much of the Greco-Roman expertise during the Dark and Middle Ages (fifth–twelfth centuries), and they expanded it to include the use of their own resources, together with Chinese and Indian herbs unknown to the Greco-Roman world. A comprehensive review of the history of medicine may be found on the website of the National Library of Medicine (NLM), United States National Institutes of Health (NIH), at http://www.nlm.nih.gov/hmd/collections/archives/index.html.

1.2 THE ROLE OF TRADITIONAL MEDICINE AND PLANTS IN DRUG DISCOVERY

Plant-based systems have continued to play an essential role in health care of many cultures [5,6], and the World Health Organization (WHO) has estimated that approximately 65% of the world's population relies mainly on plant-derived traditional medicines for their primary health care [7]. Plant products also play an important role in the health care systems of the remaining population, mainly residing in "developed" countries [7]. Of 122 compounds identified in a survey of plant-derived pure compounds used as drugs in countries hosting WHO-Traditional Medicine Centers, 80% were found to be used for the same or related ethnomedical purposes, and were derived from only 94 plant species [7]. Relevant examples are given by Fabricant and Farnsworth [8].

Probably the best example of ethnomedicine's role in guiding drug discovery and development is that of the antimalarial drugs, particularly, quinine and artemisinin. The isolation of quinine (Figure 1.1) was reported in 1820 by the French pharmacists, Caventou and Pelletier from the bark of *Cinchona* species (e.g., *Cinchona officinalis*) [9]. The bark, long used by indigenous groups in the Amazon region for the treatment of fevers, was introduced into Europe in the early 1600s for the treatment of malaria, and quinine formed the basis for the synthesis of the commonly used antimalarial drugs, chloroquine (Figure 1.1) and mefloquine, which largely replaced quinine in the mid-twentieth century. As resistance to both these drugs developed in many tropical regions, another plant having a long history of use in traditional Chinese medicine (TCM) for the treatment of fevers, *Artemisia annua* (Qinghaosu), gained prominence [10], and the discovery of artemisinin (Figure 1.1) by Chinese scientists in 1971 provided an exciting new natural product lead compound [11]. Artemisinin analogs, such as artesunate (Figure 1.1), are now used for the treatment of malaria in many countries, and many other analogs of artemisinin have been prepared in attempts to improve its activity and utility [12]. These include totally synthetic molecules with the trioxane moiety included, such as arterolane tosylate (OZ277, Figure 1.1) [13], which is in Phase II trials under Ranbaxy, artemisinin dimers [14], and the amino-artemisinin, artemisone [15].

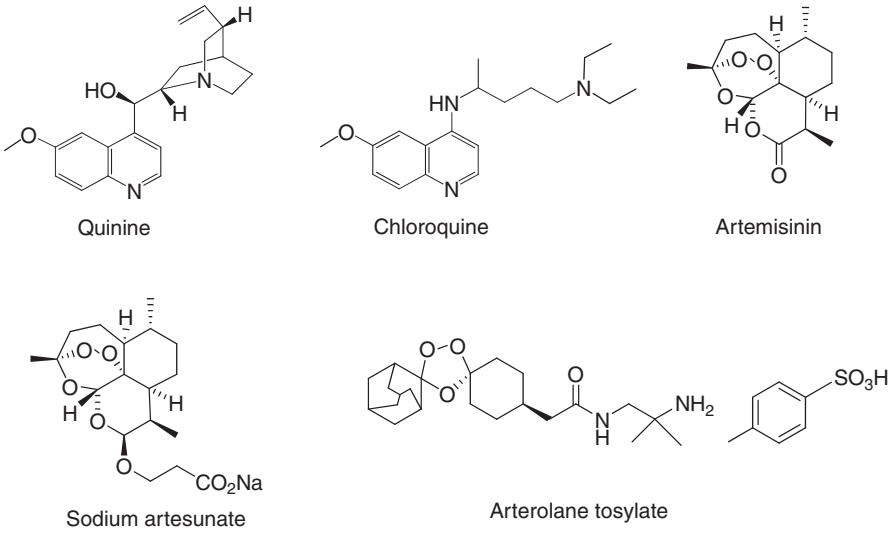

FIGURE 1.1 Natural antimalarial agents and analogs.

Resistance to artemisinin-based drugs is now being observed [16]. In order to counter this development, variations on the basic structure have been launched in combination with other antimalarials (usually variations on the chloroquine structure) such as dihydroartemisinin and piperaquine phosphate (Artekin®); artemether and lumefantrine (Coartem®); artesunate and mefloquine (Artequin®); and artesunate, sulfamethoxypyrazine, and pyrimethamine (Co-Arinate®). Currently there is one other fixed dose combination with an artemisinin derivative in Phase III clinical trials, pyronaridine/artesunate (Pyramax®) [17,18].

While artemisinin and more soluble derivatives have altered the treatment of resistant malaria, the costs of collection of sufficient quantities of the source plants is high, and the overall cost of the drugs may exceed what can be afforded by the countries where the drug is required for general treatment. In an attempt to avoid dependence on wild or even cultivated plant harvesting and thereby reduce costs, the Keasling group, in conjunction with the Gates Foundation and Amyris Pharmaceuticals, has transferred the genes from the producing plant into *Escherichia coli* and also *Saccharomyces cerevisiae*. They have successfully expressed the base terpene (amorpha-4,11-diene) and followed up with modification of the base structure both chemically, and to some extent, biochemically via P450 enzymes [19]. Titers exceeding 25 g/L of amorpha-4,11-diene have been produced by fermentation and are followed by chemical conversion to artemisinin, thereby allowing for the development of a potentially viable process to provide an alternative source of artemisinin [20].

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, from

Ephedra sinica (Ma Huang), a plant long used in traditional Chinese medicine, and the basis for the synthesis of the antiasthma 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 [9]. Although plants have a long history of use in the treatment of cancer [21], cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine, and consequently many of the claims for the efficacy of such treatment should be viewed with some skepticism [22]. Of the plant-derived anticancer drugs in clinical use, some of the best known are the so-called vinca alkaloids, vinblastine and vincristine, isolated from the Madagascar periwinkle, *Catharanthus roseus*; etoposide and teniposide which are semisynthetic derivatives of the natural product epipodophyllotoxin; paclitaxel (Taxol®), which occurs along with several key precursors (the baccatins) in the leaves of various *Taxus* species, and the semisynthetic analog, docetaxel (Taxotere®); and topotecan (hycamptamine), irinotecan (CPT-11), 9-amino- and 9-nitro-camptothecin, all semisynthetically derived from camptothecin, isolated from the Chinese ornamental tree, *Camptotheca acuminata*. These agents together with other plant-derived anticancer agents have been reviewed [23,24].

1.3 THE ROLE OF MARINE ORGANISMS IN DRUG DISCOVERY

While marine organisms do not have a significant history of use in traditional medicine, the world's oceans, covering more than 70% of the earth's surface, represent an enormous resource for the discovery of potential chemotherapeutic agents. Of the 33 animal phyla listed by Margulis and Schwartz [25], 32 are represented in aquatic environments, with 15 being exclusively marine, 17 marine and nonmarine (with 5 of these having more than 95% of their species only in marine environments), and only 1, *Onychophora*, being exclusively nonmarine. With the development of reliable scuba diving techniques enabling the routine accessibility of depths close to 40 m, the marine environment has been increasingly explored as a source of novel bioactive agents. The use of remotely operated vehicles (ROVs) permits the performance of selective deepwater collections with minimal environmental damage, but the high cost of ROV operations precludes their extensive use in routine collection operations. The systematic investigation of marine environments as sources of novel biologically active agents only began in earnest in the mid-1970s, and the rapidly increasing pace of these investigations over the recent decades has 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 [26].

While the focus of research has been on the discovery of potential new anticancer agents [23], the first marine-derived product to gain approval as a drug was Ziconotide, a non-narcotic analgesic that is currently marketed as Prialt® [27]. This compound is a constituent of combinatorial libraries of several hundred peptides that serve as the venom injected by species of the cone snail genus, *Conus*, to stun their

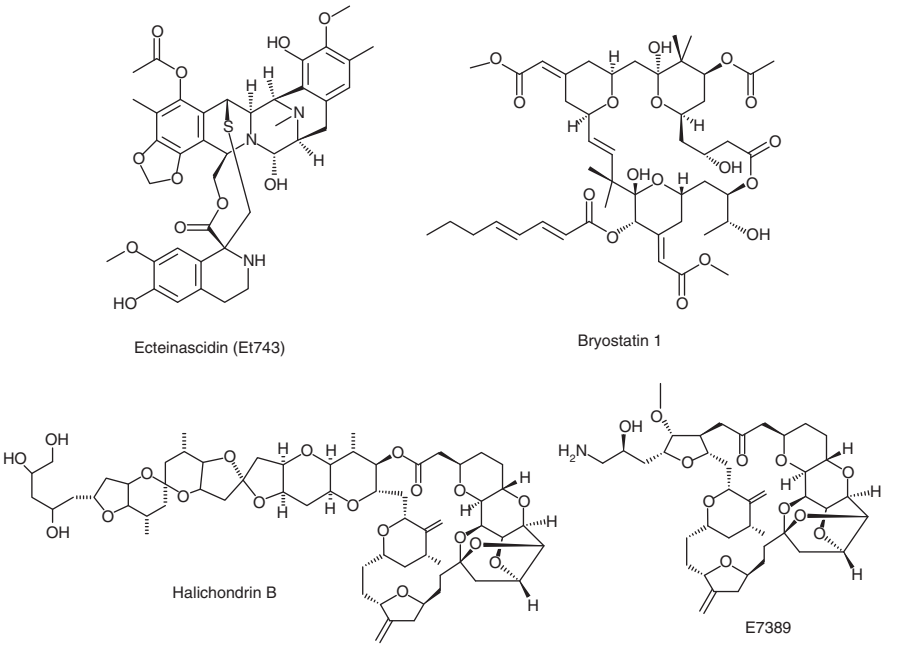

FIGURE 1.2 Some marine-derived bioactive agents.

prey prior to capture [28]. As alluded to above, however, the focus has been on the discovery of potential new anticancer agents. These include the complex alkaloid ecteinascidin 743 (Figure 1.2) isolated from the colonial tunicate *Ecteinascidia turbinata*, which was launched for the treatment of soft tissue sarcomas (STS) in Europe in late 2007 and for the treatment of relapsed ovarian cancer in Europe and the United States in 2009 under the name Yondelis®; it is also in a Phase III trial against ovarian cancer and in Phase II trials for breast, prostate, and pediatric sarcomas. The development of a semisynthetic route from the microbial product cyanosafracin B has solved the issue of compound supply, always a problem with marine-sourced materials [23,24,29]. Further examples of marine-derived anticancer agents are halichondrin B (Figure 1.2) [23,24,30], a complex polyether isolated in very low yield from several sponge sources, and bryostatin 1 (Figure 1.2), another complex macrolide originally isolated by Pettit and his collaborators from the bryozoan, *Bugula neritina* [23,24,31].

Studies on the total synthesis of halichondrin B revealed that the right-hand half of molecule retained all or most of the potency of the parent compound, and large-scale synthesis of the analog E7389 (Eribulin; Figure 1.2) provided adequate supplies for advanced preclinical and clinical development (Section 1.9.1.3). It is currently in advanced clinical trials against a range of cancers, and has shown promising activity in Phase III trials in patients with recurrent or metastatic breast cancer [32]. Bryostatin 1 has excellent antitumor activity, and despite major supply problems, enough cGMP-grade material was isolated from wild collections to provide sufficient material for

clinical trials, which indicated that the drug would be most effective in combination therapy; several Phase II trials of this nature are in progress. Synthetic studies have focused on the preparation of simpler analogs possessing comparable or better activity, particularly related to binding to some of the protein kinase C isozymes, which are the main mechanistic target of the bryostatins. The result has been the preparation of compounds, bryologs, with greater potency than bryostatin 1 in *in vitro* cell line assays [24,31]. These agents together with several other promising marine-derived anticancer agents have been reviewed [23,24].

1.4 THE ROLE OF MICROORGANISMS IN DRUG DISCOVERY: AN HISTORICAL PERSPECTIVE

With the serendipitous discovery of penicillin from the filamentous fungus, *Penicillium notatum*, by Fleming in 1929, and the observation of its broad therapeutic use in the 1940s, a new era in medicine, "The Golden Age of Antibiotics," was born, leading to the intensive investigation of Nature as a source of novel bioactive agents [33]. Microorganisms are a prolific source of structurally diverse bioactive metabolites and have led to the development of some of the most important products of the pharmaceutical industry (see Figure 1.3). These include antibacterial agents, such as the penicillins (from *Penicillium* species), cephalosporins (from *Cephalosporium acremonium*), aminoglycosides, tetracyclines, and other polyketides of many structural types (from the *Actinomycetales*); immunosuppressive agents, such as the cyclosporins (from *Trichoderma* and *Tolypocladium* species) and rapamycin (from *Streptomyces* species) (Figure 1.3); cholesterol lowering agents, such as mevastatin (compactin; from *Penicillium* species; Figure 1.3) and lovastatin (from *Aspergillus* species); and anthelmintics and antiparasitic drugs, such as the ivermectins (from *Streptomyces* species) [9].

Microbes are also a source of some of the most important cancer chemotherapeutic agents, the so-called antitumor antibiotics, which include members of the anthracycline [34], bleomycin [35], mitomycin [36], the enediynes [37], and the staurosporines [38], all isolated from various *Streptomyces* species. Clinically useful agents from these families are daunomycin (Figure 1.3) and related agents, doxorubicin, idarubicin, and epirubicin; the glycopeptidic bleomycins A_2 and B_2 (blenoxane®); the mitosanes such as mitomycin C (Figure 1.3); and the enediynes exemplified by the monoclonal antibody-linked calicheamicin conjugate, Mylotarg® (Figure 1.3).

More recent additions to the microbial antitumor armamentarium are the macrocyclic epothilones, such as epothilones A and B (Figure 1.3), isolated from myxobacteria which have a mechanism of action similar to that of Taxol, and which have been extensively studied, both from the biosynthetic and synthetic standpoints [23,24,39]. Currently, there are at least 16 molecular entities in varying stages of testing ranging from biological testing to Phase III clinical trials. Among those in clinical trials (completed, active, or recruiting) as of July, 2010, either as single agents or in combination with other agents (http://www.clinicaltrials.gov/) are ixabepilone

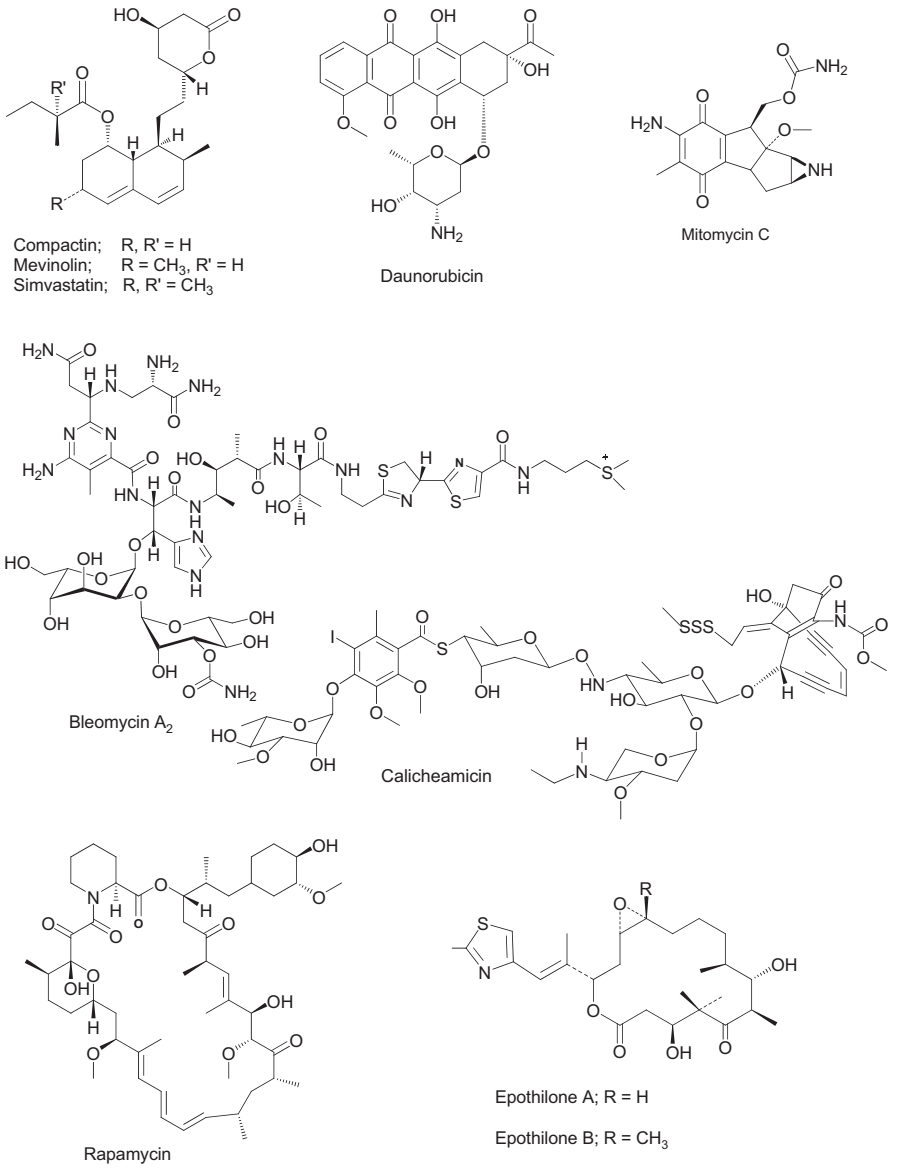

FIGURE 1.3 Microbial-derived drugs.

or 16-azaepothilone B (148 trials), which was approved in October, 2007, by the U.S. Food and Drug Administration for the treatment of aggressive metastatic or locally advanced breast cancer refractory to currently available chemotherapies; epothilone B, patupilone, or EPO-906 (54 trials); epothilone D, 9,10-didehydroepothilone D or KOS-1584 (33 trials); and sagopilone, a fully synthetic analog (14 trials completed or active).

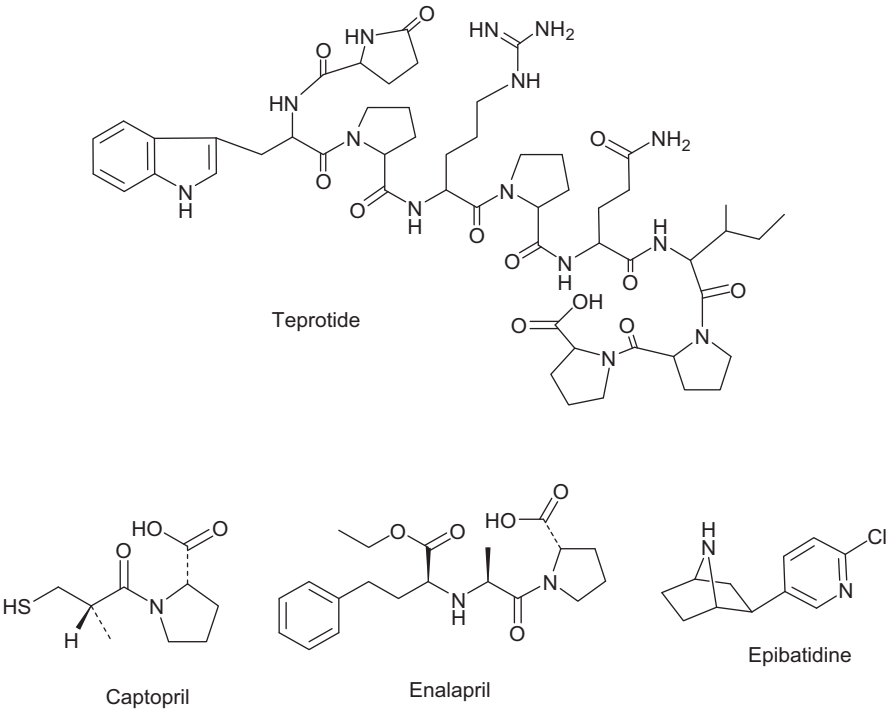

FIGURE 1.4 Leads and drugs from amphibian and reptilian sources.

1.5 OTHER SOURCES

The venom of the pit viper, *Bothrops jararaca*, yielded teprotide which led to the design and synthesis of the ACE inhibitors, captopril and enalapril (Figure 1.4) [9], used in the treatment of cardiovascular disease, while a novel class of painkillers has been developed based on epibatidine (Figure 1.4), isolated from the skin of the poisonous frog, *Epipedobates tricolor* [40]. Another significant discovery was the isolation of exendin-4 from the venom of the Gila monster, *Heloderma suspectum* [41], which led to the development of the polypeptide, exenatide, which was approved by the FDA in April 2005 for the treatment of diabetes mellitus type 2 and is marketed as Byetta.

1.6 THE IMPORTANCE OF NATURAL PRODUCTS IN DRUG DISCOVERY AND DEVELOPMENT

The drugs listed in Sections 1.2–1.5 represent but a small fraction of the total number of bioactive compounds discovered from natural sources, many of which have been

used in their original unmodified form, or which have been structurally modified, to yield effective drugs for the treatment of a wide range of diseases. Still more have served as lead compounds that have provided the basis and inspiration for the design and synthesis of a wide range of effective drugs. In a paper published in 2007 [42], Newman and Cragg analyzed the sources of new drugs over the period 01/1981–06/2006, and classified these compounds as N (an unmodified natural product), ND (a modified natural product), S (a synthetic compound with no natural product conception), S*, S*/NM (a synthetic compound with a natural product pharmacophore; /NM indicating competitive inhibition), and S/NM (a synthetic compound showing competitive inhibition of the natural product substrate). A recently updated analysis (October, 2008) using the same coding as above has indicated that, while 67% of the 1024 small molecule, new chemical entities (NCEs) are formally synthetic, 18% correspond to synthetic molecules containing pharmacophores derived directly from natural products classified as S* and S*/NM, and 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 (S/NM). Thus, only 37% of the 1024 NCEs can be classified as truly synthetic (i.e., devoid of natural inspiration) in origin (S). In certain disease categories such as anti-infectives (anti-bacterial, -fungal, -parasitic, and -viral), 68.3% are naturally derived or inspired (N; ND; S*; S*/NM; S/NM), while in the cancer treatment area 79.8% are in this category, with the figure being 62.9% if the S/NM category is excluded [24].

The brief discussion in Sections 1.1–1.6 above highlights the immense complexity and molecular diversity of natural products, and it should be clear to most observers that very few would have been discovered without the application of natural products chemistry. In addition to structural diversity, however, natural products offer another important feature of considerable interest to those involved in drug development, namely that they often possess highly selective and specific biological activities based on mechanisms of action. Two excellent examples are the HMG-CoA reductase inhibition exhibited by statins such as simvastatin (Figure 1.3), and the tubulin-assembly promotion activity of paclitaxel, neither of which would have been discovered without the natural product leads and investigation of their mechanisms of action. The bioactivity of natural products stems from the hypothesis that essentially all natural products have some receptor-binding activity; the problem is to find to which receptor a given natural product is binding [43]. Experience shows that organisms often provide investigators with complex libraries of unique bioactive constituents, analogous to the libraries of crude synthetic products initially produced by combinatorial chemistry techniques. Thus, the natural products approach can be viewed as complementary to the synthetic approach, each providing access to (initially) different lead structures. Indeed, as discussed below, combinatorial chemistry is an extremely powerful tool for the optimization of an active natural product structure (natural product lead), and the task of the natural products researcher is thus to select those initial lead compounds of pharmacological interest from the "natural combinatorial libraries" produced by extraction of organisms.

1.7 CLASSICAL NATURAL SOURCES: UNTAPPED POTENTIAL

The exceptional complexity and molecular diversity of natural products has been emphasized in earlier sections, but even more remarkable is the fact that the potential of these unique natural resources has barely been explored. Despite the intensive investigation of terrestrial flora, it is estimated that only 6% of the approximately 300,000 species (some estimates are as high as 500,000 species) of higher plants have been systematically investigated pharmacologically, and only some 15% phytochemically [8,44,45]. The potential of the marine environment as a source of novel drugs remains virtually unexplored [46], and until recently, investigations have largely been restricted to tropical and subtropical regions; however, the exploration is being expanded to colder regions including Antarctica [47–49].

The selective and reproducible production of bioactive compounds has been induced through exposure of the roots of hydroponically grown plants to chemical elicitors [50]. In addition, non-natural analogs of natural metabolites can be produced by the feeding of seedlings with derivatives of selected biosynthetic precursors, as illustrated by the production of non-natural terpene indole alkaloids related to the vinca alkaloids through the feeding of seedlings of *C. roseus* with various tryptamine analogs [51].

1.8 THE UNEXPLORED POTENTIAL OF MICROBIAL DIVERSITY

Until fairly recently, the inability to cultivate most naturally occurring microorganisms has severely restricted the study of natural microbial ecosystems, and it has been estimated that less than 1% of microorganisms seen microscopically have been cultivated. Yet, despite this limitation, an impressive number of highly effective microbially derived chemotherapeutic agents has been discovered and developed thus far. Given the observation that "a handful of soil contain billions of microbial organisms" [52], and the assertion that "the workings of the biosphere depend absolutely on the activities of the microbial world" [53], the microbial universe clearly presents a vast untapped resource for drug discovery [54]. In addition, the substantial advances in the understanding of the gene clusters encoding multimodular enzymes involved in the biosynthesis of a host of microbial secondary metabolites, such as polyketide synthases (PKSs) and/or non-ribosomal peptide synthetases (NRPSs), has enabled the sequencing and detailed analysis of the genomes of long-studied microbes, such as *Streptomyces avermitilis*. These studies have revealed the presence of additional PKS and NRPS clusters leading to the discovery of novel secondary metabolites not detected in standard fermentation and isolation processes [55]. Such genome mining has been used in the discovery of a novel peptide, coelichelin, from the soil bacterium, *Streptomyces coelicolor* [56], and this concept is further expanded on in the discussion in Section 1.8.3.

1.8.1 Improved Culturing Procedures

Relatively recent developments of procedures for cultivating and identifying microorganisms are aiding microbiologists in their assessment of the earth's full range of microbial diversity [57]. For example, using "nutrient-sparse" media simulating the original natural environment has enabled the massive parallel cultivation of gel-encapsulated single cells (gel microdroplets; GMDs) derived from microbes separated from environmental samples (seawater and soil) [58]. Thus, "the simultaneous and relatively noncompetitive growth of both slow- and fast-growing microorganisms" has been achieved, thereby preventing the overgrowth by fast-growing "microbial weeds," and resulting in the identification of previously undetected species (using 16S rRNA gene sequencing), as well as the culturing and scale-up cultivation of previously uncultivated microbes [58]. The cultivation of uncultivated members of bacteria of the divisions *Acidobacteria* and *Verrucomicrobia* from agricultural soil and from the guts of wood-feeding termites has been reported using "nutrient-sparse" agar media under hypoxic or anoxic conditions for periods over 30 days, with the addition of humic acids or their analogs, as well as quorum-signaling compounds [59]. The need for the presence of small signaling molecules, such as short peptides, in the media has also been shown to be necessary for initiating growth of the otherwise "uncultivable" strain *Psychrobacter* sp. strain MSC33 [60]. Another recent method involves the sandwiching of innocula of selected sediment samples between semipermeable membranes contained in diffusion chambers which are returned to their natural source environments for periods of 4 weeks, thereby allowing free exchange of chemicals by diffusion with the external environment [61]. This method has been adapted to a novel high-throughput technique for the *in situ* parallel cultivation and isolation of previously uncultivated microbes from a range of environments [62]. These developments have been accompanied by progress in the cultivation of marine microbes as reported in a paper on the cultivation of Gram-positive marine microbes [63]. Thus, the potential for discovery of novel bioactive agents is immense.

1.8.2 Extraction of Environmental Samples (the Metagenome)

Despite improvement in culturing techniques, greater than 99% of microscopically observed microbes still defy culture. Extraction of nucleic acids (the metagenome) from environmental samples, however, permits the identification of uncultured microorganisms through the isolation and sequencing of ribosomal RNA or rDNA (genes encoding for rRNA). A recent review covers the significant achievements thus far in this area of research [64], as well as the huge potential for the discovery of novel bioactive compounds from as yet unculturable microbes [65]. Samples from soils and seawater are currently being investigated [66,67], and whole-genome shotgun sequencing of environmental-pooled DNA obtained from water samples collected in the Sargasso Sea off the coast of Bermuda by the Venter group, indicated the presence of at least 1800 genomic species, which included 148 previously unknown bacterial phylotypes [67]. Venter et al. are also examining microbial communities in

water samples collected by the *Sorcerer II* Global Ocean Sampling (GOS) expedition, and their data predict more than six million proteins, nearly twice the number of proteins present in current databases, with some of the predicted proteins bearing no similarity to any currently known proteins, and therefore, representing new families [68]. Similar methods are also being applied to the investigation of other habitats, such as the microflora of insects [69] and marine animals [70].

Soil is reported to be the most biodiverse environment on Earth, with estimates of approximately 1000 Gbp of microbial genome sequences per gram of soil compared to the Human Genome project in which 3 Gbp were sequenced, and sequencing projects that target other microbial habitats, such as the Sargasso Sea in which 6 Gbp were sequenced [71]. The soil microbial community thus constitutes a vast resource of genes and pathways having substantial potential for the discovery of novel drug leads and generating useful information for a multitude of other processes. Thus, sequencing of the soil metagenome presents a new and ambitious challenge, which should bring considerable economic and environmental value. With this in mind, a coordinated international effort is being established aiming at combining the skills of the global scientific community to focus on sequencing and annotating the soil metagenome; the TerraGenome International Sequencing Consortium (http://www.terragenome.org/) has been launched to coordinate these efforts [71]. As a start, it is proposed to completely sequence a "reference" soil metagenome from a specific site in the United Kingdom, with the aim of generating information on several biosynthetic processes including drug discovery. Evidence that such an ambitious project is feasible and suitable tools are available is clear from a recently published report by van Elsas et al. on the Metacontrol project [72].

The cloning and understanding of the novel genes discovered through these processes, and the heterologous expression of gene clusters encoding the enzymes involved in biosynthetic pathways in viable host organisms, such as *E. coli*, should permit the production of novel metabolites produced from as yet uncultured microbes. The production of the antibiotic, pantocin A (Figure 1.5), from the bacterium, *Pantoea agglomerans*, is an example of such heterologous expression of genomic DNA [73]. The production of pantocin A by the source microbe grown in liquid culture proved to be impractical due to low titers and the complexity of the mixture of metabolites produced. Expression of a genomic DNA library isolated from *P. agglomerans* in *E. coli*, however, provided access to reasonable quantities of the small molecule antibiotics of interest [73].

1.8.3 Cryptic Clusters in Bacteria and Fungi

As has been mentioned in the introduction to Section 1.8, advances in the understanding of the gene clusters encoding multimodular enzymes involved in the biosynthesis of many microbial secondary metabolites, coupled with advances in the sequencing and detailed analysis of the genomes of long-studied microbes, such as *S. avermitilis*, has led to the discovery of novel secondary metabolites not detected in standard fermentation and isolation processes [55]. The early work on the numbers of such clusters in an individual microbe was mainly performed on the genomes of two very important *Streptomyces* species, *S. avermitilis* (where the number of putative

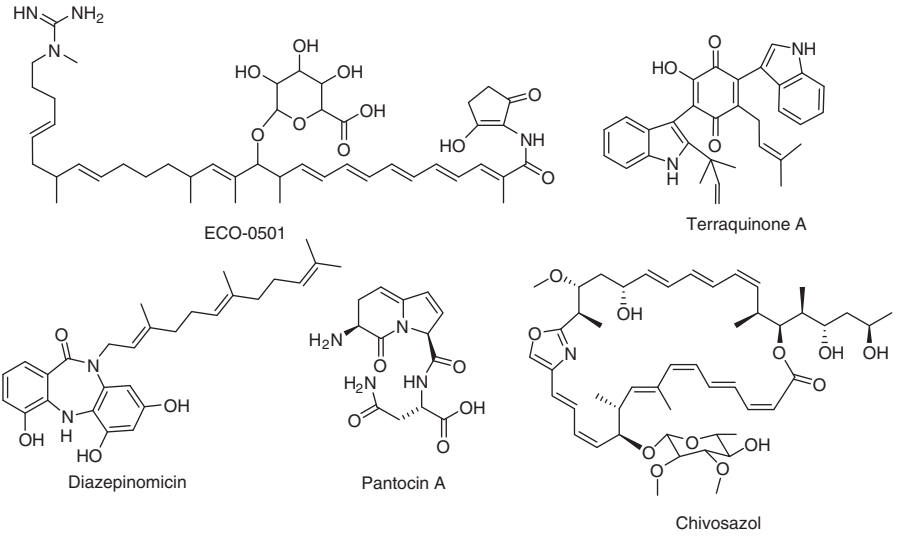

FIGURE 1.5 New compounds from genome mining.

clusters reached into the low 30s) [74,75], and *S. coelicolor* (where numbers are now reaching into the high teens to low 20s) [76]. From this pioneering work, it is now becoming evident that the genomes of the *Streptomycetes* and, by extension, Actinomycetes in general, contain large numbers of previously unrecognized secondary metabolite clusters. Thus, analysis of the genomic sequence of the well-known vancomycin producer, *Amycolatopsis orientalis* (ATCC 43491), led to the prediction of the molecular weight of the novel antibiotic ECO-0501, which following detection by high-performance liquid chromatography-mass spectroscopy (HPLC-MS), led to its isolation (Figure 1.5) [77]. The compound had a very similar biological profile to vancomycin, but it was masked by this compound. Another example illustrating the use of the same technique is the anticancer agent diazepinomicin (Figure 1.5), currently in Phase II clinical trials [78].

Genomic analyses have also been applied to the myxobacteria. The identification of the gene, *ChiR*, controlling the production of the extremely potent antifungal antibiotic, chivosazol (Figure 1.5), has been reported [79]; also discussed in this paper is the major problem of the identification and application of the transcriptional control mechanisms involved in secondary metabolite expression, whether in homologous or heterologous hosts [79]. Further work on the genetics and other aspects secondary metabolite production by myxobacteria have been reviewed by Weissman and Müller [80].

Genomic analysis of the fungus, *Aspergillus nidulans*, suggested the presence of clustered secondary metabolite genes having the potential to generate up to 27 polyketides, 14 nonribosomal peptides, 1 terpene, and 2 indole alkaloids, as well as identifying the potential controller of expression of these clusters [81]; this was demonstrated by expressing terrequinone A (Figure 1.5), a compound not previously reported from this species. Similar predictions can be made for *Aspergillus fumigatus* and *Aspergillus oryzae* based on the analysis of the potential number of secondary

metabolite clusters in these fungi [81]. A recent review has expanded the discussion on the control of secondary metabolite production in fungi [82].

1.8.4 Marine Microbes

Deep-ocean sediments are proving to be a valuable source of new actinomycete bacteria that are unique to the marine environment [63,83]. Use of a combination of culture and phylogenetic approaches has led to the description of the first truly marine actinomycete genus named *Salinispora* [63,84,85], and its members are proving to be ubiquitous, being found in concentrations of up to 10^4 per mL in sediments on tropical ocean bottoms and in more shallow waters, as well as appearing on the surfaces of numerous marine plants and animals. On culturing using the appropriate selective isolation techniques, significant antibiotic and cytotoxic activity has been observed, and has resulted in the isolation of a potent cytotoxin, salinosporamide A (Figure 1.6), a very potent proteasome inhibitor ($IC_{50} = 1.3$ nM) [86,87], currently in Phase I clinical trials as NPI-0052. The discovery and development of this compound and other salinosporamides have been reviewed [88]. More recently, the isolation and cultivation of another new actinomycete genus, named *Marinispora*, has been reported, and novel macrolides called marinomycins have been isolated [89]. Marinomycins A–D (Figure 1.6) show potent activity against drug-resistant bacterial pathogens and some melanomas [89]. A recent review discusses the isolation of these and over 60 other antitumor compounds from marine actinomycetes [83]. Recent publications on the novel and diverse chemistry of these new microbial genera include the isolation of potential chemopreventive agents, saliniketals A and B from *Salinispora arenicola* [90], while two new cyclic peptides, thalassospiramides A and B, possessing immunosuppressive activity have been isolated from a new member of the marine alpha-proteobacterium *Thalassospira* [91]. Extensive screening of extracts of actinomycete bacteria isolated and characterized from the sediment samples collected in one of the largest Norwegian fjords, the Trondheim fjord, showed strong antimicrobial activity, clearly demonstrating that actinomycetes from marine sediments in Norwegian fjords can be potential sources for the discovery of novel anti-infective agents [92].

1.8.5 Cyanophytes

Cyanophytes, which are actually prokaryotes often referred as blue-green algae in early papers, are prolific producers of bioactive secondary metabolites. Reviews by Welker and von Döhren [93], Tan [94], and Tidgewell et al. [95], provide overviews of the multiplicity of molecules isolated from either simple extraction of wild harvests or fermentation of purified organisms cultured from collections (unialgal but, especially in the case of the filamentous forms, not necessarily axenic).

The pioneering work of Moore and Patterson in Hawaii on cyanobacteria as sources of potential anticancer agents, yielded one cyanobacterial secondary metabolite, cryptophycin (Figure 1.6), which led to a large synthetic program, with a derivative, cryptophycin 52 (Figure 1.6), reaching Phase II clinical trials in cancer [96]. Currently, cryptophycin 52 is no longer in trials due to toxicity, but the

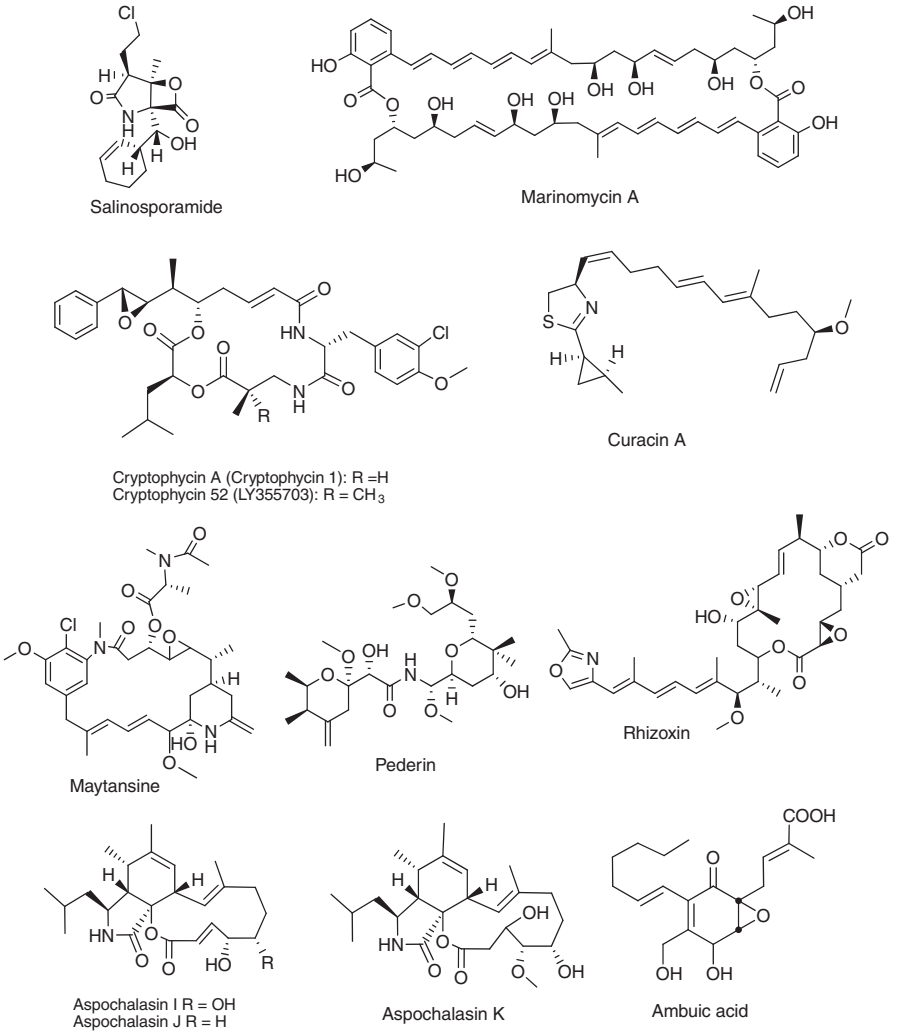

FIGURE 1.6 Bioactive compounds from relatively unexplored microbial sources.

complete biosynthetic pathway of the base molecule has been identified and cloned [97]. Later studies by the Gerwick group yielded curacin A (Figure 1.6) from *Lyngbya majuscula.* Curacin A had excellent *in vitro* cytotoxic activity and is a tubulin interactive agent, but it was effectively insoluble in any formulation that was compatible with *in vivo* testing in animals. The complete biosynthetic cluster was located and cloned by following a series of stable isotope feeding experiments that both identified the precursors and allowed the identification and cloning of the biosynthetic pathway [98]. The actual spatial production of curacin A and other secondary metabolites within *L. majuscula* in the presence of other cyanophytes has been reported, and illustrates the impact of modern instrumentation on such studies [99], and the transcription and regulation of secondary metabolism in this marine

cyanobacterium has been characterized [100]. The possibilities of *in vitro* manipulation of such a cluster have recently been reviewed by Walsh's group [101].

1.8.6 Microbial Symbionts

It is now fairly well established that many bioactive compounds isolated from various macroorganisms which can include plants, marine and terrestrial invertebrates, and even fungi, are actually metabolites synthesized by symbiotic bacteria [102–105]. These include the anticancer compounds, the maytansinoids (Figure 1.6), originally isolated from several plant genera of the Celastraceae family [106], and the pederins (Figure 1.6), isolated from beetles of the genera *Paederus* and *Paederidus*, as well as derivatives based on the pederin skeleton from several marine sponges [107–109]. These "pederine-like molecules," now numbering more than 34, are isolated from at least 8 different animal genera, and these symbiotic sources, together with a range of antitumor agents isolated from marine organisms that closely resemble bacterial metabolites, have been discussed in detail in the reviews by Piel [102,105]. In addition to documenting the wide range of structures and potential producers that have so far been identified, these articles [102,105] also elaborate on what can now be done using this information to produce novel but unnatural variations on known potential anticancer agents from these sources. For example, reaction of mycalamide A with the *PedO* gene product has generated the novel biosynthetic hybrid, 18-*O*-methylmycalamide A, which has increased cytotoxicity compared to the parent compound [110].

An interesting example of a complex symbiotic–pathogenic relationship involving a bacterium–fungus–plant interaction has been discovered in the case of rice seedling blight. The toxic metabolite, rhizoxin (Figure 1.6), originally isolated from the contaminating *Rhizopus* fungus, has actually been found to be produced by an endo-symbiotic *Burkholderia* bacterial species [111]. Rhizoxin exhibits potent antitumor activity, but its further development as an anticancer drug has been precluded by toxicity problems. Thus, in addition to offering potentially new avenues for pest control, this unexpected finding has enabled the isolation of significantly higher yields of rhizoxin as well as rhizoxin analogs through the large-scale cultivation of the bacterium independently of the fungal host [112]. This may have significant implications in the development of rhizoxin analogs with improved pharmacological properties.

As illustrated for rhizoxin above, the production of important bioactive agents by symbiotic microbes offers potentially viable solutions to the supply problem which, in particular, often hinder the advanced development of marine-derived agents. Even though most symbionts may remain uncultivated, bacterial production systems might be established by the isolation of biosynthetic genes from marine metagenomes, and expressing them in culturable bacterial hosts [113,114].

1.8.7 Plant Endophytes

As indicated in Section 1.2, plants have been relatively extensively studied as sources of bioactive metabolites, but the role of endophytic microbes that reside in the tissues between living plant cells has only recently started to receive attention. Relationships

between endophytes and their host plants may vary from symbiotic to pathogenic, and studies have revealed an interesting realm of novel chemistry [115–117]. The wide range of new bioactive molecules reported include novel wide-spectrum antibiotics, kakadumycins, isolated from an endophytic *Streptomycete* associated with the fern leafed grevillea (*Grevillea pteridifolia*) from the Northern Territory of Australia [118]; ambuic acid (Figure 1.6), an antifungal agent, which recently has been described from several isolates of *Pestalotiopsis microspora* found in many of the world's rainforests [119]; peptide antibiotics, the coronamycins, from a *Streptomyces* species associated with an epiphytic vine (*Monastera* species) found in the Peruvian Amazon [120]; and cytotoxic aspochalasins I, J, and K (Figure 1.6), isolated from endophytes of plants from the southwestern desert regions of the United States [121].

The discovery that various important anticancer agents are produced in small quantities by endophytic fungi isolated from plants is of particular significance. Examples are Taxol from *Taxomyces* [122] and many *Pestalotiopsis* species [123], as well as camptothecin [124,125], podophyllotoxin, an epimer of the precursor to the anticancer drug etoposide [126,127], vinblastine [128], and vincristine [129,130], all produced in relatively small amounts by endophytic fungi isolated from the producing plants. The fact that these compounds have been shown not to be artifacts offers the prospect for their increased production, provided the gene/gene product controlling their production by the relevant endophytes can be identified. Similar discoveries could provide an entry into greatly increased production of other key bioactive natural products.

1.8.8 Extremophiles

Extremophilic microbes (extremophiles) abound in extreme habitats, including deep-sea sediments and vents (see Section 1.8.4). Extremophiles include acidophiles (acidic sulfurous hot springs), alkalophiles (alkaline lakes), halophiles (salt lakes), piezo (baro)- and (hyper)thermophiles (deep-sea sediments and vents) [131–136], and psychrophiles (arctic and antarctic waters, alpine lakes) [137–139]. Until recently, investigations have centered on the isolation of thermophilic and hyperthermophilic enzymes (extremozymes) [140–143], but there is increasing evidence that these extreme environments are also yielding novel bioactive chemotypes.

Recent reviews highlight the promising range of compounds isolated thus far from extremophilic microbes [144,145]. Abandoned mine-waste disposal sites have yielded unusual acidophiles, which thrive in the acidic, metal-rich waters, polluted environments, which are generally toxic to most prokaryotic and eukaryotic organisms [146]. The novel sesquiterpenoid and polyketide–terpenoid metabolites, berkeleydione and berkeleytrione (Figure 1.7) showing activity against metalloproteinase-3 and caspase-1, activities relevant to cancer, Huntington's disease and other diseases, have been isolated from *Penicillium* species found in the surface waters of Berkeley Pit Lake in Montana [147–149]. An interesting observation has been the isolation of the diketopiperazine disulfide, glionitrin A (Figure 1.7), from a coculture of a *Sphingomonas* bacterial strain and an *A. fumigatus* fungal strain, both derived from coal mine drainages [150]; the compound was not produced in detectable amounts by either strain grown as a monoculture. Examples of novel compounds

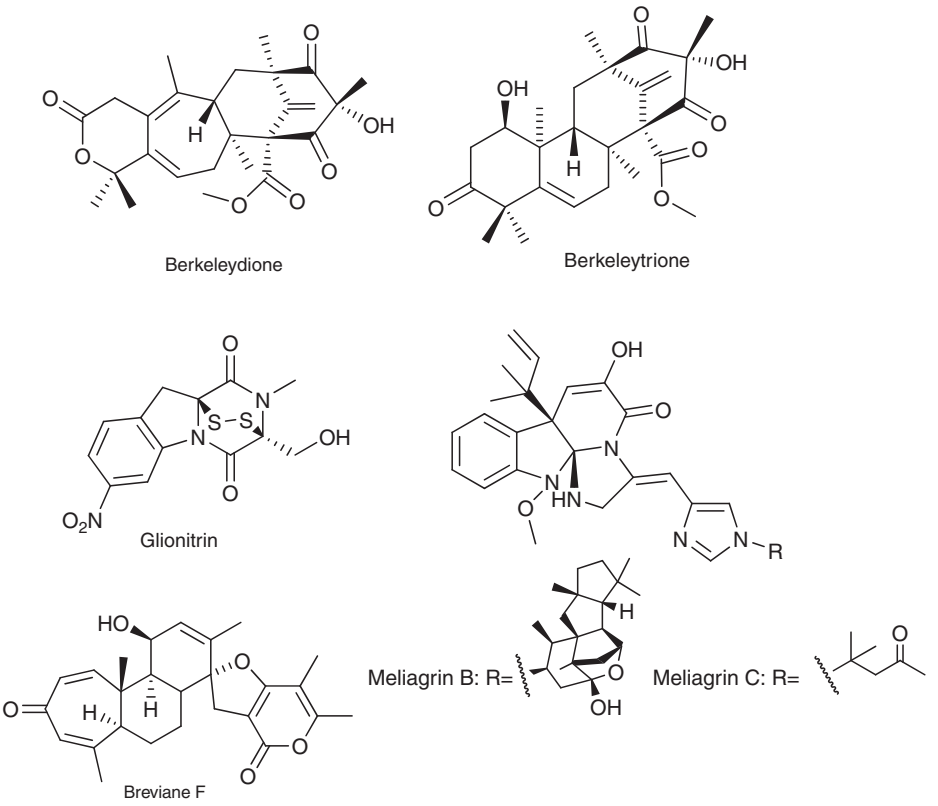

FIGURE 1.7 Novel compounds from extreme environments.

produced by deep-sea fungi (both *Penicillium* species) isolated from sediments collected at depths exceeding 5000 m, are the spiroditerpenoids, brevianes F–H (Figure 1.7) [151], and four new alkaloids, including two new meleagrin analogs, meleagrins B and C (Figure 1.7), two new diketopiperazines, roquefortines F and G, and six new diterpenes, conidiogenones B–G [152].

1.8.9 Combinatorial Biosynthesis

The substantial advances made in the understanding of the role of multifunctional polyketide synthase enzymes in bacterial aromatic polyketide biosynthesis have led to the identification of many such enzymes, together with their encoding genes. These advances have been reported and reviewed in a plethora of articles. Readers are referred to some of the reviews published during the past decade for detailed coverage [153–161], as well as to some reviews specifically covering NRPSs, which are responsible for the biosynthesis of nonribosomal peptides (NRPs) [162–165]. The rapid developments in the analysis of microbial genomes has enabled the

identification of a multitude of gene clusters encoding for polyketides, NRPs and hybrid polyketide–NRP metabolites, and have provided the tools for engineering the biosynthesis of novel "non-natural" natural products through gene shuffling, domain deletions, and mutations [155,166,167]. Results of the application of these combinatorial biosynthetic techniques to the production of novel analogs of anticancer agents such as anthracyclines, ansamitocins, epothilones, enediynes, and aminocoumarins have been reviewed by Shen et al. [168].

Some aspects related to the biosynthetic potential of myxobacteria [169], and to the production of novel "unnatural" epothilones [170] have been reviewed. An example illustrating the power of this technique is the efficient scale-up production of epothilone D, the des-epoxy precursor of epothilone B (Figure 1.3). It entered clinical trials as a potential anticancer agent under the code name of KOS-862, but has now been discontinued in favor of a congener, 9,10-didehydroepothilone D [171] known currently as KOS-1584, which is in Phase II clinical trials. The polyketide gene cluster producing epothilone B has been isolated and sequenced from two *Sorangium cellulosum* strains [172,173]. The epoxidation of epothilone D to epothilone B has been shown to be due to the last gene in the cluster, *epo*K, encoding a cytochrome P450, and heterologous expression of the gene cluster minus the *epo*K in *Myxococcus xanthus* has resulted in large-scale production of crystalline epothilone D [174].

1.9 DEVELOPMENT OF DRUGS FROM NATURAL PRODUCTS: A MULTIDISCIPLINARY PROCESS

Limited availability and structural complexity have historically been the major impediments to the development of clinically useful drugs from natural product leads. Quantities of the source biomass are often limited or, in the case of microbial sources, unculturable and the natural products themselves are often produced in trace quantities within the biomass. Advances in genomic mining and the engineering of biosynthetic pathways have revolutionized the discovery of novel natural products. These methods can also be utilized to enable large-scale production of natural products in the native or engineered organisms.

Despite several notable exceptions (e.g., adriamycin or taxanes in the antitumor area), the probability of a directly isolated natural product becoming the actual drug used for the treatment of a given disease in the future is relatively low. Natural molecules, however, can serve as lead compounds for the development of analogs, generated by combinatorial biosynthesis and/or medicinal chemistry, with optimized pharmacological properties. Evolution has selected natural products to bind to biological macromolecules and thus, natural products represent "privileged structures," [175] which are excellent templates for the synthesis of novel, biologically active, molecules. Advances in synthetic strategy and methodology are surmounting the barriers presented by the structural complexity of most natural products. Of course, evaluation and optimization of the structure–activity relationships (SARs) of the derived analogs from all these approaches requires

suitable biological assays. Thus, effective natural product–based drug discovery and development requires a truly multidisciplinary, collaborative approach.

1.9.1 Synthesis Based on Natural Products

While natural products often exhibit highly potent and selective bioactivity, they underwent evolutionary selection to serve the needs of their producing organisms not to serve as human therapeutics, and thus have not been fine-tuned to possess the potency, selectivity, and pharmacokinetic properties desired in a clinically useful drug. Optimization to improve physicochemical and pharmacokinetic properties frequently entails modification, removal or introduction of functional groups and stereocenters, or more drastic remodeling of the basic scaffold. Combinatorial biosynthetic methods offer access to unique structural diversity, but this diversity is limited by the available biosynthetic pathways of the host organism. The power of synthetic chemistry can be harnessed to access a greater extent of possible modifications and structural diversity than biosynthetic methods alone.

1.9.1.1 Derivatization and Semisynthesis. Simple functional group transformations, achieved by chemical and/or enzymatic methods, are possibly the simplest approach to optimization of a natural product lead. Large numbers of analogs can be rapidly generated by such semisynthetic approaches; however, the structural diversity of the analogs accessible by derivatization may be limited as many desired transformations cannot be accomplished due to incompatibilities with preexisting functional groups or the lack of a feasible reaction. There are numerous examples of this approach including the taxanes [176,177], camptothecins [178], and combretastatins [179].

Sometimes another, readily available natural product can serve as a starting material for the semisynthesis of a natural product of interest which itself is not readily available from biomass. The development of paclitaxel (Taxol) was severely hampered by the scarcity of *Taxus brevifolia*, whose bark was its original source. The compound supply issue and original commercial production were solved by semisynthesis from 10-deacetylbaccatin III, which is readily available from the needles of various *Taxus* species, a renewable resource. Details of the development of paclitaxel and other taxane derivatives have been comprehensively reviewed [176,177]. Another prominent example is provided by ecteinascidin 743 (Et-743, Yondelis; Figure 1.2). This complex alkaloid was discovered from the rare colonial tunicate *E. turbinata* [180,181]. The issue of compound supply for advanced studies was solved by the development of a semisynthetic route from the readily available microbial product cyanosafracin B [182; Section 1.3]. The discovery and development of ecteinascidin has been comprehensively reviewed [29,183].

1.9.1.2 Total Synthesis. Most of the dramatic advances in the field of synthetic organic chemistry are the result of the challenges posed by the total synthesis of complex natural products [184]. Adequate supply can be a serious limiting factor in the preclinical and clinical development of some naturally derived drugs, and the

focus of many top synthetic groups on devising economically feasible synthetic strategies is a very welcome development for both clinicians conducting clinical trials and patient populations. An excellent example is the marine-derived anticancer agent discodermolide, where total synthesis provided sufficient quantities for thorough clinical trials [185]. Unfortunately, these trials have now been terminated due to lack of objective responses and toxicity [186].

1.9.1.3 Diverted Total Synthesis. The process of total synthesis can often lead to the identification of the pharmacophore, the arrangement of steric and electronic features necessary to ensure optimal interaction with a biological target and trigger or block its biological response. This insight, coupled with a synthetic strategy that facilitates introduction of deep-seated structural variation, allows for the "molecular editing" of unnecessary structural complexity. Although the basic strategy had been practiced for many years by both academic and industrial groups, Danishefsky and coworkers have recently formally defined this approach and coined the term "diverted total synthesis" (DTS) [187,188]. DTS involves the synthesis of an advanced intermediate, of lesser complexity than the target natural product, which can be elaborated by different synthetic sequences to yield multiple analogs of varying complexity containing the common pharmacophore, but inaccessible from the natural product itself. Synthesis can be accomplished by both conventional medicinal chemistry or combinatorial chemistry approaches.

The development of Eribulin (E7389; Figure 1.2) from a marine-derived antitumor agent, halichondrin B, is a compelling example of the power of the DTS approach [30; Section 1.3]. Because of its extraordinary antitumor activity, halichondrin B was chosen for preclinical development in 1992. Clinical development was severely impeded due to the limited amounts of compound available from natural sources. Total synthesis studies revealed that the right-hand half of the molecule retained all or most of the potency of the parent compound, and led to the discovery of Eribulin, which is far less structurally complex, is prepared by synthesis, has greater *in vivo* stability, and possesses comparable bioactivity to, and lower toxicity than, halichondrin B [30]. Eribulin is currently in Phase III clinical trials [32] and the U.S. Food and Drug Administration (FDA) granted its manufacturer, Eisai Co., a priority review for use as treatment for advanced breast cancer. Approval for second line use against resistant breast cancer was given by the FDA in November 2011 [189].

In some instances, the original natural product may fail in clinical trials, but totally synthetic analogs continue to be developed. Thus, while clinical trials of the marine-derived anticancer agents, dolastatin 10 (Figure 1.8) and dolastatin 15, have been terminated, synthetic analogs continued in various phase of clinical trials [190]. Auristatin PE (TZT-1027 or soblidotin; Figure 1.8) is the only dolastatin analog still in clinical trials as the base molecule. There are some very interesting modifications that have been made by medicinal chemists in order to deliver this close relative of dolastatin 10 by use of monoclonal antibodies targeted at specific epitopes [191,192]. A significant number of combinations of this base molecule with varying monoclonal antibodies are currently in preclinical to Phase II clinical trials, predominately against hematologic cancers.

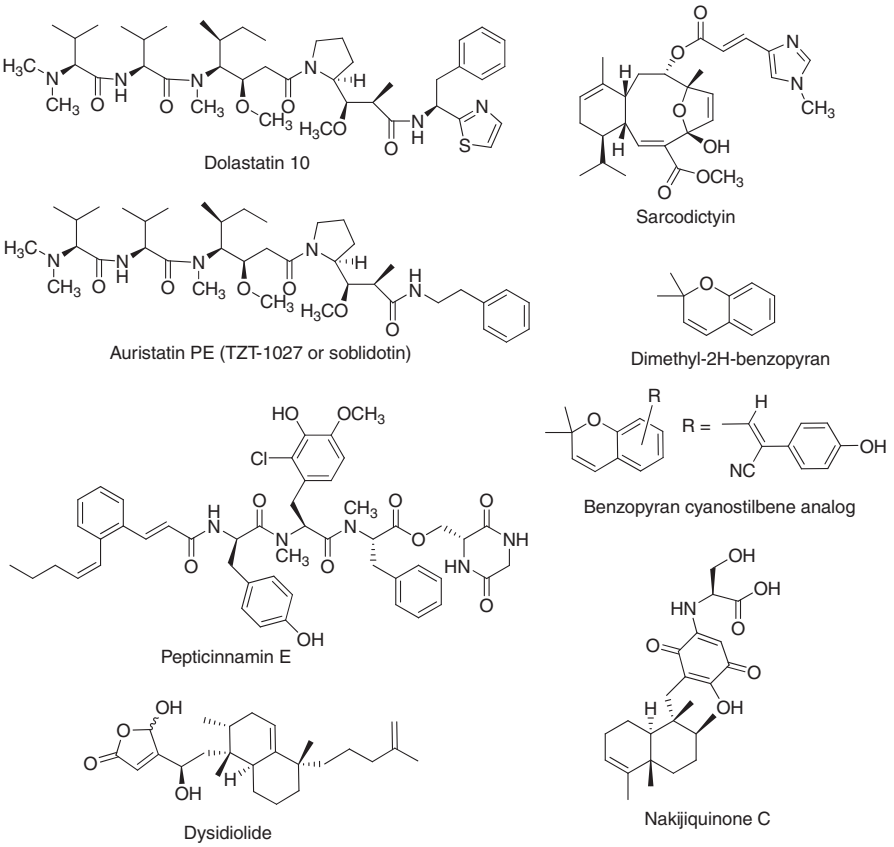

FIGURE 1.8 Synthetic and privileged natural product lead compounds and scaffolds.

Thus, Seattle Genetics has SGN-35 where the antibody is an anti-CD30 linked to auristatin PE in Phase I heading for Phase II [193], CuraGen has the antibody CR011 linked to auristatin PE in Phase II trials for metastatic breast cancer and melanoma [194] and Progenics has PSMA-ADC, a dimeric-specific PSMA antibody also conjugated to auristatin PE in Phase I against prostate cancer [195]. All of these examples are licensed from Seattle Genetics, although the exact linkages between the auristatin molecule and the antibody are subtly different in each case. A combination of the antibody trastuzumab, coupled to the maytansenoid DM1 is currently in Phase III clinical trials under the auspices of Roche, with a filing for approval expected in mid-2012.

1.9.2 Natural Product–Inspired Combinatorial Synthesis

Combinatorial chemistry is a set of techniques developed for the parallel or simultaneous synthesis of large collections of compounds (chemical libraries) for

high-throughput screening (HTS) against biological targets. The technology was rapidly embraced within the pharmaceutical industry and used to generate very large libraries of compounds with the expectation that HTS screening of vast numbers of compounds would prove to be more efficient and cost-effective than traditional approaches to drug discovery. These expectations led to the abandonment or de-emphasis of natural products research at many companies. While there are claims that combinatorial chemistry is generating new leads [196], the declining numbers of new NCEs [197] 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" [196]. As stated in that article, "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."

Approaches to the combinatorial synthesis of natural product–inspired libraries can be grouped into three basic categories although the boundaries among these are often indistinct. Combinatorial synthesis starting from known bioactive natural product scaffolds leads to libraries of natural product derivatives displaying appendage and stereochemical diversity. The second strategy, biology-oriented synthesis (BIOS) [198–200] expands on this basic concept by utilizing the structural information from natural products and their protein targets to focus on the most relevant chemical space for a particular target. The third approach, diversity-oriented synthesis (DOS) [201–204], aims to create highly diverse libraries of novel synthetic compounds with complex three-dimensional architectures that resemble natural products. DOS libraries often incorporate skeletal (scaffold) as well as appendage and stereochemical diversity. There is overlap among these approaches as well as with DTS and traditional medicinal chemical approaches to analog development.

1.9.2.1 Combinatorial Synthesis of Natural Product–Derived Libraries. Individual natural products often selectively modulate unrelated targets, a property that led to the recognition that natural product scaffolds are privileged structures as defined by Evans et al. in 1988 [175]; they have the necessary compromise of flexibility and rigidity to present functional groups in a favorable spatial arrangement to bind to biomolecular targets. As such, natural product scaffolds are obvious starting points for the application of combinatorial chemistry to prepare focused libraries of analogs for SAR studies. Nicolaou et al. [205] stated the underlying thesis 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."

Only a handful of the many recently published reports of the use of natural product scaffolds in combinatorial libraries will be cited to exemplify this approach. The synthesis of a library based on the scaffold of sarcodictyin (Figure 1.8) by Nicolaou et al. is one of the earliest examples [206]. Waldmann et al. prepared a pepticinnamin E

(Figure 1.8) library by solid-phase synthesis [207]. Solid-phase synthesis of combinatorial libraries was used to probe which regions of the epothilone A molecule are important to retention or improvement of activity [208], and combinatorial synthesis of vancomycin dimers yielded compounds with improved activity against drug-resistant bacteria [209]. Wipf et al. prepared some highly modified analogs of the antimitotic natural product curacin A (Figure 1.6), and found a simpler analog which was more potent than curacin A in inhibiting the assembly of tubulin [210]. A particularly versatile scaffold for library synthesis has been 2,2-dimethyl-2H-benzopyran (Figure 1.8); a search of the natural product literature yielded nearly 4000 analogs, with another 8000 structures identified through the inclusion of a slight modification of the search. In one example, application of solid-phase synthetic methods led to the identification and subsequent optimization of benzopyrans with a cyanostilbene substitution (Figure 1.8) that are effective against vancomycin-resistant bacteria [205,211,212].

The synthesis of combinatorial libraries based on natural product scaffolds is now a proven tool for the optimization of the known biological and pharmacokinetic properties of the parent natural product lead. It is also proving to be a potent tool for the discovery of analogs exhibiting biological activities beyond those previously associated with the parent natural product.

1.9.2.2 Biology-Oriented Synthesis of Natural Product–Inspired Libraries. BIOS is a new concept for the design of combinatorial libraries based on natural products developed by Waldmann and coworkers [198–200]. This concept is based on the recognition of fundamental and complementary properties of natural products and their protein targets. Since natural products bind both their biosynthetic enzymes and their target macromolecules, they necessarily populate biologically relevant regions of chemical space. Nature, through coevolution of natural products and their macromolecular targets, which are mainly proteins, has explored only a tiny fraction of the available chemical space. The number of three-dimensional protein folds has been shown to be even more conserved during evolution than the underlying sequences since topologically similar shapes can be formed by different sequences. Estimates of the number of proteins in humans range between 100,000 and 450,000 but the number of topologically different protein folds is actually much lower, with estimates of 600–8000 [213]. The natural product space and the protein structure space explored by Nature have to be highly complementary since they are limited in size and highly conserved. Thus, a natural product which is an inhibitor of a specific protein fold represents a biologically validated starting point for the development of analogs that may inhibit proteins with similar folds, and even allow for the discovery of specificity. These concepts are fundamentally similar to the privileged structure concept [175], but BIOS has the added dimension of using protein-folding patterns as the basis for subsequent screens.

BIOS is based upon the merger of two concepts previously developed by the Waldmann group [200]. The scaffolds of natural products can be mapped in a hierarchical manner to create a scaffold tree, a "structural classification of natural products" (SCONP) [214,215], which allows for logical pathways for the structural

simplification of scaffolds. In the second concept, "protein structure similarity clustering" (PSSC), proteins are clustered by three-dimensional shape around the ligand binding sites, regardless of sequence similarity [216–218]. The ligand of any member of a PSSC could be expected to exhibit some degree of complementarity toward other members of the PSSC and thus serve as a starting point for the development of modulators of the other members of the PSSC.

BIOS represents a refinement of combinatorial libraries based on natural product scaffolds by focusing on the most biologically relevant chemical space for the target. Furthermore, it allows the transfer of knowledge about the modulation of a target by a natural product to a whole cluster of structurally related proteins, even when those proteins catalyze mechanistically different reactions.

A combinatorial library inspired by the marine natural product dysidiolide (Figure 1.8) demonstrated the power and potential of the BIOS approach. The authors postulated that the γ-hydroxybutenolide group of dysidiolide was the major determinant of phosphatase activity. Testing of a 147-member library built around this molecule yielded a compound 10-fold more potent ($IC_{50} = 350$ nM) than the parent compound against Cdc25A [219]. In addition, other members of the library were identified with low micromolar activities against the enzymes acetylcholinesterase and 11β-hydroxysteroid dehydrogenase type 1, which fall within the same PSSC as Cdc25A [220].

A second example of the success of BIOS is the discovery of inhibitors of *Tie-2*, insulin-like growth factor 1 receptor (IGF-1R), and vascular endothelial growth factor receptor 2 (VEGFR-2 and -3). Nakijiquinone C (Figure 1.8), isolated from a marine sponge and first reported by Kobayashi et al. [221], in 1995, was shown to be an inhibitor of epidermal growth factor receptor (EGFR), c-ErbB2 and protein kinase C (PKC), in addition to having cytotoxic activity against L1210 and KB cell lines [221]. Testing of a library of 74 compounds, built around the basic nakijiquinone C structure, against a battery of kinases with similar protein domain folds, yielded seven new inhibitors with low micromolar activity *in vitro*, including one VEGFR-2 inhibitor and four inhibitors of Tie-2 kinase, a protein intimately involved in angiogenesis, and for which, at the beginning of the study, no inhibitors were known [222]. The details of the models used, the chemistry leading to the nakijiquinone-based compounds, and the ribbon structures of the kinase domain of the insulin receptor, with the corresponding homology domains of the as yet uncrystallized VEGFR-2 and *Tie-2*, have been fully reviewed [218,223].

1.9.2.3 Diversity-Oriented Synthesis of Natural Product–Like Libraries. The third approach, DOS, is both related to and fundamentally different from combinatorial approaches around natural product scaffolds. Since every member of a combinatorial library is unique, all combinatorial syntheses serve to create diversity and could be classified as DOS. In this review, we restrict the term to DOS from simple starting materials as described by Schreiber et al. in much of their pioneering work in the area.

DOS is based on the arguable premise that regions of chemical space, not defined by natural products or known drugs, may be fertile regions for discovering novel small

molecules that modulate biomacromolecules in useful ways, either as probes of function or as drug leads. The previous two approaches, based on known natural product scaffolds, aim to densely populate a specific region of chemical space, which is biologically relevant to a defined target. By contrast, DOS aims to achieve a nonfocused, diverse coverage of chemical space by the efficient and divergent synthesis of large libraries of structurally complex and structurally diverse compounds. Thus, while the molecules' structural complexity can be described as natural product-like, they are often not based on known natural product scaffolds. An in-depth discussion of DOS is outside the scope of this review and readers are referred to the excellent reviews on the subject, its relationship to natural products, and its applications to chemical genetics and drug discovery [202,204,224–230].

The most dramatic example of the power of DOS to generate novel chemical diversity is the 2009 report by Morton et al. of the synthesis of a 96-membered library based on 84 distinct molecular scaffolds [231]. Astonishingly, 65% of the scaffolds in this library are novel. When the skeletal diversity of the library was assessed by Waldmann's hierarchical scheme [214,215], the resulting scaffold tree was very similar to Waldmann's analysis of natural products. Morton et al. have not yet reported biological data for the library but the "natural product–likeness" should allow access to large regions of biologically relevant chemical space.

The synergy of combinatorial chemistry and natural products chemistry holds great potential for the discovery of new molecules populating productive regions of biologically relevant chemical space. Ultimate success will be gauged by the discovery of molecules with novel biological functions [229]. Thus far, the strategy of appending diverse substrates onto natural product scaffolds has performed remarkably well, yielding chemical probes such as secramine [232], uretupamine [233,234], and haptamide B [235].

More time is needed to critically assess the success of more recent innovations such as BIOS and DOS to deliver new small molecule probes and drugs. Ultimately, what is important is not the structural similarity of these new small molecules to natural product leads but whether they possess novel and useful biological functions.

1.10 CONCLUSIONS

While Nature, particularly plants, has long been an indispensable source of medicinal products, the vast untapped resources of the marine and microbial worlds have only recently become more readily accessible. The discovery of an impressive array of novel structure types from these sources has been reported over the past few decades, and the discovery rate and structural diversity of promising bioactive natural product leads are increasing with the rapid advances being made in collection, isolation, and structural elucidation techniques. Added to these advances, the explosion of genetic information over the past decade is leading to the advent of genetic techniques that permit the efficient isolation and expression of microbial biosynthetic cassettes, and the implementation of combinatorial biosynthetic technology and genome mining. A revised paradigm for the development of novel drugs is emerging. It is based on the

application of synthetic strategies encompassing DTS, and the elaboration of "privileged structures from Nature," or synthetic compounds modeled on such privileged structures, through use of directed combinatorial synthesis, BIOS, and DOS. The application of these refined combinatorial synthetic methodologies to the preparation and screening of focused, natural product–inspired libraries has great potential to lead to the more efficient development of novel agents and drug entities for the treatment of many disease states. The successful achievement of these goals requires the implementation of close multidisciplinary and international collaboration in both the discovery and development phases.

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A Second Edition of Anticancer Agents from Natural Products was published in November 2011 and readers may refer to this edition for the latest information discussed in references 29-31; 34-39; 96, 106, 168, 176, 178-9; 190.