Exploring Marine Resources for Bioactive Compounds

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Abstract
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Biodiversity in the seas is only partly explored, although marine organisms are excellent sources for many industrial products. Through close co-operation between industrial and academic partners, it is possible to successfully collect, isolate and classify marine organisms, such as bacteria, fungi, micro- and macroalgae, cyanobacteria, and marine invertebrates from the oceans and seas globally. Extracts and purified compounds of these organisms can be studied for several therapeutically and industrially significant biological activities, including anticancer, anti-inflammatory, antiviral, antibacterial, and anticoagulant activities by applying a wide variety of screening tools, as well as for ion channel/receptor modulation and plant growth regulation. Chromatographic isolation of bioactive compounds will be followed by structural determination. Sustainable cultivation methods for promising organisms and biotechnological processes for selected compounds can be developed, as well as biosensors for monitoring the target compounds. The (semi) synthetic modification of marine-based bioactive compounds produces their new derivatives, structural analogs and mimetics that could serve as hit or lead compounds and be used to expand compound libraries based on marine natural products. The research innovations can be targeted for industrial product development in order to improve the growth and productivity of marine biotechnology. Marine research aims at a better understanding of environmentally conscious sourcing of marine biotechnology products and increased public awareness of marine biodiversity. Marine research is expected to offer novel marine-based lead compounds for industries and strengthen their product portfolios related to pharmaceutical, nutraceutical, cosmetic, agrochemical, food processing, material and biosensor applications.

Introduction
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The maritime territory of the European Union is the world's largest with an economic zone covering 25 million km^2. The Atlantic Ocean, the Mediterranean Sea, the Aegean Sea, the Tyrrhenian Sea, the Adriatic Sea, and the brackish Baltic Sea can be regarded as the most important ones for the EU. If we count all economic activities that depend on the sea, then the EU's blue economy represents 5.4 million jobs and a gross added value of just under €500 billion per year. In all, 75 % of Europe's external trade and 37 % of trade within the EU is seaborne. The sea and the coasts are drivers of the economy (Blue Growth opportunities for marine and maritime sustainable growth; EU COM (2012) 494 final; DOI: 10.2771/43949).
Biodiversity in the seas is far from being fully explored. Most of the animal phyla are exclusive to sea environment. ◗ **Table 1** shows a classification of marine organisms exceeding 230 000 known species [1], and a predicted total number of undiscovered marine species exceeds 2 million [2].
In the last decades, the attention of the natural product researchers has been focused on the marine environment: indeed, it is a wealthy source of plants, animals and micro-organisms, which due to their adaptation to this unique habitat, produce a wide variety of primary and secondary metabolites that have demonstrated significant biological activities against, e.g., cancer and inflammation, as well as in analgesia, immunomodulation, allergy, and antiviral assays. The specific habitat where an organism is growing has influence on the chemical nature of the marine primary and secondary metabolites. The intraspecific variation can produce differences in the chemical structures of compounds and their concentrations in

Table 1 Classification of marine organisms according to Blunt and Munro [1].

Archaea and Eubacteria	Cyanobacteria (blue-green algae)	Chlorophyta (green algae)	Rhodophyta (red algae)	Phaeophyta (brown algae)	Bacillariophyta, Chrysophyta, Haptophyta	Fungi
~ 1000	~ 7500 species	~ 2000 species	~ 6000 species	~ 1500	~ 50 000 species	~ 1000
Dinoflagellata	**Porifera** (sponges)	**Cnidaria** (medusae, sea anemones, hydroids, corals)	**Platyhelminthes** (flukes, tapeworms, flatworms)	**Annelida** (true-worms)	**Bryozoa** (moss animals)	**Mollusca** (squid, mussels, snails, clams, sea hares)
~ 200 species	~ 8000 species	~ 11 000 species	~ 18 000 species	~ 10 000 species	~ 5700 species	~ 90 000 species
Echinodermata	**Crustacea**	**Hemichordata**	**Protochordata** (tunicates)	**Pisces** (fish)	**Total**	
~ 7000 species	~ 60 000 species	~ 100 species	~ 3000 species	~ 16 000 species	over 230 000 species	

Table 2 Marine or marine-derived drugs currently approved or in the pipeline.[1]

Therapeutic class	Compound	Chemical classification	Source organism
Cancer	Aplidine, plitidepsin (Aplidin®)	Depsipeptide	Tunicate Aplidium alpicans
	Brentuximab vedotin (Adcetris™)	Antibody-drug conjugate	Monomethyl auristatin E, synthetic, based on dolastatin 10, sea hare *Dollabella auricularia*/cyanobacteria
	Cytarabine, Ara-C (Cytosar-U, Depocyt)	Pyrimidine nucleoside	Sponge Cryptotheca crypta
	Elisidepsin (Irvalec®)	Depsipeptide	Synthetic, based on kahalalide F, mollusc *Elysia rufescens*
	Eribulin mesylate (Halaven®)	Macrolide	Synthetic, based on halichondrin B, sponge *Halichondria*
	Trabectedin (Yondelis®)	Alkaloid	Tunicate Ecteinascidia turbinata
	Jorumycin (Zalypsis®)	Alkaloid	Mollusc Jorunna funebris
Schizophrenia	DMXBA (GTS-21) anabaseine derivative	Alkaloid	Synthetic, based on anabaseine, marine worm *Paranemertes peregrine*
Pain	Ziconotide (Prialt®)	Peptide	Cone snail *Conus magus*
	Tetrodotoxin (Tectin®)	Alkaloid	Pufferfish Tetraodontidae

[1] http://marinepharmacology.midwestern.edu/clinPipeline.htm

different marine environments [3]. It is thus essential to study different strains of the same organism from various locations. It is worth mentioning here that biodiversity in the sea can be regarded as a synonym to the chemical diversity of the marine compounds. Over 30 000 compounds of marine origin are listed in *The Dictionary of Marine Natural Products* [1] and MarinLit database (http://pubs.rsc.org/marinlit/) at present, and about 1200 new marine compounds are reported yearly [4,5], only 5% of them having European origin. The potential of the marine environment as a source of novel drugs remains still quite unexplored.

Overview of the Drugs from the Sea
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Generally over 60% of the active principles of the pharmaceutical formulations are natural products or their synthetic derivatives or mimetics [6]. Inspection of all approved agents by FDA in the last decades clearly indicates that natural products as sources of novel structures, but not necessarily the final drug entity, are still very useful. Secondary metabolites of marine organisms are the result of millions of years of evolution and natural selection: even a single species constitutes a library of secondary metabolites that is validated for bioactivity. As a result of various enzymatic reactions, natural products have an intrinsic capacity to recognize and bind macromolecules, perturb their activity and modulate biological processes. Whereas extreme biotoxicity of some

marine bioactive compounds (e.g., maitotoxin, brevetoxin B) prevent their therapeutical application, others (e.g., okadaic acid, palytoxin, fluorescent proteins from marine organisms) are used as valuable tools in cell and molecular biology or even as drugs [7].

Major pharmaceutical companies have been exploring marine compounds for several decades, but their investments and interest ceased when synthetic combinatorial compound libraries emerged since they were simpler and more cost-efficient for the high-throughput screening. However, the combinatorial chemistry approach failed to produce enough new drug entities, and in the recent years, there has been an emergence of small biotechnology companies marketing marine-based drugs [7]. Examples of approved drugs and potential therapeutic compounds derived from marine sources are given in ◉ **Table 2**.

◉ **Table 2** shows that cancer is the main target for marine-derived drugs. Cytosine arabinoside or cytarabine (Ara-C; ◉ **Fig. 3**) is the oldest marine drug still on market, approved by FDA in 1969. Trabectedin (◉ **Fig. 1**) has been approved by EMEA for advanced soft-tissue sarcoma in 2007 and for ovarian cancer in 2009. PharmaMar is producing trabectedin semi-synthetically from the structurally related secondary metabolite of *Pseudomonas fluorescens*, cyanosafracin B [8]. Eribulin mesylate was approved by FDA in 2010 for metastatic breast cancer. Antibody-drug conjugate brentuximab vedotin was approved in 2011 for relapsed Hodgkin's lymphoma and relapsed or refractory systemic anaplastic large cell lymphoma. The related monomethyl auristatin
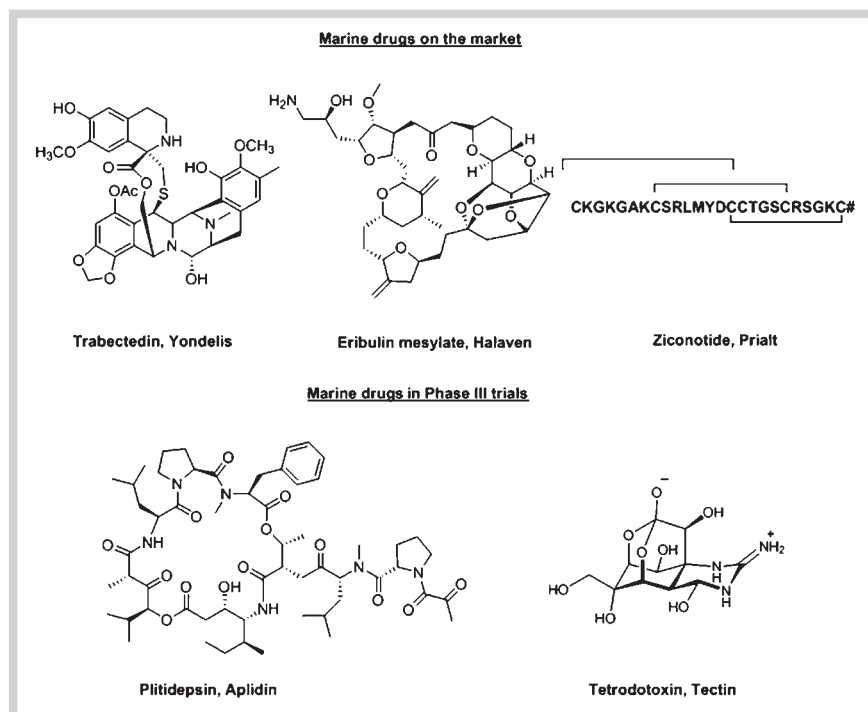
Fig. 1 Structures of marine drugs in the market and in Phase III trials.

E-linked antibody drug glembatumumab vedotin (CDX-011) has completed Phase II studies for metastatic breast cancer. Potential drug candidates for multiple myeloma are aplidine and jorumycin, currently being in Phase III and Phase II trials. Conopeptide ziconotide for severe chronic pain is an exception among the cancer drugs, and another non-cancer drug in the pipeline is synthetic anabaseine-based DMXBA [9]. It is in Phase II trials for schizophrenia and Phase I for being tested for improvements in cognition.

Large scale marine biotechnology projects have been launched recently outside the EU (e.g., Norway, South Korea), but for example in the USA, institutional funding has been decreasing in the recent years [10]. In the European Union, Horizon 2020 programme is strongly investing in marine research and innovation. Its 7th Framework Programme has several ongoing marine biotechnology projects based on drug discovery: MAREX – Exploring marine resources for bioactive compounds: from discovery to sustainable production and industrial applications (2010–2014), BAMMBO – Sustainable production of biologically active molecules of marine based origin (2011–2014), PharmaSea – Increasing value and flow in the marine biodiscovery pipeline (2012–2016), SeaBioTech – From sea-bed to test-bed: harvesting the potential of marine microbes for industrial biotechnology (2012–2016). The earlier French programme ECIMAR studied marine chemical ecology as an indicator of biodiversity, and the projects SPECIAL and SPONGES specialized on marine sponge research as well as SWAFAX and HYFFI on seaweed bioactive compounds.

Biodiversity and Ethical Aspects
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It is estimated that marine and coastal environments host about 90% of all organisms living on earth. The organisms play a crucial role in nutrients recycling, food provision (protein), and climate regulation. Moreover, marine organisms are unique sources of compounds used in medicine, biotechnology, agriculture, and many other branches. Despite the diverse values of biological diversity of marine organisms, life of many species has become threatened by human activities. Main pressures that result in biodiversity loss include unregulated exploitation of marine resources, overfishing, coastal development, invasive alien species, pollution, and global warming [11]. Becoming aware of the human-induced threats, many policymakers and scientists decided that a radical action should be undertaken to restore habitats and natural systems.

The overall consideration of ethical aspects of the marine biodiscovery process and product development is fundamental [12]. The key concepts in an ethical perspective in the process of translating marine biodiversity into industrial feasibility targeting innovative cosmetic, nutraceutical and pharmaceutical products are sustainability and safety. These key concepts are on both a conceptual and phenomenological level related to the concept of trust, for both building up and upholding trust in the outcome of the research and industrial processes on all levels. All key processes from the different aspects of research to evaluation of industrial feasibility and developing new products, testing, introducing and marketing them are aligned to a perspective of sustainability – and possibly gaining and up-holding trust. Upon this, also fair use of and equitable share of the benefits is included in the concept of sustainability in economic aspects. These aspects must be regulated by ethical and environmental guidelines as well as research-ethical and safety instructions. National regulative guidelines as well as international protocols (i.e., Convention of Biological Diversity) thus build a basic level of ethical decency. Researchers as well as persons on a general level are influenced by the non-human environment, mainly through different socially constructed institutions (states, political structures, laws, non-governmental organisations, public administration, etc.). The main aim of including a particular ethical perspective is two-fold: a vision of deepening understanding of the most important cultural conditions influencing the way humans tend to

form their picture of a good life with special attention shown to the role of health as a grounded value, and how a particular research project can communicate a trust-upholding process that acknowledges and respects the role of health in people's view of life. The concept of trust is thus based on communicating sustainability also in both research-processes aiming at attaining health and with regard to environmental issues [13].

Marine Organisms and Their Bioactive Compounds
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Marine bacteria
Marine bacteria include archaea and eubacteria, and they are an increasingly studied source for novel metabolites [4]. Archaea is an interesting group of bacteria since many of them are extremophiles, organisms that live in extreme conditions, e.g., deep sea, thermal vents, low temperature, or chemically challenging environment (salinity, pH, heavy metals). Archaean cell membranes consist of glycerol tetraethers and differ from those of eubacteria where the stabilizing element is a catenoid or hopanoid [14]. Extremophiles are a potential source not only for pharmaceutical applications but also of stable enzymes for industrial purposes [15]. Marine eubacteria consist of Gram-positive actinomycetes and bacilli, Gram-negative α-proteobacteria and γ-proteobacteria and several anoxygenic anaerobes [14]. Marine bacteria as well as marine fungi, cyanobacteria and some microalgae are living as symbionts in sediment, sponges or other invertebrates [16]. Several marine bacteria can be grown in laboratory cultures and thus only small amounts of sample are required for cultivation, or metagenomic approaches can be used to build libraries [17].
Marine bacteria produce several classes of compounds. Salinosporamide A (marizomib) is isolated from sediment-based actinomycete *Salinospora* and is currently at phase II trials as proteasome inhibitor for multiple myeloma. Around 100 novel compounds (e.g., polyketides, alkaloids, fatty acids, peptides and terpenes) are isolated from marine bacteria per year [14]. Bacterial metabolites are probably the most promising source for novel antibacterial compounds since most of the natural product-derived antibacterial drugs in the pipeline are from terrestrial actinomycetes [18]. Anthracetin [19], isolated from *Streptomyces* species is a potential antibiotic against *Bacillus anthracis* and methicillin-resistant *Staphylococcus aureus* (MRSA). Another potential antibiotic against MRSA and vancomycin-resistant enterococci is thiazolyl cyclic-peptide PM181104, isolated from sponge-associated actinobacterium strain of the genus *Kocuria* [20].

Cyanobacteria
Cyanobacteria are at the moment one of the most interesting sources of novel marine compounds [21]. Cyanobacteria have been used in medicine since as early as 1500 BC when *Nostoc* species were used in the treatment of gout, fistula, and cancer. Currently, cyanobacteria are the source of several products of medicinal value. These include anti-fungal, anti-inflammatory, anti-cancer, and anti-HIV agents [22]. ◯ **Fig. 2** shows the distribution of chemically different groups of compounds in cyanobacteria. It also displays the variety of biochemical activities of these compounds.
The most abundant group of cyanobacterial metabolites is that of oligopeptides derived through non-ribosomal peptide biosynthesis. Hundreds of structurally fully characterized or tentatively identified variants of cyanobacterial peptides or peptidic metab-

olites have been reported [23]. Both cyclic and linear peptides are known. These can be further divided into subgroups, such as microcystins, nodularins, anabaenopeptins, aeruginosins, cyanopeptolins, etc. A common characteristic for many of the peptides is specific inhibition of various mammalian and non-mammalian enzymes. Microcystins and nodularins, strong protein phosphatase inhibitors, have been considered lethal toxins for mammals and potentially harmful for many organisms, but not enough data exist for most of the remaining compounds to enable a full bioactivity or toxicology profiling.
It was postulated that apart from morphological features, modern taxonomy of cyanobacteria should include genetic, chemical, physiological and ecological characteristics of investigated species. The application of genetic methods (only) does not reflect the morphological diversity of cyanobacterial field populations. The polyphasic classification of cyanobacteria is especially important when strains tend to change phenotypically in culture. In chemotaxonomic studies of cyanobacteria, the first classification system was based on C18 fatty acids and divided the organisms according to the number and position of double bonds in the compound [24]. Fatty acid composition quite often correlated with morphological distinctions and 16S rRNA analysis. Some researchers also used accessory pigments or aromatic amino acids in classification of cyanobacteria.
Recently, the analysis of peptides, mainly those produced by the non-ribosomal peptide synthetase pathway, was used as a new promising tool in chemotaxonomy. The diversity of cyanobacterial peptides is very high and reflects great metabolic potential of the organisms. Apart from microcystins and nodularins (the cyclic oligopeptide hepatotoxins), cyanobacteria produce anabaenopeptins, cyanopeptolins, aeruginosins, microginins and microviridins [25] and probably many other unknown peptides [26]. Characteristic feature of non-ribosomal peptides is their great variability in amino acid composition and modifications in their structure [27]. Isolated strains usually produce more than one structural variant of a given peptide. The presence of specific non-ribosomal peptides is a stable feature of individual clones; their production is independent on culture conditions, which makes them especially useful in chemotaxonomic studies. Moreover, some of the compounds show biological activity, strongly depending on modifications in their chemical structure. Tandem mass spectrometry with sensitive detector (e.g., time of flight, TOF) is useful to find individual peptides or to determine peptide fingerprint characteristics for a clone or closely related clones of cyanobacteria producing bioactive compounds [28].

Microalgae including dinoflagellates
Microalgae play an important role in marine biological ecosystems, because of their photosynthetic activity. They are the major producers of biomass and organic compounds in the oceans. Marine microalgae are separated into five major divisions: Chlorophyta (green algae); Chrysophyta (golden-brown, yellow algae, and diatoms); Pyrrhophyta (dinoflagellates); Euglenophyta, and Cyanophyta (blue-green algae). The last division, Cyanophyta, is widely classified and its organisms termed as cyanobacteria and not included in the category of algae, because of their prokaryotic characteristics, despite the fact that the organisms are photosynthetic and share many algal characteristics with their eukaryotic counterparts [29]. The microscopic planktonic algae in the oceans are crucial food for filter-feeding bivalve shellfish (oysters, mussels, scallops, clams, etc.) as well as the larvae of commercially important crustaceans. In most cases, the proliferation of
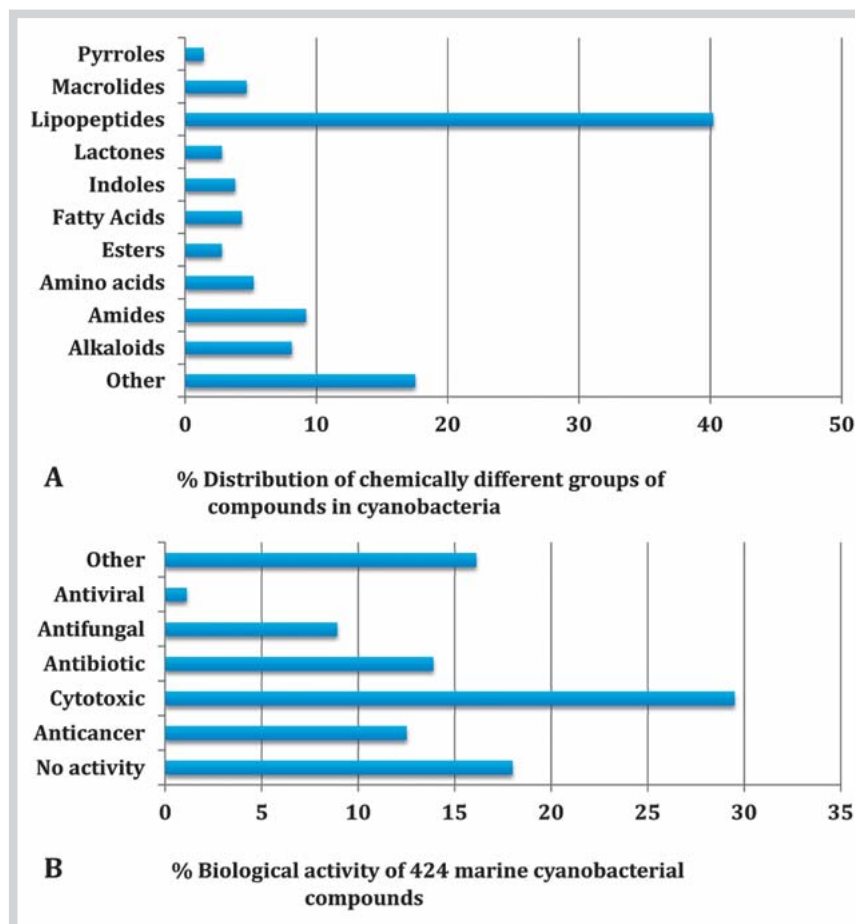
Fig. 2 An example of: **A** distribution of chemical classes of compounds in cyanobacteria and **B** their therapeutic classes [22]. (Color figure available online only.)

planktonic algae (up to millions of cells per litre) is beneficial to aquaculture; but in some situations, it can have a negative effect, causing economic losses and having major environmental and human health impacts. Among marine phytoplankton, about 300 species of dinoflagellates can sometimes proliferate in such numbers that they discolour the surface of the sea, the so-called red tides and brown tide phenomena. About 40 of these species have the capacity to produce potent toxins that can find their way through fish and shellfish to humans [30]. Harmful algal blooms can be classified into three major groups. The first is formed by non-toxic species whose proliferation under anoxic conditions results in indiscriminate killing of both fish and invertebrates. Examples of dinoflagellates that cause these problems are *Gonyaulax, Noctiluca*, and *Scrippsiella*. A second group, recently recognised as a result of the increased interest in intensive aquaculture systems, is characterized by some algal species that can seriously damage fish gills, either mechanically or through production of hemolytic substances. Examples of dinoflagellates of this group are *Prymnesium parvum, Amphidinium carteri, Chrysochromulina polylepis, Heterosigma carterae* etc. Finally, a third group is integrated by those species which produce potent toxins which can find their way through the food chain to humans, causing a variety of gastrointestinal and neurological illnesses. According to the symptoms observed in human intoxications, it is possible to consider five groups of marine toxins; PSP: paralytic shellfish poisoning (*Alexandrium, Gymnodinium* and *Pyrodinium* species*);* CFP: ciguatera fish poisoning *(Gambierdiscus toxicus,* ciguatoxin and maitotoxin); DSP: diarrhetic shellfish poisoning (*Dinophysis and Prorocentrum species,* yessotoxin, pecte-

notoxins and okadaic acid); NSP: neurotoxic shellfish poisoning (*Gymnodinium breve,* brevetoxins); ASP: amnesic shellfish poisoning (*Pseudonitzschia* species, domoic acid); and AZP: azaspiracid poisoning (*Azadinium spinosum*, azaspiracid). Dinoflagellates produce also nitrogen-containing toxins, e.g., large zooanthellatoxins and smaller saxitoxins (PSP), but smaller secondary metabolites, e.g., sterols, being a minority. Dinoflagellate toxins are being used as tools for studying cellular processes, but they have not reached the clinical studies despite promising bioactivity results. Structure determination of these extremely complex natural products requires advanced NMR techniques, and total synthesis of, e.g., maitotoxin (32 rings, 98 stereocenters), is far from being finalised and may still affect to the original assignment [31].

Macroalgae

Marine macroalgae, or seaweeds, have been used as sea vegetables, medicines, and fertilizers for centuries. Natural products extracted from seaweeds include the gelling polysaccharides agar and carrageenan (extracted from the *Rhodophyta*) and alginate (from members of the *Phaeophyta*); sometimes these are referred to as hydrocolloids or phycocolloids. The food industry exploits the gelling, water-retention, emulsifying and other physical properties of these hydrocolloids. In the biomedicine and pharmaceutical industries, alginates are used in wound dressings, production of moulds, tissue engineering, and drug delivery systems, and have a host of other applications. Carrageenans, alginates, and agaroses (the latter are prepared from agar by purification) also have several important biological activities or appli-
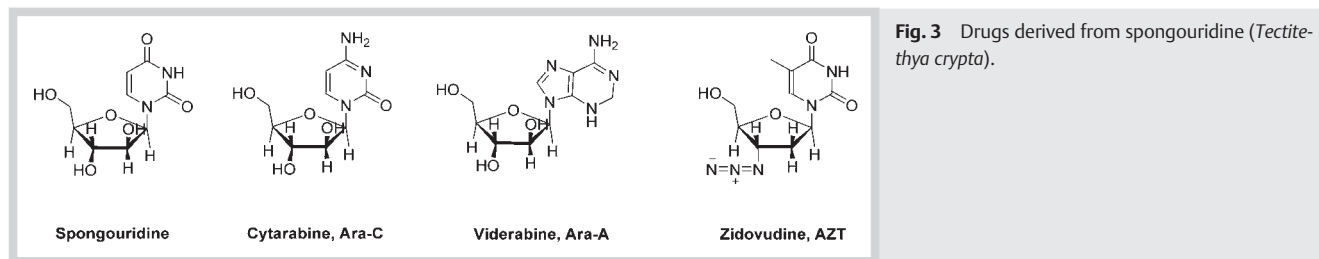
Fig. 3 Drugs derived from spongouridine (*Tectitethya crypta*).

cations in biomedicine. A large number of anticoagulant polysaccharides from marine algae have been isolated and characterized. They exert their anticoagulant activity through potentiating antithrombin III (AT III) and/or heparin cofactor II (HC II). The anticoagulant mechanism is the one by which heparin, heparin sulfate, and dermatan sulfate exert their activity. On the other hand, some algal anticoagulant polysaccharides exert anticoagulant activity through directly inhibiting fibrin polymerization and/or thrombin activity without potentiating AT III and HC II. Furthermore, new functions of algal polysaccharides have been discovered recently, as it has been shown that heparin and its derivatives have important roles in many biological processes. Algal anticoagulant polysaccharides also activate fibrinolysis system, modulate endothelial cell functions and possess anti-platelet activity [32]. Novel anticoagulant compounds have been recently isolated from fermented red alga *Pachymeniopsis elliptica* [33] and red seaweed *Lomentaria catenata* [34].

Marine organisms use chemical compounds to solve problems which also humans face. Relevant examples here are antifouling and UV sunscreen compounds [35]. Many intertidal seaweeds are exposed to dangerous levels of high-energy radiation that could overload their photosystems or cause damage to proteins and DNA; humans face a similar problem. Seaweeds are constantly faced with microbes, spores and invertebrate larvae that attempt to settle on the thallus surface; similarly, submerged man-made structures such as ships' hulls also form attractive surfaces for settlement. The mechanisms algae employ to overcome these problems (fouling, photodamage) can also be adapted for human use, resulting in new technologies (natural antifoulants, novel UV sunscreens). It is clear that investigations of appropriate natural models can provide an efficient way for the discovery and development of useful metabolites.

Marine fungi

Marine fungi belong to phyla Chytridiomycota, Oomycota, Ascomycota, Bacidomycota, Deuteromycota, and Zygomycota [14]. Like marine bacteria, marine fungi often live as symbionts in algae or marine invertebrates, especially sponges. Collection of marine fungi usually requires the collection of the host or supporting material (e.g., algae, marine invertebrates, sediment or water), which gives challenges to maintain the viability until extraction [36]. Most of the fungal species isolated from sponges belong to the genera *Aspergillus* and *Penicillium*. Around 150–200 new compounds are now isolated from marine fungi yearly [4], and the number has been increasing. The compound classes include polyketides, sesquiterpenes, alkaloids, or aromatic compounds. Although no marine fungus-based drug is currently in the pipeline, promising cytotoxic, neuroactive, antibacterial, antiviral, and antifungal activities have been found from fungi metabolites.

Marine invertebrates

Sponges (Porifera): As can be seen from ○ **Table 2**, sponges are a potential source for marine-derived drugs including eribulin mesylate, Ara-C, and discodermolide. The history starts with the discovery and subsequent identification of spongothymidine and spongouridine in 1951 from the Caribbean sponge *Tectitethya crypta* (formerly known as *Cryptotethia crypta*) [37]. Drugs developed from nucleoside spongouridine are Ara-C (○ **Fig. 3**), viderabine (or adenine arabinoside, Ara-A), an antiviral compound approved by FDA (U. S. Food and Drug Administration) in 1976 but not currently on the market, and zidovudine (or azidothymidine, AZT), one of the most popular anti-AIDS agents. The so-called pharmacodiversity can be considered to be the most substantial in sponge derived compounds [14].

Very recently sponge molecular systematics has become an alternative to morphological characterization, because the number of new sponge metabolites (297 in 2011 and 355 in 2012 [4]) increases constantly, and their structural complexity hints at a large source of new genus-specific characteristics. Presence (or absence) of a particular compound or compound family among different sponge taxa may indicate a closer phylogenetic relationship. Compound classes of sponge metabolites include terpenes, sterols, fatty acids, cyclic peptides, amino acid derivatives, nucleosides, peroxides, and alkaloids, also bromine-containing compounds are common. Another problem in chemotaxonomy is the correct assignment of a compound as sponge- and not associate-derived. When the high abundance of symbionts in sponges was discovered, it was demonstrated that sponge microsymbionts may also be the source of some of the bioactive compounds. Numerous substances bear remarkable structural similarities to bacterial compounds, implying a bacterial origin despite their high complexity.

Tunicates: Tunicates are the origin of two marine-based drugs, trabectedin and aplidine. Tunicates can be divided in three classes: ascidians (sea squirts, sea tulips), thaliaceans, and appendicularians, together comprising over 3000 species [38]. The first chemical compound directly from a marine source to enter clinical trials in 1980 was didemnin B, an anticancer agent isolated from the tunicate *Trididemnum solidum*. Although didemnin B was not successful due to its toxicity, this discovery paved the way to finding several new marine metabolites with anticancer properties. Tunicates produce structurally variable metabolites, and around 40 new structures are found yearly including macrolides, terpenes, alkaloids, and brominated aromatics [4].

Cone snails: Contrary to a common misbelief which may have discouraged some attempts of their sustainable synthetic production, not all marine bioactive compounds possess very complex chemical structures. Nowhere is the diversity of bioactive peptides more apparent than within the small peptides (conotoxins and conopeptides) present in the venom of cone snails, a family of widely distributed marine molluscs. These peptides have

evolved in ~ 500 species of fish-, mollusc- and worm-hunting cone snails for rapid prey immobilization and defence. Their small size, relative ease of synthesis, structural stability and target specificity make them important pharmacological probes. Ziconotide, a 25-residue peptide, originally isolated from *Conus magus,* is marketed for neuropathic pain under the name of Prialt®. It is estimated that over 50 000 conopeptides exist, but < 0.1 % have been characterized pharmacologically [39]. This area offers a huge opportunity for peptide-derived therapeutics employing the principles of peptidomimetics design. An orally active peptide for the treatment of neuropathic pain has been engineered from conotoxin [40]. Cone snails are one of the key organisms to be studied in various electrophysiological studies as a source of valuable new compounds.

Cnidarians: These organisms are incredibly diverse in form, and their five major groups are Anthozoa, which includes true corals, anemones, and sea pens; Cubozoa, the box jellies with potent toxins; Hydrozoa, the most diverse group with siphonophores, hydroids, fire corals, and many medusae; Scyphozoa, the true jellyfish and Staurozoa, stalked jellyfishes [41]. Yet, these diverse animals are all armed with stinging cells called nematocysts. Cnidarians are united based on the presumption that their nematocysts have been inherited from a single common ancestor [42]. Over 3000 compounds have been isolated from cnidarians in the last decade, and the majority of them are terpenoids. Sea anemones produce also fluorescent pigments, zoanthoxantins, and polypeptide toxins affecting voltage-gated ion channels.

Marine fishes

Fishes are a diverse group of living vertebrates with more than 27 000 species currently known, and over 16 000 of them are marine species [43]. Fish-based Ω-3 fatty acid ethyl esters are registered as a drug named Lovaza® in the USA or Omacor® in the EU; currently, it is the only fish-oil product approved as a drug. Oil from fresh livers of cod, *Gadus morrhua,* and from halibut, *Hippoglossus hippoglossus,* is used in vitamin A and D therapy. Squalene, present in large quantities in shark liver oil and also in plant kingdom, e.g., in olives, is used as antimicrobial, immune system enhancer, and as an intermediate in the manufacture of pharmaceuticals and an adjuvant in the vaccines [44]. Tetrodotoxin, one of the most toxic low molecular weight poisons is produced by symbiotic bacteria and is found in certain puffers, ocean sunfishes, and porcupine fishes. Tetrodotoxin is also unique in preventing the usual increase in permeability to sodium ions without affecting the outward potassium flow, and it has potential for the treatment of neuropathic pain currently in Phase III trials [45].

Cultivation and Sustainable Collection Methods
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Isolation of cyanobacteria and microalgae

Maintaining pure cultures using preservation methods is of high importance for biotechnological purposes [46]. The first step toward successful isolation is understanding and mimicking the naturally occurring environmental conditions [47]. In addition, taxonomic knowledge of the target species is very important. The second step toward successful isolation involves the elimination of contaminants, especially those that can out-compete the target species. Natural samples often contain zooplankton feeding upon algae, which can quickly consume or otherwise kill algae. Timing is of essence; some organisms die quickly, even with-

in one hour or a few hours after sampling. When samples are enriched some species quickly multiply but die suddenly, demonstrating a different aspect of timing. Application of good sterile technique is also crucial, as well as selection of proper culture medium with correct nutrient concentrations. Therefore, before isolation of cells begins, serious attention should be paid to the culture medium or media that will be used. Single cells of common weedy organisms (e.g., *Chlorella*-like organisms, *Tetraselmis*, and many diatoms) grow well when placed directly into full-strength medium.

To ensure the successful isolation of collected cyanobacteria and microalgae, state-of-the-art isolation methods must be used. For example, enrichment cultures can be established by adding enriching nutrients (culture medium, soilwater extract, or macronutrients, i.e., nitrate, ammonium, and phosphate) to the natural sample, which increases the algal growth. Single-cell isolation by micropipette can be used to remove other small cells, leaving the target organism free of contamination. On the other hand, isolation by using agar-streaking cells across agar plates is the preferred choice for many coccoid algae. Agar pour plates may need to be used in case the algae do not grow on the surface of agar plates but do grow embedded in agar (e.g., oceanic picoplankton). Isolation of microalgae may also be assisted by dragging through agar (e.g., shearing epiphytes from the filament), dilution techniques, gravity separation, atomic cell spray or by the use of phototaxis (used for example in isolation of flagellates).

Isolation and purification techniques for long-term macroalgal culture maintenance

Macroalgae is a general term for the algae that form a multicellular thallus at least in one stage of the life history, with the exception of siphonous ulvophycean algae that lack septa (e.g., *Caulerpa* and *Valonia*). In most cases they show differentiation between vegetative tissues and reproductive structures that release unicellular reproductive cells, as well as an alternation of generations. Therefore, to observe the development from unicellular reproductive cells to multicellular thallus, or to elucidate the whole life history that often cannot be observed from field-collected specimens, culture studies are necessary. Unialgal, clonal, and axenic cultures of macroalgae have become essential for studies of morphogenesis, morphological development, nutritional physiology, responses to various chemicals, crossing experiments, extracting various compounds without contamination (genomic DNA, cDNA libraries, Northern blotting, etc.), long-term strain preservation, exchange of research materials, mass culture and preparation of mariculture seed-stock. A unialgal culture is a culture that includes only one species of alga (bacteria may be present). An axenic culture is unialgal and free of bacteria. A clonal culture is a culture of a single genome set (e.g., cultures derived from a single vegetative cell or tissue or from a reproductive cell) and propagated vegetatively.

Sustainable biotechnological production

The successful search for bioactive compounds and substances is directly correlated to the increasing numbers of species being brought into culture [48]. Only the controlled maintenance of marine organisms makes their sustainable exploitation for industrial purposes possible. Many photosynthetic organisms also exhibit heterotrophic growth when nutrients are available in the absence of light. Since the profile of bioactive compounds produced may be influenced by the mode of nutrition, biomass of organisms of particular interest will be produced autotrophically,

heterotrophically, and mixotrophically (growth in which nutrients are obtained both photosynthetically and heterotrophically) so that the product profiles can be determined under each condition. Heterotrophic cultures offer the advantages of easier scale up and higher potential biomass densities than photosynthetic cultures and thus may be desirable for the production of high-value bioactive compounds. For example, most photosynthetic cultures only achieve biomass densities in the range of $1-5 \text{ g} \cdot \text{L}^{-1}$, whereas heterotrophic cultures may achieve biomass densities greater than $50 \text{ g} \cdot \text{L}^{-1}$ [49]. The productivity of microbial cultures is often improved by growing the producing organism in high cell density fed-batch culture rather than batch cultures. High cell density fed-batch cultures may be relevant for some heterotrophically produced algal compounds [50], but are not practical for photosynthetic cultures. Continuous flow culture systems can be used to improve productivity of both photo- and heterotrophic cultures.

Extraction, Separation, Isolation, and Purification of Marine Compounds

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The freeze-dried marine organisms are soaked in a solvent of medium polarity. The solvents of choice are methanol and acetone. Crude extracts from marine organisms often contain complex mixtures of a number of compounds that need several sequential purification steps. Usually the first step is a liquid solvent partitioning procedure. One of the most used is the Kupchan [51] procedure that uses solvents with increasing polarity, affording enriched extracts. MPLC on different stationary phases (adsorption chromatography with silica gel, or reverse phase chromatography with octadecylsilyl (RP8) or octylsilyl (RP18) chain phases) and exclusion chromatography on, e.g., LH-20 and LH-60 supports are effective for a first-stage fractionation. Liquid-liquid chromatography without supported stationary phases such as droplet countercurrent chromatography and centrifugal partitioning chromatography are very efficient in assuring a good level of purification of polar extracts with a complete recovery of the material, avoiding the problem of the irreversible adsorption experienced when traditional supported stationary phases are used. The last step of purification is done by HPLC, which allows obtaining compounds with a high degree of purity.

Advanced Spectroscopic Methods of Structural Determination, Stereochemical Characterization, and Chemotyping

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Mass spectrometry with new ionization techniques coupled with liquid or gas chromatography enables high-precision analysis of marine biomolecules and fingerprinting of fractions. Rapid first chemotyping of the studied biological materials and substances is essential for de-replication, the major bottleneck in the search of novel marine natural products. MS data can also be utilized in development of metagenomic methods for discovering marine natural products and their biosynthetic pathways [52].

With the advent of high field spectrometers and inverse decoupled heteronuclear correlation techniques, NMR spectroscopy has become a practical alternative to X-ray crystallography for the structure elucidation of natural products [53]. Currently, all but the most complex organic molecules are amenable to routine analysis, even with submilligram sample quantities. The structural elucidation of complex molecules by modern NMR methods is particularly useful in the natural products field, as natural products are often isolated in small amounts and do not easily crystallize. Therefore, approaches relying on "non-invasive" spectroscopic and spectrometric analysis of intact compounds are extremely appealing in this context, allowing the sample under investigation to be used for further pharmacological evaluation. Further, NMR and other spectroscopic techniques allow the sample to be preserved for further investigation.

As part of the complete structural elucidation of natural products, the determination of relative and absolute configuration is undoubtedly a key step. In fact, the full topology of the molecule has a profound impact on its biological and pharmacological properties. Nowadays several methods, mainly relying on NMR and computational approaches have provided a reliable access to the configurational assignment of even complex and flexible molecules [54]. In particular, J-based configuration analysis is a powerful yet simple technique for determining stereochemistry and conformation of a molecule [55]. Several instrumental methods exist for the determination of absolute configuration. Chirooptical methods (e.g., circular dichroism (CD), infrared (IR) and UV spectroscopy will also give structural information [56]. All of these techniques must be utilized to obtain and guarantee high-quality structural information of the isolated and purified bioactive marine compounds.

Technologies for Exploring the Bioactivity Potential of Natural Products

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Exploring large chemical libraries for biological activity is typically carried out through high-throughput screening (HTS) campaigns, which enable the study of thousands of samples in a short time-span. HTS assays can be configured to study biological responses from isolated biochemical systems containing purified receptors or enzymes, to signal transduction pathways and complex networks functioning in cellular environments. HTS is originally industry-driven technology, but in the recent past, academic input within this research field has significantly increased. This has led to innovations for improving HTS methodologies, especially in developing advanced cell-based, phenotypic screening models, which have recently been considered as key factors in improving the success of modern drug discovery [57, 58]. Many of these models employ so-called high-content screening (HCS) technologies, i.e., automated platforms enabling cellular imaging and quantitative image analysis in high-throughput format. Initially, HCS was mostly used in follow-up studies for hits identified in biochemical HTS campaigns, but due to technological advances the use of HCS in the early stages of drug discovery has expanded [59]. By using HCS as an initial screening tool, the sample libraries can be studied directly in complex cellular environment, which is considered to provide more predictive, physiologically relevant information compared to target-based approaches [60, 61].

The success of any bioactivity screening campaign is dependent on the quality of the chemical library to be screened. Historically, natural products (NPs) have constituted a rich source of lead molecules in drug discovery, but de-emphasised in industrial screening campaigns mainly due to difficulties in obtaining high-quality NP libraries suitable for large scale HTS [62]. These libraries can contain a variety of different sample types, such as crude extracts, semi-pure fractions, or purified natural compounds, which make them a challenging source for HTS. When

designing the library, advantages and disadvantages of each of these categories need to be carefully evaluated. Preparation of crude extract libraries is usually economical but demands extensive resources for later identification of bioactive components. Constructing a library based on semi-pure fractions or purified natural compounds is costly and labour-consuming but may reduce interferences encountered typically with crude extracts in HTS and also simplify the detection of actives. However, recent technological advancements made in constructing NP libraries and in follow-up studies have facilitated the use of NPs in screening campaigns. Generating a large-scale marine NP library amenable to HTS and shortening the identification of active compounds have been pursued, for example, by coupling online characterisation by MS into the process [63]. Efficient dereplication strategy (i.e., identification of previously known components) is also essential and should be implemented as early as possible in the process. This can be pursued by using metabolic profiling as a tool to evaluate the diversity and novelty of compounds present in the NPs [64, 65].

Synthetic Modification of the Marine Bioactive Compounds
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Natural compound libraries have always been an excellent source towards new drug candidates. Marine bioactive compounds can have a greater number of asymmetric atoms and increased steric complexity than any of the synthetic drugs. Natural products contain unique ring systems. Surprisingly, less than 20% of these diverse structural scaffolds are present in the current drugs on the market [66]. Interestingly, the fraction of natural compound structures with two or more "Lipinski-rule-of-five" violations is only about 10% and equal to that of the commercialised drugs. These complex structures offer challenges to synthetic chemists, but often the scope is primarily directed to the synthesis and optimization of more simple drug-like structures (MW preferably < 500 Dalton). Despite numerous brilliant examples of total synthesis of marine compounds [67, 68], it is often possible and reasonable to define the crucial structural elements required for biological activity. The methods of medicinal chemistry are useful in designing and synthesising structurally simple, potent and selective compounds with feasible and sustainable synthetic routes and at reasonable cost. The synthetic compound libraries often tend to exclude complex chemical structures although a diversity-oriented synthesis approach can lead towards more natural compound-like structures [69].

Industrial Applications of Marine Bioactive Compounds
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Pharmaceutical applications
Inflammation and cancer: Although the association between inflammation and cancer has long been suspected [70], the precise mechanism of inflammation-induced malignant transformation is not fully understood. Chronic inflammation is characterized by an ongoing and sustained release of reactive oxygen and nitrogen species and of pro- and anti-inflammatory cytokines and chemokines [71]. Such inflammatory mediators are considered as major pathogenetic factors of carcinogenic malignant transformation of human intestinal epithelial cells during chronic inflammatory diseases (i.e., ulcerative colitis). Epigenetic changes have

emerged as one of the most consistent molecular alterations in various neoplasms and have been implicated in mechanisms of cancer progression such as DNA damage and repair, apoptosis, and cell cycle control. Recently, a molecular link between inflammation and cancer has been made through the implication of NF-κB in promoting the cellular changes leading to uncontrolled growth of cancer cells by antagonizing pathways of cell death and later on changes that help tumor progression and metastasis [72]. Moreover, tumor cells produce COX-2 (cyclooxygenase, a pro-inflammatory macromolecule) that gives a further boost to inflammatory processes making the link between cancer and inflammation very interactive [73, 74]. Hence, therapeutic agents with anti-inflammatory effects could be of great benefit to inhibit cancer growth and spread [75, 76].

Ion channels: The demonstrated implication of ion channels in physiological phenomena such as neuronal excitation, excitation-contraction coupling or stimulation-secretion coupling makes it not surprising that a number of organisms have developed channel-specific toxins as mechanisms for self-defence or for capturing prey. A rich, non-exhaustive collection of natural toxins selectively target the ion channels of neurons and other cells and represent valuable tools for studying the function of cellular ion channels. One of the best-known channel toxins is tetrodotoxin [77], currently in Phase III trials for cancer-related pain. It elicits a potent and specific block of some subtypes of voltage-gated Na^+ channels responsible for action potential generation, thereby paralyzing the animals that ingest it. Ziconitide, a potent conotoxin that induces significant analgesia when delivered spinally, is a rare toxin-based commercially available drug [78]. This neuronal-specific, N-gated calcium channel blocker works at the dorsal horn and is effective in a variety of pain states. The mechanisms of action for conotoxins are further studied [79]. Despite a growing interest, the use of toxins for developing drug leads is still a niche research area.

Antimicrobial and antiviral applications: Discovery of new antimicrobial agents and characterisation of the mechanisms of action for potential leads are essential issues for modern pharmaceutical research. Today the market size for antimicrobials is huge, but the increasing number of emerging resistant bacterial strains is becoming a limiting factor in the usefulness of current drugs available. However, antimicrobial drug discovery has become highly unattractive to pharmaceutical companies mainly due to short antibacterial drug lifecycles and the acute, rather than chronic, nature of antibacterial therapy [80]. As a result, new classes of antibiotics are nowadays hardly discovered. Marine natural products have a great potential for antimicrobial applications but NP-based antibiotics in the pipeline still originate from fungi or actinomycetes [18]. One very important subcategory in antimicrobial therapy is antiviral drugs, where the availability of specific clinical treatments is still very limited. Yet, viruses are widely recognised as emerging sources of new epidemics. For example, insect-borne alphaviruses are currently spreading to more northern areas, which has been demonstrated by the spread of West Nile virus in the USA [81], and even more recently by the outbreaks of chikungunya virus in the Indian Ocean region and in Italy [82, 83]. In Finland, strains of Sindbis virus cause tick-borne encephalitis and Pogosta disease [84]. Alphaviruses are positive-strand RNA viruses and belong to the largest group of viral pathogens containing several major pathogens, such as hepatitis A, C and E viruses, rhinoviruses (major cause of common cold), coronaviruses (common cold and severe acute respiratory syndrome, SARS), and alphaviruses (Sindbis, chikungunya,

dengue fever, yellow fever, West Nile virus encephalitis, Semliki Forest), which share certain antigenic determinants. Despite their wide distribution and potential pathogenicity, neither effective chemotherapy nor commercial vaccination is available for clinical alphavirus infections.

Cosmetics

A classic example of marine compounds in cosmetic products is Estée Lauder's Resilience® that contains anti-inflammatory pseudopterosins from soft coral *Pseudopterogorgia elisabethae* [85]. The effects of harvesting of the soft corals have been studied, and it is an excellent example of sustainable exploitation of marine organisms. Clipped soft corals were branching and growing even faster than the control group [86]. Pseudopterosin A has even potential as a drug since it has been in Phase II trials as topical anti-inflammatory agent. Marine anti-inflammatory compounds will also have potential use in cosmetics, and there is an unmet need of new UV-absorbing and sustainable compounds for sunscreen applications to replace the currently used allergenic, irritating, or bioaccumulative sunscreen agents.

Nutraceuticals

Fish oils, omega-fatty acids, and marine lipids are probably the best known marine food supplements. Even one omega-3 fish oil product, Lovaza®, has been approved as a drug by FDA. Only few species (e.g., *Chlorella vulgaris* and *Spirulina pacifera*) from 40 000 species of algae have been used by the food industry [87]. Microalgae contain carotenoids, sterols, lipids, and polyphenols. Carotenoids have been produced in large scale in photobioreactors. Anti-oxidant and food dye astaxanthin can be produced effectively from *Haematococcus* biomass [88].

Biosensors

Marine biotoxins are chemical compounds that can be classified according to the acute symptoms that they present to humans and other mammals when ingested with contaminated seafood. Food safety in marine resources is especially important because of the unseen threat of microalgae. More specifically, several species of dinoflagellates and diatoms produce odorless and tasteless phycotoxins, which enter the food chain through shellfish and several other marine carnivores [89]. Syndromes caused by phycotoxins range from diarrhea, vomiting and spasms to even coma and death. Traditionally, these toxins have been detected in seafood using laborious biological and animal reference methods. The release of the European Union Directive 86/609 for animal protection to ensure progress away from animal experimentation to scientifically acceptable validated non-animal procedures started the scientific exploration for alternative methods for these animal bioassays [90,91]. Biosensor technologies are analytical tools for the recognition and measurement of a target through its association with a biological component and physiochemical detector. Therefore, a biosensor typically consists of a bio-recognition element, transducer, and an electronic system composed of a signal amplifier, processor, and display. Today, biosensors are widely known and appreciated for sensing purposes in a wide range of analytical applications [92]. The most prominent receptor molecules used are enzymes, antibodies, and aptamers [93]. Their interaction with the target of interest is converted to a measurable signal. Biosensor technology also benefits from fast growing disciplines such as micro-electronics, which results in advanced biochips by combining the knowledge of microfluidics with microelectronics [94]. Until today, various phy-

cotoxin biosensors have been made. The most common are based on antibodies and enzymes. For example, Marquette et al. developed an immunosensor for okadaic acid integrated in a fiber optic-based chemiluminescence flow injection system [95–97]. Palytoxin is a polyether marine toxin originally isolated from the zoanthid *Palythoa toxica* and is one of the most toxic nonprotein substances known. Fatal poisonings have been linked to ingestion of contaminated seafood. Its co-occurrence with other well-characterized seafood toxins (e.g., ciguatoxins) has hindered its direct associations to seafood-borne illnesses. A new Biacore T100 SPR assay for palytoxin using an anti-mouse substrate to characterize the kinetic values for a previously developed monoclonal antibody raised to palytoxin has been developed [98]. Tissue-based biolectronic sensors have also been developed: paralytic toxins were detected by investigating their effect as sodium channel blockers [99].

Agricultural applications: plant growth regulators, herbicides, and biocides

Cyanobacterial or macroalgal extracts may have use in agriculture as plant growth regulating agents, fertilizers, and as agents increasing nutrient uptake from soil or improving plant resistance. Additionally, freshwater cyanobacteria have been studied for their potential use as algal growth inhibitors. Most of the cyanobacterial herbicides inhibit electron transport in the vicinity of photosystem II. The effect of cyanobacterial extracts on photosynthesis, growth and development of higher plants and algae is worth examining. The potential use of the extracts or isolated compounds as plant growth regulating agents is useful to be assessed.

There are reports showing that algal extracts can be used in agriculture as agents increasing crops by stimulating growth, increasing nutrient uptake from soil or improving plant resistance to infections and unfavourable weather conditions. Commercial extracts made from brown alga (Maxicrop, Kelpak, Goemar, Seasol, and Algifert) have been applied to crop lands for improving yields and plant vigour. The extract from unicellular green alga *Chlorella* has been shown to stimulate chlorophyll synthesis and growth of shoots and roots of fruit-trees, vegetables, and rice. *Chlorella* extract has been approved in Japan as a growth-regulating agent. Cyanobacteria, the most dominant photosynthetic prokaryotic microorganisms, are also known to produce compounds that potentially could be used as fertilizers and plant growth regulating agents. Their beneficial effect on plants (rice and wheat) was originally attributed to nitrogen fixation. Later it was postulated that the positive influence of cyanobacteria on seed germination and growth is additionally induced by the presence of vitamins and plant hormones or compounds with plant hormone-like activity. Effects attributed to this type of activity were detected among others in *Calothrix* sp., *Hapalosiphon intricatus, Nostoc* sp., and *Arthronema africanum* [100,101].

On the other hand, some cyanobacteria, e.g., of the genera *Fischerella, Scytonema, Nostoc, Hapalosiphon*, and *Oscillatoria,* were found to produce compounds of algicidal activity directed against photosynthesis. Most of these natural herbicides inhibit electron transport in the vicinity of photosystem II. Other effects include inhibition of RNA synthesis and DNA replication. Cyanobacterial toxins are known to inhibit growth of a range of terrestrial plants; toxin uptake was correlated with lipid peroxidation in seedlings [102]. In some instances, the metabolites produced by cyanobacteria could replace synthetic phyto-regulators, toxic to non-target organisms [103]. Furthermore, the role of the compounds in

allelopathic interactions and functioning of aquatic ecosystem has been discussed [104].

Another interesting phylum is Porifera and, for example, metabolites from marine sponges may provide a promising alternative to the use of ecotoxic heavy metals in antifouling coatings. Water-soluble polymeric 3-alkylpyridinium salts isolated from the Mediterranean sponge *Reniera sarai* and 14 related synthetic analogs have shown considerable antibacterial activity against marine biofilm bacteria and may represent good candidates as natural biocides for marine technology applications [105, 106]. It has also been shown that barettin and dehydrobarettin, diketopiperazines isolated from the marine sponge *Geodia barretti*, are potent inhibitors of barnacle larvae settlement [107, 108].

Biofilms

A biofilm is any group of microorganisms in which cells stick to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm extracellular polymeric substance is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial, and hospital settings [109, 110]. Among bacteria, only a little is known about chemical defense against bacterivorous consumers. According to one hypothesis, sessile bacterial communities organized as biofilms serve as bacterial refuge from predation. By testing growth and survival of common bacterivorous nanoflagellates, evidence has been found that chemical mediated resistance against protozoan predators is common among biofilm populations in a diverse set of marine bacteria [111]. Chemical communication is essential in biofilm populations to coordinate their behavior and respond to environmental pressure. Recent research has been unravelling a complex web of chemical crosstalk mediating microbial symbiosis, competition and defense against predators and pathogens. When the molecular basis of biofilm interactions in their ecological context is understood, the potential of natural product discovery and the development of biofilm-derived biotechnologies are achievable [112].

There are certainly natural products to be found with interesting activities in antimicrobial, especially in anti-biofilm, research. Biofilms are surface-attached bacterial communities embedded in a self-produced matrix, making them much more resistant in comparison to their planktonic counterparts. The unique properties of bacterial biofilms call for the development of reliable and specific research methods, different to the ones optimized for planktonic bacteria. To fill the void of biofilm assays suitable for NP screening, a statistically robust platform of assays was developed by Skogman et al. [113]. Three important features of biofilms were targeted using three separate assays ran in sequence after each other. The effect the tested compounds have on biomass and viability can be studied at a primary screening stage. This is followed by investigating the effect the compounds have on the EPS-layer at a secondary screening stage. Hereby only so called true hits are found. This platform has been further used for a library of flavonoids [114], and also small molecule peptides [115], thus paving the way for many other natural products. Pharmaceutical, paper and pulp, and biofouling industries will benefit in future via finding new anti-biofilm compounds as the biofilm matrices are resistant for chemical agents of today.

Conclusions
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The marine environment is a rich source of biological and chemical diversity. This diversity is the source of novel chemical compounds with the potential for industrial applications as pharmaceuticals, cosmetics, nutritional supplements, molecular probes, fine chemicals, and agrichemicals. The oceans represent an untapped resource for discovery of new compounds with useful activity. Although the future potential of marine biotechnology relates to the discovery, development, and sustainable use of marine-derived compounds with health promoting applications, the needs, approaches, and opportunities apply equally to other marine bioproducts.

In maritime research, marine specimens from oceans must be collected by strictly following ethical and environmental guidelines. Most importantly, existing marine sample collections, including extract and pure compound libraries should form an integral part of the study materials. New biotechnological production techniques and green chemistry extraction techniques should be developed. Bioactive compounds should get supplied and optimised for biological assays via synthetic chemistry. The challenge facing the marine biotechnology industry in the next millenium is to identify new sources of marine bioproducts, to develop novel screening technologies, and to provide a sustainable source of supply.

Acknowledgements
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Conflict of Interest
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The authors declare no conflict of interest.

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