The influence of natural products upon drug discovery

David J. Newman,*[a]† Gordon M. Cragg[a] and Kenneth M. Snader[b]

[a] *Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville MD 20852, USA*
[b] *Pharmaceutical Resources Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville MD 20852, USA*

Received (in Cambridge) 20th December 1999

Covering: Antiquity to late 1999.

1 Introduction
2 General role of traditional medicine in drug discovery
3 Role of natural products in treatment of diseases
4 Antiinfective agents (including antimalarials)
4.1 Antibacterials: β-lactams
4.2 Antibacterials: aminoglycosides
4.3 Antibacterials: tetracyclines
4.4 Antibacterials: macrolides
4.5 Antibacterials: synergistic mixtures of streptogramins
4.6 Antifungals: general
4.7 Antifungals: lipopeptides
4.8 Antifungals: non-lipopeptides
4.9 Antimalarials
4.10 Antiviral area: general
4.11 Antiviral area: nucleoside analogues
4.12 Antiviral area: HIV protease inhibitors
5 Cardiovascular: general
5.1 Cardiovascular: the β-adrenergic amines
5.2 Cardiovascular: cholesterol lowering agents
5.3 Cardiovascular: angiotensin converting enzyme inhibitors (ACE inhibitors)
6 Pain/central nervous system: history
6.1 Pain/central nervous system: the opiates
6.2 Pain/central nervous system: the conotoxins
6.3 Pain/central nervous system: the epibatidines
7 Antineoplastics: general
7.1 Antineoplastics: plant sources
7.2 Antineoplastics: microbial sources
7.3 Antineoplastics: marine sources
8 Future trends: general
8.1 Future trends: production of 'previously unknown structures'
8.2 Future trends: the optimization of lead structures
8.3 Future trends: novel delivery systems for 'old compounds'
9 References

1 Introduction

Throughout the ages humans have relied on nature for their basic needs for the production of foodstuffs, shelters, clothing, means of transportation, fertilizers, flavors and fragrances, and, not least, medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years.[1] The first records, written on hundreds of clay tablets in cuneiform, are from Mesopotamia and date from about 2600 BC; amongst the approximately 1000 plant derived substances which they used were oils of *Cedrus* species (cedar) and *Cupressus sempevirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh), and *Papaver somniferum* (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation.

Egyptian medicine dates from about 2900 BC, but the best known Egyptian pharmaceutical record is the "Ebers Papyrus" dating from 1500 BC; this documents over 700 drugs (mostly plants, though animal organs were included together with some minerals), and includes formulae such as gargles, snuffs, poultices, infusions, pills and ointments, with beer, milk, wine and honey being commonly used as vehicles.

The Chinese *Materia Medica* has been extensively documented over the centuries, with the first record dating from about 1100 BC (Wu Shi Er Bing Fang, containing 52 prescriptions), followed by works such as the Shennong Herbal (~100 BC; 365 drugs) and the Tang Herbal (659 AD; 850 drugs). Likewise, documentation of the Indian Ayurvedic system dates from about 1000 BC (Charaka; Sushruta and Samhitas with 341 and 516 drugs respectively), and this system formed the basis for the primary text of Tibetan medicine, Gyu-zhi (Four Tantras) translated from Sanskrit during the eighth century AD.[2,3]

In the ancient Western world, the Greeks contributed substantially to the rational development of the use of herbal drugs. The philosopher and natural scientist, Theophrastus (~300 BC), in his 'History of Plants', dealt with the medicinal qualities of herbs, and noted the ability to change their characteristics through cultivation. Dioscorides, a Greek physician (100 AD), during his travels with Roman armies throughout the then 'known world', accurately recorded the collection, storage, and use of medicinal herbs, and is considered by many to be the most important representative of the science of herbal drugs in 'ancient times'. Galen (130–200 AD), who practiced and taught pharmacy and medicine in Rome, and published no less than 30 books on these subjects, is well known for his complex prescriptions and formulae used in compounding drugs. These were based on the Hippocratic theory that all illnesses were based on an imbalance of four primary 'humours' and were extremely complex at times containing dozens of ingredients (the so-called 'galenicals').

During the Dark and Middle Ages, from the fifth to the twelfth centuries, the monasteries in countries such as England, Ireland, France and Germany preserved the remnants of this Western knowledge. However, it was the Arabs who were

† *Address for correspondence*: Natural Products Branch,NCI, PO Box B, Frederick, MD, 21702, USA. E-mail: dn22a@nih.gov

This journal is © The Royal Society of Chemistry 2000

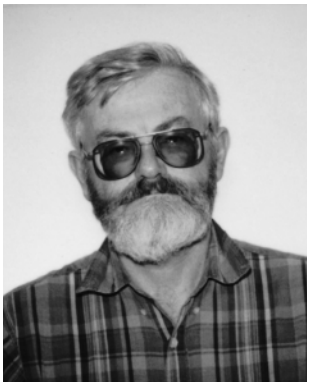
responsible for the preservation of much of the Greco-Roman expertise, and for expanding it to include the use of their own resources, together with Chinese and Indian herbs unknown to the Greco-Roman world. The Arabs were the first to establish privately owned drug stores in the eighth century, and the Persian pharmacist, physician, philosopher and poet Avicenna contributed much to the sciences of pharmacy and medicine through works, such as *Canon Medicinae*, regarded as 'the final codification of all Greco-Roman medicine'. This was subsequently superceded by the comprehensive compilation known as the *Corpus of Simples* by Ibn al-Baitar who practiced in Malaga during the Moorish occupation of Spain. This document combined the data of Dioscorides with works from the Middle and Far East.

These, and many other works, were formally codified at least in the UK by the publication in 1618 of the *London Pharmacopoeia* and the idea of 'pure' compounds as drugs may be traced to the isolation of the active principles of commonly used plants and herbs such as strychnine, morphine, atropine and colchicine in the early 1800s. These isolations were then followed by what can be considered the first commercial pure natural product, morphine, by E. Merck in 1826 and the first semi-synthetic pure drug based on a natural product, aspirin, by Bayer in 1899.[4]

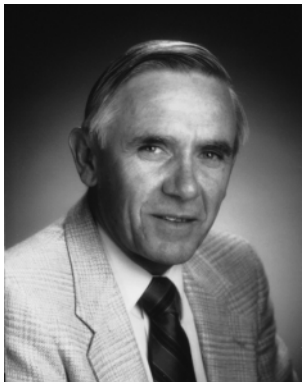
David Newman was born in Grays, Essex, UK. His initial training was as an analyst (GRIC) followed by an MSc in Organic Chemistry (University of Liverpool) and then after some time in the UK chemical industry, a DPhil in Microbial Chemistry from the University of Sussex in 1968. Following two years of postdoctoral studies on the structure of electron transport proteins at the University of Georgia, USA, he worked with Smith Kline and French in Philadelphia, PA, as a biological chemist predominantly in the area of antibiotic discovery. During this time period, he obtained an MLS in Information Sciences in 1977 from Drexel University, Philadelphia, PA. Following the discontinuance of antibiotic discovery programs at SKF, he worked for a number of US-based pharmaceutical companies in natural-products based discovery programs in anti-infectives and cancer treatments, and joined the Natural Products Branch of the NCI in 1991. He is responsible for the marine and microbial collection programs of the NCI and in concert with Gordon Cragg, for the NCI's Open and Active Repository programs. His scientific interests are in the discovery and history of novel natural products as drug leads in the anti-infective and cancer areas, in novel delivery methods for such agents and in the application of information technologies to drug discovery. In conjunction with Gordon Cragg, he has established collaborations between the National Cancer Institute and organizations in many countries promoting drug discovery from their natural resources. He has published over 50 papers and patents that are related to these interests and is both an UK Chartered Chemist and an UK Chartered Biologist.

Gordon Cragg was born in Cape Town, South Africa, and obtained his undergraduate training in Chemistry at Rhodes University before proceeding to Oxford University where he obtained his DPhil in Organic Chemistry in 1963. After two years of postdoctoral research in natural products chemistry at the University of California, Los Angeles, he returned to South Africa to join the Council for Scientific and Industrial Research. In 1966, he was appointed to the staff of the Department of Chemistry at the University of South Africa, and transferred to the University of Cape Town in 1972. In 1979, he returned to the United States to join the Cancer Research Institute at Arizona State University, working with Professor G. Robert Pettit on the isolation of potential anticancer agents from plant and marine invertebrate sources. In 1985, he moved to the National Cancer Institute in Bethesda, Maryland, and was appointed Chief of the Natural Products Branch in 1989. His major interests lie in the discovery of novel natural product agents for the treatment of cancer and AIDS. In 1991 he was awarded the National Institutes of Health Merit Award for his contributions to the development of the drug, Taxol®, and in 1998 he was elected President of the American Society of Pharmacognosy. He has established collaborations between the National Cancer Institute and organizations in many countries promoting drug discovery from their natural resources. He has published over 100 papers related to these interests.

Kenneth Snader was born in Harrisburg, PA, USA. He obtained his undergraduate training at the Philadelphia College of Pharmacy and Science and after some years in the pharmaceutical industry returned to obtain his PhD in Organic Chemistry from the Massachusetts Institute of Technology as a Walter G. Karr Fellow. He went directly to Smith Kline and French Laboratories where, after a brief period in the study of anti-inflammatory compounds he returned to natural products to oversee the antibiotics discovery chemistry group. Following the discontinuance of the antibiotic discovery program at SKF, he briefly supervised the marine natural products chemistry group at SeaPharm before he joined the Natural Products Branch of the National Cancer Institute in 1987. After a significant effort at producing enough of the drug Taxol® to complete the clinical trials of that natural product, for which he was awarded the National Institutes of Health Merit Award, he moved from the Natural Products Branch to the Pharmaceutical Resources Branch of the NCI, taking on his current responsibilities for production of GMP bulk drugs for NCI clinical trials. His scientific interests are in the discovery, identification, and large scale production of natural products for medicinal use and together with Gordon Cragg and members of the Natural Products Branch has pro moted and encouraged the discovery of new chemotherapeutic agents from natural sources. He has published over 50 papers and patents related to these interests.



David J. Newman



Gordon M. Cragg

Kenneth M. Snader

2 General role of traditional medicine in drug discovery

As mentioned above, plants have formed the basis for traditional medicine systems which have been used for thousands of years in countries such as China[5] and India.[2,6] The use of plants in the traditional medicine systems of many other cultures has been extensively documented.[7] These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care.[8,9]

Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries. Analysis of data on prescriptions dispensed from community pharmacies in the United States from 1959 to 1980 indicated that about 25% contained plant extracts or active principles derived from higher plants. Currently, at least 119 chemical substances, derived from 90 plant species, can be considered as important drugs that are in use in one or more countries.[8] Of these 119 drugs, 74% were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine.

A more recent study using US-based prescription data from 1993, demonstrated that natural products still play a major role in drug treatment, as over 50% of the most-prescribed drugs in the US had a natural product either as the drug, or as a 'forebear' in the synthesis or design of the agent.[10]

3 Role of natural products in treatment of diseases

This review will cover the following general areas of disease: microbial and some parasitic areas; neoplastic; cardiovascular and hypertension; pain/CNS and will be discussed from the aspect of the disease rather than the more customary division by source of organism. This approach was chosen because of the cross-over that is now being seen where agents originally isolated and purified as a result of their ability to inhibit one type of screen are now being utilized in other entirely different areas. The review will finish with a short section on 'Future Trends'.

Perhaps the best example of this cross-over is the work over the last 30 or so years on the microbial product, rapamycin. Originally isolated as an antifungal agent, it has just been approved as an immunosuppressive drug and is also being tested (as a derivative) as an anti-neoplastic agent. Thus the use of a disease area permits agents from all sources to be discussed in a group.

For earlier coverage of the role of natural products in drug discovery, the excellent 1995 review by Cordell should be consulted.[11]

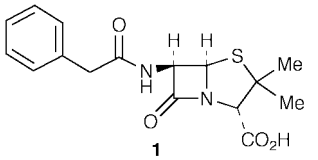
4 Antiinfective agents (including antimalarials)

We have not attempted to make the following discussion comprehensive in its coverage of the various anti-infective classes. The guiding principle has been to show areas where the natural product has led to 'improved materials by semi-synthetic modifications of a base molecule that a medicinal chemist would not even dream of making'. Thus the glycopeptides such as vancomycin, or the simple but very effective agent, chloramphenicol, are not further considered.

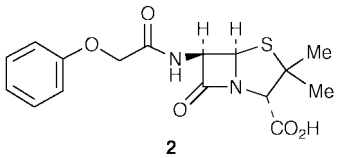
Although there were anecdotal stories of the use of poultices of rotten bread on puncture or slash wounds from the Roman wars, and from Arab medicinal treatments used during the many conflicts that the Western 'navigators' had with the Arabs from the fifteenth century onwards, no systematic method of treatment appeared in the literature for suppurating wounds caused by bacterial infections until Lister used *P. glaucum* for "curing" a large non-healing abscess in 1884 (*cf.* p. 124 in Mann[12]).

4.1 Antibacterials: β-lactams

The 'Golden Age of Antibiotics', as the time period from the 1940s to the 1970s has been christened, can be considered to have its beginning in the serendipitous discovery of penicillin by Fleming in 1928, reported in the British Medical Literature in 1929. The history of the discovery of penicillin has been told and retold and we do not intend to go over this story in detail again. An excellent reprint has just been issued of the 'History of Penicillin Production'[13] and that publication goes into the story in detail from the aspect of large scale production of penicillin G (**1**) and penicillin V (**2**). It is rather ironic that the



1

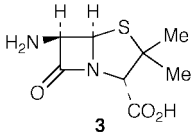


2

major impetus to the development and production of the initial β-lactams was World War II and the requirement for penicillin to be available for D-Day. In the application of the resources of the UK and the US to the problem, coupled to its importance, it can rightly be considered to be the microbiological equivalent of the Manhattan Project.

The base structure of the penicillin molecule was new to science when it was elucidated and since it acted by a mechanism that targeted a structure unique to the bacterial cell wall, it immediately became a structure '*ripe for semi-synthesis*'. With the almost immediate realization that 'microbes were smarter than man', as they produced protective enzymes, penicillinases or β-lactamases that degraded the penicillins by opening the β-lactam ring, came the requirement to produce/discover other, non-lactam antibiotics, and/or chemically modify the basic penicillin nucleus to reduce or eliminate the enzymic activity.

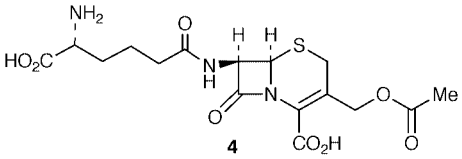
Almost all of the penicillins that have been made have started from the simple fermentation-derived product 6-amino-penicillanic acid (6-APA, **3**), which is produced by a simple



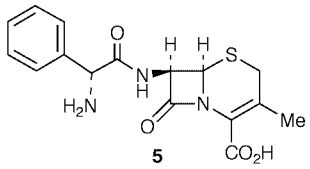
3

chemical or biochemical deacetylation from penicillin G itself. The actual number of 6APA-based structures that have been made and tested by the pharmaceutical industry and associated academic laboratories is unknown, as substantial numbers of inactive materials or compounds with only minor increases in potency were not reported in the literature, but it was well in excess of 10 000 in the late 1970s.[14]

In 1948, Brotzu[15] reported on the ring-expanded molecule, cephalosporin C (**4**), which was discovered from an isolate of a 'pseudo'-marine fungus, *Cephalosporium acremonium*, taken from a sewer outfall in Sardinia. Some of the same Oxford group that had originally worked on penicillin G, and who had seen but not isolated and purified the material at roughly the



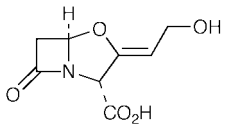
4



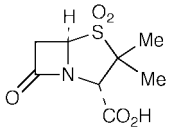
5

same time as Brotzu, now isolated a weakly active agent from the fungal extract and determined the structure of cephalosporin C.[16,17] Although very weakly antibacterial, it did not appear to be susceptible to the then known β-lactamases and this finding, coupled to the demonstration by chemists at Lilly that penicillin G could be chemically expanded to give the cephalosporin C nucleus, led to the same type of semi-synthetic effort in producing modified cephalosporins as had been seen in the case of the penicillins. An example is the first orally-available cephalosporin, cephalexin (**5**). Originally, cephalosporins were used to treat infections resistant to penicillins, but with the advent of β-lactamases that were either specific for cephalosporins, or attacked both ring systems, the search for more resistant analogues continued.

A large effort was mounted in the late 1960s and early 1970s, predominately by Beecham and Pfizer to find molecules that would have pharmacokinetic properties similar to those of penicillins but that would inhibit some or all of the common β-lactamases. Beecham were successful in producing the molecules known as clavulanates (**6**) from natural sources, and Pfizer produced the semi-synthetic molecules known as the sulbactams where the thiazole sulfur was oxidized to the sulfone **7**. In



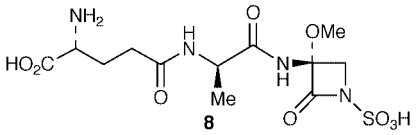
6



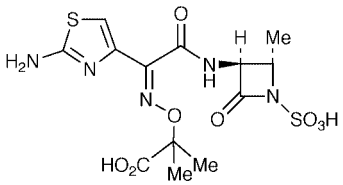
7

both cases, combination therapies were devised where mixtures, usually equimolar in nature, were administered using a common penicillin such as ampicillin or the more potent amoxicillin plus the β-lactamase inhibitor. To give an idea of the value of these patented combinations, in 1997 SKB sold over US$ 1.5 billion of Augmentin® worldwide and the US patent does not expire until 2002.[18] As yet, a corresponding cephalosporin combination has not been marketed, though from the late 1980s workers at Roche had reported on some interesting combination antibiotics where two different nuclei (β-lactams and quinolones) were linked by esters,[19] carbamates[20] or *via* a tertiary amine function[21] and reported on their stability to three common Gram-negative β-lactamases,[22] showing that only the compounds that contained the cefotaxime structure were stable to two of the three enzymes used.

The search for a 'simple β-lactam' (*i.e.*, an isolated single ring) went on for many years, predominately in synthetic chemistry laboratories. Again, Mother Nature proved to be the superior chemist when in early 1981, Imada reported on the sulfazecins[23] **8** and, within two months, Sykes *et al.* at Squibb reported that after herculean efforts, involving over 1 000 000 small-scale fermentations, they successfully isolated the monobactams including sulfazecin from aquatic organisms collected almost in their own 'backyard'.[24] This was an entertaining repetition of the search by the NRRL scientists for a high-producing *P. chrysogenum* strain that they eventually found on a rotting melon in a local fruit market in Peoria across from the



8

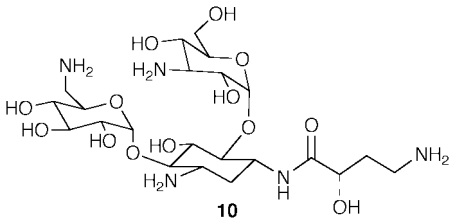


9

laboratory.[13] With the identification of the naturally occurring monobactam compounds, it became 'chemically obvious' why Nature was the best chemist. Of all the synthetic modifications made by medicinal chemists from Squibb, SmithKline and other pharmaceutical houses, none had considered that an N-sulfonic acid substituent would stabilize the β-lactam system. In a clever move, the Squibb researchers then proceeded to synthesize and patent all of the side-chain modifications that had been reported to give improved activities in the penicillin and cephalosporin series; an early compound in this series, aztreonam (Azactam®, **9**) is currently used clinically.

4.2 Antibacterials: aminoglycosides

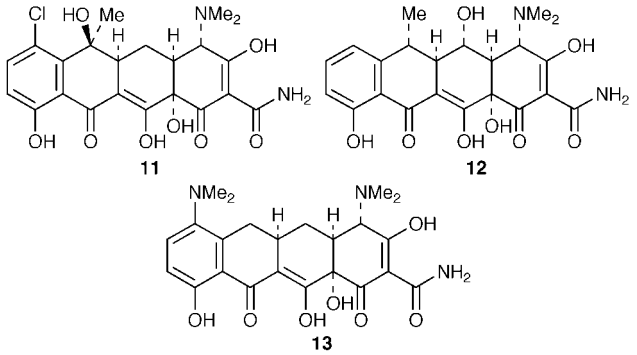
Concomitantly with the search for the penicillins, Waksman at Rutgers University commenced a search for microbial metabolites that would be active against the then (and perhaps now) major scourge, tuberculosis. In 1943, the aminoglycoside antibiotic streptomycin was isolated from *Streptomyces griseus* and, in addition to being active against *Mycobacterium tuberculosis*, it was also active against a wide range of other bacterial infections. Further work, predominately by screening metabolites from soil microbes of the *Actinomycetales*, led to the identification and isolation of a large number of antibiotics of similar structural types known generically as the aminoglycosides. These materials are mainly used against Gram-negative organisms, but due to their mechanism of action (inhibition of protein synthesis), they also exhibit true synergy with penicillins *in vivo*. Their major drawback in clinical use is the fairly rapid build-up of resistance (to a large extent, plasmid-mediated phosphorylases and acetylases) and their innate oto- and nephro-toxicity, which means that treatments have to be very carefully regulated. There have been some successful chemical modifications to produce more resistant molecules (*e.g.* amikacin, **10**), but in most cases, the isolated metabolite is used as the drug.



10

4.3 Antibacterials: tetracyclines

The next major class of metabolites to be discovered and used extensively were the tetracyclines. These were produced by various *Streptomyces* sp. and had the then unique skeleton of four linear fused rings. Although the parent molecule was not used to any great extent as an antibacterial, the chlorinated derivative, named Aureomycin® (**11**) because of its color, was.

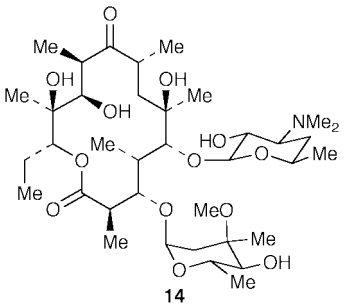


11 12 13

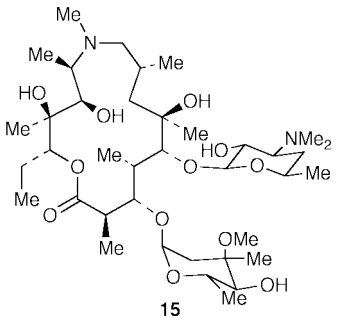
A large number of semi-synthetic tetracyclines have been made and a significant number are still in use as first line therapy, even though the parent molecule is almost fifty years old. Amongst these are doxycycline (**12**) and minocycline (**13**). Tetracycline has another 'claim to fame' in antibiotic therapy, as it was this molecule that gave its name to the '*tet* resistance pump'. In eubacteria, and in its homologous form in eukaryotes (P130 glycoprotein), this pump gives rise to the phenomenon of multidrug resistance (or MDR), where the toxic agent is pumped out as fast as it is transported in (either by diffusion or by an active ingress mechanism). Molecules based on the tetracycline molecule have since been synthesized that will block the *tet* pump, thus possibly permitting antibiotic therapy with relatively cheap tetracyclines to be used again.[25–27]

4.4 Antibacterials: macrolides

The other extremely important *Streptomyces* metabolites are the 14-membered macrolides exemplified by erythromycin (**14**). There are relatively large numbers of compounds with various



14



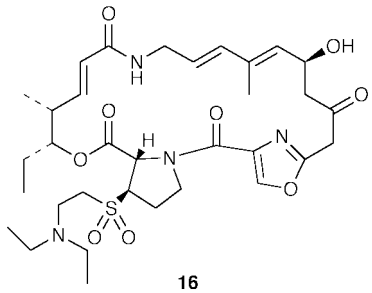
15

sizes of macrolide rings that have been isolated as a result of screening campaigns in the antibiotics industry, but, of all of them, those based on the erythronolide ring system have been the most used, even though they have to have complex salts made in order to generate oral activity. In a fairly recent development, a chemical modification has been made to the basic structure to overcome the resistance exhibited by strains from most of the important pathogens to the base molecule. These are the 'aza-macrolides' exemplified by Azithromycin® (**15**), where a nitrogen has been chemically inserted into the base macrolide ring, overcoming most if not all of the resistance shown to erythromycin.[28] The downside to such a molecule is the expense of the synthetic process, thus pricing such treatments beyond both developing and a number of developed countries.

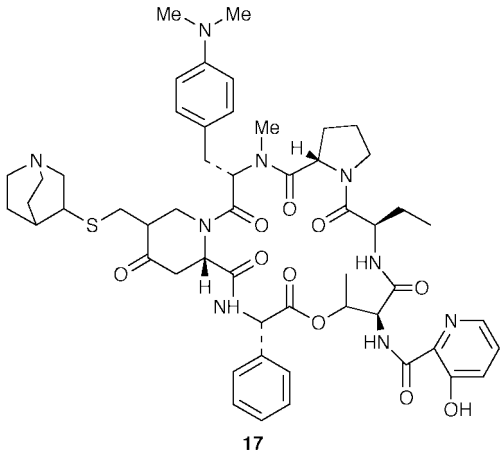
4.5 Antibacterials: synergistic mixtures of streptogramins

In the early days of antibiotic discovery, a series of synergistic mixtures were isolated from *Streptomyces* sp. and identified as being extremely potent agents against Gram-positive organisms. However, they had a major drawback, they were quite toxic in comparison to the tetracyclines and erythromycin. They were, however, developed as animal feed supplements and used extensively in the US and in Europe (virginiamycins A and B being an example).

With the advent of epidemics of methicillin-resistant *Staphylococcus aureus* (MRSA), and the potential for larger outbreaks of the glycopeptide-resistant entero- and staphylococci, came the realization that we are very close to not having any antibiotics left that will kill such resistant organisms. To make matters worse, most of the big antibiotic-discovery companies had down-sized their operations in the mid-1980s to early 1990s time frame. Rhone-Poulenc in France had had a 'franchise' on the streptogramin Pristinamycin, and in a series of elegant semi-syntheses, modified the 'A' and 'B' components to produce the water-soluble compounds, dalfopristin (**16**) (RP54476) and



16



17

quinuprisitin (**17**) (RP57669). The synergistic mixture (70 : 30 ratio) has now been approved by the US FDA under the name of Synercid® (RP59500) for use against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant entero- and staphylococci and drug-resistant *S. pneumoniae*.[29–31]

4.6 Antifungals: general

Unlike the antibacterial arena where, in relative terms, bacterial-specific targets abound, fungi, being eukaryotic in nature, have a metabolism similar to those of mammals, with differences in the t-RNA-AA-acyltransferases (only found from proteoge-

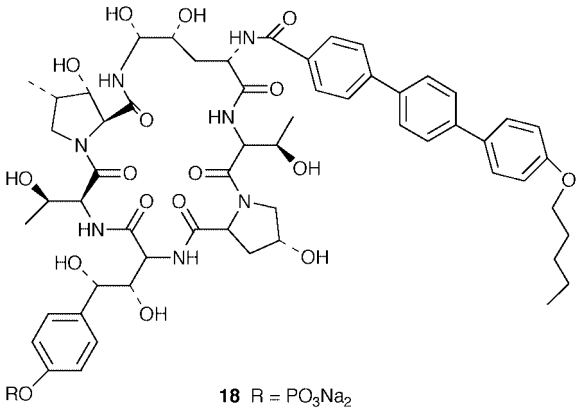
nomics studies),[32] portions of their steroid synthetase systems and their carbohydrate-based cell walls. As a result, there are few clinically viable antifungal agents, with the well known amphotericin B (*aka* 'ampho-terrible' because of its side effects) still being the gold standard against which other antifungal agents are measured.

To date, no clinically effective antibiotic directed towards the chitin synthetases has yet survived the testing process though a lot of work was performed, starting in the late 1970s, in attempts to chemically convert the peptidic antibiotics of the Nikkomycin class into viable clinical candidates against *C. albicans*. With these and similar agents, problems still arise with the specificity of this organism's peptide transport systems (*cf.* McCarthy *et al.*[33] for a discussion of these processes), thus the following discussions will be of agents directed against other targets.

4.7 Antifungals: lipopeptides

Though there have been many agents reported to have antifungal activity *in vitro*, when they were tested in relevant animal models, their ADME (absorption, distribution, metabolism and excretion) characteristics were such that significant chemistry had to be performed to obtain reasonable pharmacology.

Such operations were performed by Lilly on echinocandin in order to produce cilofungin. This compound reached Phase II clinical trials, and, following abandonment due to toxicity, further modification of the structure produced LY303366, culminating in the synthesis of its more soluble prodrug LY307853 (**18**) by converting the phenolic hydroxy to a sodium phosphate ester.[34,35]



18 R = PO_3Na_2

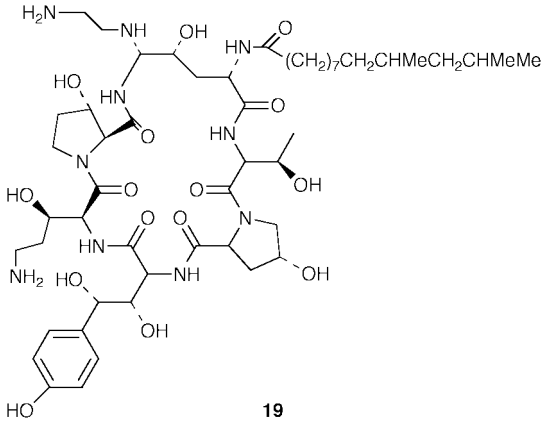
Using a similar type of compound as the starting material, pneumocandin A_0, chemists at Merck have produced MK-0991 (**19**) which likewise demonstrates good activity against the major human pathogen, *C. albicans* and other pathogenic fungi from diverse genera.[36]

4.8 Antifungals: non-lipopeptides

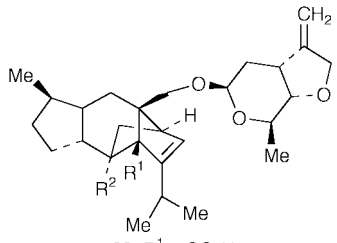
Using a high throughput screen directed against protein synthesis in *Candida*, Glaxo-Wellcome discovered that an analogue of the previously known molecule sordarin was an effective *in vitro* inhibitor, with apparent selectivity for fungal protein synthesis. Following a long program of mutation and medicinal chemistry, semi-synthetic derivatives of sordarin were produced with the analogue GM237354 (**20**) entering preclinical development.[37]

4.9 Antimalarials

The quintessential antimalarial lead was quinine, originally isolated from *Cinchona* bark, acting as the template for the

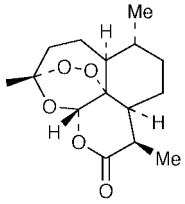


19

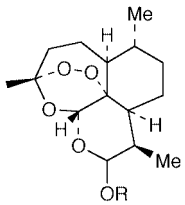


20 $R^1 = CO_2H$, $R^2 = CHO$

synthetic agents of the chloroquine/mefloquine type. With the rise of parasites resistant to these agents, came the search for other synthetic and natural product-based agents. Inspection of the data from Chinese herbal remedies led to the investigation of extracts of *Artemisia annua* (Wormwood). This particular plant had been used for centuries in China as an antimalarial[38] (known as 'Quinghaosu') and in 1972, the active agent was isolated and identified as a sesquiterpene endoperoxide named artemisinin (**21**). In a recent review, Tan *et al.*[39] reported that



21

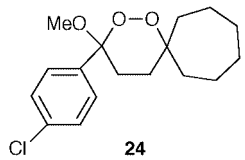


22 Artemether R = Me
23 Dihydroartemsinin R = H

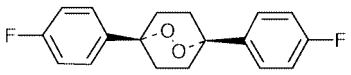
this particular agent is not limited to the species *annua*, but is found in at least two others. Using the base structure of artemisinin, many semi-synthetic compounds were made with the aim of optimizing the pharmacology of the base molecule, leading to the identification of artemether, **22**, (a relatively simple modification of the actual active metabolite, dihydroartemisinin, **23**) as a potent antimalarial agent that is now in widespread use throughout the world. Other simple modifications have been made to improve solubility and distribution but, in all cases, the active constituent ends up to be the same molecule, so these can all be considered to be 'prodrugs' of dihydroartemisinin (*cf.* Table IV in De Smet).[40]

It has been suggested from many *in vitro* and *in vivo* studies that the active principle of artemisinin resides in the peroxide bridge and that an iron-catalyzed conversion occurs to give an electrophilic free radical composed of the majority of the molecule. This compound then acylates proteins that are present in the parasite but not, to any extent, in the host.[41–43] With the recognition that the peroxy bridge is essential for activity, came the syntheses by many groups in both China and the West, of compounds containing the basic artemisinin structure. The

major group in the USA working on these compounds has been associated with the Walter Reed Army Institute of Research and the papers by Avery *et al.* should be consulted for details.[44–46] As time progressed, much simpler structures containing the required peroxy bridge were synthesized (*e.g.* **24**, **25**) and they retained nanomolar activity. Recent reviews of the work leading to such molecules have been published by Posner *et al.*[47,48]

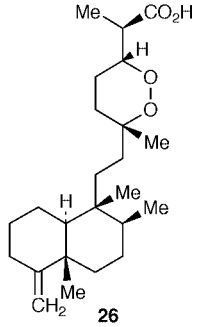


24

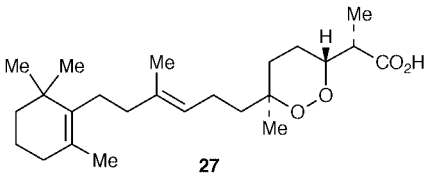


25

Natural products from marine sources also contain the peroxy-bridge found in the artemisins, examples being the norsesterterpenes sigmosceptrellin[49] (**26**) and muqubilin[50] (**27**) from the Red Sea sponges *Sigmosceptrella* and *Prianos*. Other



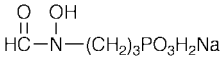
26



27

similar molecules have also been reported by the Kashman group[51] from a South African *Plakortis*. At that time, their potential as leads to antimalarials was not appreciated, though later work by Hamann's group at the University of Mississippi[52] and Konig's group at Braunschweig[53] have shown that a variety of marine-derived structures may have potential as leads to other antimalarial structures. It will be interesting to see how these molecules act as leads *versus* those from plant sources.

In a very recent paper, workers in Germany[54] have demonstrated that a very simple series of microbial metabolites, the fosmidomycins (**28**), originally reported by workers at Fuji-



28

sawa[55,56] as *Streptomyces* metabolites, have antimalarial activity both *in vitro* and *in vivo* in rodent models. These simple molecules appear to inhibit isoprenoid biosynthesis that is dependent upon the DOXP pathway (from 1-deoxy-D-xylulose 5-phosphate) rather than the more usual HMG-CoA reductase route. This discovery of a non-mammalian isoprenoid biosynthetic route in the parasite and the identification of an inhibitor may well open up an entirely new route to antimalarials, particularly as synthetic routes to this type of molecule have been published in detail[57] and they appear to lend themselves to parallel syntheses to produce a wide variety of congeners.

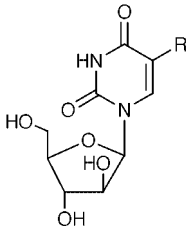
4.10 Antiviral area: general

If one thinks that there is a paucity of effective antifungal agents, then the antiviral area is positively barren by comparison, in spite of the vast number of chemical compounds, derived from natural sources, semi- or total synthesis, that have been tested for their efficacy as antiviral agents. One major reason for this is the very nature of a viral disease, in that the virus, irrespective of type, effectively takes over an infected cell and hence there are very few specific viral targets for small molecules to interact with. With the advent of molecular cloning techniques, however, the situation is changing as it is now possible to identify specific viral-related proteins, clone them, express them and then use in rapid screening systems, looking for specific interactions in the absence of the host proteins.

Rather than deal with each type of viral infection, we have elected to show how a serendipitous discovery of a series of natural products has led to the plethora of similar compounds that are now in preclincal or clinical evaluation, and in some cases, in clinical use.

4.11 Antiviral area: nucleoside analogues

From 1950 to 1956, Bergmann *et al.*[58–60] reported on two compounds that they had isolated from marine sponges, spongouridine (**29**) and spongothymidine (**30**). What was significant about these materials was that they demonstrated, for the first time, that naturally occurring nucleosides could be found using sugars other than ribose or deoxyribose.

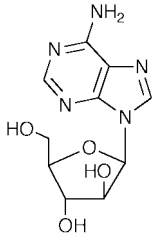


29 R = H
30 R = Me

These two compounds can be thought of as the prototypes of all of the modified nucleoside analogues made by chemists that have crossed the antiviral and anti-tumor stages since then. Once it was realized that biological systems would recognize the base and not pay too much attention to the sugar moiety, chemists began to substitute the 'regular pentoses' with acyclic entities, and with cyclic sugars with unusual substituents.

These experiments led to a vast number of derivatives that were tested extensively as antiviral and anti-tumor agents over the next thirty plus years. Suckling, in a 1991 review[61] showed how such structures evolved in the (then) Wellcome laboratories, leading to AZT and, incidently, to Nobel Prizes for Hitchens and Elion, though no direct mention was made of the original arabinose-containing leads from natural sources.

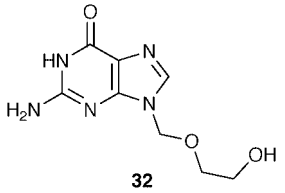
Showing that 'Mother Nature' may follow chemists rather than the reverse, or conversely that it was always there but the natural products chemists were 'slow off the mark', arabinosyladenine (Ara-A or Vidarabine®, **31**) was synthesized in 1960 as a potential anti-tumor agent,[62] but was later produced by fermentation[63] of *S. griseus* and isolated, together with spongouridine,[64] from a Mediterranean gorgonian (*Eunicella cavolini*) in 1984.



31

Of the many compounds derived from these early discoveries, some, such as Ara-A, Ara-C, Acyclovir (**32**) and later

AZT and DDI, have gone into clinical use, but most have simply become entries in chemical catalogues.

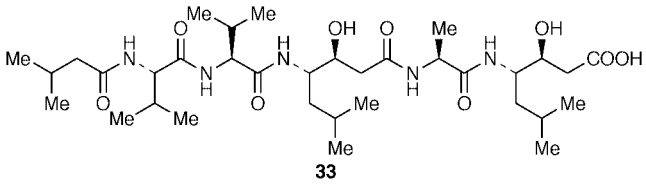


32

4.12 Antiviral area: HIV protease inhibitors

As mentioned above, there are few targets that are 'virus-only' in nature when it comes to screening, but in the case of HIV 1, a specific target is the aspartic protease that is an essential part of the processing of the viral proteins *pol* and *gag* that permit the virus to replicate in the host cell.

The initial work on this protease by the Merck group demonstrated that it was an aspartic proteinase and could be inhibited by the microbial pepsin inhibitor, pepstatin. In fact, inhibition by this peptide could be seen in both isolated enzymic and in whole cell assays.[65] Pepstatin (**33**) contains an unusual



33

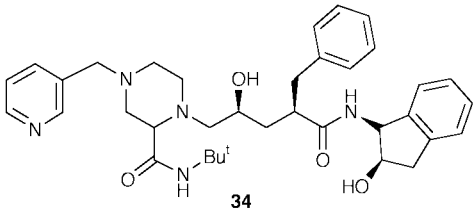
hydroxy-amino acid, statine, which can be thought of as a mimic of a putative transition state intermediate, where the hydroxy group takes the place of a water molecule that is the second substrate for the hydrolytic reaction.[66] Using this hypothesis, plus the idea that statine is acting as a dipeptide replacement, two groups, one at Wisconsin and the other at Merck, collaborated to produce renin inhibitors containing a hydroxyethylene isostere that gave activity comparable to that of pepstatin as a pepsin inhibitor.[66] Concomitantly, studies with replacement of amino acids in aspartic-proteinase substrates (in general, 6 to 8 residues in length) with statine or isosteres, led to the production of potent inhibitors of the aspartic proteinases, renin and elastase.[66]

Once the investigators realized from the pepstatin results that the activity of HIV 1 protease was due to the presence of the same (or similar) catalytic site to that of renin, then the collection of renin inhibitors was tested, leading to identification of potential HIV 1 inhibitors. Using similar techniques to those that proved fruitful with the renin and elastase systems, variations around those inhibitors, and/or others based on a short peptide that was the consensus substrate of HIV 1 protease, were synthesized using isosteric replacements for a variety of the amino acids, but, in most cases, keeping the 'statine-mimic' aligned with the geometry of the active site.

These synthetic exercises based on a natural product model have led to successful drug entities[67,68] that are now available for the clinical treatment of HIV infections, though the molecules used, such as Crixivan® (**34**), show no formal structural relationship to the original natural product inhibitor, pepstatin.[69–72]

5 Cardiovascular: general

In our usage, cardiovascular diseases will cover the following general areas. Control of the β-adrenergic nervous system which will include some aspects of respiratory function, control

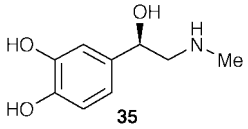


34

of cholesterol/lipid metabolism and control of angiotensin levels. The underlying physiological principle that is addressed in these areas is the homeostatic control of blood pressure.

5.1 Cardiovascular: the β-adrenergic amines

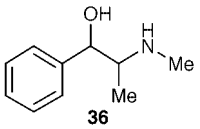
Although epinephrine (adrenaline, **35**) was discovered from natural sources (extraction of sheep adrenal glands) around the



35

turn of the 20th century and was marketed as a pharmaceutical, it was not until the investigations by Chinese scientists in Peking in the early 1920s that a reasonable source of what eventually became known as the sympathomimetic amines was identified.

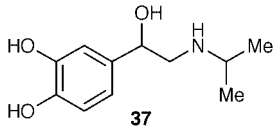
The Chinese had known of the potential of the plants *Ephedra sinaica* and *E. equisetina*[73] for millenia as treatments for asthmatic and other bronchial conditions and then, in 1923, Chen obtained pure ephedrine (**36**) from *E. sinaica* and



36

demonstrated that its physiological actions were very similar to adrenaline, causing elevation of blood pressure, plus inotropic and chronotropic actions on the heart. Following regulatory approval, it became the first in a very long line of bronchodilators/CV agents.

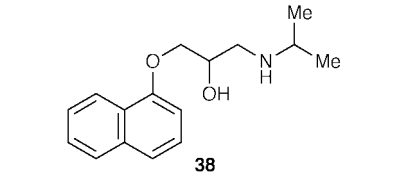
Work between the world wars led to the identification of other amino compounds based on the ephedrine basic structure, with benzedrine and methamphetamine being widely issued during WWII as stimulants. Following WWII, these compounds became tightly regulated in most jurisdictions because of their abuse potential. The next major development was the synthesis of the molecule isoprenaline (Isoprel®, **37**) by combining the *o*-



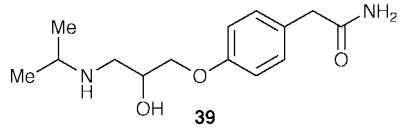
37

catechol ring with a modified amphetamine side-chain. This molecule showed excellent activity as a bronchodilator without significant action on blood pressure but it had significant cardiac stimulant effects.

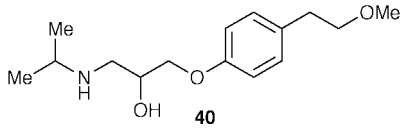
It was the subsequent work of Black (part of the studies for which he ultimately received the Nobel Prize) that demonstrated that there were two basic types of β-receptor, the β_1, which is predominately cardiac, and the β_2 which is predominately tracheal/lung. He, together with co-workers at ICI Pharmaceuticals developed the first true β-blocker, propranolol[74] (**38**). To add to the 'confusion', the compounds that were being



38



39



40

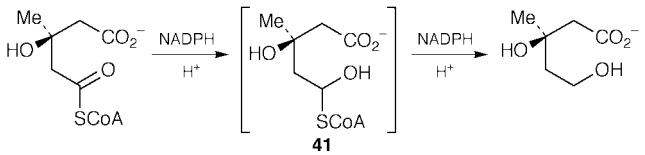
developed at that time (the middle to late 1960s through the 1980s) could have both agonist and antagonist activities and, in some cases, these activities could 'partially cross-over' and show mixed agonist/antagonist activities. Chemical manipulation around the basic structure, coupled to use of isolated receptor assay techniques has led to compounds with much better separation of the β-blocking activities such as atenolol (**39**) and metaprolol (**40**) that have no detectable intrinsic sympathomimetic activities.

Thus, starting with an agonist structure (ephedrine) that significantly affected blood pressure due to its effect on cardiac output and on release of other sympathomimetic amines, one now has available related structures that are specific blockers of such activities on cardiac tissue and are excellent 'reducers of hypertension'.

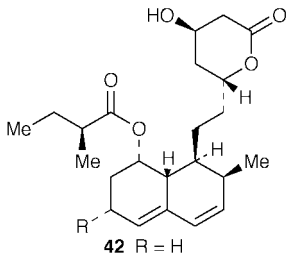
5.2 Cardiovascular: cholesterol lowering agents

Another major cause of elevated blood pressure is due to the physical blockage of the arteries by plaques of cholesterol/lipoproteins (atherosclerotic plaque). Since the human synthesizes about 50% of its requirement for cholesterol, if the synthesis can be inhibited, then a reduction in overall cholesterol may reduce the deleterious effects of this steroid.

A potential site for inhibition of cholesterol biosynthesis in eukaryotes is at the rate-limiting step in the system, the reduction of hydroxymethylglutaryl coenzyme A by HMG-CoA reductase to produce mevalonic acid (**41**). By following



41

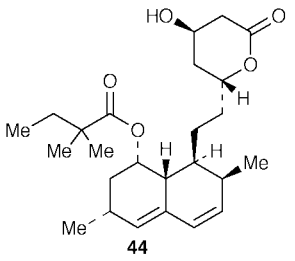


42 R = H
43 R = Me

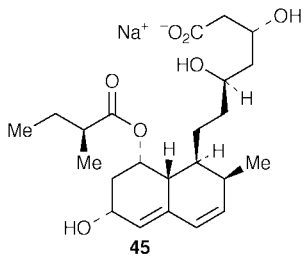
inhibition of sterol production and using fungal fermentation broths as the source of natural products, Sankyo discovered compactin (**42**) from a fermentation of *Penicillium brevicompactum* and patented it in 1975.[75,76] Compactin was also reported at the same time as an antifungal agent by Brown *et al.*[77] This material was shown to be a competitive inhibitor of the enzyme with K_is in the nanomolar range, but it was not developed further.

Using a similar assay, a homologue of compactin, mevinolin (**43**) or 7-methylcompactin, was isolated by Sankyo from *M. ruber* and reported in 1979[78,79] with a submission by Endo, under the name Monacolin K, to the Japanese Patent Office for the activity, but without a structure. Concomitantly, Merck discovered the same material from *A. terreus*, using an isolated HMG-CoA reductase assay and microbial broths as the source of test agents. Merck reported the mevinolin discovery in 1980 in a communication in the *Proceedings of the National Academy of Science*, USA[80] and after submission of both structure and findings to the Patent Office, a US Patent was issued in late 1980.[81] Following a very significant volume of work, mevinolin became the first commercialized HMG-CoA-reductase inhibitor in 1987.[82,83]

Further work using either chemical modification of the basic structure (*a la* β-lactam modifications based on 6APA) or use of biotransformation techniques led to two further compounds by converting the 2-methylbutanoate side-chain into 2,2-dimethylbutanoate, giving simvastin (**44**), or opening of the exocyclic lactone to give the free hydroxy acid, pravastatin (**45**).



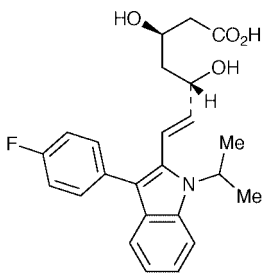
44



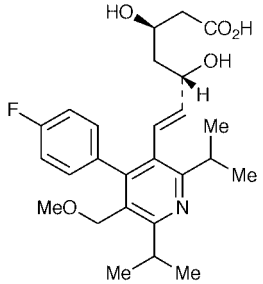
45

Data from 1997, the last year for which full sales information is available, shows that these three natural-product derived drugs are the second best-selling compounds in the world, with combined sales of US$7.53 billion.[18]

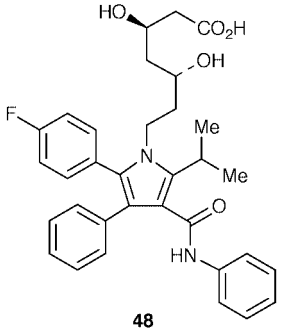
Comparison of the ring-opened lactone structure common to all of the 'statins' shows the resemblance to mevalonic acid and this recognition led to the synthesis of the three synthetic clinical products fluvastatin (**46**), cerivastatin (**47**) and atorvastatin (**48**). The first two have the dihydroxy-heptenoic acid side-



46



47



48

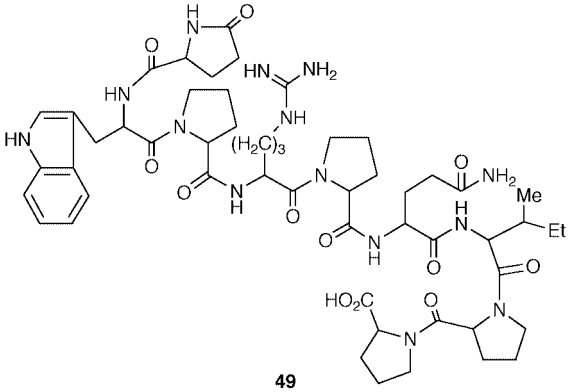
chain of the fungal-derived products linked to a lipophilic ring structure whilst the third uses the reduced form of the acid

directly linked to the heteroatom of a pyrrole ring. In the nomenclature of Cragg *et al.*,[84] these would be classified as S* compounds; synthetic in nature but derived from a natural product prototype.

5.3 Cardiovascular: angiotensin converting enzyme inhibitors (ACE inhibitors)

The angiotensinogen to angiotensin I to angiotensin II cascade is an essential mechanism in the maintenance of blood pressure in humans and it was realized that if one could inhibit the conversion of the decapeptide (angio I) into the biologically potent octapeptide (angio II), then it might be possible to control blood pressure by such compounds. In 1965, Ferreira[85] reported that fractions from the venom of the pit viper, *Bothrops jararaca*, inhibited the degradation of the mammalian nonapeptide, bradykinin. The enzyme that degraded this peptide, a dipeptidyl carboxypeptidase, was subsequently identified as having ACE activity and was, in fact, the same enzyme working with two different substrates.

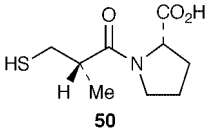
The role of ACE in hypertension was identified as a result of the work of Ondetti *et al.*[86] demonstrating that the active principle in the viper venom was a simple nonapetide, teprotide (**49**). This material had specific activity as an ACE inhibitor and



49

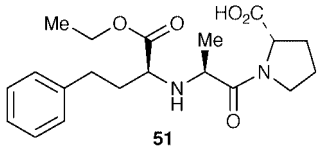
also had hypotensive efficacy in clinical trials, though, due to a lack of oral availability, it was a good lead but not a particularly good drug candidate.

With the recognition that ACE was a metallo-enzyme came the utilization of a similar carboxypeptidase (carboxypeptidase A; a monopeptidyl-carboxypeptidase; CpdA) as a surrogate model. Previous work by the Squibb group had shown that all of the peptidic inhibitors of ACE had a C-terminal proline, and by using this information, plus the fact that benzyl-succinic acid was a specific inhibitor of CpdA, they derived a series of carboxy- and mercapto-alkanoyl esters of proline that demonstrated good to excellent inhibition of ACE. One of the compounds (SQ14225), **50**, subsequently became the prototypical ACE drug, Captopril®.[86,87]

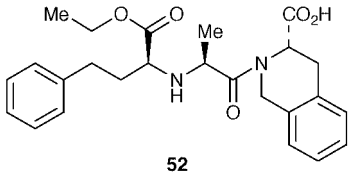


50

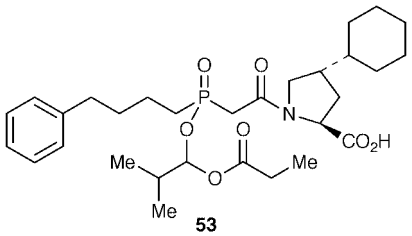
Further development of the concepts shown in this work has led to more potent ACE inhibitors as information as to ADME and the specific spatial requirements of the ACE active site(s) were further delineated. In some cases, the drugs that are used are formally prodrugs, with cleavage of carboxylic esters in the cases of Enalapril® (**51**) and Quinapril® (**52**), or of a phosphinate ester in the case of Fosinopril® (**53**), to give the active drug once in the circulation.



51



52



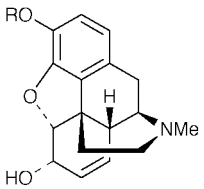
53

6 Pain/central nervous system: history

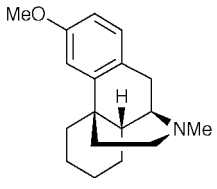
The conquest of pain was one of the major uses of medicinal plants in the Ancient World, with the possible use of the crude extract of the opium poppy (*Papaver somniferum*) dating from around 6000 years ago in Sumeria. However, the earliest undisputed reference to the use of the 'juice of the poppy' is from Theophrastus approximately 2300 years ago. The use of opium, or laudanum as it was named by Paracelsus in the sixteenth century, was popularized in both Europe and particularly in the Orient, even leading to the so-called "Opium Wars" between the UK and China in the 1840s.

6.1 Pain/central nervous system: the opiates

Dioscorides first described the method of production from the seeds of the poppy (*cf.* p. 175 in Mann[12]), and a similar method is still used today. Crude 'Opium', the dried exudate, contains about a quarter of its weight as opium alkaloids with morphine and codeine being the major components with up to 20 more distinct alkaloids including thebaine, papaverine and noscapine. Morphine (**54**) was first isolated by Serturner in 1806, followed by codeine (**55**) in 1832 by Robiquet and then the non-morphine



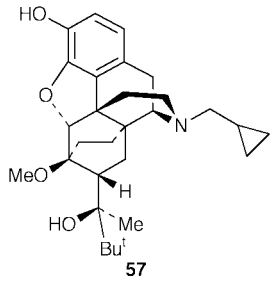
54 R = H
55 R = Me



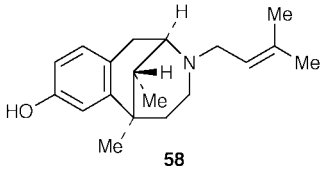
56

alkaloid papaverine by Merck in 1848. With the invention of the hypodermic needle and the availability of the purified alkaloids, the benefits and problems associated with widespread use of these alkaloids rapidly became apparent[88,89] leading to the search for potent drugs without the abuse potential. Ironically, heroin, the compound that has probably caused the most human anguish since its preparation in the UK from morphine in 1874, was marketed by Bayer from 1898 predominately as a cough suppressant. A close relative, dextromethorphan (**56**), is in fact used in most cough syrups today, but lacks the abuse potential of its chemical cousin. Interestingly, there is now evidence that morphinans (codeine and morphine at least) may be synthesized in mammalian tissues as well.[90,91]

With the recognition of the abuse potential of the opiates came the search to make compounds that mimicked morphine in pain control but lacked the abuse potential. With the exception of the semi-synthetic compound buprenorphine (**57**), which is



57



58

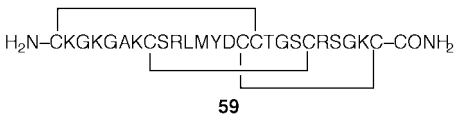
approximately 25–50 times more potent than morphine and has a lower addiction potential, none of the compounds made to date from modifications around the phenanthrene structure of morphine have exceeded the pain control properties without a concomitant addiction potential. Another interesting compound whose structure is based on that of morphine, is pentazocine (Talwin®, **58**). This is about 30% as effective as morphine, but with a much lower incidence of abuse and, in fact, will cause withdrawal symptoms in morphine addicts at high dosage due to antagonism at the morphine-active μ-receptor.

Perhaps the major advance in the understanding (at least to some molecular level) came from the work of investigators who made the then novel assumption that the complex interactions amongst this class of drugs could best be described by suggesting that there were multiple opiod receptors for these drugs.[92] Following this, in 1973 three independent groups (those of Terenius;[93] Pert and Snyder;[94] Simon, Hiller and Edelman[95]) described receptor binding sites in the mammalian nervous system. These sites were stereospecific and saturable by varying opioid drugs. Following these discoveries and confirming the suspicion that the body had a method of quelling pain, came the isolation from porcine brain by Hughes and Kosterlitz in Aberdeen in 1975 of two pentapeptides that demonstrated opioid-like effects on guinea pig ileal strips. These were named enkephalins and, following work by others reported the same year, it was realized that there are at least three distinct families of peptides that exhibit opioid-like activities, the enkephalins, the endorphins and the dynorphins.[96] Although a substantial amount of work was performed with small peptide compounds based on these relatively simple structures, none have succeeded as drugs to replace opioids as yet (though see below under conotoxins). However, these small peptides and synthetic derivatives derived from them have seen extensive use as delineators of opioid receptor sub-types in many tissues, not just those of the central nervous system. Examples of some structures are given in the review by Sibinga and Goldstein[97] and in the paper by Kramer *et al.*[98]

6.2 Pain/central nervous system: the conotoxins

The cone snails (phylum Mollusca, genus *Conus*) with 500 species known are venomous marine animals that are predominately found in the Western and South-western Pacific, though there are some species found in Californian waters. Their particular 'expertise' is that they stun/kill their prey through the use of a disposable hollow tooth that contains a multitude of peptidic toxins. One might even claim that Mother Nature invented the disposable hypodermic needle, as their method of delivery in most cases is *via* a harpoon-like hollow tooth that breaks off once in the prey.

These animals would have simply been 'just another venomous marine organism' but for the work of Olivera *et al.*[99,100] who demonstrated that the venom from each of these animals contained over thirty polypeptides ranging in size from 10 to 35 amino acids and they demonstrated that specific peptides gave specific physiologic responses in mammalian systems (and by inference, in the piscatorial arena where they were originally designed to work). From these original discoveries has come the identification of a novel class of analgesics that appear to specifically target a voltage gated Ca^{2+} channel, are very potent and have low probabilities for abuse. Olivera[101] has used the term 'Janus-ligand' for these peptidic ligands as they appear to have both a 'docking face' and a 'locking face', thus giving exquisite selectivity and sensitivity in the same molecule. The original material that has undergone clinical development, and is currently in Phase III trial/awaiting approval from the FDA, is SNX-111 (**59**) from Neurex



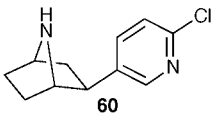
59

Corporation (now a part of Elan Pharmaceuticals). Though made synthetically, it is in fact identical to MVIIA from *Conus geographicus*.[102] Many other synthetic variants have been made and tested[102] and will probably follow SNX-111, but for other indications.

The potential for advances in other physiological areas as the properties of the myriad of other peptides are investigated is very high, showing the advantages of Nature's combinatorial chemistry approach with these molecules.

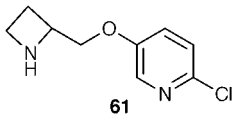
6.3 Pain/central nervous system: the epibatidines

In 1974, John Daly at the NIH isolated a small amount of a novel alkaloid from the skin of an Ecuadorian poison frog (*Epipedobates tricolor*) and demonstrated that it had excellent analgesic effects in a particular mouse model. The material could not be recollected and, without a structure, no further work was done. With the advent of new NMR techniques in the late 1980s, the 750 micrograms remaining was enough to show that the structure was a substituted chloronicotine, that was named epibatidine[103] (**60**). This molecule was synthesized and demon-



60

strated excellent inhibition of the nicotinic receptors in neurons and neuromuscular junctions but with a lack of specificity exemplified by its very low therapeutic index as referenced by Ellis from UCB Research.[104] In addition to Ellis' group at UCB Research, the Abbott group led by Decker[105–107] have shown the potential of this base molecule, and the manifold modifications that have been made have shown analgesic effects mediated *via* one or more of the nAChR (nicotinic acetylcholine receptor) subtypes in neuronal tissues. The structures shown in these papers demonstrate the relative simplicity of these agents, being predominately based on the nicotine/epibatidine locus. To date, one compound from the Abbott group, ABT594 (**61**), has



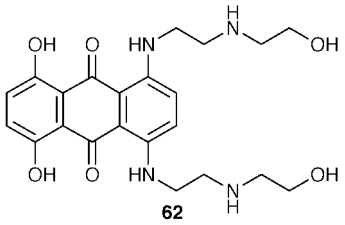
61

reached advanced preclinical status. If it moves into Phase I, it will be the first epibatidine derivative to cross that hurdle and may demonstrate that specificity as to subtype will work in man as well as mouse.

7 Antineoplastics: general

It is in the treatment of cancers and in anti-infective areas that natural products have made their major impact as templates or direct treatments. In the cancer area, of the 92 drugs commercially available prior to 1983 in the United States, or approved worldwide between 1983 and 1994, approximately 62% can be related to a natural product origin, ignoring those of biological origin such as interferon or recombinately produced cytokines.[84]

A common feature with many of the natural product-derived anticancer drugs now in clinical use is that the original natural product was too toxic. Chemists therefore, had to make semi-synthetic compounds based on the natural product structures that had the more deleterious problems of the natural product diminished by selective modification. In some cases, notably that of mitoxantrone (**62**), a totally synthetic product evolved



62

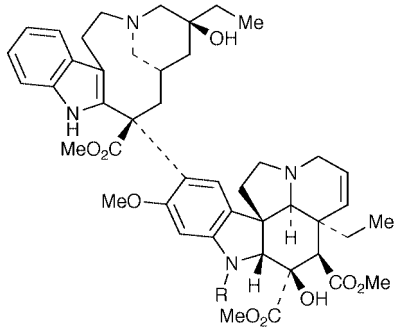
but with the attributes of two or more parent natural product structures in one molecule.[84]

7.1 Antineoplastics: plant sources

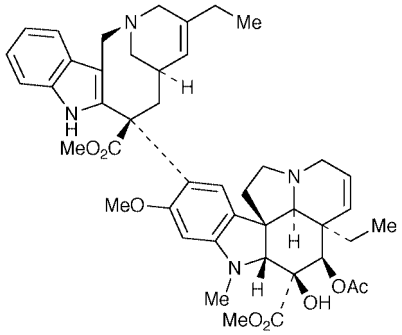
Although plants have a long history of use in the treatment of cancer,[108] many, if not all, of the claims for the efficacy of such treatment should be viewed with some skepticism because cancer, as a specific disease entity, is poorly defined in terms of folklore and traditional medicine.[109]

Amongst the best known are the so-called vinca alkaloids, vinblastine (**63**) and vincristine (**64**), isolated from the Madagascan periwinkle, *Catharanthus roseus*. *C. roseus* was used by various cultures for the treatment of diabetes, and these compounds, together with two other related active alkaloids, vinleurosine and vinrosidine, were isolated during an investigation of the plant as a source of potential oral hypoglycemic agents. Therefore, the discovery of the initial two compounds may be indirectly attributed to the observation of an unrelated medicinal use of the source plant.[109] Selective chemical modifications of these two molecules have led to two semi-synthetic compounds (from many) being approved in Europe for cancer treatment, vinorelbine (**65**) and vindesine (**66**). The former was recently approved in the US and the latter is in US clinical trials.[110]

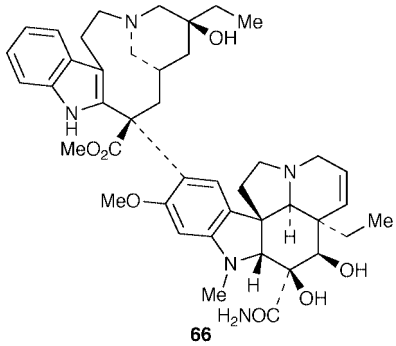
The parent of the two clinically-active agents etoposide (**67**) and teniposide (**68**) is epipodophyllotoxin. This is the naturally occurring epimer of podophyllotoxin (**69**) which was isolated as the active anti-tumor agent from the roots of various species of the genus *Podophyllum*. These plants possess a long history of medicinal use by early American and Asian cultures, including the treatment of skin cancers and warts.[109] Although podophyllotoxin was investigated at length by the NCI as a potential anti-tumor agent it was shelved due to intractable toxicity problems. Subsequent elegant work by Sandoz, prior to its merger with Ciba-Geigy, led to the synthesis from epipodophyllotoxin of etoposide and teniposide, both of which are in general clinical use. Further work by Nippon Kayaku has led to a water-soluble derivative of etoposide, NK-611 (**70**), where a dimethylamino group was placed into the sugar ring, thus giving significant water solubility; the material is now in clinical trials (*cf.* references 60 to 62 in the recent review by Wang[111]). Two recent reviews covering work at the University of North



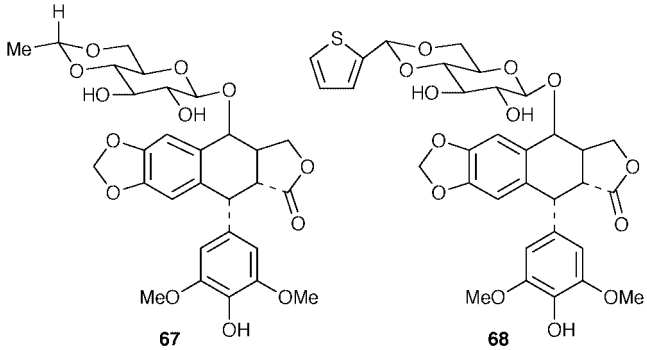
63 R = Me
64 R = CHO



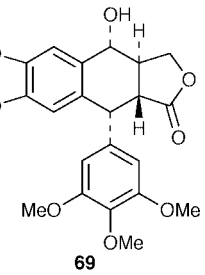
65



66

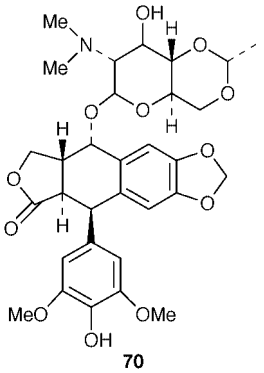


67 **68**



69

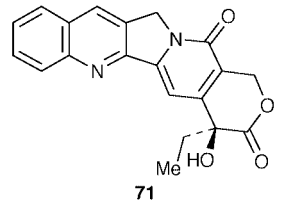
Carolina, give information on the semi-synthetic derivatives based on the podophyllotoxins[112] and the earlier review covers inhibitors of topoisomerase I and II either from natural sources or based upon natural products.[113] These reviews should be read



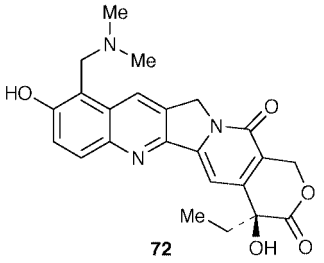
70

in conjunction with this article as further examples of where natural product-derived templates for anti-tumor agents have led to in a synthetic sense.

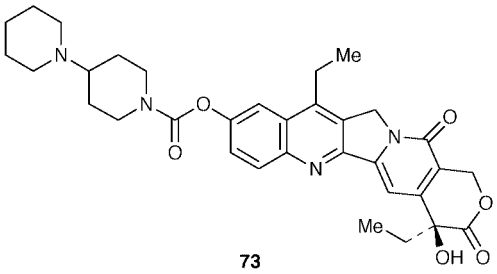
Camptothecin (**71**) was isolated from the Chinese ornamental tree *Camptotheca acuminata* by Wani and Wall[114] contemporaneously with the initial discovery of Taxol®. As the sodium salt, camptothecin was advanced to clinical trials by NCI in the



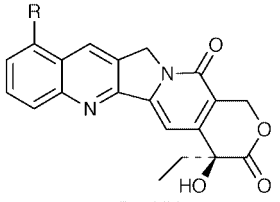
71



72



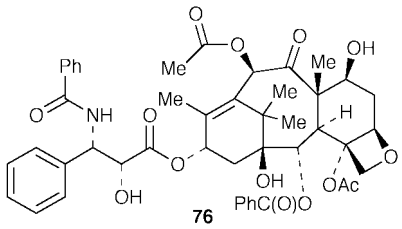
73



74 R = NH_2
75 R = NO_2

1970s, but was dropped because of severe bladder toxicity. It was resurrected as a result of its very specific biochemical activity as an inhibitor of topoisomerase I and the efforts of one of the earliest NCI National Cooperative Drug Discovery Groups (NCDDGs) involving Johns Hopkins University and the then SmithKline Beckman. From these studies eventually came the modified camptothecin, topotecan (Hycamptin®) (**72**), which was approved for use in the USA in 1996. Other groups modified the basic structure in slightly different ways,[114] leading to the approved agent irinotecan (Camptosar®) (**73**), and two others awaiting approval, 9-amino- (**74**) and 9-nitro-camptothecin (**75**).

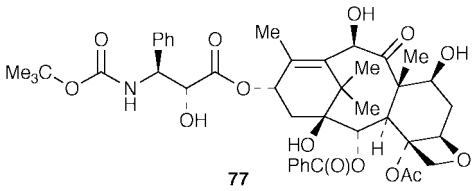
The complex diterpene Taxol® **76** (Paclitaxel) initially was isolated from the bark of *Taxus brevifolia*, collected in Washington State as part of a random collection program by the U.S. Department of Agriculture for the National Cancer



76

Institute.[115] Historically, parts of the yew tree (*T. brevifolia* and other *Taxus* species) had been used by several Native American tribes for the treatment of some non-cancerous conditions,[108] and leaves of *T. baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system,[6] with one reported use in the treatment of 'cancer'[108]

Paclitaxel, along with several key precursors (the baccatins), occurs in the leaves of various *Taxus* species, and the ready semi-synthetic conversion of the relatively abundant baccatins into paclitaxel, as well as to active paclitaxel analogs, such as docetaxel[116] (**77**), has provided a major, renewable natural

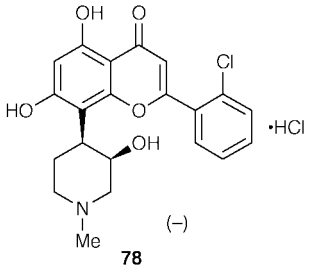


77

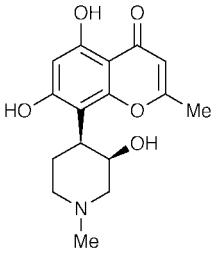
source of this important class of drugs. Using the baccatins as starting materials, many semi-synthetic taxanes have been synthesized in an effort to obtain materials that have better solubility in aqueous environments. A recent paper by Kingston *et al.* demonstrates the methods used to prepare more active 2-acyl analogues of paclitaxel[117] and covers, in its references, the many modifications that workers have made to the basic taxane skeleton in efforts to better understand the SAR of tubulin binding and activity.

The flavone, flavopiridol (**78**), is currently in Phase I/II clinical trials against a broad range of tumors.[118] While flavopiridol is totally synthetic, the basis for its novel structure is the natural product rohitukine (**79**) isolated from *Dysoxylum binectariferum.*[119] Flavopiridol has a very interesting mechanism of action in that it is an inhibitor of cyclin dependent kinases (the regulators of the G_2 to M transition in the cell cycle). This discovery has led to a series of compounds (the paullones as exemplified by structure **80**) from the NCI open compound database that, though not natural products, would not have been discovered except for the use of the natural product-derived agent as a '*seed compound*'. This story is given in detail in a recent paper by Sausville *et al.*[120] Other recent papers dealing with discovery and modification around the paullone structure are those of Schultz *et al.*[121] and Zaharevitz *et al.*[122]

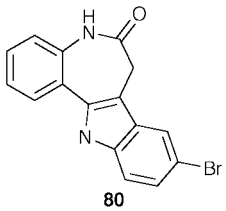
Flavopiridol is not the only CDK inhibitor discovered from a natural source and then extended to give semi-synthetic and synthetic compounds with a variety of potencies and specificities. Workers associated with Meijer's group in France have published extensively on the potential of the highly related purine analogues, olomoucine (**81**) and roscovitine (**82**) as CDK inhibitors.[123–125] These naturally occurring purine analogues are competitive inhibitors with ATP and were thus thought to bind at the ATP site in the protein. This was confirmed when the



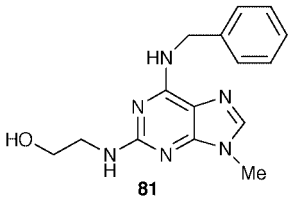
78



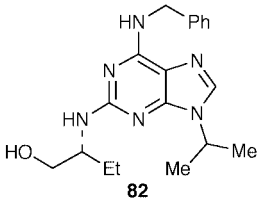
79



80

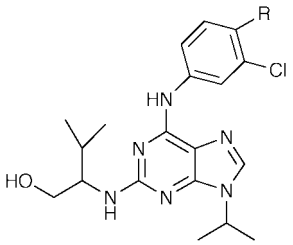


81



82

structures of the protein inhibitor complexes were solved at high resolution[126,127] confirming the results from the enzyme inhibition studies. The two natural products were not of sufficient potency for use in *in vivo* experiments and these findings led groups to use combinatorial chemistry techniques to optimize the '*natural product backbones*' of these two substituted purines. Work from Schultz' group at Berkeley was reported in 1998[128] and then more complete reviews from the same group were published in 1999.[129,130] Comparison of the structures of purvalanols A (**83**) and B (**84**) with olomoucine



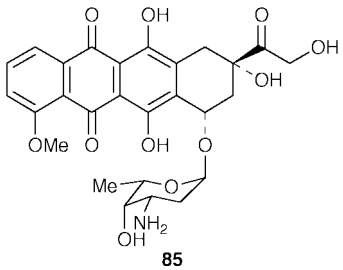
83 Purvalanol A (R = H)
84 Purvalanol B (R = CO_2H)

and roscovitine show that only very small changes in the side-chains are enough to alter the IC_{50} figures by three orders of magnitude. The interesting aspect, however, is that the base structure is effectively that of the natural products, thus demonstrating the value of using combinatorial chemistry techniques to optimize an existing active structure rather than attempting to synthesize *de novo*.

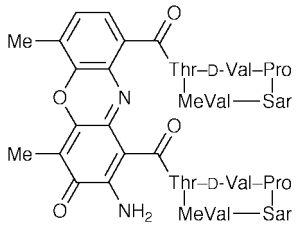
For a more general review of the history of CDK inhibitors and a discussion of structures and derivatives in addition to those mentioned above, a majority of which are natural product based, the reader should consult the recent review by Webster,[131] though, because this is such a fast-moving area, any review will be outdated by the time it is published.

7.2 Antineoplastics: microbial sources

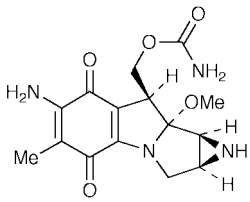
Anti-tumor antibiotics are amongst the most important of the cancer chemotherapeutic agents, which include members of the anthracycline, bleomycin, actinomycin, mitomycin and aureolic acid families.[132] Clinically useful agents from these families are the daunomycin-related agents, daunomycin itself, doxorubicin (**85**), the semi-synthetic derivatives idarubicin and epirubicin;



85



86

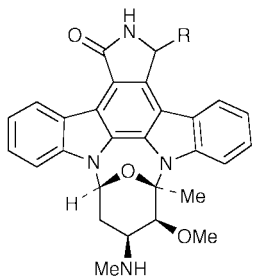


87

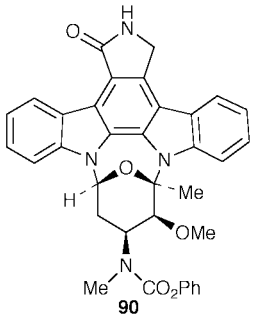
the glycopeptidic bleomycins A_2 and B_2 (blenoxane); the peptolides exemplified by dactinomycin (**86**); the mitosanes such as mitomycin C (**87**); and the glycosylated anthracenone, mithramycin. Except for the semi-synthetic compounds, all were isolated from various *Streptomyces* species.

That there is still a lot to be learned from old molecules was demonstrated by Hecht from the University of Virginia, who in a plenary lecture[133] at the November, 1999 AACR-NCI-EORTC meeting on Molecular Targets and Cancer Therapeutics demonstrated that bleomycin might well be targeting amino acid t-RNA's. He subsequently outlined a simple polymer bead-based split and pool technique that his group is using in order to produce 10^5 bleomycins within the foreseeable future in order to search for a molecule that is only t-RNA specific.

A current notable series of microbially-derived anti-tumor compounds are those directed around the staurosporin nucleus **88.** These include UCN-01 (7-hydroxystaurosporine, **89**),



88 R = H
89 R = OH

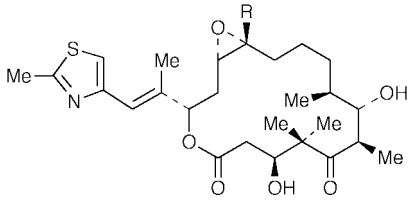


90

isolated from a *Streptomyces* species, though now made by chemical modification of staurosporin itself and many other derivatives, with the Novartis agent CGP41251[134] (**90**) and UCN-01 being in clinical trials. Novartis scientists have also recently reported that this analogue may have multiple modes of action, inhibiting angiogenesis *in vivo* in addition to its PKC inhibitory activity. If this translates into inhibition of human angiogenesis and subsequent anti-tumor activity then it distinguishes this agent from other staurosporin congeners.[135]

Perhaps the most interesting discovery from the microbial world over the last few years is the series of compounds known as the epothilones. These were originally isolated from species of myxobacteria by Hofle in Germany[136,137] and simultane-

ously by workers at Merck in the USA.[138] Various problems over patents and biological activities prevented epothilones A (**91**) and B (**92**) from being developed by companies; this

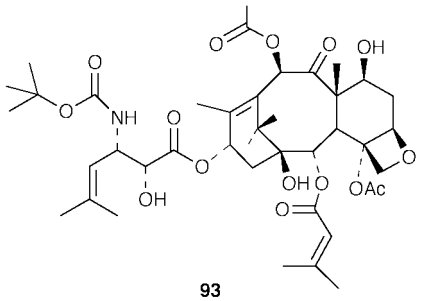


91 R = H
92 R = Me

despite the fact that the Merck group had demonstrated that they mimicked paclitaxel in their interactions with tubulin in their initial paper. Once the German group was able to assign the absolute stereochemistry,[139] then multiple groups began attempts to synthesize the epothilones.

Three groups have made major contributions to the synthesis of this class of compounds, those of Danishefsky at Sloan-Kettering who were the first to synthesize epothilone A, the second by Nicolaou at the Scripps Research Institute and the third by Schinzer in Braunschweig, close to the birthplace of the natural epothilones. Rather than go into the details of each individual group's synthetic strategies, the reader should consult the excellent recent review by Nicolaou *et al.*[140] on the epothilones. This review shows the synthetic strategies of the groups but, more importantly, shows the classical synthetic and combinatorial efforts around the structures that have led to third and fourth generation compounds now heading towards clinical development. The activity data (*cf.* Tables 7 and 8 in the Nicolaou review[140]) that are shown in these tables for some of the classical synthetically and combinatorially derived derivatives against paclitaxel-resistant cell lines are demonstrative of the power of modern synthetic chemistry when applied to an active natural product structure.

Recently, Ojima *et al.*[141] showed that a non-aromatic mimic of paclitaxel, nonataxel (**93**) was more effective than paclitaxel *in vitro* and was amenable to solution NMR analyses in order to

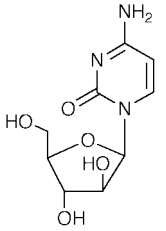


93

determine solution conformation. Using this information together with the published data for three other quite diverse antimitotic agents, discodermolide, eleutherobin and epothilone B, they were able to suggest a common pharmacophore that could account for the activities seen against tubulin by these agents and paclitaxel. A presentation by the NCI group and their collaborators[142] at the AACR-NCI-EORTC Conference on Molecular Targets and Cancer Therapeutics in November, 1999 proposed a modified pharmacophore. Their structure differed from the Ojima model by requiring the baccatin and the epothilone skeletons to maintain similar conformations permitting the required phenyl and ester linkages to be in their correct spatial relationships for maximal reaction with the binding sites. This model explained the tubulin cross-resistance profiles seen for paclitaxel-resistant cell lines.

7.3 Antineoplastics: marine sources

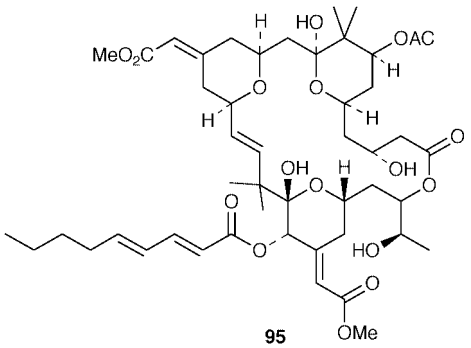
The first notable discovery of biologically-active compounds from marine sources was the serendipitous isolation of the C-nucleosides spongouridine and spongothymidine (*cf.* Antiviral section for further discussion) from the Caribbean sponge *Cryptotheca crypta* in the early 1950s. These compounds were found to possess antiviral activity, and synthetic analog studies eventually led to the development of cytosine arabinoside (Ara-C) (**94**) as a clinically useful anticancer agent approximately 15



94

years later.[143] The systematic investigation of marine environments as sources of novel biologically active agents only began in earnest in the mid-1970s. During the decade from 1977–1987, about 2500 new metabolites were reported from a variety of marine organisms. These studies have clearly demonstrated that the marine environment is a rich source of bioactive compounds, many of which belong to totally novel chemical classes not found in terrestrial sources.[144]

As yet, no compound isolated from a marine source has advanced to commercial use as a chemotherapeutic agent, though several are in various phases of clinical development as potential anticancer agents. One of these is bryostatin 1 (**95**),

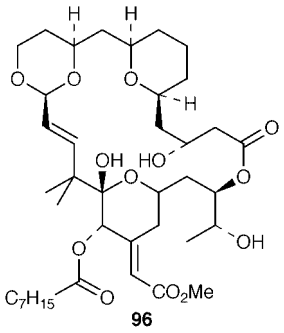


95

isolated from the bryozoan *Bugula neritina*.[145,146] This agent exerts a range of biological effects, thought to occur through modulation of protein kinase C, and has shown some promising activity against melanoma in Phase I studies,[147] and showed some stabilization of disease progression[148] in Non-Hodgkin's Lymphoma (NHL). Further Phase I/II trials are either in progress or are planned against a variety of tumors, including ovarian carcinoma and NHL, with bryostatin as both monotherapy or as an adjunct to other compounds (further information is on the NCI's web site at cancernet.nci.nih.gov).

Although bryostatin is still produced by isolation from the bryozoan, whether by aquaculture or by wild collection, work has progressed on the synthesis of simpler analogs with comparable activity. In 1988, Wender *et al.*[149] suggested a simplified pharmacophore model to explain the binding characteristics of the bryostatins to PKC. They followed this proposal up by synthesizing a series of simplified macrolide structures that they demonstrated had both PKC binding and cytotoxic activities of the same order as the bryostatins. Wender's group have reported at length on these compounds in a series of recent communications[150–154] and the structure of their most active compound (**96**) from the first series is shown.

Another marine compound that is also in Phase I/II clinical trials but under the auspices of the Spanish company Pharma-



96

Mar rather than the NCI, is ecteinascidin 743 (**97**) from the shallow water tunicate *Ecteinascidea turbinata*. Two groups, those of Rinehart *et al.*[155] and Wright *et al.*,[156] reported simultaneously on this compound from similar sources. An interesting speculation from the structure of Et743 (the usual abbreviation for ecteinascidin 743) is that the actual producing organism is a commensal microbe as the structures of these molecules resemble the saframycins[157,158] (**98**) from *Streptomyces lavendulae* and renieramycins[159] (**99**) from the Pacific sponge, *Reniera* sp.

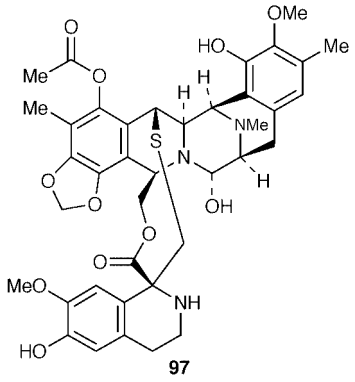
Due to the very low levels of Et743 in the tunicate and the problems of amassing enough material for large clinical trials and/or commercial use, Corey developed a synthesis of Et743 and reported it in 1996.[160] As mentioned by Corey in the discussion in this publication, Et 743 was made in 'relatively' high yield but, perhaps more importantly, the synthetic methods used opened up the potential for synthetic approaches to derivatives or to simpler compounds such as the saframycins. That this was a prescient comment was demonstrated by the publication in 1999 of simpler structures related to Et 743 made from a common intermediate in the original synthetic schema. The best compound of those reported was named phthalascidin (**100**), which demonstrated comparable activity to Et 743 in all the *in vitro* systems that it was tested in, including the NCI 60 cell line panel.[161]

The last marine examples to be discussed are materials that are still in preclinical stages of development. What is important about these compounds, however, is that they have spawned both total syntheses and then a number of combinatorial libraries that have succeeded in optimizing the *in vitro* activity of one of them. Secondly, the original compounds (in their synthetic versions) and their derivatives have been used to further delineate the binding characteristics of compounds to tubulin.

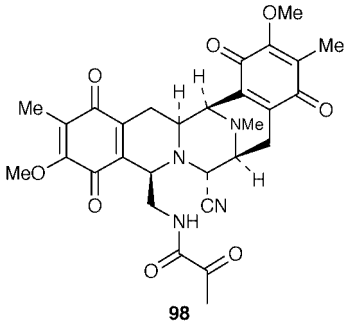
The compounds are from two series of related compounds, each containing the eunicellane carbon skeleton, a diterpene first reported in 1968 from the gorgonian octocoral *Eunicella stricta*.[162] One series was glycosylated, the eleutherobin/eleuthosides, the other being effectively their aglycone, the sarcodicytins.

The sarcodicytins (**101**) were first reported as compounds by Pietra's group, following their isolation from the Mediterranean stoloniferan coral *Sarcodictyon roseum* in 1987[163] and in 1988.[164] These reports were followed almost ten years later by an abstract from workers at Pharmacia-Upjohn presented at the 1997 American Association for Cancer Research Meeting.[165] This report demonstrated that the sarcodicytins had a paclitaxel-like action on tubulin.

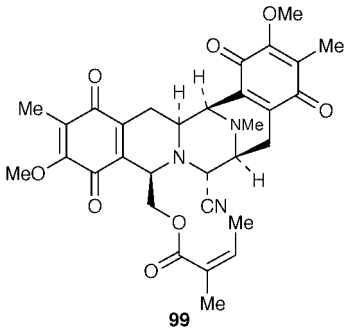
In 1994, during the intervening ten years from the original report on the sarcodicytins to that of their activity, workers in Fenical's group at The Scripps Institute of Oceanography discovered a related compound from the Western Australian coral, *Eleutherobia* sp. They named the compound eleutherobin (**102**) and showed that it contained the eunicellane skeleton but, unlike the sarcodictyins, it was glycosylated in a manner similar to that of the eleuthosides. What was unique, at that time, was its activity against tubulin where it mimicked pacilitaxel. The



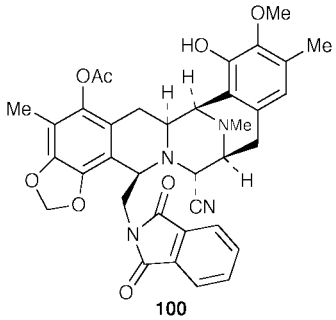
97



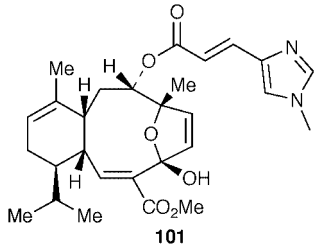
98



99



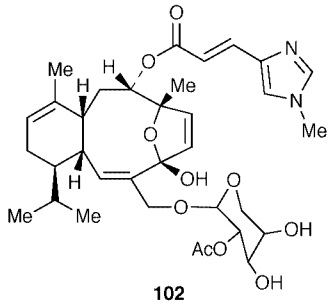
100



101

compound, and its activity, were first reported in the patent literature[166] followed by the chemical[167] and biological[168] publications.

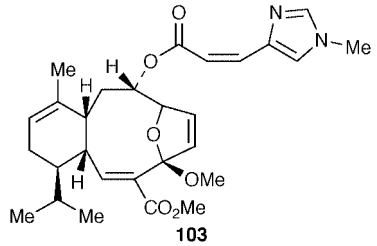
In a prime example of what the late British historian of science Derek De Solla-Price termed the 'invisible college', two excellent synthetic chemists, Nicolaou at the Scripps Research Institute and Danishevsky at Memorial Sloan Kettering suc-



102

ceeded in synthesizing both the sarcodictyins and eleutherobin. In a series of recent papers both groups described their respective synthetic methods, with Nicolaou's group being the first to press with a total synthesis of sarcodictyin A and the core of eleutherobin.[169] This publication was then followed by a very rapid series of papers from both groups.[170–174] In the middle of this series of papers, Lindel, who was one of the discoverers of eleutherobin, published a short historical review of the discovery and the relative synthetic methods used by the groups.[175]

The crux of the synthetic efforts from a drug discovery aspect, however, was the paper by Nicolaou's group that described the derivation of combinatorial libraries built around the solid and solution phase synthetic methods that they developed for the sarcodictyins.[176] In an elegant biochemical paper by Hamel *et al.*[177] the authors demonstrated the effects of one of these compounds, a compound that has some aspects of eleutherobin but without the sugar moiety (compound 1, **103)**,



103

upon binding to tubulin. In their conclusions they make two telling points:

'Like sarcodictyin A compound 1 interacts poorly with tubulin, but like eleutherobin compound 1 has substantial antiproliferative activity.'

'The activity of this agent (*compound 1*) in cells suggests that further synthetic efforts with the sarcodictyin class would yield compounds with activities greater than those of paclitaxel in cells and perhaps with tubulin as well.'

Both of these quotes show the efficacy of using combinatorial chemistry techniques around the skeleton of an *active natural product* in an attempt to optimize both molecular recognition sites and transport across cell membranes.

It is tempting to speculate that similar biochemical and chemical experiments are going on with other active compounds in this area; we will, however, have to await publication of results in either the patent or general literature to see if these speculations are correct.

8 Future trends: general

Rather than discuss each disease area from the viewpoint of future trends, it is tempting to suggest that there are areas of natural product research that are 'blooming' in-so-far as derivation of unusual or even 'un-natural' structures are concerned, and that, in the not too distant future, they will become major sources of novel agents with pharmacological promise.

8.1 Future trends: production of 'previously unknown structures'

What we are referring to is the marriage of genetic information on biochemical pathways with the manifold techniques of 'gene manipulation'. This work was pioneered by Hopwood[178] in the 1970s with initial attempts to produce hybrid antibiotics using protoplast fusion techniques in the *Streptomyces*. With the advent of recombinant DNA technology, the identification of biosynthetic pathways to antibiotics and the ability to screen large number of clones for expression of particular activities at low expression levels, these technologies have the potential to produce very unusual compounds that are true hybrids of different biosynthetic pathways.[179–183] Recently McDaniel *et al.*[184] reported on the ability to interchange modules in different type I polyketide synthases (PKSs) and Shen *et al.*[185] were able to express three genes from the other type (type II) of PKS systems. In a very recent paper Zhao *et al.*[186] reported the 'combinatorial biosynthesis' of methymycin or pikromycin aglycones coupled to the sugar from a totally unrelated antibiotic, calcheamicin. A recent review article by Hutchinson,[187] from the University of Wisconsin, who is also a pioneer in this field, should be consulted for further information on these techniques and their import in drug discovery.

In addition to the 'directed techniques' referred to above, where the biosynthetic genes are identified and then 'mixed and matched', there is another source of unusual/unknown structures that is currently being actively investigated, the 'as yet uncultured microbes'. Pioneers in this field are Colwell,[188] who described the recognition of visible but uncultured bacteria in marine environments and Handelsman and her collaborators for similar discoveries with terrestrial bacteria.[189] By isolating gross DNA from these sources, followed by clean-up, separation into eukaryotic and prokaryotic DNA pools, cloning by a variety of means in suitable hosts and then screening of the hosts for expression of novel activities and/or chemical entities a vast new area of chemical diversity may be opened up.

That these techniques are viable as sources of new structures and agents can be seen from the companies that have been set up to study these techniques and their academic linkages. Examples are Kosan Biosciences with Chaitan Khosla (University of California, Berkeley) and TerraGen Bidiversity (Julian Davies, University of British Columbia), plus two others that have changed names and affiliations over the years, Diversa and ChromoXome.

8.2 Future trends: the optimization of lead structures

In this, bioactive natural product lead structures would be identified and then new agents with improved pharmacokinetics and/or toxicology synthesized. This is 'optimization of a lead structure', but instead of using the classical medicinal chemistry techniques, rapid combinatorial/parallel synthesis methods would be applied.[190] That these are viable strategies for deriving 'new and improved' molecules can be seen from the earlier discussions under anti-tumor agents, in particular the work around paclitaxel, epothilones and sarcodictyins. What is also becoming evident is that the synthesis of complex natural products is no longer an end in itself. The molecules that are used are themselves becoming substrates for combinatorial approaches or as sources of simplified molecules, as shown by the work on bryostatins and on ecteinascidins, where simpler but biologically active molecules have been made as a result of synthetic endeavors.

8.3 Future trends: novel delivery systems for 'old compounds'

As a result of the massive screening that has been carried out in antibiotic and anti-tumor discovery programs, there are sig-

nificant numbers of very potent natural product compounds that have the required biological activity but at the expense of a very low therapeutic index. Since, under normal conditions of testing, these materials would have to be administered non-specifically (*i.e.* dosing the whole animal), a high toxicity is a significant problem. If such agents could be delivered to the tumor area on the other hand, then it might well be possible to use very toxic materials for treatment.

There are a variety of methods that can be used for such delivery systems; the two that have been studied most extensively in the past are use of liposome-encapsulated toxic agents[191] and antibody-conjugates with natural toxins[192] (*i.e.* ricin) or pure compounds such as calcheamicin and doxorubicin. A further modification would be to use a prodrug and an enzyme-coupled antibody to release the material at the site.[193] Over the last few years, another novel strategy for delivery of anticancer drugs to the tumor site has been proven clinically. This is the use of a water-soluble copolymer with a warhead attached. A driving force in this area has been the group now at the University of London's School of Pharmacy.[194] They, originally in conjunction with a polymer group at Prague and then with Farmitalia (now part of Pharmacia-Upjohn), successfully coupled doxorubicin to an *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer to produce the construct known as PK1. By adding a targeting sugar moiety to PK1, a construct (PK2) that targeted the liver was made and some preliminary imaging results from the Phase I trials have been reported.[195] PK1 is now in Phase II trials in the UK, and PK2 has just completed its first Phase I trial, also in the UK.[195–198] This work has now been extended to cover platinum complexes as well, with the first *in vivo* preclinical results being reported.[199]

Two final points:

(1) an analysis of the compounds mentioned in the 694 posters presented at the AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics meeting in November 1999, showed that of the 433 molecular entities mentioned, 264 fell into the N/ND or S* categorizations of Cragg *et al.*[84]

(2) In an ASAP paper in *Organic Letters*, Smith *et al.*[200] published their gram-scale synthesis of the tubulin-active marine natural product, discodermolide. This synthesis may well open the way to combinatorial development of a large number of related compounds for this agent, which is currently in preclinical development as an anti-tumor agent.

Thus, in closing, as can be seen from the above discussions, and as referred to by other reviewers,[201,202] 'the notification of the demise of natural products as leads to drug entities is highly premature' (*with apologies to Mark Twain*).

9 References

1 Anon, *Herbalgram*, 1998, 33.
2 S. Dev, *Environ. Health Perspect.*, 1999, **107**, 783.
3 M. Fallarino, *Herbalgram*, 1994, **31**, 38.
4 S. Grabley and R. Thiericke, *Adv. Biochem. Eng./ Biotech.*, 1999, **64**, 104.
5 H.-M. Chang and P. P.-H. But, *Pharmacology and Applications of Chinese Materia Medica*, World Scientific Publishing, Singapore, 1986, vols 1 & 2.
6 L. D. Kapoor, *CRC Handbook of Ayurvedic Medicinal Plants*, CRC Press, Boca Raton, 1990.
7 R. E. Schultes and R. F. Raffauf, *The Healing Forest*, Dioscorides Press, Portland, 1990.
8 R. Arvigo and M. Balick, *Rainforest Remedies*, Lotus Press, Twin Lakes, 1993.
9 N. R. Farnsworth, O. Akerele, A. S. Bingel, D. D. Soejarto and Z. Guo, *Bull. WHO*, 1985, **63**, 965.
10 F. Grifo, D. Newman, A. S. Fairfield, B. Bhattacharya and J. T. Grupenhoff, in *The Origins of Prescription Drugs*, ed. F. Grifo and J. Rosenthal, Island Press, Washington, D.C., 1997, p. 131.
11 G. A. Cordell, *Phytochemistry*, 1995, **40**, 1585.
12 J. Mann, *Murder, Magic and Medicine*, Oxford University Press, Oxford, 1992.
13 R. I. Mateles, *Penicillin: A Paradigm for Biotechnology*, Candida Corporation, Chicago, 1998.
14 J. R. E. Hoover, personal communication, 1980.
15 G. Brotzu, *Lav. Ist. Igiene Cagliari*, 1948.
16 G. G. F. Newton and E. P. Abraham, *Biochem. J.*, 1956, **62**, 651.
17 E. P. Abraham and G. G. F. Newton, *Biochem. J.*, 1961, **79**, 377.
18 D. J. Newman and S. A. Laird, *The Commercial Use of Biodiversity*, ed. K. ten Kate and S. A. Laird, Earthscan Publications, London, 1999, p. 333.
19 H. A. Albrecht, G. Beskid, K. K. Chan, J. G. Christenson, R. Cleeland, K. H. Deitcher, N. H. Georopapadakou, D. D. Keith, D. L. Pruess and J. Sepinwall, *J. Med. Chem.*, 1990, **33**, 77.
20 A. J. Corraz, S. L. Dax, N. K. Dunlap, N. H. Georgopapadakou, D. D. Keith, D. L. Pruess, P. L. Rossman, R. Then, J. Unowsky and C. C. Wei, *J. Med. Chem.*, 1992, **35**, 1828.
21 H. A. Albrecht, G. Beskid, J. G. Christenson, K. H. Deitcher, N. H. Georgopapadakou, D. D. Keith, F. M. Konzelmann, D. L. Pruess and C. C. Wei, *J. Med. Chem.*, 1994, **37**, 400.
22 N. H. Georgopapadakou and C. McCaffrey, *Antimicrob. Agents Chemother.*, 1994, **38**, 959.
23 A. Imada, K. Kitano, K. Kintaka, M. Muroi and M. Asai, *Nature (London)*, 1981, **289**, 590.
24 R. B. Sykes, C. M. Cimarusti, D. P. Bonner, K. Bush, D. M. Floyd, N. H. Georgopapadakou, W. H. Koster, W. C. Liu, W. L. Parker, P. A. Principe, M. L. Rathnum, W. A. Slusarchyk, W. H. Trejo and J. S. Wells, *Nature (London)*, 1981, **291**, 489.
25 T. C. Barden, B. L. Buckwalter, R. T. Testa, P. J. Petersen and V. J. Lee, *J. Med. Chem.*, 1994, **37**, 3205.
26 P. E. Sum, V. J. Lee, R. T. Testa, J. J. Hlavka, G. A. Ellestad, J. D. Bloom, Y. Gluzman and F. P. Tally, *J. Med. Chem.*, 1994, **37**, 184.
27 R. Wise and J. M. Andrews, *Antimicrob. Agents Chemother.*, 1994, **38**, 1096.
28 G. Bright, A. Nagel, J. Bordner, K. Desai, J. Dibrino, J. Nowakowska, L. Vincent, R. Watrous, F. Sciavolina, A. English, J. Retsema, M. Anderson, L. Brennan, R. Borovoy, C. Cimchowsji, J. Faiella, A. Girard, D. Gorard, C. Herbert, M. Manousos and R. Mason, *J. Antibiot.*, 1988, **41**, 1029.
29 R. G. Finch, *Drugs*, 1996, **51**, 31.
30 R. C. Moellering, P. K. Linden, J. Reinhardt, E. A. Blumberg, F. Bompart and G. H. Talbot, *J. Antimicrob. Chemother.*, 1999, **44**, 251.
31 R. L. Nichols, D. R. Graham, S. L. Barriere, A. Rodgers, S. E. Wilson, M. Zervos, D. L. Dunn and B. Kreter, *J. Antimicrob. Chemother.*, 1999, **44**, 263.
32 F. P. Tally, personal communication, 1997.
33 P. J. McCarthy, D. J. Newman, L. J. Nisbet and W. D. Kingsbury, *Antimicrob. Agents Chemother.*, 1985, **28**, 494.
34 R. Lucas, K. DeSante, B. Hatcher, J. Hemingway, R. Lachno, S. Brooks and M. Turik, *36th Intersci. Conf. Antimicrob. Agents Chemother.*, 1996, LA, USA, Abstract no. F50.
35 D. W. Denning, *J. Antimicrob. Chemother.*, 1997, **40**, 611.
36 Anon, *Scrip*, 1997, 6.
37 O. S. Kinsman, P. A. Chalk, H. C. Jackson, R. F. Middleton, A. Shuttleworth, B. A. M. Rudd, C. A. Jones, H. M. Noble, H. G. Wildman, M. J. Dawson, C. Stylli, P. J. Sidebottom, B. Lamont, S. Lynn and M. V. Hayes, *J. Antibiot.*, 1998, **51**, 41.
38 A. M. Clark, *Pharm. Res.*, 1996, **13**, 1133.
39 R. X. Tan, W. F. Zheng and H. Q. Tang, *Planta Med.*, 1998, **64**, 295.
40 P. A. G. M. De Smet, *Drugs*, 1997, **54**, 801.
41 W. Asawamahasakda, I. Ittarat, Y.-M. Pu, H. Ziffer and S. R. Meshnick, *Antimicrob. Agents Chemother.*, 1994, **38**, 1854.
42 S. R. Meshnick, *Trans. R. Soc. Trop. Med. Hyg.*, 1994, **88 (supplement 1)**, S31.
43 S. R. Meshnick, T. E. Taylor and S. Kamchonwongpaisan, *Microbiol. Rev.*, 1996, **60**, 301.
44 M. A. Avery, P. Fan, J. M. Karle, J. D. Bonk, R. Miller and D. K. Goins, *J. Med. Chem.*, 1996, **39**, 1885.
45 M. A. Avery, S. Mehrota, J. D. Bonk, J. A. Vroman, D. K. Goins and R. Miller, *J. Med. Chem.*, 1996, **39**, 2900.
46 M. A. Avery, S. Mehrota, T. L. Johnson, J. D. Bonk, J. A. Vroman and D. K. Goins, *J. Med. Chem.*, 1996, **39**, 4149.
47 G. H. Posner, X. Tao, J. N. Cumming, D. Klinedinst and T. A. Shapiro, *Tetrahedron Lett.*, 1996, **37**, 7225.
48 G. H. Posner, H. O'Dowd, P. Ploypradith, J. N. Cumming, S. Xie and T. A. Shapiro, *J. Med. Chem.*, 1998, **41**, 2164.
49 M. Albericci, J. C. Braekman, D. Daloze and B. Tursch, *Tetrahedron*, 1982, **38**, 1881.
50 Y. Kashman and M. Rotem, *Tetrahedron Lett.*, 1979, **20**, 1707.

51 A. Rudi, R. Talpir, Y. Kashman, Y. Benayahu and M. Schleyer, *J. Nat. Prod.*, 1993, **56**, 2178.
52 K. A. El Sayed, D. C. Dunbar, D. K. Goins, C. R. Cordova, T. L. Perry, K. J. Wesson, S. C. Sanders, S. A. Janus and M. T. Hamann, *J. Nat. Toxins*, 1996, **5**, 261.
53 G. M. Konig and A. D. Wright, *Planta Med.*, 1996, **62**, 193.
54 H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Turbachova, M. Eberl, J. Zeidler, H. K. Lichtenthaler, D. Soldata and E. Beck, *Science*, 1999, **285**, 1573.
55 M. Okuhara, Y. Kuroda, K. Goto, M. Okamoto, H. Terano, M. Kohsaka, H. Aoki and H. Imanaka, *J. Antibiot.*, 1980, **33**, 24.
56 E. Iguchi, M. Okuhara, M. Kohsaka, H. Aoki and H. Imanaka, *J. Antibiot.*, 1980, **33**, 18.
57 E. Ohler and S. Kanzler, *Synthesis*, 1995, 539.
58 W. Bergmann and R. J. Feeney, *J. Am. Chem. Soc*., 1950, **72**, 2809.
59 W. Bergmann and R. J. Feeney, *J. Org. Chem.*, 1951, **16**, 981.
60 W. Bergmann and D. C. Burke, *J. Org. Chem.*, 1956, **21**, 226.
61 C. J. Suckling, *Sci. Prog. Edin.*, 1991, **75**, 323.
62 W. W. Lee, A. Benitez, L. Goodman and B. R. Baker, *J. Am. Chem. Soc.*, 1960, **82**, 2648.
63 Parke Davis & Co., *UK Pat.* 1151290, 1969.
64 G. Cimino, S. De Rosa and S. De Sttefano, *Experientia*, 1984, **40**, 339.
65 P. L. Darke, C. T. Leu, L. J. Davis, J. C. Heimbach, R. E. Diehl, W. S. Hill, R. A. Dixon and I. S. Sigal, *J. Biol. Chem.*, 1989, **264**, 2307.
66 R. A. Wiley and D. H. Rich, *Med. Res. Rev.*, 1993, **13**, 327.
67 F. Gago, *IDrugs*, 1999, **2**, 309.
68 K. Z. Rana and M. N. Dudley, *Pharmacotheropy*, 1999, **19**, 35.
69 B. D. Dorsey, J. P. Vacca and J. R. Huff, in *Anti-infectives: Recent Advances in Chemistry and Structure–Activity Relationships*, Royal Society of Chemistry, London, 1997, p. 238.
70 A. H. Fray, R. L. Kaye and E. F. Kleinman, *J. Org. Chem.*, 1986, **51**, 4828.
71 T. A. Lyle, C. M. Wiscount, J. P. Guare, W. J. Thompson, P. S. Anderson, P. L. Darke, J. A. Zugay, E. M. Ermini, W. A. Schlief, J. C. Quintero, R. A. F. Dixon, I. S. Sigal and J. R. Huff, *J. Med. Chem.*, 1991, **34**, 1228.
72 J. P. Vacca, J. P. Guare, S. J. deSoloms, W. M. Sanders, E. A. Giuliani, S. D. Young, P. L. Darke, J. Zugay, I. S. Sigal, W. A. Scheif, J. C. Quintero, E. A. Emini, P. S. Anderson and J. R. Huff, *J. Med. Chem.*, 1991, **34**, 1225.
73 K. K. Chen and C. F. Schmidt, *Medicine*, 1930, **9**, 1.
74 J. W. Black and J. S. Stephenson, *Lancet*, 1962, **2**, 311.
75 A. Endo, M. Kuroda, Y. Tsujita, A. Terahara and C. Tamura, *Ger. Pat.* 2524355, 1975.
76 A. Endo, *J. Med. Chem.*, 1985, **28**, 401.
77 A. G. Brown, T. C. Smale, T. J. King, R. Hasenkamp and R. H. Thompson, *J. Chem. Soc., Perkin Trans. 1*, 1976, 1165.
78 A. Endo, *J. Antibiot.*, 1979, **32**, 852.
79 A. Endo, *J. Antibiot.*, 1980, **33**, 334.
80 A. W. Alberts, J. Chen, G. Kuron, V. Hunt, J. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Albers-Schonberg, O. Hensens, J. Hirshfield, K. Hoogsteen, J. Liesch and J. Springer, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 3957.
81 R. L. Monaghan, A. W. Alberts, C. G. Hoffman and G. Albers-Schonberg, *US Pat.* 4231938, 1980.
82 A. W. Alberts, J. S. MacDonald, A. E. Till and J. A. Tobert, *Cardiol. Drug. Dev.*, 1989, **7**, 89.
83 P. R. Vagelos, *Science*, 1991, **252**, 1080.
84 G. M. Cragg, D. J. Newman and K. M. Snader, *J. Nat. Prod.*, 1997, **60**, 52.
85 S. H. Ferreira, *Br. J. Pharmacol.*, 1965, **24**, 163.
86 M. A. Ondetti, B. Rubin and D. W. Cushman, *Science*, 1977, **196**, 441.
87 D. W. Cushman, H. S. Cheung, E. F. Sabo and M. A. Ondetti, *Biochemistry*, 1977, **16**, 5484.
88 C. E. Terry and M. Pellens, *The Opium Problem*, Bureau of Social Hygiene, New York, 1928.
89 D. F. Musto, *The American Disease*, Yale University Press, New Haven, 1973.
90 C. J. Weitz, K. F. Faull and A. Goldstein, *Nature (London)*, 1987, **330**, 674.
91 J. Donnerer, G. Cardinale, J. Coffrey, C. A. Lisek, I. Jardine and S. Spector, *J. Pharmacol. Exp. Ther.*, 1987, **242**, 583.
92 W. R. Martin, *Pharmacol. Rev.*, 1967, **19**, 463.
93 L. Terenius, *Annu. Rev. Pharmacol. Toxicol.*, 1978, **18**, 189.
94 S. H. Snyder, *Science*, 1984, **224**, 22.
95 E. J. Simon and J. M. Hiller, *Annu. Rev. Pharmacol. Toxicol.*, 1978, **18**, 371.
96 A. Goldstein, in *Opioids: Past, Present and Future*, ed. H. O. J. Collier, J. Hughes, M. J. Rance and M. B. Tyers, Taylor and Frances, London, 1984, p. 127.
97 N. E. S. Sibinga and A. Goldstein, *Annu. Rev. Immunol.*, 1988, **6**, 219.
98 T. H. Kramer, J. E. Shook, W. Kazmierski, E. A. Ayres, W. S. Wire, V. J. Hruby and T. F. Burks, *J. Pharmacol. Exp. Ther.*, 1989, **249**, 544.
99 B. M. Olivera, C. Walker, G. E. Cartier, D. Hooper, A. D. Santos, R. Schenfeld, R. Shetty, M. Watkins, P. Bandyopadhyay and D. R. Hillyard, *Ann. N.Y. Acad. Sci.*, 1999, **870**, 223.
100 C. S. Walker, D. Steel, R. B. Jacobsen, M. B. Lirazan, L. J. Cruz, D. Hooper, R. Shetty, R. C. DelaCruz, J. S. Nielsen, L. M. Zhou, P. Bandyopadhyay, A. G. Craig and B. M. Olivera, *J. Biol. Chem.*, 1999, **274**, 30664.
101 B. M. Olivera, *Mol. Biol. Cell*, 1997, **8**, 2101.
102 A. Justice, T. Singh, K. C. Gohil, K. L. Valentino and G. P. Miljanich, in *Omega Conopeptide Compositions*, USP, 5587454/1996.
103 T. P. Spande, H. M. Garrafo, M. W. Edwards, H. J. C. Yeh, L. Pannel and J. W. Daly, *J. Am. Chem. Soc*., 1992, **114**, 3475.
104 J. L. Ellis, D. Harman, J. Gonzalez, M. L. Spera, R. Liu, T. Y. Shen, D. M. Wypij and F. Zuo, *J. Pharmacol. Exp. Ther.*, 1999, **288**, 1143.
105 A. W. Bannon, M. W. Decker, M. W. Holladay, P. Curzon, D. Donnelly-Roberts, P. S. Puttfarcken, R. S. Bitner, A. Diaz, A. H. Dickenson, R. D. Porsolt, M. Williams and S. P. Arneric, *Science*, 1998, **279**, 77.
106 M. W. Decker and M. D. Meyer, *Biochem. Pharmacol.*, 1999, **58**, 917.
107 P. Curzon, A. L. Nikkel, A. W. Bannon, S. P. Arneric and M. W. Decker, *J. Pharmacol. Exp. Ther.*, 1998, **287**, 847.
108 J. L. Hartwell, *Plants Used Against Cancer*, Quarterman, Lawrence, 1982.
109 G. M. Cragg, M. R. Boyd, J. H. Cardellina II, D. J. Newman, K. M. Snader and T. G. McCloud, in *Ethnobotany and the Search for New Drugs*, Ciba Symposium No. 185, 1994, p. 178.
110 J. M. Pezzuto, *Biochem. Pharmacol.*, 1997, **53**, 121.
111 H.-K. Wang, *IDrugs*, 1998, **1**, 92.
112 K.-H. Lee, *J. Biomed. Sci.*, 1999, **6**, 236.
113 H.-K. Wang, S. L. Morris-Natschke and K.-H. Lee, *Med. Res. Rev.*, 1997, **17**, 367.
114 M. Potmeisel and H. Pinedo, *Camptothecins: New Anticancer Agents*, CRC Press, Boca Raton, 1995.
115 G. M. Cragg, S. A. Schepartz, M. Suffness and M. R. Grever, *J. Nat. Prod.*, 1993, **56**, 1657.
116 J. E. Cortes and R. Pazdur, *J. Clin. Oncol.*, 1995, **13**, 2643.
117 D. G. I. Kingston, A. G. Chaudhary, M. D. Chordia, M. Gharpure, A. A. I. Gunatilaka, P. I. Higgs, J. M. Rimoldi, L. Samala, P. G. Jaktap, P. Giannakakou, Y. Q. Jiang, C. M. Lin, E. Hamel, B. H. Long, C. R. Fairchild and K. A. Johnston, *J. Med. Chem.*, 1998, **41**, 3715.
118 M. C. Christian, J. M. Pluda, T. C. Ho, S. G. Arbuck, A. J. Murgo and E. A. Sausville, *Semin. Oncol.*, 1997, **24**, 219.
119 R. G. Naik, S. L. Kattige, S. V. Bhat, B. Alreja, N. J. de Sousa and R. H. Rupp, *Tetrahedron*, 1988, **44**, 2081.
120 E. A. Sausville, D. Zaharevitz, R. Gussio, L. Meijer, M. Louarn-Leost, C. Kunick, R. Schultz, T. Lahusen, D. Headlee, S. Stinson, S. G. Arbuck and A. Senderowicz, *Pharmacol. Ther.*, 1999, **82**, 285.
121 C. Schultz, A. Link, M. Leost, D. W. Zaharevitz, R. Gussio, E. A. Sausville, L. Meijer and C. Kunick, *J. Med. Chem.*, 1999, **42**, 2909.
122 D. W. Zaharevitz, R. Gussio, M. Leost, A. M. Senderowicz, T. Lahusen, C. Kunick, L. Meijer and E. A. Sausville, *Cancer Res.*, 1999, **59**, 2566.
123 L. Havlicek, J. Hanus, J. Vesely, S. Leclerc, L. Meijer, G. Shaw and M. Strnad, *J. Med. Chem.*, 1997, **40**, 408.
124 J. Vesley, L. Havlicek, M. Strnad, J. J. Blow, A. Donelladeana, L. Pinna, D. S. Letham, J. Kato, L. Detivaud, S. Leclerc and L. Meijer, *Eur. J. Biochem.*, 1994, **224**, 771.
125 L. Meijer, A. Borgne, O. Mulner, J. P. J. Chong, J. J. Blow, N. Inagaki, M. Inagaki, J. G. Delcros and J. P. Moulinoux, *Eur. J. Biochem.*, 1997, **243**, 527.
126 U. Schulze-Gahmen, J. Brandsen, H. D. Jones, D. O. Morgan, L. Meijer, J. Vesley and S. H. Kim, *Proteins*, 1995, **22**, 378.
127 W. F. De Azevedo, S. Leclerc, L. Meijer, L. Havlicek, M. Strnad and S. H. Kim, *Eur. J. Biochem.*, 1997, **243**, 518.
128 N. S. Gray, L. Wodicka, A.-M. W. H. Thunnissen, T. C. Norman, S. Kwon, F. H. Espinosa, D. O. Morgan, G. Barnes, S. LeClerc, L. Meijer, S.-H. Kim, D. J. Lockhart and P. G. Schultz, *Science*, 1998, **281**, 533.
129 Y. T. Chang, N. S. Gray, G. R. Rosania, D. P. Sutherlin, S. Kwon, T. C. Norman, R. Sarohia, M. Leost, L. Meijer and P. G. Schultz, *Chem. Biol.*, 1999, **6**, 361.

130 N. Gray, L. Detivaud, C. Doerig and L. Meijer, *Curr. Med. Chem.*, 1999, **6**, 859.
131 K. R. Webster, *Exp. Opin. Invest. Drugs*, 1998, **7**, 865.
132 W. O. Foye, *Cancer Chemotherapeutic Agents*, American Chemical Society, Washington, DC, 1995.
133 S. M. Hecht, *Proc. AACR-NCI-EORTC Meeting*, 1999, Abs. P3.
134 P. Thavasu, D. Propper, A. McDonald, N. Dobbs, T. Ganesan, D. Talbot, J. Braybrook, F. Caponigro, C. Hutchison, C. Twelves, A. Man, D. Fabbro, A. Harris and F. Balkwill, *Cancer Res.*, 1999, **59**, 3980.
135 D. Fabbro, E. Buchdunger, J. Wood, F. Hofmann, S. Ferrari, H. Mett, T. O'Reilly and T. Meyer, *Pharmacol. Ther.*, 1999, **82**, 293.
136 G. Hofle, N. Bedorf, H. Gerth and H. Reichenbach, *Ger. Pat.* 4138042, 1993.
137 K. Gerth, N. Bedorf, G. Hofle, H. Irschik and H. Reichenbach, *J. Antibiot.*, 1996, **49**, 560.
138 D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides and C. M. Woods, *Cancer Res.*, 1995, **55**, 2325.
139 G. Hofle, N. Bedorf, H. Steinmetz, D. Schomberg, K. Gerth and H. Reichenbach, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1567.
140 K. C. Nicolaou, F. Roschangar and D. Vourloumis, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**, 2014.
141 I. Ojima, S. Chakravarty, T. Inoue, S. Lin, He, S. B. Horowitz, S. D. Kuduk and S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 4256.
142 R. Gussio, G. Paraskevi, E. Nogales, K. H. Downing, D. Zaharevitz, D. Sackett, K. C. Nicolau and T. Fojo, *Proc. AACR-NCI-EORTC Meeting*, 1999, 130 Abs 640.
143 O. J. McConnell, R. E. Longley and F. E. Koehn, in *The Discovery of Natural Products with Therapeutic Potential*, ed. V. P. Gullo, Butterworth-Heinemann, Boston, 1994, p. 109.
144 B. K. Carte, *BioScience*, 1996, 271.
145 G. R. Pettit, C. L. Herald, D. L. Doubek, D. L. Herald, E. Arnold and J. Clardy, *J. Am. Chem. Soc.*, 1982, **104**, 6846.
146 D. J. Newman, in *Bryozoans in Space and Time, Proceedings of the 10th International Bryozoology Conference*, NIWA, Wellington, NZ, 1996, p. 9.
147 P. A. Philip, D. Rea, P. Thavasu, J. Carmichel, N. S. A. Stuart, H. Rockett, D. C. Talbot, T. Ganesan, G. R. Pettit, F. Balkwill and A. L. Harris, *J. Natl. Cancer Inst.*, 1993, **85**, 1812.
148 M. L. Varterasian, R. M. Mohammad, D. S. Eilender, K. Hulburd, D. M. Rodriguez, P. A. Pemberton, J. M. Pluda, M. D. Dan, G. R. Pettit, B. D. M. Chen and A. M. Al-Katib, *J. Clin. Oncol.*, 1998, **16**, 56.
149 P. A. Wender, C. M. Cribbs, K. F. Koehler, N. A. Sharkey, C. L. Herald, Y. Kamano, G. R. Pettit and G. M. Blumberg, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 7197.
150 P. A. Wender, J. De Brabander, P. G. Harran, J.-M. Jimenez, M. F. T. Koehler, B. Lippa, C.-M. Park and M. Shiozaki, *J. Am. Chem. Soc.*, 1998, **120**, 4534.
151 P. A. Wender, J. De Brabander, P. G. Harran, J.-M. Jimenez, M. F. T. Koehler, B. Lippa, C.-M. Park, C. Siedenbiedel and G. R. Pettit, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 6624.
152 P. A. Wender, J. De Brabander, P. G. Harran, K. W. Hinkle, B. Lippa and G. R. Pettit, *Tetrahedron Lett.*, 1998, **39**, 8625.
153 P. A. Wender, B. Lippa, C.-M. Park, K. Irie, A. Nakahara and H. Ohigashi, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1687.
154 P. A. Wender, K. W. Hinkle, M. F. Koehler and B. Lippa, *Med. Res. Rev.*, 1999, **19**, 388.
155 K. L. Rinehart, T. G. Holt, N. L. Fregeau, J. G. Stroh, P. A. Keifer, F. Sun, L. H. Li and D. G. Martin, *J. Org. Chem.*, 1990, **55**, 4512.
156 A. E. Wright, D. A. Forleo, G. P. Gunawardana, S. P. Gunasekera, F. E. Koehn and O. J. McConnell, *J. Org. Chem.*, 1990, **55**, 4508.
157 T. Arai, K. Takahashi, S. Nakahara and A. Kubo, *Experientia*, 1980, **36**, 1025.
158 T. Arai, K. Takahashi, A. Kubo, S. Nakahara, S. Sato, K. Aiba and C. Tamura, *Tetrahedron Lett.*, 1979, 2355.
159 J. M. Frincke and D. J. Faulkner, *J. Am. Chem. Soc.*, 1982, **104**, 265.
160 E. J. Corey, D. Y. Gin and R. S. Kania, *J. Am. Chem. Soc.*, 1996, **118**, 9202.
161 E. J. Martinez, T. Owa, S. L. Schreiber and E. J. Corey, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 3496.
162 O. Kennard, W. D. G. L. Riva de Sanserverine, B. Tursch and R. Bosmans, *Tetrahedron Lett.*, 1968, **9**, 2879.
163 M. D'Ambrosio, A. Guerriero and F. Pietra, *Helv. Chim. Acta*, 1987, **70**, 2019.
164 M. D'Ambrosio, A. Guerrerio and F. Pietra, *Helv. Chim. Acta*, 1988, **71**, 964.
165 M. Ciomei, C. Albanese, W. Pastori, M. Grandi, F. Pietra, M. D'Ambrosio, A. Guerrerio and C. Battistini, *Proc. Am. Assoc. Cancer Res.*, 1997, **38**, 5 (Abstract 30).
166 W. Fenical, P. R. Jensen and T. Lindel, *US Pat.* 5473057, 1995.
167 T. Lindel, P. R. Jensen, W. Fenical, B. H. Long, A. M. Casazza, J. Carboni and C. R. Fairchild, *J. Am. Chem. Soc.*, 1997, **119**, 8744.
168 B. H. Long, J. M. Carboni, A. J. Wasserman, L. A. Cornell, A. M. Casazza, P. R. Jensen, T. Lindel, W. Fenical and C. R. Fairchild, *Cancer Res.*, 1998, **58**, 1111.
169 K. C. Nicolaou, J.-Y. Xu, S. Kim, T. Ohshima, S. Hosokawa and J. Pfefferkorn, *J. Am. Chem. Soc.*, 1997, **119**, 11353.
170 X.-T. Chen, B. Zhou, S. K. Bhattacharya, C. E. Gutteridge, T. R. R. Pettus and S. J. Danishevsky, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**, 789.
171 K. C. Nicolaou, S. Kim, J. Pfefferkorn, J.-Y. Xu, T. Ohshima, S. Hosokawa, D. Vourloumis and T. Li, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**, 1418.
172 K. C. Nicolaou, J.-Y. Xu, S. Kim, J. Pfefferkorn, T. Ohshima, D. Vourloumis and S. Hosokawa, *J. Am. Chem. Soc.*, 1998, **120**, 8061.
173 K. C. Nicolaou, T. Ohshima, S. Hosokawa, F. L. van Delft, D. Vourloumis, J. Y. Xu, J. Pfefferkorn and S. Kim, *J. Am. Chem. Soc.*, 1998, **120**, 8674.
174 X.-T. Chen, S. M. Bhattacharya, B. Zhou, C. E. Gutteridge, T. R. R. Pettus and S. J. Danishevsky, *J. Am. Chem. Soc.*, 1999, **121**, 6563.
175 T. Lindel, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**, 774.
176 K. C. Nicolaou, N. Winssinger, D. Vouloumis, T. Ohshima, S. Kim, J. Pfefferkorn, J.-Y. Xu and T. Li, *J. Am. Chem. Soc.*, 1998, **120**, 10814.
177 E. Hamel, D. L. Sackett, D. Vourlanis and K. C. Nicolaou, *Biochemistry*, 1999, **38**, 5490.
178 D. A. Hopwood, *Chem. Rev.*, 1997, **97**, 2465.
179 D. E. Cane, C. T. Walsh and C. Khosla, *Science*, 1998, **282**, 63.
180 L. Katz, *Chem. Rev.*, 1997, **97**, 2557.
181 C. Khosla, *Chem. Rev.*, 1997, **97**, 2577.
182 J. Staunton and B. Wilkinson, *Chem. Rev.*, 1997, **97**, 2611.
183 C. R. Hutchinson, *Curr. Opin. Microbiol.*, 1998, **1**, 319.
184 R. M. McDaniel, A. Thamchaipenet, C. Gustafsson, H. Fu, M. Betlach and G. Ashley, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 1846.
185 Y. Shen, P. Yoon, T.-W. Yu, H. G. Floss, D. A. Hopwood and B. S. Moore, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 3622.
186 L. Zhao, J. Ahlert, Y. Xue, J. S. Thorson, D. H. Sherman and H.-W. Liu, *J. Am. Chem. Soc.*, 1999, **121**, 9881.
187 C. R. Hutchinson, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 3336.
188 R. R. Colwell, *J. Ind. Microbiol.*, 1997, **18**, 302.
189 M. R. Rondon, R. M. Goodman and J. Handelsman, *Tibtech*, 1999, **17**, 403.
190 L. H. Caporale, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 75.
191 L. D. Mayer, *Cancer. Met. Rev.*, 1998, **17**, 211.
192 E. S. Vitetta, P. E. Thorpe and J. U. Uhr, *Immunol. Today*, 1993, **14**, 252.
193 R. F. Sherwood, *Adv. Drug Del. Rev.*, 1996, **22**, 269.
194 R. Duncan, *Pharm. Sci. Technol. Today*, 1999, **2**, 441.
195 P. J. Julyan, L. W. Seymour, D. R. Ferry, S. Daryani, C. M. Boivin, J. Doran, M. David, D. Anderson, C. Christodolou, A. M. Young, S. Hesslewood and D. J. Kerr, *J. Controlled. Release*, 1999, **57**, 281.
196 V. Omelyanenko, P. Kopeckova, C. Gentry and J. Kopecek, *J. Controlled Release*, 1998, **53**, 25.
197 P. A. Vasey, S. B. Kaye, R. Morrison, C. Twelves, P. Wilson, R. Duncan, A. H. Thomson, L. S. Murray, T. E. Hilditch, T. Murray, S. Burtles, D. Fraier, E. Frigerio and J. Cassidy, *Clin. Cancer Res.*, 1999, **5**, 83.
198 A. H. Thomson, P. A. Vasey, L. S. Murray, J. Cassidy, D. Fraier, E. Frigerio and C. Twelves, *Br. J. Cancer*, 1999, **81**, 99.
199 E. Gianasi, M. Wasil, E. G. Evagorou and A. Keddle, *Eur. J. Cancer*, 1999, **35**, 994.
200 A. B. Smith III, M. D. Kaufman, T. J. Beauchamp, M. J. LaMarche and H. Arimoto, *Org. Lett.*, 1999, **1**, 1823.
201 A. L. Harvey, *Trends Pharmacol. Sci.*, 1999, **20**, 196.
202 A. L. Harvey and P. G. Waterman, *Curr. Op. Drug Discov. Develop.*, 1998, **1**, 71.