

Minireview

Marine natural products as anticancer drugs

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Abstract

The chemical and biological diversity of the marine environment is immeasurable and therefore is an extraordinary resource for the discovery of new anticancer drugs. Recent technological and methodologic advances in structure elucidation, organic synthesis, and biological assay have resulted in the isolation and clinical evaluation of various novel anticancer agents. These compounds range in structural class from simple linear peptides, such as dolastatin 10, to complex macrocyclic polyethers, such as halichondrin B; equally as diverse are the molecular modes of action by which these molecules impart their biological activity. This review highlights several marine natural products and their synthetic derivatives that are currently undergoing clinical evaluation as anticancer drugs. [Mol Cancer Ther 2005;4(2):333–42]

Introduction

An exciting “marine pipeline” of new anticancer clinical and preclinical agents has emerged from intense efforts over the past decade to more effectively explore the rich chemical diversity offered by marine life (Table 1). It is not truly known how many species inhabit the world’s oceans; however, it is becoming increasingly clear that the number of microbial species is many times larger than previously estimated, such that total marine species may approach 1 to 2 million. Whereas the oceans are vast and constitute 70% of the world’s surface, the majority of this species diversity is found in the ocean fringe. This slender land-sea interface with its high concentration of species is among the most biodiverse and productive environments on the planet. Deep ocean thermal vent communities represent another highly biodiverse and productive habitat, albeit one of limited extent. By contrast, open ocean waters are generally low in nutrients and have been likened to deserts in terms of biomass and species diversity, although recent evidence

suggests the existence of substantial microbial diversity in pelagic waters (1). It can be estimated that <1% of the earth’s surface, the narrow ocean fringe, and the known deep sea vent communities, are home to a majority of the world’s species, and thus constitute the most species rich and biologically productive regions of the world.

The intense concentration of species coexisting in these limited extent habitats necessarily makes them highly competitive and complex. Sessile macroscopic organisms such as algae, corals, sponges, and a variety of other invertebrates are in constant battle for suitable attachment space. This competition occurs both in spatial as well as temporal domains. Fish and other motile species are typically both prey and predator, and specialization of feeding habits, body shape, and behavioral characteristics are common adaptations. Nutrient, light, water current, and temperature represent additional growth limiting components, further fueling competition. As a result of this intense competition, a high percentage of species have evolved chemical means by which to defend against predation, defend against overgrowth by competing species, or conversely, to subdue motile prey species for ingestion (2). These chemical adaptations (3) generally take the form of so-called “secondary metabolites,” and involve such well-known chemical classes as terpenoids, alkaloids, polyketides, peptides, shikimic acid derivatives, sugars, steroids, and a multitude of mixed biogenesis metabolites. In addition, and unique to the marine environment, is the relatively common utilization of covalently bound halogen atoms in secondary metabolites, mainly chlorine and bromine, presumably due to their ready availability in seawater.

The past decade has seen a dramatic increase in the number of preclinical anticancer lead compounds from diverse marine life enter human clinical trials. This has occurred in part during a period of some retrenchment in the field of natural products in general and may cause some to rethink the wisdom of prematurely departing from this highly productive pursuit (4). Nevertheless, it is useful to consider the evolution of the field of marine natural products drug discovery in this context as it may help to identify future directions which will be even more successful. The earliest efforts in this field derived from the interests of marine biologists and naturalists who found a number of unique toxins that were present in diverse marine life. Cone snails inject incredibly potent peptide toxins (the conotoxins) to immobilize prey fish (5). Lionfish spines carry a lethal protein venom to the unwary (6). Zooanthids from a tide pool in Oahu, HI possess an extraordinarily toxic polyketide, “palytoxin” making them unpalatable to potential predators (7). Microalgae produce

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Table 1. Current status of marine natural products in anticancer preclinical or clinical trials

Compound (figure number)	Source organism	Chemical class	Molecular target	Current status
Ecteinascidin 743 (Fig. 4A; Yondelis)	<i>Ecteinascidia turbinata</i> (tunicate; possible bacterial source)	Tetrahydroisoquinolone alkaloid	Tubulin	Phase II
Dolastatin 10 (Fig. 3A)	<i>Dolabella auricularia</i> / <i>Symploca</i> sp. (mollusc/cyanobacterium)	Linear peptide	Tubulin	Phase II
Bryostatin 1	<i>Bugula neritina</i> (bryozoan)	Macrocyclic lactone	PKC	Phase II
Synthadotin (Fig. 3E; ILX651, dolastatin 15 derivative)	<i>Dolabella auricularia</i> / <i>Symploca</i> sp. (synthetic analogue)	Linear peptide	Tubulin	Phase II
Kahalalide F	<i>Elysia rufescens</i> / <i>Bryopsis</i> sp. (mollusc/green alga)	Cyclic depsipeptide	Lysosomes/erbB pathway	Phase II
Squalamine	<i>Squalus acanthias</i> (shark)	Aminosteroid	Phospholipid bilayer	Phase II
Dehydridemnin B (Fig. 2B; Aplidine)	<i>Trididemnum solidum</i> (tunicate, synthetic; possible bacterial/cyanobacterial source)	Cyclic depsipeptide	Ornithine decarboxylase	Phase II
Didemnin B (Fig. 2A)	<i>Trididemnum solidum</i> (tunicate)	Cyclic depsipeptide	FK-506 bp	Phase II (discontinued)
Cemadotin (Fig. 3D; LU103793, dolastatin 15 derivative)	<i>Dolabella auricularia</i> / <i>Symploca</i> sp. (synthetic analogue)	Linear peptide	Tubulin	Phase II (discontinued)
Soblidotin (Fig. 3B; TZT-1027, dolastatin 10 derivative)	<i>Dolabella auricularia</i> / <i>Symploca</i> sp. (synthetic analogue)	Linear peptide	Tubulin	Phase I
E7389 (Fig. 4D; halichondrin B derivative)	<i>Halichondria okadai</i> (sponge, synthetic)	Macrocyclic polyether	Tubulin	Phase I
NVP-LAQ824 (Fig. 1E; Psammaplin derivative)	<i>Psammaplysilla</i> sp. (sponge, synthetic)	Indolic cinnamyl hydroxamate	HDAC/DNMT	Phase I
Discodermolide	<i>Discodermia dissolute</i> (sponge)	Lactone	Tubulin	Phase I
HTI-286 (Hemiasterlin derivative)	<i>Cymbastella</i> sp. (synthetic analogue of sponge metabolite)	Linear peptide	Tubulin	Phase I
LAF-389 (Bengamide B derivative)	<i>Jaspis digonoxea</i> (sponge, synthetic)	ϵ -Lactam peptide derivative	Methionine aminopeptidase	Phase I
KRN-7000 (Agelasphin derivative)	<i>Agelas mauritianus</i> (sponge, synthetic)	α -Galacosylceramide	V α 24 + NKT cell activation	Phase I
Curacin A	<i>Lyngbya majuscula</i> (cyanobacterium)	Thiazole lipid	Tubulin	Preclinical
DMMC	<i>Lyngbya majuscula</i> (cyanobacterium)	Cyclic depsipeptide	Tubulin	Preclinical
Salinosporamide A	<i>Salinospora</i> sp. (bacterium)	Bicyclic γ -lactam- β lactone	20S proteasome	Preclinical
Laulimalide	<i>Cacospongia mycofijiensis</i> (sponge)	Macrolide	Tubulin	Preclinical
Vitilevuamide	<i>Didemnin cucliferum</i> / <i>Polysyncracion lithostrotum</i> (tunicates)	Cyclic peptide	Tubulin	Preclinical
Diazonamide	<i>Diazona angulata</i> (tunicate)	Cyclic peptide	Tubulin	Preclinical
Eleutherobin	<i>Eleutherobia</i> sp. / <i>Erythropodium caribaeorum</i> (soft corals)	Diterpene glycoside	Tubulin	Preclinical
Sarcodictyin	<i>Sarcodictyon roseum</i> (sponge)	Diterpene	Tubulin	Preclinical
Peloruside A	<i>Mycale hentscheli</i> (sponge)	Macrocyclic lactone	Tubulin	Preclinical
Salicylhalimides A and B	<i>Haliclona</i> sp. (sponge)	Polyketide	Vo-ATPase	Preclinical
Thiocoraline	<i>Micromonospora marina</i> (bacterium)	Depsipeptide	DNA-polymerase	Preclinical
Ascididemin	<i>Didemnum</i> sp. (sponge)	Aromatic alkaloid	Caspase-2/mitochondria	Preclinical
Variolins	<i>Kirkpatrickia variolosa</i> (sponge)	Heterocyclic alkaloid	Cdk	Preclinical
Lamellarin D	<i>Lamellaria</i> sp. (mollusc and various soft corals)	Pyrrrole alkaloid	Topoisomerase I / mitochondria	Preclinical
Dictyodendrins	<i>Dictyodendrilla verongiformis</i> (sponge)	Pyrrrolocarbazole derivatives	Telomerase	Preclinical
ES-285 (Spisulosine)	<i>Mactromeris polynyma</i> (mollusc)	Alkylamino alcohol	Rho (GTP- bp)	Preclinical
Dolastatin 15 (Fig. 3C)	<i>Dolabella auricularia</i> (mollusc)	Linear peptide	Tubulin	Preclinical (discontinued)
Halichondrin B (Fig. 4C)	<i>Halichondria okadai</i> (sponge)	Macrocyclic polyether	Tubulin	Preclinical (discontinued)

NOTE: See Refs. 53, 78.

incredibly potent alkaloidal neurotoxins such as saxitoxin and polyketide neurotoxins such as the brevetoxins. Early investigations gave rise to broad surveys of marine life for novel natural products with useful biological properties; however, these initial efforts clearly prioritized description of unique structural chemistry rather than discovering drugs or drug leads. Efforts became more serious and focused through a series of agency-supported programs, with the National Cooperative Drug Discovery Program of the National Cancer Institute (8) playing a key role. This inspired program recognized that the rich chemistry of marine organisms was not translating into useful drug leads largely because of poorly developed connections between academic researchers and the pharmaceutical industry. By forging collaborative interactions between groups of academic investigators and major pharmaceutical companies as well as the National Cancer Institute, a critical mass of natural product materials, modern assays and development know-how was assembled, and this has translated into several clinical trial agents (see Table 1 and examples given below).

This brief perspective is intended to showcase several marine natural products or derivatives which are advancing through anticancer clinical trials and which illustrate the success of modern academic-industry-government collaborations. Additionally, these examples have been picked, and are generally representative of, the importance of microbial processes in the generation of some of the most bioactive and potentially useful marine natural products. Indeed, marine microalgae, cyanobacteria, and heterotrophic bacteria living in association with invertebrates (e.g. sponges, tunicates, and soft corals) have been identified, or strongly suspected, as the true sources of many bioactive and useful constituents (see Table 1). Cell sorting, culture, and molecular biological methods are helping to clarify this intriguing aspect of marine metabolism as described in the conclusion of this perspective review (9).

Psammaplins from Verongid Sponges

Initial isolations of the bromotyrosine metabolite psammaplin A (Fig. 1A) from various verongid sponges (e.g., *Psammaphysilla* sp.) were reported simultaneously by several research groups in 1987. Psammaplin A, a symmetrical bromotyrosine disulfide possessing oxime moieties, was found to have potent cytotoxicity to P388 cells (IC_{50} of 0.3 $\mu\text{g}/\text{mL}$), and to co-occur with a dimeric metabolite, biprasin (Fig. 1B; refs. 10–12). Additional psammaplin compounds have since been isolated, including various sulfated and salt derivatives (psammaplins B–L), and the degraded cysteine dimer, prepsammaplin A. Several of these were found to possess potent antibacterial activity. The fact that the psammaplins have been isolated from a diversity of sponge “sources” and that brominated aromatic amino acid derivatives are common in marine bacteria suggests that these metabolites may actually derive from biosynthetic pathways of microorganisms living in association with sponges.

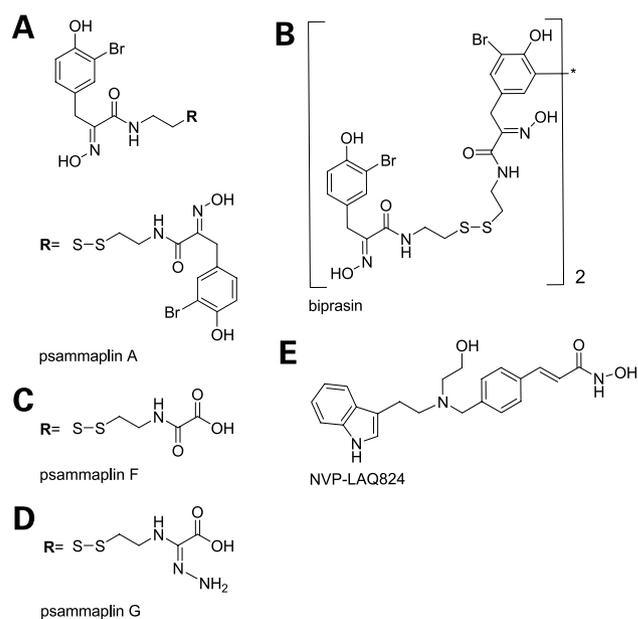


Figure 1. Structures of compounds discussed in text, including (A) psammaplin A, a Verongid sponge derived bromotyrosine disulfide with histone deacetylase and DNA methyltransferase inhibitory properties; (B) biprasin, a psammaplin A dimer; (C) psammaplin F, a selective histone deacetylase inhibitor; (D) psammaplin G, a selective DNA methyltransferase inhibitor; and (E) NVP-LAQ824, a synthetic psammaplin derivative currently in clinical trials (however, see footnote 1).

Recently, testing of known and new psammaplin metabolites as DNA methyl transferase and histone deacetylase inhibitors has been reported (13). Remarkably, psammaplin A (Fig. 1A) and biprasin (Fig. 1B) proved to be dual inhibitors of the two enzymes tested, which is a significant finding in light of the potential relationship between DNA methyl transferase and histone deacetylase as epigenetic modifiers of tumor suppressor gene activity. In addition, psammaplin F (Fig. 1C) is a selective histone deacetylase inhibitor, whereas psammaplin G (Fig. 1D) is a selective DNA methyl transferase inhibitor. Psammaplin A has also been reported to inhibit topoisomerase II (14) and aminopeptidase N with *in vitro* angiogenesis suppression (15). However, the physiologic instability of the psammaplin class has thus far precluded their direct clinical development. Nevertheless, these initial efforts inspired the development of an analogue substance, NVP-LAQ824 (Fig. 1E; ref. 16). This indolic cinnamyl hydroxamate has recently entered phase I clinical trials in patients with solid tumors or leukemia.

In preclinical studies, NVP-LAQ824 as well as several other synthetic analogues showed potent *in vitro* antitumor activity, high maximum tolerated dose (>200 mg/kg, as the monolactate) and low host toxicity in HCT116 colon and A549 human lung xenografts. Investigations using HCT116 colon, A549 lung and normal dermal human fibroblast cell lines showed that NVP-LAQ824 causes apoptosis in tumor cell lines at concentrations that induce growth arrest in the normal dermal human fibroblast cell line. Toxicity evaluation in rats identified hematopoietic and lymphatic systems as the major target organs with reversible dose dependent reduction in RBC and WBC counts and

lymphoid atrophy. These results indicated that at high doses the toxicity of NVP-LAQ824 may be similar to that of other cytotoxic agents; however, it is anticipated that this can be controlled by appropriate scheduling. In light of these findings, NVP-LAQ824 entered phase I clinical trials in patients with solid tumors or leukemia.¹

Didemnin B from a Tunicate Harboring Diverse Cyanobacterial Symbionts

Didemnin B (ref. 17; Fig. 2A), a cyclic antiproliferative depsipeptide isolated from the Caribbean tunicate *Trididemnum solidum* (18), was the first marine natural product to enter clinical trial as an antitumor agent (19). Based on a close structural resemblance of the didemnins to known cyanobacterial metabolites, Rinehart speculated that these potent cytotoxins likely derive from symbiotic cyanobacterium living in association with the tunicate (20). It showed antitumor activity against a variety of models and has been investigated in phase II clinical trials for the treatment of breast, ovarian, cervical, myeloma, glioblastoma/astrocytoma, and lung cancers. Moreover, didemnin B displays several *in vitro* biological activities, albeit with widely varying potencies (>5 orders of magnitude; ref. 21), suggesting that the activities are mediated by different mechanisms. Didemnin B (Fig. 2A) inhibits the synthesis of RNA, DNA, and proteins (22) and binds noncompetitively to palmitoyl protein thioesterase (23). Moreover, rapamycin inhibits the didemnin-induced apoptosis of human HL60 cells, suggesting activation of the FK-506 apoptotic pathway. Didemnin B perhaps modulates the activity of FK-506 binding proteins as part of its immunomodulatory process and thus leads to cell death via apoptosis (24).

Despite a variety of treatment protocols and testing against many different cancer types, the compound was simply too toxic for use, which led to the termination of trials by the National Cancer Institute in 1990. The experience gained from these trials led to the synthesis of related molecules, such as aplidine (Fig. 2B; ref. (25)). Similar to didemnin B, aplidine interferes with the synthesis of DNA and proteins and induces cell cycle arrest (26). Moreover, aplidine possesses a unique and differential mechanism of cytotoxicity that involves the inhibition of ornithine decarboxylase, an enzyme critical in the process of tumor growth and angiogenesis. Furthermore, unlike didemnin B, aplidine blocks protein synthesis at the stage of polypeptide elongation (27). This cytotoxicity is induced independently of multidrug resistance or p53 status and has shown antiangiogenic effects by decreasing the secretion of vascular endothelial growth factor (VEGF) and reducing the expression of the VEGF-r1 receptor (28, 29).

In preclinical studies, aplidine (Fig. 2B) was more active than didemnin B and displayed substantial activity against

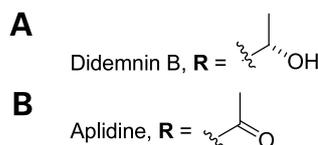
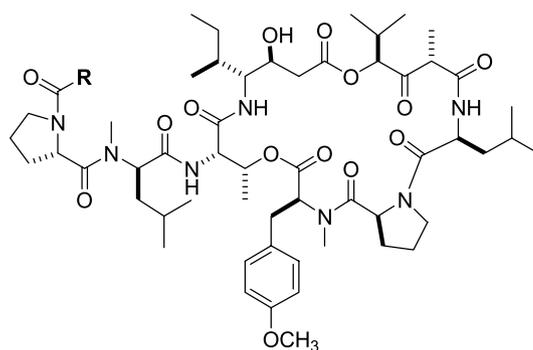


Figure 2. Structures of compounds discussed in text, including: **(A)** didemnin B, a tunicate/prochloron-derived cyclic depsipeptide previously in clinical trials and **(B)** aplidine, a drug lead for treatment of leukemia and lymphoma currently in clinical trials.

a variety of solid tumor models, including tumors noted to be resistant to didemnin B (23). Based on its preclinical activity, aplidine entered phase I clinical trials in patients with solid tumors and lymphomas. Treatment with aplidine has generally been well tolerated, with the most common adverse events being asthenia, nausea, vomiting, and transient transaminitis. Hypersensitivity reactions have also been reported. The agent does not induce hematologic toxicity, mucositis, or alopecia. The occurrence of neuromuscular toxicity with the elevation of creatine kinase levels has been dose limiting in three of these studies. Selected biopsies of affected muscles revealed muscular atrophy and loss of thick myosin filaments (27, 30). Interestingly, coadministration of L-carnitine seems to prevent and ameliorate muscular toxicity (31). Apladin, a registered trademark of aplidine (Fig. 2B), was found to selectively target and preferentially kill human leukemic cells in blood samples derived from children and adults at concentrations that are attainable in patients and well below the toxic level.² In these studies, Apladin was more selective towards leukemia and lymphoma cells than towards normal cells. In addition, the activity of Apladin was found independent of other anticancer drugs commonly used in leukemia and lymphoma, suggesting that Apladin may be effective in cases that have proved unresponsive to other agents. The success of aplidine in phase I trials has led to its current evaluation in phase II trials against solid tumors.

Dolastatin 10 from Sea Hares and Their Cyanobacterial Diets

In the early 1970s, Pettit et al. discovered the extremely potent anticancer properties of extracts from the sea hare

¹ The public literature available as of this writing indicates that NVP-LAQ824 is currently undergoing clinical evaluation; however, recent personal communications suggest that these trials have been discontinued.

² <http://www.pharmamar.com/en/pipeline/aplidin.cfm>

Dolabella auricularia. However, due to the vanishingly small abundance of the active principle (~1.0 mg/100 kg of collected organism), the structure elucidation of dolastatin 10 (Fig. 3A) took nearly 15 years to complete. The low concentrations of dolastatin 10 (Fig. 3A) in sea hares implicates a cyanobacterial diet as the origin of this bioactive secondary metabolite (32), and this was subsequently confirmed by direct isolation of dolastatin 10 from field collections of the marine cyanobacterium *Symploca* (33). Dolastatin 10 is a pentapeptide with four of the residues being structurally unique (dolavaline, dolaisoleucine, dolaproline, and dolaphenine, in addition to valine). Interestingly, at the time of its discovery, it was the most potent antiproliferative agent known with an $ED_{50} = 4.6 \times 10^{-5}$ $\mu\text{g}/\text{mL}$ against murine PS leukemia cells (34). Subsequently, dolastatin 10 was shown to be a potent noncompetitive inhibitor of *Vinca* alkaloid binding to tubulin ($K_i = 1.4 \mu\text{mol}/\text{L}$) and strongly affected microtubule assembly and tubulin-dependent guanosine triphosphate hydrolysis (35). Further work revealed that dolastatin 10 binds to the rhizoxin/maytansine binding site (ref. 36; adjacent to *Vinca* alkaloid site) as well as to the exchangeable guanosine triphosphate site on tubulin, causing cell cycle arrest in metaphase (37).

Dolastatin 10 (Fig. 3A) entered phase I clinical trials in the 1990s through the National Cancer Institute and progressed to phase II trials. Unfortunately, it was dropped from clinical trials, as a single agent, due to the development of moderate peripheral neuropathy in 40% of patients (38) and insignificant activity in patients with hormone-refractory metastatic adenocarcinoma (39) and recurrent platinum-sensitive ovarian carcinoma (40). Nevertheless, dolastatin 10 offered a logical starting point for SAR studies and synthetic drug design, ultimately leading to the analogue TZZ-1027 (Fig. 3B).

TZZ-1027 (Soblidotin; Auristatin PE; Fig. 3B) was designed with the goal of maintaining the potent antitumor activity while reducing the toxicity of the parent compound (41). TZZ-1027's structure differs from dolastatin 10 (Fig. 3A) only in the absence of the thiazole ring from the original dolaphenine residue, resulting in a terminal benzylamine moiety. Intravenous injections of TZZ-1027 in mice results in significant inhibition of P388 leukemia growth and the diminution of three solid tumor cell lines (colon 26 adenocarcinoma, B16 melanoma, and M5076 sarcoma) with equivalent or greater efficacy than dolastatin 10. Additionally, TZZ-1027 was effective in the two human xenograph models, MX-1 breast carcinoma and LX-1 lung carcinoma (42).

DNA-damaging agents are less effective against tumors with a mutant or absent *p53* gene; however, antitubulin drugs generally maintain efficacy against such tumors. Indeed, TZZ-1027 (Fig. 3B) shows equivalent potency in the *p53* normal and mutant cell lines, and hence, provides a potent therapeutic agent irrespective of *p53* status (43). TZZ-1027 also exhibits potent antitumor activity against both early and advanced stages of SBC-3/Neo and SBC-3/VEGF tumors. TZZ-1027 apparently interacts with VEGF, resulting in a significant accumulation of erythrocytes and enhanced damage to tumor vasculature. Ultimately, this cascade of

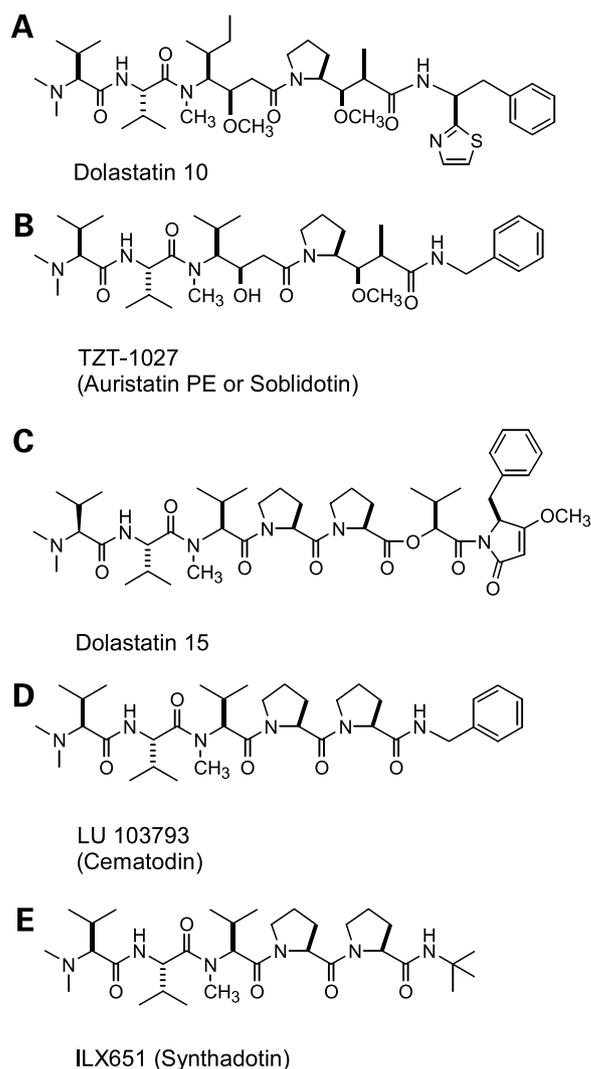


Figure 3. Structures of compounds discussed in text, including (A) dolastatin 10, a linear peptide, now known from cyanobacterial sources, that has prompted the synthesis of several important synthetic derivatives; (B) TZZ-1027 (Auristatin; Soblidotin), a dolastatin 10 derivative currently in clinical trials; (C) dolastatin 15, a linear cyanobacterial depsipeptide previously in clinical trials; (D) LU 103793 (Cematodin), a dolastatin 15 derivative previously in clinical trials; and (E) ILX651 (Synthadotin), a dolastatin 15 derivative currently in clinical trials.

events results in necrosis of the tumor due to a depletion of oxygen and essential nutrients. It is encouraging that TZZ-1027 is a potent cytotoxic and antiproliferative agent against both early and late stage SBC-3/VEGF tumors (44).

Dolastatin 15, Another Cyanobacterial Peptide Isolated from a Sea Hare

Dolastatin 15 (Fig. 3C) was also isolated from extracts of the Indian Ocean sea hare *D. auricularia* in trace amounts [6.2 mg from 1,600 kg of wet sea hare ($4 \times 10^{-7}\%$)], again strongly implicating a cyanobacterial source for this metabolite. Indeed, numerous dolastatin 15-related peptides have been

isolated from diverse marine cyanobacteria (45). Its linear depsipeptide sequence is composed of seven amino acid or hydroxyl acid residues. In initial bioassays with the National Cancer Institute's P388 lymphocytic leukemia cell line, dolastin 15 (Fig. 3C) displayed an $ED_{50} = 2.4 \times 10^{-3}$ $\mu\text{g}/\text{mL}$ (46). In contrast to dolastatin 10 (Fig. 3A), dolastatin 15 binds directly to the *Vinca* domain of tubulin (47). Obstacles to further clinical evaluation of dolastatin 15 include the complexity and low yield of its chemical synthesis and its poor water solubility. However, these impediments have prompted the development of various synthetic analogue compounds with enhanced chemical properties, including cematodin (Fig. 3D) and synthadotin (Fig. 3E).

In 1995, cematodin (LU-103793; Fig. 3D) was synthesized as a water-soluble and water-stabilized analogue of dolastatin 15 with a terminal benzylamine moiety in place of the original dolapyrrolidone. Cematodin retains the high *in vitro* cytotoxicity of the parent compound ($IC_{50} = 0.1$ $\mu\text{mol}/\text{L}$), disrupts tubulin polymerization ($IC_{50} = 7.0$ $\mu\text{mol}/\text{L}$), and induces depolymerization of preassembled microtubules. Cell cycle arrest occurs at the G_2 -M phase transition (48). Recently, cematodin underwent six phase I clinical studies with dose-limiting toxicity, including cardiac toxicity, hypertension, and acute myocardial infarction. Overall, neutropenia was the most common dose-limiting effect observed in phase I testing (30, 49). Unfortunately, phase II evaluations with malignant melanoma, metastatic breast cancer, and non-small cell lung cancer have produced no objective results to date (50–52). Therefore, current clinical evaluation of LU-103793 has been discontinued (53).

ILX-651 (Synthadotin; Fig. 3E) is an orally active third generation synthetic dolastatin 15 analogue possessing a terminal *tert*-butyl moiety (versus the original dolapyrrolidone). ILX-651 is currently in three phase II clinical trials for patients with locally advanced or metastatic non-small cell lung cancer and patients with hormone-refractory prostate cancer previously treated with Docetaxel (53).³ Results of a phase II study where ILX-651 was given daily for five consecutive days on a three week schedule in patients with inoperable locally advanced or metastatic melanoma indicate that it is "a safe, well-tolerated treatment for locally advanced and metastatic melanoma patients" (54).

Ecteinascidin-743, an Alkaloid from Tunicates Rich in Symbionts

From early surveys of marine organisms for anticancer-type activity, the aqueous extracts of the Caribbean tunicate *Ecteinascidia turbinata* were known to contain potent substances. The molecular structures of the ecteinascidin alkaloids were first deduced as complex tetrahydroisoquinolones (55, 56). Ecteinascidin-743 (ET-743; Fig. 4A) was the major metabolite, and although less potent *in vivo* than its *N*-demethyl analogue (ET-729), its cytotoxicity (IC_{50} 0.5 ng/mL versus L1210 leukemia cells),

stability and relatively high natural abundance made it most suitable for clinical development. However, mechanism of action and preclinical *in vivo* evaluation studies were hampered by a lack of material. Large-scale collections, aquaculture and synthetic efforts have all been employed (53), and culminated in the development of a semisynthesis of ET-743 from cyanosafraclin B (Fig. 4B), which was obtained in bulk through fermentation of the marine bacterium *Pseudomonas fluorescens*. Ecteinascidin's structure is consistent with a natural microbial origin (e.g., the saframycins). Indeed, there are two patents for bacterial symbionts of the tunicate *E. turbinata*. The first focuses on the isolation of the producing microbe (57), whereas the second uses 16S rDNA sequences to identify the endosymbiont as *Endoecteinascidia frumentensis*, the apparent producer of the ecteinascidins (58).

A semisynthetic approach to ET-743 (Fig. 4A) was accomplished (European brand name Yondelis, generic name trabectedin; ref. 59). ET-743 quickly progressed to phase I clinical trials after showing a high therapeutic index and potency in preclinical studies. More recently, ET-743 has been reported to bind in the minor groove of DNA to induce an unprecedented bend in the DNA helix towards the major groove (60). The multifaceted mechanism of action of ET-743 includes interference with the cellular transcription-coupled nucleotide excision repair to induce cell death and cytotoxicity which is independent of p53 status yet occurs with multidrug resistance elicitation (53, 59). Overall, advanced ovarian, breast, and mesenchymal tumors which had been heavily pretreated with platinum/taxanes showed greatest response to ET-743 in phase I trials (30, 61). In phase II trials, ET-743 was most effective in patients with refractory soft tissue sarcoma (STS), ovarian, and breast cancer. However, difficulties in establishing the drug's efficacy in STS prevented its approval in 2003. Meanwhile, European Union's Committee for Proprietary Medicinal Products has granted ET-743 (Fig. 4A) orphan drug status for the treatment of refractory ovarian cancer.

ET-743 had been previously granted orphan drug status for treatment of STS by the Committee for Orphan Medicinal Products in Europe. Phase II clinical trials in the United States and Europe continue for ovarian, STS, endometrial, breast, prostate, and non-small cell lung cancer, with notable recent success in combination drug therapy (53). At the beginning of phase II programs with protracted infusion schedules, ET-743 induced severe, life-threatening toxicities such as pancytopenia, rhabdomyolysis, and renal and hepatic failure. Baseline biliary function was identified as a reference variable to establish the eligibility of patients to receive full doses of ET-743. In addition, an intercycle peak in bilirubin and/or alkaline phosphatase indicated high risk in subsequent cycles at full dose. These results established reliable clinical variables for ET-743 dosing schedules. Interim results from phase II trials were recently presented for recurrent sarcomas (61), ovarian (62), and endometrial (63) carcinomas at the 2004 American Society of Clinical Oncology meeting. In summary, when given over 3 hours, antitumor activity of

³ <http://www.ilxon.com>

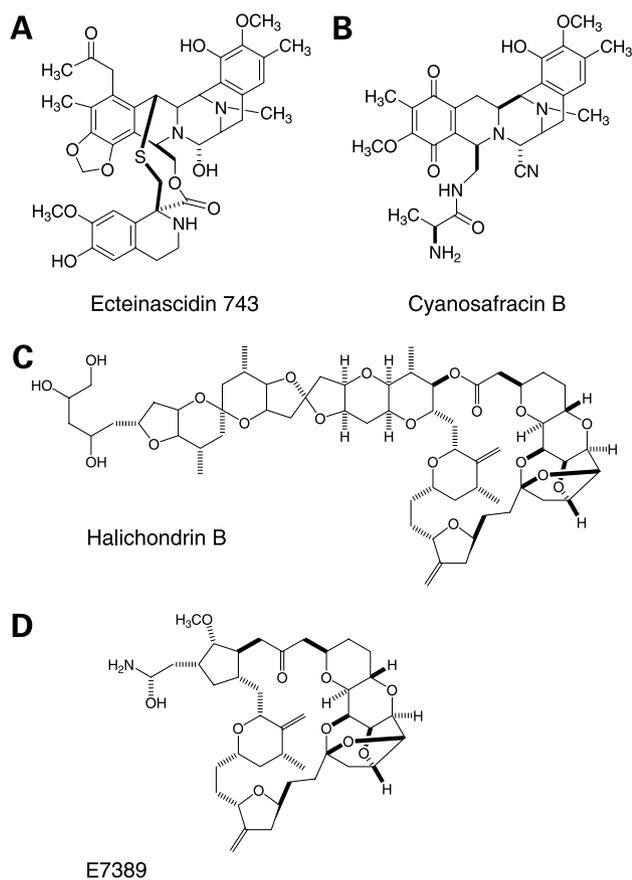


Figure 4. Structures of compounds discussed in text, including (A) ecteinascidin 743 (ET-743), a tetrahydroisoquinolone alkaloid currently in clinical trials for treatment of various cancers; (B) cyanosafraclin B, the bacterial-derived starting material used in the synthesis of ET-743; (C) halichondrin B, a complex polyether with exceptional antimetabolic activity, previously in preclinical trials; and (D) E7389, a halichondrin analogue currently in clinical trials.

ET-743 in STS is in the same range observed after infusion over 24 hours, the activity of ET-743 in ovarian cancer was confirmed with a well-tolerated weekly schedule, and ET-743 is active in endometrial carcinoma when given as a single agent in 3-hour infusions every 3 weeks, with notable toxicities being elevated alanine aminotransferase levels, neutropenia, and asthenia.

Halichondrin B, a Complex Polyether from Diverse Sponges

Some natural products, including many of those isolated from marine animals such as sponges, tunicates, and their various predators exhibit such structural complexity so as to be nearly unimaginable drug candidates. Examples include compounds such as palytoxin, maitotoxin, and the halichondrins (e.g., Fig. 4C). However, because of their phenomenal potency, even very small quantities of these agents can be valuable in a commercial sense. Palytoxin and maitotoxin are both available as research biochemicals

with natural sources yielding the commercial material. In the case of halichondrin, the exciting anticancer potential of this “sponge” metabolite has fueled an innovative chemical synthesis approach which is providing synthetic material for phase I trials.

The halichondrins were first isolated from the Japanese sponge *Halichondria okadai* by Uemura et al. and structures determined by X-ray crystallography (64). Subsequently, halichondrin B (Fig. 4C) and several natural analogues were isolated from various unrelated sponges, including *Lissodendoryx* sp., *Phakellia carteri*, and *Axinella* sp., and thus strongly suggests that this skeletal type may be constructed by an associated microorganism. A number of studies subsequently examined their mechanism of cell toxicity, and it was discovered that the halichondrins are potent tubulin inhibitors, in this case noncompetitively binding to the *Vinca* binding site and causing a characteristic G₂-M cell cycle arrest with concomitant disruption of the mitotic spindle (65, 66).

Because of their phenomenal biological activity in killing cancer cells and great structural complexity, the halichondrins rapidly became targets for chemical synthesis. The first total synthesis was completed in 1990 (67). The Kishi group focused on the synthesis of structurally simplified halichondrin analogues which retained or had enhanced biological properties, and this eventually led to the discovery of the clinical candidate E7389 (Fig. 4D). In addition to a substantial truncation of the left-hand section of halichondrin B, E7389 also possess a ketone which replaces a key destabilizing ester in the right half of halichondrin B (Fig. 4C; refs. 68, 69). Despite the roughly 35 steps and <1.0% overall yield to E7389, it remains a tenable clinical candidate because of its ultrapotency, and hence, a relatively small mass of drug is sufficient to conduct clinical trials, and ultimately, treat patients. Phase I clinical trials with E7389 (Fig. 4D) have been initiated using an accelerated dose escalation schedule to evaluate maximum tolerated dose and pharmacokinetics. Dose limiting toxicity was reached in one patient at a single dose of 0.5 mg/m², and a three-compartment model best described the plasma pharmacokinetics. Plasma levels of E7389 in excess of those required for cytotoxicity were observed in all patients for up to 72 hours, and patients with solid tumors are currently being recruited for additional phase I trials (70, 71).

Discussion and Conclusion

The above examples illustrate the intense excitement which surrounds the past decade’s achievements in the area of new anticancer leads from marine organisms. The combination of novel structures and for some, novel mechanisms of action, is translating into new methods by which to treat cancer, and ultimately, improved outcomes, particularly for patients with solid tumors of the lung, breast, colon or prostate. The last decade has seen an ever evolving strategy for the screening and discovery of new anticancer leads from nature, and this is proving effective. From former

times of evaluation of crude extracts by *in vivo* screens to current evaluation of peak or prefractionated libraries in mechanism-based assays, the level of sophistication and success has steadily improved.

Screening strategies are continuing to evolve, probing new ideas and knowledge of cancer, introducing high throughput screening and new analytic methodologies. In part, the need for this continuing evolution has been stimulated by a desire to develop novel and less toxic therapies for cancer treatment. High throughput screening methods have both enabled more sophisticated mechanism based screening, and subsequently required the move to prefractionation and peak library generation. These "prior-to-screening" purifications have the consequence of reducing the complexity of screening materials, increasing the titer of low abundance components, segregating nuisance substances into discrete fractions, and generally speeding up the time line from detection of a primary screening hit to identification of a molecular structure for the active substance. It can generally be concluded that contemporary screening protocols in natural products chemistry are using chromatographic purification steps, sometimes producing pure compounds, before biological or enzymatic bioassay. Coupled to these more effective paradigms for screening are new assays that evaluate natural products in more detailed, refined, and novel ways. For example, detailed knowledge of the cellular mechanisms controlling proliferation has yielded numerous targets for mechanism-based anticancer screens.

A long-standing and perplexing question in marine natural products chemistry has been the identification of the metabolite producing organism or potentially metabolite biotransforming organism, in systems involving an invertebrate host and symbiotic microorganisms. This has been a surprisingly difficult issue to satisfactorily answer except in a very few cases, largely because of the difficulty of growing various microorganisms separately from their hosts (72). An alternative approach that has met with some success has been to isolate the various cell populations by centrifugation, sieving, or fluorescence-activated cell sorting, and then chemically analyze these samples for metabolites of interest. For example, in the case of the sponge *Dysidea herbacea* and its symbiotic cyanobacterium *Oscillatoria spongellae*, this has been accomplished on a couple of occasions and generally supports metabolic trends observed from working with pure strains of cyanobacteria (73, 74). The cyanobacterial cells were found to contain a series of highly distinctive chlorinated peptides, previously isolated from work with the intact sponge, and which have strong structural precedence in metabolites isolated from the free-living cyanobacterium *Lyngbya majuscula* (75). Alternatively, a similar approach with the tunicate *Lissoclinum patella*, which harbors an abundance of the cyanobacterium *Prochloron* spp., yielded equivocal results for a series of distinctive cyclic peptides which were found to be associated with both the cyanobacterial and tunicate cells (76). In general, the approach of associating a specific natural product with an isolated cell type is potentially

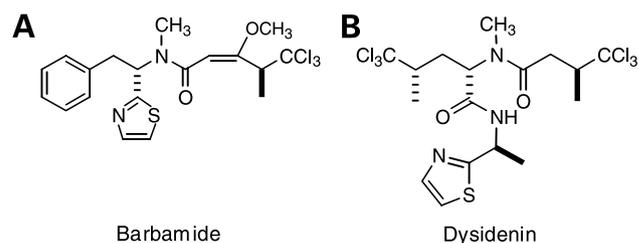


Figure 5. Structures of compounds discussed in text, including (A) barbamide, a cyanobacterial peptide containing a trichloromethyl group and (B) dysidenin, a barbamide-related compound isolated from a sponge-cyanobacterial association.

flawed as it is conceivable, and even possibly expected with microbial populations, that metabolites will be secreted and become associated with cell types other than those responsible for their production.

Hence, new approaches are needed which truly show the genetic and biochemical ability of a particular cell type to synthesize a metabolite of interest (9). A genetic approach has also been applied to this latter symbiosis which showed that *Prochloron* spp. do possess nonribosomal peptide synthetases; however, it has not been unequivocally shown that these *NRPS* genes are relevant to the biosynthesis of cyclic peptides associated with this source (77). Recent work in our laboratory has made a contribution in this regard.

Cloning of the gene cluster responsible for the biosynthesis of the chlorinated peptide barbamide (Fig. 5A) led to the identification of a gene sequence encoding the production of a putative halogenase that converts an unactivated methyl group to a trichloromethyl functionality. Because an identical trichloromethyl group is present in metabolites isolated from *D. herbacea*, typified by dysidenin (Fig. 5B), we reasoned that the symbiotic cyanobacterium *O. spongellae* should contain a related genetic element. Indeed, PCR using primers designed to conserved sections of the halogenase were successful in cloning a homologous gene from the cyanobacteria-laden sponge tissue, and this was fluorescently labeled to provide a probe of mRNA expression in intact sponge tissue. Thin sections of sponge-cyanobacteria tissue were incubated with this gene probe, washed to remove nonspecific probe binding, and visualized by fluorescent microscopy. This unequivocally established that the biosynthetic capacity to produce chlorinated peptides resided within the cyanobacterial cells.⁴ Similar methods should also be applicable to some of the important cases identified above, such as halichondrin production in diverse sponges and cyclic peptide formation in the tunicate *Lissoclinum* sp.

The productivity of the past decade in terms of discovery of new clinical anticancer leads from diverse marine life should translate into a number of new treatments for cancer in the decade to come. In turn, these successes should rekindle serious efforts to evaluate marine life for useful leads with anticancer properties. Approaches in the past

⁴ Unpublished data.

which have largely screened crude extracts for biological activity have allowed a rich harvest of "low hanging fruit." With effective prefractionation strategies broadly in utilization, a rich repertoire of diverse biological assays now available, highly effective nuclear magnetic resonance and mass spectrometry methods well suited to solving complex structures on vanishingly small quantities of a compound, and synthetic methods able to approach and be commercially tenable for exceedingly complex natural products and their derivatives, we can expect the next decade to yield an even more bountiful crop of new clinical agents from the sea!

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