Drug development from marine natural products

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Abstract | Drug discovery from marine natural products has enjoyed a renaissance in the past few years. Ziconotide (Prialt; Elan Pharmaceuticals), a peptide originally discovered in a tropical cone snail, was the first marine-derived compound to be approved in the United States in December 2004 for the treatment of pain. Then, in October 2007, trabectedin (Yondelis; PharmaMar) became the first marine anticancer drug to be approved in the European Union. Here, we review the history of drug discovery from marine natural products, and by describing selected examples, we examine the factors that contribute to new discoveries and the difficulties associated with translating marine-derived compounds into clinical trials. Providing an outlook into the future, we also examine the advances that may further expand the promise of drugs from the sea.

In 1967, a small symposium was held in Rhode Island, USA, with the ambitious title "Drugs from the Sea"[1]. The tone and theme of the meeting were somewhat hesitant, even sceptical (one paper was entitled "Dregs [*sic*] from the Sea"). The catchphrase of the symposium title has endured over the decades as a metaphor for drug development from marine natural products, and in time genuine drug discovery programmes quietly arose to fulfil that promise. The remarkably high hit rates of marine compounds in screening for drug leads were often cited as justification for the search and, indeed, notable discoveries were made of compounds that profoundly affect the cell cycle and cellular metabolism. If anything, marine natural-product chemistry has built a legacy of discovery of biomedical probes; for example, okadaic acid (a phosphatase inhibitor produced by dinoflagellates) and xestospongin C (an intracellular blocker of calcium release from a marine sponge). However, the first genuine drug from the sea was a long time coming.

Natural products, especially those from terrestrial plants and microbes, have long been a traditional source of drug molecules (for example, morphine from poppies, cardiotonic digitalis glycosides from foxgloves and penicillins from fungi). Modern pharmaceutical discovery programmes owe much to natural products. Indeed, pharmacologically active compounds from plants and microbes represent an important pipeline for new investigational drugs[2–4]. Interest in marine natural products, however, awaited refinements in technologies (mainly scuba diving) to collect the source organisms. Even so, by the late-1950s, the concept of drugs from the sea had attracted some interested. Beginning in 1951, Werner Bergmann published three reports[5–7] of unusual *arabino*- and *ribo*-pentosyl nucleosides obtained from marine sponges collected in Florida, USA. The compounds eventually led to the development of the chemical derivatives ara-A (vidarabine) and ara-C (cytarabine), two nucleosides with significant anticancer properties that have been in clinical use for decades.

The role of natural products in drug discovery has undergone many changes in the past 30 years, with a noticeable decline in participation by the major pharmaceutical companies by the mid-1990s. Nevertheless, enterprising academics, mainly partnered with industry, exploited the niche left by larger research and development efforts. The field seems to have benefited from a renaissance in the past 5 years. This has partly been driven by new developments in analytical technology, spectroscopy and high-throughput screening[8,9], and partly driven by a broad realization that competing technologies, such as combinatorial chemistry, have failed to deliver new drug leads in significant numbers. In the meantime, basic scientific research in chemistry and pharmacology of marine natural products, and directed efforts in drug development — begun in the 1970s — have finally borne fruit for marine-based drug discovery. The first drug from the sea, ziconotide (ω-conotoxin MVIIA) — a peptide originally from a tropical marine cone snail — was approved in the United States in 2004 under the trade name Prialt for the treatment of chronic pain in spinal cord injury. A second drug — the antitumour compound trabectedin

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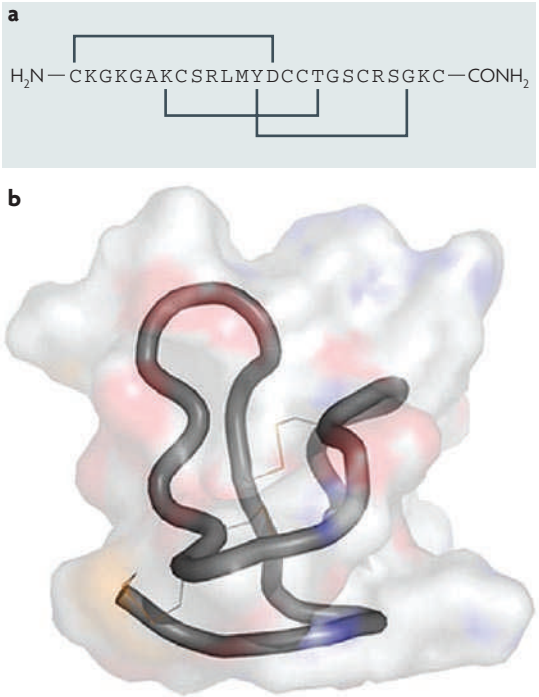


Figure 1 | **ω-Conotoxin VIIA. a** | Amino-acid sequence of the peptide ω-conotoxin MVIIA (ziconotide/Prialt; Elan Pharmaceuticals). **b** | Three-dimensional structure of the synthetic ω-conotoxin VIIA polypeptide. The cylinder represents the amide backbone of ω-conotoxin VIIA overlayed against an electrostatic potential surface. For a three-dimenational representation of ω-conotoxin MVIIA see the entry Ziconotide @ 3Dchem.com (see Further information).

(Yondelis/ecteinascidin-743/ET-743) from a tropical sea-squirt — was approved by the European Union in October 2007 for the treatment of soft-tissue sarcoma. Several other candidate compounds are presently in the pipeline, and marine natural products are being evaluated in Phase I–III clinical trials in the United States and in Europe for the treatment of various cancers. A recent tabulation of these compounds and their status can be found in REFS 2–4.

The difficulty of developing drugs from a marine source was obvious from the outset: the procurement or manufacture of quantities of rare compounds from marine sources to ensure a sustainable supply was a bottleneck. For example, the chemically versatile marine sponges, the source of many developmental compounds such as discodermolide and hemiasterlin, are primitive metazoans that live almost exclusively in marine habitats. Sponges and their microbial fauna are largely unculturable, and the valuable compounds they produce must be extracted and purified from specimens collected by hand using scuba diving from shallow to deep waters, or sometimes with the aid of submersibles equipped with robotic arms. Both of these techniques are expensive endeavours that are unwieldy and foreign to the modern pharmaceutical industry. Nevertheless, interest in the remarkable properties of marine natural products remained high enough so that inspired innovative solutions to the supply problem were proposed on a case-by-case basis, ranging from aquaculture[10] of marine invertebrates to semi-synthesis.

In this Review, selected examples serve to illustrate contemporary drug discovery and development of marine natural products — some successful, some less so. Each story begins with the discovery of a novel molecule from a marine organism, and their progression through preclinical and clinical trials (for oncology indications, with the exception of ω-conotoxin MVIIA) — mostly as the unmodified structure or as a synthetic compound modelled after the natural product. It is not intended to be comprehensive account, but to illustrate the challenges associated with moving marine-derived compounds into clinical trials. Finally, we provide an outlook to some truly revolutionary new developments in genome mining that are expected to change the way that marine-derived drugs are discovered in the future.

ω-Conotoxin MVIIA

After more than two decades of research and development, ziconotide, a synthetic form of ω-conotoxin MVIIA, became the first marine-derived drug approved by the US Food and Drug Administration (FDA). Now known under the trade name Prialt, it is approved for the specific indication of chronic pain. The venom of fish-hunting cone snails (genus *Conus*) contains a myriad of toxic peptides (conotoxins) that act synergistically to immobilize prey by targeting the neuromuscular system. The chemistry and biology of these conotoxins have been extensively reviewed[11,12]. ω-Conotoxin MVIIA (FIG. 1a), from the Pacific piscivorous marine snail *Conus magus* (FIG. 2a) has been an exceptional lead for drug development in the management of severe and chronic pain. It was originally discovered by the group of Olivera at the University of Utah, USA, in 1979 via a bioassay that elicited a characteristic shaking behaviour in mice after the peptide was administered by intracerebral injection[11]. ω-Conotoxin MVIIA is a linear 25 amino acid, polycationic peptide containing six cysteine residues linked by three disulphide bridges that stabilize its well-defined three-dimensional structure[13–15]. Its complete chemical synthesis was achieved in 1987, and N-type voltage-sensitive calcium channels (NVSCCs) were subsequently identified as its target site[16]. It potently inhibits the conduction of nerve signals (K_i value of 0.5 μM)[17] by specifically blocking the NVSCC. In the complex with NVSCC, it forms a compact folded structure with a binding loop between Cys8 and Cys15 that also contains Tyr13, an important amino-acid residue located at the binding site[18,19].

NVSCCs are found exclusively in presynaptic neurons where they regulate depolarization-induced calcium influx, which subsequently control a variety of calcium-dependent processes. NVSCCs are abundantly present in the superficial lamina of the spinal-cord dorsal horn, where they have an important role in the spinal processing of nociceptive afferent (pain signalling) activity[20,21]. The potent inhibition and highly selective affinity of ω-conotoxin MVIIA to NVSCC (K_d value of 9 pM)[22] attracted interest to develop this peptide into an antinociceptive agent.

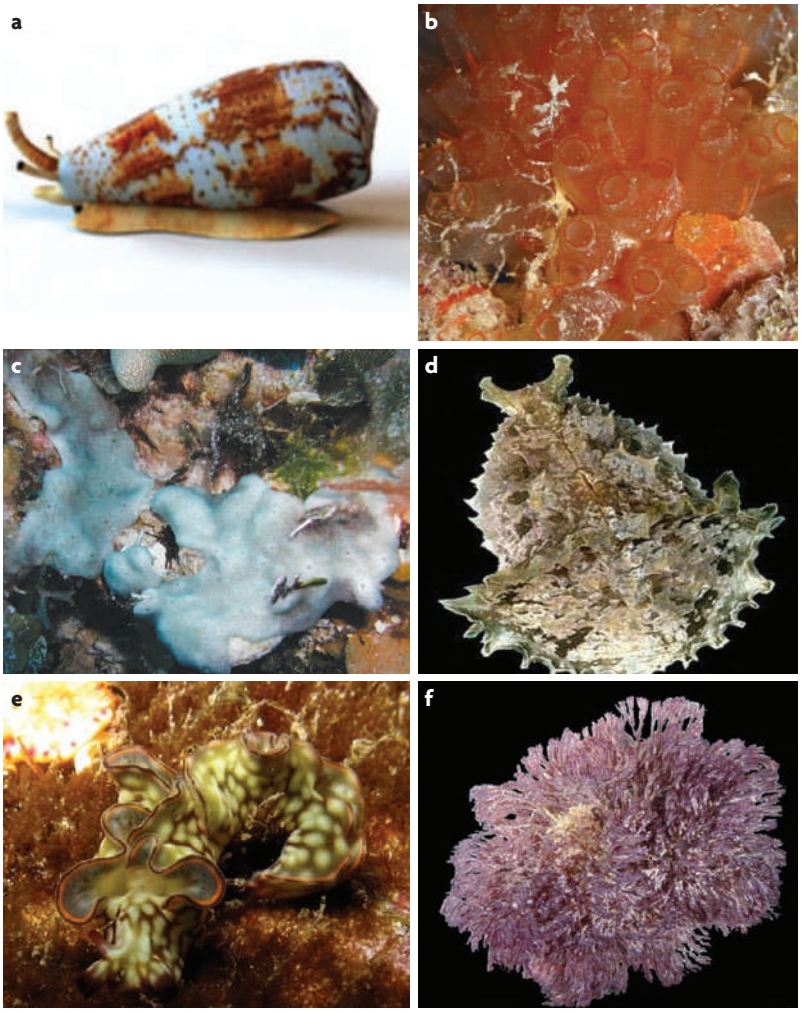


Figure 2 | **Marine invertebrates producing anticancer and analgesic drugs.** **a** | Textile cone snail *Conus magus*. **b** | The Caribbean sea-squirt *Ecteinascidia turbinata*. **c** | The Caribbean sea-squirt *Tridemnum solidum*. **d** | The sea hare *Dolabella auricularia*. **e** | The sacoglossan *Elysia rufescens* feeding on the red alga *Bryopsis* spp. **f** | The bryozoan *Bugula neritina*. Panel **a**, image reproduced with permission from Elan Pharmaceuticals, USA. Panel **b**, image courtesy of S. Lopez-Legintil, University of North Carolina, USA. Panel **d**, image reproduced with permission by W. B. Rudman, *Sea Slug Forum*, Australian Museum, Sydney, Australia. Panel **e**, image reproduced with permission by H. Flodrops, *Sea Slug Forum*, Australian Museum, Sydney, Australia. Panel **f**, image reproduced with permission by the California Academy of Sciences, USA.

Synthetic ω-conotoxin MVIIA, ziconotide (FIG. 1b), was developed and brought to the clinic by Neurex (a subsidiary of Elan Pharmaceuticals)[23]. Preliminary studies demonstrated that ziconotide has a remarkable potent antinociceptive profile in animal models with acute, persistent and neuropathic pain after intrathecal administration[24]. In a rat incisional model of post-operative pain, ziconotide demonstrated more potent (ED_{50} value of 49 pM) and longer activity than intrathecal morphine (ED_{50} value of 2.1 nM)[25].

The promising analgesic activity observed in animal studies enabled ziconotide to enter clinical trials in the United States and in Europe for the treatment of severe chronic pain. Its clinical development has been reviewed recently[26]. Ziconotide was administered to subjects by continuous infusion using an external or an implanted pump via an intrathecal catheter because of its poor tissue penetration and hypotensive effect when administered systemically.

On 22 December 2004, Elan Pharmaceuticals was finally granted FDA approval for ziconotide (an intrathecal infusion formulation) under the trade name Prialt. Two months later the European Commission approved ziconotide for the treatment of severe, chronic pain in patients who require intrathecal analgesia[27]. The approval of this synthetic form of ω-conotoxin MVIIA offers hope for several conotoxins that are currently under investigation[28] for therapeutic potential and possible clinical usage.

Ecteinascidin-743

Ecteinascidin-743 (ET-743/trabectedin) (FIG. 3a) illustrates a significant milestone in the development of marine-derived drugs. Almost 40 years after its discovery and 17 years after the publication of its structure, it became the first marine-derived anticancer drug to reach the market. Extracts of the Caribbean tunicate *Ecteinascidia turbinata* (FIG. 2b) have been reported to possess antitumour activity as early as 1969 (REF. 29); however the minute amounts of active component obtained from laborious isolation work precluded their identification for almost two decades. Finally, in 1990, Rinehart[30] and Wright[31] published the structure of the alkaloids named ecteinascidins. The structure of the most abundant active component, ET-743, and its *N*-demethyl analogue ET-729 (FIG. 3a), is comprised of three fused tetrahydroisoquinoline rings and is related to the simpler isoquinoline alkaloid saframycin A from various *Streptomyces* spp. The connection of the third tetrahydroisoquinoline ring to the base structure by a thioether bridge completes a 10-membered lactone — a distinctive structural feature of ecteinascidins. Initially, ecteinascidins were found to be cytotoxic against L1210 leukaemia cells (IC_{50} value of 0.5 ng per ml)[30] and were later shown to possess strong *in vivo* antitumour effects in various mice models bearing P388 lymphoma, B16 melanoma, M5076 ovarian sarcoma, Lewis and LX-1 human lung carcinoma, and MX-1 human mammary carcinoma xenografts[30,32]. Further studies comparing ET-743 and ET-729 indicated that they have similar potency. Subsequently, ET-743 was selected for further development because it is more abundant than ET-729 in *E. turbinata*.

Still, the yield for ET-743 from the tunicate is very low (~10 parts per million). To provide more material for *in vitro* and *in vivo* animal studies, the first multistep synthesis of the compound was completed in 1996 (0.75% yield)[33], followed by more efficient methods[34]. Although total synthesis is not practicable to provide sufficient material for clinical use, this exercise led to the novel synthetic derivative phthalascidin, which has an antiproliferative property comparable to that of ET-743 (REF. 34). Large-scale coastal aquafarming of *E. turbinata* in Europe provided a larger supply of the drug source, but yields were variable. A breakthrough was achieved by PharmaMar, the licensee of natural

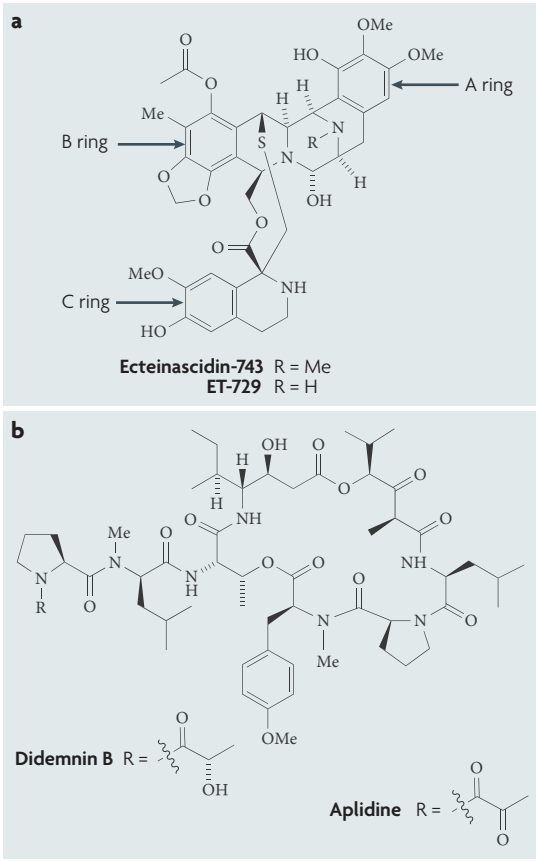


Figure 3 | **Tunicate-derived anticancer drugs.** **a** | Ecteinascidin-743 (ET-743/trabectedin; marketed under the trade name Yondelis by PharmaMar/Johnson & Johnson/OrthoBiotech), derived from *Ecteinascidia turbinata*, and its analogue ET-729. **b** | Didemnin B from *Tridemnum solidum* and aplidine (also known as dehydrodidemnin B) from *Aplidium albicans*.

ET-743, who developed a large-scale semi-synthetic method that starts with cyanosafracin B, an antibiotic that can be produced in multi-kilogram scale by fermentation of *Pseudomonas fluorescens*[35]. ET-743 was licensed by PharmaMar to Johnson & Jonhson/OrthoBiotech for drug development in the United States[36].

The mechanism of action of ET-743 is ascribed to covalent modification of DNA by guanine-specific alkylation at the N2 position, a property similar to another minor-groove alkylating agent, anthramycin. In contrast to anthramycin, ET-743 is selective for GC-rich sequences and forms an adduct with duplex DNA, which is reversible upon denaturation[37], and induces a bend in the DNA helix directed towards the major groove[38]. Molecular dynamics calculations shows protrusion of ring C of ET-743 into the minor groove and interference with DNA-binding factors[39] (FIG. 4). This partly accounts for the potency of ET-743 compared with other N2 alkylating agents such as saframycin A and anthramycin that have smaller groups corresponding to ring C. ET-743 also affects transition-coupled nucleotide excision repair and triggers cell death[39,40].

ET-743, under the trade name Yondelis, was approved for the treatment of refractory soft-tissue sarcomas by the European Commission in July 2007. Results of Phase II trials of ET-743 for Ewing's sarcoma and soft-tissue sarcomas[41], colorectal cancer[42], pretreated advanced breast cancer[43], ovarian cancer[44], and other sarcomas[45,46] have been disclosed. Phase II studies in breast cancer and advanced prostate cancer are ongoing. Concluded Phase I and II studies demonstrate that ET-743 has remarkable antitumour activity against solid tumours, in particular breast cancer and renal carcinoma, and soft-tissue sarcomas (particularly osteosarcomas, mesothelioma, leiomyosarcoma and liposarcoma). A review of these studies has been published elsewhere[47]. Gastrointestinal stromal tumours (a subclass of soft-tissue sarcomas) were found to be non-responsive to ET-743 treatment[48,49]. Preclinical studies of ET-743 combination treatments with doxorubicin[50], paclitaxel[51] or irinotecan[52] against sarcomas revealed synergistic effects between the drugs, as have Phase II combination drug studies with doxorubicin[50]. Toxicities that became dose limiting include neutropaenia and thrombocytopaenia. Hepatotoxicity, previously observed in preclinical studies, was observed in humans but could be controlled by dose adjustment. Recent findings using HepG2 human hepatocellular liver carcinoma cells revealed a cytochrome P450-mediated metabolism of ET-743 (REF. 53). It was demonstrated in rats that pretreatment with metabolism modulators such as dexamethasone and β-naphthoflavone abrogates ET-743-mediated hepatotoxicity[54], and dexamethasone–ET-473 combination drug treatment was recommended for investigation in humans[55,56].

Didemnin B and aplidine

ET-743 was not the only lead anticancer agent found from marine ascidians. Two closely related compounds from different organisms — didemnin B from the tropical *Tridemnum solidum* (FIG. 2c) and aplidine from *Aplidium albicans* collected in temperate Mediterranean waters — have been extensively investigated for more than 20 years; although clinical trials for didemnin B were stopped in the mid-1990s (see below). The chemical differences between the two compounds are minimal: they are both cyclic peptides in which didemnin B has a pyruvyl amide residue at the secondary amine of the proline residue and aplidine has a pyruvyl amide at this position (FIG. 3b). Nevertheless, the *in vitro* cytotoxicities and preclinical profiles seemed to be sufficiently different such that new clinical trials have been warranted for aplidine.

Didemnin B[47,57–59] was first isolated by the Rinehart group in 1981 from the tunicate *T. solidum*[60] and displayed antiviral and *in vivo* cytotoxic activities at nanomolar concentrations[61]. The original structure was revised in 1987 as a result of total synthesis of the first proposed structure, which revealed inconsistencies: the correct structure replaces the non-proteinogenic amino-acid residue statine with *iso*statine[62]. Complete spectroscopic characterization of didemnin B was reported[63,64] along with a definitive single-crystal X-ray structure published in 1988 (REF. 65).

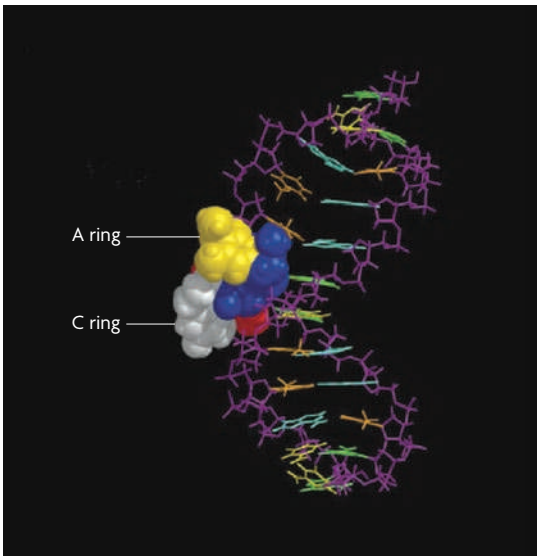


Figure 4 | **Molecular-dynamics model showing the alkylation of DNA by ET-743 at N2 of guanine in the minor groove**. The A Ring and C Ring represent the tetrahydroisoquinoline A and C rings of ET-743 (FIG. 3). Image is modified, with permission, from REF. 39 © (2001) Elsevier Science.

Precise details of the mechanism of action of didemnin B were difficult to pinpoint. Early studies showed that didemnin B inhibits palmitoyl protein thioesterase in a non-competitive manner[66,67]; although this low-affinity target did not fully account for the nanomolar cell inhibition observed with the natural product. Didemnin B induces inhibition of protein synthesis at a concentration that is commensurate with cell growth inhibition[68]. This is achieved by stabilization of aminoacyl-tRNA and prevention of EF-2-dependent translocation on the ribosome[69–72]. However, inhibition of protein synthesis does not seem to be the primary cause of apoptosis[73,74]. Apoptosis induced by didemnin B is dependent on protein tyrosine kinases and can be inhibited using protein tyrosine kinase inhibitors[75] or rapamycin, possibly through the interaction of rapamycin with the immunophilin FKBP25 (REF. 76).

As early as 1983, didemnin B was shown to be active against herpes simplex virus[77] and later against Ehrlich's carcinoma[78] in mice. Early cancer trials showed that didemnin B had minimal activity and demonstrated the requirement for co-treatment with anti-emetics[79]. *In vitro* testing established that didemnin B was active against colorectal[80], lymphatic[81] and prostate[82] cancers. Consequently, didemnin B was submitted to several Phase I[79,83,84] and Phase II clinical trials against previously treated non-small cell lung cancer (NSCLC)[85], breast cancer[86], small-cell lung cancer[87], non-Hodgkin's lymphoma[88,89], metastatic melanoma[90], glioblastoma multiforme[91], and CNS tumours[92]. However, these trials resulted in significant neuromuscular toxicity and no objective responses. Nevertheless, didemnin B showed activity in patients with advanced pretreated non-Hodgkin's lymphoma, but trials were suspended owing to onset of severe fatigue in patients[89]. Other trials showed a high incidence of anaphylaxis, and were therefore terminated[93]. All current trials of didemnim B are on hold.

While trials with didemnin B are suspended, a simple analogue of didemnin B, aplidine (dehydrodidemnin B), seems more promising. Aplidine was first reported in a 1991 patent[94] by Rinehart and is obtained from the Mediterranean tunicate *A. albicans*. Aplidine differs from didemnin B only in replacement of the *N*-lactyl side chain with a pyruvyl group. Aplidine shows similar levels of antitumour activity to didemnin B in cultured tumour cells[93], and has been shown to induce apoptosis by induction of oxidative stress[94], which triggers the pro-apoptotic receptor Fas (CD-95)[95] and induces mitochondrion-mediated apoptosis[96,97]. Aplidine also activates p38 mitogen-activated protein kinases (MAPKs) and JNK[98,99], and inhibits secretion of vascular endothelial growth factor (VEGF)[101,101]. Non-P-glycoprotein-expressing cell lines that are resistant to aplidine were shown to display temporary phosphorylation of JNK and p38 MAPKs upon exposure to aplidine, and the short duration of activation was insufficient to trigger apoptosis[102]. In relapsed–recalcitrant leukaemia cell lines, aplidine arrests the cell cycle at the G1 and G2/M phases, and induces p53-independent apoptosis[103].

Significant differential *in vitro* cytotoxicity of aplidine in primary cultured lymphocytes and in transformed cell lines was observed, which may explain why both didemnin B and aplidine show minimal haemotoxicity *in vivo*[104–107].

Aplidine has also been shown to inhibit angiogenesis in chick embryos *in vivo*[108], and to selectively disrupt β-sheet fibrils caused by prion protein sequence PrP 106–126 in a 1:1 molar ratio[109]. Phase II clinical trials with aplidine are ongoing in indications that include metastatic melanoma, multiple myeloma, non-Hodgkin's lymphoma, acute lymphoblastic leukaemia, prostate cancer and bladder cancer.

Significant challenges have been encountered during the development of didemnin B and aplidine. The supply of these two peptides from nature is limited by the difficulties of collecting sparsely distributed source organisms and the lack of feasible aquaculture conditions. Consequently, supply is entirely dependent upon multistep total synthesis of these peptides from constituent amino acids, some of which are not found in proteins. Inventive solutions to the supply problem of these and other marine natural products with therapeutic properties may ultimately determine their future use, even if clinical outcomes are favourable.

Antimitotic marine natural products

Several antitumour marine natural products, derived mainly from marine sponges or molluscs but also bryozoans and cyanobacteria, exhibit potent antimitotic properties. Several have advanced to Phase I and II clinical trials and, perhaps not coincidently, many such compounds block progression of dividing cancer cells through M phase by targeting the same protein (tubulin) in ways similar to the clinical drugs vincristine, vinblastine and paclitaxel. Although none has yet been approved

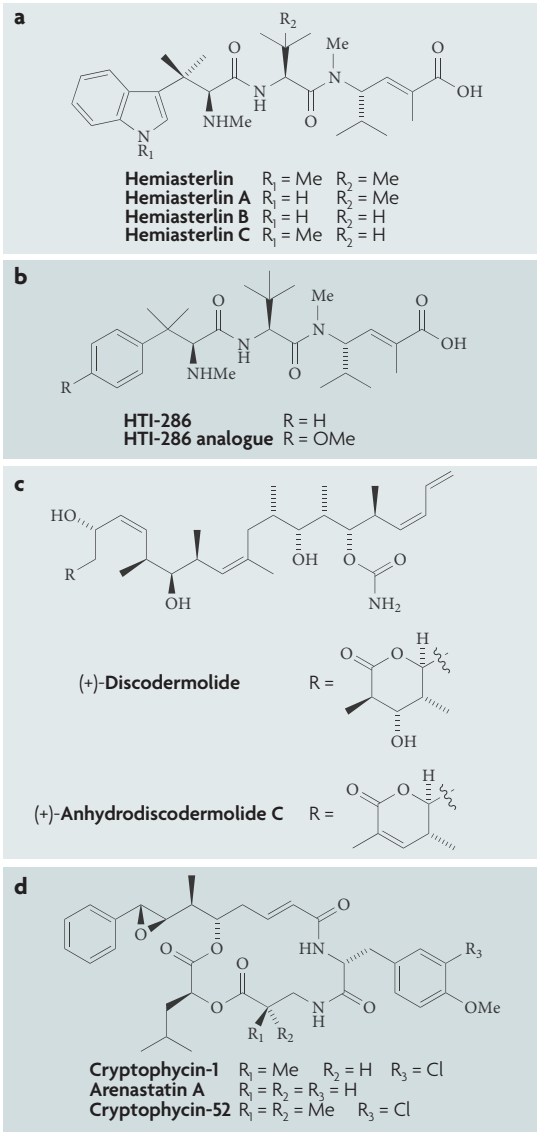


Figure 5 | **Marine-derived antimitotic compounds (part 1). a** | The hemiasterlins. All hemiasterlins showed cytotoxicity in the nanomolar range (concentrations ~1 x 10^{-9} M) against a variety of cultured human and murine cell lines. **b** | Hemiasterlin analogues, HTI-286 (SPA-110/taltobulin) and its analogue. **c** | The discodermolides, (+)-discodermolide and (+)-anhydrodiscodermolide. **d** | The cryptophycins.

as a drug, the following examples of natural products are illustrative of the wide range of chemotypes that modulate tubulin dynamics. Important differences have been reported in their mechanisms of action, and this in itself has been tremendously informative for understanding the complex interaction of microtubule proteins with antimitotic drugs.

Hemiasterlin/HTI-286. Hemiasterlin, an antimitotic tripeptide (FIG. 5a), was first isolated in 1994 by Kashman and co-workers from the sponge *Hemiasterella minor*[110]. Initial screening showed that it was highly cytotoxic with an IC_{50} value of ~0.01 μg per ml against the P388 leukaemia cell line[110]. The related isomers hemiasterlin A and B, reported by Andersen and co-workers from sponges of the genus *Auletta* and *Cymbastella* in 1995 (REF. 111), were even more potent. All hemiasterlins showed cytotoxicity in the nanomolar range (concentrations ~1 × 10^{-9} M) against a variety of cultured human and murine cell lines[111]. In 1996, an X-ray crystal structure analysis of the hemiasterlin methyl ester confirmed the linear structure of hemiasterlin and its unusual constituent amino acids[112]. A fourth analogue, hemiasterlin C, was described in 1999 (REF. 113).

The potent cytotoxicity of hemiasterlins is due to the induction of mitotic arrest in metaphase with cellular dynamics similar to those of known tubulin binders, such as the chemotherapeutics paclitaxel or vinblastine, at half-maximal effective doses (ED_{50} values) that ranged from 0.5 nM (hemiasterlin) to 28 nM (hemiasterlin B)[114]. Hemiasterlin A was shown to interfere with mitotic spindle formation at low concentrations and causes tubulin depolymerization at higher concentrations (with higher efficacy than paclitaxel or vinblastine[114]). By contrast, hemiasterlin was shown to bind to the vinca peptide binding site of β-tubulin, where it competitively inhibits its binding of the marine compound dolastatin 10 and non-competitively inhibits binding of vinblastine[110,111]. Interestingly, upon binding to tubulin, hemiasterlin induces the formation of small ring oligomers[115].

Extensive structure–activity relationship studies demonstrated that the simpler synthetic analogue of hemiasterlin, HTI-286 (SPA-110/taltobulin) (FIG. 5b), with a phenyl substituent replacing the *N*-methyltryptophan, is more potent than hemiasterlin[112]. An analogue of HTI-286 (FIG. 5b) with a *para*-methoxyl substituent on the benzene ring was even more potent[113]. Other structural elements, including the geminal β,β-dimethyl group and the *N*-methyl on the first amino-acid residue (N terminus), the isopropyl and an olefin in the homologated γ-amino acid (C terminus), including a terminal carboxylic acid or methyl ester, were essential for activity. The aryl side chain on the N terminus could be replaced synthetically by alkyl groups (for example, *tert*-butyl), while still retaining potent activity[116–120].

The stoichiometry of HTI-286 binding to an α/β-tubulin monomer was found to be one to one[121]. Binding of HTI-286 to tubulin is rapid and exothermic, but formation of oligomers is slower. Disruption of tubulin dynamics by hemiasterlin and HTI-286 takes place at low concentrations, but a full equivalent of peptide is required for depolymerization of microtubules[122]. Molecular modelling suggested that binding of both compounds takes place at the vinca peptide site of β-tubulin, near the α/β interface that is also targeted by the marine-derived tubulin inhibitors cryptophycin 1, dolastatin 10 and the fungus-derived phomopsin A[123]. Radiolabelling of protein with a photoaffinity analogue of hemiasterlin, however, indicated an exclusive binding site on α-tubulin near the α/β interface[124].

Unlike paclitaxel or vinblastine, hemiasterlin and HTI-286 are poor substrates for P-glycoproteins and maintain toxicity towards cell lines with high expression of

multidrug resistant (MDR) drug pumps. Cells resistant to either compound do not express high levels of known MDR proteins; instead it was shown that mutations on both α-tubulin and β-tubulin subunits confer resistance in most cases[125], although one study showed moderately reduced drug accumulation due to an unidentified ATP-drug pump[126]. The mutations on tubulin are not localized on the vinca peptide binding site, and do not seem to affect drug binding, but instead increase microtubule stability causing cross-resistance (3-fold to 186-fold) to other depolymerizing agents and sensitization (2-fold to 14-fold) to tubulin polymerizing agents[125].

Preclinical studies showed that HTI-286 causes tumour regression and growth inhibition of human xenografts in mice. Even cell lines expressing P-glycoprotein or resistant to paclitaxel were shown to be sensitive to HTI-286 inhibition, but required higher doses than non-resistant cell lines[127]. An open-label Phase I clinical trial of HTI-286 was completed in patients with advanced solid tumours; however, there were no objective responses and common toxicities observed included neutropaenia, nausea, alopecia and pain[128]. Phase II trials have been halted. Nevertheless, there is still interest in HTI-286 in view of recent results including high antitumour activity in androgen-dependent and androgen-independent mouse models of refractory prostate cancer, and in a newly established *in vitro* taxane-resistant prostate PC-3 cell line[129].

(+)-Discodermolide. (+)-Discodermolide[130] (FIG. 5c) was first isolated in 1990 by Gunasekera and co-workers[131] at the Harbor Branch Oceanographic Institute, Florida, USA, from the rare deep-water sponge *Discodermia dissoluta*. This sponge is found only in the Bahamas at depths of up to 300 m, although recent evidence suggests it also occurs in shallower waters. Discodermolide functions as an immunosuppressant[132,133], and induces G2/M phase cell-cycle arrest in lymphoid and non-lymphoid cells at nanomolar concentrations[134]. The structure of (+)-discodermolide was first elucidated by analysis of nuclear magnetic resonance and mass-spectrometric data[135,136], and its relative stereochemistry was provided by X-ray crystal structure analysis[135]. Significant differences in activity were found for the two enantiomers as shown by analysis of the unnatural antipode (–)-discodermolide prepared by total synthesis[137]. (–)-Discodermolide arrests cell cycle *in vitro*, albeit at higher concentrations (72–135 nM) compared with the natural enantiomer (3–80 nM). Although both enantiomers block cell cycle at S phase, their effects are not mutually competitive[138]. Subsequent synthesis produced the natural enantiomer (+)-discodermolide[139]. Owing to the exceedingly limited natural supply of (+)-discodermolide, efforts focused on developing efficient methods to synthesize the compound, and several inventive multi-step, scaled-up processes for total syntheses were developed[139–141]. These included a gram-scale synthesis[142–144] and a heroic 60 g scale preparation by workers at Novartis that provided material for Phase I clinical trials[145–149].

Investigations into the mechanism of cell-cycle arrest by (+)-discodermolide showed that the compound stabilized microtubules competitively with paclitaxel, with higher affinity for tubulin than paclitaxel[150,151]. (+)-Discodermolide is a poor substrate for P-glycoprotein and is cytotoxic to cells with tubulin mutations that confer resistance to paclitaxel[152]. Interestingly, despite their mutual competitive inhibitory activity, (+)-discodermolide and paclitaxel show strongly synergistic activity[153–155]. The cytotoxicity of (+)-discodermolide is apparent at concentrations too low to cause cell-cycle arrest, in which aberrant mitosis[156], altered induction of apoptosis[157] and a significant alteration of microtubule dynamics[158] can be observed. Similar to paclitaxel, (+)-discodermolide interferes with binding of tau protein to microtubules[159] and shows microtubule-disruption-dependent down-regulation of hypoxia-inducible transcription factor 1α (HIF1α)[160]. However, as opposed to paclitaxel, (+)-discodermolide induces accelerated senescence in A549 NSCLC cells and even a strain of A549 that has higher resistance to the compound by activating the signalling molecules plasminogen activator inhibitor, type I (PAI1; also known as SERPINE1), p66Shc and extracellular signal-regulated kinases 1 and 2 (ERK1/2)[161].

(+)-Discodermolide has further shown *in vivo* activity against HCT-116 colorectal cancer xenografts in mice. Co-treatment of mice with (+)-anhydrodiscodermolide C (FIG. 5c) and bacteriolytic treatment with *Clostridium novyi*-NT spores resulted in a rapid, complete cure of four out of five animals with a single injection[162]. A Phase I clinical study of (+)-discodermolide showed no neuropathy or neutropaenia, and demonstrated mild-to-moderate toxicity from 0.6 mg per m^2 to 19.2 mg per m^2. The pharmacokinetics of (+)-discodermolide were shown to be non-linear with recycling of (+)-discodermolide between tissues and the circulatory system[163]. At present, Novartis has discontinued Phase I trials with (+)-discodermolide owing to lack of efficacy and toxicity problems. However, potential remains for its use in combination drug therapy.

Cryptophycins. Cryptophycins are examples of marine cyanobacteria-derived tubulin-binding compounds, which, owing to extensive academic–industry collaborations, inspired the design of several synthetic analogues. Cryptophycin A was first isolated by Schwartz and co-workers in 1990 (REF. 164) from the cyanobacteria *Nostoc* spp. strain ATCC 53789. It was found to be extremely potent in mice infected with *Cryptococcus* spp. but was not pursued because of its notable toxicity. Subsequently, it was re-isolated by Moore's group in Hawaii, USA, in 1994 along with several derivative congeners from a cultured freshwater *Nostoc* spp. strain GSV 224 (REF. 165). The name was later changed to cryptophycin-1 (Crp-1; FIG. 5d). Crp-1 showed IC_{50} values of 3 pg per ml and 5 pg per ml against KB carcinoma and LoVo adenocarcinoma cells, and was active *in vivo* against xenografts of colon adenocarcinoma 38 and 51 cell lines, paclitaxel-sensitive and paclitaxel-resistant mammary adenocarcinoma M16 cells, and in mouse models of pancreatic ductal adenocarcinoma 03 (REF. 165). The related cytotoxic compound arenastatin A (FIG. 5d), isolated from the marine sponge *Dysidea arenaria*, was reported contemporaneously by Kobayashi[166], and turned

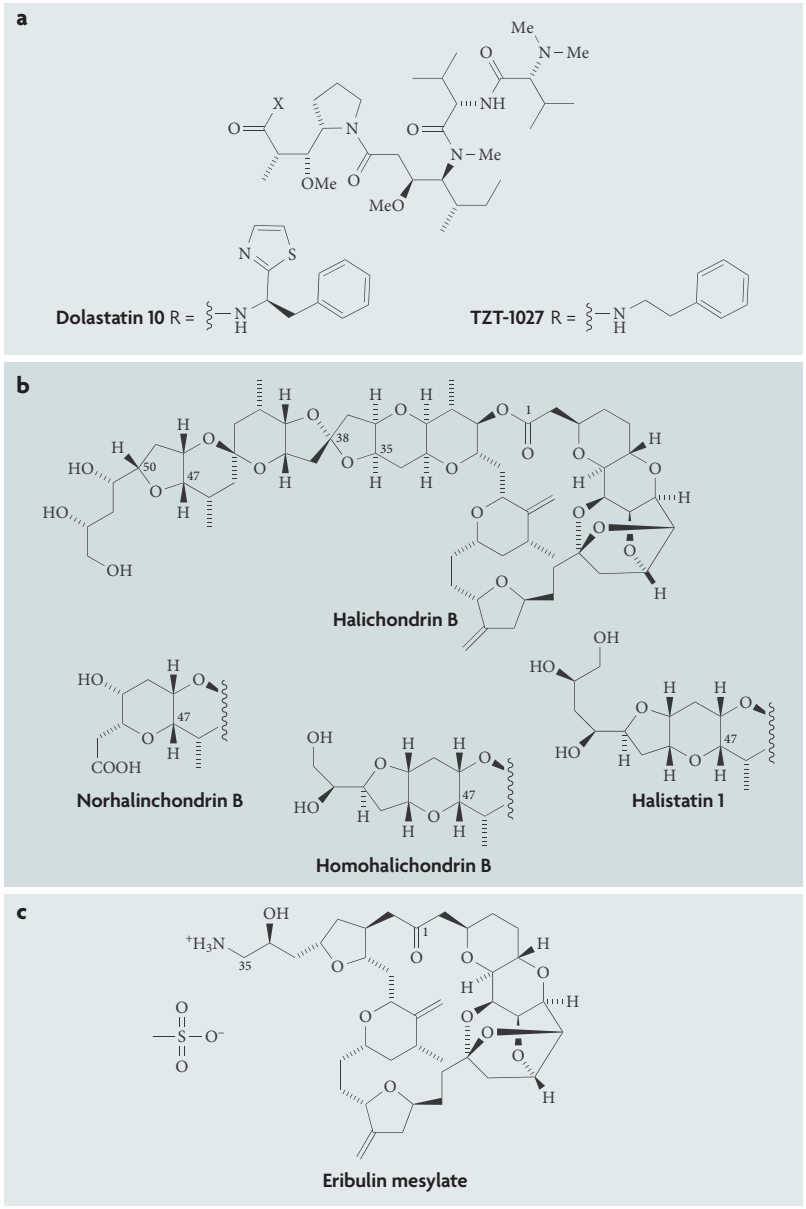


Figure 6 | **Marine-derived antimitotic compounds (part 2). a** | Dolastatin 10 and its synthetic derivative TZT-1027. **b** | The halichondrins: halichondrin B, norhalichondrin B, homohalichondrin B and halistatin 1. **c** | The halichondrin analogue eribulin mesylate (E7389).

out to be identical to Crp-24, one of the 27 cryptophycin analogues isolated by Moore. The total synthesis of Crp-1 was achieved in 1995 (REF. 167) and led to the revision of its structure (FIG. 5d). The University of Hawaii and Wayne State University, USA, licensed the natural and synthetic cryptophycins to Eli Lilly for drug development.

Cryptophycins are potent antiproliferative and antimitotic agents. The mechanism of action of cryptophycins is attributed to the destabilization of microtubule dynamics and the induction of hyperphosphorylation of the anti-apoptotic protein B-cell leukaemia/lymphoma 2 (BCL-2), triggering programmed cell death[168]. The antimitotic property is due to its tight binding to the vinca peptide binding site of tubulin — similar to hemiasterlin/HTI-286 — thereby promoting the depolymerization of microtubules[169–172]. The concentrations of cryptophycins needed to induce hyperphosphorylation of BCL-2 in human H460 NSCLC cells is three orders of magnitude higher than that of paclitaxel, and, at this concentration, the cell cycle is arrested in the G2/M phase[168].

A synthetic analogue of Crp-1, cryptophycin-52 (LY355703; FIG. 5d) was developed by Eli Lilly to improve hydrolytic stability and formulation. It is significantly less sensitive to P-glycoprotein-mediated multidrug-resistance, and 40-fold to 400-fold more potent than either vinca alkaloids or paclitaxel in the cell lines tested[173]. Cryptophycin-52 was selected for Phase I clinical trials in the late-1990s for patients with refractory solid tumours[174,175]. Dose-related toxicities included neuropathy, myalgia, cumulative long-lasting neuroconstipation and neurosensory toxicity, as well as cardiac dysrhythmia and mild alopecia. A Phase II study involving patients with Stage IIIb or IV NSCLC who were previously treated with platinum-containing chemotherapy agents was disappointing, as cryptophycin-52 failed to produce an objective response at doses (1.5 mg per m^2) high enough to induce neurological toxicity[176]. Cryptophycin-52 has been dropped from the pipeline for anticancer agents and withdrawn from clinical trials. However, new analogues, such as cryptophycin-309 and cryptophycin-249, have undergone preclinical efficacy studies, and there is sufficient interest to consider entering them into clinical trials[177].

Dolastatin 10. Collaborative endeavours of Pettit's group at the Arizona State University, USA, and the US National Cancer Institute resulted in a highly interesting compound: dolastatin 10. The discovery of the dolastatins had its beginnings in 1972 during an explorative expedition to the island state of Mauritius in the Western Indian Ocean. A series of cytotoxic peptides were isolated from the sea hare *Dolabella auricularia* found in the Indian Ocean[178] (FIG. 2d). Among these was dolastatin 10 (FIG. 6a), which exhibited outstanding *in vitro* cytotoxic activity against cancer cells. Alcohol extracts of *D. auricularia* showed exceptionally potent activity against murine NCI P388 lymphocytic leukaemia cells at subnanomolar concentrations (ED_{50} value of 4.6×10^{-5} μg per ml)[179]. A laborious bioassay-guided isolation of dolastatin 10 — made highly challenging because of extremely low yields — required a large-scale collection (~2 tonnes) of *D. auricularia* to produce the first milligram[180]! Dolastatin 10 is a novel linear depsipeptide containing four unprecedented amino acids: *N*,*N*-dimethylvaline, dolaisoleucine, dolaproine and dolaphenine (possibly biosynthesized from phenylalanine)[179]. The total synthesis of dolastatin 10 was achieved in 1989, and confirmed its absolute configuration[181].

Dolastatin 10 is a potent antimitotic agent that inhibits microtubule assembly and tubulin polymerization. It binds to tubulin at a distinct site for peptide antimitotic agents close to the vinca peptide binding site[182,183], where HTI-286 and Crp-1 bind, and consequently causes the accumulation of cells that are arrested at the mitosis stage and depleted of cellular microtubules[184]. One reason for

the high antimitotic activity of the peptide (IC_{50} value of 1.2 μM) is its prolonged intracellular retention that subsequently facilitates binding to tubulin[185].

Dolastatin 10 demonstrated *in vitro* inhibitory activity against various human cancer cell lines, including melanoma, sarcoma and ovarian cancer cells[179], and entered several Phase I and II clinical trials as a single agent. Phase I clinical trials on dolastatin 10 identified a maximum tolerated dose of 300–400 μg per m^2 for patients with advanced solid tumours, and documented granulocytopaenia as its major dose-limiting toxicity[186,187]. Dolastatin 10 progressed to Phase II clinical trials as a single agent against prostate adenocarcinoma[188], NSCLC[189], melanoma[190], colorectal cancer[191], soft-tissue sarcomas[192], breast cancer[193], and pancreaticobiliary cancers[194]. Although the toxicity profile of dolastatin 10 was acceptable, the results were disappointing as no clinically significant activity could be detected in any of the trials. The negative results of Phase II clinical trials did not justify continuation and dolastatin 10 was withdrawn from antitumour clinical trials[195]. Nevertheless, the new synthetic derivative TZT-1027 (REF. 196) (FIG. 6a) is currently being evaluated in Phase I clinical trials in Japan, Europe and the United States[197,198]. TZT-1027, which differs from dolastatin 10 in replacement of the terminal dolaphenine amino-acid residue with a simple phenethylamine group, is a potent microtubule and vascular blockade inhibitor[199], and has anticancer activity in several murine cancer models[196]. TZT-1027 and dolastatin 10 share the same mechanism of activity: they both bind near the vinca peptide site on tubulin and inhibit tubulin polymerization with equal potency (IC_{50} values of 2.2 μM and 2.3 μM, respectively). Dolastatin 10 completely displaces tubulin-bound TZT-1027 (REF. 200).

Halichondrin B and eribulin mesylate. The discovery and development of the antitumour compounds halichondrin B and its analogue eribulin mesylate is an interesting account of multidisciplinary approaches that brought a promising drug candidate to patients with cancer while addressing the perennial issue of supply that limits procurement of anticancer natural products from marine invertebrates. In 1986, Uemura and co-workers discovered halichondrin B, norhalichondrin B and homohalichondrin B (FIG. 6b) from the minor fractions of extracts of *Halichondria okadai*, the same sponge that also sequesters the phosphatase inhibitor okadaic acid[201,202]. Subsequently, halichondrin B and other halichondrins, which occur in very small concentrations (for example, halistatin 1 (REF. 203), 8.8 parts per billion; FIG. 6b) were reported from unrelated sponges: *Axinella* spp. from the western Pacific Ocean[204] and *Phakellia carteri* from the East Indian Ocean[204]. The halichondrins were shown to have potent cell growth inhibitory activity at nanomolar concentrations ($\sim 1 \times 10^{-9}$ M)[204]. Differential cytotoxicity data indicated that halichondrin B binds tubulin at the vinca peptide binding site[205,206], similar to other compounds covered in this Review. The total syntheses of halichondrin B and norhalichondrin B were reported in 1992, and required approximately 90 steps from commercially available raw materials[207]. Following favourable *in vitro* and *in vivo* data, the US National Cancer Institute advanced the compound (NCI number NSC 60935) to Phase I clinical trials in 2002.

A new source of halichondrins was discovered in a new species of deep-water sponge, *Lissodendoryx* n. sp. 1 (abbreviated here as *Lissodendoryx*), off the east coast of New Zealand's South Island in the early 1990s by the Blunt and Munro group at the University of Canterbury, New Zealand[208,209]. This fortuitous finding enabled two developments that would greatly increase the supply of this rare natural product: trawling for the sponge in its natural habitat and transplantation-aquaculture of the sponge to in-shore artificial habitats[210]. Approximately 1 tonne of *Lissodendoryx* was harvested by trawling, and subsequent isolation of halichondrin B by scaled-up isolation–purification methods provided useful quantities for preclinical studies. Meanwhile, the survival and growth of sponge explants was highly dependent upon season and water temperature, but the growing sponges did produce halinchondrins, albeit at lower yields than wild-type samples of *Lissodendoryx* (halichondrin B content ~0.4 mg per kg; total halichondrins ~1.5 mg per kg)[210]. Thus, the combined efforts of marine ecologists, governments and academic scientists and support from the US National Cancer Institute resulted in the delivery of 310 mg of halichondrin B and a comparable quantity of isohomohalichondrin B.

Although aquaculture could partially address the supply issue associated with halichondrin B, the structures of the halichondrins were too complex to make them feasible as targets for scaled-up production by total synthesis. A significant breakthrough was achieved by academic scientists in partnership with Eisai Company in Japan, its US subsidiary Eisai Corporation of North America, and the Eisai Institute, who showed truncated synthetic analogues of halichondrin B with approximately 70% of the molecular mass had equipotent activity with the natural product against tumour cells[211–213]. This work culminated in the identification and scaled-up production of the antitumour halichondrin analogue, eribulin mesylate (E7389, NCI number NSC 707389; FIG. 6c), the methanesulphonate salt of a terminal amino alcohol corresponding to the C1-C35 carbon skeleton of halichondrin B[214]. Eribulin mesylate, like halichondrin B, exhibits broad antiproliferative activity against tumour cells by binding to tubulin and arresting the cell cycle at mitosis[215]. Clinical trials of eribulin mesylate were approved in 2002, and results from Phase II trials of eribulin mesylate as monotherapy for refractory breast cancer (88 patients) were reported in 2006 (REF. 216) and more recently in heavily pretreated patients. The median duration of confirmed response was 113 days and preliminary safety data showed that the major toxicity issue was neutropaenia and leukopaenia (31 patients) with some febrile neutropaenia (two patients). In a related study with eribulin mesylate in patients with advanced breast cancer, response rates were 15% with tolerable toxicities[217]. Eribulin mesylate also showed 9.7% response rate in patients with NSCLC (10.4% in taxane-pretreated patients) and a median survival time of 9.6 months[218]. Phase III trials of eribulin mesylate for breast cancer are continuing in the United States and in Europe[219].

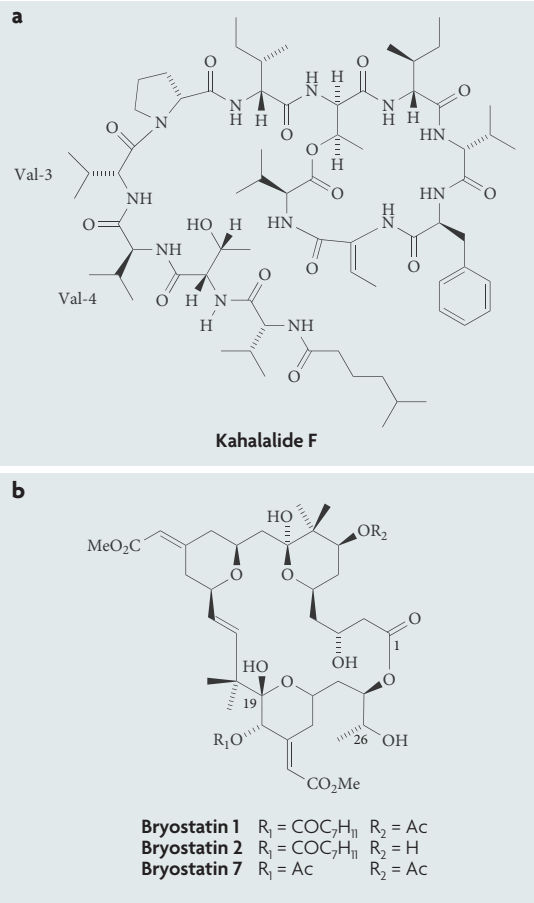


Figure 7 | **Kahalalide F and bryostatins. a** | Kahlalide F. **b** | The bryostatins.

Other antitumour marine natural products

Antimitotics are not the only antitumour compounds derived from marine organisms. Other natural products from various marine organisms, represented by a range of chemical structures and different cytotoxic or cytostatic modes of action, have entered clinical trials. To date, none has been approved as drugs.

Kahalalide F. Seasonal collections of the sacoglossan (sea slug) *Elysia rufescens* (FIG. 2e) by the Scheuer group from the University of Hawaii led to the isolation of the novel antitumour depsipeptide kahalalide F[220,221] (FIG. 7a) in 1993 in addition to other analogues[222]. Kahalalide F is a C_{75} cyclic tridecapeptide that contains several unusual amino-acid residues, including the rare *Z*-dehydroaminobutyric acid found only in a few peptides including the antibiotics cypemycin[223] and hassallidin A[224]. *E. rufesens* is a herbivorous Hawaiian opisthobranch that feeds on the alga *Bryopsis* spp.[220–222], which is the true source of kahalalide F. Accumulation of high concentrations of kahalalide F is seen in *E. rufescens* (1%) compared with the alga (0.0002%)[222], and it seems that both organisms use kahalalide F as a chemical defence against predation[225]. The relative configuration of kahalalide F was the subject of some revision. A cyclic peptide with the structure originally proposed for kahalalide F was synthesized[220,221] but it exhibited cytotoxicity an order of magnitude lower than that of the natural product. Subsequent revision of the structure — by inverting the configurations of Val3 from L- to D- and of Val4 from D- to L-, respectively — coupled with a more efficient solid-phase synthesis developed at the University of Barcelona, Spain, led to the correct structure and a product with essentially the same activity as the natural product[226,227]. Kahalalide F was licensed by the University of Hawaii to PharmaMar in the early 1990s.

The mechanism of action of kahalalide F has not yet been fully elucidated. Kahalalide F is active mainly at the lysosomal level and induces vacuolization[228], which may explain its activity on tissues that actively secrete lysosomal proteins, such as prostate cells. In human prostate cell lines and breast cancer cell lines, kahalalide F was shown to induce cell death by oncosis[229] (necrosis coupled with karyolysis and swelling). In addition, a necrosis-like process was observed in several human kahalalide-F-sensitive breast, vulval, NSCLC and hepatic and colon carcinoma cell lines[230], in which downregulation of the ERBB3 protein and inhibition of the phosphatidylinositol 3-kinase (PI3K)–AKT signalling pathway were identified as determinants of its cytotoxicity[231]. Kahalalide F also induces channel formation and consequent cell-membrane permeability in sensitive HepG2 cells[231]. It does not manifest toxicity towards murine haematopoietic progenitors and stem cells at concentrations up to 10 μM[107], and was shown to be metabolically stable[232].

A preclinical study of kahalalide F in rats reported renal toxicity using single doses at the maximum tolerated dose of 1,800 μg per m^2 administered intravenously. However, a multiple-dose regimen reduced drug-induced toxicity[233]. A Phase I study on androgen-refractory prostate cancer reported a maximum tolerated dose of 930 μg per m^2 per day, and determined a dose of 560 μg per m^2 per day for Phase II trials[234]. Out of 32 patients, one patient treated at a dose of 80 μg per m^2 had a partial response with a corresponding prostate-specific antigen reduction of at least 50%, and five patients showed stable disease. Following a Phase I trial suggesting a positive therapeutic index on advanced solid tumours[235], kahalalide F is currently in Phase II clinical trials for solid tumours including melanoma, NSCLC and hepatocellular carcinoma.

Bryostatin 1. The bryozoan *Bugula neritina* (FIG. 2f) is a common 'fouling' organism that grows in thick colonies on pier pilings and docks. In 1968, a collaborative programme between Pettit and co-workers at the Arizona State University and the US National Cancer Institute found that extracts of *B. neritina* collected from the Gulf of Mexico exhibited remarkable activity against murine P338 lymphocytic leukaemia cells[180]. Isolation and identification of the active principles was made difficult when follow-up collections of the bryozoan from different locations provided extracts with low titres of activity. Subsequent large-scale collections from the Gulf of California (Sea of Cortez) and locations along the coast of California, USA, were laboriously purified

to provide the first milligram of the active component, bryostatin 1 (FIG. 7b), the structure of which was finally solved by in 1982 by X-ray crystallographic and spectroscopic analyses[236]. Bryostatin 1 is a 26-membered macrocylic lactone, with 11 stereocentres and a unique polyacetate carbon backbone that had not been previously encountered in natural products[236]. Subsequently, 19 additional bryostatin homologues and analogues were isolated from *B. neritina* (ordinal numbering up to bryostatin 20). The main differences in the molecular structures of these compounds are substitution at C_7 and C_{20} by different acyloxy substituents[180]. Bryostatin 1 demonstrated potent *in vitro* activity against the P388 lymphocytic leukaemia cell line with an ED_{50} of 0.89 μg per ml[233]. However, the low abundance of compound (~0.01 parts per million) and variable occurrence in natural populations of the bryozoan precluded supplies of enough compound for early preclinical and clinical studies as an anticancer drug. In 1991, a novel process of large-scale isolation and purification afforded 18 g of bryostatin 1 from a collection of approximately 10,000 gallons of *B. neritina*[237]. In the meantime, the Southern California company CalBioMarine Technologies was engaged in refinement of aquaculture technology for growth and harvest, and improvement of the titre of bryostatin 1 (REF. 238). The prospects of supply of bryostatin by total synthesis were not optimistic. The unique chemical structure of bryostatin 1 posed major synthetic challenges and, as of this time, no synthesis of the more desirable bryostatin 1 has been reported, although syntheses of bryostatin 2 (REF. 239), bryostatin 3 (REFS 240,241) and bryostatin 7 (REF. 242) (FIG. 7b) have been completed together with numerous partial syntheses of its constituent molecular segments[243–245]. Computer modelling studies at Stanford University, USA, identified that the minimum pharmacophore of bryostatin 1 required the presence of oxygen atoms at C_1, C_{19} and C_{26} (REF. 246), and led to the preparation of a truncated analogue with similar levels of activity to bryostatin 2 — the so-called bryopyran — in fewer synthetic steps than required for related natural products[247,248]. Bryopyran is being considered for clinical development.

Early studies identified bryostatin 1 as a modulator of protein kinase C (PKC)[249]; however, the precise details have been difficult to define. The compound binds to the regulatory domain of PKC — the same binding site targeted by phorbol esters — at subnanomolar concentrations. Unlike phorbol esters, bryostatin 1 elicits different physiological effects and lacks tumour-promoting activity[247,248]. Short-term exposure of tumour cells to bryostatin 1 induces PKC activation, self-phosphorylation and translocation to the membrane. Subsequent prolonged exposure inhibits PKC by causing depletion from the cell, probably due to proteolysis by a proteasome[246]. Bryostatin 1 also demonstrates various other biological activities, which include modulation of the immune system[250], induction of cell differentiation[251], radioprotection[252] and synergistic interactions with other anticancer agents such as ara-C, paclitaxel, tamoxifen, auristatin PE, dolastatin, vincristine, doxorubicin and prednisone; these activities are summarized in REF. 253.

With its promising anticancer activity, bryostatin 1 has been investigated either alone or in combination with other chemotherapeutic agents in numerous clinical trials (Phase I and II) for myeloid leukaemia, lymphocytic leukaemia, melanoma, non-Hodgkin's lymphoma and NSCLC, metastatic myeloma[254], relapsed lymphoma and chronic lymphocytic leukaemia[255–257], and other refractory malignancies[258]. A review of bryostatin 1 provides key information on the history of clinical development of the drug[259]. The major dose-limiting toxicity in all cases is myalgia, which explains a direct toxic effect of bryostatin 1: muscular vasoconstriction as a result of impaired oxidative metabolism and proton efflux from the muscle cells[260]. The end results of Phase II trials of bryostatin 1 as single-agent therapy against squamous cell carcinoma of the head and neck[261], colorectal cancer[262], melanoma[263], renal cell carcinoma[264] and ovarian carcinoma[265] were discouraging, as no significant clinical effect against these tumour types was observed. Nonetheless, the results of a Phase I trial investigating co-drug therapy with 1-β-D-arabinofuranosylcytosine for acute leukaemia[266] and vincristine in B-cell malignancies[267] were promising. Patients in these trials have achieved objective remissions and prolonged stable disease. Five Phase II clinical trials were recently sponsored by the US National Cancer Institute (data from clinical trials web site; see Further information) to evaluate co-drug therapy of bryostatin 1 with rituximab, vincristine, temsirolimus, cladribine or interleukin 1 against B-cell non-Hodgkin's lymphoma, metastatic solid tumours, lymphocytic leukaemia and refractory solid tumours. Bryostatin 1 has been evaluated in combination with paclitaxel for the treatment of solid tumours and achieved superior response rates than paclitaxel alone. However, roughly one-third of patients discontinued the therapy due to myalgias[268]. Bryostatin 1 has also been reviewed in context of targeted therapies for cell-cycle checkpoint intervention[269].

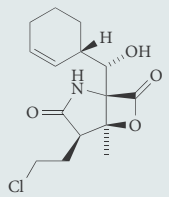
Outlook

As illustrated by the examples above, the discovery of selective and potent therapeutic activity in a rare marine natural product can drive innovative methods for its procurement, including production by aquaculture, semi-synthesis, synthesis and the development of synthetic analogues with more manageable properties. While academically inspired research has been key to marine natural product drug discovery in the past, novel approaches to translational medicine, which unite marine natural products chemists and pharmacologists with investigators in medical schools and institutes of pharmaceutical sciences, will be important in accelerating the progression of marine natural products from their discovery to the laboratory bench-top and to the clinic. Successful collaborations between academic institutes and pharmaceutical companies will continue to provide the mutual benefits that each party seeks. Academic programmes gain access capacities for sophisticated screening, pharmacological evaluation and advancement of leads to *in vivo* models, whereas

Box 1 | **Marine natural product development from bacteria**

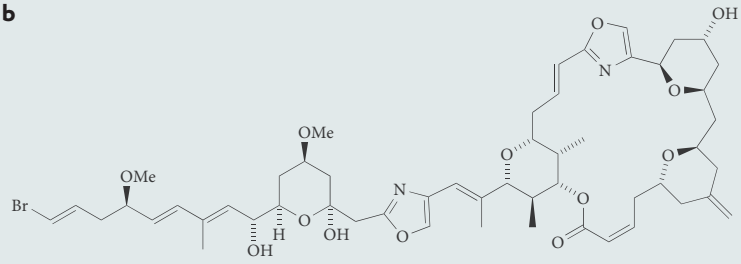
Marine bacteria have recently taken the limelight as potential sources of highly novel chemical structures and potential as drug leads. For example, the p26 proteasome inhibitor salinosporamide A (NPI-0052; **a**) was discovered by Fenical, Jensen and co-workers at the Scripps Institution of Oceanography, California, USA, in a bacterium recovered from deep-sea sediment in 2003 (REF. 278). Salinosporamide A contains an unusual β-lactone and is produced by a previously undiscovered genus of actinomycetes, a newly named family of 'salt-loving' bacteria, the *Salinispora*[279]. The mechanism of action of salinosporamide A is similar to that of the proteasome inhibitor bortezomib (Velcade; Millennium/Janssen-Cilag), which is approved for the treatment of multiple myeloma, but it overcomes resistance to bortezomib in patients with relapsed or refractory multiple myeloma[280]. Salinosporamide A has completed Phase I clinical trials for multiple myeloma under the sponsorship of Nereus Pharmaceuticals in La Jolla, California[281,282]. Furthermore, many compounds originally extracted from marine sponges are actually produced by marine bacteria. For example, the potent cytostatic agent phorboxazole A (structure 29; **b**) from a Western Australian sponge, *Phorbas* spp., that inhibits cell cycle in S-phase at subnanomolar concentrations[283–285], or the co-occurring phorbasides[286,287] may actually be produced by cyanobacteria that either live within the host, or expressed from microbial genes that have been integrated into the host sponge.

a



Salinosporamide A (NPI-0052)

b



Phorboxazole A

industry gains high-value leads while evading the high-risk associated with marine drug discovery. Major government funding can offset the high risk factor associated with marine natural products drug discovery, and new national programmes to exploit marine biotechnology and drug discovery have been launched by the governments of Germany, Ireland, Norway[9] and South Korea — but, surprisingly, less so in the USA — within the past 5 years.

But why persist at all with the pursuit of natural products for new drugs in the modern age? Many reasons have been offered as to why natural products are such good sources for drug leads, but at least one study has attempted to quantify a correlation between the drug molecules and those typically found in natural products and combinatorial chemical libraries. Combinatorial libraries are purposely synthesized in large numbers, but the structures are intentionally imbued with high randomness. A multivariate comparison of the chemical space occupied by thousands of combinatorial drug compounds with that of natural products revealed a strikingly good correlation of clinically approved drug molecules with the latter, but not the former[270]. In other words, the structures of drugs we use today more closely resemble those of natural products.

There are good reasons to be optimistic about the future. New technologies in analytical spectroscopy have pushed the limits of observation so that discovery of new molecules require only a few micrograms — a fraction of the material that was required even 10 years ago. New sources of marine natural products, such as marine bacteria, are being explored (BOX 1). Finally, there is enormous excitement and promise for drug discovery by manipulation of biosynthetic pathways in the most refractory microbes — the unculturable bacteria or metagenomic DNA from seawater and sediments. By deploying the cutting-edge tools of genetic engineering, genome mining[271] and new approaches to metagenomic mining of environmental DNA[272], it may be possible to unlock the genetic potential of millions of bacteria that occupy each millilitre of seawater or benthic sediment. Generally, pelagic bacteria are unculturable (~90%, or more correctly as yet, uncultured), unlike the soil-borne actinomycetes that historically have played an important role in drug discovery. Many compounds obtained from marine sponges, including those mentioned in this Review, are thought to arise from marine microbes that live in symbiosis with the larger host organisms. Recently, the prospects for sustainable production of pharmaceuticals from invertebrate-derived microbes have been reviewed[273], and it is clear that many obstacles must be overcome before this approach transforms into conventional technology. The potential payoff for this approach is very high. For example, biosynthesis of the antitumour drug bryostatin 1 has been traced to an as-yet uncultivated endosymbiotic γ-proteobacterium: "*Candidatus* Endobugula sertula"[274]. Most of the genes that are responsible for the biosynthesis of the drug have been recently identified and sequenced[275]. The cloning of the biosynthetic genes for bryostatin 1 and so-called heterologous expression in a bacterium more amenable to large-scale fermentation production would overcome the limitation of procurement of the drug from the ocean (which is currently limited to expensive aquaculture or field harvesting) and ensure supply. The 'golden era' of antibiotic discovery began with microbes, and it is fitting that we now return to these origins with new tools to probe the untapped, broad molecular diversity of the bacteria that populate the oceans, benthic sediments and marine invertebrates.

In ancient poems almost 3,000 years old, Homer wrote of "the wine dark sea"[276,277], an evocative phrase that still rings with mystery. Did Homer choose his words to have us empathize with a grieving Achilles in his contemplation of human suffering and untimely death by marrying two familiar metaphors: wine, the velvet hued libation and tonic to health, with the fathomless complexity and mysteries of the open seas? As is clear from the drugs from the sea discussed in this Review, the coming years may provide a glimpse of hope and cures from beneath the swell and ebb of the vast oceans.

1. Freudenthal, H. D. *Transactions of the Drugs from the Sea Symposium, University of Rhode Island, 27–29 August 1967* 1–297 (Marine Technology Society, Washington, DC, 1968).
2. Newman D. J. & Cragg G. M. Marine natural products and related compounds in clinical and advanced preclinical trials. *J. Nat. Prod.* **67**, 1216–1238 (2004).
3. Newman D. J. & Cragg G. M. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* **70**, 461–477 (2007).
4. Newman, D. J. & Cragg, G. M. in *Marine Anticancer Compounds in the Era of Targeted therapies* 1st edn (ed. Chapner, B.) (Permanyer Publications, Barcelona, 2008).
5. Bergmann, W. & Feeney R. J. Contributions to the study of marine products. XXXII. The nucleosides of sponges. I. *J. Org. Chem.* **16**, 981–987 (1951).
6. Bergmann, W. & Burke, D. C. Contributions to the study of marine products. XL. The nucleosides of sponges. IV. Spongosine. *J. Org. Chem.* **22**, 226–228 (1956).
7. Bergmann, W. & Stempien, M. F. Contributions to the study of marine products. XLIII. The nucleosides of sponges. V. The synthesis of spongosine. *J. Org. Chem.* **22**, 1575–1557 (1957).
8. Koehn, F. E. & Carter, G. T. The evolving role of natural products in drug discovery. *Nature Rev. Drug Discovery* **4**, 206–220 (2004).
This important, prescient review from scientists in a large pharmaceutical drug discovery programme presents the technical and logistical challenges for natural products discovery.
9. Jarvis, L. M. Liquid gold mine. *Chem. Engin. News* **85**, 22–28 (2007).
10. Mendola, D. in *Drugs from the Sea* (ed. Fusetani, N.) 120–133 (Karger, Basel, 2000).
11. Olivera, B. M. *et al.* Peptide neurotoxins from fish-hunting cone snails. *Science* **230**, 1338–1343 (1985).
12. Terlau, H. & Olivera, B. M. *Conus* venoms: a rich source of novel ion channel-targeted peptides. *Physiol. Rev.* **84**, 41–68 (2004).
13. Olivera, B. M., Miljanich, G. P., Ramachandran, J. & Adams, M. E. Calcium-channel diversity and neurotransmitter release — the ω-conotoxins and ω-agatoxins. *Ann. Rev. Biochem.* **63**, 823–867 (1994).
14. Chung, D., Gaur, S., Bell, J. R., Ramachandran, J. & Nadasdi, L. Determination of disulfide bridge pattern in ω-conopeptides. *Int. J. Pept. Protein Res.* **46**, 320–325 (1995).
15. Price-Carter, M., Hull, M. S. & Goldenberg, D. P. Roles of individual disulfide bonds in the stability and folding of an ω-conotoxin. *Biochemistry* **37**, 9851–9861 (1998).
16. Olivera, B. M. *et al.* Neuronal calcium-channel antagonists — discrimination between calcium-channel subtypes using ω-conotoxin from *Conus magus* venom. *Biochemistry* **26**, 2086–2090 (1987).
17. Yeager, R. E., Yoshikami, D., Rivier, J., Cruz, L. J. & Miljanich, G. P. Transmitter release from presynaptic terminals of electric organ: inhibition by the calcium channel antagonist ω *Conus* toxin. *J. Neurosci.* **7**, 2390–2396 (1987).
18. Atkinson, R. A., Kieffer, B., Dejaegere, A., Sirockin, F. & Lefevre, J. F. Structural and dynamic characterization of ω -conotoxin MVIIA: the binding loop exhibits slow conformational exchange. *Biochemistry* **39**, 3908–3919 (2000).
19. Kim, J. I., Takahashi, M., Ohtake, A., Wakamiya, A. & Sato, K. Tyr13 Is essential for the activity of ω -conotoxin MVIIA and GVIA, specific N-type calcium channel blockers. *Biochem. Biophys. Res. Commun.* **206**, 449–454 (1995).
20. Gohil, K., Bell, J. R., Ramachandran, J. & Miljanich, G. P. Neuroanatomical distribution of receptors for a novel voltage-sensitive calcium-channel antagonist, SNX-230 (ω -conopeptide MVIIC). *Brain Res.* **653**, 258–266 (1994).
21. Bowersox, S. S. *et al.* Selective N-type neuronal voltage-sensitive calcium channel blocker, SNX-111, produces spinal antinociception in rat models of acute, persistent and neuropathic pain. *J. Pharmacol. Exp. Ther.* **279**, 1243–1249 (1996).
22. Kristipati, R. *et al.* Characterization of the binding of ω -conopeptides to different classes of non-L-type neuronal calcium channels. *Mol. Cell. Neurosci.* **5**, 219–228 (1994).
23. Jones, R. M. *et al.* Composition and therapeutic utility of conotoxins from genus *Conus*. Patent status 1996–2000 *Exp. Opin. Ther. Patents* **11**, 603–623 (2001).
24. Bowersox, S. S. & Luther, R. Pharmacotherapeutic potential of ω-conotoxin MVIIA (SNX-111), an N-type neuronal calcium channel blocker found in the venom of *Conus magus*. *Toxicon* **36**, 1651–1658 (1998).
25. Wang, Y. X., Pettus, M., Gao, D., Phillips, C. & Scott Bowersox, S. Effects of intrathecal administration of ziconotide, a selective neuronal N-type calcium channel blocker, on mechanical allodynia and heat hyperalgesia in a rat model of postoperative pain. *Pain* **84**, 151–158 (2000).
26. Miljanich, G. P. Ziconotide: neuronal calcium channel blocker for treating severe chronic pain. *Curr. Med. Chem.* **11**, 3029–3040 (2004).
27. Garber, K. Peptide leads new class of chronic pain drugs. *Nature Biotech.* **23**, 399 (2005).
28. Heading, C. E. *Conus* toxins: targets and properties. *IDrugs* **7**, 1011–1016 (2004).
29. Sigel, M. M. *et al.* in *Food–Drugs from the Sea: Proceedings* (ed. Youngken, H. W. Jr) 281–294 (Marine Technology Society, Washington, DC, 1969).
30. Rinehart, K. L. *et al.* Ecteinascidin-729, Ecteinascidin-743, Ecteinascidin-745, Ecteinascidin-759a, Ecteinascidin-759b, and Ecteinascidin-770 — potent antitumor agents from the Caribbean tunicate *Ecteinascidia turbinata*. *J. Org. Chem.* **55**, 4512–4515 (1990).
31. Wright, A. E. *et al.* Antitumor tetrahyrodisoquinoline alkaloids from the colonial ascidian *Ecteinascidia turbinata*. *J. Org. Chem.* **55**, 4508–4512 (1990).
32. Sakai, R., Rinehart, K. L., Guan, Y. & Wang, A. H. Additional antitumor ecteinascidins from a Caribbean tunicate: crystal structures and activities *in vivo*. *Proc. Nat. Acad. Sci. USA* **89**, 11456–11460 (1992).
33. Corey, E. J., Gin, D. Y. & Kania, R. S. Enantioselective total synthesis of ecteinascidin 743. *J. Am. Chem. Soc.* **118**, 9202–9203 (1996).
34. Martinez, E. J. & Corey, E. J. A new, more efficient, and effective process for the synthesis of a key pentacyclic intermediate for production of ecteinascidin and phthalascidin antitumor agents. *Org. Lett.* **2**, 993–996 (2000).
35. Cuevas, C. *et al.* Synthesis of ecteinascidin ET-743 and Phthalascidin Pt-650 from cyanosafracin B. *Org. Lett.* **2**, 2545–2548 (2000).
36. Aune, G. J., Furuta, T. & Pommier, Y. Ecteinascidin 743: a novel anticancer drug with a unique mechanism of action. *Anticancer Drugs* **13**, 545–555 (2002).
37. Pommier, Y. *et al.* DNA sequence- and structure-selective alkylation of guanine N2 in the DNA minor groove by ecteinascidin 743, a potent antitumor compound from the Caribbean tunicate *Ecteinascidia turbinata*. *Biochemistry* **35**, 13303–13309 (1996).
38. Zewail-Foote, M. & Hurley, L. H. Ecteinascidin 743: a minor groove alkylator that bends DNA toward the major groove. *J. Med. Chem.* **42**, 2493–2497 (1999).
39. Zewail-Foote, M. *et al.* The inefficiency of incisions of ecteinascidin 743-DNA adducts by the UvrABC nuclease and the unique structural feature of the DNA adducts can be used to explain the repair-dependent toxicities of this antitumor agent. *Chem. Biol.* **8**, 1033–1049 (2001).
40. Takebayashi, Y. *et al.* Antiproliferative activity of ecteinascidin 743 is dependent upon transcription-coupled nucleotide-excision repair. *Nature Med.* **7**, 961–966 (2001).
An excellent technical paper describing the excision repair inhibition mechanism that is unique to ET-743.
41. Di Leo, P. *et al.* Final results of a phase II trial of 3-HR infusion trabectedin in patients with recurrent sarcomas. *Ann. Oncol.* **17**, 167–167 (2006).
42. Paz-Ares, L. *et al.* Phase II study of trabectedin in pretreated patients with advanced colorectal cancer. *Clin. Colorectal Cancer* **6**, 522–528 (2007).
43. Zelek, L. *et al.* A phase II study of Yondelis® (trabectedin, ET-743) as a 24-h continuous intravenous infusion in pretreated advanced breast cancer *Br. J. Cancer* **94**, 1610–1614 (2006).
44. Sessa, C. *et al.* Trabectedin for women with ovarian carcinoma after treatment with platinum and taxanes fails. *J. Clin. Oncol.* **23**, 1867–1874 (2005).
45. Yovine, A. *et al.* Phase II study of ecteinascidin-743 in advanced pretreated soft tissue sarcoma patients. *J. Clin. Oncol.* **22**, 890–899 (2004).
46. Jimeno, J. *et al.* Therapeutic impact of ET-743 (Yondelis; trabectidin), a new marine-derived compound, in sarcoma. *Curr. Op. Orthopaed.* **14**, 419–428 (2003).
47. Jimeno, J. *et al.* Progress in the clinical development of new marine-derived anticancer compounds. *Anticancer Drugs* **15**, 321–329 (2004).
48. Ryan, D. P. *et al.* A phase II and pharmacokinetic study of ecteinascidin 743 in patients with gastrointestinal stromal tumors. *Oncologist* **7**, 531–538 (2002).
49. Blay, J. Y. *et al.* A phase II study of ET-743/trabectedin ('Yondelis') for patients with advanced gastrointestinal stromal tumours. *Eur. J. Cancer* **40**, 1327–1331 (2004).
50. Meco, D. *et al.* Effective combination of ET-743 and doxorubicin in sarcoma: preclinical studies. *Cancer Chemother. Pharmacol.* **52**, 131–138 (2003).
51. Takahashi, N., Li, W. W., Banerjee, D., Scotto, K. W. & Bertino, J. R. Sequence-dependent enhancement of cytotoxicity produced by ecteinascidin 743 (ET-743) with doxorubicin or paclitaxel in soft tissue sarcoma cells. *Clin. Canc. Res.* **7**, 3251–3257 (2001).
52. Riccardi, A. *et al.* Combination of trabectedin and irinotecan is highly effective in a human rhabdomyosarcoma xenograft. *Anticancer Drugs* **16**, 811–815 (2005).
53. Brandon, E. F. A. *et al. In-vitro* cytotoxicity of ET-743 (Trabectedin, Yondelis), a marine anti-cancer drug, in the Hep G2 cell line: influence of cytochrome P450 and phase II inhibition, and cytochrome P450 induction. *Anticancer Drugs* **16**, 935–943 (2005).
54. Donald, S. *et al.* Comparison of four modulators of drug metabolism as protectants against the hepatotoxicity of the novel antitumor drug yondelis (ET-743) in the female rat and in hepatocytes *in vitro*. *Cancer Chemother. Pharmacol.* **53**, 305–312 (2004).
55. Donald, S. *et al.* Complete protection by high-dose dexamethasone against the hepatotoxicity of the novel antitumor drug yondelis (ET-743) in the rat. *Cancer Res.* **63**, 5902–5908 (2003).
56. Beumer, J. H., Schellens, J. H. & Beijnen, J. H. Hepatotoxicity and metabolism of trabectedin: a literature review. *Pharmacol. Res.* **51**, 391–398 (2005).
57. Chun, H. G. *et al.* Didemnin B. The first marine compound entering clinical trials as an antineoplastic agent. *Invest. New Drugs* **4**, 279–284 (1986).
58. Vera, M. D. & Joullie, M. M. Natural products as probes of cell biology: 20 years of didemnin research. *Med. Res. Rev.* **22**, 102–145 (2002).
59. Rinehart, K. Antitumor Compounds from Tunicates. *Med. Res. Rev.* **1**, 1–27 (2003).
60. Rinehart, K. L. Jr., Gloer, J. B., Cook, J. C., Mizsak, S. A. & Scahill, T. A. Structures of the didemnins, antiviral and cytotoxic depsipeptides from a Carribean tunicate. *J. Am. Chem. Soc.* **103**, 1857–1859 (1981).
61. Rinehart, K. L. Jr *et al.* Didemnins: antiviral and antitumor depsipeptides from a caribbean tunicate. *Science* **212**, 933–935 (1981).
62. Rinehart, K. L. Jr *et al.* Total synthesis of didemnins A, B, and C. *J. Am. Chem. Soc.* **109**, 6856–6848 (1987).
63. McKee, T. C., Ireland, C. M., Lindquist, N. & Fenical, W. The complete spectral assignment of didemnin B and nordidemnin B. *Tetrahedron Lett.* **30**, 3053–3056 (1989).
64. Gloer, J. B. *Structures of the Didemnins.* Thesis, Univ. Illinois (1983).
65. Hossain, M. B. *et al.* Crystal and molecular structure of didemnin B, an antiviral and cytotoxic depsipeptide. *Proc. Natl Acad. Sci. USA* **85**, 4118–4122 (1988).
66. Meng, L., Sin, N. & Crews, C. M. The antiproliferative agent didemnin B uncompetitively inhibits palmitoyl protein thioesterase. *Biochemistry* **37**, 10488–10492 (1998).
67. Crews, C. M., Lane, W. S. & Schreiber, S. L. Didemnin binds to the protein palmitoyl thioesterase responsible for infantile neuronal ceroid lipofuscinosis. *Proc. Natl Acad. Sci. USA* **93**, 4316–4319 (1996).
68. Li, L. H. *et al.* Mechanism of action of didemnin B, a depsipeptide from the sea. *Cancer Lett.* **23**, 279–288 (1984).
69. SirDeshpande, B. V. & Toogood, P. L. Mechanism of protein synthesis inhibition by didemnin B *in vitro*. *Biochemistry* **34**, 9177–9184 (1995).
70. Ahuja, D. *et al.* Inhibition of protein synthesis by didemnin B: how EF-1a mediates inhibition of translocation. *Biochemistry* **39**, 4339–4346 (2000).
71. Marco, E., Martin-Santamaria, S., Cuevas, C. & Gago, F. Structural basis for the binding of didemnins to human elongation factor eEF1A and rationale for the potent antitumor activity of these marine natural products. *J. Med. Chem.* **47**, 4439–4452 (2004).

72. Crews, C. M., Collins, J. L., Lane, W. S., Snapper, M. L. & Schreiber, S. L. GTP-dependent binding of the antiproliferative agent didemnin to elongation factor 1 α. *J. Biol. Chem.* **269**, 15411–15414 (1994). **Illustrates the 'chemical biological' approach to the identification of protein targets of natural product drugs, and the importance of discriminating high- and low-affinity binding partners (see also reference 67)**
73. Beidler, D. R., Ahuja, D., Wicha, M. S. & Toogood, P. L. Inhibition of protein synthesis by didemnin B is not sufficient to induce apoptosis in human mammary carcinoma (MCF7) cells. *Biochem. Pharmacol.* **58**, 1067–1074 (1999).
74. Erba, E. *et al.* Cell cycle phase perturbations and apoptosis in tumour cells induced by aplidine. *Br. J. Cancer* **86**, 1510–1517 (2002).
75. Johnson, K. L., Vaillant, F. & Lawen, A. Protein tyrosine kinase inhibitors prevent didemnin B-induced apoptosis in HL-60 cells. *FEBS Lett.* **383**, 1–5 (1996).
76. Johnson, K. L. & Lawen, A. Rapamycin inhibits didemnin B-induced apoptosis in human HL-60 cells: evidence for the possible involvement of FK506-binding protein 25. *Immunol. Cell Biol.* **77**, 242–248 (1999).
77. Weed, S. D. & Stringfellow, D. A. Didemnins A and B. Effectiveness against cutaneous herpes simplex virus in mice. *Antiviral Res.* **3**, 269–274 (1983).
78. Urdiales, J. L., Morata, P., Nunez De Castro, I. & Sanchez-Jimenez, F. Antiproliferative effect of dehydrodidemnin B (DDB), a depsipeptide isolated from Mediterranean tunicates. *Cancer Lett.* **102**, 31–37 (1996).
79. Dorr, F. A., Kuhn, J. G., Phillips, J. & von Hoff, D. D. Phase I clinical and pharmacokinetic investigation of didemnin B, a cyclic depsipeptide. *Eur. J. Cancer Clin. Oncol.* **24**, 1699–1706 (1988).
80. Scheithauer, W., Moyer, M. P., Clark, G. M. & Von Hoff, D. D. Application of a new preclinical drug screening system for cancer of the large bowel. *Cancer Chemother. Pharmacol.* **21**, 31–34 (1988).
81. Grubb, D. R., Wolvetang, E. J. & Lawen, A. Didemnin B induces cell death by apoptosis: the fastest induction of apoptosis ever described. *Biochem. Biophys. Res. Commun.* **215**, 1130–1136 (1995).
82. Geldof, A. A., Mastbergen, S. C., Henrar, R. E. & Faircloth, G. T. Cytotoxicity and neurocytotoxicity of new marine anticancer agents evaluated using *in vitro* assays. *Cancer Chemother. Pharmacol.* **44**, 312–318 (1999).
83. Stewart, J. A., Low, J. B., Roberts, J. D. & Blow, A. A phase I clinical trial of didemnin B. *Cancer* **68**, 2550–2554 (1991).
84. Maroun, J. A., Stewart, D., Verma, S. & Eisenhauer, E. Phase I clinical study of didemnin B. *Invest. New Drugs* **16**, 51–56 (1998).
85. Shin, D. M. *et al.* Phase I/II clinical trial of didemnin B in non-small-cell lung cancer: neuromuscular toxicity is dose-limiting. *Cancer Chemother. Pharmacol.* **29**, 145–149 (1991).
86. Benvenuto, J. A. *et al.* Phase II clinical and pharmacological study of didemnin B in patients with metastatic breast cancer. *Invest. New Drugs* **10**, 113–117 (1992).
87. Shin, D. M. *et al.* Phase II clinical trial of didemnin B in previously treated small cell lung cancer. *Invest. New Drugs* **12**, 243–249 (1994).
88. Goss, G. *et al.* Didemnin B in favorable histology non-Hodgkin's lymphoma: a phase II study of the National Cancer Institute of Canada Clinical Trials Group. *Invest. New Drugs* **13**, 257–260 (1995).
89. Kucuk, O. *et al.* Phase II trail of didemnin B in previously treated non-Hodgkin's lymphoma: an Eastern Cooperative Oncology Group (ECOG) Study. *Am. J. Clin. Oncol.* **23**, 273–277 (2000).
90. Hochster, H., Oratz, R., Ettinger, D. S. & Borden, E. A phase II study of Didemnin B (NSC 325319) in advanced malignant melanoma: an Eastern Cooperative Oncology Group study (PB687). *Invest. New Drugs* **16**, 259–263 (1999).
91. Mittelman, A. *et al.* Phase II clinical trial of didemnin B in patients with recurrent or refractory anaplastic astrocytoma or glioblastoma multiforme (NSC 325319). *Invest. New Drugs* **17**, 179–182 (1999).
92. Taylor, S. A. *et al.* Phase II study of didemnin B in central nervous system tumors: a Southwest Oncology Group study. *Invest. New Drugs* **16**, 331–332 (1999).
93. Nuijen, B. *et al*. Pharmaceutical development of anticancer agents from marine sources. *Anticancer Drugs* **11**, 793–811 (2000).
94. Rinehart, K. L. & Lithgow-Bertelloni, A. M. Dehydrodidemnin B. WO9104985 (A1) (1991).
95. Garcia-Fernandez, L. F. *et al.* Aplidin induces the mitochondrial apoptotic pathway via oxidative stress-mediated JNK and p38 activation and protein kinase C. *Oncogene* **21**, 7533–7544 (2002).
96. Gajate, C., An, F. & Mollinedo, F. Rapid and selective apoptosis in human leukemic cells induced by Aplidine through a Fas/CD95- and mitochondrial-mediated mechanism. *Clin. Cancer Res.* **9**, 1535–1545 (2003).
97. Grubb, D. R., Ly, J. D., Vaillant, F., Johnson, K. L. & Lawen, A. Mitochondrial cytochrome *c* release is caspase-dependent and does not involve mitochondrial permeability transition in didemnin B-induced apoptosis. *Oncogene* **20**, 4085–4094 (2001).
98. Cuadrado, A. *et al.* Aplidin induces apoptosis in human cancer cells via glutathione depletion and sustained activation of the epidermal growth factor receptor, Src, JNK, and p38 MAPK. *J. Biol. Chem.* **278**, 241–250 (2003).
99. Cuadrado, A., Gonzalez, L., Suarez, Y., Martinez, T. & Munoz, A. JNK activation is critical for Aplidin-induced apoptosis. *Oncogene* **23**, 4673–4680 (2004).
100. Broggini, M. *et al.* Aplidine, a new anticancer agent of marine origin, inhibits vascular endothelial growth factor (VEGF) secretion and blocks VEGF–VEGFR-1 (flt-1) autocrine loop in human leukemia cells MOLT-4. *Leukemia* **17**, 52–59 (2003).
101. Biscardi, M. *et al.* VEGF inhibition and cytotoxic effect of aplidin in leukemia cell lines and cells from acute myeloid leukemia. *Ann. Oncol.* **16**, 1667–1674 (2005).
102. Losada, A., Lopez-Oliva, J. M., Sanchez-Puelles, J. M. & Garcia-Fernandez, L. F. Establishment and characterisation of a human carcinoma cell line with acquired resistance to Aplidin. *Br. J. Cancer* **91**, 1405–1413 (2004).
103. Erba, E. *et al.* Effect of aplidine in acute lymphoblastic leukaemia cells. *Br. J. Cancer* **89**, 763–773 (2003).
104. Albella, B. *et al. In vitro* toxicity of ET-743 and aplidine, two marine-derived antineoplastics, on human bone marrow haematopoietic progenitors. comparison with the clinical results. *Eur. J. Cancer* **38**, 1395–1404 (2002).
105. Baker, M. A., Grubb, D. R. & Lawen, A. Didemnin B induces apoptosis in proliferating but not resting peripheral blood mononuclear cells. *Apoptosis* **7**, 407–412 (2002).
106. Bresters, D. *et al. In vitro* cytotoxicity of aplidin and crossresistance with other cytotoxic drugs in childhood leukemic and normal bone marrow and blood samples: a rational basis for clinical development. *Leukemia* **17**, 1338–1343 (2003).
107. Gomez, S. G., Bueren, J. A., Faircloth, G. T., Jimeno, J. & Albella, B. *In vitro* toxicity of three new antitumoral drugs (trabectedin, aplidin, and kahalalide F) on hematopoietic progenitors and stem cells. *Exp. Hematol.* **31**, 1104–1111 (2003).
108. Taraboletti, G. *et al.* Antiangiogenic activity of aplidine, a new agent of marine origin. *Br. J. Cancer* **90**, 2418–2424 (2004).
109. Perez, M., Sadqi, M., Munoz, V. & Avila, J. Inhibition by Aplidine of the aggregation of the prion peptide PrP 106–126 into β-sheet fibrils. *Biochem. Biophys. Acta* **1639**, 133–139 (2003).
110. Talpir, R., Benayahu, Y., Kashman, Y., Pannell, L. & Schleyer, M. Hemiasterlin and geodiamolide TA: two new cytotoxic peptides from the marine sponge *Hemiasterella minor* (Kirkpatrick). *Tetrahedron Lett.* **35**, 4453–4456 (1994).
111. Coleman, J. E., de Silva, E. D., Kong, F., Andersen, R. J. & Allen, T. M. Cytotoxic peptides from the marine sponge *Cymbastela* sp. *Tetrahedron* **51**, 10653–10662 (1995).
112. Coleman, J. E., Patrick, B. O., Andersen, R. J. & Rettig, S. J. Hemiasterlin methyl ester. *Acta Cryst. Sec. C* **C52**, 1525–1527 (1996).
113. Gamble, W. R. *et al.* Cytotoxic and tubulin-interactive hemiasterlins from *Auletta* sp. and *Siphonochalina* spp. sponges. *Bioorg. Med. Chem.* **7**, 1611–1615 (1999).
114. Anderson, H. J., Coleman, J. E., Andersen, R. J. & Roberge, M. Cytotoxic peptides hemiasterlin, hemiasterlin A and hemiasterlin B induce mitotic arrest and abnormal spindle formation. *Cancer Chemother. Pharmacol.* **39**, 223–226 (1997).
115. Bai, R., Durso, N. A., Sackett, D. L. & Hamel, E. Interactions of the sponge-derived antimitotic tripeptide hemiasterlin with tubulin: comparison with dolastatin 10 and cryptophycin 1. *Biochemistry* **38**, 14302–14310 (1999).
116. Nieman, J. A. *et al.* Synthesis and antimitotic/cytotoxic activity of hemiasterlin analogues. *J. Nat. Prod.* **66**, 183–199 (2003).
117. Zask, A. *et al.* Synthesis and biological activity of analogues of the antimicrotubule agent *N*,β,β-trimethyl-L-phenylalanyl-N^1-[(1*S*,2*E*)-3-carboxy-1-isopropylbut-2-enyl]-N^1,3-dimethyl-L-valinamide (HTI-286). *J. Med. Chem.* **47**, 4774–4786 (2004).
118. Zask, A. *et al.* D-piece modifications of the hemiasterlin analog HTI-286 produce potent tubulin inhibitors. *Bioorg. Med. Chem. Lett.* **14**, 4353–4358 (2004).
119. Yamashita, A. *et al.* Synthesis and activity of novel analogs of hemiasterlin as inhibitors of tubulin polymerization: modification of the A segment. *Bioorg. Med. Chem. Lett.* **14**, 5317–5322 (2004).
120. Niu, C. *et al.* Tubulin inhibitors. Synthesis and biological activity of HTI-286 analogs with B-segment heterosubstituents. *Bioorg. Med. Chem. Lett.* **14**, 4329–4332 (2004).
121. Lo, M. C. *et al.* Probing the interaction of HTI-286 with tubulin using a stilbene analogue. *J. Am. Chem. Soc.* **126**, 9898–9899 (2004).
122. Krishnamurthy, G. *et al.* Biophysical characterization of the interactions of HTI-286 with tubulin heterodimer and microtubules. *Biochemistry* **42**, 13484–13495 (2003).
123. Mitra, A. & Sept, D. Localization of the antimitotic peptide and depsipeptide binding site on β-tubulin. *Biochemistry* **43**, 13955–13962 (2004).
124. Nunes, M. *et al.* Two photoaffinity analogues of the tripeptide, hemiasterlin, exclusively label α-tubulin. *Biochemistry* **44**, 6844–6857 (2005).
125. Poruchynsky, M. S. *et al.* Tumor cells resistant to a microtubule-depolymerizing hemiasterlin analogue, HTI-286, have mutations in α- or β-tubulin and increased microtubule stability. *Biochemistry* **43**, 13944–13954 (2004).
126. Loganzo, F. *et al.* Cells resistant to HTI-286 do not overexpress P-glycoprotein but have reduced drug accumulation and a point mutation in α-tubulin. *Mol. Cancer Ther.* **3**, 1319–1327 (2004).
127. Loganzo, F. *et al.* HTI-286, a synthetic analogue of the tripeptide hemiasterlin, is a potent antimicrotubule agent that circumvents P-glycoprotein-mediated resistance *in vitro* and *in vivo*. *Cancer Res.* **63**, 1838–1845 (2003).
128. Ratain M. J. *et al.* Phase 1 and pharmacological study of HTI-286, a novel antimicrotubule agent: correlation of neutropenia with time above a threshold serum concentration. *Proc. Am. Soc. Clin. Oncol.* **22**, 516 (2003).
129. Hadaschik, B. A. *et al.* Targeting prostate cancer with HTI-286, a synthetic analog of the marine sponge product hemiasterlin *Int. J. Cancer*: **122**, 2368–2376 (2008).
130. Kalesse, M. The chemistry and biology of discodermolide. *ChemBiochem* **1**, 171–175 (2000).
131. De Souza, M. V. (+)-discodermolide: a marine natural product against cancer. *Scientific World Journal* **4**, 415–436 (2004).
132. Longley, R. E., Caddigan, D., Harmody, D., Gunasekera, M. & Gunasekera, S. P. Discodermolide — a new, marine-derived immunosuppressive compound. I. *In vitro* studies. *Transplantation* **52**, 650–656 (1991).
133. Longley, R. E., Caddigan, D., Harmody, D., Gunasekera, M. & Gunasekera, S. P. Discodermolide — a new, marine-derived immunosuppressive compound. II. *In vivo* studies. *Transplantation* **52**, 656–661 (1991).
134. Longley, R. E., Gunasekera, S. P., Faherty, D., McLane, J. & Dumont, F. Immunosuppression by discodermolide. *Ann. NY Acad. Sci.* **696**, 94–107 (1993).
135. Gunasekera, S. P., Gunasekera, M., Longley, R. E. & Schulte, G. K. Discodermolide: a new bioactive polyhydroxylated lactone from the marine sponge *Discodermia dissoluta*. *J. Org. Chem.* **55**, 4912–4915 (1990).
136. Gunasekera, S. P., Gunasekera, M., Longley, R. E. & Schulte, G. K. Discodermolide: a new bioactive polyhydroxylated lactone from the marine sponge *Discodermia dissoluta* [Erratum to document cited in CA113[9]:75187b]. *J. Org. Chem.* **56**, 1346 (1991).
137. Nerenberg, J. B., Hung, D. T., Somers, P. K. & Schreiber, S. L. Total synthesis of the immunosuppressive agent (–)-discodermolide. *J. Am. Chem. Soc.* **115**, 12621–12622 (1993).
138. Hung, D. T., Nerenberg, J. B. & Schreiber, S. L. Distinct binding and cellular properties of synthetic (+)- and (–)-discodermolides. *Chem. Biol.* **1**, 67–71 (1994).

A good example of enantioselective bioactivity of natural and unnatural antipodes of marine-derived drugs.
139. Harried, S. S., Lee, C. P., Yang, G., Lee, T. I. & Myles, D. C. Total synthesis of the potent microtubule-stabilizing agent (+)-discodermolide. *J. Org. Chem.* **68**, 6646–6660 (2003).
140. Paterson, I. & Lyothier, I. Total synthesis of (+)-discodermolide: an improved endgame exploiting a Still-Gennari-type olefination with a C1-C8 β-ketophosphonate fragment. *Org. Lett.* **6**, 4933–4936 (2004).
141. Paterson, I. *et al.* A second-generation total synthesis of (+)-discodermolide: the development of a practical route using solely substrate-based stereocontrol. *J. Org. Chem.* **70**, 150–160 (2005).
142. Smith, A. B. 3rd, Kaufman, M. D., Beauchamp, T. J., LaMarche, M. J. & Arimoto, H. Gram-scale synthesis of (+)-discodermolide. *Org. Lett.* **1**, 1823–1826 (1999).
143. Smith, A. B. 3rd *et al.* Evolution of a gram-scale synthesis of (+)-discodermolide. *J. Am. Chem. Soc.* **122**, 8654–8664 (2000).
144. Smith, A. B. 3rd, Freeze, B. S., Brouard, I. & Hirose, T. A practical improvement, enhancing the large-scale synthesis of (+)-discodermolide: a third-generation approach. *Org. Lett.* **5**, 4405–4408 (2003).
145. Mickel, S. J. *et al.* Large-scale synthesis of the anti-cancer marine natural product (+)-discodermolide. Part 1: synthetic strategy and preparation of a common precursor. *Org. Proc. Res. Dev.* **8**, 92–100 (2004).
146. Mickel, S. J. *et al.* Large-scale synthesis of the anti-cancer marine natural product (+)-discodermolide. Part 2: synthesis of fragments C1–6 and C9–14 *Org. Proc. Res. Dev.* **8**, 101–106 (2004).
147. Mickel, S. J. *et al.* Large-scale synthesis of the anti-cancer marine natural product (+)-discodermolide. Part 3: synthesis of fragment C15–21 *Org. Proc. Res. Dev.* **8**, 107–112 (2004).
References 145–147 describe the challenge for production, met by a tour de force multi-step, total synthesis, of a complex, rare marine-derived drug. The synthesis of 65 g of discodermolide shows what is possible when the target is highly desirable.
148. Mickel, S. J. *et al.* Large-scale synthesis of the anti-cancer marine natural product (+)-discodermolide. Part 4: preparation of fragment C7–24 *Org. Proc. Res. Dev.* **8**, 113–121 (2004).
149. Mickel, S. J. *et al.* Large-scale synthesis of the anti-cancer marine natural product (+)-discodermolide. Part 5: linkage of fragments C1–6 and C7–24 and finale. *Org. Proc. Res. Dev.* **8**, 122–130 (2004).
150. Hung, D. T., Chen, J. & Schreiber, S. L. (+)-Discodermolide binds to microtubules in stoichiometric ratio to tubulin dimers, blocks taxol binding and results in mitotic arrest. *Chem. Biol.* **3**, 287–293 (1996).
151. ter Haar, E. *et al.* Discodermolide, a cytotoxic marine agent that stabilizes microtubules more potently than taxol. *Biochemistry* **35**, 243–250 (1996).
152. Kowalski, R. J. *et al.* The microtubule-stabilizing agent discodermolide competitively inhibits the binding of paclitaxel (Taxol) to tubulin polymers, enhances tubulin nucleation reactions more potently than paclitaxel, and inhibits the growth of paclitaxel-resistant cells. *Mol. Pharmacol.* **52**, 613–622 (1997).
153. Martello, L. A. *et al.* Taxol and discodermolide represent a synergistic drug combination in human carcinoma cell lines. *Clin. Cancer Res.* **6**, 1978–1987 (2000).
154. Giannakakou, P. & Fojo, T. Discodermolide: just another microtubule-stabilizing agent? No! A lesson in synergy. *Clin. Cancer Res.* **6**, 1613–1615 (2000).
155. Honore, S. *et al.* Synergistic suppression of microtubule dynamics by discodermolide and paclitaxel in non-small cell lung carcinoma cells. *Cancer Res.* **64**, 4957–4964 (2004).
156. Chen, J. G. & Horwitz, S. B. Differential mitotic responses to microtubule-stabilizing and -destabilizing drugs. *Cancer Res.* **62**, 1935–1938 (2002).
157. Broker, L. E. *et al.* Late activation of apoptotic pathways plays a negligible role in mediating the cytotoxic effects of discodermolide and epothilone B in non-small cell lung cancer cells. *Cancer Res.* **62**, 4081–4088 (2002).
158. Honore, S. *et al.* Suppression of microtubule dynamics by discodermolide by a novel mechanism is associated with mitotic arrest and inhibition of tumor cell proliferation. *Mol. Cancer Ther.* **2**, 1303–1311 (2003).
159. Kar, S., Florence, G. J., Paterson, I. & Amos, L. A. Discodermolide interferes with the binding of tau protein to microtubules. *FEBS Lett.* **539**, 34–36 (2003).
160. Escuin, D., Kline, E. R. & Giannakakou, P. Both microtubule-stabilizing and microtubule-destabilizing drugs inhibit hypoxia-inducible factor-1α accumulation and activity by disrupting microtubule function. *Cancer Res.* **65**, 9021–9028 (2005).
161. Klein, L. E., Freeze, B. S., Smith, A. B. & Horwitz, S. B. The microtubule stabilizing agent discodermolide is a potent inducer of accelerated cell senescence. *Cell Cycle* **4**, 501–507 (2005).
162. Smith, A. B. 3rd *et al.* Discodermolide analogues as the chemical component of combination bacteriolytic therapy. *Bioorg. Med. Chem. Lett.* **15**, 3623–3626 (2005).
163. Mita, A. *et al.* A phase I pharmacokinetic (PK) trial of XAA296A (Discodermolide) administered every 3 wks to adult patients with advanced solid malignancies. *J. Clin. Oncol.* **22**, 2025 (2004).
164. Schwartz, R. E. *et al.* Pharmaceuticals from cultured algae. *J. Ind. Microbiol.* **5**, 113–124 (1990).
165. Trimurtulu, G. *et al.* Total structures of cryptophycins, potent antitumor depsipeptides from the blue-green-alga *Nostoc* sp strain Gsv-224. *J. Am. Chem. Soc.* **116**, 4729–4737 (1994).
166. Kobayashi, M. *et al.* Arenastatin A, a potent cytotoxic depsipeptide from the Okinawan marine sponge *Dysidea arenaria*. *Tetrahedron Lett.* **35**, 7969–7972 (1994).
167. Barrow, R. A. *et al.* Total synthesis of cryptophycins — revision of the structures of cryptophycin-A and cryptophycin-C. *J. Am. Chem. Soc.* **117**, 2479–2490 (1995).
168. Lu, K., Dempsey, J., Schultz, R. M., Shih, C. & Teicher, B. A. Cryptophycin-induced hyper-phosphorylation of Bcl-2, cell cycle arrest and growth inhibition in human H460 NSCLC cells. *Cancer Chemother. Pharmacol.* **47**, 170–178 (2001).
169. Mooberry, S. L., Taoka, C. R. & Busquets, L. Cryptophycin 1 binds to tubulin at a site distinct from the colchicine binding site and at a site that may overlap the vinca binding site. *Cancer Lett.* **107**, 53–57 (1996).
170. Kerksiek, K., Mejillano, M. R., Schwartz, R. E., George, G. I. & Himes, R. H. Interaction of cryptophycin 1 with tubulin and microtubules. *FEBS Lett.* **377**, 59–61 (1995).
171. Smith, C. D. & Zhang, X. Q. Mechanism of action of cryptophycin — interaction with the vinca alkaloid domain of tubulin. *J. Biol. Chem.* **271**, 6192–6198 (1996).
172. Panda, D., Himes, R. H., Moore, R. E., Wilson, L. & Jordan, M. A. Mechanism of action of the unusually potent microtubule inhibitor cryptophycin 1. *Biochemistry* **36**, 12948–12953 (1997).
173. Wagner, M. M. *et al. In vitro* pharmacology of cryptophycin 52 (LY355703) in human tumor cell lines. *Cancer Chemother. Pharmacol.* **43**, 115–125 (1999).
174. Sessa, C. *et al.* Phase I and pharmacological studies of the cryptophycin analogue LY355703 administered on a single intermittent or weekly schedule. *Eur. J. Cancer* **38**, 2388–2396 (2002).
175. Stevenson, J. P. *et al.* Phase I trial of the cryptophycin analogue LY355703 administered as an intravenous infusion on a day 1 and 8 schedule every 21 days. *Clin. Cancer Res.* **8**, 2524–2529 (2002).
176. Edelman, M. J. *et al.* Phase 2 study of cryptophycin 52 (LY355703) in patients previously treated with platinum based chemotherapy for advanced non-small cell lung cancer. *Lung Cancer* **39**, 197–199 (2003).
177. Liang, J. *et al.* Cryptophycins-309, 249 and other cryptophycin analogs: Preclinical efficacy studies with mouse and human tumors. *Investig. New Drugs* **23**, 213–224 (2005).
178. Pettit, G. R. The dolastatins. *Fortschr. Chem. Org. Naturst.* **70**, 1–79 (1997).
179. Pettit, G. R. *et al.* The isolation and structure of a remarkable marine animal antineoplastic constituent — Dolastatin 10. *J. Am. Chem. Soc.* **109**, 6883–6885 (1987).
180. Pettit, G. R. Progress in the discovery of biosynthetic anticancer drugs. *J. Nat. Prod.* **59**, 812–821 (1996).
181. Pettit, G. R. *et al.* The absolute-configuration and synthesis of natural (–)-Dolastatin-10. *J. Am. Chem. Soc.* **111**, 5463–5465 (1989).
182. Bai, R., Pettit, G. R. & Hamel, E. Dolastatin-10, a powerful cytostatic peptide derived from a marine animal — inhibition of tubulin polymerization mediated through the vinca alkaloid binding domain. *Biochem. Pharmacol.* **39**, 1941–1949 (1990).
183. Bai, R., Pettit, G. R. & Hamel, E. Binding of dolastatin-10 to tubulin at a distinct site for peptide antimitotic agents near the exchangeable nucleotide and vinca alkaloid sites. *J. Biol. Chem.* **265**, 17141–17149 (1990).
184. Bai, R., Friedman, S. J., Pettit, G. R. & Hamel, E. Dolastatin-15, a potent antimitotic depsipeptide derived from *Dolabella auricularia*: interaction with tubulin and effects on cellular microtubules. *Biochem. Pharmacol.* **43**, 2637–2645 (1992).
185. Verdier-Pinard, P., Kepler, J. A., Pettit, G. R. & Hamel, E. Sustained intracellular retention of dolastatin 10 causes its potent antimitotic activity. *Mol. Pharmacol.* **57**, 180–187 (2000).
186. Pitot, H. C. *et al.* Phase I trial of dolastatin-10 (NSC 376128) in patients with advanced solid tumors. *Clin. Cancer Res.* **5**, 525–531 (1999).
187. Madden, T. *et al.* Novel marine-derived anticancer agents: a phase I clinical, pharmacological, and pharmacodynamic study of dolastatin 10 (NSC 376128) in patients with advanced solid tumors. *Clin. Cancer Res.* **6**, 1293–1301 (2000).
188. Vaishampayan, U. *et al.* Phase II study of dolastatin-10 in patients with hormone-refractory metastatic prostate adenocarcinoma. *Clin. Cancer Res.* **6**, 4205–4208 (2000).
189. Krug, L. M. *et al.* Phase II study of dolastatin-10 in patients with advanced non-small-cell lung cancer. *Ann. Oncol.* **11**, 227–228 (2000).
190. Margolin, K. *et al.* Dolastatin-10 in metastatic melanoma: a phase II and pharmokinetic trial of the California Cancer Consortium. *Invest. New Drugs* **19**, 335–340 (2001).
191. Saad, E. D. *et al.* Phase II study of dolastatin-10 as first-line treatment for advanced colorectal cancer. *Am. J. Clin. Oncol.* **25**, 451–453 (2002).
192. von Mehren, M. *et al.* Phase II trial of dolastatin-10, a novel anti-tubulin agent, in metastatic soft tissue sarcomas. *Sarcoma* **8**, 107–111 (2004).
193. Perez, E. A. *et al.* Phase II trial of dolastatin-10 in patients with advanced breast cancer. *Invest. New Drugs* **23**, 257–261 (2005).
194. Kindler, H. L. *et al.* Phase II trials of dolastatin-10 in advanced pancreaticobiliary cancers. *Invest. New Drugs* **23**, 489–493 (2005).
195. Newman, D. J. & Cragg, G. M. Marine natural products and related compounds in clinical and advanced preclinical trials. *J. Nat. Prod.* **67**, 1216–1238 (2004).
196. Kobayashi, M. *et al.* Antitumor activity of TZT-1027, a novel dolastatin 10 derivative. *Jpn. J. Cancer Res.* **88**, 316–327 (1997).
197. Schoffski, P. *et al.* Phase I and pharmacokinetic study of TZT-1027, a novel synthetic dolastatin 10 derivative, administered as a 1-hour intravenous infusion every 3 weeks in patients with advanced refractory cancer. *Ann. Oncol.* **15**, 671–679 (2004).
198. de Jonge, M. J. *et al.* Phase I and pharmacokinetic study of the dolastatin 10 analogue TZT-1027, given on days 1 and 8 of a 3-week cycle in patients with advanced solid tumors. *Clin. Cancer Res.* **11**, 3806–3813 (2005).
199. Hashiguchi, N. *et al.* TZT-1027 elucidates antitumor activity through direct cytotoxicity and selective blockade of blood supply. *Anticancer Res.* **24**, 2201–2208 (2004).
200. Natsume, T. *et al.* Characterization of the interaction of TZT-1027, a potent antitumor agent, with tubulin. *Jpn. J. Cancer Res.* **91**, 737–747 (2000).
201. Hirata, Y. & Uemura, D., Halichondrins — antitumor polyether macrolides from a marine sponge. *Pure App. Chem.* **58**, 701–710 (1986).
202. Uemura, D. *et al.* Norhalichondrin A: an antitumor polyether macrolide from a marine sponge. *J. Am. Chem. Soc.* **107**, 4796–4798 (1985).
203. Pettit, G. R. *et al.* Isolation and structure of halistatin 1 from the eastern Indian Ocean marine sponge *Phakellia carteri*. *J. Org. Chem.* **58**, 2538–2543 (1993).
204. Pettit, G. R. *et al.* Antineoplastic agents. 219. Isolation and structure of the cell growth inhibitory constituents from the western Pacific marine sponge *Axinella* sp. *J. Med. Chem.* **34**, 3339–3340 (1991).

205. Bai, R. L. *et al.* Halichondrin B and homohalichondrin B, marine natural products binding in the vinca domain of tubulin. Discovery of tubulin-based mechanism of action by analysis of differential cytotoxicity data. *J. Biol. Chem.*, **266**, 15882–15889 (1991).
206. Dabydeen, D. A. *et al.* Comparison of the activities of the truncated halichondrin B analog NSC 707389 (E7389) with those of the parent compound and a proposed binding site on tubulin. *Mol. Pharmacol.* **70**, 1866–1875 (2006).
207. Aicher, T. D *et al.* Total synthesis of halichondrin B and norhalichondrin B. *J. Am. Chem. Soc.* **114**, 3162–3164 (1992).
208. Hart, J. B., Lill, R. E., Hickford, S. J. H., Blunt, J. W. & Munro, M. H. G. in *Drugs from the Sea* (ed. Fusetani, N.) 134–153 (Karger, Basel, 2000).
209. Gravalos, D. G., Lake, R., Blunt, J. W., Munro, M. H. G. & Litaudon, M. S. P. Halichondrins: cytotoxic polyether macrolides. EP0572109 (B1) (1993).
210. Munro, M. H. G. *et al.* The discovery and development of marine compounds with pharmaceutical potential. *J. Biotechnol.* **70**, 15–25 (1999).
211. Stamos, D. P., Sean, S. C. & Kishi, Y. New synthetic route to the C14-C38 segment of halichondrins. *J. Org. Chem.* **62**, 7552–7553 (1997).
212. Wang, Y., Habgood, G. J., Christ, W. J., Kishi, Y., Littlefield, B. A. & Yu, M. J. Structure–activity relationships of halichondrin B analogues: modifications at C30-C38. *Bioorg. Med. Chem. Lett.*, **10**, 1029–1032 (2000).
213. Littlefield, B. A. *et al.* Macrocyclic analogs and methods of their use and preparation. WO9965894 (A1) (1999).
214. Yu, M. J., Kishi, Y. & Littlefield, B. A. in *Anticancer Agents from Natural Products* (eds Cragg, G. M., Kingston, D. G. I. & Newman, D. J.) 241–265 (Taylor and Francis, Boca Raton, 2005).
215. Towle, M. J. *et al. In vitro* and *in vivo* anticancer activities of synthetic macrocyclic ketone analogues of halichondrin B. *Cancer Res.* **61**, 1013–1021 (2001).
216. Forero, J. B. *et al.* E7389, a novel anti-tubulin, in patients with refractory breast cancer. *J. Clin. Oncol.* **24** (Suppl. 18), 653 (2006).
217. Blum, J. L. *et al.* Phase II study of eribulin mesylate (E7389) halichondrin B analog in patients with refractory breast cancer. *J. Clin. Oncol.* **25** (Suppl. 18), 1034 (2007).
218. Spira, A. I., *et al.* Phase II study of eribulin mesylate (E7389), a mechanistically novel inhibitor of microtubule dynamics, in patients with advanced non-small cell lung cancer (NSCLC). *J. Clin. Oncol.* **25** (Suppl. 18), 7546 (2007).
219. PR Newswire. Eribulin Mesylate Demonstrated Anti-Tumor Activity in Heavily Pretreated Patients With Advanced Breast Cancer. *PR Newswire web site* [online], <http://www.prnewswire.co.uk/cgi/news/release?id=227511> (2008).
220. Hamann, M. T. & Scheuer, P. J. Kahalalide F: a bioactive depsipeptide from the Sacoglossan mollusk *Elysia rufescens* and the green alga *Bryopsis* sp. *J. Am. Chem. Soc.* **115**, 5825–5826 (1993).
221. Goetz, G., Yoshida, W. Y. & Scheuer, P. J. The absolute stereochemistry of kahalalide F. *Tetrahedron* **55**, 7739–7746 (1999).
222. Hamann, M. T., Otto, C. S., Scheuer, P. J. & Dunbar, D. C. Kahalalides: bioactive peptide from a marine mollusk *Elysia rufescens* and its algal diet *Bryopsis* sp. *J. Org. Chem.* **61**, 6594–6600 (1996).
223. Minami, Y. *et al.* Structure of cypemycin, a new peptide antibiotic. *Tetrahedron Lett.* **35**, 8001–8004 (1994).
224. Neuhof, T. *et al.* Hassallidin A, a glycosylated lipopeptide with antifungal activity from the cyanobacterium *Hassallia* sp. *J. Nat. Prod.* **68**, 695–700 (2005).
225. Becerro, M. A., Goetz, G., Paul, V. J. & Scheuer, P. J. Chemical defenses of the sacoglossan mollusk *Elysia rufescens* and its host alga *Bryopsis* sp. *J. Chem. Ecol.* **27**, 2287–2299 (2001).
226. Lopez-Macia, A., Jimenez, J. C., Royo, M., Giraet, E. & Albericio, F. Synthesis and structure determination of kahalalide F. *J. Am. Chem. Soc.* **123**, 11398–11401 (2001).
227. Bonnard, I., Manzanares, I. & Rinehart, K. L. Stereochemistry of kahalalide F. *J. Nat. Prod.* **66**, 1466–1470 (2003).
228. Garcia-Rocha, M., Bonay, P. & Avila, J. The anti-tumoral compound Kahalalide F acts on cell lysosomes. *Cancer Lett.* **99**, 43–50 (1996).
229. Suarez, Y. *et al.* Kahalalide F, a new marine-derived compound, induces oncosis in human prostate and breast cancer cells. *Mol. Cancer Ther.* **2**, 863–872 (2003).
230. Janmaat, M. L., Rodriguez, J. A., Jimeno, J., Kruyt, F. A. E. & Giaccone, G. Kahalalide F induces necrosis-like cell death that involves depletion of ErbB3 and inhibition of Akt signaling. *Mol. Pharmacol.* **68**, 502–510 (2005).
231. Sewell, J. M. *et al.* The mechanism of action of Kahalalide F: variable cell permeability in human hepatoma cell lines. *Eur. J. Cancer* **41**, 1637–1644 (2005).
232. Sparidans, R. W. *et al.* Chemical and enzymatic stability of a cyclic depsipeptide, the novel, marine-derived, anti-cancer agent kahalalide F. *Anticancer Drugs* **12**, 575–582 (2001).
233. Brown, A. P., Morrissey, R. L., Faircloth, G. T. & Levine, B. S. Preclinical toxicity studies of kahalalide F, a new anticancer agent: single and multiple dosing regimens in the rat. *Cancer Chemother. Pharmacol.* **50**, 333–340 (2002).
234. Rademaker-Lakhai, J. M. *et al.* Phase I clinical and pharmacokinetic study of Kahalalide F in patients with advanced androgen refractory prostate cancer. *Clin. Cancer Res.* **11**, 1854–1862 (2005).
235. Ciruelos, C. *et al.* A phase I clinical and pharmacokinetic (PK) study with Kahalalide F (KF) in patients (pts) with advanced solid tumors (AST) with a continuous weekly (W) 1-hour iv infusion schedule. *Eur. J. Cancer* **38** (Suppl.), S33 (2002).
236. Pettit, G. R. *et al.* Isolation and structure of bryostatin-1. *J. Am. Chem. Soc.* **104**, 6846–6848 (1982).
237. Schaufelberger, D. E. *et al.* The large-scale isolation of bryostatin-1 from *Bugula neritina* following current good manufacturing practices. *J. Nat. Prod.* **54**, 1265–1270 (1991).
238. Rouhi, M. A. Supply issues complicate trek of chemicals from sea to market. *Chem. Eng. News* **73**, 42–44 (1995).
239. Evans, D. A. *et al.* Total synthesis of bryostatin 2. *J. Am. Chem. Soc.* **121**, 7540–7552 (1999).
240. Ohmori, K. *et al.* Total synthesis of bryostatin 3. *Angew. Chem. Int. Ed. Engl.* **39**, 2290–2294 (2000).
241. Ohmori, K. Evolution of synthetic strategies for highly functionalized natural products: a successful route to bryostatin 3. *Bull. Chem. Soc. Jap.* **77**, 875–885 (2004).
242. Kageyama, M. *et al.* Synthesis of bryostatin-7. *J. Am. Chem. Soc.* **112**, 7407–7408 (1990).
243. Blanchette, M. A. *et al.* Synthesis of bryostatins.1. construction of the C(1)-C(16) fragment. *J. Org. Chem.* **54**, 2817–2825 (1989).
244. Voight, E. A., Seradi, H., Roethle, P. A. & Burke, S. D. Synthesis of the bryostatin 1 northern hemisphere (C1-C16) via desymmetrization by ketalization/ring-closing metathesis. *Org. Lett.* **6**, 4045–4048 (2004).
245. DeBrabander, J. & Vandewalle, M. Towards the asymmetric synthesis of bryostatin 1. *Pure Appl. Chem.* **68**, 715–718 (1996).
246. Isakov, N., Galron, D., Mustelin, T., Pettit, G. R. & Altman, A. Inhibition of phorbol ester-induced T-cell proliferation by bryostatin is associated with rapid degradation of protein-kinase-C. *J. Immunol.* **150**, 1195–1204 (1993).
247. Wender, P. A. *et al.* Synthesis of the first members of a new class of biologically active bryostatin analogues. *J. Am. Chem. Soc.* **120**, 4534–4535 (1998).
248. Wender, P. A. *et al.* Modeling of the bryostatins to the phorbol ester pharmacophore on protein kinase C. *Proc. Natl Acad. Sci. USA* **85**, 7197–7201 (1988).
249. Hennings, H. *et al.* Bryostatin 1, an activator of protein kinase C, inhibits tumor promotion by phorbol esters in SENCAR mouse skin. *Carcinogenesis* **8**, 1343–1346 (1987).
250. Berkow, R. L. *et al. In vivo* administration of the anticancer agent bryostatin 1 activates platelets and neutrophils and modulates protein kinase C activity. *Cancer Res.* **53**, 2810–2815 (1993).
251. Hu, Z. B., Ma, W. L., Uphoff, C. C., Lanotte, M. & Drexler, H. G. Modulation of gene-expression in the acute promyelocytic leukemia-cell line Nb4. *Leukemia* **7**, 1817–1823 (1993).
252. Grant, S., Pettit, G. R. & McCrady, C. Effect of bryostatin-1 on the *in vitro* radioprotective capacity of recombinant granulocyte macrophage colony-stimulating factor (Rgm-Csf) toward committed human myeloid progenitor cells (Cfu-Gm). *Exp. Hematol.* **20**, 34–42 (1992).
253. Mutter, R. & Wills, M. Chemistry and clinical biology of the bryostatins. *Bioorg. Med. Chem.* **8**, 1841–1860 (2000).
254. Propper, D. J. *et al.* A phase II study of bryostatin 1 in metastatic malignant melanoma. *Br. J. Cancer* **78**, 1337–1341 (1998).
255. Varterasian, M. *et al.* Phase I trial of bryostatin 1 in relapsed lymphoma and CLL. *Blood* **88**, 2269 (1996).
256. Varterasian, M. L. *et al.* Phase II study of bryostatin 1 in patients with relapsed multiple myeloma. *Invest. New Drugs* **19**, 245–247 (2001).
257. Varterasian, M. L. *et al.* Phase II trial of bryostatin 1 in patients with relapsed low-grade non-Hodgkin's lymphoma and chronic lymphocytic leukemia *Clin. Cancer Res.* **6**, 825–828 (2000).
258. Grant, S. *et al.* Phase Ib trial of bryostatin 1 in patients with refractory malignancies. *Clin. Cancer Res.* **4**, 611–618 (1998).
259. Clamp, A. & Jayson, G. C. The clinical development of the bryostatins. *Anticancer Drugs* **13**, 673–683 (2002).
260. Hickman, P. F. *et al.* Bryostatin 1, a novel antineoplastic agent and protein-kinase-C activator, induces human myalgia and muscle metabolic defects — a P-31 magnetic-resonance spectroscopic study. *Br. J. Cancer* **72**, 998–1003 (1995).
261. Pfister, D. G. *et al.* A phase II trial of bryostatin-1 in patients with metastatic or recurrent squamous cell carcinoma of the head and neck. *Invest. New Drugs* **20**, 123–127 (2002).
262. Zonder, J. A. *et al.* A phase II trial of bryostatin 1 in the treatment of metastatic colorectal cancer. *Clin. Cancer Res.* **7**, 38–42 (2001).
263. Bedikian, A. Y. *et al.* Phase II evaluation of bryostatin-1 in metastatic melanoma. *Melanoma Res.* **11**, 183–188 (2001).
264. Pagliaro, L. *et al.* A phase II trial of bryostatin-1 for patients with metastatic renal cell carcinoma. *Cancer* **89**, 615–618 (2000).
265. Clamp, A. R. *et al.* A phase II trial of bryostatin-I administered by weekly 24-hour infusion in recurrent epithelial ovarian carcinoma. *Br. J. Cancer* **89**, 1152–1154 (2003).
266. Cragg, L. H. *et al.* Phase I trial and correlative laboratory studies of bryostatin I (NSC 339555) and high-dose 1-β-D-arabinofuranosylcytosine in patients with refractory acute leukemia. *Clin. Cancer Res.* **8**, 2123–2133 (2002).
267. Dowlati, A. *et al.* Phase I and correlative study of combination bryostatin 1 and vincristine in relapsed B-cell malignancies. *Clin. Cancer Res.* **9**, 5929–5935 (2003).
268. Ajani, J. A. Multi-center Phase II study of sequential paclitaxel and bryostatin-1 (NSC 339555) in patients with untreated, advanced gastric or gastroesophageal junction adenocarcinoma. *Invest. New Drugs* **24**, 353–357 (2006).
269. Schwartz, G. K. & Shah, M. A. Targeting the cell cycle: a new approach to cancer therapy. *J. Clin. Oncol.* **23**, 9408–9421 (2005).
270. Feher, M. & Schmidt, J. M. Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. *J. Chem. Inf. Comput. Sci.* **43**, 218–227(2003).
271. Udwary, D. W. *et al.* Genome sequencing reveals complex secondary metabolome in the marine actinomycete *Salinispora tropica*. *Proc. Natl Acad. Sci. USA* **104**, 10376–10381 (2007).
272. Newman, D. J. & Hill, R. T. New drugs from marine microbes: the tide is turning. *J. Ind. Microbiol. Biotechnol.* **33**, 539–544 (2006).
273. Piel, J. Bacterial symbionts: prospects for the sustainable production of invertebrate-derived pharmaceuticals. *Curr. Med. Chem.* **13**, 39–50 (2006).
274. Hildebrand, M. *et al. bryA*: An unusual modular polyketide synthase gene from the uncultivated bacterial symbiont of the marine bryozoan *Bugula neritina*. *Chem. Biol.* **11**, 1543–1552 (2004).
275. Sudek, S. *et al.* Identification of the putative bryostatin polyketide synthase gene cluster from "*Candidatus* Endobugula sertula", the uncultivated microbial symbiont from the marine bryozoan *Bugula neritina*. *J. Nat. Prod.* **70**, 67–74 (2007).
276. Homer, *The Iliad*, (~ 7th century, B.C). Fagles, R. (translation). Penguin Books (1990).
277. Homer, *The Odyssey* (~ 7th century B. C.). Fagles, R. (translation). Penguin Books (2006).
278. Feling, R. H. *et al.* Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinospora*. *Angew. Chem. Intl. Ed.* **42**, 355–357 (2003).

279. Maldonado, L., Fenical, W., Goodfellow, M., Jensen, P. R. & Ward, A. C. *Salinispora* gen nov., sp. nov., *Salinispora arenicola* sp. nov., and *S. tropica* sp. nov., obligate marine actinomycetes belonging to the family *Micromonosporaceae*. *Internat. J. System. Appl. Microbiol.* **55**, 1759–1766 (2005).
280. Chauhan, D. *et al*. A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. *Cancer Cell* **8**, 407–419 (2005).
281. Chauhan, D. *et al*. Combination of proteasome inhibitors bortezomib and NPI-0052 trigger *in vivo* synergistic cytotoxicity in multiple myeloma. *Blood* **111**, 1654–1664 (2008).
282. Fenicala, W. et al. Discovery and development of the anticancer agent salinosporamide A (NPI-0052). *Bioorg. Med. Chem*. 5 Nov 2008 (doi:10.1016/j.bmc.2008.10.075).
This article, and several within the same issue of the journal, chronicle the discovery of key marine antitumour drugs by the original investigators and illustrates issues of procurement, scale-up, production and clinical investigations.
283. Searle, P. A. & Molinski, T. F. Phorboxazoles A and B — potent cytostatic macrolides from marine sponge *Phorbas* sp. *J. Am. Chem. Soc.* **117**, 8126–8131 (1995).
284. Searle, P. A., Molinski, T. F., Brzezinski, L. J. & Leahy, J. W. Absolute configuration of phorboxazoles A and B from the marine sponge *Phorbas* sp.1. macrolide and hemiketal rings. *J. Am. Chem. Soc.* **118**, 9422–9423 (1996).
285. Molinski T. F. Absolute configuration of phorboxazoles A and B from the marine sponge, *Phorbas* sp.2. C43 and complete stereochemistry. *Tetrahedron Lett.* **37**, 7879–7880 (1996).
286. Skepper, C. K., MacMillan, J. B., Zhou, G.-X., Masuno, M. N. & Molinski, T. F. Chlorocyclopropane macrolides from the marine sponge *Phorbas* sp. Assignment of the absolute configurations of phorbasides A and B by quantitative CD. *J. Am. Chem. Soc.* **129**, 4150–4151 (2007).
287. MacMillan, J. B., Xiong-Zhou, G., Skepper, C. K. & Molinski, T. F. Phorbasides A-E, cytotoxic chlorocyclopropane macrolide glycosides from the marine sponge Phorbas sp. CD determination of C-methyl sugar configurations. *J. Org. Chem.* **73**, 3699–3706 (2008).

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