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Marine Natural Products. Key Advances to the Practical Application of this Resource in Drug Development

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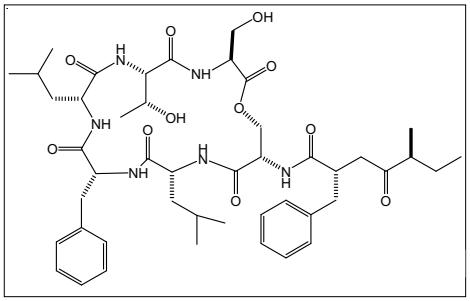
Abstract: Over a half century has passed since the first discovery of spongothymidine and spongouridine. These sponge-derived natural products initiated a chain of events that has resulted in countless lives saved from viral infections and cancer. In addition these humble marine products inspired the critical evaluation of marine life for the production of novel chemistry and new drug leads. The resulting natural products chemistry from these efforts is unprecedented in regard to structural complexity and biological activity. The drug leads based on marine natural products have however created unique challenges in scaleable production and structural optimization to evaluate toxicity and enhance biological activity. In this report we have focused our discussion on the progress toward the development of general methodologies for the production, optimization and development of rare but promising marine natural product drug leads. Key developments discussed briefly include sourcing, lead optimization, molecular biology and phylogeny, marine microbial culture, and tools for structure assignment.

Keywords: Alkaloids · Marine natural products · Peptides · Polyketides · Terpenoids

1. Introduction

Estimates suggest that secondary metabolites generated by plants and microbes have either directly or indirectly contributed to as many as 75% of our treatments for cancer and infectious diseases. Among the least explored of the global natural product diversity is undoubtedly the chemically defended organisms of the marine environment. The oceans are populated with a wealth of animal, plant and microbial life that has successfully assembled unprecedented small molecule diversity with pharmaceutical, agrochemical and research applications. The invertebrates and microbial communities of a coral reef are constantly engaged in chemical warfare yielding some of the most sophisticated as well as the most highly bioactive metabolites known to humankind. In a period of time as short as a decade we have witnessed the complete loss of the expression of the metabolite kahalalide A (shown below) a once common and abundant but not particularly active secondary metabolite from the kahalalide class of which F is the most promising.[1] In the same period of time, new and previously unpublished metabolites from this class appear to be biosynthesized replacing kahalalide A.[2] This serves as a reminder that the bioactive secondary metabolites we characterize today are products of bioorganic evolution which may or may not withstand the test of time. This also suggests that the relationship between the macro-organisms of a coral reef and their associated bacteria is driven by the ability of the associated microbe to quickly respond to changes in the environment with modified and optimized secondary metabolism for the benefit of the host.

The number of marine animals is estimated to be over 2,000,000 and countless microorganism species are available for both discovery and optimization.[3] Recent molecular studies have shown that microbial diversity in the oceans and likely also in other environments is much greater than previous estimates based on conventional molecular techniques that overlooked microbes present at low abundance. There are many highly diverse species present at low abundance that constitute a 'rare biosphere' that is largely unexplored[4] and provides a huge resource for bioprospecting. The Sorcerer II Global Ocean Sampling Expedition led by Craig Venter has shown by a metagenomic approach that, even with the discovery of more than 6 million predicted proteins from water samples collected through the northwest Pacific and eastern tropical Pacific, the rate of discovery of new protein families remains almost linear, im-



Kahalalide A structure

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plying that we are very far from discovering all protein families.[5] With the advent of innovative new approaches to culturing microbes and the use of molecular approaches to harness their genetic potential, the vast biological diversity present in marine microbes is now much more accessible for screening for bioactivity.

A particularly significant feature of animals in the marine environment is a greater diversity at higher taxonomic levels combined with a unique relationship with a sometimes complex assemblage of associated microorganisms. This biological diversity coupled with a sophisticated relationship with microorganisms has resulted in a vast and unprecedented array of small molecule diversity incorporating elements not readily available to terrestrial species. Sourcing of marine natural products provides a practically unlimited supply of unusual molecules from marine invertebrates (phyla: Annelida, Arthropoda, Brachiopoda, Bryozoa, Chordata, Cnidaria, Echinodermata, Hemichordata, Mollusca, Nematoda, Platyhelminthes, Porifera); algae, (phyla: Chlorophycota, Chromophycota, Chromophyta, Cyanophycota, Euglenophycota, Rhodophycota) and from microorganisms (bacteria, fungi and protozoa).[6] The seemingly endless and constantly evolving diversity of marine animals coupled with the estimation that only a half of one percent of the marine animals have been evaluated for their generation of antineoplastic constituents,[7] and even fewer have been examined to discover agents against cardiovascular, infectious, metabolic and neurological diseases leaves a tremendous resource for drug discovery and development.

An emerging frontier in natural products discovery is the investigation of the microbial communities associated with marine invertebrates and algae from which important compounds have been isolated. In addition to serving as a reservoir of novel microbes for drug screening programs, there is increasing evidence that in some cases these compounds are likely to be produced by symbiotic or associated microbes rather than by the invertebrates themselves.[8] Examples in which the microbial origin of compounds isolated from invertebrates has been thoroughly studied include the sponge *Theonella swinhoei* (theopalauamide and swinholide A)[9] and the bryozoan *Bugula neritina* (bryostatins).[10] In cases where symbiotic microbes rather than the invertebrates themselves are true producers of compounds of interest, these microbes may be amenable to culture and growth in fermentation systems to provide a sustainable and economic source of compounds of interest.

Despite the tempting opportunities, the development of products from the ocean has been slow due to a number of significant logistical and technical challenges. The first key technology to move the field of natural products chemistry forward was the development of the self-contained underwater breathing apparatus (SCUBA). Further improvements to SCUBA have resulted in Closed Circuit Underwater Breathing Apparatus (CCUBA) which now provides access to diverse and remote locations with depths as great as –150 m (see Fig. 1). CCUBA allows an investigator to extend the depth and time spent on a dive significantly beyond the limits imposed by diving with conventional SCUBA equipment. CCUBA systems utilize high-pressure oxygen and diluent gas cylinders, a carbon dioxide scrubber, a closed breathing loop, oxygen sensors, electronic/manual gas control systems, and a computer to interface the unit with the ambient environment. With CCUBA it has now relatively easy to dive to –150 m with dive times of as long as eight hours. This is accomplished through the efficient use of breathing gases that optimize the use of oxygen and minimize the decompression debt imposed by the inert diluent gases. Gas mixtures are controlled by a dive computer that adjusts the gas mixture to maintain a constant partial pressure for oxygen (PO_2), which is established and adjusted by the diver at anytime throughout the dive. The computer then calculates decompression times based on depth, time, PO_2 and mixed diluent gases (nitrogen and helium).

Fig. 1. A diver (Dr. Steve Bobzin) at –100 m using closed circuit diving technologies to recover marine samples

Small manned research submersibles provide another tool for accessing novel marine invertebrates and their associated microbiota. Manned submersibles provide access to intact, undamaged species from crevices and allow targeted collections of unusual specimens not possible by dredging or collection by other surface-deployed devices.

The environmental conditions (light, temperature and pressure) change dramatically in going from the surface to –150 m resulting in rapid changes in both the species diversity as well as the size of the animals. Often, the size of the animal increases with depth, associated with the absence of season storm surges common under shallower conditions. This significantly benefits natural products studies which are limited by supply of materials for a complete preclinical evaluation and optimization. With extended bottom times and greater depths it is also possible to make much more thorough and diverse collections and has resulted in our characterization of over thirty new natural products including several new ring systems from just a single sample of *Myrmekioderma styx* discussed below. The invertebrates' capability to continue to grow and regenerate biomass allows them to be harvested and later re-harvested yielding natural product starting materials for optimization rather than depending entirely on total synthesis. The diverse and unusual chemistry of the oceans offers a unique opportunity to further modify and optimize these rare and unusual structural classes in an attempt to fashion drugs for emerging and remerging diseases as well as those that still remain a challenge to treat after centuries and perhaps even millennia of investigation. Listed below are examples in which natural products have led to the development of novel classes of drug leads. In cases like the nucleosides, extensive synthetic studies and medicinal chemistry have resulted in significant contributions to treatments of HIV-1 and other viral diseases. In most examples of bioactive marine natural products a considerable effort to optimize the structure, synthetic methods, sustainable sourcing and target(s) identification studies remains to be completed providing both opportunities and challenges to identify drug leads from this resource.

2. Novel Leads from the Deep

A deep water collection of the sponge *M. styx* yielded an unprecedented number of new bioactive sesquiterpenes and diterpenes (Fig. 2 and 3). The 5,6,7-tricarbocyclic diterpenes, cyanthiwigin C and two epoxide analogues were first reported from a Venezuelan sponge *M. styx* by Sennett *et al.* in 1992, along with a linear diterpene, styxenol.[11] Six linear diterpenes from a *M. styx* collected from the Caribbean have been reported by Albrizio and coworkers.[12,13] The cyanthiwigin type diterpenes (cyanthiwigins A–D) were also reported from a Jamaican sponge *Epipolasis reiswigi*,[14] and the antifouling sesquiterpenes, curcuphenol and curcudiol[15] were reported from *M. styx* by Tsukamoto *et al.*[16] These diterpenes share the same tricarbocyclic skeleton as the cyanthins which are metabolites from the bird's nest fungus *Cyanthus* sp. and as result were named 'cyanthiwigins'.[3] This detailed examination of a deep-reef collec-

tion of the Jamaican sponge *M. styx* yielded twenty-six new diterpenes called cyanthiwigins E–AD and two novel sesquiterpenes named styxone A and B as well as a new lactone named styxlactone.[17,18] Many of the new metabolites including the styxones, styxlactone and the new diterpene cyanthiwigin AC and AD represent new ring systems. This type of diversity in secondary metabolism is not uncommon from sponges, however this number of new metabolites in a single report is quite unusual and illustrates how deeper collections can yield a wealth of very exciting new chemistry for drug discovery and development. The identification of the cyanthiwigins from a variety of unrelated species (including a bird's nest fungus) suggests that there may be a microbial link to their production. This raises the possibility of culturing the producer microbe which would be most valuable for the sustainable production should the metabolites show any practical application as drugs.

In addition, this deep reef collection of *M. styx* is an interesting example of a crude extract which showed significant biological activity, however the bioassay guided isolation yielded a suite of metabolites with activity significantly reduced when compared to the parent extract. As a result an investigation involving potential synergistic interactions showed that indeed cyanthiwigin B, which was isolated in good yields and not active, could be recombined with curcuphenol to significant increase the activity of curcuphenol against *Mycobactrium tuberculosis* and other bacteria.[19] Microbial transformation studies of the marine diterpene cyanthiwigin B using two actinomycete cultures, *Streptomyces* NRRL 5690 and *Streptomyces spheroides*, metabolized cyanthiwigin B to new metabolites. *Streptomyces* NRRL 5690 transformed cyanthiwigin B to three new compounds cyanthiwigins AE, AF, AG, (Fig. 4) and the known cyanthiwigin R (Fig. 3). *S. spheroides* transformed cyanthiwigin B to cyanthiwigins S, E, and AE. Each of these microbial

Fig. 2. *Didiscus oxeata* shown above and a related species *Myrmekioderma styx* are common on deep slopes in the Caribbean and have yielded an unprecedented number of new metabolites

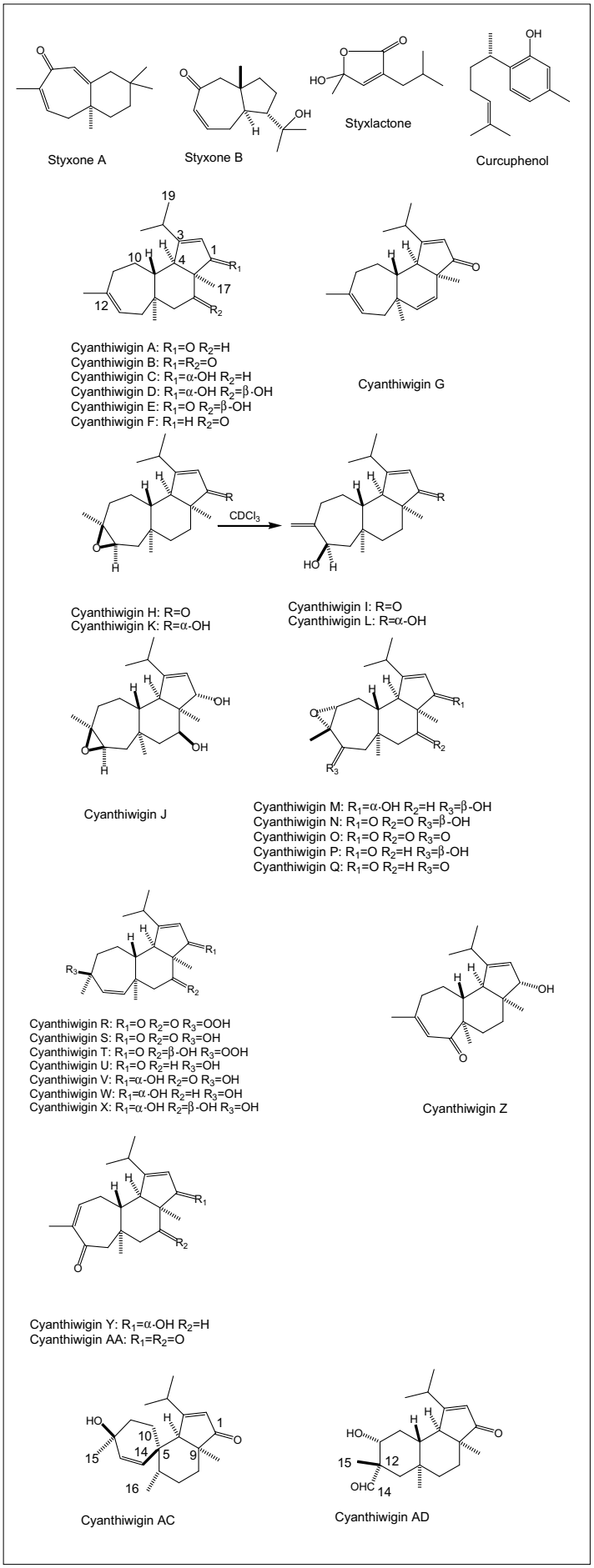


Fig. 3. An unprecedented number of new sesquiterpenes and diterpenes from a deep reef collection of *Myrmekioderma styx* are shown above. Cyanthiwigin AC and AD represent new carbon skeletons.

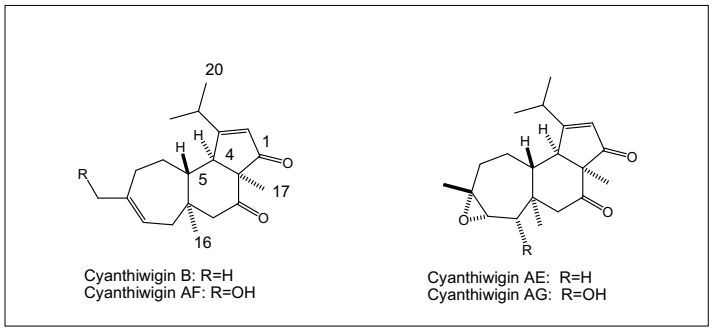


Fig. 4. Additional new cyanthiwigin metabolites generated from microbial metabolism studies

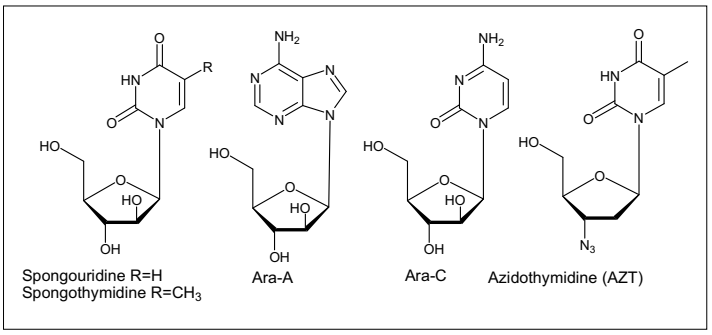


Fig. 5. Marine-derived and semisynthetic nucleosides

metabolized derivatives of cyanthiwigin B exhibited the ability to increase the antimicrobial activity of curcuphenol.

Our hypothesis for this diverse consortium of secondary metabolites is that a bacterium or fungi associated with this sponge is likely responsible for the biosynthesis of cyanthiwigin B and perhaps a few other related metabolites while yet another microbe is likely responsible for the production of curcuphenol. Then the sponge and remaining microbial community (including perhaps actinomycetes) is responsible for the biotransformation of the parent diterpene into the suite of molecules associated with the invertebrate. The combination of the sesquiterpene (curcuphenol) and diterpene metabolites yields a broader spectrum and more potent extract than possible with just a single class of secondary metabolites. A sponge's ability to harbor a diverse assemblage of microorganisms would most certainly play a role in the diverse chemistry reported from this group of marine invertebrates.

2.1. *Nucleosides*

The nucleosides represent one of the few examples of a drug class that have successfully developed into a field of their own. Although the importance of this class of compounds in the design and synthesis of drugs may appear intuitively obvious, the genesis of this field shares a unique common lineage with marine natural products. In the early 1950s Bergmann and coworkers reported the structure of spongouridine and spongothymidine from the Caribbean sponge *Cryptotheca crypta*.[20–22] There are a number of interesting aspects to the discovery of the sponge-nucleosides which exemplify the sequence of events common in natural products chemistry and drug discovery. First it is highly noteworthy that the original natural product leads spongouridine and spongothymidine never became useful drugs and this is common with the lead compounds from marine sources. These molecules however obviously possess good drug-like properties and inspired over 50 years of synthesis and pharmacology by many different groups of investigators to yield a series of clinically useful drugs. Several of the most closely related nucleosides include ara-C (Cytarabine) also called spongocytidine and ara-A (Vidarabine) also referred to spongoadenosine (Fig. 5). It does not appear as though either ara-A or ara-C have ever been reported from a sponge but were synthetic products utilizing the same atypical configuration of the carbohydrate (arabinose) as found in the original spongouridine and hence the trivial names. Both ara-A and ara-C do however have significant clinical utility and reveal how important although labor intensive the synthesis, semisynthesis and detailed investigation of natural product analogs are once a lead has been discovered. Unfortunately the medicinal chemistry of most marine natural products is not as straightforward as the nucleosides and as a result a significant bottleneck has evolved in which there is backlog of promising marine natural product leads and a dearth of medicinal chemistry studies around the vast majority of these leads. As a result, significant advances in marine chemistry are certain to be associated with the microbiology required to provide inexpensive, bulk, optically pure starting materials for medicinal chemistry studies. There is another significant aspect of marine drug discovery illustrated by the nucleosides. In the case of ara-A or spongoadenosine the molecule has been reported from a gorgonian (rather than a sponge to make the trivial nomenclature particularly confusing)[23] an actinomycete[24] and from total synthesis.[25] This is perhaps not so surprising and a general goal of marine natural products drug discovery is to utilize invertebrates as a source for prototypes followed by the identification of a microbial or a synthetic source for sustainable production. It is however rather unusual that in this case of ara-A the retro process occurred and the molecule was first reported as a synthetic product and much more recently from an invertebrate.

2.2. *Manzamines*

The manzamines are a unique group of polycyclic marine-derived alkaloids first reported in 1986 by Higa's group from the Okinawan sponge *Haliclona* sp.[26] These compounds represent a highly novel group of arene-alkaloids and possess an apparent Diels-Alder generated tetra- or pentacyclic ring system, which is attached to a β-carboline moiety through a Pictet-Spengler reaction with the aldehyde related to ircinal A as proposed by Baldwin and Whitehead.[27] Since the original report of manzamine A well over sixty manzamine-type alkaloids have been identified from nine different sponge genera (four orders).[28,29] The occurrence of these alkaloids from a diversity of unrelated sponges generated speculation that these metabolites maybe of microbial origin. The most impressive biological characteristic of this unusual group of alkaloids is their potent activity against several infectious diseases including malaria. Globally malaria accounts for over a million deaths each year from approximately 120 million annual infections.[30] There is speculation that these numbers could be quite conservative. The most dangerous malaria parasite, *Plasmodium falciparum* which causes cerebral malaria, could easily spread to the central or northern regions of Europe and North America as a product of global climate change and a serious absence of control of the disease in the tropics.

Several other significant opportunistic infections such as those caused by *M. tuberculosis* and *Toxoplasma gondii* also appear susceptible to the manzamine alkaloids.[31] The pathogenic synergy of many infectious diseases with HIV-1 increases the great need for new drug leads to control these infectious bacteria and parasites.[32] For example, the incidence of tuberculosis infection in HIV-1 positive cases is 50 fold over HIV-1 negative individuals and a similar case is true of *T. gondii*.[33] One-third of the global human population conceals latent tuberculosis, which generates more than three million mortality cases annually. The increasing incidence of tuberculosis and toxoplasmosis is also confounded by the emergence of drug-resistant and multidrug resistance to *M. tuberculosis* as well as the lack of any current chemotherapy that

targets the latent form of *T. gondii*. An array of manzamine-related molecules along with the unprecedented manzamine dimer, neo-kauluamine have been identified from an extremely common genus of Indo-Pacific sponge called *Acanthostrongylophora* (Fig. 6).[34,35] This sponge and its probable associated microbial community has been a virtual treasure trove of analogs for the manzamines to aid in our establishment of preliminary structure–activity relationships (SAR).

Fig. 6. The manzamine producing *Acanthostrongylophora*

In vitro analyses of several manzamine analogs against *T. gondii* show that manzamine A displayed 70% inhibition of the parasite at 0.1 μM concentrations without host cell toxicity. The activity is significantly increased at concentrations of 1 and 10 μM even though it was accompanied by an increase in host–cell toxicity. As a result manzamine A was selected for *in vivo* analysis since it was the most active and least toxic *in vitro*. A daily intraperitoneal (i.p.) dose of 8 mg/kg of manzamine A, for eight consecutive days, beginning on day 1 following the infection, prolonged the survival of SW mice to 20 days, as compared with 16 days for the untreated control. Additional manzamine isolations, SAR and optimized dosing studies will be of significant value to improve the *in vivo* efficacy of the manzamines against *T. gondii*. Most manzamines, with the exception of neo-kauluamine, induced 98–99% inhibition of *M. tuberculosis* (H37Rv) with an MIC <12.5 μg/ml. Manzamine A, E and 8-hydroxymanzamine A exhibit MIC endpoints of 1.56, 3.13 and 3.13 μg/ml, respectively.

Manzamine A and the closely related 8-hydroxy manzamine A were assayed *in vivo* against *Plasmodium berghei* with a single i.p. dose of 100 μmoles/kg and exhibited no apparent toxicity. 8-Hydroxymanzamine A efficiently reduced parasitemia with an increase in the average survival days of *P. berghei*-infected mice (9–12 days), as compared with: untreated controls (2–3 days), mice treated with artemisinin (2 days) and chloroquine (6 days). Three 50 μmoles/kg i.p. doses of manzamine A were found to be curative and totally cleared the parasite and two oral doses (100 μmoles/kg) provided a notable reduction of parasitemia. The pharmacokinetic properties of manzamine A and possibly the rest of the manzamines with its rapid onset of action (2 h) followed by continuous sustainable bioavailability provides effective parasite eradication in rodents.[36] These data indicate that manzamines are more active than the currently available antimalarial drugs artemisinin and chloroquine. A continuing challenge of the manzamine class is cytotoxicity. The manzamine dimer *neo*-kauluamine[31] is cytotoxic with an IC_{50} of 1.0 μg/ml, against human lung and colon carcinoma cells as compared with the first manzamine dimer kauluamine, which also showed significant cytotoxicity.[37]

The initial evaluation of the manzamine alkaloids in murine models showed that these antimalarial leads are more effective than the currently used drugs, artemisinin and chloroquine. The *in vivo* improvement in potency despite similar *in vitro* activity is certain to be in part due to the longer half-life.[38] The manzamines are clearly valuable candidates for further investigation and development as promising leads against malaria and perhaps other serious infectious diseases. The need to develop antimalarials from novel structural classes and with unique mechanisms of action is exceptionally important for the long term and sustainable control of drug resistant malaria. Fig. 7 shows how few structural classes exist for the control of malaria and how novel the manzamine class is relative to other antimalarial drugs. This class of compounds remains under extensive investigation in our laboratory in order to optimize biological activity. The natural products discussed above combined with analogs based on Winkler's total synthesis reveal that modifications to the bottom aliphatic region of the alkaloids consistently results in a loss of biological activity.[39,40] Optimization using ircinal and the Pictet-Spengler reaction proved valuable in improving antimycobacterial activity but resulted in a loss of malaria activity.[41] A study which evaluated analogs of the manzamine class against Alzheimer's disease revealed that this group of molecules shows significant potential in the control of neurodegenerative disease by targeting GSK3 and other kinases with selectivity.[42] This kinase selectivity could explain in part the activity against the *P. falciparum*. A collaborative effort between the authors of this paper revealed that the manzamine class may also have immune suppressive activity.[43] The immune suppression activity adds to the value of the class in regard to a lead for the control of organ rejection, however initially raised concerns regarding a possible involvement of a target which would make the class a development liability for any application. Fortunately the SAR data to date strongly indicates that an analogue optimized for one of the disease targets discussed above results in reduced activity in the remaining group.

2.3. *Bengamides*

About 20 years ago the first two members of bengamides were isolated from the marine sponge *Jaspis cf. coriacea*, collected off the coast of the Fiji Islands.[44] The *in vivo* antitumor activity against human breast carcinoma xenografts in mice and the unique *in vitro* cytotoxicity profile triggered the isolation of new members of this class of marine natural products from a variety of morphologically related sponges.[45] Bengamide A and O were the most potent analogs against MDA-MB-435 human mammary carcinoma cells (Fig. 8). The absolute configuration of all chiral centers could be established by total synthesis and by thorough spectroscopic analyses.[46] A medicinal chemistry effort led to the identification of potent synthetic analogs with general antiproliferative activity.[47] Three structural changes to bengamide B were introduced: removal of the N-methyl group, replacement of the iso-propyl alkane moiety by *tert*.-butyl and the inversion of the 6-hydroxy group. Replacement of the fatty acid moiety by shorter cyclic hydrocarbons and aromatic rings reduced the potency against carcinoma cells, maintained the *in vivo* activity but showed markedly improved solubility. LAF389 was the most potent candidate of the cycloalkyl series *in vivo*, with 29% tumor regression at 100 μmol/kg and minimal body weight loss. This compound has been selected for development as an anticancer agent. In order to allow for large-scale supply a convergent synthesis of LAF389 has been developed, starting with natural products containing all necessary stereocenters.[48] The initial synthesis comprising >30 steps could be optimized to less than 14 steps and scaled up in the pilot plant. The bengamides cause cell cycle arrest at G0/G1 and G2/M interfaces after apoptosis but their detailed mode of action is still unknown. A proteomics-based approach to identify signaling pathways affected by LAF389 was done after initial tests failed to show any activity in DNA binding, topoisomerase binding, microtubule assembly or proteasome inhibition.[49,50] H1299 human small cell lung carcinoma and A549 human non-small cell lung carcinoma cells were treated with LAF389 and analyzed for altered mobility of proteins on two dimensional gel electrophoresis. Analysis of one altered protein, 14-3-3γ, indicated retention of amino-terminal methionine, suggesting the involvement of methionine aminopeptidases (MetAps). LAF389 indeed inhibited MetAp1 and MetAp2 with IC_{50} = 800 nM.

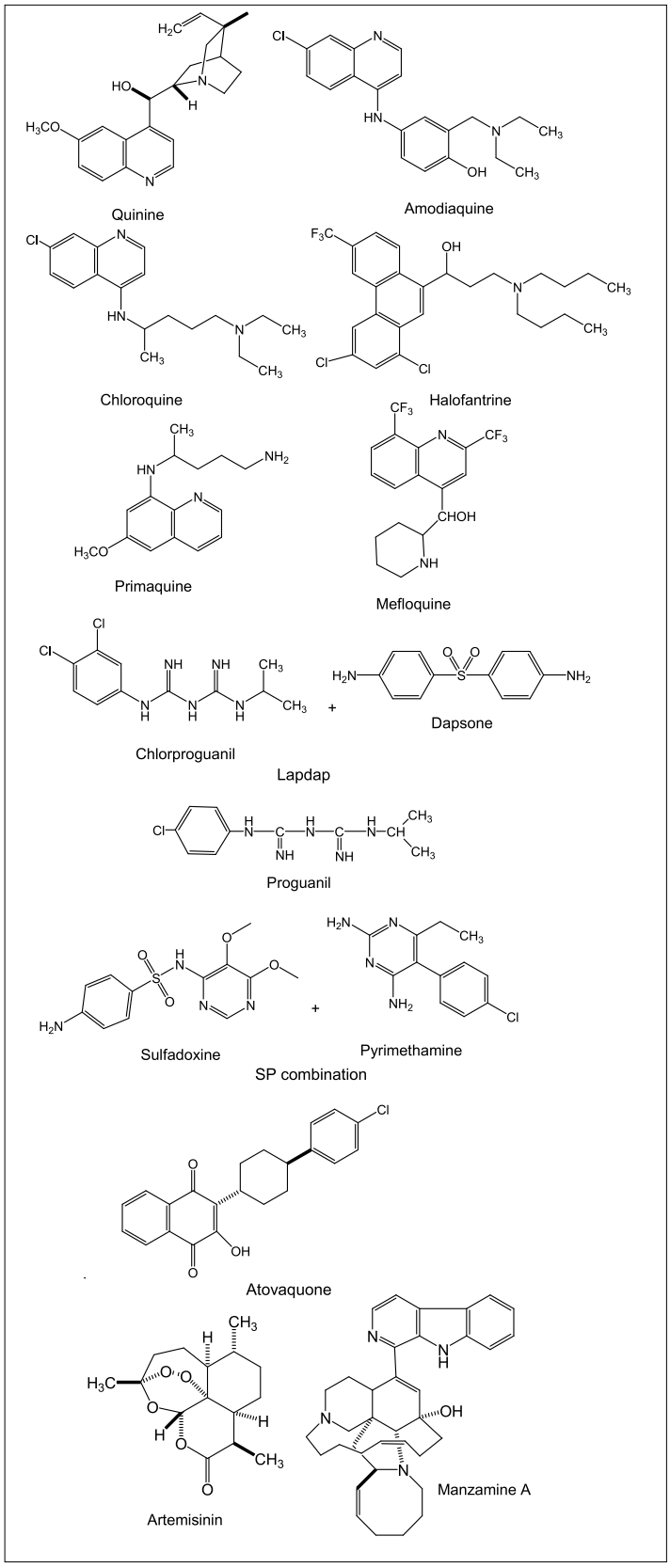


Fig. 7. Antimalarial drugs, drug combinations and drug leads. Manzamine A represents a marine natural product preclinical lead with a completely novel antimalarial pharmacophore

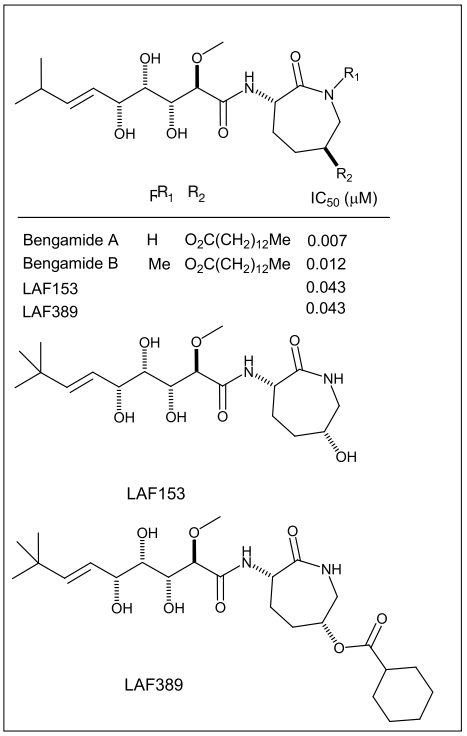


Fig. 8. *In vitro* anti-proliferative activity on MDA-MB-435 human mammary carcinoma cells

In parallel, fumagillin, a known irreversible inhibitor of MetAp2, has been tested (IC_{50} = 30 nM). The X-ray structure of human MetAp2 in complex with LAF153, the hydrolytic metabolite of LAF389, showed a very similar binding mode as the substrate derived inhibitor (3*R*)-amino-(2*S*)-hydroxyl-heptanoyl-Ala-Leu-Val-Phe-OMe. Fumagillin covalently binds to His-231 residue in the active site of MetAp2. siRNA depletion of MetAp2 blocked processing of 14-3-3γ in tumor epithelial as well as endothelial cells. Since endothelial cells depleted of MetAp2 by siRNA treatment were still sensitive to growth inhibition by LAF389 or fumagillin it became evident that MetAp2 could not be the relevant target for the antiangiogenic effects of bengamides. MRI studies of tumors from animals treated with LAF389 showed inhibition of tumor vascularization. A phase I clinical trial with 33 patients has been performed to evaluate the safety and pharmacokinetic profile of LAF389.[51] The compound was well tolerated up to 15 mg/day, given iv.

2.4. *Discodermolides*

Regulation of α,β-tubulin dimer assembly is currently one of the most intensively studied concepts in oncology research, especially since the successful introduction of paclitaxel (Taxol®) in cancer chemotherapy.[52] Besides paclitaxel, a range of natural products have been identified that bind to tubulin and affect assembly of microfibers. Two modes of action can be distinguished: i) depolymerization of microtubules, causing blockage of the mitotic spindle (vinblastine, colchicines), ii) promotion of tubulin polymerization, leading to abnormal mitosis and subsequent apoptosis (paclitaxel, epothilone, laulimalide, eleutherobine, sarcodictyins, dictyostatin, cyclostreptin). Discodermolide is a novel polyketide natural product binding to tubulin. It has been first isolated from an extract of the deep sea sponge *Discodermia dissoluta*.[53] It is one of the most potent microtubule stabilizing agents and inhibits tumor cell growth *in vitro* and *in vivo*. The growth of ovarian and colon carcinoma cells that overexpress P-glycoprotein and the growth of drug-resistant cells is inhibited by discodermolide at low nanomolar concentrations.[54] Discodermolide also retains activity in tumor cells containing mutated tubulin resistant to

paclitaxel. These findings suggest that discodermolide has properties distinct from paclitaxel. As an obvious step, both compounds were tested for synergistic effects. In a mouse model using human ovarian carcinoma xenografts the combination treatment (paclitaxel 20 mg/kg, discodermolide 5 mg/kg) yielded 40% tumor regression, whereas single drug treatments resulted in minimal growth suppression.[55]

Discodermolide is produced in small quantities by the sponge (2 mg/kg dry weight) and the amount isolated from the natural source did not allow for adequate testing and clinical evaluation. In view of the high potency of discodermolide and the small amount required for treatment a total synthesis has been elaborated aiming at 60 g of drug substance for phase I clinical trials.[56] The entire synthesis took 20 months to complete, on average one step every two weeks. Despite the synthetic effort by many groups it was not possible so far to identify analogs with equal or improved *in vivo* potency. A recent attempt to explore NMR structural information of discodermolide and dictyostatin led to the synthesis of a hybrid between these two compounds.[57,58] The cyclic derivative exhibited IC_{50} = 170–400 nM against colon, breast and non-small lung cancer cells, representing a reduction of activity by factors of 10 to 20 compared to discodermolide (Fig. 9). This encouraging result can be attributed to the resemblance of the cyclic hybrid structure to the bioactive conformation of discodermolide.

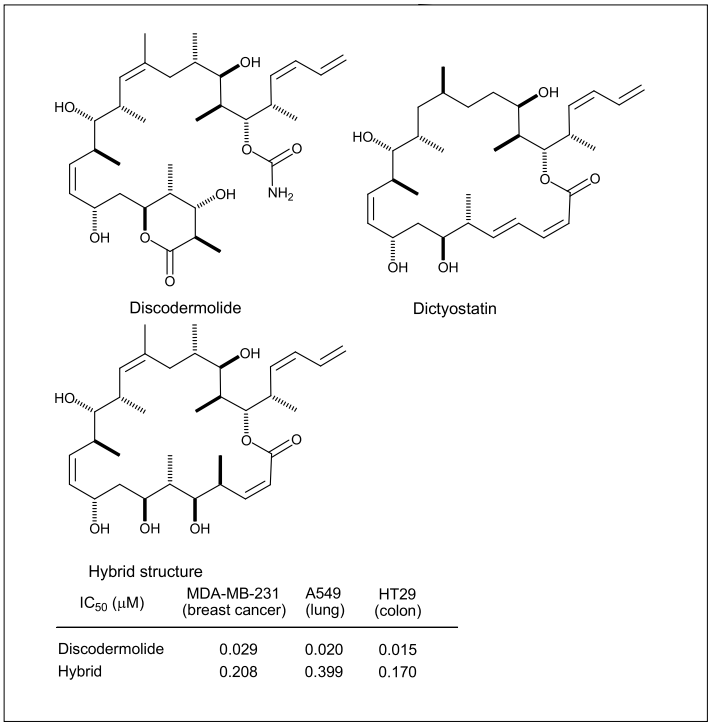
3. Future Directions in Bacterial Production/Expression and Semisynthesis

The examples discussed above clearly reveal that the oceans are a well-established resource for novel drug leads with unique opportunities for optimization and development. A significant remaining challenge is the cost-effective production of the countless marine structural classes that are not practical synthetic targets. For products in which the cost of goods is a significant issue for development and synthesis is likely to be expensive, the production from associated bacteria is beginning to show significant promise.[59] In addition Schmidt *et al.* have recently been successful in the heterologous expression of the biosynthetic pathways of marine invertebrate products in *Escherichia coli*.[60] This landmark achievement illustrates that the production of marine drug leads first discovered in invertebrates can be successfully heterologously expressed in a host and produced through fermentation. In this example the cyclic peptides know as the patellamides (shown in Fig. 10) were successfully expressed from the biosynthetic gene cluster isolated from a cyanobacterial symbiont.

The reported yields of patellamide produced through heterologous expression is 20 μg/l and will require significant yield improvements to be practical, however the expression of a complicated secondary metabolite in *E. coli* represents a remarkable achievement and promising step forward. This is particularly significant for the discovery and development of novel marine natural products as treatments for malaria and other neglected diseases. The patellamide (*pat*) pathway shown below was successfully amplified from DNA using a wild type producing reef strain while no products were amplified from a closely related but non-producing strain. The point at which epimerization of amino acids from L to D occurs during the biosynthesis of the patellamides remains unclear. The proposed biogenesis for the thiazole and oxazolines are shown below in Scheme 1 along with the points of cyclization of the peptide. These same types of thiazole or thiazoline containing peptides have also been isolated from the marine mollusk, *Pleurobranchus forskali*.[61] In this case the peptide is likely to be the result of accumulation or sequestration from a dietary ascidian adding an additional layer of complication in regard to determining the true source for natural products characterized from marine invertebrates.

4. Additional Reviews of Marine Natural Products

There are numerous outstanding reviews covering the literature of marine natural products and as a result we have focused this review on the improvements in technologies that continue to enhance our ability to utilize this resource effectively. Some previous reviews organized by topic areas include: Newman, Cragg and Snader's natural products as sources of new drugs over the period 1981–2002[62] and natural products from marine invertebrates and microbes as modulators of antitumor targets,[63] marine natural products from marine invertebrates and sponge-associated fungi;[64,65] marine natural products as therapeutic agents (patent literature);[66] genomic screening to identify novel marine antimicrobial peptide;[67] marine natural products as anti-infective agents by Donia and Hamann;[68]



IC_{50} (μM)	MDA-MB-231 (breast cancer)	A549 (lung)	HT29 (colon)
Discodermolide	0.029	0.020	0.015
Hybrid	0.208	0.399	0.170

Fig. 9. Discodermolide, dictyostatin and the hybrid structure. Human cancer cell growth inhibition by discodermolide and hybrid, 50% growth inhibition concentration after 72 h.[58]

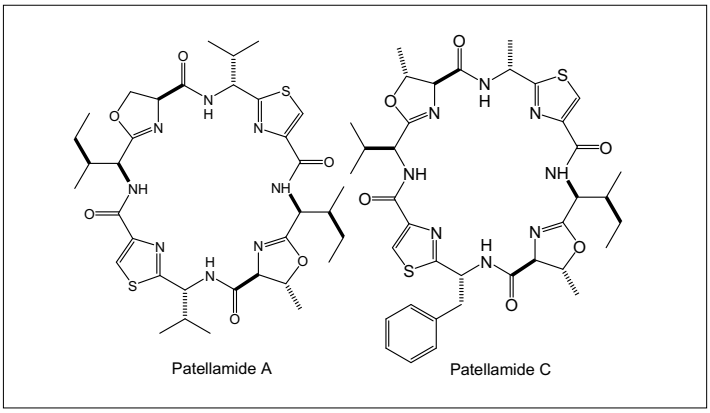
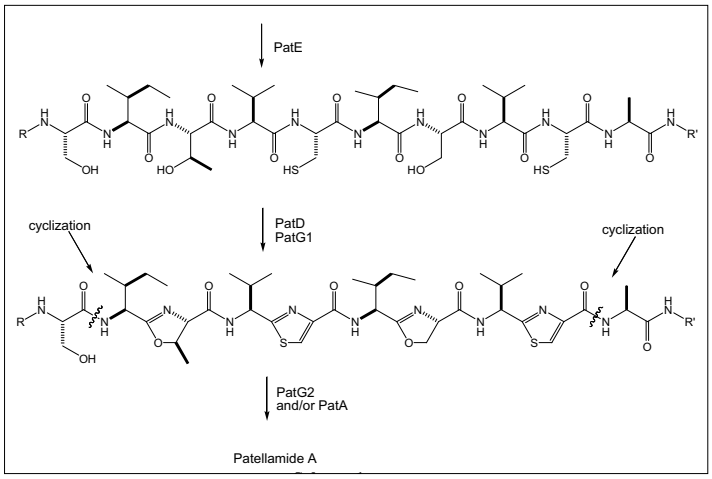


Fig. 10. Patellamides are examples of marine invertebrate products in which the pathway has been successfully expressed in bacteria



Scheme 1. Proposed biosynthetic pathway to the patellamides

and mining marine microorganisms as a source of new antimicrobials and antifungals by Bernan, Greenstein and Carter.[69] Reviews covering peptides include antimicrobial peptides from marine invertebrates by Tincu and Taylor;[70] and bioactive peptides from marine sources: pharmacological properties and isolation procedures by Anseris and Garatei.[71] Additional recent reviews deal with antimycobacterial natural products[72] and naturally occurring peroxides from marine sponges with antimalarial and antifungal activities[73] and antifungal compounds from marine organisms;[74] marine natural products as lead anti-HIV agents by the groups of Hamann and Schinazi;[75] anti-HIV activity from marine organisms by Tziveleka, Vagias and Roussis;[76] and antiviral marine natural products by Gustafson, Oku and Milanowski.[77] Anti-inflammatory metabolites from marine sponges[78] and Ziconotide: neuronal calcium channel blocker for treating severe chronic pain.[79] For complete reviews of marine pharmacology please see Mayer and Hamann's marine pharmacology in 1999–2004: compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, anthelmintic, anti-inflammatory, antiplatelet, antiprotozoal and antiviral activities; affecting the cardiovascular, endocrine, immune, and nervous systems; and other miscellaneous mechanisms of action.[80–83] The importance of natural products from marine invertebrates and microbes as modulators of antitumor targets was reviewed by Newman and Cragg.[84] The antileukemial potential of marine natural products based on work at the National Cancer Institute was recently reviewed.[85] This is far from an exhaustive list but is a helpful place to start if the reader is interested in further exploring this field.

5. Conclusion

The oceans clearly hold a tremendous potential for the discovery of new drugs as well as the creation of novel or optimized chemotypes with a variety of potential therapeutic applications. If you consider the impact that several small nucleosides have had on HIV and AIDS, other viral diseases and cancer since their original discovery it becomes very apparent that a primary value of natural products and marine natural products in particular are novel chemotypes for optimization. This is best revealed in the references above by Newman and Cragg from NCI which very nicely illustrates the key role of natural products in the genesis of the majority of our commonly prescribed drugs while only rarely is the unoptimized natural product the best drug lead. In the examples where the unmodified natural product plays a significant role often other factors including cost of goods and the absence of extensive synthetic optimization contribute to the importance of natural drug products. As a result it is quite clear that cost-effective supplies of complex marine natural product scaffolds combined with synthesis and semisynthesis will provide many of the important drugs of the future. Nucleoside chemistry in particular has grown into a field in itself and many of other marine natural product chemotypes with biological activity and good drug-like properties are certain to follow this same path once a viable drug can be generated from the original discovery of the marine prototype. We have shown a few key examples in this review of novel marine chemotypes with activity against cancer, infectious diseases as well as illustrated their significant promise for neurological diseases. The rational and efficient application of this biological reservoir depends on the technology to collect, rapidly purify and characterize and modify these secondary metabolites. The recent advances in underwater life support systems combined with ever improving tools in molecular phylogeny, analytical and synthetic chemistry have made it possible to utilize these resources for the creation of tremendous numbers of unusual and bioactive molecules. While the development of drugs from natural products has not experienced quantum leaps since the development of SCUBA to revolutionize the application of these resources, there are constant incremental improvements that increase efficiency and open doors to the complex and intriguing chemistry of the oceans. The molecules from the ocean clearly mirror the complexity of the environment from which these metabolites originate.

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