The discovery and development of marine compounds with pharmaceutical potential

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Received 12 October 1998; received in revised form 1 December 1998; accepted 22 December 1998

Abstract

An assessment of the current status of marine anticancer compounds is presented along with a case study on the aquaculture of Lissodendoryx n. sp. 1, a sponge that produces the antimitotic agents halichondrin B and isohomohalichondrin B. The use of polymer therapeutics to enhance the properties of marine natural products is considered. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Biodiversity; MarinLit; Sponge aquaculture; Anticancer; Antimitotic; Polymer therapeutics

1. The discovery phase

In contrast to work on terrestrial natural products the first serious work on studying marine natural products started just 50 years ago with the pioneering work of Bergman (e.g. Bergman and Feeney, 1951). While the difficulties of collecting marine samples cannot be underestimated, a large number of easily accessible marine samples are available simply by shore-wading. That the opportunity was not seriously grasped until the 1940s is possibly a commentary on the difficulties of isolation and purification of marine natural products with the limited techniques available at that time. However, since the 1940s the field has blossomed and matured. In 1997 there were 713 papers published on marine natural products. This is out of a total of 10 311 papers recorded in MarinLit, a database dedicated to the marine natural products literature (MarinLit, 1998). At the time of the mid-year release of the 1998 version of MarinLit, 484 new papers had been included. From the marine literature it is the Porifera that have been the most studied phylum followed closely by the Cnidaria, Chromophycota, Rhodophycota, Mollusca, Chordata and the Echinodermata (MarinLit, 1998).
Over the years some distinct trends have emerged in the study of marine natural products. One has been the emphasis on the discovery of new bioactive natural products. Initial work by Bergman was undoubtedly curiosity-driven, but it was his discovery of the biologically-active, pharmaceutically important and novel arabino-nucleosides from the sponge *Cryptotethya crypta* that sparked interest in marine natural products and served to highlight the biomedical potential of the field (Bergman and Feeney, 1951). With advances in chromatographic techniques for dealing with polar compounds along with better analytical and structural elucidation technology an increasing proportion of the compounds isolated have shown cytotoxic properties (suggestive of potential antitumour compounds). In an early review (Munro et al., 1987) covering the marine literature up to early 1986, 185 bioactive compounds were reported. In 1993 a review (Schmitz, 1994) covering the next 5 years commented on an additional 400 compounds. A survey of MarinLit reveals that this trend has continued with some 46% of all cytotoxic compounds in the database having been reported since 1993.

As a source of bioactive compounds with pharmaceutical potential how well does the marine environment compare with the more traditional areas such as terrestrial microorganisms and plants? The best comparative data is that published by Garson based on statistical data from the US National Cancer Institute (NCI) screening programme provided by Dr Peter Murphy. This clearly indicated that marine invertebrates are a preferred source due to the much higher incidence of significant cytotoxic activity (Garson, 1994) (Fig. 1). If those screening data for marine animals are in turn examined on a phylum basis certain phyla (e.g. Porifera, Bryozoa, Chordata) have a higher incidence of bioactivity with the trend becoming very obvious as species with very significant bioactivity (IC₅₀ < 2 µg ml⁻¹) are selected (Fig. 2).

As the data in Fig. 1 suggest, the sampling of oceanic life-forms enhances the probability of discovering species from natural sources with potential anticancer activities. This can be rationalised as a sampling strategy which accesses the widest range of phyla. Greater than 70% of all recorded living species belong to the animal kingdom.
While only 28% of the animals are aquatic these in fact represent >90% of the animal phyla (May, 1988). When searching for bioactivity the total number of samples collected is important, but it is not as important as sampling across phyla. Put simplistically, the probability of finding a bioactive species (a ‘hit’) can be expressed as:

\[
\text{‘Hits’} \approx \text{Samples} \times \text{‘Biodiversity of Samples’} \times \text{Assays}
\]

Clearly, the greater the number of samples assayed in the greatest number of possible assays will enhance the probability of finding useful compounds. With the advent over the last decade of high throughput screening (HTS) the number of assays that can be applied to any sample has gone up by between one and two orders of magnitude. However, the key term in the expression is ‘biodiversity of samples’. This is a qualitative factor relating to the number of unique structural classes of compounds sampled (adapted from Devlin, 1997). By sampling across phyla the probability of finding unique classes of compounds is higher than by sampling many species within one phylum. The factor associated with ‘biodiversity of samples’ is probably higher for a random collection of marine organisms than from any other source and perhaps explains why marine samples offer greater opportunity for the discovery of unique compounds with pharmaceutical potential.

2. Current status of bioactive marine natural products

In spite of the advances in computer-assisted drug design, in molecular biology and gene therapy there is still a pressing need for new drugs to counteract drug-resistant pathogens like, for instance, the mycobacterium that causes tuberculosis, or multi-drug resistant cancers, or even disease states such as Alzheimers which is of pressing concern as the age demographics of the Western World change. What of value has emerged from the marine resource over the past 50 years that would justify the investment of time and money and optimism in this field? In the area of cancer there have been valuable discoveries. Likewise in the area of inflammatory diseases (Fenical, 1996), while probably the first marine-based drug that will be marketed is in the area of
intractable pain management (Olivera, 1997). However, for the purposes of this overview only the cancer leads will be commented on.

Progress in the cancer area is summarised in Table 1. Of the four compounds now in clinical trials two are derived from tunicates. These are ecteinascidin 743 and aplidine (dehydrodidemnin B). The other compound, dolastatin 10, originated from a bryozoan. Didemnin B, another tunicate-derived compound closely related to aplidine, has previously been in clinical trials and reached as far as phase 2 as had bryostatin 1 before being withdrawn in late 1998.

At a preclinical phase are halichondrin B, a sponge metabolite, and kahalalide F from a mollusc followed by a range of compounds from a variety of sources, but all with potent in vivo activities against a range of cancer cell lines. From the mechanism of action of these marine metabo-

### Table 1
**Testing status of anticancer marine metabolites**

<table>
<thead>
<tr>
<th>Status</th>
<th>Compound (origin/activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>–</td>
</tr>
<tr>
<td>Phase III</td>
<td>–</td>
</tr>
<tr>
<td>Phase II</td>
<td>Ecteinascidin 743 (tunicate/antimitotic)</td>
</tr>
<tr>
<td>Phase I</td>
<td>Aplidine (dehydrodidemnin B) (tunicate/protein synthesis inhibitor)</td>
</tr>
<tr>
<td>Pre-clinical</td>
<td>Halichondrin B (sponge/antimitotic)</td>
</tr>
<tr>
<td></td>
<td>Kahalalide F (mollusc/-)</td>
</tr>
<tr>
<td>In vivo active</td>
<td>Aplyalronine (sea hare/actin)</td>
</tr>
<tr>
<td></td>
<td>Thiocoralline (marine microorganism/RNA inhibition)</td>
</tr>
<tr>
<td></td>
<td>Isohomohalichondrin B (sponge/antimitotic)</td>
</tr>
<tr>
<td></td>
<td>Discodermolide (sponge/antimitotic)</td>
</tr>
<tr>
<td></td>
<td>Sarcodictyins (soft coral/antimitotic)</td>
</tr>
<tr>
<td></td>
<td>Eleutherobins (soft coral/antimitotic)</td>
</tr>
<tr>
<td></td>
<td>Spongistatins/Altohyrtins/Cinchyrolide (sponge/antimitotic)</td>
</tr>
<tr>
<td></td>
<td>Lamellarin N (mollusc/tunicate/sponge/antimitotic)</td>
</tr>
<tr>
<td></td>
<td>Cryptophycins (blue green alga/antimitotic)</td>
</tr>
</tbody>
</table>

lites it is very obvious that the marine environment has been an excellent source of antimitotic agents. Cancer chemotherapy exploits differences between normal and malignant cells. Ideally, total selectivity between the cell types is required, but has not been achieved. The high proliferation rate of cancer cells is one area that is targeted when looking for cytotoxic agents. Compounds that block mitosis (anti-mitotic agents) which occurs during cell proliferation have become some of the most important anticancer agents, e.g. taxol, vincristine. Antimitotic agents can be divided into four categories depending on which particular step in the microtubule polymerisation/depolymerisation steps that they inhibit (as indicated in Table 2) (Avila, 1997).

Two of these antimitotic agents are already in clinical trials and many of the others listed are being actively investigated as potential anticancer agents. Undoubtedly, some will proceed further to clinical trials. There seems little doubt now that one, or more, of these potent marine natural products (or synthetic analogues derived from them) will emerge in the future as new, therapeu-

### Table 2
**Marine-derived antimitotic agents**

<table>
<thead>
<tr>
<th>Type</th>
<th>Marine compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I (colchicine site)</td>
<td>Halichondrin B</td>
</tr>
<tr>
<td>Type II (GTP binding site)</td>
<td>Isohomohalichondrin B</td>
</tr>
<tr>
<td></td>
<td>Spongistatins/Altohyrtins/Cinchyrolide</td>
</tr>
<tr>
<td></td>
<td>Dolastatin 10</td>
</tr>
<tr>
<td>Type III (microtubule stabilisation)</td>
<td>Eleutherobin</td>
</tr>
<tr>
<td>Type IV (microtubule network disorganisation)</td>
<td>Sarcodictyins</td>
</tr>
<tr>
<td></td>
<td>Discodermolide</td>
</tr>
<tr>
<td></td>
<td>Ecteinascidin 743</td>
</tr>
<tr>
<td></td>
<td>Lamellarin N</td>
</tr>
</tbody>
</table>
3. The development phase

Given the difficulty in synthesising many of the marine natural products, perhaps the most significant hindrance to their development as drugs, or industrial biocides, is their limited supply. The marine resource offers the biological diversity for sampling in the discovery-phase of new drug development. What is less attractive about marine macroorganisms is the lack of knowledge about obtaining either the organism in bulk, or sourcing the key compound by a routine method. There is no routine, easy source of material for scale-up such as seeding out plantations, or fermentation on a 50,000 L scale as applies to plant, or microbial products. No matter how attractive a biological profile a compound might possess, unless an adequate supply stream can be generated the compound will remain of novelty value only. For example, at the NCI if an adequate initial supply of the compound can be obtained an in vivo active compound can proceed as far as Decision Network IIA (DNIIA), an advanced point in that organisation’s preclinical evaluation of anticancer compounds, but unless arrangements can then be made for the purchase or supply of the compound in bulk the compound will not proceed to the next step, DNIIIB. Halichondrin B is a good example of a compound in that situation.

When a marine natural product succeeds in the development phases, techniques for large-scale commercial supply need to be employed immediately in order to maximise the patent, or license investment. Realistically, the bulk supply of bioactive compounds can only be achieved by harvesting from natural origins, by aquaculture/fermentation, or by synthesis. Neither tissue culture nor genome transfer from the producing organism to an appropriate vector can be considered as viable supply options at least for the foreseeable future. All but one of the compounds listed in Table 1 have been synthesised (kahalide F), but one has to distinguish between an ‘academic’ synthesis and an ‘industrial’ synthesis. The former is where the goal is simply to synthesise the compound, preferably being the first to do so, and by a novel and elegant route. In the latter case the aim is to provide a viable, low-cost synthesis with as few steps as possible. Not all compounds lend themselves readily to synthesis on a large scale. This is usually due either to the complexity of the skeleton, the number of stereogenic centres, or a combination of both. Other compounds, like those of peptide origin, are more amenable to synthesis and compounds such as dolastatin 10 fall into this category. Supply from natural sources by careful harvesting works well for rapidly growing and abundant species. One example of this is the supply of the tunicate-based antimitotic ecteinascidin 743. In other cases where the source organism is rare, or grows only in extreme conditions, then aquaculture might be the only way of obtaining sufficient compound. When an assessment¹ is made of the potential supply source of the current antitumour compounds (Table 1) the following pattern emerges (Table 3).

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¹ A personal judgement specialist (MHGM and JWB) assisted by Dr Johnathan Morris, a special synthetic chemist.
Of the compounds in clinical trials, adequate supplies have been obtained from natural sources by harvesting with good husbandry practices, by aquaculture, or by total synthesis. Of the candidates at the pre-clinical phase it would be possible to obtain adequate supplies of halichondrin B by aquaculture methods. This is the first time that a Porifera secondary metabolite has become accessible by aquaculture. The same comment also applies to the production of ecteinascidins and bryostatins, metabolites from the phyla Chordata and Bryozoa, respectively.

4. Production of halichondrin B by aquaculture

In work in New Zealand over the past 14 years ~ 6000 samples have been collected and assessed for biological potential. The samples have been mainly sponges and tunicates from sites ranging from Antarctica, through the sub-Antarctic islands, and around the coast line of New Zealand up to the Northern tip of the North Island. The most promising candidate discovered to date is the sponge Lissodendoryx n. sp. 1. The bioactive components in this sponge are a series of compounds belonging to the halichondrin B family, which have now been found in a total of four sponges (the others are Halichondria okadai (Hirata and Uemura, 1986), an Axinella sp. and Phakellia carteri (Pettit et al., 1991, 1993)). Halichondrin B is being assessed by the NCI, while isohomohalichondrin B, a related compound (Litaudon et al., 1994), will be developed independently in Europe by PharmaMar SA.

Lissodendoryx n. sp. 1 is a rare, deep water species (~ 80 to ~ 100 m) found exclusively off the Kaikoura Peninsula (Page and Battshill, 1998). An extensive environmental survey, conducted using an ROV and a benthic camera, established that the 'sponge field' was only ~ 5 km² with the mean biomass and abundance of sponge estimated to be 69 ± 21 g m⁻² with 1.1 ± 0.1 individuals per m² over the sponge field. This survey gave an estimated total biomass of the Lissodendoryx sponge of just 289 ± 90 t total (Dumdei et al., 1998; Page and Battershill, 1998) and established quite unambiguously that the halichondrins could never be supplied on a commercial scale by collection from the wild. However, based on the results of the survey a permit was obtained and a collection of 1 t made, from which the halichondrins were isolated (310 mg of halichondrin B (NCI) and a comparable amount of isohomohalichondrin B) providing sufficient mass for the initial preclinical trials only.

To establish a supply option for the halichondrins the New Zealand National Institute of Water and Atmospheric Research (NIWA), in collaboration with the University of Canterbury and the NCI, have carried out aquaculture feasibility trials at various scales on Lissodendoryx n. sp. 1. There were early indications that one mode of Lissodendoryx reproduction was by fragmentation and advantage was taken of this observation. It was established that small explants were capable of extremely rapid growth (up to 5000% within 1 month) given the correct conditions (Battershill and Page, 1996). Preliminary experiments also established that use of a scallop lantern was feasible as a support and an ‘analysis of variance’ model was adopted to allow examination of variability of growth and target metabolite production patterns in response to the following factors: season, location, site (within location), and depth. The experiments were run over a period of 18 months. It was quickly apparent that summer transplants were not successful and that while significant growth of the explants was observed at the deep sites this was short-lived, as all sponges succumbed to bryozoan overgrowth and pathogen attack. Mortality was over 95%. In contrast, the winter explants (April) at all sites were generally successful (mortality less than 15%), especially at the greater depths, with the same previously observed growth rates observed through to December, when a decline was observed as fouling again became excessive. The mortality rate of the sponges was high in summer, especially at shallower depths, and a critical temperature of 18°C has been identified above which the sponge will not survive (Dumdei et al., 1998).

The next most important question after getting the sponges to grow and survive was whether or not the halichondrins were being produced under these conditions. Samples of the biomass were
taken at regular intervals across all sites and extracted and examined by bioactivity assays and HPLC for the presence of the various halichondrins. From the bioactivity profiles it could be established that halichondrins were being produced and by HPLC analysis of five bulk samples from two sites at differing depths the production and profile of the halichondrin production was established. Wild samples of the sponge typically contain ~400 µg kg⁻¹ of halichondrin B, ~200 µg kg⁻¹ of homohalichondrin B and ~900 µg kg⁻¹ of isohomohalichondrin B. The overall halichondrin content of the cultured sponge was not as high as that of the wild sponge, and the production of the individual halichondrins was site dependent (see Fig. 3) (Dumdei et al., 1998). The relative production of halichondrin B in the cultured samples was generally higher than that found for homohalichondrin B and isohomohalichondrin B and, depending on the site, the figure for the total production of halichondrins per kg of sponge ranged from 30 to 60% of that found in the wild sponge at four of the sites surveyed. This is a significant rate of production, especially when rate of growth is taken into account.

These initial experiments showed that the sponge could be grown successfully in small-scale trials and that the halichondrins continued to be biosynthesised, even after several years of culture. There was a need, however, to scale-up experimentation to a level that would simulate commercial production conditions. This was carried out in late 1997 using a variety of culture methods. The selection of culture methods was dictated by consideration of those approaches that could ultimately be most readily converted to a large-scale mechanised operation. Wild samples of Lissodendoryx n. sp. 1 were collected from the sponge field off Kaikoura and immediately transferred to aerated buckets and flown by light aircraft to Wellington before deployment. Explants (8 cm³) were allocated at random into the following treatments: lantern, tray, bag or disc, all at 10 m depth. The lanterns used were commercially available scallop lanterns 1 m in diameter, with ten tiers supported by wire hoops and covered in a 1.5 cm nylon mesh. Tray culture represented a prototype sponge deployment system which essentially held explants in a vertically aligned nylon mesh sandwich. Bag treatments similarly represented a prototype for a continuous stocking type culture system and finally, discs held sponges in a clutch-type culture array where explants were suspended on ropes without any surrounding mesh. The explants were monitored for three months. Growth and mortality were measured in situ. Fig. 4 shows the changes in volume (directly proportional to weight in this species).
Sponges cultured in trays, or on discs did not grow well. In both cases, fouling became a significant problem as the plastic component of the support structures appeared to promote settlement of fouling species, predominantly *Bugula flabellata*. The best initial growth rate was observed in lantern cultures, but after 26 days sponges were observed to lose weight and condition. It was apparent that the enclosed nature of the apparatus retarded growth after a certain size was obtained and that the lanterns also soon became severely fouled. The bag-type culture system proved to be the best both in terms of promoting fast growth, as well as maintaining growth. Explants quickly grew through the mesh and were soon able to grow uninhibited, using the overgrown bag for support. This culture system was also the most readily adaptable to a mechanised system and could be developed to a large-scale stocking-type culture system, not unlike traditional mussel-seeding aquaculture.

This suite of experiments was carried out in summer. This is usually a period through which the sponge does not grow in nature and hence represents a worst-case example of the potential of sponge aquaculture. None the less it was established that in less than 26 days sponge explants could quadruple their volume and weight. In other experiments, carried out at the same time but at different localities, explants were observed growing to 120 cm³ in less than 30 days, clearly indicating that there will be locations which are more suitable for growing sponges (Battershill, unpublished data).

These results show that aquaculture of sponges is a viable and reliable option for creating extractable biomass. Very preliminary estimates of biomass production suggested that the annual production of 5 t of sponge per 100 m of longline is achievable (Dumdei et al., 1998). These estimates were based on use of scallop lanterns, good husbandry to reduce overgrowth, two harvests a year, mortality restricted to < 50% and growth of 8 cm³ explants to ~ 300–400 g. The use of stocking-type systems as opposed to scallop lanterns will influence both the likely yields as well as the economics of production. The economics of production is likely to be very competitive as the mechanised technology is already available for seeding and harvest using stocking type systems. In addition, sponges may be readily grown with other species such as mussels in successful polyculture operations.

If the halichondrins progress satisfactorily through preclinical trials over the next year, gram quantities of halichondrin B and isohomohali-
chondrin B will be required. This equates to tonnes of sponge which can only be supplied by aquaculture. Harvesting at that scale from the limited wild-stock is not possible. The supply of this quantity of the compounds will give the opportunity to estimate more accurately the actual production parameters and economics of the production of *Lissodendoryx* n. sp. 1 by aquaculture. Should either halichondrin B, or isohomohalichondrin B then proceed through clinical trials and become established as pharmaceuticals the estimated amount of each compound required annually is ~ 5 kg. This estimate is based on the potency of the halichondrins in in vivo animal trials and projected dose regimes and corresponds to the annual production of at least 5000 t of *Lissodendoryx* n. sp.

5. Future trends

The development of a successful pharmaceutical requires that attention also be paid to delivery of the drug as well as supply. The antiproliferative properties of today’s anticancer compounds will never overcome solid tumours because of the sensitivity of the surrounding tissue to the fatal effects of exposure to the compounds. This limits the use of high concentrations. What is required are alternative approaches that facilitate the specific targeting of tumours. One approach, known as polymer therapeutics, is a rapidly growing multidisciplinary field requiring the combined talents of organic chemists, polymer chemists, pharmacologists and oncologists (Duncan, 1992). The concept behind polymer therapeutics is shown schematically in Fig. 5. The drug is attached via a biodegradable linker to a water soluble polymer. In other, optional approaches, specific targeting residues can also be added.

Polymer therapeutics not only offers improved pharmacokinetic properties, but better targeting of tumour tissue and higher selectivity. The basis for this better targeting and selectivity operates by what is known as the ‘enhanced permeability and retention’ effect (EPR) which leads to higher concentrations of the anticancer agent within the tumour. In vivo trials have established the success of this approach. Two such drugs currently undergoing phase I/II clinical trials are PK1 and PK2 where the anticancer drug doxorubicin has been attached via a tetrapeptide linker to a water-soluble hydroxypropyl-methacrylamide (HPMA) polymeric backbone. The tetrapeptide linker was designed to resist peptidase activity in the bloodstream, but be susceptible to lysomal enzymatic hydrolysis following the transfer by endocytosis to the interior of the target tumour cells (Duncan et al., 1996).

Polymer therapeutics is an ideal approach to enhancing the value of marine toxins. Compounds like halichondrin B are already established by in vivo trials as effective agents which can be transferred intravenously to remote sites within the test animal and inhibit the growth of a range of human tumour types. Any modifications that can enhance pharmacokinetic properties, reduce required plasma concentrations and exhibit enhanced selectivity can only be considered advantageous. To this end we are working in collaboration with the NCI and the London School of Pharmacy on the development of a polymeric therapeutic based on the halichondrin skeleton. An amino derivative of the halichondrin skeleton has been synthesised and converted into a polymeric form comparable to PK1 and is currently undergoing in vitro testing against a range of human tumour cell lines.

![Fig. 5. Schematic showing basic features of a polymer therapeutic drug.](image-url)
Ultimately, this approach will be applied to other marine toxins such as the mycalamides (Perry et al., 1988), the discorhabdins (Perry et al., 1986), pateamine (Northcote et al., 1991), calyculinamides and swinholides (Dumdei et al., 1997) that we have isolated from New Zealand marine organisms. The interest in the swinholides and calyculinamides will centre on the attempt to develop these two toxins into in vivo active polymeric drugs. Both classes of compound have potent activities in vitro, but are inactive in vivo. The swinholides disrupt the formation of actin filaments while the calyculinamides are protein phosphatase 2A inhibitors. These biological profiles complement the antimitotic properties of our initial polymeric drug based on aminohalichondrin.

6. Conclusion

The sea offers a rich source of biodiversity from which a series of potential drugs, particularly in the area of cancer chemotherapy, have already been discovered. That aspect of the drug development process is dominated by chemists, zoologists and biochemists. The development phase, where there is the pressing need for supply of these compounds, will need strong leadership from the marine biotechnologists as not all marine-based drugs will be able to be synthesised, or obtained by fermentation technology. There will be a need for new, innovative approaches to the aquaculture of many species from phyla that traditionally have not been subject to aquaculture. For those compounds that do become marketable drugs there will exist the challenge to grow the producing organisms commercially by aquaculture.

The chemists will continue to find new leads. Can the biotechnologists produce the compounds economically? That is the challenge for the future.

References


