Impact of Natural Products on Developing New Anti-Cancer Agents†

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1. Introduction

Throughout the ages, Nature has catered to the basic needs of humans, not the least of which is the provision of medicines for the treatment of a wide spectrum of diseases. Plants, in particular, have played a dominant role in the development of sophisticated traditional medicine systems. Egyptian medicine dates back to 2900 BCE, but the best known record is the "Ebers Papyrus", which dates from 1500 BCE and documents over 700 drugs, mostly of plant origin. Records documenting the uses of approximately 1000 plant-derived substances in Mesopotamia date from around 2600 BCE, and many are still used today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation.[1] Extensive documentation of the Chinese Materia Medica has occurred over the centuries,[2] with the first record dating from about 1100 BCE (Wu Shi Er Bing Fang, containing 52 prescriptions), being 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]

In the ancient Western World, the Greeks and Romans made substantial contributions to the rational development of the use of herbal drugs, with Dioscorides, a Greek physician (100 CE), accurately recording the collection, storage, and use of medicinal herbs during his travels with Roman armies throughout the then "known world", and Galen (130−200 CE), a practitioner and teacher of pharmacy and medicine in Rome, being well-known for his complex prescriptions and formulas used in compounding drugs. The preservation of much of the Greco−Roman expertise during the Dark and Middle Ages (5th−12th centuries) may be attributed to the Arabs, who expanded it to include the use

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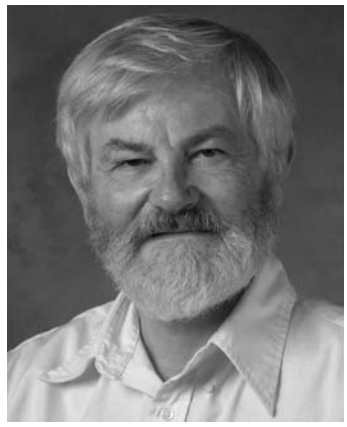


David Newman is the current chief of the Natural Products Branch (NPB) in the Developmental Therapeutics Program at the National Cancer Institute in Frederick, MD. Born in Grays, Essex, U.K., in 1939, he received a M.Sc. in synthetic organic chemistry from the University of Liverpool in 1963. Following time as a synthetic chemist at Ilford, Ltd., he joined the ARC's Unit of Nitrogen Fixation at the Universities of London and Sussex, as a research assistant in metallo-organic chemistry, transferring to the microbial biochemistry group in early 1966 as a graduate student and being awarded a D.Phil. in 1968 for work on microbial electron transport proteins from *Desulfovibrio*. He moved to the United States in 1968 as a postdoc at the Biochemistry Department at the University of Georgia working on protein sequencing of *Desulfovibrio* ferredoxins and then in 1970 joined SK&F in Philadelphia as a biological chemist. At SK&F, most of his work was related to antibiotic discovery, and in 1985, when the antibiotic group was dissolved, he left SK&F. For the next six years, he worked in marine and microbial discovery programs at Air Products, SeaPharm, and Lederle, and then in 1991, he joined the NPB as a chemist responsible for marine and microbial collection programs. He was given the NIH Merit Award in 2003 for this work, and following Gordon Cragg's retirement from the position of Chief, NPB, at the end of 2004, he was acting chief until appointed chief in late 2006. He is the author or coauthor of over 120 papers, reviews, and book chapters (and an editor, with Gordon Cragg and David Kingston, of *Anticancer Agents from Natural Products*) and holds 18 patents mainly on microbial products.

Gordon Cragg obtained his undergraduate training in chemistry at Rhodes University, South Africa, and his D. Phil. (organic chemistry) from Oxford University. After two years of postdoctoral research at the University of California, Los Angeles, he returned to South Africa to join the Council for Scientific and Industrial Research. In 1966, he joined the Chemistry Department at the University of South Africa, and he transferred to the University of Cape Town in 1972. In 1979, he returned to the U.S.A. to join the Cancer Research Institute at Arizona State University, working with Professor G. R. Pettit. In 1985, he moved to the National Cancer Institute (NCI), National Institutes of Health (NIH), in Bethesda, Maryland, and was appointed Chief of the NCI Natural Products Branch in 1989. He retired in December, 2004, and is currently serving as an NIH Special Volunteer. His major interests lie in the discovery of novel natural product agents for the treatment of cancer and AIDS, with an emphasis on multidisciplinary and international collaboration. He has given over 100 invited talks at conferences in many countries worldwide and has been awarded NIH Merit Awards for his contributions to the development of the anti-cancer drug Taxol (1991), leadership in establishing international collaborative research in biodiversity and natural products drug discovery (2004), and contributions to developing and teaching NIH technology-transfer courses (2004). In 1998−1999, he was President of the American Society of Pharmacognosy and was elected to Honorary Membership of the Society in 2003, and he was named as a Fellow of the Society in 2008. In November, 2006, he was awarded the "William L. Brown Award for Plant Genetic Resources" by Missouri Botanical Garden, which also named a recently discovered Madagascar plant in his honor, *Ludia craggiana*. He has established collaborations between the NCI and organizations in many countries, promoting drug discovery from their natural resources. He has published over 150 papers related to these interests.

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 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.

The continuing and essential role played by plant-based systems in the healthcare of many different cultures has been extensively documented,[5,6] and the World Health Organization (WHO) has estimated that approximately 65% of the world's population rely mainly on plant-derived traditional medicines for their primary health care. Plant products, however, also play an important role in the health care systems of the remaining population, mainly residing in developed countries.[7] In a survey of plant-derived pure compounds used as drugs in countries hosting WHO−Traditional Medicine Centers, 80% of 122 such compounds identified were found to be used for the same or related ethnomedical purposes and were derived from only 94 plant species.[7,8]

Plants have a long history of use in the treatment of cancer. Hartwell, in his review of plants used against cancer, lists more than 3 000 plant species that have reportedly been used in the treatment of cancer.[9] In many instances, however, the "cancer" is undefined or reference is made to conditions such as "hard swellings", abscesses, calluses, corns, warts, polyps, or tumors, to name a few; these symptoms would generally apply to skin, "tangible", or visible conditions, and may indeed sometimes correspond to a cancerous condition. Many of the claims for efficacy in the treatment of cancer 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. This is in contrast to other plant-based therapies used in traditional medicine for the treatment of afflictions such as malaria and pain, which are more easily defined, and where the diseases are often prevalent in the regions where traditional medicine systems are extensively used.[10]

Despite the above statement, the discovery of several effective anti-cancer agents from plants may be attributed, directly or indirectly, to a history of use of the relevant plant in traditional medicine. Thus, the first plant-derived agents to advance into clinical use, the so-called vinca alkaloids vinblastine (VLB; **1**; Scheme 1) and vincristine (VCR; **2**; Scheme 1) (section 5.2), were isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don, used by various cultures for the treatment of diabetes.[11] The plant was actually being investigated as a source of potential oral hypoglycemic agents, but the serendipitous observation of the reduction of white blood cell counts and bone marrow depression in rats led to the isolation of VLB and VCR. A more direct link to traditional use is apparent in the discovery of podophyllotoxin

Paul Grothaus earned a B.S.Chem. from Creighton University in 1977 and his Ph.D. from Purdue University in 1983, where he completed the first enantiospecific total synthesis of a trichothecene mycotoxin, anguidine. His education was followed by a postdoctoral stint at the University of Washington. In 1984, he joined the Natural Products Group in the Plant Sciences Division of Monsanto Agricultural Company, where he investigated the synthesis and structure−activity relationships of agriculturally useful natural products. In 1988, he became the head of chemistry at Hawaii Biotech, Inc., in Aiea, Hawaii, where he worked on drug discovery based upon both terrestrial and marine natural product leads. In 2002, he joined the Medicinal Chemistry department of Celera Genomics, Inc., in South San Francisco, where he became an Associate Director of Medicinal Chemistry in 2005. Research at Celera focused on development of protease and kinase inhibitors and the development of activity-based probes for chemical proteomics studies. In 2007, he joined the Natural Products Branch of the National Cancer Institute in Frederick, MD. Dr. Grothaus is the author or coauthor of 22 papers, reviews, and book chapters and holds 5 patents.

(**3**; Scheme 1), a stereoisomer of epipodophyllotoxin, the precursor to the semisynthetic anti-cancer agent, etoposide (**4**; Scheme 1) (section 5.2). A long history of medicinal use, including the treatment of skin cancers and warts, led to the investigation of *Podophyllum peltatum* Linnaeus (commonly known as the American mandrake or Mayapple), resulting in the isolation of podophyllotoxin as the active agent from the roots.[12] Following extensive research, etoposide was developed as a clinically active agent.[13]

As mentioned in section 5.5.4, the indirubins (e.g., **5**; Scheme 1), currently in preclinical development, were initially identified as the major active components of the traditional Chinese medicine, Danggui Longhui Wan, which has been used for many years to treat chronic myleogenous leukemia (CML) in China.[14] One further example is flavopiridol (**6**; Scheme 1) (section 5.5.1). Though it is totally synthetic, the basis for its novel structure is a natural product, rohitukine (**7**; Scheme 1), isolated by chemists at Hoechst India Ltd. in the early 1990s from *Dysoxylum binectariferum* Hook. f., which is phylogenetically related to the Ayurvedic plant, *D. malabaricum* Bedd., used for rheumatoid arthritis. Rohitukine was isolated as the constituent responsible for anti-inflammatory and immunomodulatory activity. A synthetic campaign performed for structure−activity studies resulted in flavopiridol, the only compound from over 100 analogues synthesized that was found to possess tyrosine kinase activity and potent growth inhibitory activity against a series of breast and lung carcinoma cell lines.[15]

2. Continuing Role of Nature in Drug Discovery

In 2007, Newman and Cragg published[16] the third in their series of analyses of the sources of drugs, covering the period from 01/1981 to the middle of 2006 and showing the sources of the listed 974 small molecule drugs. The analysis demonstrated the continuing and valuable contributions of Nature as a source not only of potential chemotherapeutic agents but also of lead compounds that have provided the basis and inspiration for the semisynthesis or total synthesis of effective new drugs. In this analysis, the drugs were classified as N (an unmodified natural product); ND (a modified natural product); S (a synthetic compound with no natural product conception); S/NM (a synthetic compound showing competitive inhibition of the natural product substrate); S* (a synthetic compound with a natural product pharmacophore); and S*/NM (a synthetic compound with a natural product pharmacophore showing competitive inhibition of the natural product substrate).

In Chart 1, an updated analysis is shown, using the same coding and extending the period of time to the middle of October 2008, so that the chart now covers from January 1981 to the middle of October 2008 and covers 1024 new chemical entities, an increase of 50 small molecules in the two years. From the current data, 67% of the compounds are formally synthetic, but the analysis indicates that 18% of these correspond to the S* and S*/NM classes (NP pharmacophore) and 13% fall into the S/NM class (model a natural product inhibitor of the molecular target of interest, or mimic, as they are competitive inhibitors, the endogenous substrate of the active site). Thus, as with the 2007 analysis, the proportion of truly synthetic (i.e., devoid of natural product inspiration and coded as S) is still at 37%. In considering disease categories, 68.3% of anti-infectives (antibacterials, -fungal, -parasitic, and -viral) were classified as naturally derived or inspired (N; ND; S*; S*/NM; S/NM), while in the cancer treatment area, 79.8% were in these categories, with the figure dropping to 62.9% if the S/NM category is excluded.

3. Why Nature?

3.1. Some Examples of the Potential Role of Natural Products in Nature

While the contributions of natural secondary metabolites to modern medicine are abundantly clear, why these inherently biologically active compounds are actually produced by organisms still remains a topic of some debate. Though initially they were regarded as waste products, further research has revealed that organisms have evolved over eons to produce these complex and often toxic chemicals for purposes of defense, communication, and predation.

Plants, insects, and marine invertebrates utilize natural products as a means whereby they defend themselves chemically against predation and consumption (e.g., herbivory). A fascinating example is provided by the pupae of the coccinellid beetle, *Epilachna borealis*, which exert a chemical defensive mechanism against predators through the secretion from their glandular hairs of droplets containing a library of hundreds of large-ring (up to 98 members) macrocyclic polyamines, with the simplest example having the generic formula shown (**8**; Scheme 2).[17] The use of three simple (2-hydroxyethylamino)alkanoic acid precursors in the building of these libraries provides clear evidence that combinatorial chemistry was pioneered and widely used in Nature for the synthesis of biologically active compound libraries.

Plants and sessile marine organisms (e.g., corals) release toxic compounds that suppress the growth of neighboring

Scheme 1. Anti-Cancer Agents Derived from Plants Used in Traditional Medicine

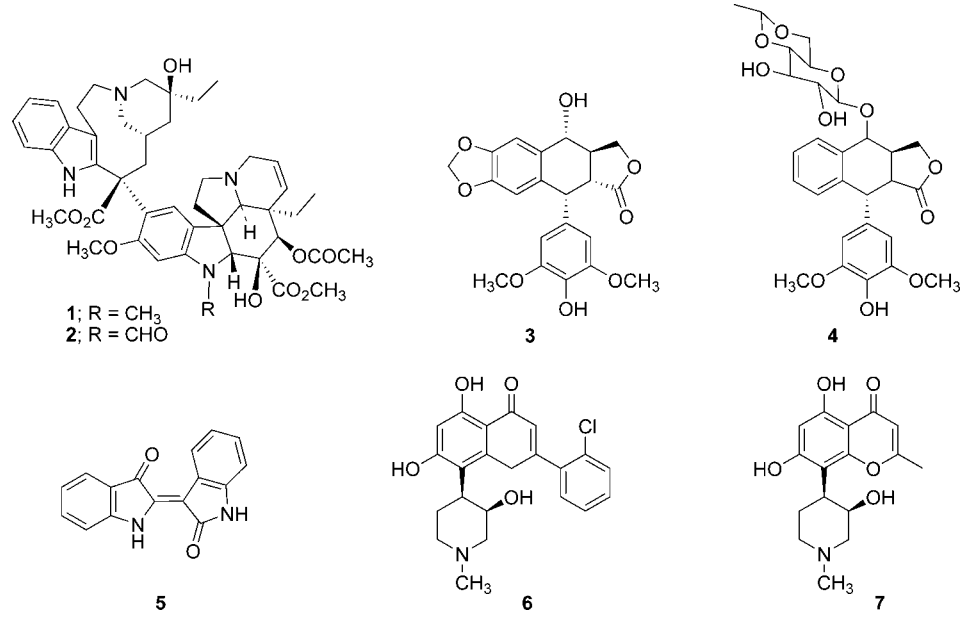
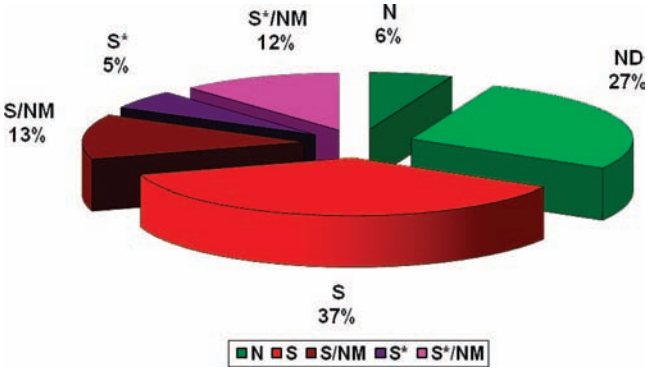
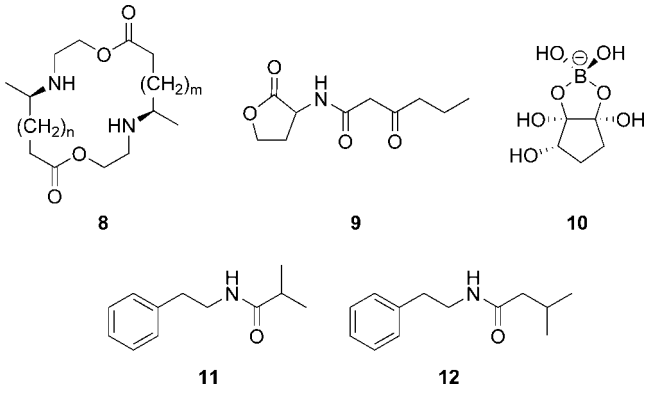


Chart 1. Small Molecule New Chemical Entities 01/1981−10/2008, By Source ($N = 1024$)



Scheme 2. Secondary Metabolites in Chemical Defense and Quorum Sensing



species. For plants, this process is known as allelopathy.[18,19] Similarly, microorganisms produce and excrete antimicrobial toxins as a means of killing sensitive strains of the same or related species.[20]

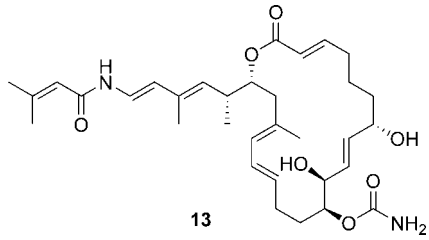
The cell-to-cell signaling mechanism, known as quorum-sensing, exerted by bacteria to control their density of population growth and biofilm formation, involves the excretion of so-called quorum-sensing compounds. The best studied are the acyl homoserine lactones (AHLs), with compounds, including *N*-3-oxohexanoyl-l-homoserine lactone (**9**; Scheme 2) and a previously unidentified furanone boronate diester that appears to be a universal signal (**10**; Scheme 2), from *Vibrio fisheri* being examples. These compounds signal the activation of genes promoting virulence, spore formation, biofilm formation, and other phenomena.[21,22] Very recently, another variation on interruption of Gram-negative aggregates by Gram-positive nontoxic secondary metabolites (phenylethylamides **11** and **12**; Scheme 2), by interfering with quorum-sensing, was reported by Teasdale et al.[23] This is the first time that such compounds had been identified. In addition to such actions, microbes can also produce simple molecules that will disperse biofilms, as demonstrated by Davies and Marques in late 2008 when they demonstrated that the pseudomonad metabolite *cis*-2-decenoic acid was a nanomolar disrupter of cross-kingdom biofilms.[24] In addition to simple signaling in normal settings in soils, quorum-sensing may also be one of the causes of significant infection in long-term patients due to the recruitment of many different microbes and the subsequent production of resistant biofilms on in-dwelling catheters and prosthetic devices.

Species of the cone snail genus, *Conus*, inject venom composed of combinatorial libraries of several hundred peptides to stun their prey prior to capture,[25] and the venom may also be used for defense against predators. One component of this combinatorial mixture has been developed as Ziconotide, a non-narcotic analgesic, currently marketed as Prialt.[26]

3.2. Classical Nonmicrobial Natural Sources: Untapped Potential

The classification and documenting of terrestrial flora have been intensively investigated, with estimates of the number of higher plant species ranging from 300 000 to as high as 500 000. In terms of pharmacological and phytochemical investigation, however, estimates are as low as 6% and 15%, respectively.[8,27,28] Furthermore, the marine environment remains virtually unexplored as a potential source of novel drugs,[29,30] and until recently, the investigation had largely been restricted to tropical and subtropical regions. Exploration, however, has expanded to colder regions, and a recent

Scheme 3. Palmerolide, a Natural Product from a Novel Polar Marine Source



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significant discovery is the isolation of the cytotoxic macrolide palmerolide A (**13**; Scheme 3) from an Antarctic tunicate,[31] with total synthesis leading to a revision of the original structure.[32]

The power of Nature as applied to plant secondary metabolite production can be augmented through the use of chemical elicitors and selected derivatives of biosynthetic precursors. Thus, exposure of the roots of hydroponically grown plants to chemical elicitors induces the selective and reproducible production of bioactive compounds,[33] while the feeding of seedlings of *Catharanthus roseus* with various tryptamine analogues has resulted in the production of nonnatural terpene indole alkaloids related to the vinca alkaloids.[34]

3.3. Microorganisms. Unexplored Genomic Potential

The term "microorganism" is often taken to mean only prokaryotic single-celled organisms that do not have an organized nucleus (no nuclear membrane enclosing their chromosomes); however, we are using the term to mean any single-celled organism (including the prokaryotic cyanophytes, be they unicellular or filamentous). We also include the eukaryotic fungi (yeast or filamentous) and the vast number of genera and species that fall under the "catch-all name" of the *Protista*, which includes the dinoflagellates, very well-known for their extremely potent polyether toxins. The biosynthesis of these toxins has been the object of significant scientific debate since the original proposal of synchronized epoxide opening by Nakanishi in 1985,[35] now shown to be fundamentally correct, and very recently thoroughly reviewed by Gallimore.[36]

3.3.1. Potential of the "Metagenome"

With the current ability to cultivate only a vanishingly small number of naturally occurring microorganisms, the study of either terrestrial or marine natural microbial ecosystems has been severely limited. As a result, it has been estimated that less than 1% of microorganisms seen microscopically have been cultivated. Nevertheless, despite this limitation, a most impressive number of highly effective microbially derived chemotherapeutic agents has been discovered and developed. Given the observation that "a handful of soil contains billions of microbial organisms",[37] and the assertion that "the workings of the biosphere depend absolutely on the activities of the microbial world",[37,38] the microbial universe clearly presents a vast untapped resource for drug discovery.

Uncultured microorganisms present in environmental samples, however, can be identified through the extraction of nucleic acids (the metagenome) from such environmental samples, as well as the isolation and sequencing of rRNA or rDNA (genes encoding for rRNA). Samples from soils and seawater are currently being investigated.[39,40] Application of whole-genome shotgun sequencing to environmental-pooled DNA obtained from water samples collected in the Sargasso Sea off the coast of Bermuda by the Venter group, has indicated the presence of at least 1 800 genomic species, which included 148 previously unknown bacterial phylotypes.[40] The Venter group is also examining microbial communities in water samples collected by the Sorcerer II Global Ocean Sampling (GOS) expedition, and they have reported that 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.[41] Similar methods are also being applied to the investigation of other habitats, such as the microflora of insects[42] and marine animals.[43]

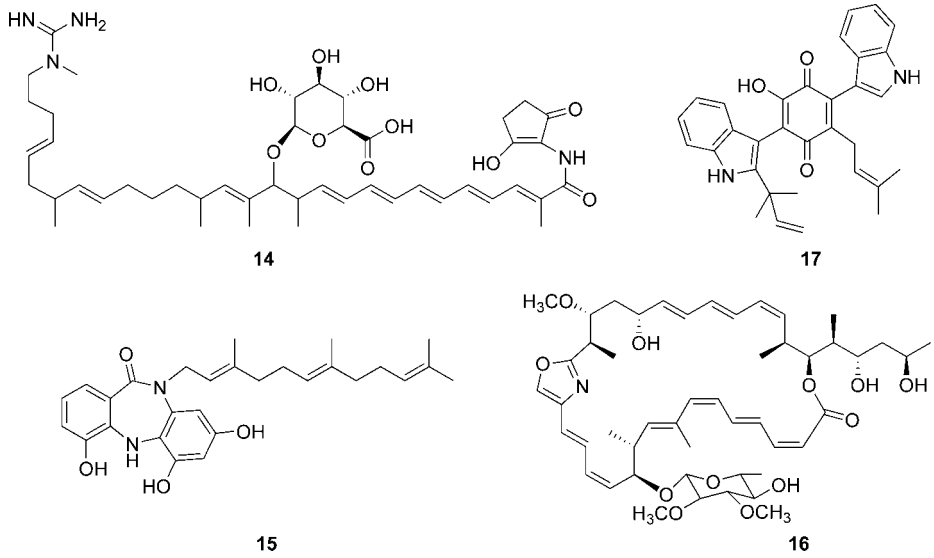
Two recent reviews giving up to date information on the manifold structures that can be found by expression of environmental DNA,[44,45] together with a very recent review demonstrating the metabolite pathway diversity from metagenomic libraries,[46] provide many more examples of the value of this type of investigation. In addition to these examples, very recently, a proposal has been put forward by the TerraGenome International Sequencing Consortium (www.terragenome.org) to completely sequence the "metagenome" of soil at a specific site in the U.K., with the aim of generating information on biosynthetic processes that involve not only drug discovery but a multitude of other processes. That such an ambitious project is feasible and the tools are available can be seen from the recently published report on the Metacontrol project by van Elsas et al.[47]

3.3.2. Cryptic Clusters in Bacteria and Fungi

In the past few years, because of the great advances in studying the genomes of microbes (currently of only some of the taxa implied by that term) and the continuing advances in understanding the structure of genes and their corresponding products (the proteins encoded by the DNA), there has come the recognition of the following problems: (a) there are many more putative biosynthetic clusters present than originally deduced from conventional methods of fermentation and/or extraction and (b) the knowledge of the control of expression of these "cryptic clusters" is in its infancy. The early work on the numbers of such clusters in an individual microbe was mainly carried out on the genomes of two very important *Streptomyces* species, *S. avermitilis* (where the number of putative clusters reached into the low 30s)[48,49] and *S. coelicolor* (where numbers are now reaching into the high teens to low 20s).[50]

From this pioneering work of the Omura[48,49] and Hopwood[50] groups, 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. This is exemplified by the investigation of the genome of the well-known vancomycin producer, *Amycolatopsis orientalis* (ATCC 43491); the isolation of the novel antibiotic ECO-0501 (**14**; Scheme 4) was only achieved through use of the genomic sequence to predict the molecular weight and then looking for the molecule directly by high-performance liquid chromatography−mass spectroscopy (HPLC−MS). The compound had a very similar biological profile to vancomycin, but it was masked by this compound.[51] That this technique

Scheme 4. New Compounds from Genome Mining



will work for anti-cancer agents as is shown by diazepinomicin (**15**; Scheme 4), currently in phase II clinical trials, which was found by the same technique as used in the case of ECO-0501 and by the same company, which is now known as Thallion Pharmaceuticals.[52] Though it was first reported four years earlier from a marine-sourced bacterium by the Wyeth group,[53] Thallion, then known as Ecopia, had already patented the agent.

Genomic analyses have now also been applied to the myxobacteria, and the identification and utilization of *ChiR*, the gene controlling production of chivosazol (**16**; Scheme 4), an extremely potent anti-fungal antibiotic, have been reported.[54] Also discussed in this paper is a major problem occurring in secondary metabolite expression, whether in homologous or heterologous hosts, namely, the identification and application of the transcriptional control mechanisms involved. Further work on the genetics of the myxobacteria and their secondary metabolites has recently been reported by German groups who are the scientific descendants of the Höfle and Reichenbach laboratories.[55−59]

A recently reported genomic analysis of the fungus *Aspergillus nidulans* predicts the presence of potential gene products controlling metabolite production.[60] The analysis not only suggested the presence of clustered secondary metabolite genes having the potential to generate up to 27 polyketides, 14 nonribosomal peptides, one terpene, and two indole alkaloids, but also identified the potential controller of expression of these clusters; this was demonstrated by expressing terrequinone A (**17**; Scheme 4), a compound not previously reported from this species.[54] Similar predictions can be made for *A. fumigatus* and *A. oryzae* as a result of the analysis of the potential number of secondary metabolite clusters in these fungi.[60] The discussion on control of secondary metabolites in fungi has been expanded in a recent review.[61]

In the next series of subsections under this major heading, we will discuss the findings and potential of some of the major groupings of microbes from the aspect of secondary metabolites that have potential or actual possibilities for development as anti-cancer agents. These sections are not organized by formal taxonomy but more on the lines of what microbial organism(s) natural products isolation chemists have investigated, often due to their accessibility.

3.3.3. Cyanophytes

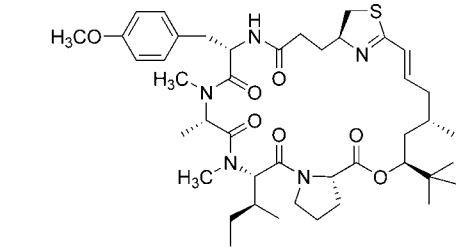
These organisms, often known as blue−green algae in the earlier literature, are actually prokaryotes and are some of the most prolific producers of bioactive secondary metabolites (in a multiplicity of pharmacologic areas) yet identified. It is possible that, in due course, they may actually outstrip the myxobacteria in terms of the raw numbers of novel agents discovered. In order to give an idea 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), one need only consult the recent reviews by Welker and von Dohren[62] or Tan,[63] or the recent papers showing the extremely potent potential anti-cancer agents apratoxin E[64] (**18**; Scheme 5) and coibamide A[65] (**19**; Scheme 5), both isolated from wild collections.

As a result of the pioneering work of Moore and Patterson in Hawaii on cyanobacteria as sources of potential anti-cancer agents,[66] one cyanobacterial secondary metabolite cryptophycin (**20**; Scheme 5) led to a large synthetic program, with a derivative, cryptophycin 52 (**21**; Scheme 5), reaching phase II clinical trials in cancer under the auspices of Lilly. Currently, cryptophycin 52 is no longer in trials due to toxicity, but the complete biosynthetic pathway of the base molecule has been identified and cloned.[67]

A few years later in 1994, Gerwick, then at Oregon State University, reported that the new compound curacin A (**22**; Scheme 5) was a potential anti-cancer lead.[68] However, curacin A was effectively insoluble in any formulation that was compatible with in vivo testing in animals, but it had excellent cytotoxic activity in vitro and was a tubulin interactive agent similar to the cryptophycins in its mechanism, i.e., depolymerization rather than stabilization of tubulin. The complete 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.[69]

The actual spatial production of curacin A and other secondary metabolites within *L. majuscula* in the presence of other cyanophytes has been the focus of some very recent reports from the Gerwick group that should be consulted in order to see what is now feasible with modern instrumenta-

Scheme 5. New Compounds from Cyanophytes



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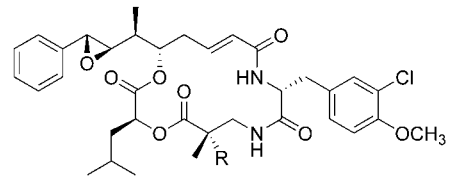

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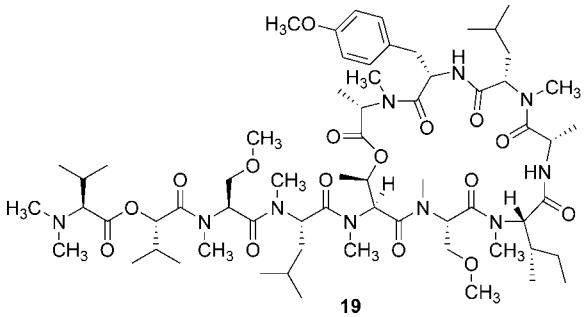
21; R = CH_3



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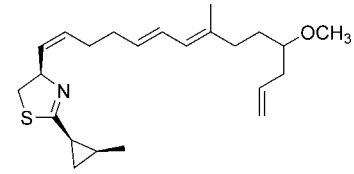

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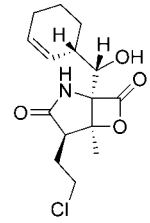
tion.[70] The possibilities of in vitro manipulation of such a cluster have recently been reviewed by Walsh's group.[71]

Free living cyanophytes are not the only producers of interesting chemical compounds that may have future utility as anti-cancer agents. Evidence that compounds, originally considered to be from marine invertebrates but now shown to be from "symbiotic" cyanophytes, has recently been reported. Expression of the active peptides in *E. coli* provides yet another possibility for production of these clusters, further expanding their potential.[72]

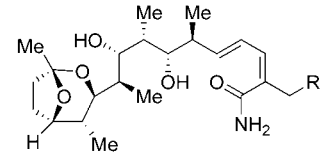
3.3.4. Marine Microbes (Non-Cyanophytes)

The relatively recent investigation of deep ocean sediments, particularly by the Fenical group at the Center for Marine Biotechnology and Biomedicine at the Scripps Institute of Oceanography, has led to the discovery of an increasing number of new actinomycete bacteria that are unique to the marine environment.[73] Use of a combination of culture and phylogenetic approaches have led to the description of the novel marine actinomycete genus now known as *Salinospora*[73,74] whose members are proving to be ubiquitous, occurring 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. Use of appropriate selective culturing and isolation techniques has led to the observation of significant antibiotic and cytotoxic activity and has resulted in the isolation of a potent cytotoxin, salinosporamide A (**23**; Scheme 6), a very potent proteasome inhibitor ($IC_{50} = 1.3$ nM),[75] currently in phase I clinical trials and heading for phase II. Other recent discoveries include saliniketals A and B (**24** and **25**; Scheme 6), potential chemopreventive agents isolated from *Salinispora arenicola*[76] and the marinomycins, novel macrolides isolated from another new actinomycete genus named *Marinispora*, with marinomycins A (**26**; Scheme 6) to D showing potent activity against drug-resistant bacterial pathogens and some melanomas.[77]

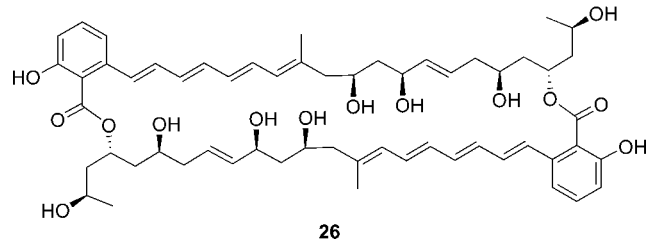
Scheme 6. New Compounds from Marine Microbes (Non-Cyanophytes)



23



24; R = H
25; R = OH

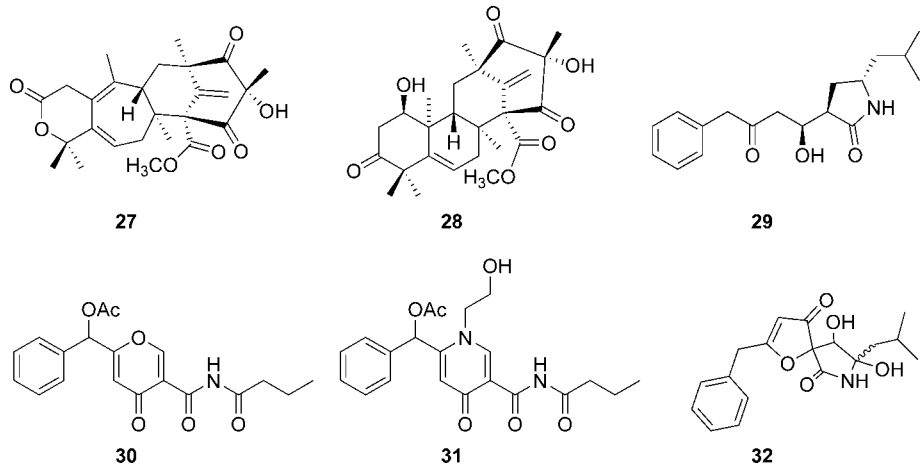


26

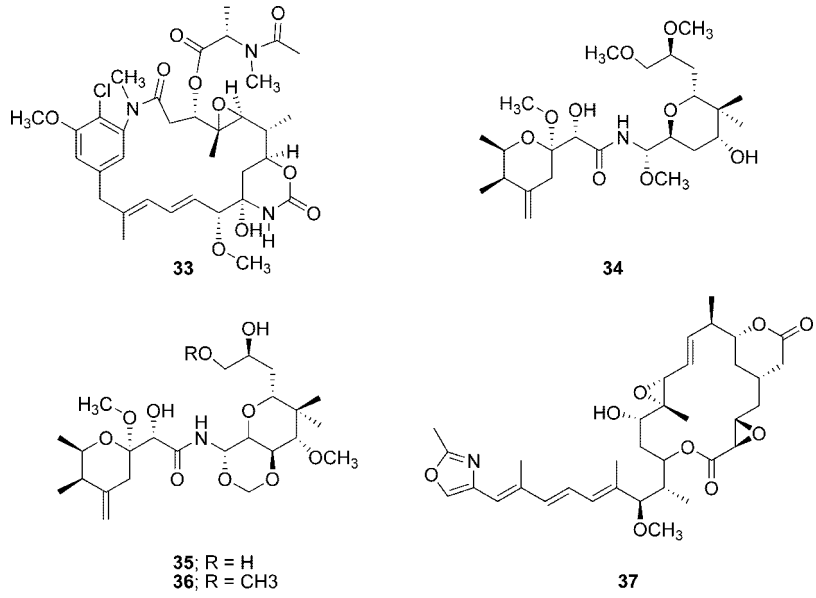
3.3.5. Extremophiles

Extremophilic microbes (extremophiles) abound in extreme habitats, and include acidophiles (acidic sulfurous hot springs), alkalophiles (alkaline lakes), halophiles (salt lakes), piezo (baro)- and (hyper)thermophiles (deep-sea vents),[78−82] and psychrophiles (arctic and antarctic waters, alpine lakes).[83] While research has centered on the isolation of thermophilic and hyperthermophilic enzymes (extremozymes),[84−88] there is little doubt that these extreme environments will also yield novel bioactive small molecule chemotypes. Unusual acidophiles that thrive in the acidic, metal-rich waters, polluted environments have been isolated from abandoned mine-waste disposal sites that are generally toxic to most prokaryotic and eukaryotic organisms.[89] *Penicillium* species found in the contaminated surface waters of Berkeley Pit Lake in Montana have yielded the novel sesquiterpenoid and polyketide−terpenoid metabolites, berkeleydione (**27**, Scheme 7), berkeleytrione (**28**; Scheme 7), and berkeleyamides A−D (**29**−**32**; Scheme 7), showing activity against metallopro-

Scheme 7. New Compounds from Extreme Environments



Scheme 8. Examples of Novel Natural Products from Microbial Symbionts



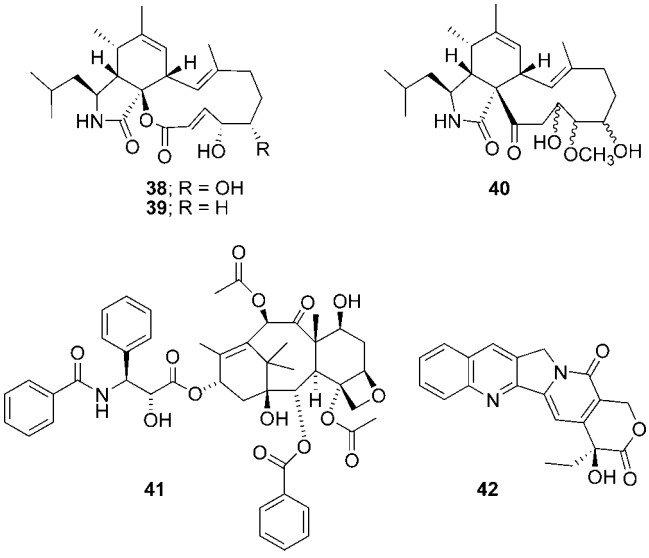
teinase-3 and caspase-1, activities relevant to cancer, Huntington's disease, and other diseases.[89−92]

3.3.6. Microbial Symbionts

There is mounting evidence that many bioactive compounds isolated from various macro-organisms, which can include plants, marine, terrestrial invertebrates, and even fungi, are actually metabolites synthesized by symbiotic bacteria.[93] These include the maytansinoids (**33**; Scheme 8), anti-cancer compounds originally isolated from several genera of the Celastraceae plant family,[94] and pederin (**34**; Scheme 8), isolated from beetles of the genera *Paederus* and *Paederidus* as well as derivatives based on the pederin skeleton from several marine sponges.[95−97] These "pederine-like molecules" now number more than 34 from at least 8 different animal genera, and full details on these symbiotic sources together with a discussion of the range of anti-tumor agents isolated from marine organisms that closely resemble bacterial metabolites,[29] are given in the 2004 review by Piel;[93] an updated review from the same author covering the literature to the end of 2007 was also recently published.[98] These articles should be consulted in order to see the wide range of structures and potential producers that have so far been identified, and to see what can now be done using this information to produce novel but unnatural variations on known potential anti-cancer agents from these sources. For example, reaction of mycalamide A (**35**; Scheme 8) with the *PedO* gene product has generated the novel biosynthetic hybrid, 18-*O*-methylmycalamide A (**36**; Scheme 8), which has increased cytotoxicity compared to the parent compound.[99]

The discovery of a bacterium−fungus−plant interaction occurring in the case of rice seedling blight provides an interesting example of an even more complex symbiotic−pathogenic relationship. The toxic metabolite, rhizoxin (**37**; Scheme 8), originally isolated from a *Rhizopus* fungus contaminating rice seedlings, has actually been found to be produced by the endo-symbiotic bacterial species, *Burkholderia rhizoxina*,[100,101] and the gene cluster encoding rhizoxin biosynthesis has been identified.[102] Rhizoxin has been reported to exhibit potent anti-tumor activity, but its further development as an anti-cancer drug has been precluded by toxicity problems.[103] Isolation and large-scale fermentation of *Burkholderia rhizoxina* in pure culture have resulted in a significantly elevated (10× higher) production of rhizoxin as well as rhizoxin analogues in considerably improved yields,[101] and this may have significant implications in development of agents with improved pharmacological properties.

Scheme 9. Natural Products from Endophytes



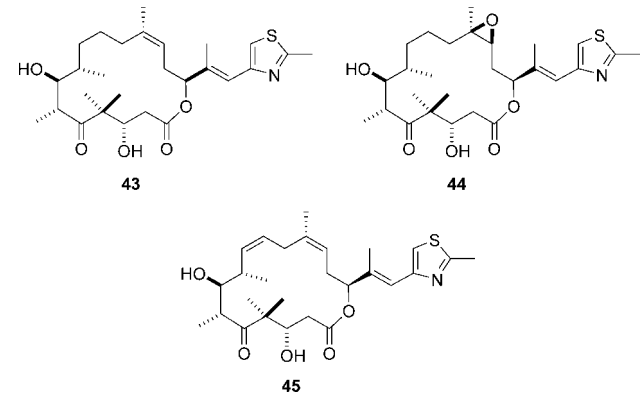
3.3.7. Plant Endophytes

As mentioned above (sections 1 and 3.2), 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 receiving attention. The relationships between endophytes and their host plants may vary from symbiotic to pathogenic, and studies are revealing an interesting realm of novel chemistry.[104−106] Among the wide range of new bioactive molecules reported are peptide antibiotics, the coronamycins (structure not determined), isolated from a *Streptomyces* species associated with an epiphytic vine (*Monastera* species) found in the Peruvian Amazon,[107] and the cytotoxic aspochalasins I, J, and K (**38**, **39**, and **40**; Scheme 9), isolated from endophytes of plants from the southwestern desert regions of the United States.[108] The discovery that various important anti-cancer agents are produced in small quantities by endophytic fungi isolated from plants is of particular significance. Examples include Taxol (**41**; Scheme 9) from *Taxomyces*[109] and many *Pestalotiopsis* species,[110] as well as camptothecin (**42**; Scheme 9),[111,112] podophyllotoxin (**3**; Scheme 1), an epimer of the precursor to the anticancer drug etoposide (**4**; Scheme 1),[113,114] vinblastine (**1**; Scheme 1),[115] and vincristine (**2**; Scheme 1),[116,117] all produced by endophytic fungi isolated from the original source 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.

3.3.8. Combinatorial Biosynthesis

The substantial advances made in the understanding of the role of multifunctional polyketide synthase enzymes (PKSs) in bacterial aromatic polyketide biosynthesis have led to the identification of many such enzymes, together with their encoding genes.[118−121] The same applies to nonribosomal peptide synthases (NRPS) responsible for the biosynthesis of nonribosomal peptides (NRPs).[120] Through the analysis of microbial genomes, a multitude of gene clusters encoding for polyketides, NRPs, and hybrid polyketide−NRP metabolites have been identified, and the tools have been

Scheme 10. Natural Product Analogues Produced by Combinatorial Biosynthesis



provided for engineering the biosynthesis of novel "non-natural" natural products through gene shuffling, domain deletions, and mutations.[120,122] The application of these combinatorial biosynthetic techniques to the production of novel analogues of anti-cancer agents, such as the anthracyclines, ansamitocins, epothilones, enediynes, and aminocoumarins, has recently been reviewed by Shen et al.[123]

The power of this technique is exemplified by the efficient scale-up production of epothilone D (**43**; Scheme 10), the des-epoxy precursor of epothilone B (**44**; Scheme 10). It was the most active of the epothilone series against some selected cell lines in vivo when first synthesized by Danishefy's group in 1998 under the name desoxyepothilone B,[124] and entered clinical trials as a potential anti-cancer agent under the code name of KOS-862. It has now been discontinued in favor of a congener, 9,10-didehydroepothilone D[125] (**45**; Scheme 10) 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.[126,127] 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 P_{450}, and heterologous expression of the gene cluster minus the *epo*K in *Myxococcus xanthus* has resulted in large-scale production of crystalline epothilone D.[128]

4. Development of Drugs from Natural Products

Historically, the major impediments to the development of a natural product lead have been limited availability and structural complexity. Natural products are often produced in trace quantities, and biomass is limited or, in the case of microbial sources, unculturable. As discussed in section 3, the discovery of novel natural products has been revolutionized by advances in genomic mining and the engineering of biosynthetic pathways. These methods can also be utilized to enable large-scale production of natural products in the native or engineered organisms.

While the probability of a directly isolated natural product (e.g., adriamycin or taxanes in the anti-tumor area) being the actual drug used for the treatment of a given disease in the future is relatively low, these natural molecules can serve as lead compounds for the development of analogues, generated by combinatorial biosynthesis and/or combinatorial chemistry, with optimized pharmacological properties. Recent advances in synthetic methodology and strategy are surmounting the barriers presented by the structural complexity of most natural products. In addition, natural products

have been evolutionarily selected to bind to biological macromolecules and, thus, represent "privileged structures",[129] which are excellent templates for the synthesis of novel, biologically active, natural product-like molecules. Of course, suitable biological assays for evaluation of the structure−activity relationships (SARs) of the optimization products are required for all these approaches, and thus, a truly multidisciplinary, collaborative approach is required for effective natural product-based drug discovery and development.

4.1. Synthesis Based on Natural Products

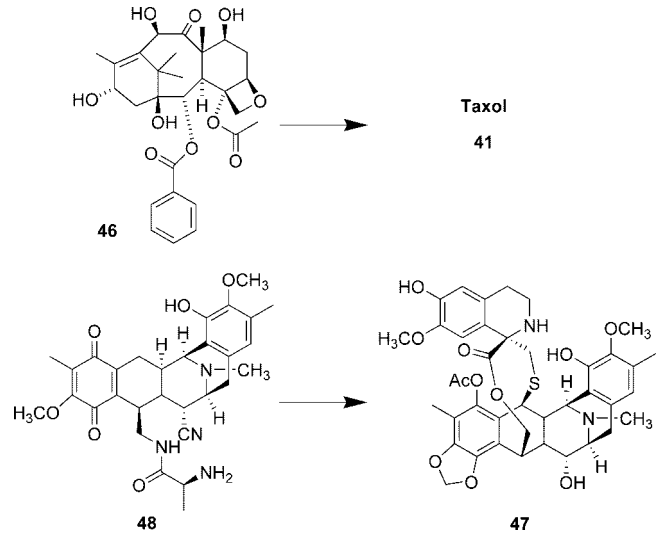
While natural products often exhibit highly potent and selective bioactivity, they did not undergo evolutionary selection to serve as human therapeutics and, thus, have not been fine-tuned to possess the potency, selectivity, and pharmacokinetic properties desired in a clinically useful drug. Optimization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

4.1.1. Derivatization and Semisynthesis

Possibly the simplest approach to optimizing such a lead is to modify the natural product by simple functional group transformations. This can be achieved by chemical and/or enzymatic methods. Large numbers of analogues can be rapidly generated by such semisynthetic approaches; however, many desired transformations cannot be accomplished due to incompatibilities with pre-existing functional groups or the lack of a feasible reaction. Thus, the structural diversity of the analogues accessible by derivatization is limited. There are numerous examples of this approach including taxanes,[130,131] camptothecins,[132] and combretastatins.[133]

Although the natural product of interest may not be readily available from biomass, sometimes another natural product that can serve as a starting material for the semisynthesis of the target is readily available. The development of paclitaxel (Taxol, **41**, Scheme 9) was severely hampered by the scarcity of its original source, the bark of *Taxus brevifolia*. The compound supply issue and original commercial production were solved by semisynthesis from 10-deacetylbaccatin III (**46**; Scheme 11), which is readily available from the needles of various *Taxus* species, a renewable resource. Details of this and the development of other taxane derivatives have been comprehensively reviewed.[130,131] Another prominent example is provided by the complex alkaloid ecteinascidin 743 (Et-743, Yondelis) (**47**; Scheme 11), discovered from the colonial tunicate *Ecteinascidia turbinata*,[134,135] The issue of compound supply for advanced studies was solved by the development of a semisynthetic route from the microbial product cyanosafracin B (**48**; Scheme 11).[136] This and other aspects of the discovery and development have been comprehensively reviewed.[137,138]

Scheme 11. Natural Products Produced by Semisynthesis



4.1.2. Total Synthesis

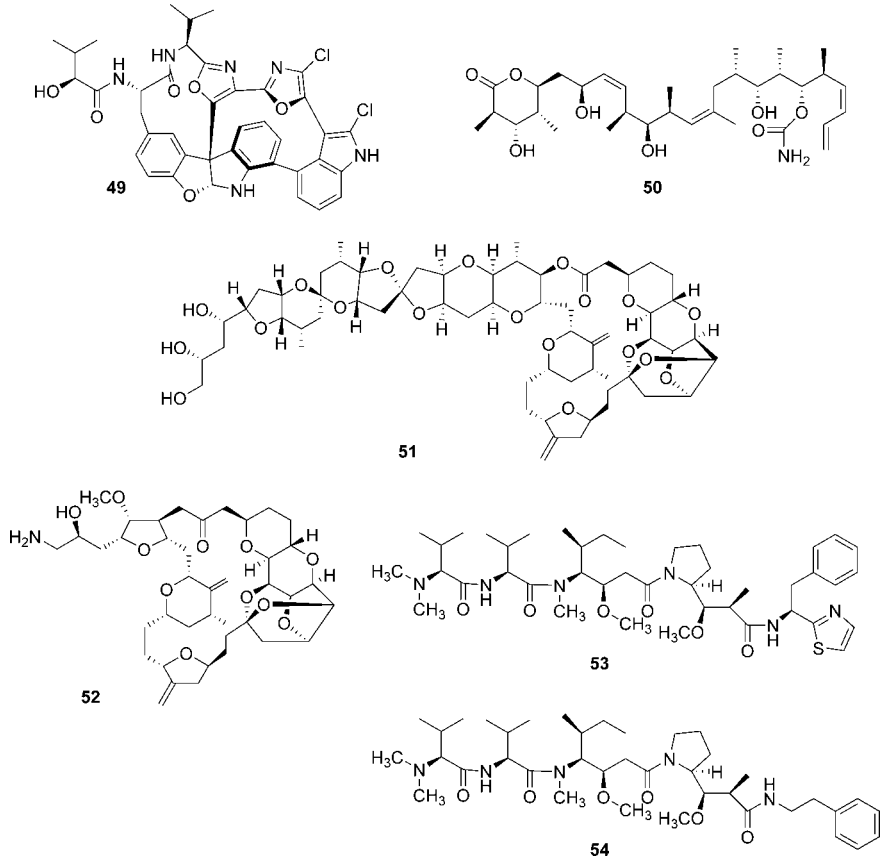
The total synthesis of complex natural products has long posed challenges to the top synthetic chemistry groups worldwide and has led to dramatic advances in the field of organic chemistry.[139] In some instances, as noted in section 3.2 regarding the cytotoxic macrolide palmerolide A (**13**; Scheme 3), total synthesis has led to a revision of the original published structure;[32] another notable example is that of the marine-derived anti-tumor compound, diazonamide A (**49**; Scheme 12).[140]

Significant strides have been made in the synthesis and structural modification of drugs that are difficult to isolate in sufficient quantities for development. 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 (**50**; Scheme 12), where total synthesis provided sufficient quantities for thorough clinical trials. Unfortunately, these have now been terminated due to lack of objective responses and toxicity.[141,142]

4.1.3. Diverted Total Synthesis

The process of total synthesis can often lead to the identification of the pharmacophore, the substructural portion of the molecule bearing the essential features necessary for activity. This knowledge, combined with a synthetic strategy that allows for the introduction of deep-seated structural variations, allows for the "molecular editing" of unnecessary structural complexity. In some instances, this has resulted in the synthesis of simpler analogues having similar or better activity than the natural product itself. Although the term "Diverted Total Synthesis" (DTS) was recently coined by Danishefsky and co-workers to describe this approach,[143,144] the basic strategy had been previously practiced by many academic and industrial groups. 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 analogues of varying complexity containing the common pharmacophore. Synthesis can be accomplished by both conventional medicinal chemistry or combinatorial chemistry approaches.

Scheme 12. Products of Total Synthesis and Diverted Total Synthesis



One of the most notable examples is that of the marine-derived anti-tumor agent, halichondrin B (**51**; Scheme 12), where total synthetic studies revealed that the right-hand half of the molecule retained all or most of the potency of the parent compound, and the analogue, E7389 (Eribulin) (**52**; Scheme 12), is currently in phase III clinical trials.[145] Eribulin 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.

In some instances, clinical trials of the original natural product may fail, but totally synthetic analogues continue to be developed. Thus, while clinical trials of the marine-derived anti-cancer agents, dolastatin 10 (**53**; Scheme 12) and dolastatin 15, have been terminated, synthetic analogues are in various phases of clinical trials.[146] Only auristatin PE (TZT-1027 or soblidotin) (**54**; Scheme 12) is still in clinical trials as the base molecule, and there are some very interesting modifications that have been made by medicinal chemists in order to deliver this close relative to dolastatin10 by use of monoclonal antibodies targeted at specific epitopes.[147,148] Currently, a significant number of combinations of this base molecule with varying monoclonal antibodies are in preclinical to phase II clinical trials predominately against hematologic cancers.

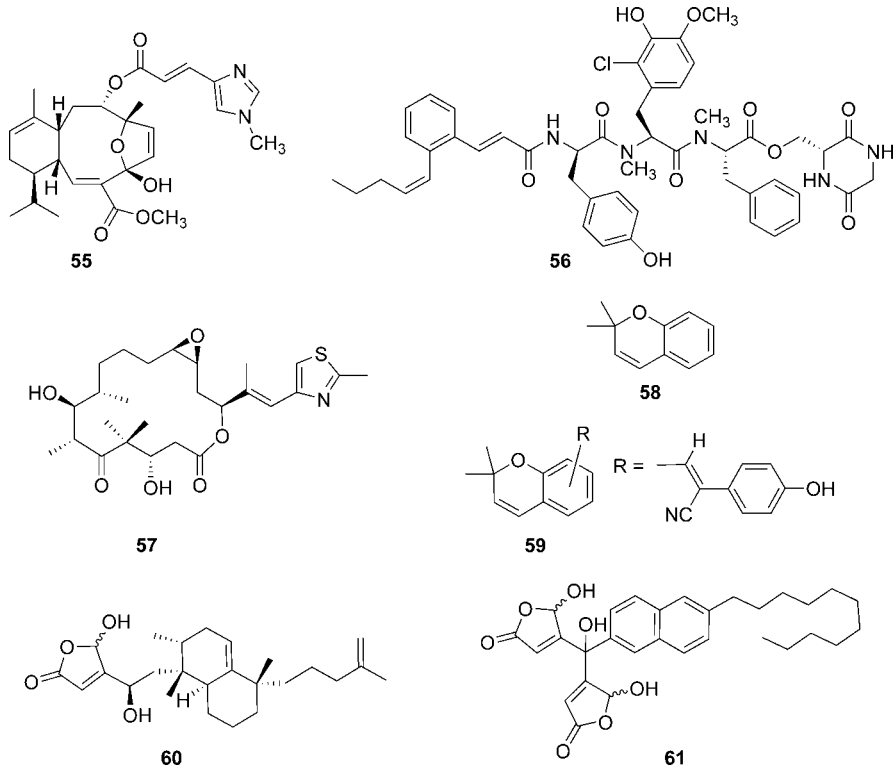
Thus, Seattle Genetics has SGN-35 where the antibody is an anti-CD30 linked to auristatin E in phase I heading for phase II,[149] CuraGen has the antibody CR011 linked to auristatin E in phase II trials for metastatic breast cancer and melanoma,[150] and Progenics has PSMA-ADC, a dimeric-specific PSMA antibody also conjugated to auristatin E in phase I against prostate cancer.[151] In all of these examples, although the exact linkages between the auristatin molecule and the antibody are subtly different, all are licensed from Seattle Genetics.

4.2. Natural Product-Inspired Combinatorial Synthesis

Combinatorial chemistry is a set of techniques developed for the simultaneous or parallel 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. Expectations that HTS screening of vast numbers of compounds would prove to be more efficient and cost-effective than traditional approaches to drug discovery 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,[152] the declining numbers of new New Chemical Entities (NCEs)[153] indicate that the use of de novo combinatorial chemistry approaches to drug discovery over the past decade has been disappointing, with some of the earlier libraries being described as "poorly designed, impractically large, and structurally simplistic".[152] 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".

Combinatorial synthesis of natural product-inspired libraries covers a spectrum of approaches. These can be grouped into three basic categories, although the distinctions among these are often indistinct. Synthesis based on bioactive natural product scaffolds leads to libraries of natural product derivatives displaying appendage and stereochemical diver-

Scheme 13. Natural Product-Inspired Combinatorial Synthesis



sity. Biology-oriented synthesis (BIOS)[154−156] 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),[157−160] aims to create highly diverse libraries of novel synthetic compounds that resemble natural products in that they incorporate complex three-dimensional architectures. 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 analogue development.

4.2.1. Combinatorial Synthesis of Natural Product-Derived Libraries

Since natural products bind both their biosynthetic enzymes and their target macromolecules, they necessarily populate biologically relevant regions of chemical space. 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;[129] 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, they are obvious starting points for the application of combinatorial chemistry to prepare focused libraries of analogues for SAR studies. Nicolaou[161] 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."

In recent years, many published reports of the use of natural product scaffolds in combinatorial libraries have appeared in the literature. Only a handful will be cited to exemplify this approach. One of the earliest examples was the synthesis of a library around the sarcodictyin (**55**; Scheme 13) scaffold by Nicolaou et al.[162] Waldmann et al. prepared a pepticinnamin E (**56**; Scheme 13) library by solid-phase synthesis.[163] Solid-phase synthesis of combinatorial libraries of epothilones (epothilone A, **57**; Figure 13) was used to probe regions of the molecule important to retention or improvement of activity,[164] and combinatorial synthesis of vancomycin dimers yielded compounds with improved activity against drug-resistant bacteria.[165] Wipf et al. prepared some highly modified analogues of the anti-mitotic natural product curacin A (**22**; Scheme 5), and found a simpler analogue that was more potent than curacin A in inhibiting the assembly of tubulin.[166] 2,2-Dimethyl-2H-benzopyran (**58**; Scheme 13) has proven to be a particularly versatile scaffold for library synthesis; a search of the natural product literature yielded nearly 4 000 analogues, with another 8 000 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 (**59**; Scheme 13) that are effective against vancomycin-resistant bacteria.[161,167,168]

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 analogues exhibiting biological activities beyond those previously associated with the parent natural product.

4.2.2. Biology-Oriented Synthesis of Natural Product-Inspired libraries

Waldmann has developed a new concept for the design of combinatorial libraries based on natural products that he calls BIOS.[154−156] This concept is based on the recognition of fundamental and complementary properties of natural

products and their protein targets. Nature, through evolution of natural products, has explored only a tiny fraction of the available small-molecule chemical space. The same holds true for the biological targets of natural products, which are mainly proteins. The number of three-dimensional protein folds have 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; the number of topologically different protein folds is actually much lower, with estimates of 600−8 000.[169] Since both the natural product space and the protein structure space explored by Nature are limited in size and highly conserved, these structure spaces have to be highly complementary. Thus, a natural product that is an inhibitor of a specific protein fold represents a biologically validated starting point for the development of closely related structures 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,[129] but BIOS has the added dimension of using protein folding patterns as the basis for subsequent screens.

BIOS is based upon two concepts previously developed by the Waldmann group. The scaffolds of natural products can be mapped in a hierarchical manner to create a scaffold tree, a "structural classification of natural products" (SCONP).[170,171] This 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.[172−174] Merging these two concepts led to the BIOS approach.[156] 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.

The success of the BIOS approach was demonstrated by a combinatorial library inspired by the marine natural product dysidiolide (**60**; Scheme 13). Postulating 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 (**61**; Scheme 13) 10-fold more potent (IC_{50} = 350 nM) than the parent compound against Cdc25A.[175] 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.[176] A second example of the success of BIOS, 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), is discussed in section 5.5.5.

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.

4.2.3. 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 libraries 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 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 that is biologically relevant to a defined target. By contrast, DOS aims to achieve a diverse and nonfocused coverage of chemical space by the efficient and divergent synthesis of large libraries of structurally complex and structurally diverse compounds. Thus, while the molecules 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. Readers are referred to the excellent reviews on the subject, its relationship to natural products, and its applications to chemical genetics and drug discovery.[158,160,177−183]

The most dramatic example of the power of DOS to generate novel chemical diversity is the recent report by Morton et al. of the synthesis of a 96-membered library based on 84 distinct molecular scaffolds.[184] Astonishingly, 65% of the scaffolds in this library are novel. When the skeletal diversity of the library was assessed by Waldmann's hierarchical scheme,[170,171] the resulting scaffold tree was very similar to Waldmann's analysis of natural products. Morton et al. report no biological data for the library, but the "natural product-likeness" should allow access to large regions of biologically relevant chemical space.

The authors can think of no better words to describe the current state produced by the synergy of combinatorial chemistry and natural products chemistry than those given in a recent review by Cordier et al.[182] *"The success of using natural products to inspire diversity-oriented synthesis can ultimately only be gauged by the discovery of new biologically active molecules: not close derivatives of natural products with related functions, but molecules, which through populating productive regions of biologically relevant chemical space, have novel biological functions. In this regard, the strategy of appending natural product scaffolds with diverse substituents has performed remarkably well, yielding chemical probes such as secramine,[185] uretupamine,[186,187] and haptamide B.[188] It is not yet possible to assess the success of the more recent innovations in diversity-oriented synthesis: in a few years' time, with the benefit of hindsight, it will be possible to assess critically the ability of these new approaches to deliver new small molecule tools for use in chemical genetic studies. It is not the structural similarity of small molecules to natural products that is ultimately important: it is discovery of valuable small molecule tools with biological functions which known natural products do not possess."*

5. Nature: A Major Source of Molecular and Mechanistic Diversity in Cancer Chemotherapy

A list of all anti-cancer drugs currently in clinical use and classified according to their source is given in Table 1.

Table 1. All Anti-Cancer Drugs (1940s−12/2007) (Organized Alphabetically by Generic Name within Source)

generic name	year introduced	reference[a]	page	Source[b]
131I-chTNT	2007	I 393351		B
H-101	2005	DNP 19	46	B
aldesleukin	1992	ARMC 25	314	B
alemtuzumab	2001	DNP 15	38	B
bevacizumab	2004	ARMC 40	450	B
celmoleukin	1992	DNP 06	102	B
cetuximab	2003	ARMC 39	346	B
denileukin diftitox	1999	ARMC 35	338	B
interferon alfa2a	1986	I 204503		B
interferon alfa2b	1986	I 165805		B
interferon, gamma-1a	1992	ARMC 28	332	B
interleukin-2	1989	ARMC 25	314	B
mobenakin	1999	ARMC 35	345	B
nimotuzumab	2006	DNP 20	29	B
panitumumab	2006	DNP 20	28	B
pegaspargase	1994	ARMC 30	306	B
Rexin-G[c]	2007	I 34634		B
rituximab	1997	DNP 11	25	B
tasonermin	1999	ARMC 35	349	B
teceleukin	1992	DNP 06	102	B
tositumomab	2003	ARMC 39	364	B
trastuzumab	1998	DNP 12	35	B
aclarubicin	1981	I 090013		N
actinomycin D	1964	FDA		N
angiotensin II	1994	ARMC 30	296	N
arglabin	1999	ARMC 35	335	N
asparaginase	1969	FDA		N
bleomycin	1966	FDA		N
carzinophilin	1954	Japan Antibiotics		N
chromomycin A3	1961	Japan Antibiotics		N
daunomycin	1967	FDA		N
doxorubicin	1966	FDA		N
leucovorin	1950	FDA		N
masoprocol	1992	ARMC 28	333	N
mithramycin	1961	FDA		N
mitomycin C	1956	FDA		N
neocarzinostatin	1976	Japan Antibiotics		N
paclitaxel	1993	ARMC 29	342	N
palictaxel nanoparticles[d]	2005	DNP 19	45	N
paclitaxel nanoparticles[e]	2007	I 422122		N
pentostatin	1992	ARMC 28	334	N
peplomycin	1981	I 090889		N
sarkomycin	1954	FDA		N
solamargine (aka BEC)	1987	DNP 03	25	N
trabectedin	2007	I 139221		N
streptozocin	Pre-1977	Carter		N
testosterone	Pre-1970	Cole		N
vinblastine	1965	FDA		N
vincristine	1963	FDA		N
Kunecatechins[f]	2006	DNP 20	24	NB
sinecatechins[f]	2007	I 283701		NB
alitretinoin	1999	ARMC 35	333	ND
amrubicin HCl	2002	ARMC 38	349	ND
belotecan hydrocholoride	2004	ARMC 40	449	ND
calusterone	1973	FDA		ND
cladribine	1993	ARMC 29	335	ND
cytarabine ocfosfate	1993	ARMC 29	335	ND
dexamethasone	1958	FDA		ND
docetaxel	1995	ARMC 31	341	ND
dromostanolone	1961	FDA		ND
elliptinium acetate	1983	I 091123		ND
epirubicin HCI	1984	ARMC 20	318	ND
estramustine	1980	FDA		ND
ethinyl estradiol	pre-1970	Cole		ND
etoposide	1980	FDA		ND
exemestane	1999	DNP 13	46	ND
fluoxymesterone	pre-1970	Cole		ND
formestane	1993	ARMC 29	337	ND
fosfestrol	pre-1977	Carter		ND
fulvestrant	2002	ARMC 38	357	ND
gemtuzumab ozogamicin	2000	DNP 14	23	ND
goserelin acetate	1987	ARMC 23	336	ND
hexyl aminolevulinate	2004	I 300211		ND
histrelin	2004	I 109865		ND
hydroxyprogesterone	pre-1970	Cole		ND
idarubicin hydrochloride	1990	ARMC 26	303	ND
irinotecan hydrochloride	1994	ARMC 30	301	ND
ixabepilone	2007	I 293356		ND
leuprolide	1984	ARMC 20	319	ND
medroxyprogesterone acetate	1958	FDA		ND
megesterol acetate	1971	FDA		ND
methylprednisolone	1955	FDA		ND
methyltestosterone	1974	FDA		ND
miltefosine	1993	ARMC 29	340	ND
mitobronitol	1979	FDA		ND
nadrolone phenylpropionate	1959	FDA		ND
norethindrone acetate	pre-1977	Carter		ND

Table 1. Continued

generic name	year introduced	reference[a]	page	Source[b]
pirarubicin	1988	ARMC 24	309	ND
prednisolone	pre-1977	Carter		ND
prednisone	pre-1970	Cole		ND
temsirolimus	2007	I 218793		ND
teniposide	1967	FDA		ND
testolactone	1969	FDA		ND
topotecan HCl	1996	ARMC 32	320	ND
triamcinolone	1958	FDA		ND
triptorelin	1986	I 090485		ND
valrubicin	1999	ARMC 35	350	ND
vapreotide acetate	2003	I 135014		ND
vindesine	1979	FDA		ND
vinorelbine	1989	ARMC 25	320	ND
zinostatin stimalamer	1994	ARMC 30	313	ND
amsacrine	1987	ARMC 23	327	S
arsenic trioxide	2000	DNP 14	23	S
bisantrene hydrochloride	1990	ARMC 26	300	S
busulfan	1954	FDA		S
carboplatin	1986	ARMC 22	318	S
carmustine (BCNU)	1977	FDA		S
chlorambucil	1956	FDA		S
chlortrianisene	pre-1981	Boyd		S
cis-diamminedichloroplatinum	1979	FDA		S
cyclophosphamide	1957	FDA		S
dacarbazine	1975	FDA		S
diethylstilbestrol	pre-1970	Cole		S
flutamide	1983	ARMC 19	318	S
fotemustine	1989	ARMC 25	313	S
heptaplatin /SK-2053R	1999	ARMC 35	348	S
hexamethylmelamine	1979	FDA		S
hydroxyurea	1968	FDA		S
ifosfamide	1976	FDA		S
lenalidomide	2005	DNP 19	45	S
levamisole	pre-1981	Boyd		S
lobaplatin	1998	DNP 12	35	S
lomustine (CCNU)	1976	FDA		S
lonidamine	1987	ARMC 23	337	S
mechlorethanamine	1958	FDA		S
melphalan	1961	FDA		S
mitotane	1970	FDA		S
nedaplatin	1995	ARMC 31	347	S
nilutamide	1987	ARMC 23	338	S
nimustine hydrochloride	pre-1981	Boyd		S
oxaliplatin	1996	ARMC 32	313	S
pamidronate	1987	ARMC 23	326	S
pipobroman	1966	FDA		S
porfimer sodium	1993	ARMC 29	343	S
procarbazine	1969	FDA		S
ranimustine	1987	ARMC 23	341	S
razoxane	pre-1977	Carter		S
semustine (MCCNU)	pre-1977	Carter		S
sobuzoxane	1994	ARMC 30	310	S
sorafenib mesylate	2005	DNP 19	45	S
thiotepa	1959	FDA		S
triethylenemelamine	pre-1981	Boyd		S
zoledronic acid	2000	DNP 14	24	S
anastrozole	1995	ARMC 31	338	S/NM
bicalutamide	1995	ARMC 31	338	S/NM
bortezomib	2003	ARMC 39	345	S/NM
camostat mesylate	1985	ARMC 21	325	S/NM
dasatinib	2006	DNP 20	27	S/NM
erlotinib hydrochloride	2004	ARMC 40	454	S/NM
fadrozole HCl	1995	ARMC 31	342	S/NM
gefitinib	2002	ARMC 38	358	S/NM
imatinib mesilate	2001	DNP 15	38	S/NM
lapatinib ditosylate	2007	I 301036		S/NM
letrozole	1996	ARMC 32	311	S/NM
nafoxidine	pre-1977	Carter		S/NM
nilotinib hydrochloride	2007	I 386178		S/NM
sunitinib maleate	2006	DNP 20	27	S/NM
tamoxifen	1973	FDA		S/NM
toremifene	1989	ARMC 25 319		S/NM
aminoglutethimide	1981	FDA		S*
azacytidine	pre-1977	Carter		S*
capecitabine	1998	ARMC 34	319	S*
carmofur	1981	FDA		S*
clofarabine	2005	DNP 19	44	S*
cytosine arabinoside	1969	FDA		S*
decitabine	2006	DNP 20	27	S*
doxifluridine	1987	ARMC 23	332	S*
enocitabine	1983	ARMC 19	318	S*
floxuridine	1971	FDA		S*
fludarabine phosphate	1991	ARMC 27	327	S*
fluorouracil	1962	FDA		S*
ftorafur	1972	FDA		S*
gemcitabine HCl	1995	ARMC 31	344	S*

Table 1. Continued

generic name	year introduced	reference[a]	page	Source[b]
mercaptopurine	1953	FDA		S*
methotrexate	1954	FDA		S*
mitoxantrone HCI	1984	ARMC 20	321	S*
nelarabine	2005	DNP 19	45	S*
thioguanine	1966	FDA		S*
uracil mustard	1966	FDA		S*
abarelix	2004	ARMC 40	446	S*/NM
bexarotene	2000	DNP 14	23	S*/NM
pemetrexed	2004	ARMC 40	463	S*/NM
raltiterxed	1996	ARMC 32	315	S*/NM
tamibarotene	2005	DNP 19	45	S*/NM
Temozolomide	1999	ARMC 35	350	S*/NM
vorinostat	2006	DNP 20	27	S*/NM
bcg live	1990	DNP 04	104	V
hpv vaccine (Merck)	2006	DNP 20	26	V
hpv vaccine (GSK)	2007	I 309201		V
melanoma theraccine	2001	DNP 15	38	V

[a] The reference codes are as follows: ARMC, Annual Reviews of Medicinal Chemistry, Academic Press, San Diego, Volume, Page; DNP, Drug News and Perspectives, Prous Science, Barcelona, Volume, Page; I ######, Identification number in the Prous Integrity Database; FDA, FDA listing of approved drugs; Japan Antibiotics, personal communication from Prof. Morimasa Yagisawa, Keio University; Boyd, Boyd, M. R. In *Current Therapy in Oncology*, Niederhuber, J., Ed., Decker: Philadelphia, PA, 1993, p 11; Carter, Carter, S. K., Bakowski, M. T., Hellman, K. *Chemotherapy of Cancer*, Wiley: New York, 1977, p 350; Cole, Cole, W. H. *Chemotherapy of Cancer*, Lea and Febiger: Philadelphia, PA, 1970, p 349. [b] Source classifications for small molecules are given in section 2. Table 1 includes the following codes for other drugs: B (biologicals), NB (Natural Product/Botanical), and V (vaccines). [c] No generic name; this is the trade name. [d] Abraxane (entirely different from below in particle source and approved in U.S.A.). [e] Nanoxel (entirely different from above in particle source and approved in India). [f] These botanical agents are approved for sale by FDA or equivalent with a disease indication.

Readers are referred to Newman and Cragg's 2007 review[16] for detailed references. In the sections below, after briefly reviewing the various methodologies used in anti-tumor screening, we have provided an overview of the chemotherapeutic agents currently in clinical use or development for the treatment of cancer. Our discussion of these agents is divided into subsections based on their mechanisms of action. Information on ongoing clinical trials may be found at http://www.cancer.gov/Search/SearchClinicalTrialsAdvanced.aspx, and readers are referred to this site for details.

5.1. Anti-tumor Screening

A given organism provides the investigator with a complex library of unique bioactive constituents, analogous to the library of synthetic compounds produced by combinatorial chemistry techniques. The two approaches can be seen as complementary to each other, with each providing access to different lead structures. The task of the natural products researcher is to select those compounds of pharmacological interest through bioassay-guided fractionation of the "natural combinatorial libraries" produced by extraction of organisms, and then to collaborate in the optimization and development of the lead natural product structure. As mentioned in section 4, the successful development of effective new drugs requires suitable assays to guide not only the discovery of a bioactive lead but also the evaluation of analogues developed through optimization of the lead.

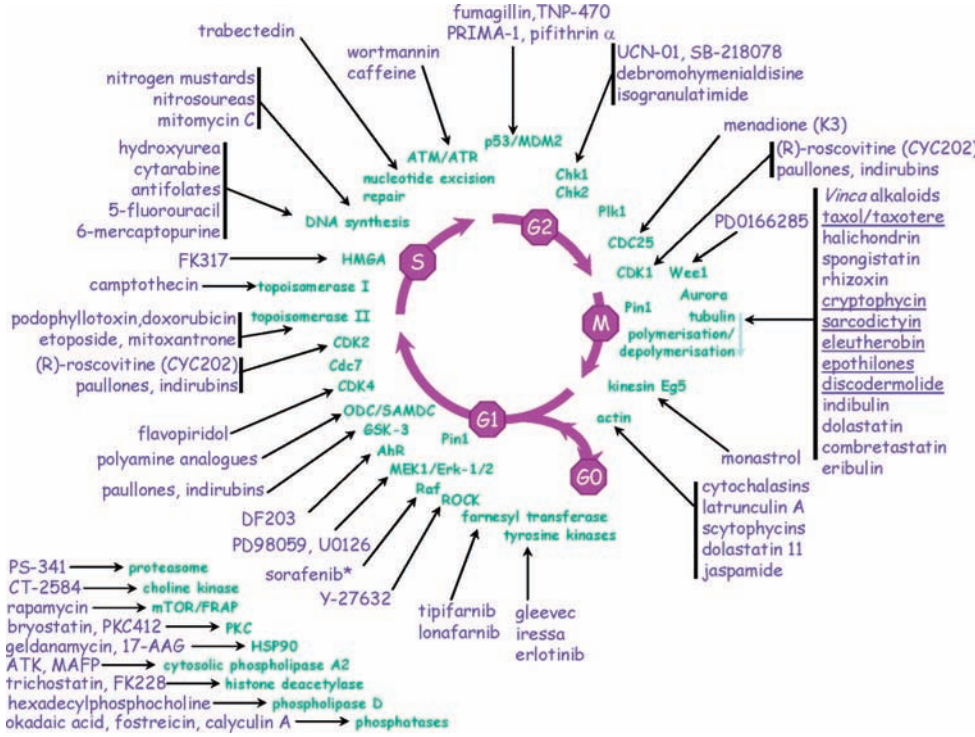
5.1.1. Molecular Target Assays

Natural products frequently possess highly selective and specific biological activities. A striking illustration is the influence of natural products on many of the molecular processes operative in cell cycle progression. Details may be found at the Web site of the Roscoff Biological Station (http://www.sb-roscoff.fr/CyCell/Frames80.htm), which covers diagrams originally published by Meijer[189] on natural products and the cell cycle, with a modified version shown below in Chart 2.

In the early days of natural products research, new compounds were simply isolated at random, or at best, by the use of simple broad-based bioactivity screens based on antimicrobial or cytotoxic activities. Although these screens did result in the isolation of many bioactive compounds,[190] they are considered to be too nonspecific for discovery of the next generation of drugs. Fortunately, a large number of robust and specific biochemical and genetics-based screens using transformed cells, a key regulatory intermediate in a biochemical or genetic pathway, or a receptor−ligand interaction (often derived from the explosion in genomic information since the middle 1990s) are now in routine use. These screens will permit the more precise detection of bioactive compounds in the complex matrices that are natural product extracts. Importantly, these assays provide preliminary data about the mechanism of action (MOA) early in the discovery process. Knowledge of the putative MOA at this stage can be a valuable discriminator in the prioritizing process.

This new generation of biochemical and genetic screens are highly automated, high-throughput assays (upward of 50 000 assay points per day in a number of cases). The resultant screening capacity at many companies is significantly larger than the potential input from in-house chemical libraries. Since screening capacity is no longer the rate-limiting step, many major pharmaceutical companies became very interested in screening natural products (either as crude extracts or as prefractionated "peak libraries") as a low-cost means of discovering novel lead compounds. A good illustration, though not an anti-cancer compound, was the discovery at Merck Research Laboratories of a new antibiotic, platensimycin, through the testing of a library of 250 000 natural product extracts in a custom-designed assay involving an engineered strain of *Staphylococcus aureus* incorporating the fatty acid synthase pathway enzyme, FabF.[191] Platensimycin is a selective FabF inhibitor and exhibits in vitro activity against several drug-resistant bacteria. High-throughput assays are becoming less expensive, and such assays are moving from the industrial or industrial−academic consortium-based groups to academia in general, with specific expression systems being employed as targets for natural product lead discovery.[177] The application of new techniques, including new fluorescent assays, NMR, affinity chroma-

Chart 2. Natural Products and the Cell Cycle[a]



[a] Reprinted with permission from Meijer, L. Le Cycle de division cellulaire et sa régulation. *Oncologie* **2003**, *5*, 311. Copyright 2003 Springer.

tography, and DNA microarrays, has led to significant advances in the effectiveness of high-throughput screening.[192,193]

5.1.2. Cell-Based Assays

While some of the molecular target screens alluded to in section 5.1.1 may involve use of transformed cells, the National Cancer Institute's (NCI) 60 cell-line cytotoxicity screen for anti-tumor agents represents a more traditional cell-based screen. It has been described in detail[194] and, although this is not a true receptor-based screen, it has now been developed into a system whereby a large number of molecular targets within the cell lines may be identified by informatics techniques, and refinements are continuing. Information as to the current status of the screens involved can be obtained from the following URL: http://dtp.nci.nih.gov/screening.html.

An assay based on differential susceptibility to genetically modified yeast strains has been described[195] and has led to many screens based upon genetically modified yeasts, but at times, the low permeability of the unmodified yeast cell wall to chemical compounds has been overlooked. Thus, data from such screens, particularly those designed with gene deletions, must be carefully scrutinized since a large number are based upon hosts without a modified cell wall. In addition, there are simple but robust assays that can be used by workers in academia, particularly in developing countries, who do not have access to, or may not need, high-throughput screens. Examples are the brine shrimp and potato disk assays.[196,197]

5.1.3. In vivo Assays

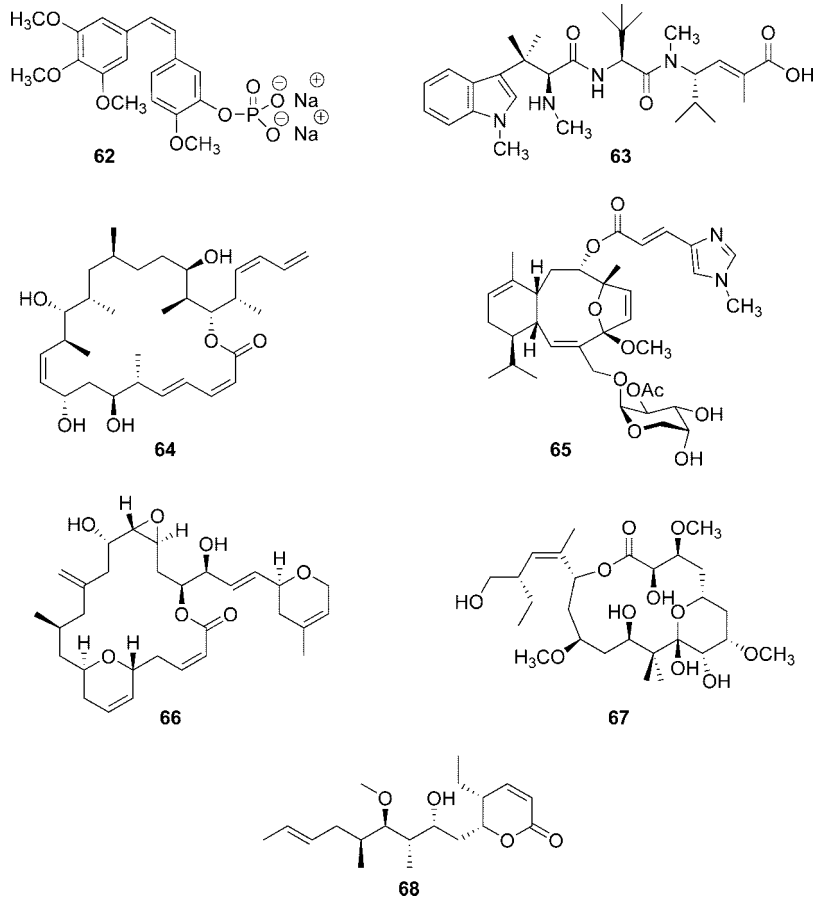
Further evaluation of the antitumor potential of natural products identified from molecular target or traditional cell-based assays requires the use of in vivo assays. The NCI uses the Hollow Fiber Assay[198] as a relatively inexpensive in vivo prescreen to prioritize compounds for testing in the more definitive human tumor xenograft models.[199] Traditionally, xenograft models were not employed until the bioactive components had been obtained in pure form. Currently, both the Hollow Fiber Assay and xenograft models are being utilized for the prioritization of natural product extracts for fractionation at NCI. Information on the regular in vivo assays currently used by the NCI, including a detailed description of the protocol used in the Hollow Fiber Assay, can be obtained from the following URL: http://dtp.nci.nih.gov/screening.html.

Once the bioactive component has been obtained in pure form, either as a novel structure or as a known compound exhibiting previously unreported activity, it must then be tested in a series of biological assays to determine its efficacy, potency, toxicity, and pharmacokinetics. These assays will help to determine the priority of the compound's spectrum of activity within the portfolio of compounds that a group may be assessing for advanced development as either drug candidates or leads thereto.

5.2. Tubulin Interactive Agents (TIAs)

The majority of the TIAs in development through to 2003, from preclinical studies up to clinical use, have been discussed in detail in a 2004 review by two of the authors,[200] and also more recently (2005) in the book *Anticancer Agents from Natural Products*.[201] The TIAs covered in that volume include taxanes (Taxol, **41**; Scheme 9),[130] epothilones (Epo A, **57**; Scheme 13),[13] and discodermolide (**50**; Scheme 12),[202] which act as promoters of polymerization of tubulin heterodimers to microtubules, leading to mitotic arrest through suppression of dynamic changes in microtubule functions. Other chapters are devoted to combretastatins (e.g., CA-4 phosphate, **62**; Scheme 14),[133] vinca alkaloids (**1**, **2**; Scheme 1),[11] maytansinoids (**33**; Scheme 8),[94] dolastatins (e.g.,

Scheme 14. Tubulin Interactive Agents



dolastatin 10, **53**; Scheme 12),[146] halichondrins (**51**; Scheme 12),[145] and hemiasterlins (e.g., hemiasterlin A, **63**; Scheme 14),[203] which act through inhibition of tubulin heterodimer polymerization. The coverage also includes agents derived from or synthetically modeled on those initial structures in order to develop drug candidates with improved solubilities, pharmacodynamics, or metabolic patterns, compared with the original natural products. Besides this review, the interested reader should consult the book chapters cited, together with the references given therein, for a discussion of the multiplicity of structures that have been developed from natural product lead compounds. Another detailed discussion of the marine-derived TIAs mentioned above (discodermolide, dolastatins, halichondrins, and hemiasterlins) is presented in a review of natural products from marine invertebrates and microbes as modulators of antitumor targets.[137] Other agents discussed in that review include dictyostatin (**64**; Scheme 14), diazonamide (**49**; Scheme 12), eleutherobin (**65**; Scheme 14), laulimalide (**66**; Scheme 14), and peloruside (**67**; Scheme 14), which all act in a similar manner to the taxanes, though not usually at the same binding site(s).

While most TIAs act either as reversible inhibitors or promoters of tubulin heterodimer polymerization as mentioned above, pironetin (**68**; Scheme 14), an α,β-unsaturated δ-lactone derived from a *Streptomyces* species, is the only TIA identified so far that acts through covalently binding to the α-tubulin chain.[204] The binding occurs at Lys,[352] an amino acid located at the entrance of a small pocket in α-tubulin that faces the β-tubulin of the next dimer.[205]

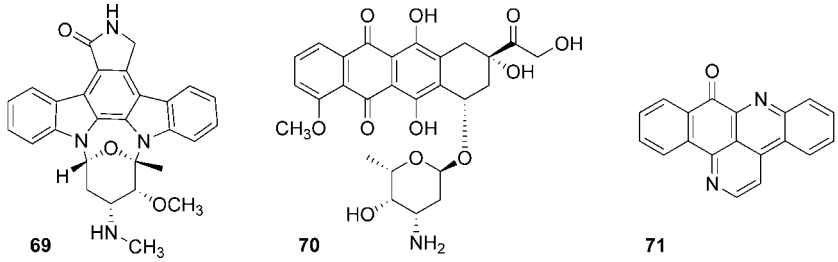
In addition to targeting a different site, pironetin has a unique, far less complicated structure than the other TIAs. This makes it an attractive starting point for the synthesis of analogues based upon its unique scaffold. An initial attempt was disappointing because all reported analogues exhibited decreased biological activities.[206] Waldmann's group used their BIOS methodology, described in section 4.2.2, to synthesize a focused library of 50 α,β-unsaturated δ-lactones that yielded several unique modulators of cell-cycle progression.[207] No additional derivatives of pironetin have yet been reported as candidate leads.

Perhaps the only surrogate for the "value" of the taxane skeleton as a drug or lead thereto would be the sales figures on a worldwide basis and an idea of the number of distinct compounds that are currently in clinical trials ranging from phase I through preregistration. Using the worldwide sales figures from the Prous Integrity database, the total sales in US $ for the three year period 2006−2008 are just under $10B, with the majority coming from sales of docetaxel. In addition, there are currently 14 taxane-based drug candidates in clinical trials worldwide, 4 in phase III or in preregistration, 8 in phase II, and 2 in phase I, with a number in the preclinical stages.

5.3. Inhibitors of Topoisomerases I and II

In early 2004, Cragg and Newman reviewed new developments in the field of topoisomerase inhibitors in a special issue of the *Journal of Natural Products* honoring Drs. Monroe Wall and Mansukh Wani, the codiscoverers of both Taxol and camptothecin.[200] The history of camptothecin (**42**; Scheme 9) is presented in that review. Although the majority of new topoisomerase I inhibitors are based on the camptothecin pharmacophore,[132] various derivatives of the protein

Scheme 15. Topoisomerase Inhibitors



kinase inhibitor staurosporine (**69**; Scheme 15) inhibit both topoisomerase I and II.[208] The anthracyclines are another class of important drugs that act via inhibition of topoisomerase II, with doxorubicin (**70**; Scheme 15) being a prime example of the many members of this class.[209] It should be pointed out, however, that almost all of the clinically useful compounds of this chemical class were developed as a result of their cytotoxic activities and without prior knowledge of this mechanism of action.[209] Likewise, the clinically active podophyllotoxin derivative, etoposide (**4**; Scheme 1), was developed by the then Sandoz company through modification of *epi*-podophyllotoxin without prior knowledge of the mechanism.[12] It is interesting to note that podophyllotoxin acts as an inhibitor of tubulin polymerization, whereas etoposide acts on topoisomerase II. Although etoposide is a commonly used anti-cancer drug, acquired drug-resistance and poor water solubility remain serious problems, and extensive research is being devoted to the production of a new generation of clinical trial candidates.[12]

A subsequent paper has reviewed the anticancer activity of some new topoisomerase inhibitors that include six topoisomerase I, twelve topoisomerase II, and six dual topoisomerase inhibitors, many of which are derivatives of natural products.[210] A second paper[211] has reported on an analogue, AK-37 (**71**; Scheme 15), of a marine-derived pyridoacridine that stabilizes the topoisomerase I cleavable complex in a manner comparable to that of 9-nitro-camptothecin. AK-37 was in phase III clinical trials for the treatment of pancreatic cancer in combination with gemcitabine, but the current status is unclear as the New Drug Application (NDA) for this indication was withdrawn in late 2007. For those interested in reading further, the wide variety of structures and activities of pyridoacridines has been reviewed.[212]

Using similar arguments to the "value" of the taxane skeleton, in the case of the topoisomerase I inhibitors exemplified by the camptothecins, data on the sales of the initial two approved for human use, irinotecan and topotecan over the period 2006−2008, show that almost $4B was spent on treatment with these agents in those three years and the figure would probably be above the $4B level if data on the sales of the third approved camptothecin-based drug, belotecan, were available. In addition to these sales figures, there are currently two compounds in phase III, nine in phase II, and ten in phase I trials all based on the camptothecin skeleton, plus there are a number of other, non-camptothecin molecules with the same nominal mechanism of action in early clinical trials as a result of the initial identification of this particular mechanism as the reason for the cytotoxicity of the base molecule.

When one tries to evaluate molecules with topoisomerase II activity using the criterion of sales, it is extremely difficult because of the fact that all of the anthracyclines were discovered to exhibit this particular mechanism of action well after the initial examples were approved for clinical use. However the influence of such a mechanism can be seen by inspection of the number of agents that are either totally synthetic (predominately quinolone derivatives) or based upon/are natural products that are in phase I to phase III trials with this mechanism. Currently over 50 topoisomerase II inhibitors are listed in Integrity. Of the 17 that have already launched, 6 are either natural products or modifications thereof. Of those in clinical trials, 16 are natural products or based upon an NP skeleton, including such well-known structures as tafluposide (podophyllotoxin; **3**; Scheme 1) in phase I and sabarubicin (doxorubicin; **70**; Scheme 15) and becatecarin (staurosporine; **69**; Scheme 15) in phase II.

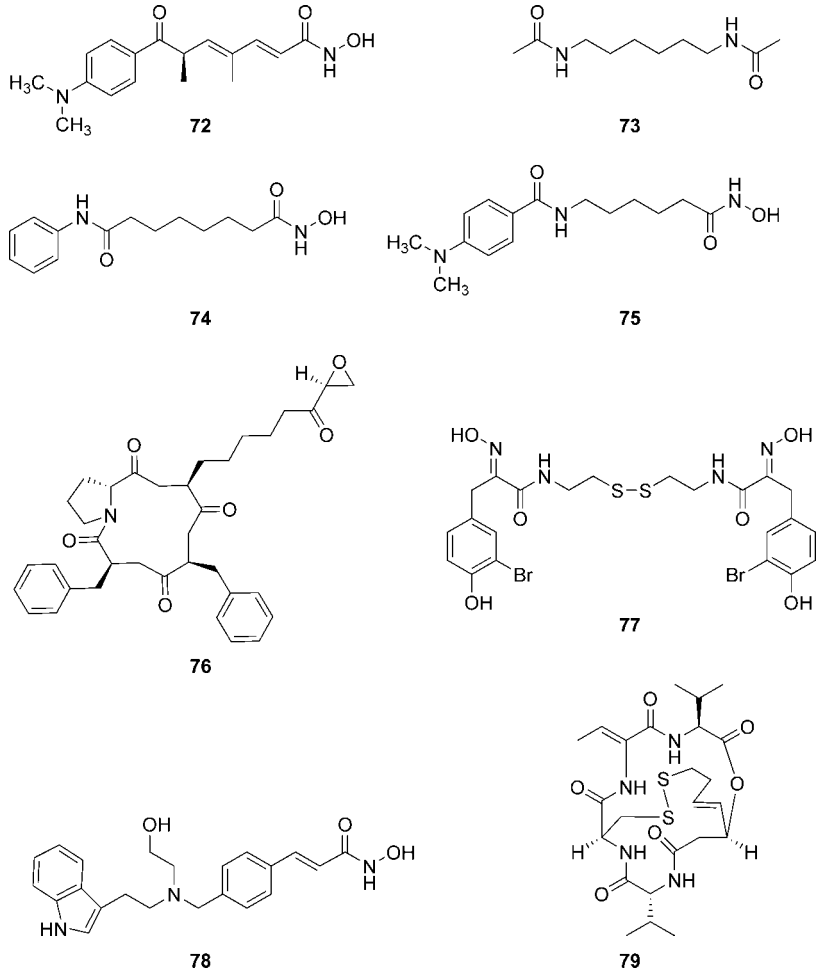
5.4. Inhibitors of Histone Deacetylases (HDACs)

The role of histone deacetylases (HDACs) in the regulation of gene expression, oncogenic transformation, and cellular differentiation and the promotion of angiogenesis is discussed by Kingston and Newman[213] and references cited therein. Suffice it to say, the inhibition of HDAC activity can exert a significant role in suppression of the neoplastic process.

HDAC inhibitors have been described as tripartite: an enzyme-binding group, frequently aromatic; a hydrophobic spacer group; and an inhibitor group.[214−216] Trichostatin A (TSA) (**72**; Scheme 16) clearly demonstrates such a system, with the structure mimicking the Lys side chain of the substrate (the "linker"), the inhibitory end being the zinc-chelating hydroxamic acid, and the aromatic enzyme binding group being the 4-dimethylamino-benzoyl group. This molecule, together with its congeners (trichostatins B, C and D), was first isolated as an antifungal agent,[217] and approximately a decade later they were found to have potent differentiation-inducing and antiproliferative activities in Friend erythroleukemia cells. Subsequently, TSA demonstrated potent in vitro and in vivo inhibition (nanomolar range) of class I and class II HDACs, with a slight selectivity for HDAC1 and HDAC6 compared to HDAC4. The *S* enantiomer of TSA was inactive, and neither enantiomer had any activity against the class III enzymes. The full mechanism of action has not yet been elucidated, but a large series of effects were observed in signal transduction systems, including induction of apoptosis when healthy and tumor cells from many different sources were treated with this agent.[218]

Identification of the basic structural features of TSA and its initial activities led to research on the synthesis of compounds that were more stable and had improved water solubility. Prior research with hexamethylene bisacetamide (HMBA) (**73**; Scheme 16), belonging to a family of molecules known as hybrid polar compounds (HPCs), demonstrated that it induced hyperacetylation of histone H4 in healthy keratinocytes, as well as in squamous cell

Scheme 16. HDAC Inhibitors



carcinoma derived from these cells, but did not inhibit their growth in vitro and induced a wide variety of other pathway modulations.[219] The high doses of HMBA required for in vivo activity resulted in toxicity and led to cessation of development, but these results, combined with knowledge of the basic structure of TSA, led to the development of a series of second-generation HPCs, which were tested as HDAC inhibitors. The lead compound from these studies, suberoylanilide hydroxamic acid (SAHA) (**74**; Scheme 16), was approved in 2006 as vorinostat (Zolinza) and still is currently in over 40 clinical trials (phases I, II, and III), either as a single agent or in combination with other agents, against a variety of refractory tumors, both solid and leukemic in Nature, including a phase II study of an oral formulation.[220] Efforts to resolve the problems of low yields of (*R*)-TSA from natural sources and difficulties in achieving its total synthesis have resulted in a simple four-step strategy being devised for the synthesis of achiral amide analogues of the natural product. The analogues consisted of a hydroxamate function, a benzamide, and an aliphatic spacer, with maximal inhibitory activity being observed with a five-carbon linker chain.[221] The resulting lead compound was 6-(4-dimethylaminobenzoyl)aminocaproic acid hydroxamide (**75**; Scheme 16), and though the anti-tumor and cell transduction activities of these compounds have been reported, no in vivo data has yet been published.[222]

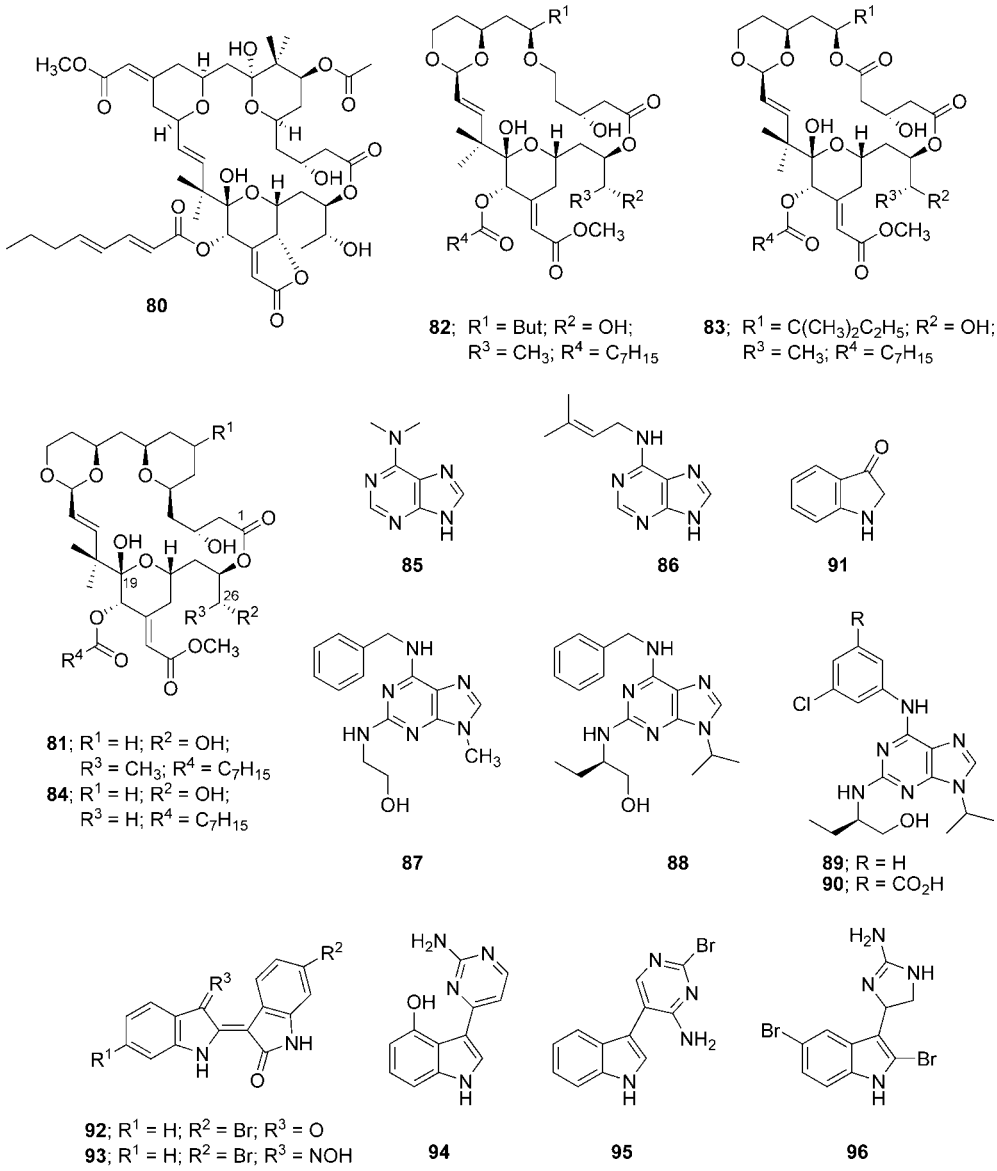
The natural product trapoxin (**76**; Scheme 16) was reported to be an irreversible inhibitor of HDACs in 1993, but in contrast to TSA, it was found to demonstrate some selectivity against class I and class II HDACs, inhibiting HDAC1 and HDAC4 but not HDAC6.[223] Combination of structural features of trapoxin, TSA, and another potent HDAC inhibitor, the marine natural product psammaplin A (**77**; Scheme 16), resulted in the de novo synthesis of NVP-LAQ-824 (dacinostat) (**78**; Scheme 16), which inhibits HDAC and the proliferation of cancer cell lines at low nanomolar concentrations; it showed efficacy in a number of solid tumor xenograft models, advancing to phase I clinical trials in 2002,[224,225] but was discontinued by Novartis in 2005. The full history of its evolution has been reviewed.[226]

The microbially-derived depsipeptide, FR-901228 (romidepsin) (**79**; Scheme 16), which was originally identified as a result of its potent antitumor activity, is now known to be active in signal transduction as a result of its HDAC activity[227] and is currently in phase III clinical trials, with recent publications reporting the solid-phase synthesis of analogues that have good in vitro activity.[228] A recent review by Paris et al.[229] should be consulted for further details of HDAC inhibitor evolution in addition to the articles referred to earlier in the section under specific agents.

5.5. Protein Kinase Inhibitors

Several agents that have advanced into clinical trials or commercial use in recent years have either been derived directly from Nature or incorporate key structural features from natural products. Thus the development of Gleevec can be traced back to ATP-mimicry, with its history briefly reviewed by Newman et al.,[230] and the history of Iressa is similar.

Scheme 17. Protein Kinase Inhibitors



5.5.1. Flavopiridol

The flavone, flavopiridol (Alvocidib) (**6**; Scheme 1), is totally synthetic, but, as discussed in section 1, its novel structure is based on the natural product rohitukine (**7**; Scheme 1) isolated from *Dysoxylum binectariferum*. Flavopiridol was originally considered to be an inhibitor of cyclin-dependent kinases (the regulators of the G_2 to M transition in the cell cycle) and was entered into phase I and then phase II clinical trials against a broad range of tumors.[15] It has now been reported to be a very potent inhibitor of CDK-7 and -9, the kinases primarily responsible for promoting RNAP II (RNA polymerase II) activity, thus involving these agents in the transcription process. The molecular targets/interactions involved in the transcription processes and flavopiridol interactions have been reviewed.[231,232] Currently (early 2009), the compound is reported to be in nine clinical trials ranging from phase I to phase II covering leukemias, lymphomas, and solid tumors either as single agent or in combination with other anticancer agents in the NCI's clinical trial Web site and is reported in the Prous Integrity database to be in phase III trials under the auspices of Sanofi−Aventis.

5.5.2. Bryostatins

The bryostatins are a class of highly oxygenated macrolides, and the multiyear program that culminated in the isolation and purification of (currently) 20 bryostatin structures has been well-documented by a variety of authors over the years.[233−240] These reviews may be consulted for the experimental details that indicated that the bryostatins have signal transduction activities. In particular, bryostatin 1 (**80**; Scheme 17) has been the focus of preclinical and clinical studies; details on the clinical trials of bryostatin 1 have been recently reviewed.[240]

While the total synthesis of bryostatin 1 is not a feasible process for the production of this agent, three of the naturally occurring bryostatins, bryostatins 7,[241] 2,[242] and 3,[243] have been synthesized, and their syntheses and the syntheses of other partial bryostatin structures, including bryostatin 1, have been reviewed;[237,239,244] these reviews should be consulted for specific details of reaction schemes and comparisons of routes. Very recently, Trost and Dong reported a much more concise synthetic method for the synthesis of bryostatin 16.[245] None of these methods, however, are viable for the large-scale production of any of the bryostatins for further

development. However, analytical studies by the Wender group of the potential binding site of the phorbol esters on PKC as a guide to the design of a simpler analogue of these agents[246] were expanded to bryostatin 1[247] and led to the production of simpler bryostatin analogues known colloquially as "bryologs" that maintained the putative binding sites at the oxygen atoms at C_1 (ketone), C_{19} (hydroxyl), and C_{26} (hydroxyl). These molecules (**81**, **82**; Scheme 17) demonstrated nanomolar binding constants when measured in displacement assays of tritiated phorbol esters, with the figures being in the same general range as those for bryostatin 1, and had activities in in vitro cell line assays close to those demonstrated by bryostatin 1 itself.[248−251] Introduction of a second lactone gave a compound (**83**; Scheme 17) with 8 nM binding affinity and an ED_{50} of 113 nM against the murine leukemia P388 cell line,[252] and use of different fatty acid esters gave compounds exhibiting binding affinities for PKC isozymes in the 7−232 nM range depending upon the fatty acid used.[253] A further simple modification involving removal of a methyl group in the C_{26} side chain gave a compound (**84**; Scheme 17) that had a binding affinity to PKC at the picomolar level[254] and demonstrated greater potency than bryostatin 1 in in vitro cell line assays. Improved syntheses of the bryologs might well permit further exploration of these analogues.[255,256]

5.5.3. Adenine Derivatives

The observation that substituted purines, particularly 6-dimethylaminopurine (6-DMAP) (**85**; Scheme 17) and isopentenyladenine (**86**; Scheme 17), from *Castanea* species, showed weak inhibition of cyclin-dependent kinase 1 (CDK1)/cyclin B, which led to the search for other purine-derived compounds.[257] Another plant secondary metabolite originally isolated from the cotyledons of the radish, and subsequently named olomucine (**87**; Scheme 17), demonstrated an improved efficacy ($IC_{50} = 7\ \mu M$) and selectivity for cyclin dependent kinases (CDKs) and, to some extent, MAP kinases, by direct competition with ATP. Olomucine, which earlier had been synthesized,[258] disproved the existing dogma that no specific kinase inhibitors could be found for ATP-binding sites since they would be swamped by the presence of excess of ATP. Further development of this series using combinatorial chemistry techniques led to roscovitine (Selicicib) (**88**; Scheme 17), and finally to purvalanol A (**89**; Scheme 17) and purvalanol B (**90**; Scheme 17). Like flavopiridol, olomucine and roscovitine are very potent inhibitors of CDK-7 and -9. The purvalanols demonstrated improved potency, with IC_{50} values in the 4−40 nM range, compared to 450 nM for roscovitine.[259] The *R*-isomer of roscovitine is currently in phase II under the auspices of Cyclacel with reports of clinical trials in Europe. Although some beneficial effects are observed with signal transduction inhibitors (STIs) alone, complete or partial responses tend only to be demonstrated when sequential treatments of STI/cytotoxin are used, so also with *R*-roscovitine, sequential treatment with cytotoxins is being used and/or considered.

5.5.4. Indigo and the Indirubins

Hydroxylation of indole in the 3-position, presumably by a suitable cytochrome P_{450}, gives a product that is tautomeric with the 3-keto analogue, indoxyl (**91**; Scheme 17), and various levels of oxidation then lead to a mixture of indigo, indirubin (**5**; Scheme 1), and their isomers, which is commonly used as the source of indigo dyestuffs, a mixture obtained from the plant *Isatis tinctoria* found to contain an indigo precursor.[260] Although usually considered to be plant products, indigo and the indirubins have been reported from four nominally independent sources: a variety of plants,[260] a number of marine mollusks, usually belonging to the *Muricidae* family of gastropods,[261] natural or recombinant bacteria,[262] and human urine.[263]

The indirubins have been identified as the major active components of the traditional Chinese medicine formulation known as Danggui Longhui Wan, which has been used for many years to treat CML in China.[14] Of importance from both a natural product and a pharmacological perspective, the indirubins were recognized as being inhibitors of several CDKs and potent inhibitors of glycogen synthase kinase-3 (GSK-3).[264] Included in this study were 6-bromoindirubin (**92**; Scheme 17), first isolated from Nature from the mollusk *Hexaplex trunculus*,[257] and its chemically modified oxime derivative BIO (**93**; Scheme 17), and these two compounds demonstrated an at least 5-fold specificity versus CDK1/cyclin B and/or CDK/p25, as well as significantly greater specificity against a wide range of other kinases. Significantly, GSK-3 is also an important target in both Alzheimer's disease and type 2 diabetes, and although indole derivatives have not been reported as being associated with pharmacological intervention in these specific disease areas, their potential must be considered quite high. The treatment potential for inhibitors of GSK-3, including a listing of other natural product-related structures serving as possible inhibitors in these disease states, has recently been reviewed.[265] Using the same basic suite of compounds, it was demonstrated that indirubins serve as ligands for the "orphan receptor" known as the aryl hydrocarbon receptor (AhR).[266] No other natural ligands have yet been identified for AhR, even though, contrary to earlier beliefs, it has existed for over 450 million years. Indole-containing compounds, however, had been suggested as natural ligands for AhR slightly earlier.[267] Full details of the chemistry involved, and SARs established using X-ray crystallography and molecular modeling techniques, have been published.[268]

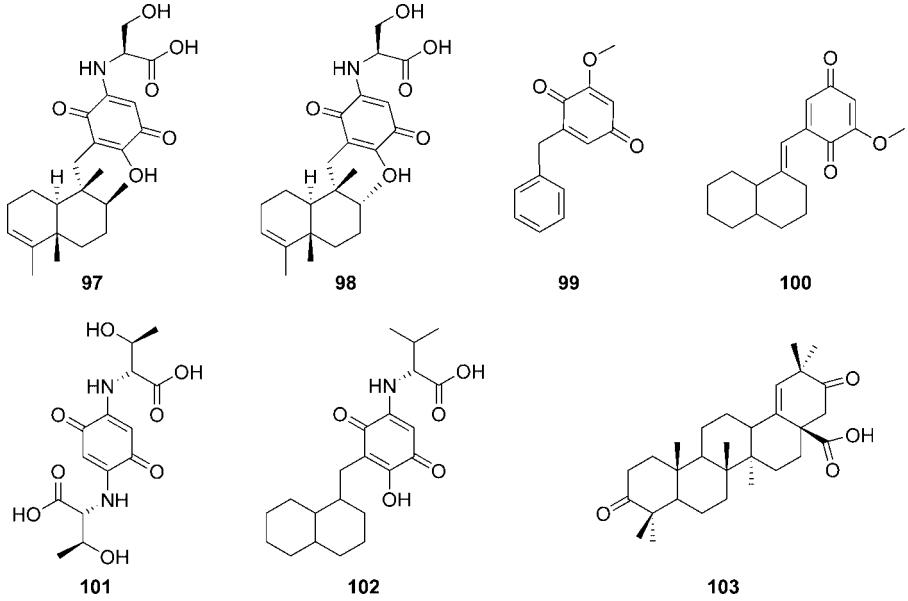
Among other natural products with indirubin-like kinase inhibitory activities are the meridianins (e.g., meridianin A; **94**; Scheme 17), a group of halogenated indole derivatives that are closely related to the base structures of the psammopemmins (e.g., psammopemmin A; **95**; Scheme 17) and discodermindole (**96**; Scheme 17). The psammopemmins and discodermindol were isolated from sponges, whereas the meridianins were isolated from the ascidian *Aplidium meridianum*.[269]

5.5.5. BIOS-Derived Kinase Inhibitors

Significant effort has been, and continues to be, devoted to the development of novel kinase inhibitors through the "fitting of structures to the ATP-binding sites", and this approach has been quite successful at producing structures for clinical trials.[270] In an alternative approach that did not initially concentrate on the specifics of the ATP-binding site, the Waldman group successfully utilized BIOS to search for kinase inhibitors.

Nakijiquinone C (**97**; Scheme 18), isolated from a marine sponge and first reported by Kobayashi et al.[271] 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

Scheme 18. Protein Kinase Inhibitors Discovered by BIOS



lines.[271] 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 (**98**; Scheme 18) and four inhibitors of Tie-2 kinase (**99**-**102**; Scheme 18), a protein intimately involved in angiogenesis and for which, at the beginning of the study, no inhibitors were known.[272] During the study, the first natural product inhibitor of Tie-2 kinase was reported[273] (**103**; Scheme 18) from the plant *Acacia aulacocarpa*, and a set of four papers from another research group demonstrated the activity of synthetic pyrrolo[2,3-*d*]pyrimidines as inhibitors of the same class of kinases.[274−277] 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.[174,278]

5.6. Inhibitors of Heat Shock Protein 90

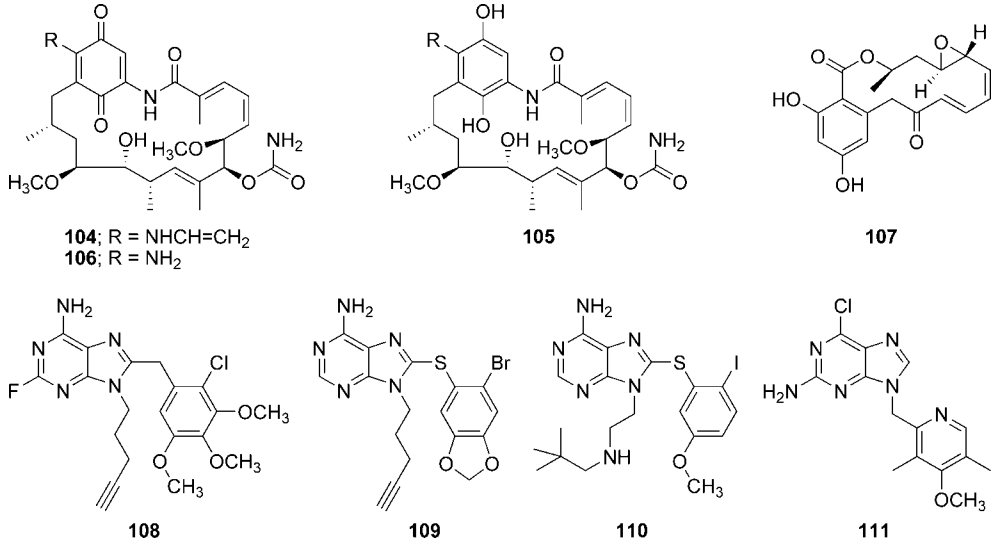
Heat shock protein 90 (Hsp90) is a chaperone protein that plays an important role in stabilizing the conformation of many cell-signaling proteins and maintaining their function. In this respect, many oncogenic proteins are more dependent on Hsp90 than their normal counterparts, and hence, Hsp90 plays an important role in maintaining transformation and increasing the survival and growth tendency of cancer cells. It has also been shown to exist in an activated form in cancer cells while existing in a latent inactive form in normal cells, thus making it an attractive target for chemotherapy in cancer and other diseases, such as neurodegenerative diseases.[279] Advances in the development of Hsp90 inhibitors have been reviewed.[280,281]

5.6.1. Ansamycins: Geldanamycin (GA) derivatives

The development of the ansamycins leading to the 17-substituted analogues has been reviewed.[282] This review highlights the significant differences in the macrocyclic ring stereochemistries reported in the literature for what is nominally the same molecule. These differences are not simply due to a complete stereochemical inversion around the ring, where the relative stereochemistries are maintained, but are quite different renditions from different research groups and should be noted when referring to different papers. 17-Allylaminogeldanamycin **(**17-AAG; tanespimycin) (**104**; Scheme 19) entered clinical trials as the first example of a signal transduction modulator in 1999 under the auspices of the NCI and was subsequently licensed to Kosan (now absorbed by Bristol Myers Squibb) for development. Currently (January 2009), there are two derivatives of geldanamycin in phase III trials, 17-AAG (tanespimycin) and its probable metabolic intermediate, the 18,21-dihydro-derivative IPI-504 or retaspimycin (**105**; Scheme 19) (Infinity Pharmaceuticals),[283] and what may well be the actual active metabolite,[284] 17-aminogeldanamycin (**106**; Scheme 19), is in phase I trials with Infinity. In addition, there is a semisynthetic modification of Macbecin I that is under development by Biotica in conjunction with GSK but whose structure has not yet been divulged.

Two apparent anomalies in the interactions of GA derivatives and radicicol (monorden) (**107**; Scheme 19) with Hsp90 have been under intensive study. The first anomaly is that, despite the fact that both healthy and tumor cells require Hsp90 for cellular function, they respond differently to these drugs, and the second is the fact that the affinity of these drugs for recombinant Hsp90 (rHsp90) is much lower than the levels required for responses in tumor cell lysates. The higher binding affinity for Hsp90 in tumor lysates has been attributed to the existence of other co-chaperones in tumor cells that are not expressed in healthy cells, and this effect was demonstrated by the addition of such proteins to rHsp90.[285] In addition, X-ray crystal studies have demonstrated that the structure of GA in the unbound form has a *trans*-configuration at the amide bond between the benzoquinone and the rest of the ansa ring, whereas when bound to Hsp90, GA displays the *cis*-configuration at this center.[286] Similarly, Jez et al. reported that the closely related GA derivative 17-DMAG requires both a macrocyclic ring conformational change and a *trans*−*cis* isomerization of the amide bond in order to bind to Hsp90.[287] The tumor selectivity, however, is still a subject of investigation.[288]

Scheme 19. Hsp90 Inhibitors



5.6.2. Non-Ansamycin Inhibitors

Supply problems associated with GA derivatives and radicicol, together with GA toxicity problems, led Chiosis et al. to propose use of a simple substituted adenine derivative as a potential base molecule. Significantly, the proposal was based on considering which particular substructures might provide ATP-mimics with improved binding characteristics, rather than on computerized modeling. Thus, knowledge of the requirements of the ATP-binding pocket of Hsp90, and demonstration that a small molecule could function as a cytostatic agent,[289] provided the intellectual stimulus for designing the purine-based PU class of compounds.[290−292] Rational changes in the substituents in both rings and alteration of the length and rigidity of the linker gave rise to PU24FCl (**108**; Scheme 19),[293] which, although not the most active in the series, was utilized to further investigate Hsp90 inhibition in both healthy and tumor cells. The extensive effects exhibited by both healthy and tumor tissues when exposed to the compound have been reported,[294] and, as with 17-AAG and GA, PU24FCl exhibited at least 10- (brain, pancreas, lung) to 50-fold (heart, kidney, liver) lower affinity for Hsp90s from healthy tissues as compared with those from transformed cells. Later studies have shown that replacement of the methylene bridge with sulfur gives 8-arylsulfanyl adenine derivatives (e.g., **109**; Scheme 19) of greater potency,[295] while introduction of an ionizable amino group in the N(9) side chain improved both the water solubility and potency of the compounds to give orally active agents (e.g., **110**; Scheme 19).[296,297] Finally, by extending the Chiosis concept further, Conforma (now Biogen−Idec) scientists derived CNF2024 (**111**; Scheme 19) which is now in phase II clinical trials.[298]

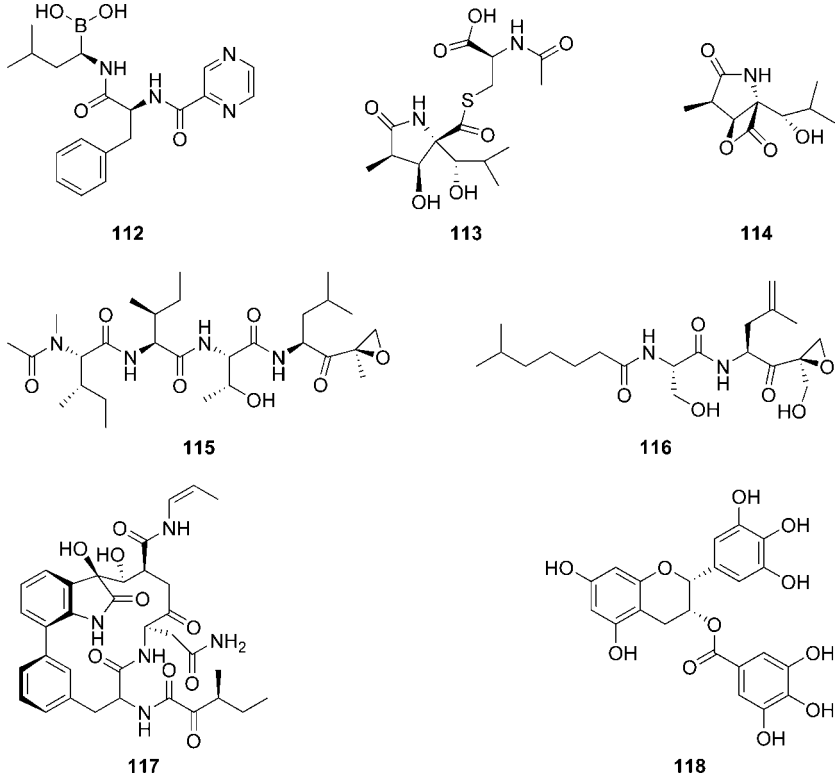
5.7. Proteasome Inhibitors

The proteasome is a multi-enzyme complex involved in the ubiquitin−proteasome pathway control of cell-cycle progression, in the termination of signal transduction cascades, and in the removal of mutant, damaged, and misfolded proteins. As such, it is a promising therapeutic target, and the background to this aspect has been reviewed.[299−302] The synthetic dipeptidyl peptide boronate, bortezomib (Velcade) (**112**; Scheme 20), is the first clinical drug that uses this MOA,[303−305] and the development of this compound, which is based upon a natural product-based structure that inhibited chymotrypsin, has been described by the original inventor.[306]

There are, however, a significant number of other compounds from Nature, and their derivatives, that have led to a greater understanding of the intricacies of this multienzyme complex. The 20S proteasome in mammals has three closely linked proteolytic activities, which are termed trypsin-, chymotrypsin-, and caspase-like from their substrate profiles, though the complex only acts as a concerted whole; individual activities are not demonstrable. In fact, if the chymotrypsin-like activity is inhibited by a suitable compound, then a large reduction in the rate of protein degradation is observed, but if the sites corresponding to the other nominal activities are modified, the overall rate of hydrolysis of proteins is not significantly changed. Because of the substrate specificity of chymotryptic sites, most inhibitors are hydrophobic, whereas in the case of the other two active sites, their "peptide-based" substrates/inhibitors tend to be charged. As a result, almost all of the proteasome inhibitors tend to have chymotrypsin-like activities with some overlapping, but weaker, effects on the other sites.

In 1991, the microbial metabolite lactacystin (**113**; Scheme 20) was reported to induce neuritogenesis in neuroblastoma cells,[307] and this was followed by reports[308,309] demonstrating that radiolabeled lactacystin selectively modified the β5(X) subunit of the mammalian proteasome and irreversibly blocked activity. In subsequent studies, it was demonstrated[310,311] that the actual inhibitor in vitro was the β-lactone, *clasto*-lactacystin-β-lactone (**114**; Scheme 20), and that this substance was formed spontaneously when lactacystin was exposed to neutral aqueous media. The parent compound and other analogues have been synthesized, and the authors suggested that *clasto*-lactacystin-β-lactone should be named omuralide (**114**; Scheme 20).[312,313] The marine bacterial metabolite salinosporamide A (section 3.3.4) (**23**; Scheme 6) demonstrates activity as a cytotoxic proteasome inhibitor[97] and has been synthesized.[314] Compared to omuralide, salinosporamide is uniquely functionalized and has a cyclohexene ring replacing the isopropyl group found at the C(5)-position in omuralide. The isopropyl group in omuralide is essential for activity, as is the chloro substituent in salinosporamide A. Salinosporamide A interacts with the 20S proteasome by forming a covalent link with the chymotryptic-like threonine hydroxyl; the X-ray of the bound molecule

Scheme 20. Proteasome Inhibitors and DNA Interactive Agents



was published in 2006.[315] This molecule is being developed by Nereus Pharmaceuticals and currently is in phase I clinical trials against refractory lymphomas and myelomas, as well as various solid tumors.

The epoxyketone microbial metabolites epoxomicin (**115**; Scheme 20) and eponemycin (**116**; Scheme 20) exhibited cytotoxic activities as a result of proteasome inhibition,[316,317] being the most selective proteasome inhibitors reported to date. There are reports of other natural products active as proteasome inhibitors but with different mechanisms to those described previously. Thus, the cyclic peptide TMC-95-A (**117**; Scheme 20), isolated from *Apiospora montagnei* is a potent chymotrypsin-like inhibitor, but with activity against the other sites as well,[318] apparently binding noncovalently to active sites through an array of hydrogen bonds. (−)-Epigallocatechin 3-gallate (**118**; Scheme 20) is a potent covalent inhibitor of the 20S proteasome, apparently due to acylation of the active site threonines through threonine cleavage of the ester linkage in EGCG.[319]

5.8. DNA Interactive Agents (non-Topoisomerase I and II Inhibitors)

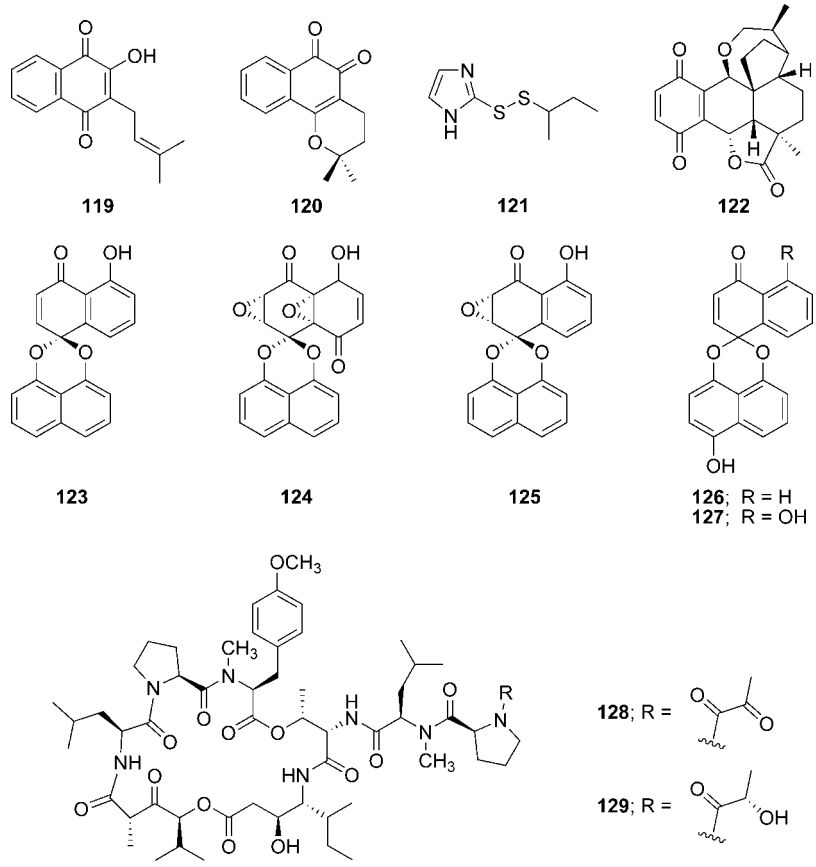
The complex alkaloid ecteinascidin 743 (Et-743, Yondelis) (**47**; Scheme 11), discovered from the colonial tunicate *Ecteinascidia turbinata*,[134,135] was found to have a unique mechanism of action, binding to the minor groove of DNA and interfering with cell division, the genetic transcription processes, and DNA repair machinery.[320,321] There has been a considerable number of reports published in the literature giving possibilities as to the MOA(s) of ecteinascidin 743 when tumor cells are treated in vitro. A significant problem with some of the reports is that the concentration(s) used in the experiments are 10 orders of magnitude greater than those that demonstrate activity in vivo. These levels are in the low nM to high pM range and, thus, care should be taken when evaluating published work on the MOA of this compound. The discovery and development of ecteinascidin 743 have been comprehensively reviewed.[137,138]

Under the name Yondelis, ecteinascidin 743 has been granted Orphan Drug designation in Europe and the U.S.A., was approved by the EMEA in late September, 2007, and was launched in Europe later that year for the treatment of soft tissue sarcomas (STS).[322] It is currently in phase II and III trials in ovarian metastatic breast and prostate cancers, as well as pediatric sarcomas.

5.9. Agents That Activate Caspase and/or Induce Apoptosis

The relatively simple naphthoquinone β-lapachol (**119**; Scheme 21) is a well-known compound obtained from the bark of the lapacho tree, *Tabebuia avellanedae*, and other species of the same genus that are native to South America. β-Lapachol and other plant components are extensively used as ethnobotanical treatments in the Amazonian region, and β-lapachol was advanced to clinical status by the National Cancer Institute (NCI) in the 1970s. It was later withdrawn due to unacceptable levels of toxicity, but its close relative β-lapachone (**120**; Scheme 21) has demonstrated interesting molecular target activity, with one mechanism of action being the induction of apoptosis in transformed cells.[323] Evidence of its involvement in transcription processes has been reported, demonstrating that the agent induced activation of caspase-3, inhibition of NFκB, and subsequent downregulation of *bcl*-2.[324] Currently, β-lapachone (ARQ501) is in phase II clinical trials in the U.S.A. for advanced solid tumors, and further information on the background of these agents may be obtained from a 2004 review.[325]

Scheme 21. Caspase Activators, Apoptosis Inducers, and Inhibitors of HIF and Miscellaneous Targets



5.10. Inhibitors of Hypoxia Inducible Factor (HIF)

Hypoxia inducible factor 1 (HIF-1) is composed of two subunits, an oxygen-sensitive inducible factor (HIF-1α) and the constitutive HIF-1β [also known as AhR nuclear translocator (ARNT)], which may prove to be an important target in diseases that have a hypoxic component such as cancer (where the interior of a tumor is anoxic compared with the outer surfaces), heart disease, and/or stroke. The involvement of HIF proteins with a variety of inhibitors (not necessarily direct inhibition, but alteration of transduction pathways upstream and downstream) have been reviewed, and included in the review are well-known materials with natural product "backgrounds", such as Taxol, vincristine, 2-methoxyestradiol, rapamycin, GA, quinocarmycin, and the IP3K inhibitors wortmannin and LY-294002.[326]

Of significance from a natural product perspective was the initial realization that inhibition of thioredoxin reductase 1 (TRX-1) may act indirectly on HIF-1α. By comparing the NCI 60 human cancer cell line cytotoxicity profile of a known TRX-1 inhibitor and phase II clinical candidate, PX-12 (**121**; Scheme 21), with the profiles of a range of compounds in the NCI screening database, the fungal natural product pleurotin (**122**; Scheme 21) was identified as exhibiting a similar killing pattern to PX-12.[327] Research on a focused combinatorial library of naphthoquinone acetals based upon palmarumycin CP1 (**123**; Scheme 21), which included diepoxins (e. g., diepoxin, **124**; Scheme 21) and deoxypreussomerins (e. g., deoxypreussomerin A, **125**; Scheme 21), indicated that they possessed potent cytotoxicity, but their potential targets were unidentified at that time.[328] Palmarumycin CP1, however, was later shown to have inhibitory activity comparable to that of pleurotin in the TRX-1 assay, with IC_{50} values in the 170−350 nM range, and it was demonstrated that certain aspects of the base structure, in particular the enone system, were required for activity in this assay.[329] Evidence for direct inhibition of HIF-1α by both pleurotin and PX-12 helped to demonstrate that the cytotoxicity of these compounds, and hence palmarumycin CP1, was likely due to HIF-1α interactions.[330] Further palmarumycins isolated from extracts of the fermentation broth of an unidentified ascomycete from Costa Rica failed to show activity in the assays used but provided important SAR information.[331] This information led in turn to further modifications of the base structure, yielding the simple analogues S-11 (**126**; Scheme 21) and S-12 (**127**; Scheme 21), which exhibited biological activities comparable to pleurotin in both the thioredoxin enzyme (TRX-1) system and (most importantly) in the cytotoxicity assays.[331] Thus, a fairly complex interaction of results from several different research groups has led to promising candidates for further biological studies, including in vivo experiments that are planned and will be reported in due course.

5.11. Miscellaneous Target Inhibitors

There are a number of agents, particularly from marine sources, whose initial molecular targets have been identified, though it is highly probable that, over the next few years, these initial targets will be refined as methods and other information becomes available.

One such compound, aplidine, is an agent with multiple targets. Formally dehydrodidemnin B (**128**; Scheme 21), it was first reported in a patent and then referred to in a 1996 paper on structure−activity relationships among the didemnins.[332] In 1996, the anti-tumor potential was reported by PharmaMar scientists; the total synthesis was reported in

a patent application in 2000, and the patent was issued in 2002. The compound was advanced into phase I clinical trials in 1999 under the trade name of Aplidin for the treatment of both solid tumors and non-Hodgkin's lymphoma, and published details through early 2004 are given by Newman and Cragg[29] together with discussion as to the mechanisms of action that might be relevant. Details of the progress of this drug through preclinical and clinical development have been reviewed.[29,138,333]

It should be noted that the clinical trials of a very close aplidine analogue, didemnin B (**129**; Scheme 21), were discontinued because of the toxicities observed, including significant immunosuppression. In contrast, evidence for a lack of myelosuppression by aplidine was reported using a murine competitive repopulating model as the test system,[334] and no hematological toxicity has been observed clinically.[138] It is very interesting both chemically and pharmacologically that the removal of two hydrogen atoms, i.e., conversion of the lactyl side chain to a pyruvyl side chain, appears to significantly alter the toxicity profile, as this is the only formal change in the molecule when compared to didemnin B. However, the comments on dosage regimens should be taken into account when such comparisons are made in the future.[335]

6. Future Prospects

Nature has been a source of medicinal products for millennia, and during the past century, many useful drugs have been developed from natural sources, particularly plants. It is clear that Nature will continue to be a major source of new drug leads. The drug potential of the marine environment remains relatively unexplored, but it is becoming increasingly evident that the realm of microorganisms offers a vast untapped potential. With the advent of genetic techniques that permit the isolation and expression of biosynthetic cassettes, microbes and their marine invertebrate hosts may well be the new frontier for natural products lead discovery. Plant endophytes also offer an exciting new resource, and research continues to reveal that many of the important drugs originally thought to be produced by plants are probably products of an interaction with endophytic microbes residing in the tissues between living plant cells. This has been further accentuated by the recent report of the isolation of hypericin from an endophytic fungus from *Hypericum perforatum*.[336] Effective drug development will depend on multidisciplinary collaboration embracing natural product lead discovery and optimization through the application of total and diversity-oriented synthesis and combinatorial chemistry and biochemistry, combined with good biology. The impressive number of anti-cancer drugs that are derived from natural sources are discussed in terms of their mechanisms of action, and as can be seen from these discussions, natural products from all sources still have the potential to lead chemists of all types into areas of drug discovery and development that would never have been considered if the "privileged structures from Nature" had not been isolated, purified, and used as probes of cellular and molecular mechanisms. In spite of the discussions in the early-to-late 1990s concerning the vast potential of combinatorial chemistry as a discovery tool, it is now quite evident that this technique, except in the very special cases of peptides and nucleosides (which are actually "privileged structures" in their own right), is not the panacea that it was thought to be. However, the application of combinatorial synthetic methodology to elaborate around a skeleton from a privileged structure, and the extensions shown by DTS, DOS, and BIOS, demonstrates that the use of all these methodologies has great potential to lead to novel agents and drug entities in many disease states.[337]

7. References

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