Reviews

Recent Natural Products Based Drug Development: A Pharmaceutical Industry Perspective

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The search for new pharmacologically active agents obtained by screening natural sources such as microbial fermentations and plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. A recent review pointed out that approximately 60% of the antitumor and antiinfective agents that are commercially available or in late stages of clinical trials today are of natural product origin.[1] Historically, the majority of the natural product-based drugs including cyclosporine, paclitaxel, and camptothecin derivatives were first discovered by traditional cell-based in vitro assays (antibacterial, antifungal, antiviral, antiparasitic, or cytotoxic assays) before their real molecular biological targets were identified. These cellular biological responses of natural products are most probably associated with inherent properties of secondary metabolites for the defense of their producing organisms (e.g., fungus against bacteria: β-lactams; fungus against other fungi: echinocandin; agents acting at the cell cycle to inhibit the proliferation of foreign organisms: bryostatin).[2] Thus, most traditional cell-based in vitro assays can be viewed as detection methods of the fundamental and unique phenomena of living organisms. As a result, antiinfective and antitumor targets have been, historically, the effective research area for natural products screening programs. Today, with the advent of genomics research and newer molecular biology tools for developing bioassays, more sophisticated biological assays in addition to cell-based assays are being employed routinely in the drug discovery paradigm. Consequently, in recent years a notable number of natural product-derived agents, such as pravastatin, lovastatin, and FK-506, has been discovered by employing mechanism-based screening approaches involving cellular or biochemical targets in their assay design. In addition, a large number of natural products, especially plant-derived drugs, continues to be discovered on the basis of traditional or empirical local medical practices.[3]

Over the years, however, interest in the pharmaceutical industry in natural products research has been a somewhat cyclical phenomenon. Most recently, as the lead compound generation and drug discovery processes have been significantly impacted by emerging approaches such as advanced genomics, high-throughput screening, combinatorial chemistry and biology, and computer-assisted de novo drug design, there is an emerging perception that chemical diversity, especially among small organic molecules, may no longer be in short supply, and thus, the role that natural products have played historically in lead generation may start to diminish. This review is intended to summarize, mostly from the perspective of pharmaceutical industry, a number of important natural product-based drug developments in the past 10−15 years, which have led to therapeutically useful agents either in current clinical use or in various stages of clinical trial. It is hoped that this review will be able to address the recent contributions of natural products research to overall drug discovery and development and the role that this field will play in future. The discussion that follows is divided into five major disease areas with brief examples of interesting natural product molecules provided for each.

1. Infectious Disease Area

The increasing clinical importance of drug-resistant bacterial pathogens has lent additional urgency to antibacterial research. Important developments of antibacterial agents over recent years have been related to some well-known natural product classes such as β-lactams (e.g., cephalosporins), tetracyclines (e.g., glycylcyclines), macrolides (e.g., erythromycin and rifamycin analogues), spectinomycins, and glycopeptides (e.g., vancomycin and teicoplanin analogues). In the antifungal area, many chemistry and formulation studies have also been undertaken on known natural product classes such as polyenes (e.g., amphotericin B and nystatin analogues) and nucleosides (e.g., nikkomycin Z). Studies on these well-known chemotypes are well documented elsewhere[4] and are therefore not included in this review.

Streptogramins are obtained by fermentation from modified strains of *Streptomyces* spp. The particular feature of this class is that it is made up of two groups of components that act in synergy. This family of antibiotics inhibits protein synthesis by interfering with the ribosomal function. Two antibiotics of this family, pristinamycin and virginiamycin, are both utilized clinically in Europe; a lack of water solubility limits their wider use. Rhone-Poulenc Rorer's new injectable

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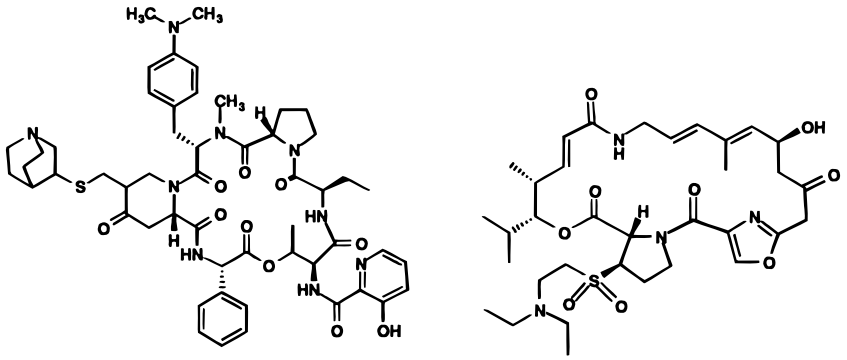
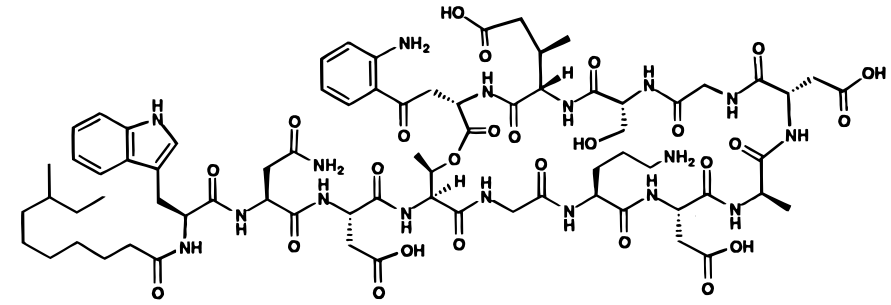
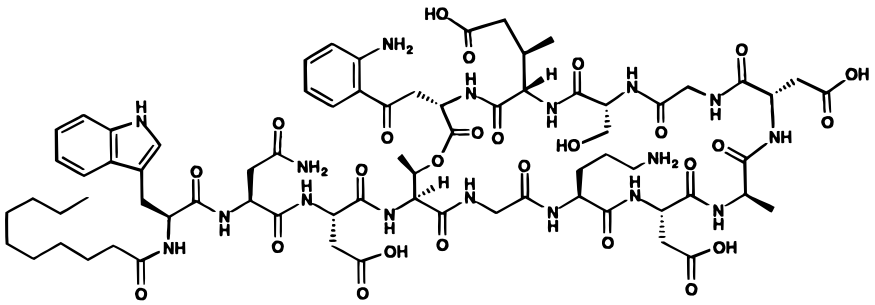


Quinupristin Dalfopristin

Figure 1.



A21978C complex (A21978C$_1$ structure shown)

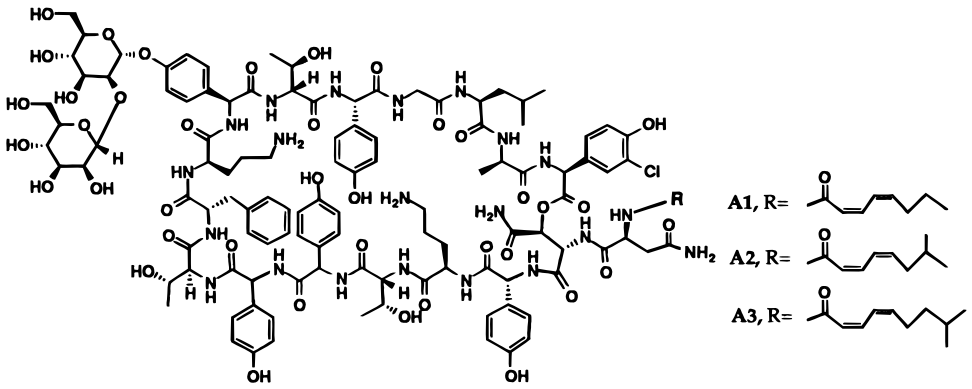


Daptomycin

Figure 2.

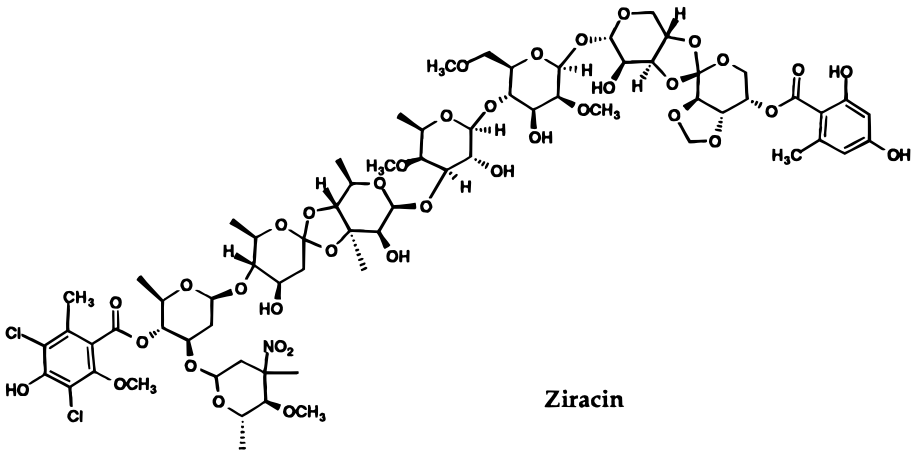
antibiotic RP-59500 was designed to overcome these limitations. It contains water-soluble, semisynthetic dalfopristin (RP-54476) and quinupristin (RP-57669) combined in a ratio of 70:30 (Figure 1). RP-59500 was found to be active against methicillin-resistant and erythromycin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, and its MIC_{90} value was comparable to those of clarithromycin and azithromycin. Phase III clinical trials of RP-59500 under the name synercid have been completed, and the New Drug Application (NDA) to the U.S. FDA was filed in September 1997 for the treatment of a variety of severe infections including bacteremia, skin infections and pneumonia caused by Gram-positive bacteria, such as vancomycin-resistant *Enterococcus faecium* (VREF), methicillin-resistant *S. aureus* (MRSA), methicillin-resistant *S. epidermidis* (MRSE), and drug-resistant *Streptococcus pneumoniae* (DRSP).[4] In February 1998, the FDA's Anti-Infective Drugs Advisory Committee recommended its approval.

Eli Lilly's A21978C complex is a family of lipopeptide antibiotics produced by *Streptomyces roseosporus*. Each member of this family has the same 13 amino acid peptide core but differs in the structure of the fatty acids that acylate the *N*-terminus. A21978C complex demonstrated excellent activity against Gram-positive bacterial infections; however, acute toxicity was augmented with a small increase in the chain length of the lipid side chain. To overcome this problem, the fatty acid acyl group was removed by incubation of the A21978C complex with *Actinoplanes utahensis* to release the peptide nucleus. New analogues of A21978C were synthesized by reacylation of the *N*-terminus. Of the analogues prepared, the *n*-decanoyl analogue (LY-146032) or daptomycin gave the best survival in the mouse acute toxicity test[5] (Figure 2). Although the antibacterial mechanism of action of daptomycin is not fully understood, it is believed that the drug acts by inhibiting the synthesis of lipoteichoic acid, a component of the bacterial cell wall, as well as by disrupting the cell membrane.[5] Because of its new mechanism of action, daptomycin is active against pathogens, including vancomycin-resistant *Enterococci* (VRE) and MRSA, resistant to other antibiotics. Eli Lilly took intravenous



Ramoplanin

Figure 3.



Ziracin

Figure 4.

daptomycin through phase II clinical development as a treatment for bacterial infections of the skin and soft tissue, bacteremia, and endocarditis. While the drug displayed efficacy, doses that were high enough to fully eradicate microbes from the deep tissues of the body brought unacceptable muscle toxicity. Due to the unacceptable therapeutic window, Eli Lilly halted the clinical development in 1991. In November 1997, Cubist Pharmaceuticals acquired the rights to develop, manufacture, and market daptomycin from Eli Lilly. Cubist plans to resubmit an IND in mid-1998 and initiate pivotal phase III trials in late 1998 or early 1999 on i.v. daptomycin for the treatment of infections caused by VRE, MRSA, and other antibiotic resistant microbes.

Ramoplanin (A-16686, MDL-62198), a complex of three glycolipodepsipeptides A1 (minor), A2 (major), and A3 (minor), was discovered from *Actinoplanes* sp. at Marion Merrell Dow's Lepetit Research Center in Italy (Figure 3). The complex is active against a broad range of Gram-positive bacteria including MRSA and MRSE. Experimental data also demonstrated antiplaque activity and inhibitory activity against bacterial cell wall synthesis. Since ramoplanin inhibits the peptidoglycan biosynthesis at a step earlier than that of vancomycin, it is thus highly active against VRE.[6] Oral administration of ramoplanin to healthy volunteers was well tolerated and highly effective in reducing the burden of Gram-positive fecal flora. Unfortunately, nephrotoxicity limits its clinical utility.[7] An improved analogue of this class may have the potential for treatment of infections due to VRE.

Oligosaccharide antibiotics of the everninomicin class have been known since 1964, but none were developed for human medical use because of their nephrotoxicity. Ziracin (Sch-27899), an everninomicin analogue discovered in 1979, has shown both potent antibacterial activity against MRSA and VRE and low toxicity compared with other members in the class, but the mechanism of action is unknown (Figure 4). Since the late 1980s, Schering-Plough has revived interest in this compound and has developed a new injectable formulation for clinical investigation.[8] In phase I clinical studies, ziracin has been safe and well-tolerated, and it is currently in phase II clinical trials.

The echinocandin class of lipopeptides, discovered in the 1970s, from fungi are potent fungicidal agents under development for broad-spectrum antimycotic therapy. These compounds inhibit synthesis of the vital cell wall polymer, 1,3-β-D-glucan. Echinocandins have been chemically modified to produce semisynthetic analogues with improved pharmacological properties. One of these, cilofungin, a highly hydrophobic compound, reached phase II clinical trials but was then abandoned because of toxicity associated with the formulation vehicle. Several second-generation semisynthetic leads, namely, Eli Lilly's LY-303,366 (and prodrug LY-307,853) derived from echinocandin B and Merck's L-743,872 (MK-0991) derived from pneumocandin A_o, have shown promising in vitro and in vivo activities against *Candida*

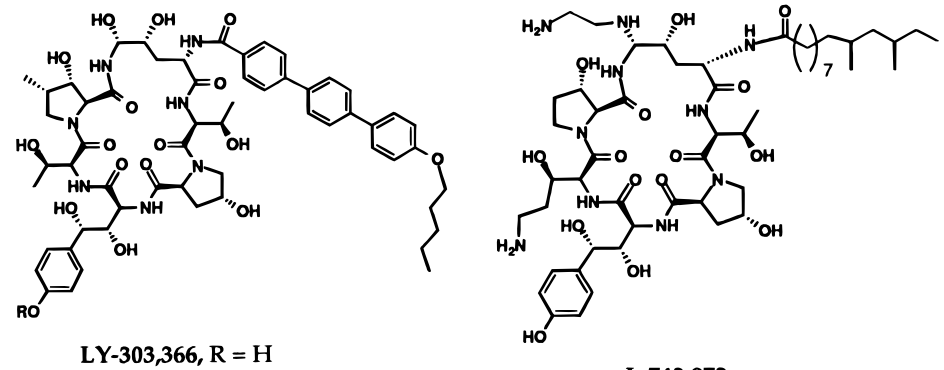


Figure 5.

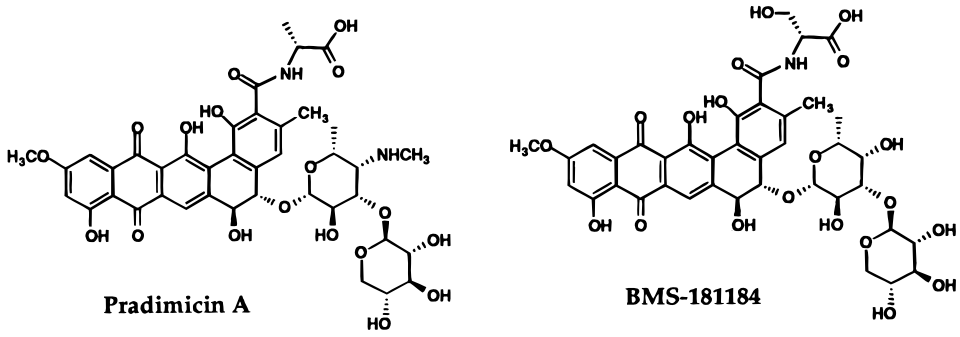


Figure 6.

albicans, *Aspergillus fumigatus*, *Pneumocystis carinii*, *Saccharomyces cerevisiae*, and other fungi[9] (Figure 5). Recently, MK-991 was also shown to be effective against *Histoplasma capsulatum*, a common opportunistic infection in AIDS patients. In phase I clinical trials, a single oral dose of LY-303,366 was well tolerated and provided plasma levels well in excess of the MIC_{90} values for *C. albicans*.[10] Results from phase I trials of MK-991, administered once daily intravenously, indicate that it is generally well tolerated, and the compound has progressed into phase II clinical studies.[11]

Pradimicins, a group of benzonaphthacene quinones substituted with a D-amino acid and a disaccharide side chain, are potent broad-spectrum antifungal compounds discovered at the Bristol-Myers Squibb research laboratories in Tokyo by a high-volume screen for activity against *C. albicans*. BMS-181184, a water-soluble analogue of pradimicin A, was successfully produced by the fermentation of *Actinomadura* sp. in the presence of D-serine and D-cycloserine, both as alanine racemase inhibitors[12] (Figure 6). The fungicidal action is believed to be due to the binding of pradimicins to the saccharide portion of the fungal cell surface mannoprotein, leading to the leakage of intracellular potassium and severe morphological alterations.[13] BMS-181184 was brought into phase I clinical trials for the parenteral treatment of systemic fungal infections.

Glaxo-Wellcome's high-throughput screening for inhibitors of *C. albicans* protein synthesis resulted in the discovery of GR135402, a new sordarin analogue from *Graphium putredinis*, possessing promising potency and selectivity for fungal vs mammalian protein synthesis. Sordarin was first isolated in the 1960s, and is known to have fungistatic activity, but is only moderately active and has a limited spectrum against a battery of fungal organisms. Through a program of mutasynthesis,

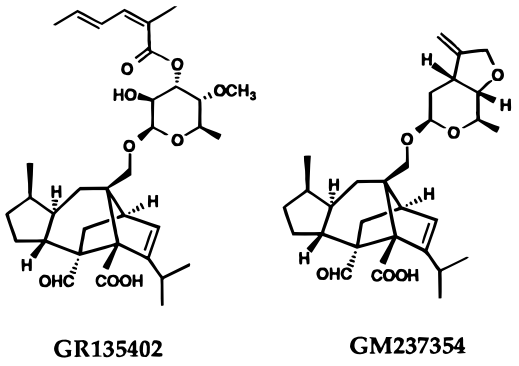
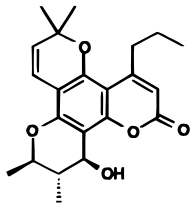


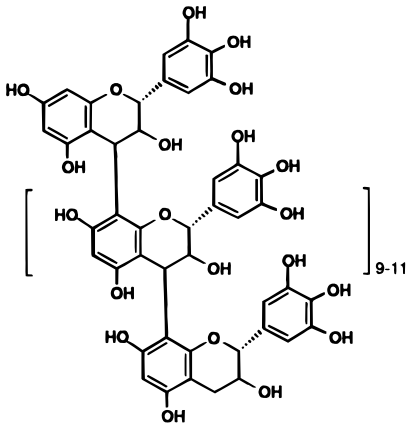
Figure 7.

biotransformation, and medicinal chemistry, a series of sordarin derivatives were prepared at Glaxo-Wellcome, and some of them exhibited potent in vitro and in vivo antifungal activity and a broad spectrum of action. This ultimately yielded the semisynthetic analogue GM237354. It is believed to have a novel mode of action by inhibiting the elongation step of yeast protein synthesis. It had no primary effect on RNA synthesis[14,15] (Figure 7). The compound has been progressed to exploratory development.

Calanolide A is a reverse-transcriptase inhibitor discovered from the Malaysian rainforest tree, *Calophyllum lanigerum* by the U.S. National Cancer Institute (Figure 8). In vitro studies of (+)-calanolide A have demonstrated the compound to be effective against HIV-1, including strains resistant to AZT and other non-nucleoside reverse-transcriptase inhibitors. It also exhibited synergistic anti-HIV activity in combination with nucleoside reverse-transcriptase inhibitors, including AZT, ddI, and ddC.[16] In 1995, MediChem Research, Inc., exclusively licensed the calanolides from the NCI for further development and commercialization. To



Calanolide A

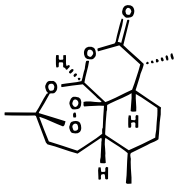


SP-303

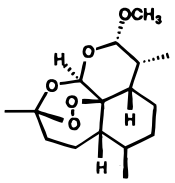
Figure 8.

overcome the difficulty with the supply of calanolide A from natural sources, MediChem subsequently accomplished the total synthesis. In June 1997, Sarawak MediChem Pharmaceuticals, Inc., a joint venture between MediChem Research and the state of Sarawak, Malaysia, began clinical development of calanolide A as a potential treatment for AIDS and HIV infection. A single-center 7-month U.S. phase Ia clinical trial of calanolide A was started to assess the safety and tolerability of the compound.

Since being founded, Shaman Pharmaceuticals, based in South San Francisco, CA, has explored plant-derived therapeutic agents based on knowledge provided by indigenous people living in tropical regions. SP-303, a mixture of natural oligomeric proanthocyanidins up to molecular weight 2100 Da, was isolated from the latex of a Latin America plant *Croton lechleri* (Figure 8). Traditional uses of this medicinal herb include treatments for respiratory and gastrointestinal disorders. SP-303 was shown to have activity against the herpes virus and was also active against a variety of other DNA and RNA viruses. Virend, a topical formulation of SP-303, was brought into phase II clinical studies for treatment of recurrent genital herpes in combination with acyclovir, the current standard of care. But the trials were later suspended as results revealed no additional benefit over using oral acyclovir alone. Provir, an oral formulation of SP-303, proved to be safe and well tolerated in a phase I clinical trial but ineffective in early phase II studies for respiratory syncytial virus (RSV), since there was no adequate absorption by the patients. For treating diarrhea, however, low absorption is a benefit. Subsequently, a phase II open-label study of patients with travelers' diarrhea (secretory or watery diarrhea) was initiated that demonstrated the efficacy of Provir in symptomatic relief through restoration of normal bowel function and prevention of recurrences. No



Artemsinin (qinhaosu)



Artemether

Figure 9.

significant adverse events were reported. Provir attacks the underlying cellular mechanism responsible for secretory diarrhea by inhibiting the chloride ion secretion in the small intestine and the subsequent accumulation of fluid in the intestine. Excess secretion of chloride, spurred by bacterial toxins from organisms such as *Escherichia coli*, causes a buildup of water, resulting in diarrhea and eventual dehydration. Provir has been designated a fast-track product for regulatory review by the U.S. FDA. A single pivotal 300-patient phase III trial was initiated for treatment of diarrhea in patients with AIDS, and the study is intended to serve as the basis for an NDA submission.[17−18]

Ancient Chinese medical texts written 2000 years ago describe the herb *Artemisia annua* as a remedy for malaria. From this indication, in the late 1960s Chinese researchers initiated evaluation of various extracts. Bioassay-guided isolation yielded the new antimalarial compound artemisinin (qinhaosu) (Figure 9). Artemisinin possesses a unique endoperoxide group that distinguishes it from the old generation of antimalarial drugs. It is effective in treating chloroquine-resistant cases and other severe cases without major toxicity. Artemether, a synthetic analogue of artemisinin, has been developed in the People's Republic of China. Two recent comparative clinical studies suggested that artemether is as effective as quinine in the treatment of severe malaria.[19] Artemether, manufactured by Kunming Pharmaceutical Factory of China and a few European pharmaceutical companies, is available as tablets and an intramuscular injectable form in oil in more than 25 malaria-endemic countries and is included in the model list of essential drugs of the World Health Organization (WHO). Novartis with its Chinese partners is undertaking phase III clinical studies with a combination of oral artemether and benflumetol in several institutions worldwide.[20]

Merck's antiparasitic drug, ivermectin, discovered from a *Streptomyces* sp. in the late 1970s, belongs to the avermectin group of macrolides. Avermectins have been the subject of intense scientific and commercial interest, since they possess potent activity against both nematode and arthropod parasites of livestock. Ivermectin, a mixture of the 22,23-dihydroavermectins B1a (80%) and B1b (20%), has been used in many countries in the world for the treatment and control of parasites in cattle, horses, sheep, pigs and dogs. For many years, ivermectin was the biggest-selling veterinary drug[21] (Figure 10). It also has been used as a human medicine since 1987 to treat onchocerciasis (river blindness), and more than 5.2 million people have received the drug through WHO's Onchocerciasis Control Program and other programs. River blindness is prevalent in many countries in Africa as well as South and Central America. The parasitic worm is transmitted via the bite of the blackfly. New immature parasites produced in

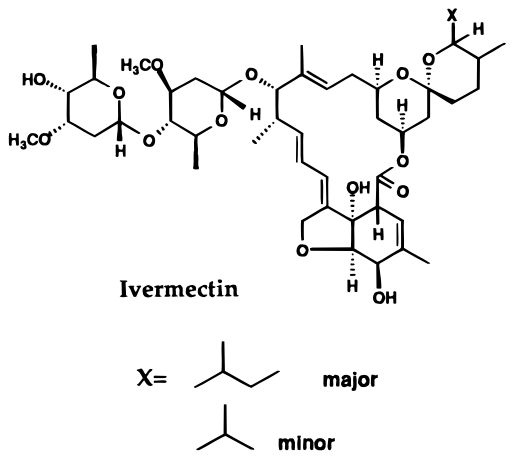


Figure 10.

the human body cause intense skin itching, and can also migrate to the eyes, causing inflammation and blindness. Ivermectin also demonstrated efficacy in clinical trials for treatment of strongyloidiasis, a parasitic infection in the small intestine and a common disease in many tropical countries. The infection can persist for many years, causing abdominal pain, diarrhea, and an increased number of eosinophils in the blood. In immunocompromised individuals, the infection can spread and be fatal. As a result, ivermectin was approved in December 1996 by the U.S. FDA for two human parasitic diseases—strongyloidiasis and onchocerciasis.[22]

2. Neurological Disease Area

The dramatic effects of ergot (*Claviceps purpurea*) and its constituent indole alkaloids when ingested by humans has been recognized for nearly two centuries. To varying degrees, these agents act as partial agonists or antagonists at α-adrenergic, serotoninergic, and dopaminergic receptors. The complexity of their actions limits the therapeutic uses of natural ergot alkaloids, but clinical applications of their analogues exists for the treatment of migraine, Parkinson's disease, and postpartum hemorrhage. Recent discovery efforts have mostly focused on dopamine receptor agonism aiming at the treatment of Parkinson's disease. Among the new generation of ergot alkaloid analogues is cabergoline, a potent, selective, and long-lasting dopamine D2 receptor agonist. This was launched recently in several European countries and the U.S. by Pharmacia & Upjohn for the symptomatic treatment of Parkinson's disease, as adjuvant therapy with levodopa, a dopa-decarboxylase inhibitor, to control daily fluctuations in motor performance (Figure 11). It also allows reductions in levodopa dosage.[23] Carbergoline is being investigated as a monotherapy for the treatment of newly diagnosed Parkinson's patients to delay the onset of the disease. In addition, the compound is indicated for the treatment of hyperprolactinaemic disorders due to pituitary tumors or idiopathic disease.[24] A similar partial dopamine D2 receptor agonist, terguride, was launched in Japan in 1994 by Nippon Schering[25] (Figure 11). Terguride reduces spontaneous locomotor behavior and induces marked contralateral circling behavior in animals. It is marketed for the treatment of schizophrenia and Parkinson's disease.

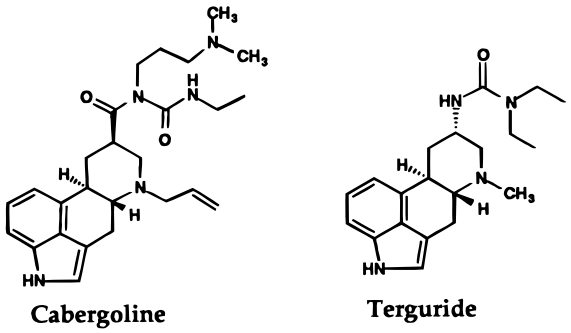
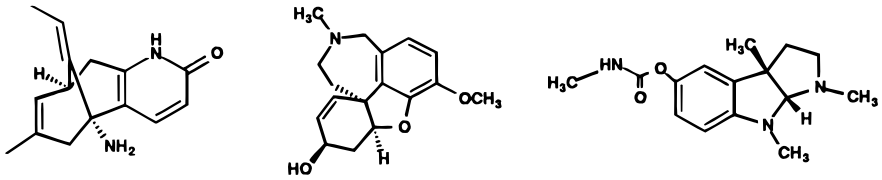


Figure 11.

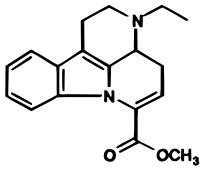
For several centuries, elderly people in some parts of mainland China have brewed tea from the leaves of the club moss (*Huperzia serrata*) for improvement of their memory. In the early 1980s, Chinese scientists isolated huperzine A from this traditional medicine as a potent, reversible, and selective inhibitor of acetylcholinesterase (Figure 12). Because huperzine is produced at very low levels in nature, a total synthesis has been developed. On the basis of animal studies and early clinical trials carried out in the People's Republic of China in healthy humans and patients with dementia, oral huperzine A proved to be a promising candidate for the treatment of cholinergic-related neurodegenerative disorders such as Alzheimer's disease (AD). In a prospective, multicenter, double-blind trial with 103 patients, huperzine A was found to be safe and superior to placebo and induced improvement in memory cognition and behavior in about 58% of patients with AD.[26] Several international biomedical institutions have confirmed the efficacy of huperzine A on primates. It appears that huperzine A is a more selective and less toxic acetylcholinesterase inhibitor than those available as AD therapies, such as Eisai's donepazil and Warner-Lambert's tacrine. Clinical trials in the U.S. are in the planning stage.[27]

Galanthamine is a long-acting, centrally active competitive cholinesterase inhibitor, a natural product originally isolated from Caucasian snowdrops (*Galanthus nivalis*) in the 1950s (Figure 12). Throughout the region that spans Bulgaria, Turkey and the Caucasus maintains, *Galanthus* species have been used for hundreds of years in traditional medicine to treat painful neurological conditions such as facial neuralgia, with such treatment being essentially topical. Most of the early seminal research on galanthamine was carried out in Bulgaria and the USSR during the 1960s, but the information obtained was not readily available to the medical community outside the Eastern Bloc at that time. Furthermore, galanthamine was available as a natural product only in limited amounts from Bulgarian and Turkish sources. These reasons have long hampered the proper investigation of the drug despite its promising pharmacological properties.[28] New studies have found that galanthamine is well tolerated during long-term treatment. Galanthamine under the name of Nivalin is already approved in Austria for AD and is marketed there and in Germany for other indications such as facial neuralgia. The U.K. drug firm, Shire Pharmaceuticals Ltd., with Janssen Pharmaceutical NV as development and marketing partner, recently carried out phase II trials of galanthamine in patients with senile dementia of the Alzheimer's type. The study has



Huperzine Galanthamine Physostigmine

Figure 12.



Vinconate

Figure 13.

shown a statistically significant improvement in cognitive performance. The apparent absence of adverse effects on the liver compares this drug favorably with tacrine. Shire is conducting simultaneous phase III trials of the drug in European countries.[29]

The plant alkaloid, physostigmine, was first isolated in the nineteenth century and has long been known as an anticholinesterase agent (Figure 12). In November 1997, Forest Labs and Bayer filed a U.S. NDA for physostigmine (Synapton) and a similar submission to the European Community as a twice-daily treatment for AD. The pivotal Synapton clinical trial consists of four studies in more than 2000 patients. In long-term extension trials, some patients have been treated with the drug for up to 6 years. The drug showed significant statistical benefits measured by both the AD Assessment Scale-Cognitive Subscale (ADAS-Cog), an assessment of memory and learning, and the CIBIC-Plus method, a clinician's opinion-based evaluation, plus caregiver input.[30]

In addition to the utility of vinca (*Catharanthus*) alkaloids in antitumor therapy, many clinical investigations have indicated beneficial effects on cerebral circulation due to this class of compounds, suggesting their potential in the treatment of cerebrovascular disease. It is obvious that significant structural modifications are needed in order to minimize the potent cytotoxicity, a serious pitfall for CNS agents. Tokyo Tanabe's vinconate was designed to address the problem; this novel vinca alkaloid derivative can easily penetrate the blood−brain barrier and distribute into the brain (Figure 13). Experimental results have indicated that vinconate facilitates phosphatidylinositol turnover by stimulating muscarinic receptors, facilitating the coupling of muscarinic receptors and G protein.[31] After a significant time in clinical trials, the NDA for vinconate has now been filed in Japan for the treatment of cerebral ischemia, cerebral hemorrhage, and as a cerebral metabolic enhancer.

Cholecystokinin (CCK), a polypeptide hormone occurring in numerous molecular forms, is found in the periphery that affects gut function, digestive processes, and feeding behavior. CCK also occurs in the brain, where it acts as a neurotransmitter and neuromodulator. Asperlicin, a nonpeptide CCK-A antagonist (IC_{50}, 1.4 uM) isolated from a fungal fermentation broth, was discovered in Merck's screening program using a CCK-binding assay. Many synthetic analogues were made to increase the potency, selectivity, and oral activity, with the primary focus on functionizing the 1,4-benzodiazepine core of asperlicin. Among them, devazepide (MK-329), an orally active CCK-A antagonist (IC_{50} CCK-A 0.08 nM), and L-365,260, an orally active CCK-B antagonist (IC_{50} CCK-B 2.0 nM), were selected as clinical development candidates for the potential treatment of anxiety and panic disorders. However, both compounds suffered from limitations of solubility and bioavailability in phase I and II trials (Figure 14). A second generation of benzodiazepine CCK antagonists is currently being sought to overcome these disadvantages.[32,33] More recent work by Ferring Research Institute and Yamanouchi Pharmaceutical has focused on orally active CCK-B antagonists for the treatment of gastrointestinal−esophageal reflux and other gastrointestinal disorders. A number of new 1,4-benzodiazepin-2-one-based gastrin/CCK-B receptor antagonists related to the archetypal analogue L-365,260 has been synthesized, and these were shown to be potent and selective ligands for the gastrin/CCK-B receptor. Among them, YF-476 further demonstrated extremely potent dose-dependent effects in functional in vivo models of the pentagastrin-induced gastric acid secretion in rats and Heidenhain pouch dogs, and it has excellent oral bioavailability (Figure 14). YF-476 is currently under clinical investigation for the treatment of gastro-esophageal reflux disease.[34]

Apomorphine, a semisynthetic opium alkaloid, has long been known for its erectile activity at the effective dose of 2−6 mg (Figure 15). Physicians discovered the effect over 100 years ago, but found the drug, at a much higher dose (ca. 200 mg), to be more suitable for poison victims as an emetic (to induce vomiting) because it also caused serious nausea and vomiting. As a dopamine D2 agonist, apomorphine represents a significant advance in the treatment of well-developed motor fluctuations in Parkinson's disease through subcutaneous injections or infusions.[35] Apomorphine exerts its erectile effect at the central nervous system. Recently, Takeda Abbott Pharmaceuticals (TAP) developed a fast-acting sublingual formulation of apomorphine for treatment of male erectile dysfunction. The product is in Phase III trials, with regulatory filings expected for early 1999. It has been reported that the sublingual formulation, which is under patent protection minimizes the nausea-inducing effect of apomorphine.[36]

Effective and potent analgesics, especially for chronic pain, are urgently needed by the medical community. There are 30−40 million people in the U.S. with moderate to severe pain that is not controlled by common analgesics such as aspirin or ibuprofen. Thousands of patients with intense and unrelenting pain,

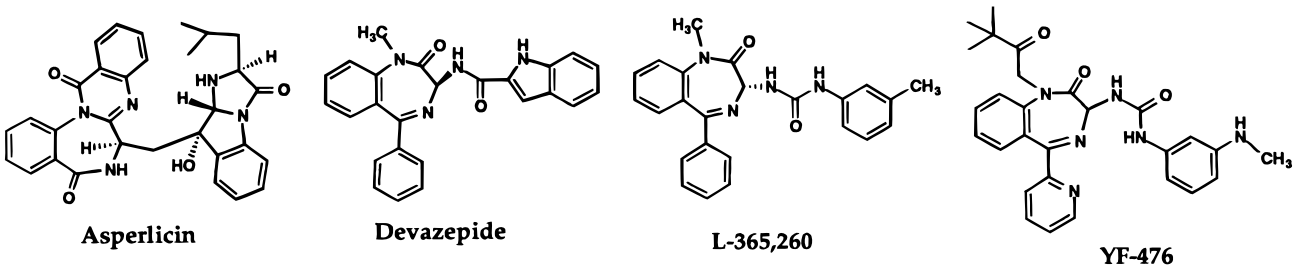


Figure 14.

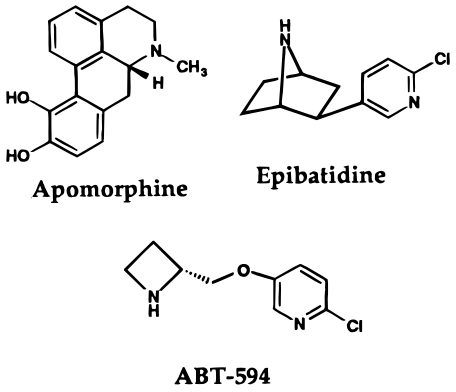


Figure 15.

such as that resulting from cancer or injury, have to depend on morphine despite its side effects. In 1976, Daly and co-workers at the U.S. National Institute of Diabetes and Digestive and Kidney Diseases found that an extract from the skin of an Ecuadorean frog, *Epipedobates tricolor,* could block pain 200 times more effectively than morphine and that the action was not mediated via the interaction with opioid receptors. When the structure of this potent alkaloid epibatidine was determined in 1992, its resemblance to nicotine triggered intensive chemical and biological interest (Figure 15). The analgesic action of epibatidine via a neuronal nicotinic acetylcholine receptor (nAChR), however, is accompanied by severe adverse effects such as paralysis, seizures, and even death, precluding its use as a human analgesic agent.[37] Researchers at Abbott Laboratories realized that the structure of epibatidine was close to a group of neuronal nAChR ligands synthesized in their discovery program related to Alzheimer's disease. After screening more than 500 compounds for their analgesic potential, the lead compound, ABT-594, was found to have antinociceptive properties equal in efficacy to those of morphine across a series of diverse animal models representing acute thermal, persistent chemical, and neuropathic pain states. In contrast to morphine, repeated treatment with ABT-594 did not appear to elicit opioid-like withdrawal or physical dependence (Figure 15). Thus, ABT-594 may be an analgesic that lacks the problems associated with opioid analgesia, and represents a new kind of pain reliever. ABT-594 has progressed to phase I trials in Europe for assessment of its safety in humans.[38]

3. Cardiovascular and Metabolic Disease Areas

An earlier example of the mechanism-based high-volume screen is the use of HMG CoA reductase as a biological target. The extensive screening and isolation chemistry studies by Endo and co-workers at Sankyo led to the discovery of mevastatin (compactin), with inhibitory activity against the enzyme, in 1976. This was the first of a novel class of compounds now known as statins used for the treatment of hyperlipoproteinemia. Compactin was isolated from a culture of *Penicillium* sp.,[39] and Sankyo's selective microbial hydroxylation method converted compactin into the more active pravastatin (Figure 16). Subsequently from a screening program at Merck for folic acid biosynthesis inhibitors for use as antibacterial/antiprotozoal agents, an interesting active *Aspergillus terreus* culture was discovered. The active extract was found to be also a potent inhibitor of HMG CoA reductase after testing in various biochemistry assays. In November 1978, lovastatin was discovered to be responsible for the activity (Figure 16). Pravastatin and lovastatin are proven to be reversible, competitive inhibitors of binding of the natural substrate, HMG CoA, to the enzyme. Since the discovery of pravastatin and lovastatin, extensive studies on these agents have continued well into today and have led to the introduction of several newer statin analogues such as simvastatin of Merck to the market. The statins have emerged as remarkable frontline therapy in reducing the risks of hypercholesterolemia and coronary heart disease.[40] Several large clinical trials have revealed profound therapeutic and preventive benefits of the statin class. In 1995, the first landmark primary prevention clinical trial, the West of Scotland Coronary Prevention Study (WOSCOPS) using Bristol-Myers Squibb's pravastatin (Pravacol) which included patients without established coronary heart disease, demonstrated that pravastatin reduces the risk of a first heart attack by 31% in patients with high cholesterol. A new secondary prevention trial, the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) study, is the first cholesterol-lowering trial to show a reduction in total mortality, strokes, and heart attacks in people who have heart disease. The LIPID study, which enrolled more than 9000 men and women with average or below average cholesterol levels who had either already suffered a heart attack or had a history of unstable angina, demonstrated that pravastatin significantly reduced the risk of death from any cause by 23%, stroke by 20%, death from coronary heart disease by 24%, and fatal and nonfatal heart attack by 29%. In the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) trial, Merck's lovastatin (Mevacor) has shown a significant reduction in the risk of myocardial infarction by 35% and unstable angina by 34% in healthy low-risk patients without coronary heart disease or raised cholesterol levels. While the LIPID trial is seen as confirmation of the benefits of statin treatment in patients who already

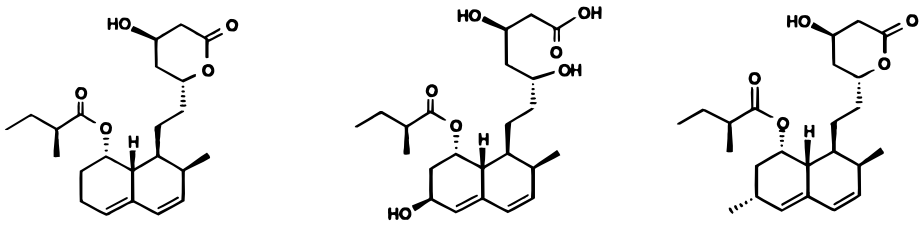


Figure 16.

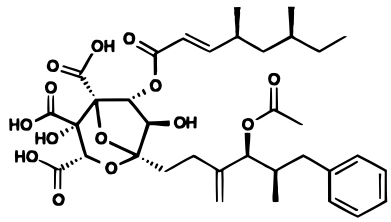


Figure 17.

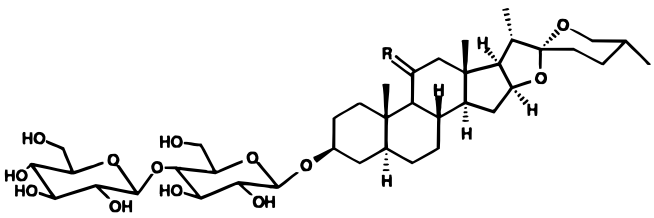


Figure 18.

have heart disease, the AFCAPS/TexCAPS trial extends eligibility for statin treatment to more patients who were not at particularly high risk of developing heart disease. In March 1998, the U.S. FDA cleared pravastatin for use in reduce the risk of stroke or transient ischemic attack, otherwise known as ministroke, in post heart attack patients who have normal cholesterol levels. The U.S. FDA also cleared simvastatin to reduce stroke in post heart attack patients with high cholesterol. The statins have indeed become one of the most successful examples of natural products developed as drugs in recent years.[41]

Squalene synthase (SQS) is the first enzyme involved in the conversion of farnesyl pyrophosphate (FPP) to squalene in the cholesterol biosynthetic cascade. Inhibition of this enzyme is considered potentially advantageous over HMG-CoA reductase inhibition, since it does not affect the biosyntheses of other isoprenoids essential for cell growth (e.g., ubiquinone). Glaxo and Merck virtually simultaneously discovered a group of fungal metabolites that exhibit extremely potent inhibition of SQS; they are known collectively as either squalestatins or zaragozic acids. Animal studies have demonstrated that some of the analogues are orally active in rodent models in inhibition of hepatic cholesterol synthesis and lowering of serum cholesterol. Several brief reports on the toxicity of zaragozic acids have been published, and both zaragozic acids A and C caused hepatotoxicity after periods of 1−2 weeks in dogs.[42] Synthetic manipulations of most of the functional groups in the core structure have been carried out. Further preclinical studies are aimed at the selection of a desirable development candidate (Figure 17).

In addition to inhibiting cholesterol biosynthesis, reducing dietary cholesterol intake by decreasing absorption at the intestinal wall exists as an alternative method for reducing low-density lipoprotein cholesterol (LDL-C), an important risk factor of atherosclerotic coronary heart disease. It is known that natural and synthetic saponins inhibit cholesterol absorption and reduce plasma cholesterol levels in experimental animals, and are, therefore, of potential pharmacological utility in the treatment of hypercholesterolemia. The synthetic saponin, β-tigogenin cellobioside (tiqueside; CP-88818), was first prepared at Pfizer as a prototypical form of a nonsystemic cholesterol-lowering agent and

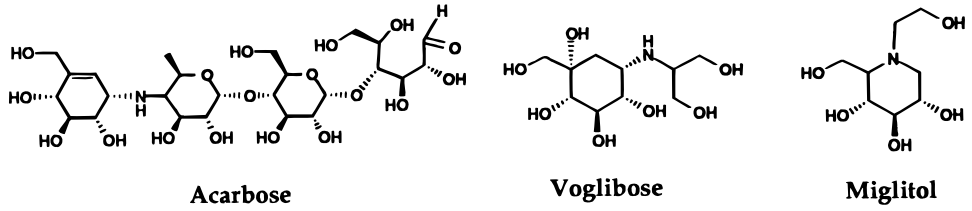


Figure 19.

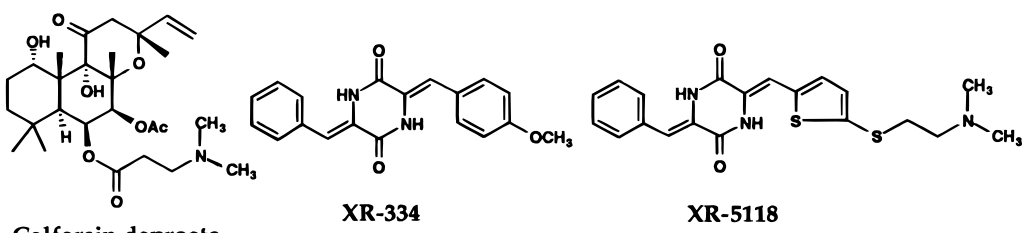


Figure 20.

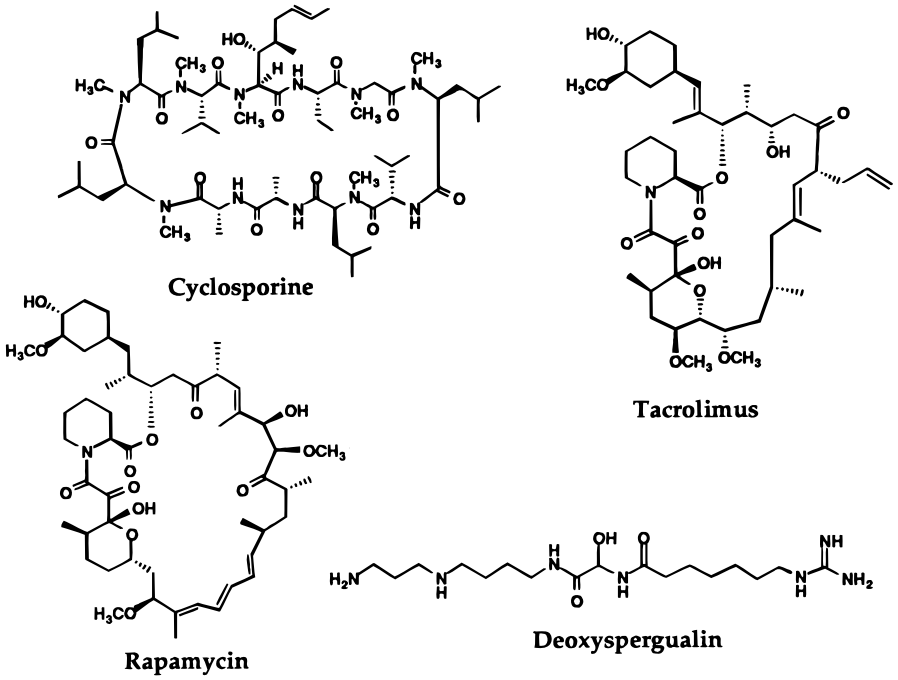


Figure 21.

is taken orally with meals. It was found that CP-88818 lowers plasma LDL/VLDL (very low density lipoprotein) cholesterol in a variety of both cholesterol-fed and chow-fed animals without affecting bile acid metabolism (Figure 18). The mechanism of action is reported to involve sequestration of both dietary and endogenous biliary cholesterol, but molecular targets of this class of compounds are still unknown. CP-88818 has been effective clinically, but the gram-quantity doses make it less attractive. CP-148623 (pamaqueside), a more potent lead compound in this class, was subsequently prepared (Figure 18). Phase I and II clinical studies have shown that pamaqueside is safe and effective, and it inhibits cholesterol absorption by 35−40% in normolipidemic individuals, with a resulting 10−12% decrease in LDL-C at 300 mg b.i.d.[43,44]

In the antidiabetes area, the past decade has witnessed the market introduction of several α-glucosidase inhibitors derived from natural products. Acarbose, a complex of oligosaccharides isolated from *Actinoplanes* sp., was discovered at Bayer from a search for α-glucosidase enzyme inhibitors (Figure 19). By inhibiting α-glucosidase, acarbose decreases the release of glucose from ingested carbohydrate and slows the increase of food-induced blood glucose levels. Acarbose is now approved in Germany, Japan, the U.S., and other countries and has been used as adjuvant therapy in diabetes.[45,46] Several known aminoglycoside and pseudo-oligosaccharide antibiotics were also found to exhibit potent inhibitory effects against intestinal α-glucosidase. Takeda's voglibose is the synthetic analogue of valiolamine, a *Streptomyces* aminocyclitol; voglibose was introduced in Japan in 1993 for the treatment of diabetes and obesity, and it is much more potent and has fewer side effects than acarbose[47] (Figure 19). Miglitol is a hydroxyethyl derivative of 1-deoxynojirimycin synthesized at Bayer; satisfactory phase III results were obtained, and Bayer has submitted the NDA of miglitol for regulatory approval for the treatment of type I/II diabetes and expects to market it in 1998[48] (Figure 19).

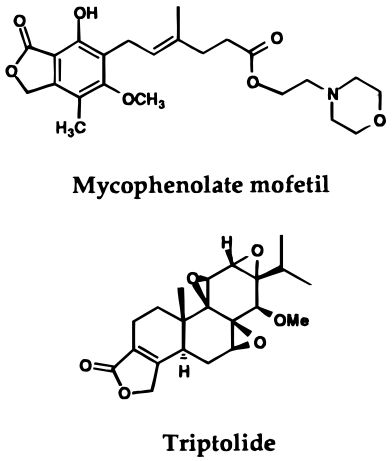
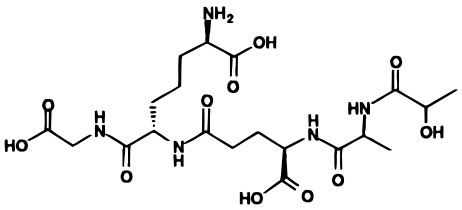


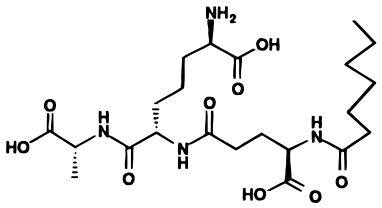
Figure 22.

Shaman Pharmaceuticals, in collaboration with Lipha/Merck and Ono Pharmaceuticals, has focused its discovery of oral antihyperglycemic agents for the treatment of Type II diabetes primarily on the screening of medicinal plants in animal models.[49] A number of orally active natural products were shown to reduce blood glucose in these in vivo models of Type II diabetes. In October 1997, Shaman filed the IND (Investigational New Drug) status for SP-134101 (structure not available), the first clinical candidate to emerge from its antidiabetes discovery effort. Preclinical data have demonstrated that the compound lowers blood glucose and triglycerides and also lowers blood pressure in a hypertensive animal model. Phase I clinical trials for SP-134101 began in January 1998.

Forskolin (colforsin) is a diterpene natural product isolated from the Indian plant *Coleus forskohlii* at Hoechst's research laboratories in India. The compound was first found to have blood pressure lowering and cardioactive properties. Later, forskolin was found as

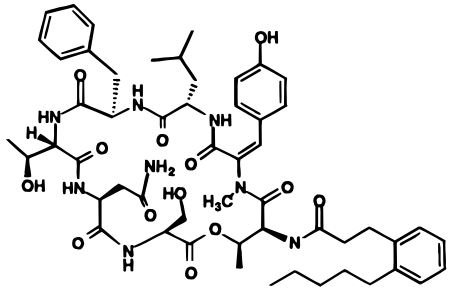


FK-156

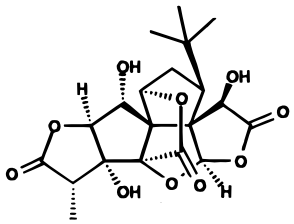


FK-565

Figure 23.



FK-224

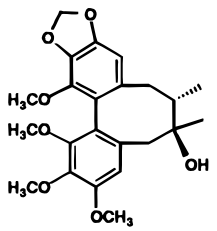


Ginkgolide B

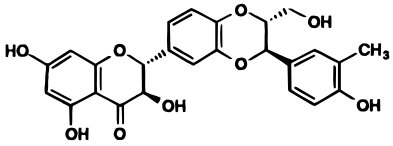
Figure 24.

a potent adenylate cyclase activator.[50] Nippon Kayaku's semisynthetic efforts generated a water-soluble forskolin derivative, colforsin daproate (NHK-477) (Figure 20). The compound was shown to have reversible effects on the respiratory, circulatory, and autonomic nervous systems. Preliminary clinical trials of colforsin daproate demonstrated beneficial hemodynamic effects in heart failure patients. Colforsin daproate was then brought into phase III clinical trials in Japan for treatment of cardiac insufficiency and phase II trials for treatment of asthma.[51]

Plasminogen activator inhibitor 1 (PAI-1) binds to and inhibits endogenous tissue plasminogen activator (tPA), reducing the fibrinolytic and clot-clearing activity of blood. Epidemiological evidence shows that PAI-1 is a risk factor in thrombotic disease, with elevated levels of PAI-1 present in patients with thrombotic disorders, such as deep vein thrombosis, unstable angina, and myocardial infarction. Thus, inhibition of PAI-1 activity in vivo can result in enhanced thrombolysis and a reduction in reocclusion. However, so far there are no suitable small-molecule agents that can block PAI-1 activity available besides monoclonal antibodies and peptides. Extensive screening efforts for anti PAI-1 activity were conducted at Xenova, a British natural product drug discovery company. A known *Streptomyces* diketopiperazine, XR-334, was found to be active in the screen. Medicinal chemistry studies further led to the generation of a series of XR-5118 analogues (Figure 20). Xenova and its partners have reported the correlation between inhibition of PAI-1 by these analogues and dissolution of clots in a preclinical model and demonstrated that XR-5118 binds to PAI-1, reduces plasma PAI-1 activity levels, promotes endogenous fibrinolysis, and reduces post-thrombolysis thrombus growth in rabbits. XR-5118 is thus considered as the first nonpeptide compound with significant anti-PAI-l activity in vivo and serves as a template for the development of more potent anti-PAI-1 agents with acceptable pharmacokinetic properties as suitable candidates of human clinical trials.[52] In February 1998, Eli Lilly and Xenova announced the formation of a strategic partnership for the development of small-molecule inhibitors of PAI-1 as potential antithrombotic drugs for chronic use.

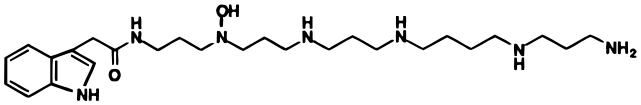


Gomisin A

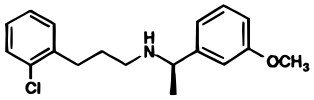


Silybin

Figure 25.



Agatoxin 489



Norcalcin

Figure 26.

4. Immunological, Inflammatory, and Related Disease Areas

It is widely claimed that the recent increase of successful organ transplantations is largely due to the effective use of cyclosporine. Since the introduction of cyclosporine A (Sandimmune), as many as 10 new

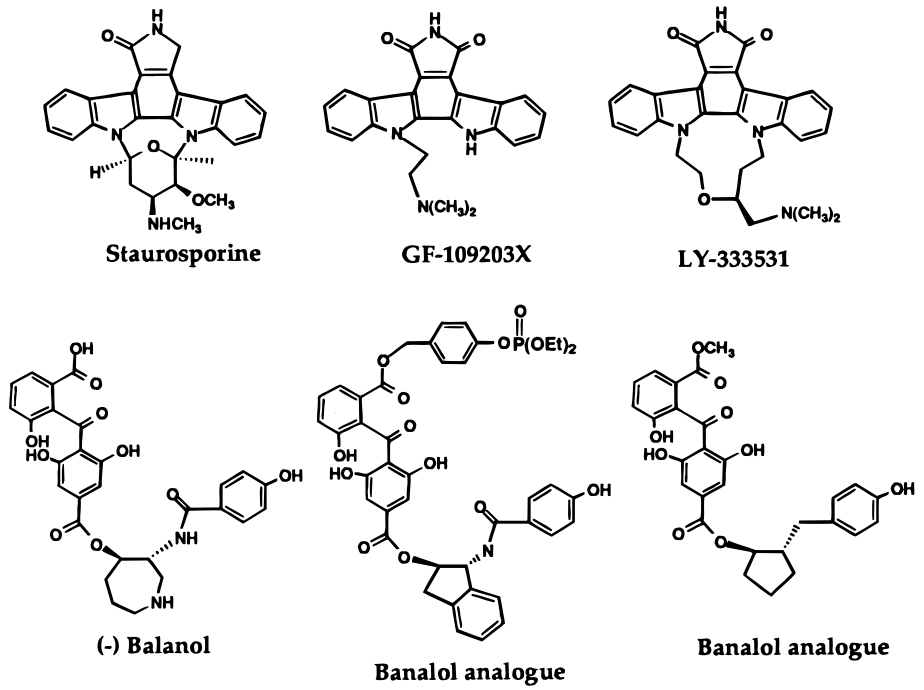


Figure 27.

nonpeptide drugs have been brought into various stages of clinical and preclinical development. Natural products have thus become a rich source of immunosuppressive agents for organ transplantation. With the approval of tacrolimus (FK-506) and other new agents, the outlook for successful organ transplantation is even more promising. Cyclosporine, a fungal cyclic oligopeptide discovered at Sandoz, and sirolimus (rapamycin), a macrolide antibiotic, discovered at Wyeth-Ayerst, were originally recognized as antifungal agents in the 1970s. In the 1980s, their remarkable immunosuppressant activities were recognized and vigorously pursued (Figure 21). Rapamycin is currently being investigated in clinical trials for prophylaxis of renal transplant rejection. Its mechanism of action is the blockage of response of T and B cells to cytokines, thereby preventing cell-cycle progression in G1 and consequently cell proliferation. The drug exhibits synergy with cyclosporine in vitro as well as in animal and clinical studies. In phase II trials, the combination of rapamycin−cyclosporine therapy reduced the frequency of acute rejection episodes and permitted withdrawal of concomitant corticosteroid therapy.[53] Tacrolimus, a macrolide antibiotic, was discovered at Fujisawa by a mechanism-based screening method, suppression of the mouse mixed lymphocyte reaction, followed by the inhibition assay of interleukin (IL) 2 production.[54] Cyclosporine, tacrolimus, rapamycin, and their analogues, acting as molecular glue, form macromolecular complexes through their binding proteins. The complexes of cyclosporine and tacrolimus selectively inhibit T-cell proliferation by blocking cytokine (particularly IL-2) synthesis at the transcriptional level. However, the complex of rapamycin, acting at a more distal point in the lymphocyte activation pathway, blocks the activity of cyclins, which regulate cell division. Cyclosporine and tacrolimus are now approved worldwide, and rapamycin, currently in phase III clinical studies, is near its regulatory approval[55] (Figure 21). SDZ RAD, a rapamycin analogue,

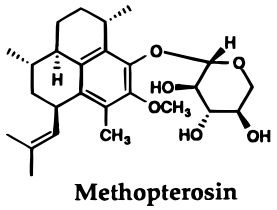


Figure 28.

was recently brought into phase I clinical development by Norvartis as an orally active immunosuppressant. Similar to rapamycin, SDZ RAD inhibits vascular change and acts synergistically with cyclosporine A in animal models, indicating potential for the prevention of chronic rejection.[56] Gusperimus (deoxyspergualin), a natural product discovered by Nippon Kayaku initially as an antitumor agent, was found to act at the G_o phase of the T cell cycle, as the cell prepares for division, and interferes the APC (antigen-presenting cell)-TCR (T cell receptor) interaction. Gusperimus was launched in 1994 in Japan as an immunosuppressant for the treatment of kidney transplant rejection. In North America, Bristol-Myers Squibb conducted phase III trials for the treatment of graft rejection and phase I/II trials for rheumatoid arthritis[55,57] (Figure 21).

Mycophenolic acid, a phenolic fungal metabolite with known antitumor, antiviral, and immunosuppressive effects, is an inhibitor of two enzymes, inosine monophosphate dehydrogenase (IMP-DH) and guanylate (GMP) synthetase. These enzymes are involved in the generation of GDP, GTP, and dGTP but are not involved in the salvage pathway of purine biosynthesis. Since lymphocytes, unlike other cells, do not utilize the salvage pathway, treatment with mycophenolic acid and its morpholinoethyl ester, mycophenolate mofetil (RS-61442, Roche-Syntex), a prodrug that is rapidly converted to mycophenolic acid, reduces the guanine nucleotide pool in lymphocytes, leading to the inhibition of DNA synthesis (Figure 22). Mycophenolate mofetil

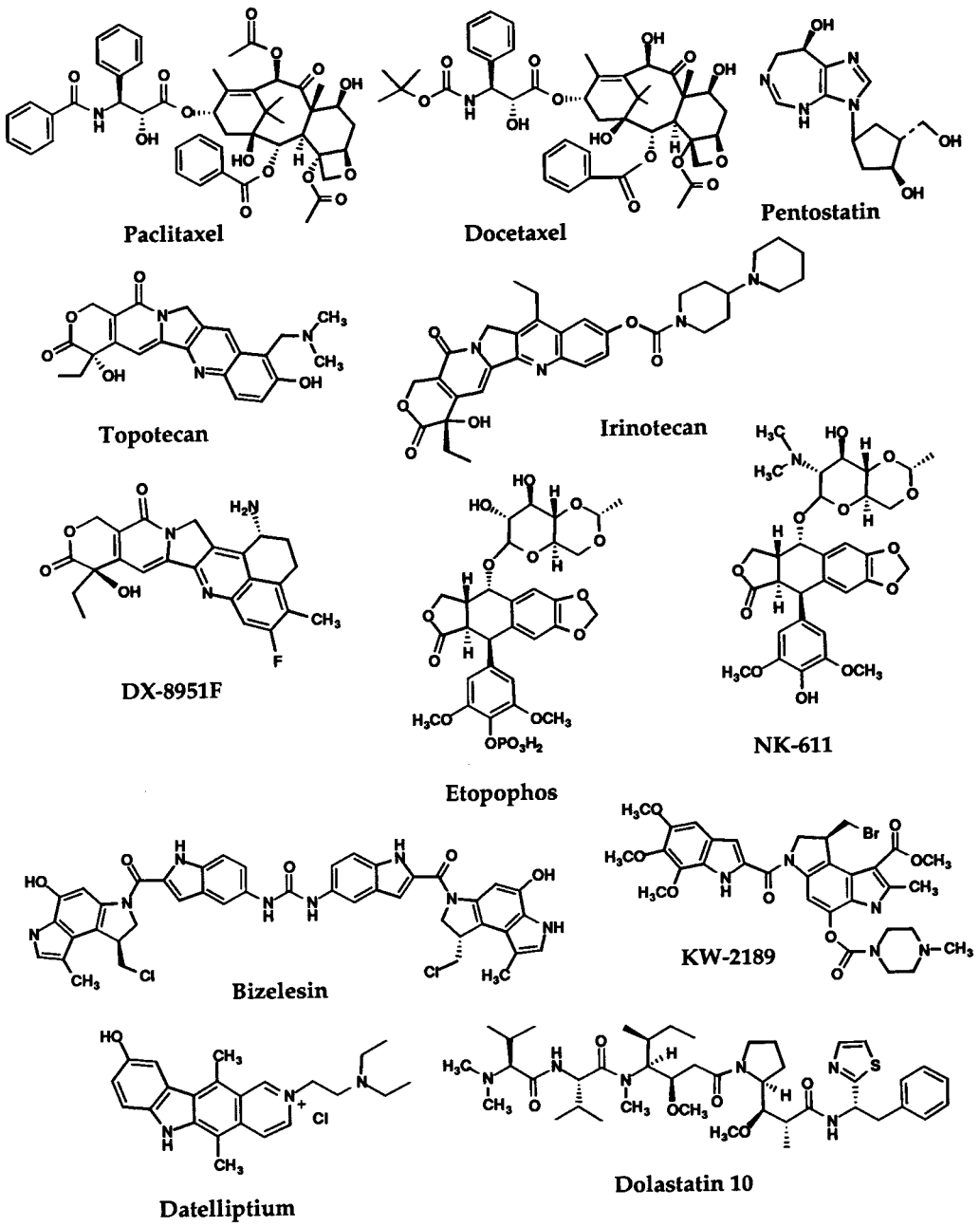


Figure 29.

was brought into clinical development and approved for the prevention of acute renal allograft rejection when given in combination with cyclosporine and steroids. Several studies also demonstrated that the agent is effective in the treatment of refractory rejection in renal, heart, and liver transplantation recipients and may have efficacy in the treatment of chronic rejection as well.[57]

Triptolide is a major active component isolated from the Chinese plant *Tripterygium wilfordii*, a plant traditionally used for treatment of rheumatoid arthritis. A variety of formulations, including capsules, topical liquids, and patches of the plant extract, were developed in mainland China and are shown to be effective in the treatment of patients with inflammatory and autoimmune diseases. Triptolide was demonstrated to significantly inhibit arthritis in animal models. The immunosuppressive effect is mediated by inhibition of IL-2 receptor expression and interference with IL-2 signal transduction. The compound was also found to have potent cytotoxicity (Figure 22). Clinical development of injectable triptolide is being conducted at the Fujian Acadamy of Medical Science in Fuzhou and several other medical institutions in the People's Republic of China. A more desirable analogue is also being sought.[58]

The immunostimulating peptide FK-156, a metabolite of *Streptomyces* sp., was discovered through its antitumor activity against murine P388 leukemia by Fujisawa. The activity appeared to be a host-mediated effect. FK-565, a synthetic tripeptide analogue of FK-156, was shown to act by phagocyte, especially macrophage, activation, as it induced IL-6 and granulocyte macrophage colony-stimulating factor (GM-CSF) release from lipopolysaccharide-stimulated peritoneal macrophages, and augmented natural killer cells, macrophage, and T cells in vivo. FK-565 was brought into phase II clinical

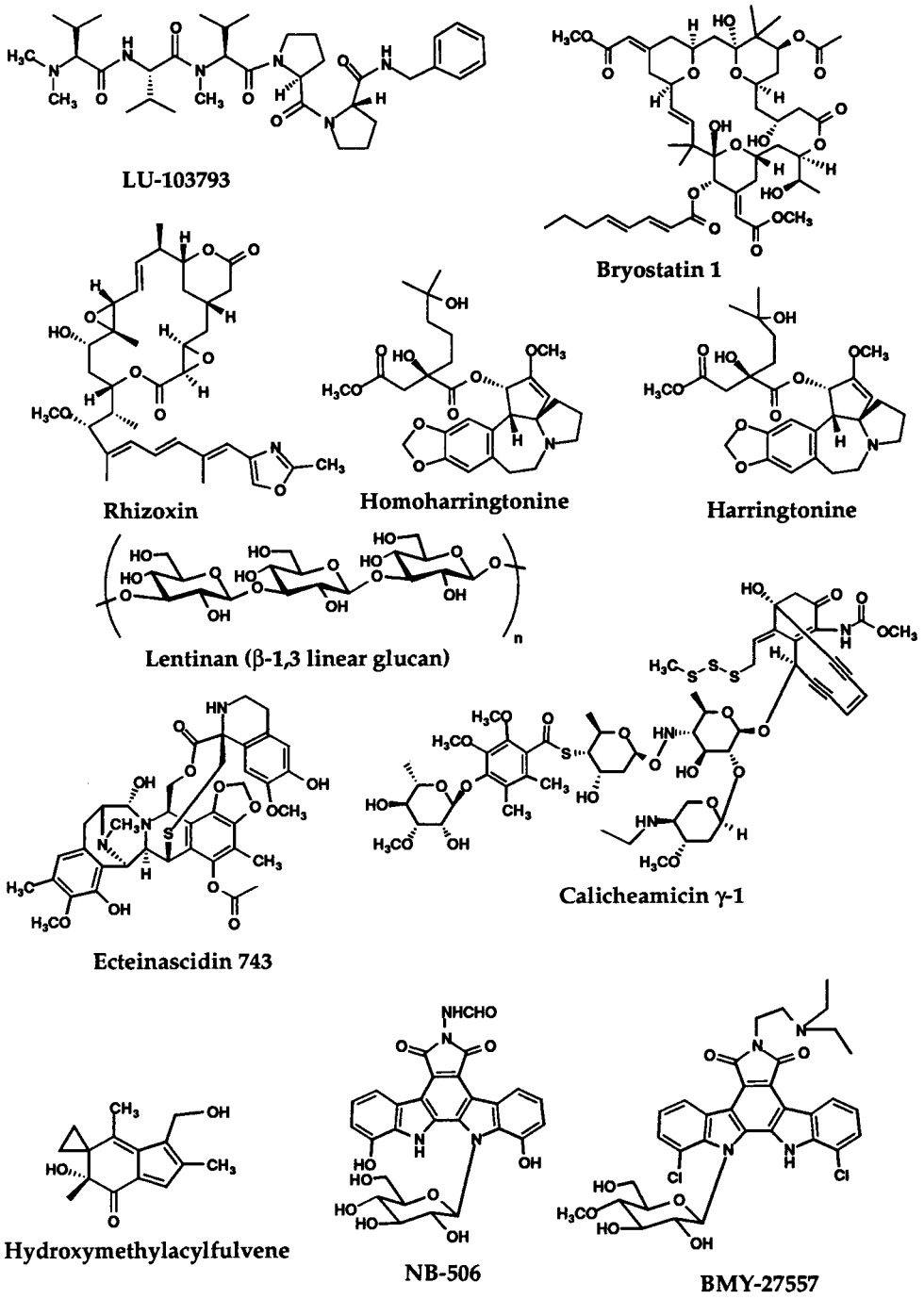


Figure 30.

trials in the U.S. for the treatment of cancer and AIDS[59] (Figure 23).

Fujisawa's FK-224 (FR-155224), a dual antagonist of both substance P and neurokinin A (NKA) receptors, is the tetrahydrogenated derivative of WS9326A, a cyclic depsiheptapeptide from *Streptomyces violaceoniger* discovered through a substance P binding assay (Figure 24). Results from in vivo studies in various animal models suggested that FK-224 may have greater potential in the treatment of respiratory diseases such as asthma and bronchitis. Phase II trials in Japan and Europe with FK-224 administered as an aerosol have shown a significant antiasthmatic effects.[60,61]

The Chinese tree *Ginkgo biloba* has been used therapeutically for thousands of years. More recently, extracts of the leaves have become available in many European countries as over-the-counter products for the treatment of cerebral vascular insufficiency and tinnitus. The dried ripe seeds of *G. biloba* have been used in traditional medicine as an antiasthmatic. Tebonin, one of the plant leaf extracts, is marketed for dementia and is among the most prescribed medicines in Germany, with annual sales of approximately 200 million U.S. dollars. Recently, the first randomized placebo-controlled trial of the plant extract, using current standard methods of measuring cognitive function, has shown that the drug does appear to have some benefit in dementia.[62] Ginkgolides, a class of unique diterpene cagelike molecules, are among the natural products isolated from the leaves. It was found in the late 1980s that ginkgolides represent a group of highly selective platelet-activating factor (PAF) receptor antagonists.

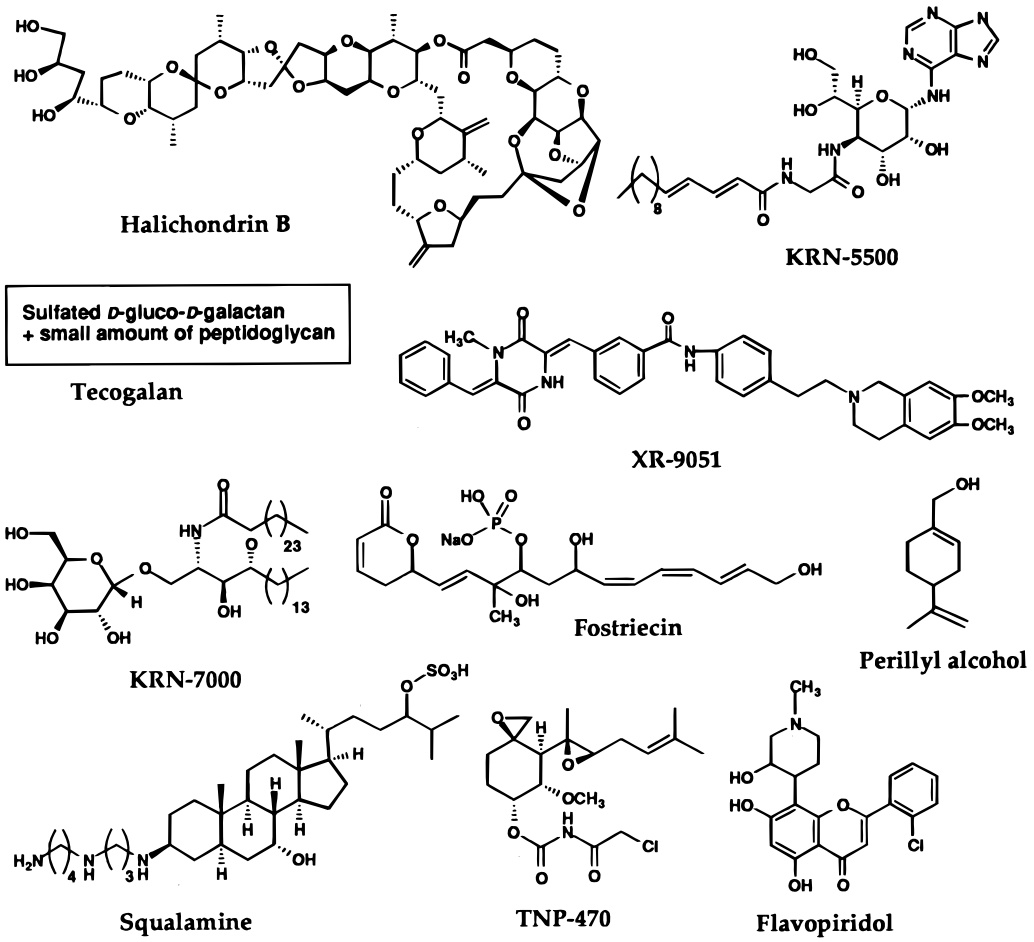


Figure 31.

Among them, ginkgolide B (BN-52021) has been advanced to phase III clinical trials by the French pharmaceutical company Ipsen-Beaufour for the treatment of septic shock in patients with severe sepsis caused by Gram-negative bacterial infections (Figure 24). Good results were also found in inflammatory and autoimmune disorders.[63,64]

Gomisin A is a lignan derivative isolated from the dry fruits of *Schisandra chinensis*, a traditional Chinese medicine used for treatment of liver intoxication (Figure 25). Gomisin A was found to protect against hepatocarcinogenesis and liver damage in various animal models. The mechanism of action includes induction of hepatic metabolizing enzyme systems, increase in the proliferation of the endoplasmic reticulum of liver cells, and antioxidant activity. Advanced preclinical studies on Gomisin A and its analogues were conducted to explore its potential as a human hepatoprotectant by Tsumura in Japan.[65]

Another hepatoprotectant, Idb-1016, is also derived from a plant source, namely, the fruits of *Silybum marianum*. Idb-1016 (silipide) is a complex of the flavonolignan antioxidant, silybin, and phosphatidylcholine (Figure 25). It is currently in phase III clinical development as an antihepatotoxic agent by Inverni Della Beffa (Synthelabo) in Italy.[66]

Hyperparathyroidism is a growing medical concern with bone loss, bone pain, muscle weakness, and other chronic symptoms. In hyperparathyroidism, excess parathyroid hormone triggers pathological changes in bone and in the kidney, thereby increasing the level of calcium in the blood. Calcimimetic compounds activate calcium receptors leading to suppression of secretion of parathyroid hormone, which in turn reduces the level of serum calcium. Norcalcin (NPS *R*-568), a calcium receptor agonist of NPS Pharmaceuticals, arose from its screening program of natural and synthetic products against calcium receptor binding on parathyroid cells. NPS initiated the program with natural products having polycations such as aminoglycosides and polyamines and utilized a unique library of invertebrate venoms isolated from wasps and spiders, leading to a group of early calcium receptor agonists such as agatoxin 489 and other arylalkylamines. As a result of further structure−activity relationship studies, a lead compound, norcalcin, was discovered and subsequently brought into clinical development (Figure 26). Results of preliminary phase I/II clinical trials for hyperparathyroidism indicated that norcalcin is safe and well tolerated by all volunteers. It also had the desired effects of decreasing plasma parathyroid hormone and serum calcium concentrations and in raising levels of calcitonin in kidney dialysis patients with hyperparathyroidism. Further phase II clinical studies in the U.S. were carried out by NPS' licensee, Amgen. Results have indicated that norcalcin's low oral bioavailability and liver-based metabolism contributed to variable plasma levels of the drug. In September 1997, NPS and Amgen decided to initiate new clinical trials in 1998 on a second-generation calcimimetic compound of norcalcin

Table 1. Natural Products-Based Antitumor Drugs

name	mode of action/activity	development status	ref
paclitaxel (plant diterpene)	promotes tubulin assembly, stabilize microtubules	launched 1993, first line ovarian cancer, first-line nonsmall-cell lung cancer, also for breast and Kaposi's sarcoma	79
docetaxel (paclitaxel analogue)	promote tubulin assembly, stabilize microtubules	approved in U.S., 1996, second line breast cancer	80
pentostatin (*Streptomyces* metabolite)	adenosine deaminase inhibitor	launched 1991, hairy cell leukemia	81
topotecan (camptothecin analogue)	topoisomerase I inhibitor	approved in U.S. in 1996, second line ovarian cancer	82
irinotecan (camptothecin analogue)	topoisomerase I inhibitor, good water solubility	launched in Japan in 1994, approved in 1996 in U.S., colorectal, lung, and ovarian cancers	83
DX-8951F (camptothecin analogue)	topoisomerase I inhibitor, claimed to have greater potency than topotecan and irinotecan	phase I in U.S.	84
etopophos (podophyllotoxin analogue)	topoisomerase II inhibitor, water soluble	approved in 1996, lung cancer	85
NK-611 (podophyllotoxin analogue)	topoisomerase II inhibitor, good water solubility	phase II, lung cancer	86
bizelesin (dimeric CC-1065 analogue)	binds to the minor groove of DNA and alkylates adenine N3 (sequence specific)	phase I	87
KW-2189 (duocarmycin analogue)	binds to the minor groove of DNA and alkylates DNA (sequence specific)	phase II	88
datelliptium (ellipticine analogue)	DNA damage mediated by inhibiting topoisomerase II, water soluble	phase II	89
dolastatin 10 (linear tetrapeptide from sea hare)	inhibits microtubule assembly and binds to tubulin at the vinblastine site	phase I	90
LU-103793 (dolastatin 15 analogue)	inhibits microtubule assembly and bind to tubulin at the vinblastine site, water soluble	phase I	90, 91
bryostatin 1 (macrolide from marine bryozoan)	partial agonist of protein kinase C	phase I−II, in U.S., U.K., melanoma	90
rhizoxin (fungal metabolite)	antimitotic and binds to tubulin at the vinblastine site	phase II	91
homoharringtonine, harringtonine (plant alkaloids)	possibly involved in apoptosis	phase III in China, early phase in U.S., leukemia	92
lentinan (polysaccharide from mushroom)	immunostimulant	launched in Japan for gastric cancer with UFT (tegafur and uracil mixture), phase II in U.S.	93
CMB 401 or CDP671 (recombinant monoclonal antibody conjugate of calicheamicin γ−1)	reduction of enediyne triggers generation of biradical, causing sequence selective DNA scission	phase II for ovarian, breast, and lung cancers	94−95
BMY-27557 (rebeccamycin analogue)	indolocarbazole type inhibitor of topoisomerase I, DNA intercalator	phase I	96
NB-506 (BE−13793C analogue)	indolocarbazole type inhibitor of topoisomerase I, DNA polymerase and RNA polymerase inhibitor	phase I−II	97−99
ecteinascidin 743 (isolated from marine tunicate *Ecteinascidia turbinata*)	alkylating agent, DNA minor grove-, guanine-specific	phase I	100
tecogalan (sulfated polysaccharide−peptidoglycan from *Arthrobacter* sp.)	angiogenesis inhibitor	phase I−II, adjunct antitumor agent	101
hydroxymethylacylfulvene (sesquiterpene illudin analogue from mushroom)	cytotoxic	phase I	102
halichondrin B (macrocyclic polyether from sponge)	antimitotic, binds to tubulin, inhibit microtubule assembly	preclinical	78
XR-9051 (analogue of *Streptomcyces* diketopiperazine XR-334)	multiple drug resistance (MDR)-modulating agent	phase I, adjunct tumor therapy with cytotoxic agent	103−104
KRN-5500 (spicamycin derivative)	cytotoxic	phase I, gastrointestinal cancers	105−106
KRN-7000 (monoglycosylceramide)	immunostimulatory and antimetastatic activity	phase I, liver cancer	107
fostriecin (polyene lactone from *Streptomcyces*)	DNA topoisomerase II inhibitor	phase I	108
TNP-470 (derivative of fungal metabolite fumagillin)	angiogenesis inhibitor, inhibits growth factor-induced proliferation of endothelial cells	phase III for Kaposi's sarcoma and other cancers	109−111
squalamine (aminosterol antibiotic isolated from the shark)	angiogenesis inhibitor, prevents growth factor-induced endothelial cell proliferation	phase I trial in combination with cytotoxic agents	112
flavopiridol (synthetic flavone related to plant alkaloid rohitukine)	cyclin dependent kinase (cdk1, cdk2, cdk4, cdk7) inhibitor	phase I	113
perillyl alcohol (limonene analogue)	cytotoxic	phase II for advanced carcinomas of the breast, prostate and ovary	114

Table 2. Competitiveness: Natural Products vs Synthetic and Combinatorial Libraries

competing area	characteristics of natural products research	enhancement of competitiveness of natural products
lead generation time	• hit begins with unknown mixture • bioassay guided fractionation relates to unknown factors (interfering/false positive materials, low concentration of active compound) • usual time frame of isolation and structure elucidation: ~6 months, almost same as lifetime of high-throughput screening	• improve diversity and quality of sample source • improve screen suitability to natural products • reduce incidence of false positive and interfering materials • accelerate dereplication • automate and standardize early isolation steps • shorten the time frame of isolation and structure elucidation to 3 month
lead novelty	• qualitatively distinct and complementary to synthetic and combinatorial libraries • often new active chemotype	• a clear advantage • a clear advantage
analogue development	• some natural anti-microbials, cytotoxics directly qualify as drug candidates • starting material sometimes not easily available • defined and complex 3D structure, not an easy synthetic target	• antimicrobial, antitumor (e.g., cell cycle) areas are still the strength of natural products research program • large fermentation • plant cell culture • aquaculture, mariculture • biotransformation, precursor−analogue directed biosynthesis, mutasynthesis, analogue production by genetic engineering
intrinsic value	• novelty and often have potency • especially benefit the therapeutic areas where no valid synthetic leads exist	• a clear advantage • a clear advantage, many natural product derived drugs are considered as disease area's first: e.g., statins, cyclosporine, and FK-506

that has better bioavailability. Kirin Brewery, NPS' licensee in Asia, is also to conduct phase I trials in Japan.[67−70]

Protein kinase C (PKC), a family of closely related serine and threonine kinases, is a key element in the signal transduction pathway. Overactivation of some PKC isoenzymes has been postulated to occur in several disease states such as cancer, inflammation, viral infection, immune and CNS disorders, and cardiovascular malfunction. Selective inhibition of overactivated PKC isoenzymes may offer a unique therapeutic approach to these diseases.[71] Staurosporine, an indolocarbazole-type *Streptomyces* metabolite, was identified as a potent PKC inhibitor in 1986 but was found to be nonselective to various protein kinases. However, it remained the most potent prototype lead until the discovery of (−)-balanol, a selective PKC inhibitor. GF-109203X (Go6850) was synthesized at Godecke Preclinical Research in Germany in a search for staurosporine analogues with improved kinase selectivity. GF-109203X proved to be a highly potent and selective inhibitor against PKC. The efficacy of GF-109203X was demonstrated by in vivo studies using various inflammatory animal models[72] (Figure 27). Further preclinical studies are continuing for selection of a desirable clinical development candidate. Recently PKC, especially the β isozyme, was found to be involved in vascular complications such as diabetic retinopathy. Eli Lilly's LY-333531, a new macrocyclic analogue of staurosporine, was discovered to be a potent and specific inhibitor of the PKC β isozyme (Figure 27). When administered orally, LY-333531 normalized the glomerular filtration rate, albumin excretion rate, and retinal circulation in diabetic rats in a dose-responsive fashion in parallel with its PKC inhibitory activity.[73,74] The development of LY-333531 thus points to a promising future for the further development of PKC inhibitors as clinically useful agents for PKC-mediated human diseases. (−)-Balanol is a novel fungal metabolite that was discovered as one of the most potent naturally occurring PKC inhibitors by Sphinx Pharmaceuticals[75] and later by Roche's Research Center in Japan.[76] Total synthesis and systematic structural modifications of balanol were carried out for the development of promising analogues with therapeutic applications. Four to five hundred balanol analogues were prepared; replacement of the azepine ring with other ring systems, modification of the carboxyamide functionality, and prodrug design at the benzophenone carboxylic acid moiety resulted in several interesting lead compounds with potent enzyme and cellular activity during the continuing lead optimization study at Sphinx Pharmaceuticals, now a part of Eli Lilly[71] (Figure 27).

Methopterosin (OAS-1000 or VM-301) is a semisynthetic analogue of pseudopterosin, a marine natural product isolated from a Gorgonian coral (Figure 28). The antiinflammatory action of this compound was studied in PMA (phorbol myristate acetate)-induced mouse ear edema and DNCB (2,4-dinitrochlorobenzene)-induced dermatitis in guinea pigs, and the activity was attributed to its inhibition of leukotriene B4 from neutrophils.[77] The compound is under clinical development by VIMRx for wound healing, including full and partial thickness wounds.

5. Oncological Disease Area

Identification of agents active against human cancers has been largely dependent upon the screening of natural products, and their analogue development in experimental tumors. Historically, natural products have been invaluable as agents to regulate the cell-cycle, leading to fruitful achievements in the antitumor area in particular. With a better understanding of many molecular details of the cycle, natural products are likely to offer continued promise for the future. Since the newly discovered antitumor natural products were the subject of a recent excellent review,[78] the following section is intended only to briefly tabulate the status of significant examples of natural product drug development from the perspective of the pharmaceutical industry (Table 1, Figures 29−31). Recent drug development efforts have produced new generations or improved

versions of historically well-known antitumor agents, such as the vinca alkaloids (e.g., vinorelbine), anthracyclines, mitomycin analogues (e.g., KW-2149, BMS-181174), bleomycin analogues (e.g., liblomycin) and others; however, these aspects will not be discussed in this review.

Conclusion

As a recent visible trend, pharmaceutical companies, especially those research-based major global players, are placing unprecedented emphasis on innovative drug discovery as the primary driver of industry growth. These companies are aiming at shortening their discovery timelines by half and tripling the number of novel chemical entities delivered to their development process. According to a recent Anderson Consulting analysis,[115] in order to keep pace with an annual industry growth rate of 10%, major global pharmaceutical companies will need to launch three to five significant new chemical entities (NCEs or new products) per year. However, between 1990 and 1994, the top companies launched, on average, only 0.45 NCEs per year. More importantly, despite the escalating R&D efforts in the pharmaceutical industry, there is an urgent need to identify novel, active chemotypes as leads for effective drug development in many therapeutic areas. For example, in the antiinfective area in the past several years, the rapid emergence and spread of resistant nosocomial and community-acquired pathogens has generated a great threat to public health worldwide; yet, for these reemerging and other new infectious diseases there are no effective therapies available today. In the oncology area, there are great unmet medical needs in the treatment of solid neoplasms such as cancers of the breast, lung, colon, pancreas, and liver and the need to develop novel cancer chemotherapeutic agents to overcome the development of multidrug resistance, which increasingly occurs in the chemotherapy of various tumor types.

The major achievements of natural products research of the past 10−15 years summarized in this review have clearly demonstrated that natural products represent an unparalleled source of molecular diversity to drug discovery and development and are indeed complementary to those emerging molecular sources, such as combinatorial libraries. On the other hand, natural products research as a part of drug discovery effort faces increasing challenges; the most significant challenge to this field is how to improve its competitiveness with synthetic and combinatorial libraries; the disadvantage relative to synthetic and combinatorial libraries can be the time taken to isolate and characterize active compounds from the complex mixtures that are characteristic of natural product extracts. Table 2 is intended to analyze these challenges and possible ways for enhancing the competitiveness of natural products research. Meeting the challenges will require scientists working in this field to embrace bold new research strategies and processes and change the research culture into a high-performance paradigm. As a result, natural product research should continue to be a very fruitful endeavor and an active contributing force in the quest for innovative and productive drug discovery and development.

References and Notes

(1) Cragg, G. M.; Newman, D. J.; Snader, K. M. *J. Nat. Prod.* **1997**, *60*, 52−60.
(2) Hung, D. T.; Jamison, T. F.; Schrieber, S. L. *Chem. Biol.* **1996**, *3*, 623−639.
(3) Farnsworth, N. R.; Akerele, O.; Bingel, A. S.; Soejarto, D. D.; Guo, Z. *Bull. WHO* **1985**, *63*, 965−981.
(4) Nicas, T. I.; Zeckel, M. L.; Braun, D. K. *Trends Microbiol.* **1997,** *5*, 240−249.
(5) Canepari, P.; Boaretti, M.; del Mar Lleo, M.; Satta, G. *Antimicrob. Agents Chemother.* **1990**, *34*, 1220−1226.
(6) Collins, L. A.; Eliopoulos, G. M.; Wennerstein, C. B.; Ferraro, M. J.; Moellering, R. C. *Antimicrob. Agents Chemother.* **1993**, *37*, 1364−1366.
(7) Romeo, B.; Kaschube, M.; Cavenaghi, L.; Borgonovi, M.; Jaumac, B.; Monteil, H.; Gardner, S.; Seppala, A. *33rd Intersci. Conf. Antimicrob. Agents Chemother.* New Orleans, LA, Oct 17−20, 1993, Abstr. No. 448.
(8) Patel, M. *Natural Products Discovery, New Technology to Increase Efficiency and Speed*, Coronado, CA, Mar 17−18, 1997, Abstr. No. 9.
(9) Denning, D. W. *J. Antimicrob. Chemother.* **1997**, *40*, 611−614.
(10) Lucas, R.; DeSante, K.; Hatcher, B.; Hemingway, J.; Lachno, R., Brooks, S.; Turik, M. *36th Intersci. Conf. Antimicrob. Agents Chemother.* New Orleans, LA, Sep 15−18, 1996, Abstr. No. F50.
(11) *Scrip* **1997**, *2197*, 6.
(12) Furumai, T.; Saitoh, K.; Kakushima, M.; Yamamoto, S.; Suzuki, K.; Ikeda, C.; Kobaru, S.; Hattori, M.; Oki, T. *J. Antibiot.* **1993**, *46*, 265−274.
(13) Ueki, T.; Numata, K.; Sawada, Y.; Nishio, M.; Ohkuma, H.; Toda, S.; Kamachi, H.; Fukagawa, Y.; Oki, T. *J. Antibiot.* **1993**, *46*, 455−464.
(14) Kinsman, O. S.; Chalk, P. A.; Jackson, H. C.; Middleton, R. F.; Shuttleworth, A.; Rudd, B. A. M.; Jones, C. A.; Noble, H. M.; Wildman, H. G.; Dawson, M. J.; Stylli, C.; Sidebottom, P. J.; Lamont, B.; Lynn, S.; Hayes, M. V. *J. Antibiot.* **1998**, *51*, 41−49.
(15) *Drugs Future* **1997**, *22*, 1221−1225.
(16) Currens, M. J.; Gulakowski, R. J.; Mariner, J. M.; Moran, R. A.; Buckheit, R. W., Jr.; Gustafson, K. R.; McMahon, J. B.; Boyd, M. R. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 645−651.
(17) Ubillas, R.; Jolad, S. D.; Bruening, R. C.; Kernan, M. R.; King, S. R.; Sesin, D. F.; Barrett, M.; Stoddart, C. A.; Flaster, T. *Phytomedicine* **1994**, *1*, 77−106.
(18) Orozco-Topete, R.; Sierra-Madero, J.; Cano-Dominguez, C.; Kershenovich, J.; Ortiz-Pedroza, G.; Vazquez-Valls, E.; Garcia-Cosio, C.; Soria-Cordoba, A.; Armendariz, A. M.; Teran-Toledo, X.; Romo-Garcia, J.; Fernandez, H.; Rozhon, E. J. *Antiviral Res.* **1997**, *35*, 91−103.
(19) van Hensbroek, M. B.; Onyiorah, E.; Jaffar, S.; Schneider, G.; Palmer, A.; Frenkel, J.; Enwere, G.; Forck, S.; Nusmeijer, A.; Bennett, S.; Greenwood, B.; Kwiatkowski, D. *New Eng. J. Med.* **1996**, *335*, 69−75.
(20) de Vries, P. J.; Dien, T. K. *Drugs* **1996**, *52*, 818−836.
(21) Davies, H. G., Green, R. H. *Nat. Prod. Rep.* **1986**, 117−121.
(22) *Scrip* **1996**, *2187*, 18−19.
(23) Uitti, R. J.; Ahlskog, J. E. *CNS Drugs* **1996**, *5*, 369−388.
(24) *Scrip* **1997**, *2221*, 20.
(25) *Drugs Future* **1994**, *19*, 422−424.
(26) *Drugs Future* **1996**, *21*, 651−652.
(27) Skolnick, A. A. *JAMA* **1997**, *277*, 776.
(28) Rainer, M. *CNS Drugs* **1997**, *7*, 89−97.
(29) *Scrip* **1996**, *2152*, 23.
(30) *Scrip* **1997**, *2288*, 19.
(31) *Drugs Future* **1994,** *19*, 427−428.
(32) Murphy, M. G.; Sytnik, B.; Kovacs, T. O. G.; Mertz, H.; Ewanik, D.; Shingo, S.; Lin, J. H.; Gertz, B. J.; Walsh, J. H. *Clin. Pharmacol. Ther.* **1993**, *54*, 533−539.
(33) Bock, M. G.; DiPardo, R. M.; Mellin, E. C.; Newton, R. C.; Veber, D. F.; Freedman, S. B.; Smith, A. J.; Patel, S.; Kemp, J. A.; Marshall, G. R.; Fletcher, A. E.; Chapman, K. L.; Anderson, P. S.; Freidinger, R. M. *J. Med. Chem.* **1994**, *37*, 722−724.
(34) Semple, G.; Ryder, H.; Rooker, D. P.; Batt, A. R.; Kendrick, D. A.; Szelke, M.; Ohta, M.; Satoh, M.; Nishida, A.; Akuzawa, S.; Miyata, K. *J. Med. Chem.* **1997**, *40*, 331−341.
(35) Hughes, A. J. *Drugs* **1997**, *53*, 195−205.
(36) *Scrip* **1997**, 2277, 22.
(37) Daly, J. W. *J. Nat. Prod.* **1998**, *61*, 162−172.
(38) Bannon, A. W.; Decker, M. W.; Holladay, M. W.; Curzon, P.; Donnelly-Roberts, D.; Puttfarcken, P. S.; Bitner, R. S.; Diaz, A.; Dickenson, A. H.; Porsolt, R. D.; Williams, M.; Arneric, S. P. *Science* **1998**, *279*, 77−80.
(39) Endo, A.; Kuroda, M.; Tanzawa, K. *FEBS Lett.* **1976**, *72*, 323−326.
(40) Kathawala, F. G. *Med. Res. Rev.* **1991,** *11*, 121−146.
(41) *Scrip* **1997**, *2286*, 16−17.
(42) Nadin, A.; Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1996,** *35*, 1622−1656.

(43) DeNinno, M. P.; McCarthy, P. A.; Duplantier, K. C.; Eller, C.; Etienne, J. B.; Zawistoski, M. P.; Bangerter, F. W.; Chandler, C. E.; Morehouse, L. A.; Sugarman, E. D.; Wilkins, R. W.; Woody, H. A.; Zaccaro, L. M. *J. Med. Chem.* **1997**, *40*, 2547−2554.
(44) Harris, W. S.; Windsor, S. L.; Newton, F. A.; Gelfand, R. A. *Clin. Pharmacol. Ther.* **1997,** *61*, 385−389.
(45) Truscheit, E.; Frommer, W.; Junge, B.; Mueller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem.* **1981**, *93*, 738−748.
(46) Strupczewski, J. D.; Ellis, D. B.; Allen, R. C. *Ann. Rep. Med. Chem.* **1991**, *26*, 297−313.
(47) Fukase, H. *Yuki Gosei Kagaku Kyokaishi* **1997**, *55*, 920−925.
(48) *Drugs Future* **1995,** *20*, 1286.
(49) Luo, J. In *Natural Products II, New Technology to Increase Efficiency and Speed*; Sapienza, D. M., Ed.; IBC Library Series no. 982; International Business Communications: Southborough, MA, 1998; pp 119−145.
(50) Seamon, K. B. *Ann. Rep. Med. Chem.* **1984**, *19*, 293−310.
(51) Tatee, T.; Narita, A.; Narita, K.; Izumi, G.; Takahira, T.; Sakurai, M.; Fujita, A.; Hosono, M.; Yamashita, K.; Enomoto, K.; Shiozawa, A. *Chem. Pharm. Bull.* **1996**, *44*, 2274−2279.
(52) Friederich, P. W.; Levi, M.; Biemond, B. J.; Charlton, P.; Templeton, D.; van Zonneveld, A. J.; Bevan, P.; Pannekoek, H.; tenCate, J. W. *Circulation* **1997**, *96*, 916−921.
(53) Kelly, P. A.; Gruber, S. A.; Behbod, F.; Kahan, B. D. *Pharmacother.* **1997**, *17*, 1148−1156.
(54) Kino, T.; Hatanaka, H.; Hashimoto, M.; Nishiyama, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1987**, *40*, 1249−1225.
(55) Maggon, K. K. *Drug News Perspect.* **1994**, *7*, 389−401.
(56) *Scrip* **1997**, *2246*, 20.
(57) Sievers, T. M.; Rossi, S. J.; Ghobrial, R. M.; Arriola, E.; Nishimura, P.; Kawano, M.; Holt, C. D. *Pharmacother.* **1997**, *17*, 1178−1197.
(58) *Drugs Future* **1995**, *20*, 742−743.
(59) *Drugs Future* **1994**, 19, 243−247.
(60) Murai, M.; Morimoto, H.; Maeda, Y.; Kiyotoh, S.; Nishikawa, M.; Fujii, T. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 403−408.
(61) Takeya, T. *Pure Appl. Chem.* **1996**, *68*, 875−880.
(62) Le Bars, P. L.; Katz, M. M.; Berman, N.; Itil, T. M.; Freedman, A. M.; Schatzberg, A. F. *JAMA*, **1997**, *278*, 1327−1332.
(63) Dhainaut, J. F.; Tenaillon, A.; Le Tulzo, Y.; Schlemmer, B.; Solet, J. P.; Wolff, J.; Holzapfel, L.; Zeni, F.; Dreyfuss, D.; Mira, J. P. *Crit. Care Med.* **1994**, *22*, 1720−1728.
(64) *Drugs Future* **1995**, *20*, 690−691.
(65) *Drugs Future* **1995**, *20*, 524.
(66) *Drugs Future* **1995**, *20*, 307.
(67) Nemeth, E. F.; Brown, E. M.; Hebert, S. C.; Van Wagenen, B. C.; Balandrin, M. F.; Fuller, F. H.; Del Mar, E. G. World Patent, WO 94/18959, Sep 1, 1994, pp 201.
(68) Nemeth, E. F.; Van Wagenen, B. C.; Balandrin, M. F.; Del Mar, E. G.; Moe, S. T. World Patent, WO 95/11221, Apr 27, 1995, p 132.
(69) Nemeth, E. F.; Steffey, M. E.; Hammerland, L. G.; Hung, B. C.; Van Wagenen, B. C.; Del Mar, E. G.; Balandrin, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4040−4045.
(70) *Scrip* **1996**, *2281*, 23.
(71) Hu, H. *Drug Discov. Today* **1996**, *1*, 438−447.
(72) Jacobson, P. B. *J. Pharm. Exp. Ther.* **1995**, *275*, 995−1002.
(73) Jirousek, M. R.; Gillig, J. R.; Gonzalez, C. M.; Heath, W. F.; McDonald, J. H., III; Neel, D. A.; Rito, C. J.; Singh, U.; Stramm, L. E. *J. Med. Chem.* **1996**, *39*, 2664−2671.
(74) Ishii, H.; Jirousek, M. R.; Koya, D.; Takagi, C.; Xia, P.; Clermont, A.; Bursell, S.; Kern, T. S.; Ballas, L. M. *Science* **1996**, *272*, 728−731.
(75) Kulanthaivel, P.; Hallock, Y. F.; Boros, C.; Hamilton, S. M.; Janzen, W. P.; Ballas, L. M.; Loomis, C. R.; Jiang, J. B.; Katz, B.; Steiner, J. K.; Clardy, J. *J. Am. Chem. Soc.* **1993**, *115*, 6452−6453.
(76) Ohshima, S.; Yanagisawa, M.; Katoh, A.; Fujii, T.; Sano, T.; Matsukuma, S.; Furumai, T.; Fujiu, M.; Watanabe, K.; Yokose, K.; Arisawa, M.; Okuda, T. *J. Antibiot.* **1994**, *47*, 639−647.
(77) Haimes H. B.; Glasson, S. S.; Harlan, P. M. *Inflammation Res.* **1995**, *44*, Suppl. 3W13/17.
(78) Cragg, G. M.; Newman, D. J.; Weiss, R. B. *Seminar Oncol.* **1997**, *24*, 156−163.
(79) deFuria, M. D. *Phytomedicine* **1997**, *4*, 273−282.
(80) Rowinsky, E. K. *Annu. Rev. Med.* **1997**, *48*, 353−374.
(81) *Drugs Future* **1995**, *20*, 735−736.
(82) Markman, M. *Seminar Oncol.* **1997**, *24*, S5−1.
(83) *Drugs Future* **1995**, *20*, 307.
(84) *Scrip* **1997**, *2257*, 13.
(85) *Drugs Future* **1995**, *20*, 942−943.
(86) *Drugs Future* **1995**, *20*, 205−206.
(87) *Drugs Future* **1994**, *19*, 781−783.
(88) *Drugs Future* **1995**, *20*, 1284−1285.
(89) Ohashi, M.; Oki, T. *Exp. Opin. Ther. Pat.* **1996**, *6*, 1285−1294.
(90) Pettit, G. R. *J. Nat. Prod.* **1996**, *59*, 812−821.
(91) Raynaud, F. I.; Judson, I. R. *Drugs Future* **1996**, *21*, 65−69.
(92) *Drugs Future* **1995**, *20*, 621−622.
(93) Gordon, M.; Guralnik, M.; Kaneko, Y.; Mimura, T.; Goodgame, J.; DeMarzo, C.; Pierce, D.; Baker, M.; Lang, W. *J. Med.* **1995**, *26*, 193−207.
(94) Hinman, L.; Yarranton, G. *Ann. Rep. Med. Chem.* **1993**, *28*, 237−246.
(95) *Scrip* **1996**, *1290*, 20.
(96) Cleary, J.; Tutsch, K. D.; Berlin, J.; Arzoomanian, R. Z.; Alberti, D.; Feierabend, C.; Simon, K.; Huston, P.; Steward, J.; Wilding, G. *Proc. Am. Assoc. Cancer Res.* **1996**, *27*, 164.
(97) Yoshinari, T.; Matsumoto, M.; Arakawa, H.; Okada, H.; Noguchi, K.; Suda, H.; Okura, A.; Nishimura, S. *Cancer Res.* **1995**, *55*, 1310−1315.
(98) Arakawa, H.; Iguchi, T.; Morita, M.; Yoshinari, T.; Kojiri, K.; Suda, H.; Okura, A.; Nishimura, S. *Cancer Res.* **1995**, *55*, 1316−1320.
(99) *Scrip* **1996**, *2177*, 20.
(100) *Scrip* **1997**, *2227*, 14.
(101) Eckhardt, S. G.; Burris, H. A.; Eckardt, J. R.; Weiss, G.; Rodrigues, G.; Rothenberg, M.; Rinaldi, D.; Barrington, R.; Kuhn, J. G.; Masuo, K.; Sudo, K.; Atsumi, R.; Oguma, T.; Hayashi, L.; Fields, S.; Smetzer, L.; Von Hoff, D. D. *Ann. Oncol.* **1996**, *7*, 491−496.
(102) *Chem. Eng. News* **1997**, Feb. 10, p 8.
(103) Ashworth, P. A.; Hunjan, S.; Pretswell, I. A.; Ryder, H.; Brocchini, S. J. World Patent, WO 96/20180, July 4, 1996, p 65.
(104) *Scrip* **1997**, *2185*, 9.
(105) *Scrip* **1997**, *2241*, 14.
(106) Kamishohara, M.; Kawai, H., Sakai, T.; Kataoka, S.; Isoe, T.; Yoshioka, E.; Mochizuki, J.; Uchida, T.; Otake, N. 1995. *Proc. Am. Assoc. Cancer Res.* **1995**, *36*, 6, Abstr. 2364.
(107) Hoshi, A.; Castaner, J. *Drugs Future* **1996**, *21*, 152−154.
(108) de Jong, R. S.; de Vries, E. G. E.; Mudler, N. H. *Anticancer Drugs* **1997**, *8*, 413−418.
(109) *Drugs Future* **1995**, *20*, 1176−1178.
(110) Yanase, T.; Tamura, M.; Fujita, K.; Kodama, S.; Tanaka, K. *Cancer Res.* **1993**, *53*, 2566−2570.
(111) Twardowski, P.; Gradishar, W. J. *Curr. Opin. Oncol.* **1997**, *9*, 584−589.
(112) *Scrip* **1996**, *2175*, 19.
(113) Sedlacek, H. H.; Czech, J.; Naik, R.; Kaur, G.; Worland, P.; Losiewicz, M.; Parker, B.; Carlson, B.; Smith, A.; Senderowicz, A.; Sausville, E. *Int. J. Oncol.* **1996**, *9*, 1143−1168.
(114) Evans, E.; Arneson, D.; Kovatch, R.; Supko, J. *Proc. Am. Assoc. Cancer Res.* **1995**, *36*, 6, Abstr. 2180.
(115) *Scrip Mag.* **1997**, Nov, 35−38.

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