Review articles

Therapeutic agents from the sea: biodiversity, chemo-evolutionary insight and advances to the end of Darwin's 200th year

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Key words
Marine animals, biology, ecology, pharmacology-marine, drugs, malignancy, general interest

Abstract

(Newman DJ, Cragg GM, Battershill CN. Therapeutic agents from the sea: biodiversity, chemo-evolutionary insight and advances to the end of Darwin's 200th year. *Diving and Hyperbaric Medicine*. 2009;39(4):216-25.)
Drugs from the sea? Darwin may not have considered this concept when he was thinking about mechanisms that drove diversification of life on earth. In recognition of his 200th year, and celebration of the publication in 1859 of his *On the origin of species*, we review the global status of marine biodiscovery in medicinal fields, with a focus on the South Pacific. Furthermore, in the Darwinian spirit, we touch on putative evolutionary drivers and the chemical ecology of the successful leads. We argue that, for the relatively limited investment in effort to date, the success of marine leads as therapeutics promotes enhanced focus on marine biodiversity as a source of useful medicinal agents. The simple prime argument in support of this is the fact that we can exploit over four billion years of evolution in combinatorial chemistry in marine organisms, directed at relevant and effective biological activity.

Drugs from the sea

The beginning of marine biodiscovery and the vision of marine-derived drugs on the market can be traced to discoveries by Bergmann and the subsequent identification of spongothymidine and spongouridine in the early 1950s from the Caribbean sponge *Tethya crypta*.[1–3] The subsequent explosive discovery of compounds is described in the citations by Suckling and Newman et al.[4,5] These discoveries led to the identification of a close analogue, cytosine arabinoside, as a potent antileukaemic agent that was commercialised subsequently as Ara-C. Other closely related compounds such as adenine arabinoside (Ara-A), an antiviral compound later also found in the Mediterranean gorgonian *Eunicella cavolini*, and azidothymidine (AZT), can be traced back to this initial discovery.

The advent of scuba techniques approximately 60 years ago and their subsequent utilization by natural-products chemists, and biologists working closely with them, led to questions as to how marine sessile organisms defend themselves against predation, competition and disease. Very early on, the chemical diversity, complexity and novelty of marine extracts were appreciated and since Bergmann's discoveries, exciting modes of biological activity of relevance to humans have been reported.[6] In this, the 200th year celebrating Darwin's birth and his insight into biodiversity and evolutionary process, we should not be surprised that potent, chemotherapeutically relevant chemical leads can be readily discovered from marine organisms for a substantial range of clinical applications. These compounds are secondary metabolites with many natural functions selected for in an evolutionary context; they represent the front-line defence for most marine organisms, particularly in those sedentary, soft-bodied filter feeders where cellular challenge from pathogenic micro-organisms is ever-present.

The metabolites need to be potent because of immediate dilution on being exuded, highly targeted in mode of action (conservation in the metabolic process minimises generalist bioactivity), and they need to get into the cells of other organisms to effect a response (Figure 1). Such qualities are also the attributes of effective drugs. Furthermore, when considering the lowest metazoans, possessing essentially the same basic biochemical pathways as higher vertebrates, there has been over 800 million years of evolutionary-scale experimentation resulting in a highly varied and flexible repertoire of biologically active chemistry.[7] However, the evolution of marine invertebrates and algae cannot be considered in isolation, since many marine organisms are likely to be influenced by, or share or benefit from the biochemistry of microbial symbionts. Thus, exploration of the full range of marine micro- and macro-diversity arguably harnesses four billion years of selective pressure directing biosynthesis of defensive metabolites toward functional biological activity. The result is sophisticated chemical flexibility producing an amazing array of compounds and compound classes.[8,9] These exciting biological activities, once discovered and fine tuned with medicinal chemistry to therapeutic targets, will be the drugs of the future.[10–12]

Biodiversity equates to chemical diversity

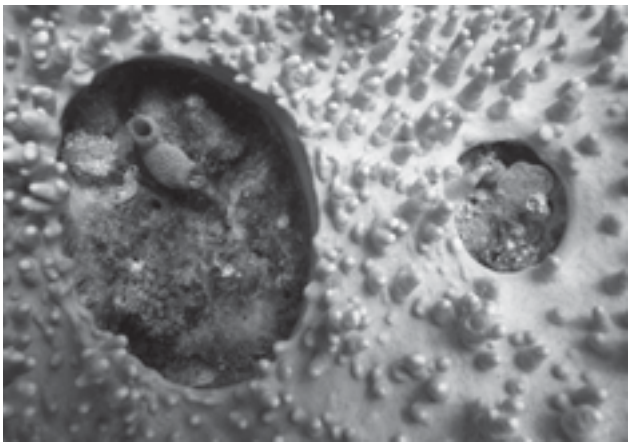
There is a rich and growing library of review articles focusing on the sources of new drugs and their properties. A few of the more prominent and relevant to the topic

at hand are cited above. Natural product-derived drugs have been used in all therapeutic areas: as anti-malarials, anti-virals, anti-fungals, anti-tuberculosis treatment, anti-HIV agents, anti-inflammatory agents, for osteoporosis, Alzheimer's disease and other neurological diseases and disorders.[13–15] They are also the source of many biomedicinal tools and have been used extensively as probes in medicinal research.[16] Synthesising across many review papers, over 75% of all drugs used as anti-cancer, anti-infective and anti-cholesterolaemic agents come from or have their synthetic origin, be it the scaffold, warhead or concept, in nature. This is because, even though natural products arise from a limited selection of simple biochemical building blocks and biosynthetic pathways, the diversity of structure and function created under almost limitless natural recombination far exceeds that possible in synthetic libraries.[17]

Furthermore, this process is guided by selective pressure at the very coal-face of survival for almost all benthic marine species including micro-organisms, as it is through their 'defensive chemistry' that they either survive to reproduce, or die. Increasingly we realise that this chemistry has many roles, from causing the eliciting organism to be toxic or distasteful, to more subtle biological acitivities such as mediating rapid cellular growth and loss of adhesion (by which sponges, for example, can slough off settling epizoic organisms such as bryozoans) or to cause cells in encroaching species to cease to divide, thereby effecting a competitive standoff and maintenance of occupied reef territory (Figure 1).

It does not take much imagination to visualise applied uses for such compounds on the understanding that the molecules are designed to be effective on multicellular animal life. Therefore, sampling biodiversity across a full phylogenetic range will yield diverse new chemical classes and novel biologically active compounds. Over 90% of all macro-organism phyla on the planet occur on the seafloor, equating to an unprecedented molecular diversity of secondary metabolites, enzymes and biochemical pathways.[18] This diversity pales into insignificance when compared to that of marine micro-organisms, where recent work on metagenomic diversity in seafloor sediments or in the water column suggests a limitless biological resource with, for example, over 20,000 microbial entities in one litre of seawater and an estimated 36 x 10^{30} cells in the oceans.[19]

Figure 1
Zones of growth inhibition in the soft coral *Lobophytum* sp. surrounding *Didemnum molle* (a green ascidian, left) and *Lissoclinum patella* (a white translucent ascidian, right) on a shallow-water coral reef (courtesy E. Evans-Illidge, AIMS)



Marine natural products

The body of research and development of drugs is too large to effectively review here; hence the remainder of the paper will focus largely on anti-cancer drugs and marine natural products. It is, however, interesting to review briefly the role of marine natural products in drug-development programmes for other important therapeutic areas.

Arguably the world's number-one killer is malaria, with 1–2 million deaths a year and 300–500 million new clinical cases reported annually. Of 621 terrestrial plant species screened, 82 (13.2%) were found to have significant anti-malarial activity (IC_{50} <10ug.mL^{-1}).[13] Although the total number of marine extracts screened was not stated, but is likely to be much less, 27 compounds that have anti-malarial activity were reported.[13] These compounds were isolated from sponges, gorgonians, octocorals and ascidians, also from cyanophytes and red algae, and included compounds such as sesquiterpenes, diterpenes, peptides, glycosides and alkaloids. Of note are the manzamine alkaloids from symbionts of the Pacific sponge *Petrosia* sp., that are currently under development.[10,13,20] The species that elicit these compounds are invariably ones that are found in densely encrusting communities or are even epizoic in habit. They are known to possess rich bacterial symbiotic populations. They would likely come into contact with pathogens (fungal and other infections) and would exist in conditions where competition for space was heavy. An arsenal of biologically active chemistry for defence would be expected.

Natural products are growing in prominence as anti-HIV agents in an expanding search for treatments for this, the world's fourth biggest killer, with over 60 million people having been infected by 2005. Alkaloids, coumarins, flavonoids, lignans, phenolics, quinones, saponins, terpenes and sterols, xanthones, carbohydrates, peptides and proteins have all been sourced from nature. Of 122 compounds reported in a 2005 review as being of considerable interest in the development of anti-HIV agents, 19 were from marine sources, 15 being sponge-derived compounds.[21] The relatively low number of marine candidates to date may reflect the traditional focus on terrestrial plant-derived compounds, given these leads make up the sources of almost all of the other compounds reviewed, rather than superior efficacy of terrestrial over marine leads. Nevertheless, given

the probable sampling bias, marine and particularly sponge-derived leads are prominent. Again on examining the habitats and growth characteristics of the source marine species, we find that they are predominantly species that are soft-bodied and either occupy densely encrusted habitats where forms of chemical defence rather than structural armament are commonplace or where the host sponge harbours diverse bacterial communities.

Naturally occurring anti-mycobacterial drug leads have also traditionally been sought from terrestrial plant sources, although again there is growing interest in marine leads.[14] Of 91 lead compounds reviewed including alkaloids, flavonoids, terpenoids, steroids, phenols and peptides, 40 were plant derived, 22 from terrestrial fungi and eight synthetic. Despite relatively little focus to date, marine leads account for 22% of the anti-mycobacterial leads of current interest.[14] The marine leads listed come almost exclusively from gorgonians and ascidians, with the most active compound a marine pyridoacridine coming from a *Didemnum* species. All of the ascidian leads are species that are aggressively epizoic in nature, overgrowing and often killing their host, frequently causing secondary fungal and other infections in the process. Therefore, some self-defence against the collateral damage of overgrowth activity would be expected. It would be useful to test the hypothesis that the compounds elicited in this competitive process are the same ones that we find of use for human treatment.

Marine natural products as anti-cancer agents

AGENTS IN CURRENT CLINICAL TRIALS

The following review represents a selection of the more prominent marine natural product drug leads currently in the clinic; it is not exhaustive. Full details may be found in papers by Newman, Cragg and others cited below. In keeping with the thrust of this article, we annotate this summary with comments as to the natural origin of the target molecules.

Bryostatins

In 1968, the US National Cancer Institute (NCI) commissioned a large-scale (for those days) collection of the bryozoan *Bugula neritina* for chemical workup. The aqueous isopropanol extract was tested for intrinsic activity as an antitumor agent in the then current P388 and L1210 murine leukaemia *in vivo* models. The extract was found to be inactive against L1210, but using the P388 model at the same concentration gave a 68% increase in life span.[22] After further painstaking and difficult research, the compound was purified and identified as bryostatin 3, one of a series of closely related compounds that now number twenty.[23,24]

Subsequent work identified two other geographic areas where significant (in relative terms) quantities of bryostatin 1 could be isolated from *B. neritina* colonies. By 1990, there was enough cGMP-grade (current Good Manufacturing Practice) material to commence systematic clinical trials, though prior to this time frame, small quantities of bryostatin1 had been supplied to a variety of collaborators so that basic biochemical studies and initial clinical trials could be performed in the United Kingdom.

From these studies, it was shown that bryostatins bind to the same receptors as the tumour-promoting phorbol esters, the protein kinase C isozymes, but have little or no tumour-promoter activity. To date, bryostatin 1 has been studied in more than 80 human clinical trials, usually as a single agent. It has become evident that this is not the optimal treatment regimen, with improved responses being reported for combination studies with fludarabine at the Phase I level. Combination studies with biological agents, such as interleukin-2 or granulocyte macrophage-colony stimulating factor, the nucleoside derivative cytarabine or other cytotoxic agents such as paclitaxel, vincristine or cisplatin, are currently in progress. These combinations are being tested against various forms of leukaemia as well as against other carcinomas.[25] (For data from NCI clinical trials, see their website <http://clinicaltrials.gov>.)

Bryostatin is also in the early stages of being assessed as an anti-Alzheimer's drug, with Phase I trials to be initiated soon. Supply remains an issue for this compound, synthesis being difficult in the extreme. Of significance here is the identification of the gene cluster that would produce the 'hypothetical bryostatin precursor, bryostatin 0'.[26] If this gene cluster can be expressed in a heterologous host (currently the genetic source is the as yet uncultured symbiont *Candidatus endobugula sertula*), then the production of significant quantities of base structural material may be possible. *B. neritina* is a cosmopolitan fouling bryozoan, found on most wharf pilings and ships' hulls around the world. It is an aggressive coloniser, but it does not always possess the bryostatin metabolites; samples tested from Australia and New Zealand possessed no bryostatins. Haygood and colleagues are currently working on the wider chemical ecology of this species.

Dolastatin derivative TZT-1027 (auristatin PE or soblidotin)

The original compounds, the 'dolastatins' were first sourced from *Dollabella* nudibranchs. As a result of the synthetic processes developed in early studies on dolastatin 10, many derivatives of the dolastatins were synthesized, with TZT-1027 entering Phase I clinical trials in Europe, Japan and the USA. This compound is also known as auristatin PE and soblidotin. It has had a fairly chequered career thanks to the machinations of the pharmaceutical industry. Initial Phase I and II clinical trials were terminated, but currently a new series of Phase I trials has commenced. Of interest is that the compound is also a vascular disrupting agent, causing the vasculature within tumours to collapse.[27] Currently it is in three clinical trials, from Phase I to Phase III, using auristatin PE linked to specific monoclonal antibodies.

Dolastatin derivative ILX651 (synthadotin)

As in the case of soblidotin, this compound has also had a chequered career as companies were bought and sold. It is orally active and had advanced to Phase II trials in a variety of cancers, but those trials ceased. It then entered into a Phase I trial in the USA against solid tumours. Recently it was reported that ILX651, in fact, might be a relatively weak prodrug for the functionally active tasidotin C-carboxylate which is 10–30 times more potent in an *in vitro* assay of purified microtubule dynamics.[28] However, the compound has recently been withdrawn from trial. Although a moot point now, the source of the dolastatins has recently been shown to be microbial (as with the bryostatins), with a report of the direct isolation of dolastatin 10 from a marine cyanobacterium known to be grazed on by *Dolabella auricularia*.[29] Of relevance in a biodiscovery sense, the nudibranch acts as a useful sequestering agent of these and many other compounds. Without the nudibranch, these compounds, which occur in low concentration in benthic symbiotic associations, might not have been discovered.

Kahalalide F

This cyclic depsipeptide was isolated from the Sacoglossan mollusc *Elysia rufescens* following grazing by the mollusc on a green macroalga *Bryopsis* sp.[30] It was synthesized in a very efficient manner using solid-phase peptide techniques and in 2000 entered Phase I clinical trials in Europe for the treatment of androgen-independent prostate cancer.

There are a variety of mechanisms attributed to this compound. It was known to target lysosomes, suggesting selectivity for tumour cells with high lysosomal activity, such as prostate tumours.[31] Kahalalide F was shown to induce cell death via 'oncosis' (the progression of cellular processes leading to necrotic cell death) possibly initiated by lysosomal membrane depolarization in both prostate and breast cancer cell lines.[32] Then in 2005, HepG2 cells were reported to demonstrate significant alterations in their membrane permeability with cell swelling/blebbing, implying specific interactions with membranes and/or proteins at 300nM.[33] It has also been reported to induce a necrosis-like cell death involving inhibition of protein kinase B signalling and depletion of ErbB3. ErbB3 may well be a marker for progress against suitable tumour types, and an ErbB3 kinase inhibitor may well increase efficacy.[34] Recently a Patent Cooperation Treaty international application was successfully filed by another group claiming production of kahalalide F and other derivatives from a *Vibrio* species isolated from *Bryopsis* and *Elysia rufesens*, implying that the invertebrate obtains the producing microbe from the alga and then maintains it as a symbiont.[35] Thus, there is a potential renewable source of these agents by use of fermentation.

Aplidine

This compound, formally known as dehydrodidemnin B, was first reported in a patent application in 1989, with a UK patent issued in 1990, and then referred to in a paper on the structure-activity relationships amongst the didemnins.[17] The initial work on aplidine, its entry into Phase I and II trials and the preferred method of synthesis were described in detail in 2004.[36] At that time, the dose-limiting toxicity was muscle pain, responsive to either dose limitation or addition of carnitine, which increased the maximum tolerated dose by 40%. Several Phase II clinical trials are now underway in Europe for acute lymphoblastic leukaemia, lymphoma, multiple myeloma, prostate and bladder cancer. The precise mechanism of activity (MOA) of this agent is not yet known, but it appears to block vascular endothelial growth factor (VEGF) secretion and blocks the corresponding VEGF-VEGF-receptor-1 (also known as *flt-1*) autocrine loop in leukaemic cells.[37] Aplidine and kahalalide F are also being studied in psoriasis targets.

Ecteinascidin 743 (Yondelis®)

Although antitumour activity from the ascidian *Ecteinascidia turbinata* had been reported as early as 1969, it was not until 1990 that the structure of the most active component, known as Et743 from the absorption maximum, was published.[38,39] The yield from natural sources was very low, and in order to provide enough material to perform basic *in vitro* and *in vivo* studies on the MOA, considerable amounts of the ascidian had to be collected from areas around the Caribbean. The compound was subsequently synthesized in a chemical *tour de force* and a refined process reported that produced Et743 in higher yields.[40]

The requirement for supply of material for further pre-clinical and clinical development, included large-scale wild collections, and aquaculture both on land and in sea, but for late cGMP-grade clinical and commercial supply, an elegant 21-step semi-synthesis from the marine *Pseudomonas fluorescens* metabolite cyanosafracin B was devised. This was feasible in spite of a low overall yield of 1.4% because the starting material could only be obtained on a large scale by fermentation.[36]

Several reports over the last few years give some indication of the likely MOA(s) for Et743 when tumour cells are treated *in vitro.* A major problem has been that the concentrations used in these experiments were often orders of magnitude greater than those physiologically relevant *in vivo.* Since the latter levels are in the low nanomolar to high picomolar range, care should be taken when evaluating published work on the MOA of this compound. At *in vivo* concentrations, the MOAs of Et743 have been shown to include effects on the transcription-coupled nucleotide excision repair process and interaction between the Et743 DNA adduct and DNA transcription factors, in particular the NF-Y factor. Recently pharmacogenomic analyses have identified a series of genes involved in the sensitivity of tumour cells to this agent.[34] Prior to the establishment of mechanisms of action, the compound was placed into human clinical trials and in 2001,

Et743 was licensed under the generic name trabectedin (brand name Yondelis®). Details of the trials and methods were reported in a 2005 review.[36]

As a result of these earlier trials, Et743 was pre-registered in the European Union (EU) and granted orphan drug status for sarcoma by the European Commission's Committee for Orphan Medicinal Products of the European Agency for the Evaluation of Medicinal Products (EMEA). Following a somewhat chequered path through the EMEA, the compound was approved within the EU in late 2007 for commercialization for the treatment of sarcoma, becoming the first 'direct from the sea' compound to reach that goal as an antitumor treatment. Since 2007, there have been six clinical trials listed on the NCI trials website, plus at least four others in Europe in the Prous Integrity® database. It is hoped that these will lead in due course to approvals for use in the treatment of other types of cancer. Again, the source organism is a very successful cosmopolitan biofouling species, and the elicited chemistry of interest to medicine is likely to have beneficial properties in nature. The chemical ecology of *E. turbinata* is the topic of much interest.

Zalypsis (PM-00104)

This compound is a synthetic derivative of jorumycin (from a mollusc) or the renieramycins (from sponges), and entered Phase I clinical trials from 2005 in both the USA and Europe. It is reported from genomic analyses to have a similar mechanism to that of Et743 in terms of inducing double-stranded breaks in DNA, though, to date, details have only been presented as a meeting abstract.[41] It will be interesting to follow this compound in comparison to Et743.

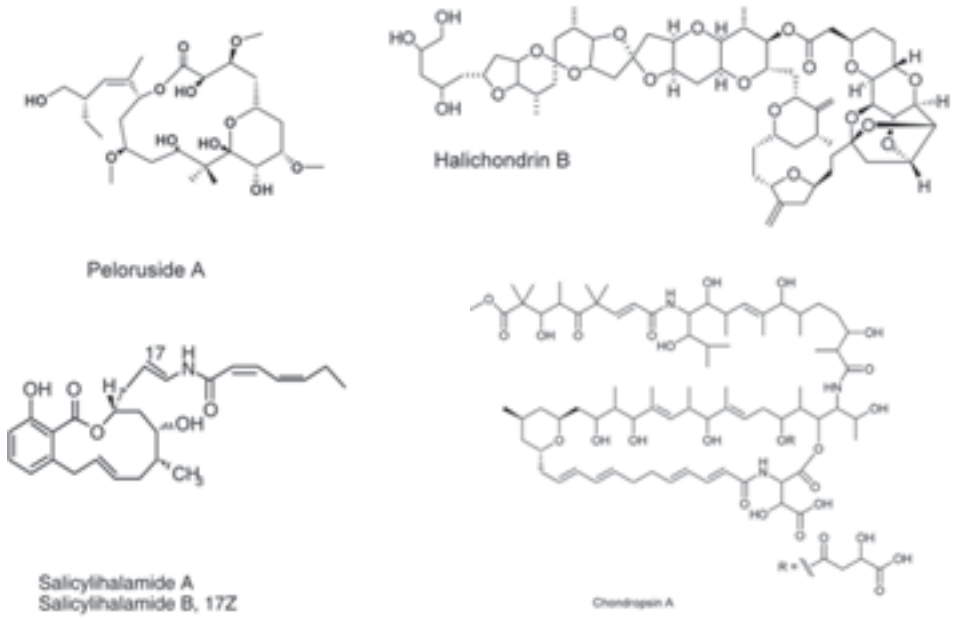
Eribulin (E-7389)

This agent, modelled from the naturally occurring antitubulin compound, halichondrin B (Figure 2) arose from another synthetic tour de force utilizing the synthetic method for halichondrin B combined with the realization that the active part of the molecule resided in the macrolide ring (approximate molecular weight (MW) 600; right-hand end of molecule shown in Figure 2) and not in the 'tail' (the remaining 400 of the overall 1000 MW).[42] Over 200 different molecules have been synthesized, the modified, truncated, macrocyclic ketone (E-7389) being chosen as the candidate compound.

This molecule, like its parent, is a tubulin-interactive agent with very potent activity at the nanomolar level *in vitro* and recent modelling studies suggest that it binds at or close to the 'vinca site'.[43] Since tubulin is a dynamic dimer and no high resolution X-ray crystallographic structure exists, the 'putative binding sites' on the tubulin molecule are defined by displacement binding assays. Thus the 'vinca site' is the site that the vinca alkaloids bind to and can be displaced from by other compounds. Similarly, the 'laulimalide site' (see later) is different from the vinca site, as laulimalide can be displaced by other agents, but not by the vinca alkaloids. This is a biochemical concept that is used in many assays for beta-agonist binding sites.

Halichondrin B, the source molecule, is one of at least nine other halichondrin molecules, variations on the halichondrin scaffold but with widely varying biological activities. Originally found in small quantities in a Japanese *Halichondria* species, the bulk of the preparative

Figure 2
A sample of South Pacific marine natural products that have demonstrated anti-tumour activity



work was carried out on halichondrin B extracted from the sponge *Lissodendoryx* sp., discovered by Munro and Blunt in partnership with the NCI. Halichondrin B and its sister molecules are biosynthesised by *Lissodendoryx* sp. (and probably its symbionts) continuously, but with seasonal peaks and in heightened yields when the sponge is challenged with competitors, (such as didemnid ascidians, Battershill CN, unpublished data). During a programme to produce enough sponge for extraction to progress pre-clinical trials, aquaculture-produced sponge was found to elicit halichondrin B (as well as the other halichondrins) even after three years in 'in-sea' culture, suggesting a very stable association with symbionts in a biosynthetic partnership. Heightened yields of halichondrin B on exposure to competing species or in response to damage suggest these metabolites are defensive in function.

Salinosporamide A (NPI-0052)

This compound, in addition to having an unusual structure, is also the first of what may well be a future wave of compounds to enter clinical trials. It was produced by a marine-derived streptomycete of an entirely new genus and species, *Salinispora tropica*. It is also unusual in that it has gone from initial discovery to clinical trial in only four years, a testament to the researchers at Scripps Oceanographic Institution and Nereus Pharmaceutical in San Diego.[44]

Over the last 20 or so years, there has been much discussion on whether a number of the agents found in marine invertebrates had 'microbe(s) in their background', such as using genomic information in the cases of bryostatin and Et743 and by direct isolation of microbes from the *Bryopsis*

Table 1
Status of marine-derived natural products in clinical and preclinical trials (updated from reference 12)

Compound	Source	Habitat/ecology	Development	Status
Didemnin B	*Tridemnum solidum#*	fouling	Phase II (cancer)	dropped mid-90s
Dolastatin 10	*Dolabella auricularia* (marine microbe; cyano)	sequestered	Phase I/II (cancer)	no further trials
Girolline	*Pseudaxinyssa cantharella*	lagoonal sponge community	Phase I (cancer)	discontinued
Bengamide	*Jaspis* sp.#	dense encr., sediment	Phase I (cancer)	Novartis, discontinued
Cryptophycins	*Nostoc* sp., *Dysidea arenaria#*	dense encr., sediment	Phase I (cancer)	Lilly, now Sanofi-Aventis
Bryostatin 1	*Bugula neritina#*	fouling	Phase II (cancer)	one trial going
Ecteinascidin	*Ecteinascidia turbinate#*	dense encrusting	Phase II/III (cancer)	approved as Yondelis®
Aplidine	*Aplidium albicans#*	dense encrusting	Phase II (cancer)	PharmaMar
E7389 (Hali B)	*Lissodendoryx* sp.	sediment	Phase I (cancer)	Eisai
Discodermolide	*Discodermia dissoluta*	microbial symbionts	Phase I (cancer)	Novartis, discontinued
Kahalalide F	*Eylsia rufescens, Bryopsis* sp.#	sequestered?	Phase II (cancer)	licensed to PharmaMar
Spisulosine	*Spisula polynyma*	surf clam	Phase I (cancer)	discontinued
HTI-286	*Cymbastella* sp.#	dense encrusting	Phase II (cancer)	Wyeth, work stopped
KRN-7000	*Agelas mauritianus*	sponge gardens	Phase I (cancer)	
Squalamine	*Squalus acanthias*	spiny dogfish	Phase II (cancer)	macular degeneration
Laulimalide	*Cacospongia mycofijiensis*	dense encrusting	preclinical (cancer)	
Curacin A	*Lyngbya majuscula#*	sediment, fouling	preclinical (cancer)	
Vitilevuamide	*Didemnum cucliferum, Polysyncraton lithostrotum#*	dense encrusting	preclinical (cancer)	
Diazonamide	*Diazona angulata*	dense encr. (cave)	preclinical (cancer)	
Eleutherobin	*Eleutherobia* sp.#	dense encrusting	preclinical (cancer)	
Sarcodictyin	*Sarcodictyon roseum*	dense encr./fouling	preclinical (cancer)	
Peloruside A	*Mycale hentscheli#*	dense encr., fouling	preclinical (cancer)	licenced to Reata
Salicylihalimide	*Haliclona* sp.	dense encrusting	preclinical (cancer)	
Thiocoraline	*Micromonospora marina*	isolated from sea sand	preclinical (cancer)	
Variolins	*Kirkpatrickia variolosa*	dense encrusting	preclinical (cancer)	
Dictyodendrins	*Dictyodendrilla verongiformis*	dense encrusting	preclinical (cancer)	licensed to Taiho
Manoalide	*Luffariaella variabilis#*	dense encr., sediment	Phase II	discontinued
IPL-576,092	*Petrosia contignata*	sponge garden	Phase II	licensed to Aventis
Ziconotide	*Conus magus*	toxin	Phase III (pain)	approved for pain
CGX-1160+	*Conus geographus, catus, victoriae*	toxin	Phase I (pain)	

Epizoic habit; encr. - encrusting

Figure 3
Phylogenetic origin of anti-cancer leads currently in the clinic or in late phase pre-clinical trial with the US National Cancer Institute[12]
Phyla: CY Cyanophyta; CHL Chlorophyta; RHO Rhodophyta; PHA; Phaeophyta; POR Porifera; BRY Bryozoa; COE Coelenterata; MOL Mollusca; ECH Echinoida: CRU Crustacea; ASC Ascidiacea.

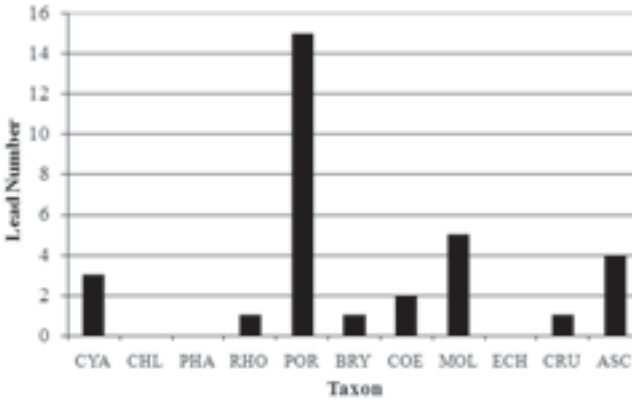
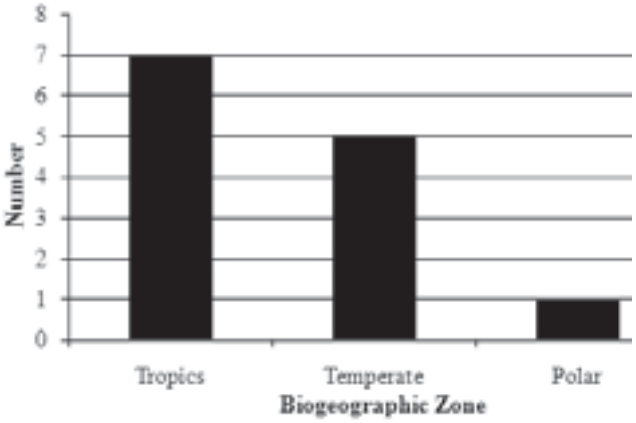


Figure 4
Source biogeographic regions for the marine cancer leads currently in late phase pre-clinical trial or in the clinic



alga in the case of kahalide F. Fenical and Jensen, at Scripps, proposed, however, that there were deep-sea, free-living microbes that could be cultivated and novel agents produced by modifications of methods used for other microbial flora. This hypothesis has since proved to be correct.[44]

The structure of salinosporamide A is reminiscent of the terrestrial bacterial product omuralide, a known proteasome inhibitor, and such activity was reported in the original publication.[44] The compound had an unusual chlorine substitution and within a year or so, two research groups had synthesized the base molecule.[45,46] In addition, the necessary cGMP product for clinical trials could be produced by fermentation in a saline environment, the first time that this task had been performed successfully on any scale with a marine-sourced microbe. During these runs, several other salinosporamide derivatives were isolated and other secondary metabolites were further explored. The compound entered Phase I clinical trials in 2006, initially against solid tumours and leukaemias and, in 2007, a Phase I trial against multiple myeloma was initiated.

There is an updated 'marine pharmacology website' that is kept relatively up to date as to the current (usually within the last six months) status of marine natural products as drugs (<http://marinepharmacology.midwestern.edu/>).

Ecological and phylogenetic trends

An updated summary of marine leads in late-phase pre-clinical trials or in the clinic as of 2004 is reproduced from Cragg and Newman in Table 1.[12] Details have been added (where known) about the habitat or habits of the source organism. In most cases, compounds of interest are derived from species found in densely encrusted communities where competition for space is high, hence biosynthesis of allelopathic and immunosuppressive compounds likely. Alternatively, the source organisms are species from fouling communities in high sediment environments where pathogenic attack is commonplace. They are also species that do not have highly elaborated skeletal armament, but tend to be soft-bodied and, where known, are host to a diverse array of symbionts.

For anti-cancer leads, sponges (Porifera) are clearly the lead taxon (Figure 3), possibly as they have need for subtle forms of chemical defence. For an organism defending space, as most benthic encrusting species need to do, it is to their advantage not to kill encroaching species outright but to inhibit their growth such that a standoff is maintained. Otherwise in killing the neighbour, the space created if not immediately occupied by oneself, may be colonised by a more competitively aggressive occupant. In surveys of reef systems around the world from the tropics to the poles, standoff competitive outcomes represent 95% of the encounters, frequently with a chemically maintained no-growth zone between neighbours in evidence (Figure 1). Therefore, elicited defensive compounds act to halt cellular replication in target tissues and appear to have similar functions when addressed to human cancer cells. Marine and sponge metabolites have been shown to be active through all stages of the cell cycle, resulting in cell stasis, which is why they have such strong interest to drug-development agencies.[10]

While there have been attempts to identify high-yielding biogeographic regions (as Figure 4 might suggest), the pattern of decreasing incidence of anti-tumour active leads with increasing latitude possibly reflects more the greater effort that has gone into tropical collection compared to the polar regions than any true biogeographical distribution. However, the Australasian region appears to produce a disproportionately large number of leads, given the relatively

low level of collection activity to date (Table 1). From a collection of approximately 1,500 samples in New Zealand, there are three candidate compounds in clinical or late phase clinical trial (halichondrin B derivatives, variolins, and peloruside A, Figure 2). From Australian collections of 3,000 samples, three vacuolar-ATPase active compounds made it to the Federal Register (chondropsin A, salicylihalamide A and lobatamide A, Figure 2). Until recently these had not been progressed due to issues of availability, both in terms of physical supply and legal provenance; now both solved. Australia is also the source of manoalide and a number of conotoxins, as well as a large number of other biologically active metabolites such as the phorboxazoles. It is clear that the South Pacific is a rich source of novel, biologically active compounds and most nations in the region are currently developing legislative and collaborative research mechanisms to enhance discovery of useful leads.

The future

As can be seen from the number of comments about the involvement of microbes in the production of materials obtained from marine invertebrates, and the discovery of marine-derived Gram-positive microbes from the Actinomycetales, it is becoming evident that a large proportion, perhaps even the majority, of chemical compounds of interest isolated from marine sources are produced by unicellular organisms (eubacteria, archaea or eukarya). These are then possibly modified by the host invertebrate in some cases, rather than being the direct product of the invertebrate. When one realizes that over 50% of the body mass of the *Porifera* (sponges) is composed of microbes, this does not seem quite such a leap of faith. There are some interesting reviews on sponge-associated microbes that are worth reading by a wider audience than just those working in the microbial field.[47,48] The relevance of the macro-organism is its role as the discoverable source of the target compound, even if that is biosynthesised by the microbe. Furthermore, evidence suggests that such biosynthesis may not occur without some stimulus from the host or particular micro-environmental conditions.

Finally to demonstrate the potential of the *Porifera*, in particular, the story of peloruside is quite instructive in that it is now known to be produced by a variety of sponges of the genus *Mycale*. Depending on its location around New Zealand, different mixtures of peloruside with other secondary metabolites are found.[49] As with *Lissodendoryx* sp. and halichondrin B, and indeed with two Australian leads examined to date; salicylihalamide A from *Haliclona* sp. and manoalide compounds from *Luffariella variabilis*, the yields of the target metabolites may vary with season. This variation is repeated through the years, and the yields have been maintained in aquaculture in either as good as or above naturally occurring levels, indicating a stable biosynthetic system.[50–52] Chemico-ecological studies suggest some environmental influence, but further work examining the chemical micro-ecology is needed to fully appreciate the role of these metabolites in nature and how they are biosynthesised. An outcome of such work will be novel production options including metagenomic expression and perhaps even semi-synthetic improvement in the range of metabolites produced, with heightened specificity for new therapeutic targets.[53] The complex structures of these molecules (Figure 2) has been a key issue limiting investment in and updating of natural marine products as drug candidates. Breakthroughs in sustainable and economic supply of these leads will herald a new age of drug discovery sourced from the sea.

To further highlight the potential of the South Pacific, the lead compound peloruside A is demonstrating some very interesting synergistic activities with taxoid-site drugs and apparently binds at the so-called 'laulimalide site' on the tubulin dimer but does not exhibit synergy with the latter compound.[54] Major joint studies are being performed on the synthesis of peloruside A, including investigation of other synthetic routes. Further details as to the intrinsic activities/MOA of this agent will be very interesting to follow as they come to light.[55]

When one couples these data with the explosion in secondary metabolite-cluster recognition resulting from current research in the microbial world (even fungi have clustered secondary metabolite genes), then the potential for novel compounds and the ability to produce them via fermentative means, perhaps using surrogate hosts, should be considered seriously. This would remove the major perceived hindrance to studies of marine-derived compounds by the larger pharmaceutical houses.

The reader will have noticed that the pathway to drug development is a frustratingly tortuous one with entry in and out of pre-clinical and clinical trial. In true Darwinian style, the passage of a drug lead through to the market is a highly selective process where only the 'fittest' compounds will survive. Marine natural product leads will always be weighed at every step against synthetic products and leads from terrestrial sources. The scales to date have been biased against marine sources because of the economics and sustainability of the target compound supply and issues surrounding their legal provenance (Access and Benefit Sharing Agreements with source countries). Synthetic leads are clearly desirable on these counts, if they work. Marine leads, however, do work and many are highly specific in their activities because, as we have argued, they were designed expressly for relevant biological function.

The two major issues, supply and provenance, that have plagued marine natural products drug discovery over the last 30 years, have now been effectively resolved. Firstly most countries have established legal frameworks compliant with the Convention of Biological Diversity Guidelines for Access to Biodiversity and the fair and equitable benefit share of any revenue from successes. Secondly synthetic and semi-synthetic chemistry has evolved to a point where

supply is no longer an impassable hurdle. As indicated above, the future is likely to see metagenomic expression and manipulations as an increasingly common technique for lead development and supply into the market. We see an exciting future for marine natural products as drugs.

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