

Exploring the global animal biodiversity in the search for new drugs - marine invertebrates

Dennis RA Mans*

Department of Pharmacology, Anton de Kom University, Paramaribo, Suriname

Abstract

Man has probably used since his existence plants and plant-derived compounds for his health care and well-being. This has led to the development of life-saving drugs for treating a multitude of conditions including infectious, cardiovascular, malignant, and diabetic disease. More recently, the amazing biodiversity represented by the world's oceans have been realized to represent an equally and exceptionally rich source of valuable bioactive compounds. The majority of marine organisms have an evolutionary history that dates back to the Cambrian, some 500 million years ago. Since then, these organisms have dealt successfully with competitors and predators by developing a unique arsenal of highly effective secondary metabolites for their defense, reproduction, and communication. Many of these chemicals affect metabolic pathways that are common to humans and are involved in critical physiological functions. Thus, these substances often possess meaningful pharmacological properties. For these reasons, marine organisms may represent precious resources for developing drug candidates, cosmetics, nutritional supplements, and molecular probes for improving our well-being. This paper addresses the significance of bioactive compounds from marine invertebrates to the development of new drugs.

Introduction

Historically, terrestrial plants and microorganisms have been important natural sources for developing new medicines [1,2]. In fact, approximately 75% of the twenty most commonly used hospital drugs, and approximately 20% of the hundred most prescribed drugs are derived from natural sources [3]. A few examples are antibiotics such as penicillin and streptomycin derived from the fungus *Penicillium chrysogenum* (Trichocomaceae), and aminoglycosides such as gentamicin and tobramycin from *Streptomyces* and *Micromonospora* bacteria, respectively; the narcotic morphine and the antitussive codeine derived from the opium poppy *Papaver somniferum* L. (Papaveraceae); the cardiotonic digoxin from the foxglove *Digitalis purpurea* L. (Plantaginaceae); the antimalarial quinine derived from the bark of *Cinchona* spp (Rubiaceae); and the skeletal muscle relaxant tubocurarine from the stem of *Chondrodendron tomentosum* Ruiz & Pavón (Menispermaceae) that is used for general anesthesia [1,2].

These and many other examples underscore the importance of plant-derived compounds - particularly those from tropical rain forests - to new drug discovery and development activities [1,2]. However, the world's oceans may represent an equally important resource for discovering and developing new therapeutics. This statement is based on two important pieces of evidence. Firstly, the oceans cover more than 70% of the earth's surface and contain more than 200,000 species of invertebrates and algae as well as an unknown number of microorganisms, only a relative handful of which has been studied [4]. Secondly, the relatively small number of marine organisms evaluated to date - mostly algae and invertebrates - has already yielded thousands of chemical compounds that have found medical applications ranging from algae-based skin care products [5,6] to established antineoplastic agents such as cytarabine [7].

This paper provides the rationale for marine drug discovery and development, addresses a number of bioactive compounds from marine invertebrates, elaborates about their mechanisms of action, focuses on

their potential clinical usefulness and applicability, and reflects on the prospects for new discovery and development activities based on the exploration of these compounds. The compounds have been grouped on the basis of the taxonomic groups ([sub]phyla) in which they have been encountered (Table 1).

Background

Why do marine species including invertebrates produce such an abundance of compounds with unusual chemical structures and unique mechanisms of action upon which molecular modeling and chemical synthesis of new drugs can be based? An important part of the answer to this question lies in the fact that many of them are sessile organisms that live in densely populated habitats where competition for space and resources is intense. Examples are reef-building corals, sponges, sea fans, bryozoans, and tunicates. Indeed, a superficial examination of a coral reef habitat reveals an astonishing density of such plants and animals. All of them compete with each other for food and space, must avoid attack by predators and being fouled or overgrown, need to reproduce successfully, and must ward off microbial infections. For these purposes, they have developed an exceptional arsenal of secondary metabolites for their defense, reproduction, and communication [8,9].

Some of these chemicals are toxic or noxious, conferring protection against predators; others suppress the growth and reproduction of rivals, thus preventing their overgrowth and crowding out and allowing

Correspondence to: Prof. Dr. Dennis R.A. Mans, Department of Pharmacology, Faculty of Medical Sciences, Anton de Kom University of Suriname, Kernkampweg 5, Paramaribo, Suriname, Tel/Fax: 597 441071; **E-mail:** dennis_mans@yahoo.com; dennis.mans@uvs.edu

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Table 1. Bioactive compounds with potential clinical applicability derived from a number of marine species of invertebrates grouped according to the phyla in which they have been encountered.

Phylum	Species (Family)	Compound	(Potential) clinical application(s)
Porifera	<i>Topsentipachastrelloides</i> Topsent, 1892 (Halichondriidae)	Bisindole alkaloids	Against bacterial infections including those caused by MRSA
	<i>Petrosiacontignata</i> Thiele, 1899 (Petrosiidae)	Contignasterol	Against asthma and hemodynamic disorders
	<i>Stylolellaaurantium</i> Kelly-Borges & Bergquist, 1988 (Halichondriidae)	Debromohymenialdisine	Against cancer, Alzheimer's disease, and osteoarthritis
	<i>Discodermiadissoluta</i> Schmidt, 1880 (Theonellidae)	(+)-Discodermolide	Against cancer
	<i>Halichondria okadaï</i> Kadota, 1922 (Halichondriidae)	Halichondrins	Against cancer
	<i>Pseudaxinyssacantharella</i> Lévi, 1983 (Halichondriidae)	Girolline	Against malaria
Cnidaria	<i>Pseudopterogorgiaelisabethae</i> Bayer 1961 (Gorgoniidae)	Pseudopterოსins	Against skin irritation, as analgesics, for wound-healing, and against inflammation
	Jellyfish-associated marine fungi	-	Against microbial infections
Nemertea	<i>Paranemertes peregrina</i> Coe, 1901 (Emplectonematidae)	Anabaseine	Against Alzheimer's disease
Mollusca	<i>Dolabellaauricularia</i> Lightfoot, 1786 (Aplysiidae)	Dolastatin 10	Against cancer
	<i>Elysia rufescens</i> Pease, 1871 (Plakobranchidae)	Kahalalide F	Against cancer and skin conditions
	<i>Spisulapolyynyma</i> Stimpson, 1860 (Mactridae)	Spisulosine	Against cancer
	<i>Comus</i> spp. Linnaeus, 1758 (Conidae)	Ziconotide	Against chronic pain
Bryozoa	<i>Bugulaneritina</i> Linnaeus, 1758 (Bugulidae)	Bryostatin 1	Against cancer and Alzheimer's disease
Tunicata	<i>Lissoclinum</i> sp. (Didemnidae)	Mandelalides	Against cancer
	<i>Ecteinascidiaturbinata</i> Herdman, 1880 (Perophoridae)	Trabectedin	Against cancer
	<i>Aplidium albicans</i> Milne-Edwards, 1841 (Polyclinidae)	Plitidepsin	Against cancer
Hemichordata	<i>Cephalodiscus gilchristi</i> Ridewood, 1908 (Cephalodiscidae)	Cephalostatin 1	Against cancer

them to maintain space; and still others serve as natural defenses against microbial infections [8,9]. As many of these chemicals also interact with enzymes and metabolic pathways involved in human diseases, they often represent valuable lead compounds for the development of new drugs for treating human diseases [8,9]. If, for example, a certain sponge produces a chemical that prevents a coral from invading its space by interfering with the coral's proliferation and cell division, that compound may also be effective in inhibiting the uncontrolled growth of cancer in humans. In fact, several marine-derived chemical compounds have been developed as antineoplastic agents, while some are under preclinical or clinical evaluation as a treatment for cancer. And more comprehensive studies on the bioactivities of these substances may well open the door to the development of many other medical applications ranging from novel antivirals to drugs against Alzheimer's disease.

Bioactive compounds from Porifera

The phylum Porifera (sponges) is evolutionarily considered the oldest group of existing animals, and probably represents one of the most important sources of marine natural products for developing new drugs. Their sessile life-style and abundant diversity - roughly 2,800 different species - were probably the driving evolutionary forces to develop their unique defensive toxins which have potent pharmacological activities. Notably, exploration of sponges as sources of new drugs led in the 1950s to the identification of a number of unusual arabinose nucleosides in *Tectitethya crypta* (Tethyidae) in the waters off Bimini Islands, Bahamas [10], and the subsequent development of the antimetabolite cytarabine (cytosine arabinoside, ara-C, or Cytosar®), an essential component of combination chemotherapeutic regimes for treating several hematological malignancies [7]. Further exploration of marine sponges for their medicinal potential led to the identification of some highly interesting bioactive compounds including bisindole alkaloids, contignasterol, debromohymenialdisine, (+)-discodermolide, halichondrins, and girolline.

Bisindole alkaloids

The bisindole alkaloids *cis*-3,4-dihydrohamacanthin B and

bromodeoxytopsentin have been isolated from the South African sponge *Topsentia pachastrelloides* Topsent, 1892 (Halichondriidae). Interest in these sponge metabolites arose following their potent and diverse pharmacological activities - including antimicrobial activity - in various preclinical models [11]. Further exploration of *cis*-3,4-dihydrohamacanthin B and bromodeoxytopsentin revealed potent and selective interference with pyruvate kinase (PK), a key hub protein in the interactome of methicillin-resistant *Staphylococcus aureus* (MRSA) as well as inhibition of the growth of *S. aureus* and MRSA *in vitro* [12,13]. MRSA - also known as the 'superbug' - is an exceptionally drug-resistant pathogenic bacteria that is responsible for a considerable number of fatalities in many public healthcare facilities throughout the world where resistant bacteria are selected due to pressure generated by the overuse of antibiotics [14,15].

Since PKs are evolutionarily highly-conserved rate-limiting enzymes for the irreversible conversion of phosphoenolpyruvate into pyruvate during glycolysis, they have been recognized as unprecedented targets for mechanistically novel antibiotics [16]. Indeed, targeting MRSA PK may represent a novel strategy for developing much needed new antibiotics against this drug-resistant pathogen [12]. This prospect not only spurred further research on natural marine bisindole alkaloids [17,18], but also stimulated efforts directed at the synthesis of analogues with original chemical structures [19,20]. So far, however, the potent MRSA PK inhibition recorded *in vitro* could not be translated to meaningful MRSA antibacterial activity *in vivo* [18]. Still, the identification of these lead compounds holds the promise for the development of efficacious antibiotics against MRSA and other multidrug-resistant bacteria.

Contignasterol

The polyhydroxysteroid contignasterol (IZP-94005) was first isolated from the sponge *Petrosia contignata* Thiele, 1899 (Petrosiidae) in Papua New Guinea in the early 1990s [21]. This compound prevented histamine release by rat peritoneal mast cells induced by IgE, presumably by inhibiting phospholipase C activity [22]. Furthermore, contignasterol significantly inhibited acute antigen-induced

bronchoconstriction *in vitro* as well as *in vivo* [23], and prevented allergen-induced plasma protein exudation in the tracheobronchial tree of sensitized guinea pigs [24]. All these observations pointed towards an important role for contignasterol as an anti-allergic, more specifically, anti-asthmatic drug. In addition, contignasterol inhibited platelet aggregation suggesting anti-thrombolytic activity [25]. Based on these and other observations, contignasterol is now being evaluated as a candidate for treating respiratory and hemodynamic disorders [25].

Debromohymenialdisine

Debromohymenialdisine is an alkaloid originally isolated from the marine sponge *Stylotella aurantium* Kelly-Borges & Bergquist, 1988 (Halichondriidae). This organism is encountered in the shallow waters around the Republic of Palau, an island country in the western Pacific Ocean. Debromohymenialdisine inhibited cancer cell growth by a novel mechanism of action, *viz.* selective blockage of two kinases involved in the G₂ checkpoint [26]. This led to massive apoptosis in some cancer types [27] which was much more pronounced *in vivo* than *in vitro* [26] but the induction of only moderate cytotoxicity as well as radioprotection to normal cells [26-29]. For all these reasons, debromohymenialdisine is undergoing evaluation as an anticancer agent [30]. As aberrant kinase activities have also been linked to Alzheimer's disease [31], debromohymenialdisine is being tested as a remedy against this condition [32]. In the meantime, this compound has been licensed for commercial development as a possible treatment for osteoarthritis as it slowed joint and cartilage deterioration in laboratory animals [33].

(+)-Discodermolide

(+)-Discodermolide is a natural polyketide that was isolated in 1990 from the Bahamian deep-sea sponge *Discodermia dissoluta* Schmidt, 1880 (Theonellidae) (Figure 1) [34]. Preclinical studies showed that (+)-discodermolide potently inhibited the proliferation of several cultured (multidrug-resistant) cancer cell lines [35] by stabilizing their microtubules, leading to arrest of cell division in the M phase of the cell cycle [36]. The drug showed promising activity against pancreatic cancer and various other (inherently) drug-resistant malignancies [37], as well as appreciable synergy with paclitaxel in lung cancer [38]. Other noteworthy properties of (+)-discodermolide



From: www.bu.edu

Figure 1. The deep-sea sponge *Discodermia dissoluta* (Theonellidae)

are immunosuppressive activity [39] and potent acceleration of cell senescence [40]. For these reasons, clinical studies have been initiated to evaluate (+)-discodermolide for its efficacy against solid tumors, paclitaxel-resistant tumors, and/or in combination with paclitaxel.

Halichondrins

Halichondrins are polyether macrolides that were first isolated from the Japanese sponge *Halichondria okadai* Kadota, 1922 (Halichondriidae) [41]. Initial studies with halichondrin B revealed extraordinary *in vitro* and *in vivo* anticancer activity [42] by potent binding to tubulin at a site close to, but distinct from the vinca site, leading to alteration of tubulin depolymerization [43]. However, further studies were hindered by a lack of the natural product. For this reason, research efforts were redirected to the deep-water Pacific sponge *Lissodendoryx* sp. (Coelosphaeridae) that was available at sufficiently large quantities to yield halichondrins to continue their evaluation and development. This resulted in the isolation of isohomohalichondrin that showed encouraging preclinical anticancer activity [44]. This compound also prevented microtubule assembly [45] and caused delayed S phase progression, mitotic block, and apoptosis in cancer cell lines [46]. Isohomohalichondrin B as well as a number of its synthetic analogues [47] are currently in clinical trials in patients with a wide range of malignancies.

Girolline

Girolline (or girodazole) is a 2-aminoimidazole derivative isolated from the New Caledonian sponge *Pseudaxinyssa cantharella* Lévi, 1983 (Halichondriidae) [48]. This compound was of interest as a potential anticancer agent, as it inhibited protein synthesis in eukaryotic cells at the termination steps rather than at the initiation or chain elongation steps like other known inhibitors such as emetine and homoharringtonine [49]. However, further development of girolline as an anticancer agent was discontinued when phase I clinical trials showed that it caused severe hypertension [50]. Fortunately, additional studies on girolline's bioactivity demonstrated inhibition of the growth of *Plasmodium falciparum* *in vitro* and *in vivo* as well as *in vitro* synergistic activity with chloroquine [51]. These observations suggest that girolline may represent a lead compound for new drugs to fight malaria.

Bioactive compounds from Cnidaria

The phylum Cnidaria contains over 10,000 species of aquatic, mostly marine animals and includes, among others, sessile sea anemones and corals as well as motile jellyfish and box jellies. These organisms are also called nettle-bearers because they attack their prey with their poisonous stinging cells called cnidoblasts. The venom of some species is extremely potent and their stings are very painful and can even be fatal to humans. The Cnidaria have yielded a few compounds with important biological activity such as the pseudopterosins. Furthermore, at least one species of Cnidaria harbors fungi that synthesize antimicrobials, presenting the opportunity of a novel way to produce these substances.

Pseudopterosins

The pseudopterosins are tricyclic diterpenepentose glycosides isolated from the sea whip *Pseudopteroergorgia elisabethae* Bayer 1961 (Gorgoniidae), a Caribbean soft coral species (Figure 2) [52]. These compounds possess notable anti-inflammatory and analgesic properties that exceed the potencies of existing cyclooxygenase-inhibiting non-steroidal anti-inflammatory drugs [53-55]. They not only represent a new class of natural products [52], but also act through



Figure 2. The sea whip *Pseudopterogorgia elisabethae* (Gorgoniidae)
From: reefcorner.com

a novel mechanism that involves prevention of eicosanoid biosynthesis by inhibiting phospholipase A2, 5-lipoxygenase, and cyclooxygenase activity, and by preventing degranulation of leukocytes and the subsequent liberation of lysosomal enzymes [53,56,57]. For these reasons, extracts from *P. elisabethae* have been incorporated in a line of Estée Lauder cosmetic products for treating skin irritation [58], while particularly pseudopterins A to D are being evaluated for their use as analgesics as well as wound-healing and anti-inflammatory drugs [59-61].

Jellyfish-associated marine fungi

The jelly fish *Nemopilema nomurai* Kishinouye, 1922 (Rhizostomatidae) - also known as Nomura's jellyfish - is probably one of the largest cnidarians in the world, growing up to 2 meters in diameter and weighing up to 200 kilograms [62]. It is most often encountered in the waters of China, Korea, and Japan where it appears since 2003 in massive numbers, probably as a result of the favorable condition in these waters [63]. *N. nomurai* is toxic type of giant jellyfish [63]; its sting is painful and has been associated with adverse cardiovascular [64] as well as cytotoxic and hemolytic effects [65].

More recently, ethyl acetate extracts from cultivable fungi living in association with *N. nomurai* were found to exhibit meaningful activity towards the pathogenic bacteria *Staphylococcus aureus* (Staphylococcaceae) and *Salmonella enterica* (Enterobacteriaceae) and the plant pathogenic fungi *Rhizoctonia solani* (Ceratobasidiaceae) and *Botrytis cinerea* (Sclerotiniaceae) [66]. Importantly, the yield and type of the antimicrobials varied along with the cultivation conditions [66]. These observations suggest that *N. nomurai* - considered of low nutritional and economic value - may become a novel reservoir for the production of antibacterial and antifungal compounds.

Bioactive compounds from Nemertea

The Nemertea comprise a phylum of soft-bodied worm-like invertebrate animals also known as ribbon worms because of their unsegmented and smooth body. About 1,400 species are known, the majority of which are marine, but some are freshwater, and a few live on land [67]. Most nemerteans are carnivores feeding on annelids, clams, and crustaceans [67]. For this purpose, many possess a proboscis that turns inside-out to emerge just above the mouth, coils around the

prey, paralyzes it by sticky, venomous secretions, and draws it into its mouth [68]. Others have a stylet, a calcareous barb, with which they stab the prey many times to inject toxins and digestive secretions after which it is swallowed whole [68]. The venom is also used as a feeding deterrent against predation [69]. An important bioactive compound from nemertean venom is anabaseine.

Anabaseine

Anabaseine is a nicotinoid alkaloid that was isolated in the early 1970s from various nemertine worms including *Paranemertes peregrina* Coe, 1901 (Emplectonematidae) (Figure 3) [70]. However, anabaseine had already been synthesized in the laboratory in the 1930s as an analogue of nicotine, and has more recently been found as a venom in the ant species *Nemertines* and *Aphaenogaster* [69]. The venom acts as an agonist of acetylcholine on peripheral neuromuscular nicotinic receptors [71]. In vertebrates, however, it stimulates neuromuscular nicotinic receptors in the central and peripheral nervous system [71], causing depolarization of neurons and release of both dopamine and norepinephrine [71]. As such, it has the potential as a treatment of cognitive function loss. A synthetic anabaseine analogue, DMXBA (GTS-21) has exhibited memory-enhancing effects in recipients [70]. Both anabaseine and DMXBA are under clinical evaluation as a treatment for Alzheimer's disease.

Bioactive compounds from Mollusca

With over 130,000 living species, the Mollusca comprise the second largest animal phylum after the Arthropoda. They have an evolutionary history extending back to the Precambrian, roughly 555 million years ago. This phylum includes slugs, snails, squids, cuttlefish, octopuses, as well as a large variety of marine shellfish such as clams, mussels, and oysters. Arthropods are the most common marine animals, occupying virtually every niche in the oceans. Exploration of this phylum has led to the identification of various unusual structures with exciting biological activities such as dolastatin 10, kahalalide F, spissulosine, and ziconotide.

Dolastatin 10

The linear pentapeptide dolastatin 10 was isolated from the marine sea hare *Dolabella auricularia* Lightfoot, 1786 (Aplysiidae) which



Figure 3. The nemertine worm *Paranemertes peregrina* (Emplectonematidae)
From: soundwaterstewards.org

can be found in the Indian Ocean as well as the Western and North-western Pacific [72]. However, this compound is not actually produced by *D. auricularia* but by blue-green algae grazed by this sea hare [73]. Dolastatin 10 binds to the vinca/peptide region of tubulin resulting in inhibition of microtubule assembly, the formation of tubulin aggregates, and disruption of mitosis [74]. It also induces tumor cell apoptosis by targeting the oncoprotein bcl-2 that is overexpressed in certain cancers [75]. Phase II clinical trials with single-agent dolostatin 10 against a wide range of tumors did not show meaningful antineoplastic activity [76]. Still, this agent and several analogues are currently under evaluation in combination drug regimens against difficult-to-treat malignancies such as malignant melanoma as well as renal and pancreatic cancer [77].

Kahalalide F

Kahalalide F is so far the only known member of the kahalide family of peptides and has been isolated from the Hawaiian herbivorous marine sacoglossan mollusk *Elysia rufescens* Pease, 1871 (Plakobranchidae) [78,79]. Like dolastatin 10, the mollusc produces kahalalide F as a secondary metabolite from substances in its diet of (green) algae [78,79]. Kahalalide F acts as a cytotoxin that disrupts lysosomal membranes in (tumor) cells, thereby initiating apoptosis [80,81]. It also interferes with the expression of genes involved in DNA replication and cell proliferation thereby inhibiting tumor spread and growth [80,81]. The drug showed promise against a broad range of tumors and is in clinical trial in patients with, among others, prostate cancer [82,83]. It is also being tested for its therapeutic efficacy against several dermatological conditions [82].

Spisulosine

Spisulosine is a sphingoid-type base that was first isolated from the North Arctic surf clam *Spisula polynyma* Stimpson, 1860 (Mactridae) [84]. This substance exhibited potent cytotoxicity in preclinical studies [84,85] through an unprecedented mechanism of action: the induction of changes in the shape of the cytoskeleton [85]. Thus, spisulosine prevented the formation of actin stress fibers in cultured cells - probably by decreasing the activity of Rho proteins - resulting in perturbation of cell adhesion, migration, and morphogenesis [85] and eventually apoptosis [86]. Spisulosine is currently under clinical evaluation as an antitumor agent [87]. However, *S. polynyma* reaches reproductive maturity only at an age of 5 to 8 years which hampered a regular supply of spisulosine from this source. For this reason, efforts are being dedicated to the production of a more rapidly growing variant of *S. polynyma* that can be maintained in aquaculture in order to commercially produce sufficient spisulosine for drug development purposes [88].

Ziconotide

Ziconotide (or SNX-111 or Prialt®) is a synthetic form of a newly described chemical family of short peptides (25 to 30 amino acids in length) called conotoxin peptides [89], and is probably among the most exciting marine natural compounds ever identified. Conotoxin peptides were first extracted from the venom of the predatory cone snails *Conus geographicus* Linnaeus, 1758, and *Conus magus* Linnaeus, 1758 (Conidae) (Figure 4) [90]. These snails live in the coral reefs surrounding Australia, Indonesia, and the Philippines, and hunt and kill prey using the powerful neurotoxins in their venom [91]. Each species of cone snail produces its own cocktail of unique venoms containing dozens of nerve toxins, some of which instantly shock the prey while others cause paralysis [91]. Ziconotide was molded on the



Figure 4. The predatory marine cone snail *Conus geographicus* (Conidae)
From: www.duikvakanties.net

basis of the peptides found in cone snail venom due to their potential to act as pain-killing drugs [92].

Ziconotide is administered through intrathecal injection because it does not cross the blood-brain barrier [93]. It precisely blocks N-type calcium channels in pain-transmitting nerve cells, rendering them unable to transmit pain signals to the brain [93,94] while the rest of the nervous system continues to function properly [93,94]. Obviously, this represents an important advantage of ziconotide over currently available opiate pain killers. Of note, ziconotide's efficacy in treating pain exceeds that of morphine by fifty to several thousand times [93,94], but because it is non-addictive it may be suitable for long-term use. Ziconotide also does not cause the dreadful side-effects of morphine such as sedation and respiratory depression [93,94]. As a result, this drug has been approved for the management of chronic pain in patients suffering from among others, AIDS and cancer.

Bioactive compounds from Bryozoa

The phylum Bryozoa comprises approximately 5,000 species of colony-forming aquatic invertebrate animals that build networks of branching tubes attached to the sea bed. Bryozoans are also known as moss animals because of the similarity of the branching colonies with moss. The small animals attach to aquatic plants, woody debris, stones, and even snail shells, and are often responsible for biofouling on ships' hulls, docks, marinas, and offshore structures. They typically extend a crown of retractable tentacles lined with cilia (the lophophore) into the current to sieve small drifting food particles out of the water. Most bryozoans produce deterrent chemicals that protect them from predation by making them unpalatable to their attackers [95]. Several of such compounds belong to the group of bryostatins [95].

Bryostatin 1

Bryostatin 1 is a member of a group of twenty different macrolide lactones first isolated from extracts of the bryozoan marine invertebrate *Bugula neritina* Linnaeus, 1758 (Bugulidae) (Figure 5) [95]. Together with other bryostatins, this compound probably defends the highly vulnerable larval stages of *B. neritina* from fish predation [95]. Due to its capacity to potently modulate protein kinase C activity [96], bryostatin 1 has been evaluated as an anticancer agent [96]. In preclinical studies, it inhibited cell growth and angiogenesis, stimulated cell differentiation and apoptosis, and acted synergistically with various other anti-cancer drugs [96]. However, when tested as a single agent in phase II trials in tumor patients, it demonstrated only moderate activity while toxicities

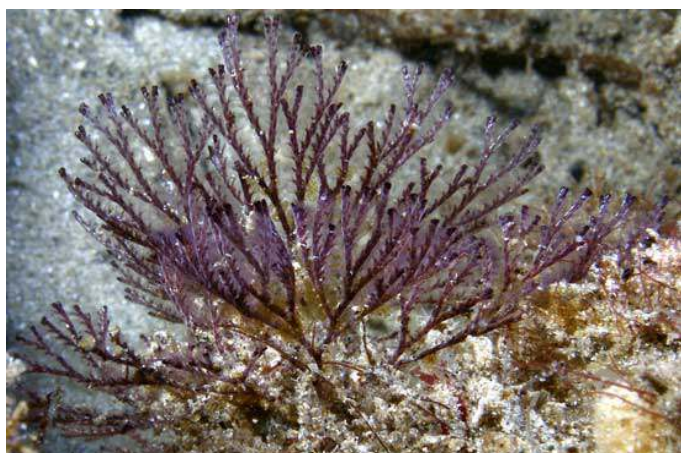


Figure 5. The bryozoan *Bugula neritina* (Bugulidae)
From: www.roboastra.com

were substantial [96]. For these reasons, research focus has shifted to combinations of bryostatin 1 with other antitumor agents [96].

Bryostatin 1 also produced very promising results in enhancing memory in animal models. It increased the duration of memory retention of a sea snail by over 500% [97], improved learning capacity in laboratory animals [98] including a transgenic mouse model of Alzheimer's disease [99], and alleviated brain damage in ischemically brain-injured rats [100]. These observations signify potential therapeutic applications for bryostatins other than those related to neoplastic disease.

Bioactive compounds from Tunicata

The Tunicata comprise a subphylum of about 2,150 species of marine invertebrates with a unique outer covering or 'tunic' that is formed from proteins and carbohydrates and serves as an exoskeleton. Various species are known as sea squirts (because they contract their bodies sharply and squirt out water when disturbed), sea pork or sea liver (because of the resemblance of their dead colonies to pieces of meat), or sea tulips (because of their colorful bodies on slender stalks). Tunicates have a water-filled, sac-like body and two tubular openings through which they draw in and expel water for filter-feeding. Some species accumulate large amounts of vanadium, lithium, iron, niobium, or tantalum in vacuoles in the presence of sulphuric acid, and deposit these substances just below the outer surface of their tunic in order to deter predation [101]. Others produce distasteful organic compounds as chemical defenses against predators [102]. Such compounds - among others, mandelalides, trabectedin, and plitidepsin - have potentially useful medicinal properties.

Mandelalides

Mandelalides A to D are variously glycosylated, unusual polyketide macrolides isolated in 2012 from the newly described South African tunicate *Lissoclinum* sp. (Didemnidae) [103]. Strikingly, mandelalides A and B exhibited cytotoxicity at nanomolar concentrations in human tumor cell lines [103]. However, these compounds could only be obtained at too low quantities from the tunicate to allow studies on their mechanism(s) of action and molecular targets (s). For this reason, several successful efforts have been undertaken to provide a reliable synthetic supply of particularly mandelalide A, the most active compound of this series of marine macrolides [104-107]. This was expected to open the door for biochemical and pharmacological

studies with these promising cytotoxic compounds.

Unfortunately, the previously reported potent biological activity of the naturally-occurring mandelalides A and B [103] could not be reproduced with synthetic mandelalide A [104,107]. So far, the reasons for this discrepancy are not known. However, applying shotgun metagenomic sequencing of DNA extracted from *Lissoclinum* sp, it could be deduced that the mandelalides were not produced by *Lissoclinum* sp. but by a bacterial symbiont of the tunicate belonging to the phylum Verrucomicrobia [108]. It is hoped that this discovery will shed more light on the precise configurations of the bioactive mandelalides in order to continue the further development of this exciting class of marine compounds.

Trabectedin

Trabectedin (or ecteinascidin 743, ET-743, or Yondelis[®]) is a tetrahydroisoquinoline alkaloid that was originally isolated from the Caribbean sea squirt *Ecteinascidia turbinata* Herdman, 1880 (Perophoridae) (Figure 6) found in the West Indies around the roots of intertidal and subtidal mangroves [84]. Subsequent studies found that the compound is in fact produced by *Candidatus Endoecteinascidia frumentensis*, a microbial symbiont of the tunicate [109]. Preclinical and early clinical studies showed appreciable activity of trabectedin against a range of tumor types including soft tissue sarcomas [110,111]. This occurs through a unique mechanism of action: the production of superoxide near the nuclear DNA of tumor cells resulting in DNA backbone cleavage and tumor cell apoptosis [112]. Importantly, only cells engaged in active gene transcription undergo apoptosis [112], suggesting that trabectedin has substantial specificity to cancer cells which in general exhibit greatly accelerated transcription and translation rates when compared to normal cells.

Trabectedin also interfered with the *mdr1* gene [113] that encodes for the plasma membrane-bound P-glycoprotein which confers multidrug resistance to cancer cells by actively transporting anticancer drugs out of the cells. This suggests a role for trabectedin as a key ingredient in combination chemotherapy regimens to prevent tumor cells from developing resistance to the other drugs. Trabectedin has been granted orphan status against advanced soft tissue sarcomas [114] and is currently undergoing clinical trials for the treatment of various other malignancies [115].



Figure 6. The Caribbean sea squirt *Ecteinascidia turbinata* (Perophoridae)
From: bioweb.uwlax.edu

Plitidepsin

Plitidepsin (or dehydrodidemnin B or Aplidin®) is a member of a class of cyclic depsipeptides that can be extracted from the Mediterranean sea squirt *Aplidium albicans* Milne-Edwards, 1841 (Polyclinidae) [116] but is currently produced by chemical synthesis [117]. Plitidepsin exhibited marked anticancer properties in preclinical studies [118,119]. These effects are believed to occur through multiple mechanisms including rapid and persistent activation of apoptosis [120] and interruption of the tumor cell cycle at the G₁-G₂ border [121], as well as inhibition of ornithine decarboxylase receptor and vascular endothelial growth factor receptor expression involved in the growth and vascularization of certain tumor types [121]. Notably, plitidepsin seemed to exhibit a high specificity for tumor cells and to induce low toxicity to the normal tissues [120,121]. The drug has been granted orphan status in Europe in 2003 for treating acute lymphoblastic leukemia [122] and is being developed for the potential treatment of a variety of cancers [123].

Bioactive compounds from Hemichordata

The Hemichordata comprise a relatively small phylum (only a few hundred species) of worm-like marine invertebrates [124]. They feed on small particles of organic matter either as filter feeders or as substrate eaters [124], and can be found both in shallow coastal waters and in the deeper seas [124]. Their body can be distinguished into a short proboscis, a collar which may bear tentacles, and a trunk which contains the digestive and reproductive organs [124].

Hemichordata have provided important insights into the evolution of the Deuterostomia because of their pharyngeal gill slits [124,125]. Some of these marine are also known to produce and accumulate various toxic substances - such as halogenated phenols and pyrroles [126] - that may or may not deter predators [126]. More recently, cephalostatin 1 has emerged as a highly interesting bioactive compound from a marine tube worm.

Cephalostatin 1

Cephalostatin 1 is a bis-steroidal pyrazine alkaloid isolated from the small hemichordate *Cephalodiscus gilchristi* Ridewood, 1908 (Cephalodiscidae) [127] that can be encountered at the South Africa's temperate southern coast [128]. Evaluation in the *in vitro* screen of the US National Cancer Institute showed that cephalostatin 1 is an extremely potent inhibitor of cell proliferation, inhibiting tumor cell growth at subnanomolar to nanomolar concentrations [127,129]. Furthermore, its unique cytotoxicity profile in this screen suggested a novel mechanism of action that turned out to involve activation of anew, unusual pathway for apoptosis: the selective induction of Smac/DIABLO (second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with a low isoelectric point) as a mitochondrial signaling molecule [127,130,131]. Notably, this process occurred without the release of cytochrome c from the mitochondria [127,130,131] but required caspase 9 activation [127] following stimulation by endoplasmic reticulum stress-associated caspase 4 [129].

These observations suggest that cephalostatin 1 may be particularly useful for treating chemo-resistant malignancies. For this reason, this compound is now in preclinical development. Unfortunately, the availability of this compound as well as other cephalostatins from *C. gilchristi* - their only known natural source - is extremely limited, which restricts their further evaluation and subsequent preclinical development.

Future prospects and concluding remarks

The extraordinary biological and chemical diversity of marine invertebrates addressed in this paper illustrates the potential of this largely untapped source for the discovery and development of new therapeutics. However, this enterprise poses unprecedented challenges to molecular biologists, pharmacologists, clinical chemists, and chemical ecologists who are active in this comparatively new scientific field. Apart from technological, financial, and regulatory issues, many of these challenges are related to the supply issue: how can the sustainable use of these resources be ensured? Indeed, the regular supply of raw material is a major limiting factor for the further pharmaceutical development of many marine-derived compounds.

Often, the bioactive compound of interest is present in trace amounts in the organism, and supply from wild harvest poses many logistic problems and usually does not provide enough of the compound for even preclinical studies. For instance, the sponge *D. dissoluta* must be harvested at a minimum depth of thirty-three meters to obtain the relative modest content of 0.002% of the light-sensitive drug discodermolide [34], and roughly one tonne of animals is needed to extract one gram of trabectedin [84] or bryostatin [95] while about five grams are needed for a clinical study [84,95].

Attempts to solve these problems through total synthesis have often proved difficult due to the structural complexity of the compounds of interest [132]. More success has been achieved with hemi-synthesis starting from a more readily available natural precursor [133] and the synthesis of structurally simpler analogues with a similar biological profile and in some cases greater pharmacological potency [134,135]. Controlled harvesting, aquaculture of the source organism, *in vitro* production through cell culture, and transgenic production have also proved feasible alternatives to obtain certain bioactive marine compounds in sufficient quantities [136,137].

More recently, an increasing number of natural products from higher animals have been found to ultimately derive from a bacterial symbiont. A few examples have been given throughout this paper [73,78,108]. These 'misses' could for an important part be attributed to genome sequencing and genomics that largely relied on cultivated clonal cultures. The application of metagenomics - the direct genetic analysis of genomes contained within an environmental sample - provides access to the functional gene composition of entire microbial communities [138-140]. Thus, metagenomics may help uncovering the enormous functional gene diversity in marine samples, aiding to the identification and production of drug candidates from microbial origin for pre-clinical and clinical development.

These advances, together with new technologies to explore the oceans of our planet (such as probes, sensors, and miniaturized tools that can function in harsh environments) will one day help to explore marine invertebrates and other deep sea resources to their full potential. Undoubtedly, this will lead to the discovery and development of unprecedented new medical treatments, nutritional supplements, pesticides, cosmetics, and other commercial products for the benefit of mankind.

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