Appl Microbiol Biotechnol (2002) 59:125–134
DOI 10.1007/s00253-002-1006-8

MINI-REVIEW

P. Proksch · R. A. Edrada · R. Ebel

Drugs from the seas – current status and microbiological implications

Received: 4 February 2002 / Revised: 12 March 2002 / Accepted: 13 March 2002 / Published online: 12 June 2002

Abstract The oceans are the source of a large group of structurally unique natural products that are mainly accumulated in invertebrates such as sponges, tunicates, bryozoans, and molluscs. Several of these compounds (especially the tunicate metabolite ET-743) show pronounced pharmacological activities and are interesting candidates for new drugs primarily in the area of cancer treatment. Other compounds are currently being developed as an analgesic (ziconotide from the mollusc *Conus magus*) or to treat inflammation. Numerous natural products from marine invertebrates show striking structural similarities to known metabolites of microbial origin, suggesting that microorganisms (bacteria, microalgae) are at least involved in their biosynthesis or are in fact the true sources of these respective metabolites. This assumption is corroborated by several studies on natural products from sponges that proved these compounds to be localized in symbiotic bacteria or cyanobacteria. Recently, molecular methods have successfully been applied to study the microbial diversity in marine sponges and to gain evidence for an involvement of bacteria in the biosynthesis of the bryostatins in the bryozoan *Bugula neritina*.

Introduction

Nature has continuously provided mankind with a broad and structurally diverse arsenal of pharmacologically active compounds that continue to be utilised as highly effective drugs to combat a multitude of deadly diseases or as lead structures for the development of novel synthetically derived drugs that mirror their models from nature. Traditionally, higher plants and, since the discovery of the penicillins, terrestrial microorganisms have proven to be the richest sources of natural drugs that are indispensable especially for the treatment of fatal diseases such as cancer. Well-known examples of plant-derived anti-cancer drugs include paclitaxel (taxol), from *Taxus brevifolia*; etoposide (vepesid), derived by partial synthesis from the lignan podophyllotoxin isolated from *Podophyllum peltatum*; and irinotecan (campthosar), which was obtained by optimizing the structure of the alkaloid camptothecin from *Camptotheca acuminata.* Examples from bacterial sources include doxorubicin (adriamycin) and bleomycin from various *Streptomyces* strains.

Serious attempts to tap the vast potential of marine organisms as sources of bioactive metabolites that may be directly utilised as drugs or serve as lead structures for drug development started in the late 1960s. The discovery of sizeable quantities of prostaglandins, which had just been discovered as important mediators involved in inflammatory diseases, fever and pain, in the gorgonian *Plexaura homomalla* by Weinheimer and Spraggins in 1969 is usually considered as the "take-off point" of any serious search for "drugs from the sea" (Weinheimer and Spraggins 1969) (note, however, that unusual nucleosides which had been isolated from marine sponges already in the 1950s served as lead structures for the development of nowadays commercially important anti-viral drugs such as ara-A and the anticancer drug for leukemia, ara-C).

From 1969–1999 approximately 300 patents on bioactive marine natural products were issued. From humble beginnings the number of compounds isolated from various marine organisms has virtually soared and now exceeds 10,000 (MarinLit 2001), with hundreds of new compounds still being discovered every year (Faulkner 2000b; Faulkner 2002). Through the combined efforts of marine natural products chemists and pharmacologists a number of promising compounds have been identified that are either already at advanced stages of clinical trials (most of them in the treatment of cancer) (Table 1) or have been selected as promising candidates for extended preclinical evaluation (Faulkner 2000a).

It is interesting to note that the majority of marine natural products currently in clinical trials or under pre-

P. Proksch (✉) · R.A. Edrada · R. Ebel
Institut für Pharmazeutische Biologie der
Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1,
Geb. 26.23, 40225 Düsseldorf, Germany
e-mail: proksch@uni-duesseldorf.de

Table 1 Selected marine natural products currently in clinical trials (updated from Fusetani 2000)

Source	Compounds	Disease area	Phase of clinical trials	References
Conus magnus (cone snail)	Ziconotide	Pain	III	Osenbach and Harvey (2001)
Ecteinascidia turbinata (tunicate)	Ecteinascidin 743	Cancer	II/III	Delaloge et al. (2001; Villalona-Calero et al. 2002)
Dolabella auricularia (sea hare)	Dolastatin 10	Cancer	II	Vaishampayan et al. (2000)
Dolabella auricularia (sea hare)	LU103793[a]	Cancer	II	Smyth et al. (2001)
Bugula neritina (bryozoan)	Bryostatin 1	Cancer	II	Varterasian et al. (2001; Blackhall et al. 2001)
Tridemnum solidum (tunicate)	Didemnin B	Cancer	II	Mittelman et al. (1999)
Squalus acanthias (shark)	Squalamine lactate	Cancer	II	Bhargava et al. (2001)
Aplidium albicans (tunicate)	Aplidine	Cancer	I/II	Gomez et al. (2001)
Agelas mauritianus (sponge)	KRN7000[b]	Cancer	I	Kikuchi et al. (2001)
Petrosia contignata (sponge)	IPL 576,092[c]	Inflammation/asthma	I	Coulson and O'Donnell (2000)
Pseudopterogorgia elisabethae (soft coral)	Methopterosin[d]	Inflammation/wound	I	Mayer et al. (1998)
Luffariella variabilis (sponge)	Manoalide	Inflammation/psoriasis	I	De Rosa et al. (1998)
Amphiporus lactifloreus (marine worm)	GTS-21[e]	Alzheimer/schizophrenia	I	Kem (2000)

[a] Synthetic analogue of dolastatin 15
[b] Agelasphin analogue (α-galactosylceramide derivative)
[c] Synthetic analogue of contignasterol (IZP-94,005)
[d] Semisynthetic pseudopterosin derivative
[e] Also known as DMXBA, 3-(2, 4-dimethoxybenzylidene)-anabaseine

clinical evaluation is produced by invertebrates such as sponges, tunicates, molluscs or bryozoans (Table 1) but not by algae. This is in sharp contrast to the terrestrial environment where plants by far exceed animals with regard to the production of bioactive natural products (also called secondary metabolites). The wealth of bioactive metabolites isolated from soft-bodied, sessile or slow-moving marine invertebrates that usually lack morphological defence structures such as spines or a protective shell is no coincidence but reflects the ecological importance of these constituents for the respective invertebrates. It has been repeatedly shown that chemical defence through accumulation of toxic or distasteful natural products is an effective strategy to fight off potential predators (e.g. fishes) or to force back neighbours competing for space (Proksch and Ebel 1998; Proksch 1999; McClintock and Baker 2001).

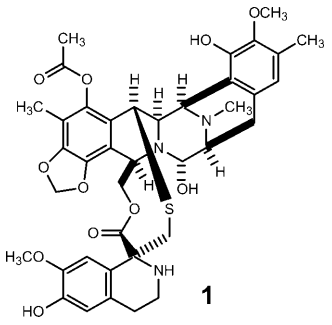
Taking the described ecological importance of marine natural products into consideration, it comes therefore hardly as a surprise that the majority of drug candidates from the sea has so far been isolated from invertebrates that thrive in tropical (for example as inhabitants of coral reefs) or subtropical seas where the grazing pressure by predators such as fishes is higher than in any other ecosystem of the world. On tropical coral reefs fish have been estimated to bite the bottom in excess of 150,000 times per m^2 and day (Carpenter 1986). Under these severe selective pressures only those organisms will survive that can rely on effective means of chemical defence. In many cases compounds that protect their invertebrate producers from predators or that help to fight off neighbours (e. g. didemnin B; Lindquist et al. 1992) have also attracted attention in pharmacological assays that are aimed at drug discovery (Table 1). Thus, organisms that thrive in spite of pronounced biotic pressures can to some degree be expected to contain metabolites that are also of interest for drug prospectors searching the oceans.

Current status of drugs from the sea

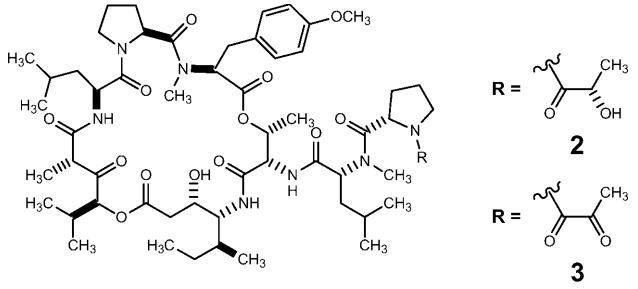
Of the marine natural products (or analogues) that are currently under clinical investigation as potential new anti-cancer drugs (Table 1) the marine alkaloid ecteinascidin 743 (ET-743) (Scheme 1) is by far the most advanced compound. ET-743 is in the late stages of phase II clinical trials as an anti-cancer drug and is expected to enter the drug market in Europe already by the end of 2002 or in early 2003. The drug has a broad-spectrum anti-tumour activity and is especially effective against solid tumours such as sarcomas and breast cancer (Valoti et al. 1998). So far over 1,000 patients have been treated with ET-743 in hospitals in Europe and in the USA. The molecular mode of action of this promising compound has been elucidated in detail. The alkaloid was shown to be a minor-groove alkylator of DNA (Zewail-Foote and Hurley 1999; Minuzzo et al. 2000) and to cause inhibition of MDR1 gene transcription (Jin et al. 2000), the latter being responsible for the well-known phenomenon of multi-drug resistance (MDR), which causes tumours to become insensitive to anti-cancer drugs and is a severe obstacle for chemotherapy. ET-743 furthermore elicits non-p53-mediated apoptosis in tumour cells.

The natural source of ET-743 is the tunicate *Ecteinascidia turbinata*, which occurs naturally in the Caribbean

Scheme 1



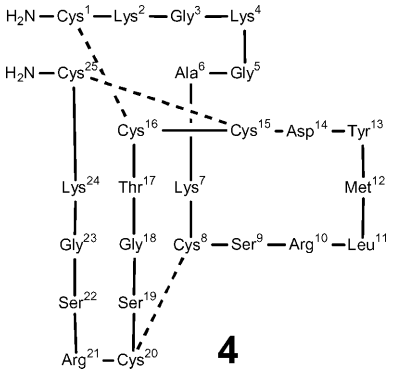
Schemes 2, 3



Sea and at other sites such as the Bahamas or the Florida Keys. Tunicates have repeatedly been shown to be interesting sources of chemically unique and strongly bioactive metabolites (Rinehart 2000). In addition to alkaloids such as ET 743 and others, these delicate animals accumulate structurally unusual depsipeptides including the didemnins from the Caribbean tunicate *Tridemnum solidum* (Sakai et al. 1996). The didemnins, especially didemnin B, provoked interest already back in the 1980s due to their pronounced anti-tumour activity which can be traced back to their interference with protein synthesis (Ahuja et al. 2000a, b). Didemnin B eventually proceeded to phase II clinical trials. Its further development as an anti-cancer drug, however, was recently cancelled due to hepatotoxic side effects of the compound. Dehydrodidemnin B (also called aplidine) (Schemes 2, 3), isolated from the Mediterranean tunicate *Aplidium albicans*, could prove to be a favourable substitute for didemnin B (Depenbrock et al. 1998). Aplidine appears to be less toxic and even more effective than didemnin B (five to six times more active) (Geldof et al. 1999) with a broad spectrum activity both in vitro and in vivo against various types of cancer diseases such as colorectal, lymphoma, thyroid and renal cancers (Rinehart 2000). It furthermore shows anti-angiogenic activity in experimental models. Aplidine is concluding phase I clinical trials and is scheduled for phase II trials.

Whereas the stronghold of marine natural products that have entered clinical trials is clearly in the area of cancer chemotherapy (Table 1), the oceans hold further promising compounds that might lead to new drugs in other important indications such as pain or inflammation. In this context the pain-killing marine natural prod-

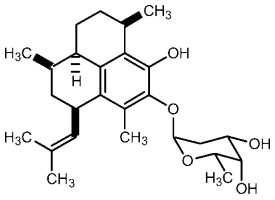
Scheme 4



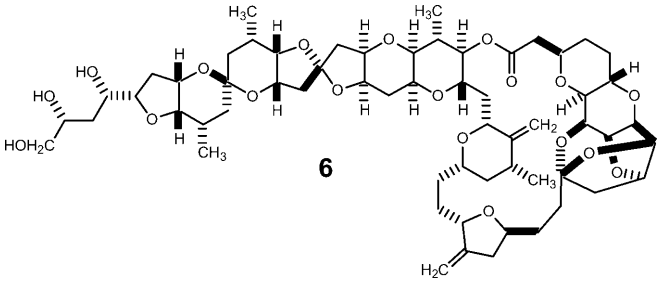
uct ω-conotoxin MVIIA (SNX-111) (Scheme 4) is a noteworthy success story. It has successfully completed phase III clinical trials for two therapeutic applications: to alleviate pain associated with malignant diseases (cancer and AIDS) and as an analgesic for nonmalignant neuropathic pain (Olivera 2000). The compound will be shortly marketed in the USA under the generic name ziconotide (the manufacturer received an approval letter from the FDA on June 28, 2000). Ziconotide is a 25-amino-acid linear peptide exhibiting three disulfide bonds; it occurs along with other peptides in the venom of the predatory Indo-Pacific marine mollusc *Conus magus* (Olivera 2000). *C. magus* and other *Conus* species are fish-hunting molluscs that use their venom to paralyse their prey (Kohn 1956). The venom of *Conus* snails can be deadly also for humans who happen to get stung while handling the molluscs. The remarkable analgesic activity of ziconotide (the compound proved to be 1,000 times more active than morphine in animal models of nociceptic pain) is due to the blockage of calcium channels (McCleskey et al 1987; Olivera 2000). Synaptic transmission requires Ca^{2+} entry for neurotransmitter release. The compound probably inhibits neurotransmitter release and thus communication from incoming sensory fibers and spinal cord neurons which transmit the signals to the brain (Olivera 2000). In contrast to the classic analgesic morphine, no tolerance to ziconotide (which would require progressively higher doses of the analgesic during long-term treatment for the same level of pain relief) was observed. Following the discovery of this *Conus* peptide in the late 1970s, no efforts were spared to develop more potent synthetic analogues. However, ziconotide proved to be superior to any structural analogue prepared by medicinal chemists.

The Caribbean gorgonian *Pseudopterogorgia elisabethae* is an example of another field of application of marine natural products which is not directly concerned with drug discovery but nevertheless holds considerable economic potential: Extracts of *P. elisabethae* show anti-inflammatory activity and are nowadays used as an ingredient for cosmetic skin care products. The activity of the extract is due to unusual diterpene glycosides, the so-called pseudopterosins – as exemplified by pseudopte-

Scheme 5



5



6

Scheme 6

rosin E (Scheme 5) – which inhibit phospholipase A_2 (Mayer et al. 1998; Look et al. 1986a, 1986b), the key enzyme for the biosynthesis of inflammatory eicosanoid mediators. A simple derivative of pseudopterosin is currently in phase I clinical trials as a potential new anti-inflammatory agent.

The supply issue

A serious obstacle to the ultimate development of most marine natural products that are currently undergoing clinical trials or that are in preclinical evaluation is the problem of supply. The concentrations of many highly active compounds in marine invertebrates are often minute, sometimes accounting for less than 10^{-6}% of the wet weight. For example, in order to obtain approximately 1 g of the promising anti-cancer agent ET-743, close to 1 metric tonne (wet weight) of the tunicate *E. turbinata* has to be harvested and extracted (Mendola 2000).

In other cases, such as for the halichondrins (e.g halichondrin B, Scheme 6), which are powerful cytostatic polyketides of sponge origin, the ratio of biomass to yield of product is even less favourable. In order to obtain as little as 300 mg of a mixture of two halichondrin analogues, 1 metric tonne of the sponge *Lissodendoryx* sp. had be collected and extracted (Hart et al. 2000).

This already causes considerable difficulties and delays in clinical studies where gram quantities of compounds are generally needed but will prove to be an overwhelming obstacle once one of these compounds is licensed as a drug. For example, provided that the halichondrins make it to the market as new anti-cancer drugs the annual need for these compounds is estimated to be in the range 1–5 kg per year, which corresponds to roughly 3,000–16,000 metric tonnes of sponge biomass per year (Hart et al. 2000). It is obvious that such large amounts of biomass of either sponges, tunicates or other pharmacologically promising marine invertebrates can never be harvested from nature without risking extinction of the respective species. Alternative strategies for an environmentally sound and economically feasible supply of marine natural products are therefore needed.

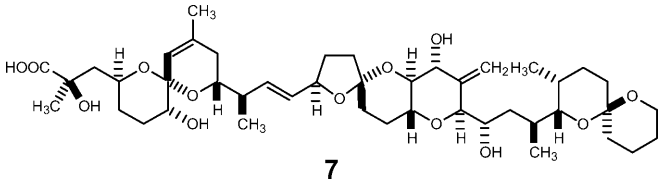
With the exception of the *Conus* toxin ziconotide which, due to its peptide nature, can be obtained in virtually unlimited amounts through synthesis, most other marine natural products of interest are highly complex molecules, such as ET-743, the bryostatins or halichondrins, that are rich in centres of asymmetry. For these latter compounds, economically feasible strategies of chemical synthesis do not exist at present and are not likely to be developed in the foreseeable future. However, in the case of ET-743, a partial synthetic route starting from the biotechnologically available cyanosafracin B (see below) has been developed (Cuevas et al. 2000).

Mariculture of sponges, tunicates and bryozoans with the aim of securing a steady supply of compounds has long been neglected. Recently, however, considerable progress has been made in this area and the bryozoan *Bugula neritina* (the source of the bryostatins) (Mutter and Wills 2000) as well as the tunicate *E. turbinata* are already accessible through mariculture (Mendola 2000). The obtained yields of biomass, however, are still far from those that will be needed once one of these compounds has finally entered the drug market. Furthermore, mariculture of invertebrates in tanks or in the sea, just like the more conventional mariculture of shrimps and fishes, is subject to uncertainties such as destruction due to storms or diseases which will make any predictability of the year to year harvest difficult.

In view of the discussed problems related to the important supply issue the provocative question arises whether sponges, tunicates, bryozoans and other invertebrates are the true producers of the various bioactive compounds discussed or whether these natural products (or at least some of them) are rather of microbial origin. The answer to this question, which is one of the key issues in modern marine natural products research, will have far-flung consequences and (if eventually answered to the positive) could open up entirely new approaches for the production of “drugs from the sea” using biotechnological methods.

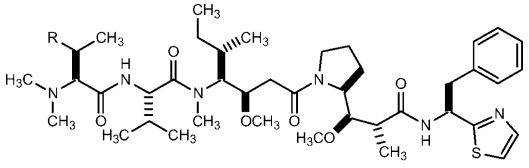
Are microorganisms the true sources of compounds from marine invertebrates?

Most if not all marine invertebrates harbour microorganisms that include bacteria, cyanobacteria and fungi within their tissues where they reside in the extra- and intracellular space (e.g. Vacelet and Donadey 1977; Wilkinson 1992). In some cases these associated microorganisms may constitute up to 40% of the biomass for example of sponges such as the Mediterranean *Aplysina aerophoba* (Vacelet 1975; Friedrich et al. 1999). On the other hand



7

Scheme 7



8 R = CH_3
9 R = H

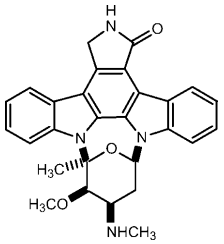
Schemes 8, 9

many invertebrates are filter feeders and consume microorganisms from the inhaled sea water by phagocytosis. The relationships of marine invertebrates and marine microorganisms that may serve as food or that live either permanently or temporarily inside of marine macroorganisms are highly complex and far from being understood (Wilkinson 1992; Steinert et al. 2000; Hentschel et al. 2000).

Microorganisms not only serve as food for filter feeders or (in the case of cyanobacteria and chemoautotrophic bacteria) enrich the diet of their hosts by carbon and nitrogen fixation but may perhaps also be involved in the biosynthesis of natural products that are recovered for example from sponges. Sponges of the genus *Halichondria* such as *H. okadai* or *H. melanodocia* provide a well-known example of the importance of microalgae for the typical natural products recovered from these invertebrates. Both *Halichondria* species contain the protein phosphatase inhibitor okadaic acid (Scheme 7) (Tachibana et al. 1981). Okadaic acid, however, was later shown to be produced by dinoflagellates of the genus *Prorocentrum* (Murakami et al. 1982) and is now considered to be of dietary origin rather than a "true" sponge metabolite in terms of its biosynthetic origin. In the case of okadaic acid the evidence for a dietary origin is further corroborated by inspection of the unique structural elements of the compound. Okadaic acid is a typical polycyclic ether derivative that shares important structural features with other polycyclic ethers such as brevetoxin A or zooxanthellatoxin A which are likewise produced by dinoflagellates (Shimizu 2000).

The case of okadaic acid appears to be no exception. Circumstantial chemical evidence for a microbial origin of natural products isolated from marine macroorganisms exists for numerous invertebrates. For example, symplostatin 1, a close structural analogue of dolastatin 10 (Schemes 8, 9) isolated from the marine mollusc *Dolabella auricularia* and currently in phase II clinical

Scheme 10



10

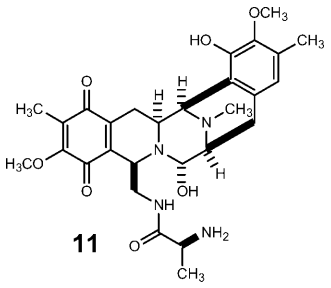
trials (Table 1), was found to be a metabolite of the blue-green alga *Symploca hydnoides* (Harrigan et al. 1998). Further evidence for a dietary origin of dolastatins in *Dolabella auricularia* was provided when the same dolastatin derivatives that had previously been isolated from the sea hare were detected in free-living cyanobacteria, such as dolastatin 10 detected in the marine cyanobacterium *Symploca* species VP642 (Luesch et al. 2001).

Staurosporine (Scheme 10), recently isolated from the Micronesian tunicate *Eudistoma toealensis* and its predatory flatworm *Pseudoceros sp*. (Schupp et al. 1999), was so far known only from actinomycetes such as *Saccharothrix aerocolonigenes* subsp. *staurorosporea* (formerly known as *Streptomyces staurosporeus*). Like dolastatin 10, staurosporine derivatives have received considerable interest due to their pronounced cytotoxic activity.

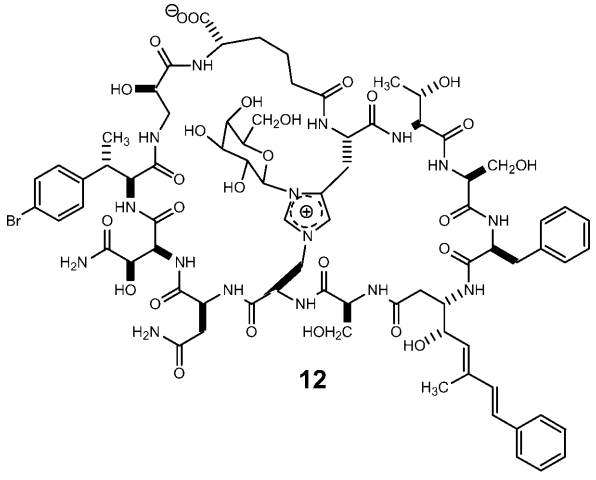
A close inspection of the structural features of ET-743 (Scheme 1) from the tunicate *E. turbinata* (Table 1) reveals striking similarities to safracin B (Scheme 11), a metabolite of *Pseudomonas fluorescens* (Ikeda et al. 1983). This chemical similarity is in fact so pronounced that biotechnologically available cyanosafracin B provides a commercially feasible precursor for a partial synthesis of ET-743 (Cuevas et al. 2000). Based on the discussed close chemical similarities or even identity of highly unusual natural products from marine invertebrates and microorganisms, it is tempting to assume that, rather than reflecting a mere chemical coincidence, compounds such as dolastatin 10, staurosporine or even ET-743 are introduced into the respective invertebrates through the food chain or originate from symbiotic (*sensu lato*) bacteria or microalgae.

Even more convincing evidence for an involvement of microorganisms in natural product synthesis has recently been compiled for the tropical sponges *Dysidea herbacea* and *Theonella swinhoei*. Specimens of *D. herbacea* from the Great Barrier Reef in Australia contain the sesquiterpenes spirodysin and herbadysidolide as well as the chlorinated amino acid derivative 13-demethylisodysidenin (Unson and Faulkner 1993). The sponge tissue is furthermore loaded with the cyanobacterial symbiont *Oscillatoria spongeliae* (Berthold et al. 1982) which comprises close to 50% of the cellular volume (Bewley and Faulkner 1998). Following disruption of the sponge tissue and fixation of the dissociated cells with formaldehyde or glutaraldehyde the cyanobacterial cells were separated from other cells with the aid of a

Scheme 11

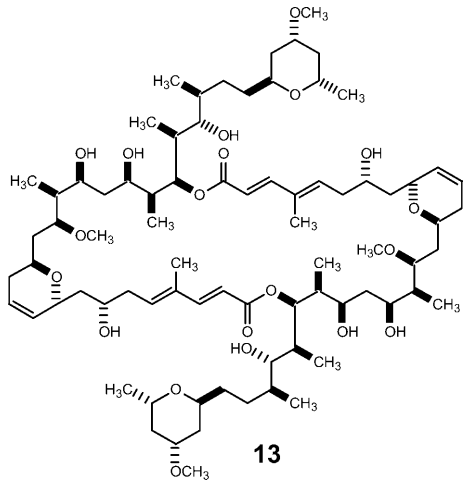


11



12

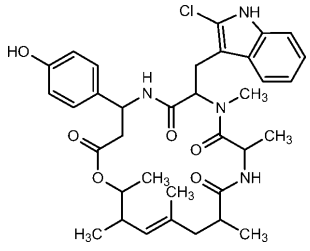
Scheme 12



13

Scheme 13

Scheme 14



14

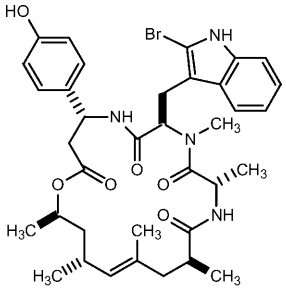
cell sorter using the characteristic cholorophyll fluorescence for detection. The amino acid derivative 13-demethylisodysidenin was the major natural product identified in the fluorescent cell fraction whereas the nonfluorescent cells (sponge cells and bacteria) contained spirodysin and herbadysidolide (Unson and Faulkner 1993).

The sponge *Theonella swinhoei* collected in the Philippines or in Micronesia contains the cyclic peptide theopalauamide (Scheme 12) and the macrolide swinholide A (Scheme 13). Both compounds show pronounced biological activities. Whereas theopalauamide shows antifungal activity the macrolide swinholide A is strongly cytotoxic. Using differential centrifugation several cellular fractions could be obtained from the tissue of *T. swinhoei*. Theopalauamide was detected in filamentous bacteria whereas swinholide A was found in a fraction containing mostly unicellular bacteria as evidenced by spectroscopic (^{1}H NMR) and microscopic analysis of the various fractions obtained (Bewley et al. 1996; Bewley and Faulkner 1998). Using 16S rDNA gene sequencing the filaments that contained the cyclic peptide were shown to belong among the δ-proteobacteria as a sister taxon to Myxococcales. The theopalauamide-containing symbiont has been assigned the taxonomic status "*Candidatus* Entotheonella palauensis" (Schmidt et al. 2000). Attempts to culture this bacterium, however, have so far not met with success.

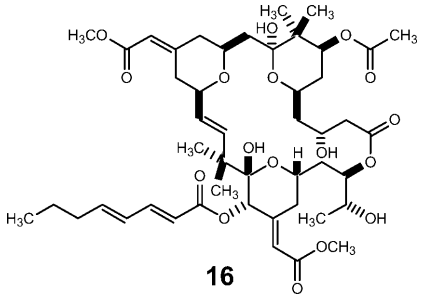
Although myxobacteria have been intensively studied by natural products chemists and have already yielded a plethora of new natural products belonging to various biogenetic classes, some of them exhibiting pronounced biological activities as exemplified by the tubulin-interacting epothilone (Reichenbach and Höfle 1993) reports on marine-derived myxobacteria so far are scarce (see for example, Iizuka et al. 1998). Nevertheless, apart from the above-mentioned symbiont in *Theonella*, further myxobacterial secondary metabolites exhibit pronounced structural similarities to natural products isolated from marine sponges. Perhaps the most striking example is the close relation of the cyclodepsipeptide chondramide D (Scheme 14) from *Chondromyces crocatus* (Jansen et al. 1996) to jasplakinolide (also designated as jaspamide) (Scheme 15) from various *Jaspis* spp. marine sponges (Crews et al. 1986; Zabriskie et al. 1986).

In the past, characterisation of the microbial communities of sponges and other marine invertebrates relied primarily on the culturability of the respective species and on their observation in situ using electron microscopy. Both approaches are severely limited. Whereas the morphological plasticity of bacteria does not match their taxonomic diversity, the number of bacterial strains obtained for example from sponges is usually exceedingly small compared to the real microbial diversity as exemplified by the Mediterranean sponge *A. aerophoba* (Hentschel et al. 2001). This sponge is especially rich in

Scheme 15



15



16

Scheme 16

bacteria. The bacterial concentration in *A. aerophoba* exceeds that of the surrounding sea water by two to three orders of magnitude. Judging from microscopic analysis, up to 40% of the sponge biomass consists of bacteria and cyanobacteria. Less than 1% of these bacteria, however, could be cultured using the standard ZoBell medium (Friedrich et al. 2001).

A major breakthrough in the characterisation of the microbial community present in sponges was achieved by applying molecular methods such as fluorescence in situ hybridisation (FISH) using group-specific 16S-rRNA-targeted oligonucleotide probes (Friedrich et al. 1999, 2001; Hentschel et al. 2001; Webster et al. 2001). One of the first studies using this approach was again conducted with *A. aerophoba* and its sibling species *A. cavernicola* (also from the Mediterranean) (Friedrich et al. 1999, 2001). The bacterial profiles of both species proved to be very similar. No Archaea were detected. Among the Bacteria the δ-proteobacteria were most abundant, followed by the high-GC gram-positive bacteria, the γ-proteobacteria and the *Bacteroides* cluster. The application of a planctomycete-specific FISH probe gave signals with a comparable intensity to those of δ-proteobacteria (Friedrich et al. 2001).

The bacterial community associated with *A. aerophoba* and *A. cavernicola* is remarkably stable. Transplantation of specimens of *A. cavernicola* from a depth of 40 m to 15 m caused no significant changes in the bacterial composition even over an experimental period of ca. 3 months (Thoms et al., in preparation). A similarly stable relationship of sponge and associated bacteria was observed when specimens of *A. aerophoba* were kept over a week in aquaria with sea water that had been supplemented with antibiotics (Friedrich et al. 2001). The similarity of the bacterial communities in *A. aerophoba* and *A. cavernicola* corresponds to similarities in the natural product profiles of both sponges which are characterised by brominated alkaloids as major constituents (Ebel et al. 1997). At present it is unknown whether these observations are merely coincidental or whether bacteria are really involved in the alkaloid biosynthesis of their hosts.

In the case of the bryozoan *B. neritina*, which is the source of the well-known bryostatins (e.g. bryostatin Schemes 1, 16), however, recent evidence is in favour of a bacterial origin of these macrocyclic lactones (Davidson et al. 2001) which are believed to be biosynthesized by a polyketide synthase. Through several lines of evidence it could be demonstrated that the uncultivated γ-proteobacterial symbiont "*Candidatus* Endobugula sertula" (Haygood and Davidson 1997) is probably involved in the biosynthesis of the bryostatins. Laboratory colonies of *B. neritina* when treated with antibiotics showed reduced numbers of symbionts and a likewise reduced bryostatin content. Polyketide synthase type I (PKS-I) gene fragments could be cloned from the *B. neritina* symbiont association and a corresponding RNA probe was shown to bind specifically to the symbionts but not to cells of the bryozoan or to other bacteria.

Conclusions and outlook

As discussed in this review marine natural products continue to be a structurally diverse and pharmacologically most interesting source of bioactive metabolites. Some of them hold great potential for the development of new and much needed drugs primarily in the treatment of cancer. For example, the cone snail toxin MVIIA has already successfully passed phase III clinical trials and will soon be available as an analgesic, marketed as ziconotide.

Based on striking chemical similarities of numerous highly bioactive compounds from marine invertebrates and from microorganisms, on localisation studies of natural products in invertebrates and on recent discoveries using molecular tools evidence for a microbial origin (either through the food chain or as a result of the biosynthetic capacities of symbiotic microorganisms) of some of these metabolites is accumulating.

Isolation and cultivation of suspected microbial producers of bioactive natural products either from the surrounding sea water or from the tissue of invertebrates through careful design of special media could provide a much needed answer to the pressing supply problem that is currently presented by many pharmacologically interesting compounds and hampers their further development as a drug. If bacteria are indeed the producers of bioactive metabolites of interest, transfer of gene clusters responsible for the biosynthesis of the respective natural products to a vector suitable for large-scale fermentation could perhaps provide an alternative strategy thereby avoiding the foreseeable difficulties in culturing symbiotic bacteria. In the case of marine natural products that

arise through the polyketide pathway, like the bryostatins and others, this ambitious goal may perhaps be realised within the next couple of years. For many other marine natural products, however, their biosynthetic origin is either completely unknown or only poorly understood (Kerr 2000). Obviously, efforts to characterise the biosynthesis of a given compound will be markedly changed if it is believed to be of microbial origin, due in part to the differences in genomic resources available for microorganisms on the one hand, and for (higher) metazoa on the other hand.

While chemistry has dominated the first three decades of marine natural products research resulting in the isolation and structure identification of thousands of new metabolites, it appears that increased input from marine biology, microbiology and molecular biology is now needed in order to generate an equally sound knowledge with regard to the biochemical and genetic mechanisms underlying the expression of natural products. With the advance of this knowledge, the ultimate goal of supplying sufficient quantities of "drugs from the sea" for pharmaceutical development without destroying valuable natural resources may ultimately be reached.

Acknowledgements Continued support of our research on bioactive marine natural products through the Bundesministerium für Bildung und Forschung (BMBF), the Deutsche Forschungsgemeinschaft (DFG) and the Fonds der Chemischen Industrie is gratefully acknowledged. Furthermore we wish to thank Dr. U. Hentschel and Prof. J. Hacker (both University of Würzburg) for stimulating discussions.

References

Ahuja D, Geiger A, Ramanjulu JM, Vera MD, SirDeshpande B, Pfizenmayer A, Abazeed M, Krosky DJ, Beidler D, Joullié MM, Toogood PL (2000a) Inhibition of protein synthesis by didemnins: cell potency and SAR. J Med Chem 43: 4212–4218

Ahuja D, Vera MD, SirDeshpande BV, Morimoto H, Williams PG, Joullié MM, Toogood PL (2000b) Inhibition of protein synthesis by didemnin B: how EF-1 mediates inhibition of translocation, Biochemistry 39: 4339–4346

Berthold RJ, Borowitzka MA, Mackay MA (1982) The ultrastructure of *Oscillatoria spongeliae,* the blue-green algal endosymbiont of the sponge *Dysidea herbacea*. Phycologia 21: 327–335

Bewley CA, Faulkner DJ (1998) Lithistid sponges: Star performers or hosts to the stars? Angew Chem Int Ed 37: 2162–2178

Bewley CA, Holland ND, Faulkner DJ (1996) Two classes of metabolites from *Theonella swinhoei* are localized in distinct populations of bacterial symbionts. Experientia 52: 716–722

Bhargava P, Marshall JL, Dahut W, Rizvi N, Trocky N, Williams JI, Hait H, Song S, Holroyd KJ, Hawkins MJ (2001) A phase I and pharmacokinetic study of squalamine, a novel antiangiogenic agent, in patients with advanced cancers. Clin Cancer Res 7: 3912–3919

Blackhall FH, Ranson M, Radford JA, Hancock BW, Soukop M, McGown AT, Robbins A, Halbert G, Jayson GC (2001) A phase II trial of bryostatin 1 in patients with non-Hodgkin's lymphoma. Br J Cancer 84: 465–469

Carpenter RC (1986) Partitioning herbivory and its effects on coral algal communities. Ecol Monogr 56: 345–365

Coulson FR, O'Donnell SR (2000) The effects of contignasterol (IZP-94,005) on allergen-induced plasma protein exudation in the tracheobronchial airways of sensitized guinea-pigs in vivo. Inflamm Res 49: 123–127

Crews P, Manes LV, Boehler M (1986) Jasplakinolide, a cyclodepsipeptide from the marine sponge, *Jaspis* sp. Tetrahedron Lett 27: 2797–2800

Cuevas C, Pérez M, Martin MJ, Chicharro JL, Fernández-Rivas C, Flores M, Francesch A, Gallego P, Zarzuelo M, de la Calle F, Garcia J, Polanco C, Rodriguez I, Manzanares I (2000) Synthesis of ecteinascidin ET-743 and phthalascidin Pt-650 from cyanosafracin B. Org Lett 2: 2545–2548

Davidson SK, Allen SW, Lim GE, Anderson CM, Haygood MG (2001) Evidence for the biosynthesis of bryostatins by the bacterial symbiont "*Candidatus* Endobugula sertula" of the bryozoan *Bugula neritina*. Appl Environ Microbiol 67: 4531–4537

De Rosa M, Giordano S, Scettri A, Sodano G, Soriente A, Pastor PG, Alcaraz MJ, Paya M (1998) Synthesis and comparison of the antiinflammatory activity of manoalide and cacospongionolide B analogues. J Med Chem 41: 3232–3238

Delaloge S, Yovine A, Taamma A, Riofrio M, Brain E, Raymond E, Cottu P, Goldwasser F, Jimeno J, Misset JL, Marty M, Cvitkovic E (2001) Ecteinascidin-743: a marine-derived compound in advanced, pretreated sarcoma patients–preliminary evidence of activity. J Clin Oncol 19: 1248–1255

Depenbrock H, Peter R, Faircloth GT, Manzanares I, Jimeno J, Hanauske AR (1998) In vitro activity of aplidine, a new marine-derived anti-cancer compound, on freshly explanted clonogenic human tumor cells and hematopoietic precursor cells. Br J Cancer 78: 739–744

Ebel R, Brenzinger M, Kunze A, Gross HJ, Proksch P (1997) Wound activation of pro-toxins in the marine sponge *Aplysina aerophoba*. J Chem Ecol 23: 1451–1462

Faulkner DJ (2000a) Highlights of marine natural products chemistry (1972–1999). Nat Prod Rep 17: 1–6

Faulkner DJ (2000b) Marine pharmacology. Antonie van Leeuwenhoek 77: 135–145

Faulkner DJ (2002) Marine natural products. Nat Prod Rep 19: 1–48

Friedrich AB, Merkert H, Fendert T, Hacker J, Proksch P, Hentschel U (1999) Microbial diversity in the marine sponge *Aplysina cavernicola* (formerly *Verongia cavernicola*) analyzed by fluorescence in situ hybridization (FISH). Mar Biol 134: 461–470

Friedrich AB, Fischer I, Proksch P, Hacker J, Hentschel U (2001) Temporal variation of the microbial community associated with the Mediterranean sponge *Aplysina aerophoba*. FEMS Microbiol Ecol 1301: 1–9

Fusetani N (ed) (2000) Drugs from the sea. Karger, Basel, pp 1–5

Geldof AA, Mastbergen SC, Henrar REC, Faircloth GT (1999) Cytotoxicity and neurocytotoxicity of new marine anticancer agents evaluated using in vitro assays. Cancer Chemother Pharmacol 44: 312–318

Gomez SG, Faircloth G, Lopez-Lazaro L, Jimeno J, Bueren JA, Albella B (2001) In vitro hematotoxicity of Aplidine on human bone marrow and cord blood progenitor cells. Toxicol In Vitro 15: 347–350

Harrigan GG, Luesch H, Yoshida WY, Moore RE, Nagle DG, Paul VJ, Mooberry SL, Corbett TH, Valeriote FA (1998) Symplostatin 1. A dolastatin 10 analogue from marine cyanobacterium *Symploca hydnoides*. J Nat Prod 61: 1075–1077

Hart JB, Lill RE, Hickford SJH, Blunt JW, Munro MHG (2000) The halichondrins: Chemistry, biology, supply and delivery. In: Fusetani N (ed) Drugs from the sea. Karger, Basel, pp 134–153

Haygood MG, Davidson SK (1997) Small-subunit rRNA genes and in situ hybridization with oligonucleotides specific for the bacterial symbionts in the larvae of the bryozoan *Bugula neritina* and proposal of "*Candidatus* Endobugula sertula." Appl Env Microbiol 63: 4612–4616

Hentschel U, Steinert M, Hacker J (2000) Common molecular mechanisms of symbiosis and pathogenesis. Trends Microbiol 8: 226–231

Hentschel U, Schmid M, Wagner M, Fieseler L, Gernert C, Hacker J (2001) Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. FEMS Microbiol Ecol 35: 305–312

Ikeda Y, Matsuki H, Ogawa T, Munakata T (1983) Safracins, new antitumor antibiotics. II. Physicochemical properties and chemical structures. J Antibiot 36: 1284–1289

Iizuka T, Jojima Y, Fudou R, Yamanaka S (1998) Isolation of myxobacteria from the marine environment. FEMS Microbiol Lett 169: 317–322

Jansen R, Kunze B, Reichenbach H, Höfle G (1996) Chondramides A – D, new cytostatic and antifungal cyclodepsipeptides from *Chondromyces crocatus* (Myxobacteria): Isolation and structure elucidation. Liebigs Ann 2: 285–290

Jin S, Gorfajn B, Faircloth G, Scotto KW (2000) Ecteinascidin 743, a transcription-targeted chemotherapeutic that inhibits MDR1 activation. Proc Natl Acad Sci USA 97: 6775–6779

Kem WR (2000) The brain?7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: studies with DMXBA (GTS-21). Behav Brain Res 113: 169–181

Kerr RG (2000) Biosynthesis of bioactive marine natural products. In: Atta-ur-Rahman (ed) Studies in natural products chemistry, vol 21. Elsevier, Amsterdam, pp 293–328

Kikuchi A, Nieda M, Schmidt C, Koezuka Y, Ishihara S, Ishikawa Y, Tadokoro K, Durrant S, Boyd A, Juji T, Nicol A (2001) In vitro anti-tumour activity of α-galactosylceramide-stimulated human invariant Vα24+NKT cells against melanoma. Br J Cancer 85: 741–746

Kohn AJ (1956) Piscivorous gastropods of the genus *Conus*. Proc Natl Acad Sci USA 42: 168–171

Lindquist N, May ME, Fenical W (1992) Defense of ascidians and their conspicuous larvae: adults vs. larval chemical defenses. Ecol Monogr 62: 547–568

Look SA, Fenical W, Matsumoto GK, Clardy J (1986a) The pseudopterosins: a new class of antiinflammatory and analgesic diterpene pentosides from the marine sea whip *Pseudopterogorgia elisabethae* (Octocorallia). J Org Chem 51: 5140–5145

Look SA, Fenical W, Jacobs RS, Clardy J (1986b) The pseudopterosins: anti-inflammatory and analgesic natural products from the sea whip *Pseudopterogorgia elisabethae*. Proc Natl Acad Sci USA 83: 6238–6240

Luesch H, Moore RE, Paul VJ, Mooberry SL, Corbett TH (2001) Isolation of dolastatin 10 from the marine cyanobacterium *Symploca* species VP642 and total stereochemistry and biological evaluation of its analogue symplostatin 1. J Nat Prod 64: 907–910

MarinLit, Version September 2001. A marine literature database produced and maintained by the Department of Chemistry, University of Canterbury, New Zealand

Mayer AMS, Jacobson PB, Fenical W, Jacobs RS, Glaser KB (1998) Pharmacological characterization of the pseudopterosins: novel anti-inflammatory natural products isolated from the Caribbean soft coral, *Pseudopterogorgia elisabethae*. Life Sci 62: PL401–PL407

McCleskey EW, Fox AP, Feldman D, Cruz LJ, Olivera BM, Tsien RW, Yoshikami D (1987) ω-conotoxins: Direct and persistent blockade of specific types of calcium channels in neurons but not muscle. Proc Natl Acad Sci USA 84: 4327–4331

McClintock JB, Baker BJ (eds) (2001) Marine chemical ecology. CRC, Boca Raton, Florida

Mendola D (2000) Aquacultural production of bryostatin 1 and ecteinascidin 743. In: Fusetani N (ed) Drugs from the sea. Karger, Basel, pp 120–133

Minuzzo M, Marchini S, Broggini M, Faircloth G, D'Incalci M, Mantovani R (2000) Interference of transcriptional activation by the antineoplastic drug ecteinascidin-743. Proc Natl Acad Sci USA 97: 6780–6779

Mittelman A, Chun HG, Puccio C, Coombe N, Lansen T, Ahmed T (1999) Phase II clinical trial of didemnin B in patients with recurrent or refractory anaplastic astrocytoma or glioblastoma multiforme (NSC 325319). Invest New Drugs 17: 179–182

Murakami Y, Oshima Y, Yasumoto T (1982) Identification of okadaic acid as a toxic component of a marine dinoflagellate *Prorocentrum lima*. Bull Jp Soc Sci Fish 48: 69–72

Mutter R, Wills M (2000) Chemistry and clinical biology of the bryostatins. Bioorg Med Chem 8:1841–1860

Olivera BM (2000)?-conotoxin MVIIA: >From marine snail venom to analgesic drug. In: Fusetani N (ed) Drugs from the sea. Karger, pp 74–85

Osenbach RK, Harvey S (2001) Neuraxial infusion in patients with chronic intractable cancer and noncancer pain. Curr Pain Headache Rep 5: 241–249

Proksch P (1999) Chemical defense in marine ecosystems. In: Wink M (ed) Functions of plant secondary metabolites and their exploitation in biotechnology. Academic, Sheffield, pp 134–154

Proksch P, Ebel R (1998) Ecological significance of alkaloids from marine invertebrates. In: Roberts MF, Wink M (eds) Alkaloids, biochemistry, ecology and medicinal applications Plenum, New York, pp 379–394

Reichenbach H, Höfle G (1993) Biologically active secondary metabolites from myxobacteria. Biotech Adv 11: 219–277

Rinehart KL (2000) Antitumor compounds from tunicates. Med Res Rev 20: 1–27

Sakai R, Rinehart KL, Kishore V, Kundu B, Faircloth G, Gloer JB, Carney JR, Namikoshi M, Sun F, Hughes RG, Gravalos DG, de Quesada TG, Wilson GR, Heid RM (1996) Structure-activity relationships of the didemnins. J Med Chem 39: 2819–2834

Schmidt EW, Obraztsova AY, Davidson SK, Faulkner DJ, Haygood MG (2000) Identification of the antifungal peptide-containing symbiont of the marine sponge *Theonella swinhoei* as a novel δ-proteobacterium, "*Candidatus* Entotheonella palauensis". Mar Biol 136: 969–977

Schupp P, Wray V, Eder C, Schneider P, Herderich M, Paul V, Proksch P (1999) Staurosporine derivatives from the ascidican *Eudistoma toealensis* and its predatory flatworm *Pseudoceros* sp. J Nat Prod 62: 959–962

Shimizu Y (2000) Microalgae as a drug source. In: Fusetani N (ed) Drugs from the sea. Karger, Basel, pp 30–45

Smyth J, Boneterre ME, Schellens J, Calvert H, Greim G, Wanders J, Hanauske A (2001) Activity of the dolastatin analogue, LU103793, in malignant melanoma. Ann Oncol 12: 509–511

Steinert M, Hentschel U, Hacker J (2000) Symbiosis and pathogenesis: Evolution of the microbe-host interaction. Naturwissenschaft 87: 1–11

Tachibana K, Scheuer PJ, Tsukitani Y, Kikuchi H, Van Engen D, Clardy J (1981) Okadaic acid: A cytotoxic polyether from two marine sponges of the genus *Halichondria*. J Am Chem Soc 103: 2469–2471

Unson MD, Faulkner DJ (1993) Cyanobacterial symbiont biosynthesis of chlorinated metabolites from *Dysidea herbacea* (Porifera). Experientia 49: 349–353

Vacelet J (1975) Etude en microscopie électronique de l'association entre bactéries et spongiaires du genre *Verongia* (Dictyoceratida). J Microsc Biol Cell 23: 271–288

Vacelet J, Donadey C (1977) Electron microscope study of the association between some sponges and bacteria. J Exp Mar Ecol 30: 301–314

Vaishampayan U, Glode M, Du W, Kraft A, Hudes G, Wright J, Hussain M (2000) Phase II study of dolastatin-10 in patients with hormone-refractory metastatic prostate adenocarcinoma. Clin Cancer Res 6: 4205–4208

Valoti G, Nicoletti MI, Pelligrino A, Jimeno J, Hendriks H, D'Incalci M, Faircloth G, Giavazzi R (1998) Ecteinascidin-743, a new marine natural product with potent antitumor activity on human ovarian carcinoma xenografts. Clin Can Res 4: 1977–1983

Varterasian ML, Pemberton PA, Hulburd K, Rodriguez DH, Murgo A, Al-Katib AM (2001) Phase II study of bryostatin 1 in patients with relapsed multiple myeloma. Invest New Drugs 19: 245–247

Villalona-Calero MA, Eckhardt SG, Weiss G, Hidalgo M, Beijnen JH, van Kesteren C, Rosing H, Campbell E, Kraynak M, Lopez-Lazaro L, Guzman C, Von Hoff DD, Jimeno J, Rowinsky EK (2002) A phase I and pharmacokinetic study of ecteinascidin-743 on a daily x 5 schedule in patients with solid malignancies. Clin Cancer Res 8: 75–85

Webster NS, Wilson KJ, Blackall LL, Hill RT (2001) Phylogenetic diversity of bacteria associated with the marine sponge *Rhopaloeides odorabile*. Appl Environ Microbiol 67: 434–444

Weinheimer, AJ, Spraggins, RL (1969) The occurrence of two new prostaglandin derivatives (15-epi-PGA_2 and its acetate, methyl ester) in the gorgonian *Plexaura homomalla*. Chemistry of coelenterates. XV. Tetrahedron Lett 15: 5185–5188

Wilkinson CR (1992) Symbiotic interactions between marine sponges and algae. In: Reisser W (ed) Algae and Symbioses. Biopress, Bristol, pp 112–151

Zabriskie TM, Klocke JA, Ireland CM, Marcus AH, Molinski TF, Faulkner DJ, Xu C, Clardy JC (1986) Jaspamide, a modified peptide from a *Jaspis* sponge, with insecticidal and antifungal activity. J Am Chem Soc 108: 3123–3124

Zewail-Foote M, Hurley LH (1999) Ecteinascidin 743: A minor groove alkylator that bends DNA toward the major groove. J Med Chem 42: 2493–2497