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Pharmacological Developments Obtained from Marine Natural Products and Current Pipeline Perspective

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Marine organisms represent a new extensive source for bioactive molecules. They have the potential to provide new therapeutic alternatives to treat human diseases. In this paper, we describe and discuss a variety of isolated and semisynthetic molecules obtained from marine sources. These compounds are in phase II, phase III and at the commercialization stage of new drug development. A description of the mechanism of action, dosage used and side effects are also reported. The positive results obtained from these studies have triggered the development of new studies to evaluate the prospects for utilization of marine organisms.

Keywords: Marine natural products, pharmacology, drugs, action mechanism.

Introduction

The oceans cover about 75% of the surface of the Earth. They embrace all the Kingdoms of nature allowing a dense and complex biotic diversity to flourish. In fact, it is estimated that hundreds of thousands of species dwell in the oceans and most of them are unknown.

Out of this great variety of marine species, sessile and soft body organisms are the most important for producing bioactive molecules. Sessile organisms settle on the seafloor to grow, while soft body organisms do not have this requirement. The former type is evolutionary disadvantaged because they are not able to escape from their predators. However, while soft body organisms can run away from predators, they do not possess an external skeleton (shell) to protect themselves. These organisms have evolved and they produce a wide range of chemical substances, in which most of them function as secondary metabolites. These are used mainly as mediators for communication, reproduction and adaptation processes. They are also used as a defense mechanism to counter predator attacks.

Current developments and advances in chemistry and isolation methods have made it possible to discover and isolate the secondary metabolites produced by sessile and soft body organisms. Several of these molecules have been used, such as for human drugs, for example Cytarabine® and Vidarabine® [1]. The goal of this paper is to review secondary metabolites isolated from different marine organisms that can be potentially used as human medicines.

The first reported structure of a marine product was made in 1880 by Krukenberg [2,3]. He isolated and characterized several steroidal derivatives, mainly from echinoderms and sponges [4-6]. Two years later, he published the chemical structure of some pigments, and isolated a uridine nucleotide from several marine organisms [7-9]. The work of Krukenberg inspired new researchers to investigate and isolate new molecules from marine organisms, such as cholesterol-related compounds and some marine pigments [10]. In this case, the most salient work was the isolation of spongosterin (**1**) and spongosterol (**2**) from the sponge *Suberites domuncula* [11,12]. These were the beginnings of investigations into marine natural products chemistry, which has resulted in the discovery of new chemical structures and new bioactive substances [2].

In 1944, Pratt and collaborators reported the biological activity of aqueous extracts prepared from microalgal cell cultures of *Chlorella*. These extracts presented antimicrobial activity against *Staphylococcus aureus, Streptococcus pyrogenes, Bacillus subtilis, Bacterium coli* and *Pseudomonas pyocyanea* [13]. A year later, other scientists studied the biological activity of eukaryotic organisms of greater structural complexity, such as macro-algae, coelenterates, and cephalopods. These studies were focused mainly on the toxicity and antimicrobial activity of either organic extracts or exudates produced by these organisms [14-17]. The isolation of bioactive molecules from marine extracts was made mainly by crystallization and re-crystallization techniques, which rendered mainly nucleotide-like molecules (**3**, **4**) [18].

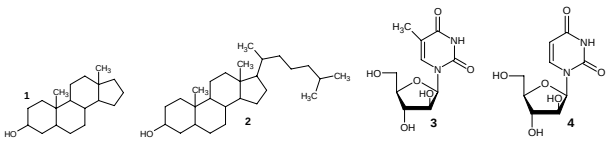


Figure 1: First molecules isolated from marine natural products.

No major progress was made in this field until the mid-1950s when Stahl introduced thin layer chromatography (TLC) as a reliable, reproducible and good resolving analytical technique for the separation of complex mixtures [19,20]. The development of different chromatographic techniques increased the number of structurally-complex molecules obtained from bioactive natural extracts. For example, the study of Bergmann and Burke led to the isolation of spongothymidine (**3**, Ara-T) and spongouridine (**4**, Ara-U). These nucleotides were obtained from the acetone extract of the sponge *Crypotethia cripta* by chromatography [21]. The promising anticancer activity of these molecules made them the first marine molecules for pharmacological development [22]. Large amounts of these nucleotide derivatives were produced and the need for developing an efficient synthesis of structural analogues emerged. As a result, researchers synthesized chemical analogues such as Ara-A (**5**), Ara-C (**6**) and zidovudine (**7**) [23,24]. ARA-C proved to be a potent tumor inhibitor in animal and human models [25,26].

In the last ten years more than one thousand reports of bioactive molecules isolated from marine natural organisms have been published. (Figures 2 and 3). A

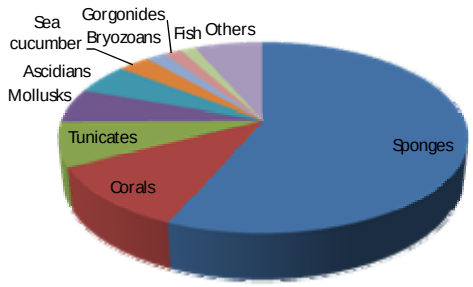


Figure 2: Percentage of bioactive compounds obtained from marine species (from 1998 to 2008) [27-39].

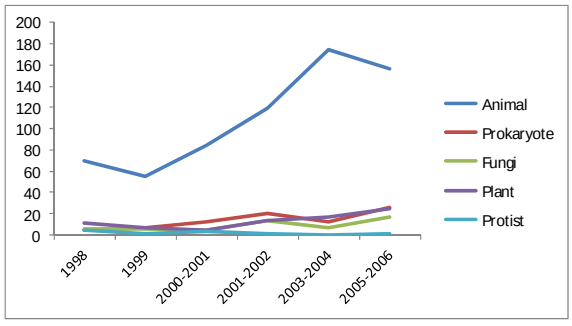


Figure 3: Comparison of the number of reports of bioactive marine natural products made in the 1998-2006 period [27-39].

description of the most representative molecules isolated from marine organisms and other compounds obtained from marine sources are explained in this review; they are arranged according to their clinical and pharmacological phase.

Ara C (Cytarabine)

Ara C is considered to be the first synthetic molecule associated with a marine source. It was marketed by Pharmacia Laboratories & Upjohn Company in 1969, and was approved by the Food and Drug Administration (FDA) for the treatment of leukemia under the trade name of Cytosar-U® in the same year [40].

Ara C (Cytarabine, 1β-D-arabinofuranosyl cytosine) is a colorless crystalline solid, soluble in water and moderately soluble in ethanol and chloroform. It is used as an antineoplastic agent intravenously, intrathecally or subcutaneously. In the bloodstream it is rapidly converted to cytosine arabinoside triphosphate. This form damages DNA during cell division between phases G1 to S [41,42].

Cytarabine is available in injectable solutions up to 100 mg/mL. It is combined with other anticancer drugs and administrated intrathecally at a dose of 100 mg/m^2/day by a continuous infusion for 1 to 7 days, or intravenously to 100 mg/m^2 every 12 hours during the same period of treatment. This drug has a low bioavailability if administered orally. For example, less than 20% of the oral dose of cytarabine is absorbed in the gastrointestinal tract losing its therapeutic effectiveness. Studies with tritium radiolabeled cytarabine showed maximum plasma peak times of 20 and 60 minutes after subcutaneous and intramuscular injections, respectively [43].

This drug, if infused intravenously, produces relatively constant plasma concentrations from 8 to 24 hours. The intravenous dose shows a biphasic elimination. The initial phase has a half-life of 10 minutes. In this phase, from 70 to 80% of the administered dose is metabolized in the liver by the deoxycytidine kinases and other nucleotide kinases transforming Ara-C into the nucleotide triphosphate, an effective DNA polymerase inhibitor. The second phase has a longer half-life of between 1 to 3 hours. Ara-C is also metabolized by the kidneys, gastrointestinal mucosa, granulocytes and other tissues. About 10% is eliminated unchanged via urine [43]. The reported side effects include pain, bleeding, anemia, nausea, vomiting, diarrhea, anorexia, gritty eyes, mouth ulcers, dysgeusia, and an increase in uric acid levels in blood [44,45]. Currently, this molecule is being evaluated along with other anticancer drugs, in order to enhance its therapeutic effectiveness [46,47].

Ara-A (Vidarabine)

Ara-A (9-β-D-ribofuranosyl-adenine) is a compound closely related to Ara-C. It is a potent antiviral drug synthesized and marketed by Burroughs Welcome

Laboratories (nowadays, GlaxoSmithKline). This compound was isolated from the gorgonian *Eunicella cavolini* [48]. Ara-A monophosphate proved to have activity *in vitro* and *in vivo* against type 1 and 2 herpes with IC_{50} doses of 135 and 145 mg/mL, respectively [49]. These results led to FDA approval in 1978 for the treatment of herpes infection [50]. The mechanism of action involves blocking of viral DNA synthesis. Vidarabine is phosphorylated to Ara-ATP by kinases, and is able to inhibit DNA polymerase by a competitive inhibition of adenosine bases (dATP) preventing viral DNA synthesis [51].

Vidarabine is active against poxivirus, rhabdovirus, hepadnavirus and some carcinogenic RNA viruses. It is generally used in topical formulations at a 3% concentration for the treatment of keratoconjunctivitis and recurrent superficial keratitis caused by HVH-1 and HVH-2 herpes. Nowadays, this drug has been replaced by other nucleotide derivatives with greater potency and bioavailability, such as acyclovir (**8**), valacyclovir, and famciclovir [52].

The successful discovery and synthesis of active compounds has encouraged the search for new therapeutic molecules isolated from marine organisms. For this reason, the U.S. National Institute of Health (NIH) founded the "Computer Retrieval of Information on Scientific Projects (CRISP)" database. This has compiled more than 555 research projects related to the search for new molecules from marine sources since 1972.

Ziconotide (ω-conotoxin, Prialt®)

The cone snails are a large genus of gastropod mollusks, all of which are believed to be actively venomous predators. The venom is synthesized in a long tubular duct from which it is squeezed by a muscular bulb, and injected into the prey through a hollow radular tooth. These cones then extend their proboscis above the substrate and wiggle it. A fish so baited may be stung in the mouth and paralyzed almost instantly. The venoms contain peptide toxins that attack various critical targets in the neuromuscular system of the prey; these toxins were called conotoxins. The conotoxins were originally isolated from the marine cone snail *Conus magnus* and *C. geographus*. Chemically, they are small basic molecules (<15 amino acids), with from one to three disulfide bridges [53].

Conotoxins are classified pharmacologically into five types based on their cellular receptor [53]. The α-conotoxins bind the acetylcholine receptors of neuromuscular synapses, and are made up of smaller peptides (13-15 amino acids long). The μ-conotoxins block the voltage-dependent sodium channels of the muscle cells, and are composed of from 20 to 22 amino acids. The ω-conotoxins target the neuronal calcium channels. The mechanism of action of the other two conotoxins (δ and μ) is less well known. The δ-conotoxins block mollusk and vertebrate neuronal sodium channels. The μ-conotoxins block the mollusk neuron calcium channels [54]. Ziconotide is a synthetic peptide derived from ω-conotoxin MVIIA, with a molecular weight of 2639 Da (**9**). Ziconotide represents the first innovative product to treat severe pain since the discovery of morphine more than 200 years ago. Ziconotide is effective for the treatment of neuropathic, nociceptive, and mixed neuropathic/nociceptive pain. Ziconotide was approved by the FDA in 2004 to treat chronic pain and nowadays is commercialized by Elan Corporation, under the trade mark of Prialt® [55].

The mechanism of action of Ziconotide is associated with the N-type presynaptic calcium channel blockage located on the presynaptic terminal of dorsal horn C fibers. This reduces the excitation ability of the neurotransmitters in the afferent portion of the terminal nerves, thereby reducing release of pain-relevant neurotransmitters, such as glutamate and neuropeptides, from the primary afferent nerve terminals into the synaptic cleft [56]. It does not bind to the opioid receptors, and hence it does not cause the addiction and breathing problems generated by the opioids. Morphine and related drugs act on the μ-opioid receptor linking to this calcium channel via a G protein–coupled mechanism. The efficacy of ziconotide in providing pain relief has been demonstrated in several trials, prompting its addition to the 2007 Polyanalgesic Consensus Conference panel list of level 1 drugs. The panel recommended ziconotide as a viable alternative for patients who cannot tolerate intrathecal administration of morphine and/or hydromorphone [55].

This medication can be taken intrathecally at a dose from 1.2 (0.05 μg/h) to 2.4 μg/day (0.1 μg/h) under continuous patient monitoring. The dosage may be increased by up to 2.4 μg per day (0.1 μg/h) at intervals no more than two to three times per week up to a recommended maximum of 19.2 μg per day (0.8 μg/h). However, despite having demonstrated efficacy, Ziconotide is associated with several adverse effects. The most commonly reported side effects are memory impairment, dyskinesia, nausea, ataxia, drowsiness, vomiting, diarrhea and asthenia. Other adverse effects reported in clinical trials and case studies include elevated creatinine kinase levels, sedation, nausea, headache, lightheadedness, depression, confusion and emotional distress, with certain symptoms possibly correlated with the rate of infusion [43,55].

Ecteinascidin-743 (Trabectedin, Yondelis®)

This compound is found in extracts of the tunicate *Ecteinascidia turbinata.* The first study of the biological activity of these extracts against murine leukemia was reported in 1969 [57]. Twenty-one years later, Rinehart and Wright were able to elucidate the structure of the tetrahydroisoquinoline alkaloids named Ecteinascidin (ET). These compounds are responsible for the therapeutic activity of *E. turbinata* [58,59]. ET-743 (**10**) proved to be

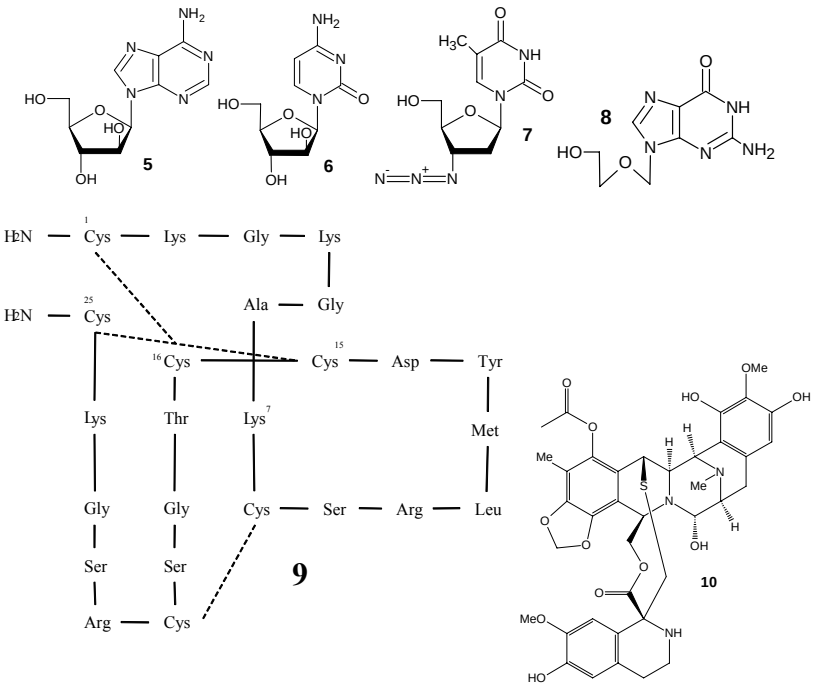


Figure 4: Marine-derived drugs in phase III and IV (post marketing surveillance).

the most bioactive alkaloid out of the six Ecteinascidin-type alkaloids isolated. It has an i*n vitro* IC_{50} of 0.5 ng/mL against L1210 leukemia cells, and an *in vivo* IC_{50} of 15 μg/kg in mouse models with lymphoma P388. However, this compound presents a low isolation ratio ($1x10^{-4}$% w/w), which limits research ability to pursue more *in vivo* studies against various cell lines.

The novel and unique structure of ecteinascidins consists of a monobridged pentacyclic skeleton composed of two fused tetrahydroisoquinoline rings (subunits A and B) linked to a 10-membered lactone bridge through a benzylic sulfide linkage. Most ecteinascidins have an additional tetrahydroisoquinoline or tetrahydro-b-carboline ring (subunit C) attached to the rest of the structure through a spiro ring [60]. In 1996, the first successful attempt to synthesize ET-743 was achieved. This stepwise synthesis was made by employing 23 different steps, starting from the α,β-unsaturated malonic ester of 2-(benzyloxy)-3-methyl-4,5-(methylenedioxy) benzaldehyde [61]. ET-743 is a structure homologous to safracine-B, an antibacterial agent, which is a by-product from the fermentation of *Pseudomonas fluorescens*. Advances in biotechnology allowed the synthesis of ET-743 from cianosafracine-B, avoiding chemical synthesis and having a significant cost reduction and large scale production [62]. Other synthetic routes have been devised by Cuevas and Francesch [60].

ET-743 (Trabectedin), patented by the Spanish PharmaMar laboratory under the tradename of Yondelis®, was approved for marketing in Europe. Subsequently, PharmaMar licensed this product to Ortho Biotech Products for commercialization in the United States. In 2001, Trabectedin was granted orphan drug status in Europe for recurrent soft tissue sarcoma by the European Medicines Agency (EMEA), and the same drug status by the FDA for the same indication in 2004 [63]. In 2007, Trabectedin was approved by the EMEA to treat soft tissue sarcoma in the special case of adult patients where either conventional therapy with anthracyclines and ifosfamide has been ineffective, or in patients who are allergic to those medications [43,60].

The mechanism of action of Trabectedin is complex and involves the blockage of several steps of cell division. Trabectedin is an alkylating agent which reacts with the amine groups at the N-2 position of guanine-rich sequences in the minor groove of DNA. As a consequence, the DNA helix turns toward the major groove, producing a conformational change preventing DNA from replication. Furthermore, Trabectedin acts on the cell cycle, stimulating the S phase of mitosis and blocking the cycle at the G_2 phase. This blockage is accompanied by an increase in the production of p53 tumor suppressor gene. Trabectedin also stimulates the endogenous cellular response of the patients. Other studies have suggested that Trabectedin generates cellular apoptosis due to the blockage of the cell cycle. This drug also prevents the aggregation of the cell microtubules. However, this mechanism does not involve tubulin inhibition, as occurs with Taxol [64].

Trabectedin is marketed as a powder for reconstitution in infusion solutions. The recommended dose is 1.5 mg/m^2 administered by central intravenous infusion over 24 hours, every 21 days. The EMEA recommends administering 30 mg of IV dexamethasone, 20 min prior to the administration of Trabectedin. This will prevent nausea

and has hepatoprotective effects. Because of the complex endogenous reactions of this drug, it is necessary to monitor the liver, kidney and blood functions in all patients. Studies conducted on 569 patients during the phase III trials of this drug determined that 91% of the patients had side effects including nausea, fatigue, vomiting, anorexia, neuthropenia, and increased transaminase blood levels. These patients were undergoing treatment for soft tissue sarcoma, breast cancer, osteosarcoma, ovarian cancer, melanoma and renal cell carcinoma [43].

Based on the preclinical results, trabectedin is also being developed for ovarian, prostate, lung, breast and pediatric cancers. Trabectedin activity in Phase II clinical trials after standard platinum-based therapy prompted a large, randomized Phase III trial. This may result in a commercialization request for trabectedin for ovarian cancer in the future [60].

IPL 576,092 and IPL 512,602 (contignasterol derived)

In 1992, the isolation of contignasterol (**11**, named IZP-94005) from the marine sponge *Petrosia contignata* was reported [65], and in 2002 its absolute configuration was elucidated [66]. *In vitro* pharmacological evaluation of contignasterol showed its ability to inhibit histamine liberation in rat peritoneal cells stimulated with anti-IgE [67]. I*n vivo* trials conducted on ovalbumin sensitized Guinea pigs (*Cavia porcellus*) also demonstrated the inhibition of histamine in the skin. IZP-94005 was shown to be a good candidate as an anti-asthma drug, since it maintains inhalation volume of Guinea-pigs after inhaling 200 μg/kg of IZP-94005 and subsequent stimulation with ovalbumin. IZP-94005 acted to prevent severe inflammatory processes in these animals [68].

The search for contignasterol structural analogs led researchers to synthesize other bioactive steroids. The most important was IPL576,092, which is structurally less complex but has good anti-inflammatory activity (**12**). An oral dose of 5 mg/kg of IPL 576,092 caused an 80% inflammation reduction. However, an oral dose of 50 mg/kg of contignasterol produced a reduction of only 60% in a rodent model with allergen-induced lung inflammation. The mechanism of action of IPL576,092 is associated with the inhibition of some mediators of the inflammatory process, such as tumor necrosis factor-alpha (TNFα), hexosamides, prostaglandin D_2 (PGD_2), and interleukin 5 (IL5). This mechanism is different from that of glucocorticoids [69].

The promising results found in animal studies led Inflazyme-Aventis laboratories to develop Phase II studies in humans. These were conducted at doses between 100 to 200 mg of IPL576,092 in 17 patients for one week. A second generation of leukocyte selective anti-inflammatory drugs (LSAD) was synthesized by Aventis Laboratories, such as IPL512,602, code named AVE 0547 (**13**) [70,71].

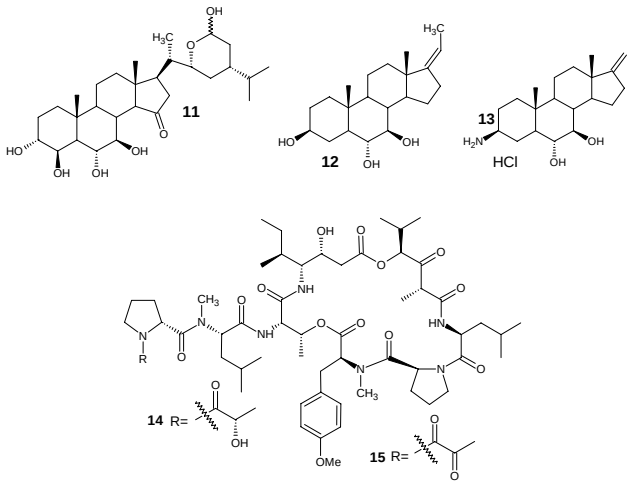


Figure 5: Marine organism-derived drug candidates under pharmacological studies phase II/III.

Recent Phase IIb studies in humans showed that IPL576,092 was effective for the treatment of patients with moderate to severe asthma symptoms [72].

Didemnin B and Aplidin

Didemnins A, B and C were isolated in 1981 from the tunicate *Tridemnum solidum* [73]. The most important depsipeptide is didemnin B (**14**), which has the most potent *in vitro* activity in preventing DNA and RNA virus replication. didemnin B is also highly cytotoxic to L1210 leukemia cells and shows a protective action for normal rat cells *in vivo* against the B16 melanoma and P388 leukemia cell lines at a concentration of 0.0011 μg/mL [74]. The chemical structure of didemnin B was finally confirmed in 1988 by X-ray crystallography of a single crystal [75].

Didemnin B inhibits DNA and RNA synthesis and is a non-competitive inhibitor of the palmitoyl-protein-thioesterase enzyme-substrate complex (PPT1) and PPt1-palmitoyl-CoA. The inhibition constant (K_i) of this complex is 92 nM [76,77]. This molecule has the ability to block the cell cycle in any phase at doses from100 to 300 ng/mL. Furthermore, at low doses (3-10 ng/mL) it blocks only the G1 and S phases of the cell cycle. This dose-response relationship demonstrates the complexity of the cell blocking mechanism shown by this molecule. Didemnin B and cyclosporines inhibit prolactin since they bind to the Nb2 node of the lymphoma cells in the G1 phase. In addition, didemnin B has shown a complex apoptotic activity at 1 μM concentration in less than 2 hours after IV administration. This mechanism involves the activation of the caspase pathway and the inhibition of the human FKBP25 protein. The high complexity of the mechanism of action is responsible for the wide range of anticancer activity, because didemnin B is active against more than 60 types of cell lines and several *in vivo* models, for example, metastatic breast cancer, non-Hodgkin´s lymphoma, and malignant melanoma [78].

Didemnin B is considered to be the first molecule extracted from a marine organism that reached clinical trials. Phase II studies were conducted in 1991 at doses ranging from 1.6 to 6.3 mg/m^2 depending on the conditions tested [78]. However, due to reported cases of toxicity and possible deaths, didemnin B was discarded as a reliable therapeutic alternative [79].

In 1991, a structural derivative of didemnin B, named dihydrodidemnin B or Aplidin®(**15**), was isolated from the tunicate *Aplidium albicans*. The main structural difference between Aplidin and didemnin B lies in the alkyl chain. Didemnin B possesses an N-lactyl chain, whereas, Aplidin has a pyruvyl substituent [80]. As a result, Aplidin has better anticancer activity than its predecessor, didemnin B. Phase I and II studies were initiated in Canada, Spain, France and the UK for the treatment of solid tumors and non-Hodgkin lymphomas. These studies were conducted on 200 patients at a dose of 5 mg/m^2 given by IV infusion taken according to two regimens (3 and 24 hours)[81]. The main side effect reported was muscle soreness, which was compensated for by the concomitant administration of carnitine. This amino acid allows for increasing the therapeutic dose up to 7 mg/m^2, and hence improving the therapeutic activity [82].

At the tissue level, the drug inhibits endothelial growth factor (VEGF) in cancer cells and also binds to VEGF receptor-1 preventing cell detachment and generating apoptosis [83]. The minimum dose required to produce apoptosis is 20 nM. The mechanism of action involves blockage of the serine/threoline kinases JNK (Jun N-terminal kinases) and the P38 MAPK protein kinase at the end of the G1 phase. Aplidin causes oxidative stress in cells reducing the glutathione levels and increasing Src kinase activity, protecting the normal cells against cancer cells growing [84]. In recent years, it has shown positive results for the treatment of different types of melanoma, non-Hodgkin's lymphoma, and colorectal, kidney and thyroid cancers [85].

Aplidin was classified as an orphan drug by the EMEA (2003) and FDA (2004) to treat acute lymphoblastic leukemia (ALL) and multiple myeloma (MM), respectively. The IP dose of 0.6 mg/Kg administered every 4 days in 3 schemes, repeated every 21 days showed growth inhibition of solid tumors in 48% of the patients [86,87]. Aplidin has also been effective in patients with advanced thyroid and renal cell carcinoma, showing low signs of toxicity [88,89]. Currently, it is in the final stage of phase II studies.

Dolastatins and synthetic derivatives

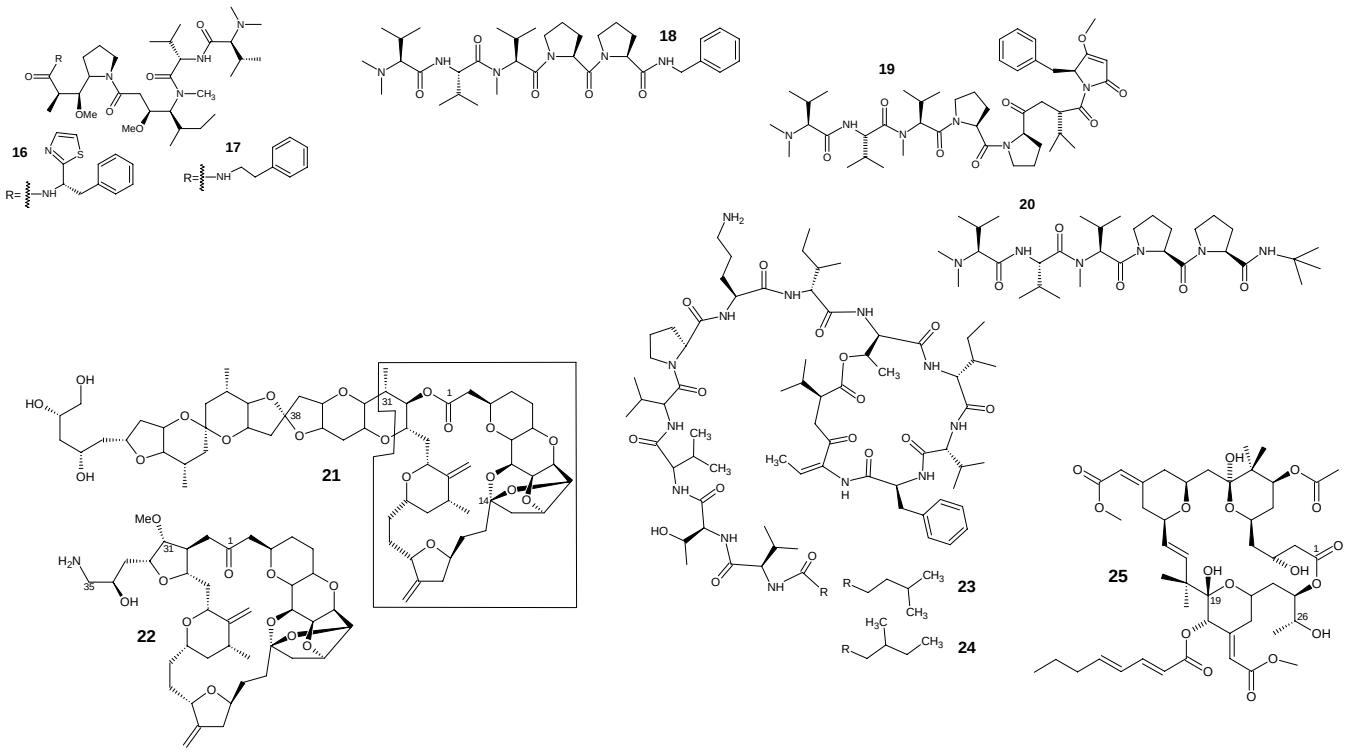
Pettit and collaborators conducted studies on the aqueous and ethanol extracts of the mollusk *Dolabella auricularia* found in eastern Africa. The extracts had a potent cytotoxic activity against the P-388 leukemia cell line [90]. Dolastatin is one of the peptides isolated from the aqueous and ethanol extracts. It is highly active *in vitro* against the P-388 cancer cell line and B16 murine melanoma at a concentration of 11 μg/Kg [91]. Due to the low yield of these peptides (≈1.0 mg/100 Kg of mollusk), it took about 15 years to elucidate the structure of dolastatin (**16-20**). Recently, it was found that the cyanobacterial genus *Symploca* was responsible for producing it. This bacterium is usually ingested and incorporated intact in the tissue of the mollusk [92].

Type 10 Dolastatin (D-10, **16**) is the most active pentapeptide against the PS murine leukemia cell (ED_{50}= 4.6x10^{-5} μg/mL) [93]. The mechanism of action involves the inhibition of microtubules and tubulin polymerization through non-competitive inhibition. This mechanism is similar to that shown by Vinca alkaloids and its derivatives having a K_i of 1.4 μmol/L. During the inhibition of microtubules, dolastatin is hydrolyzed into guanine triphosphate, blocking the cell cycle in metaphase [94,95]. D-10 has shown *in vitro* inhibitory activity against melanoma cell lines, ovarian cancer and sarcoma [93]. The efficient mass production of this peptide on a large scale is still a major problem. In 1990, phase I studies of this compound were conducted by the National Cancer Institute (NCI) [96]. Recent studies determined a human maximum dose ranging from 300 to 400 μg/m^2 and found neutropenia as the main side effect in 33% of the patients evaluated [97].

D-10 was evaluated in phase II studies against prostate adenocarcinoma [98] and metastatic melanoma at an IV dose of 400 μg/m^2 at three week intervals [99]. Since the results were not positive, this compound is no longer in development as an antitumor agent. As a result, new synthetic analogs of D-10, such as TZT-1027 (**17**, Soblidotin®, D-10 derivative), were created [100].

TZT-1027 is more effective than D-10 in treating mice tumors. For example, an IV and IP dose of 2 mg/kg inhibits murine solid tumors and a panel of 26 cell line of colon-adenocarcinoma, B16-melanoma and M5076-sarcoma. TZT-1027 is more effective than cisplatin, vincristine, E7010 and 5-fluorouracil (5-FU) in treating the above mentioned tumors. TZT-1027 also has the same cellular mechanism of inhibition as D-10. Phase I studies were developed in the United States, Europe and Japan [100]. Phase II studies determined the therapeutic dose ranging from 1.8 to 2.1 mg/m^2, administered weekly [101,102]. Larger doses led to side effects such as neutropenia, myalgias and constipation [103].

D-15 (**18**) is another antineoplastic agent formed by seven amino acids and isolated from the marine mollusk *Dolabella auricularia* in a yield of 4x10^{-7}%. This compound is produced by cyanobacteria of the genus *Symploca*, which form part of the mollusk diet. The NCI reported an *in vitro* effective dose of 2.4x10^{-3} μg/mL against the P388 cell line of lymphocytic leukemia [104].



Figures 6: Marine organism-derived drug candidates under phase II studies.

The mechanism of action of D-15 involves blockage of the vinca alkaloids domain on the β-tubulin surface [105]. However, since this drug has a very low aqueous solubility, several synthetic analogs such as LU 103793 (**19**, Cematodin, D-15 derivative) and ILX651 (**20**, synthadotin, D-15 derivative) have been produced.

Phase I and phase II studies demonstrated that LU 103793 (**19)** has a good bioavailability and low toxicity compared with D-10 and D-15. However, this molecule was subsequently discarded as an antineoplastic agent because results were not conclusive in the therapeutic range from 2.5 to 10 mg/m^2 [106,107].

ILX651 (Tasidotin®) is a third generation D-15 derivative. The mechanism of action involves blockage of the microtubules assembling and hence, increases the *lag* phase in microtubule assembly [108]. It has a metabolite derivative called tasidotin-C-carboxylate, which is about 10 to 30 times more potent than ILX651 having an *in vitro* IC_{50} of 4 nM [109]. Preclinical studies revealed activity against melanomas, sarcomas and leukemia, and lung, colon, ovary, prostate and kidney cancers [110]. Phase I studies determined the optimal therapeutic dose from 28 to 34 mg/m^2 after an IV infusion for 30 min. Common side effects at the high dose are neutropenia, diarrhea, vomiting, high blood levels of transaminases and pyrexia [108]. Genzyme Corporation has conducted phase II studies in adult patients with melanoma, lung and prostate cancers, and found positive results [111]. This molecule is in the third stage of phase II studies and is currently being tested in more than 80 patients in the United States.

Halichondrin B (H-B)

Halichondrin B (**21**) is a macrolide isolated from the Japanese sponge *Halichondria okadai*, with a yield of 2x10^{-3}%. It has *in vitro* cytotoxic activity against B-16 melanoma cells (IC_{50} 0.093 ng/mL) and antineoplastic activity against B-16 melanoma, P-388 and L-1210 leukemia cells of mice [112]. H-B has also been isolated from other sponges such as *Axinella* sp., *Phakelia carteri* and *Lissodendoryx* sp. [107]. In 1992, H-B was successfully synthesized after a painstaking process involving 90 steps [113]. The mechanism of action also involves blockage of the binding site for the Vinca alkaloids, D-10 and its analogues. It also inhibits GTP hydrolysis and the nucleotide exchange mechanism, blocking the cancer cell cycle in the G2 phase [114]. H-D is active against a wide variety of cancer cell lines, melanomas, sarcomas, and lung and colon cancers *in vivo* [115]. Since H-B is a very complex molecule, structure-activity relationship (SAR) studies were conducted to find the section of the molecule responsible for the antineoplastic action. It was concluded that the lactone portion of the C1-C38 carbons behaves as a pharmacophore in the molecule. Thus, the synthesis of less complex analogues has been undertaken [116]. As a result, ER-076349 (NSC 707390), later called E7389 (Eribulin®, **22**) was synthesized. In this molecule, the lactone ester group in the C1 position of H-B was replaced by a ketone group, and the methyl group at the C31 position was

replaced by a methoxy group and a primary amine. These structural changes increased the biological activity of E7389 [117].

E7389 has the same mechanism of action as H-B, having a better affinity for the Vinca alkaloid-binding site. For this reason, it has better *in vitro* and *in vivo* activity against more than 60 cell lines tested by the NIH. In 2002 Esai Inc performed phase I and phase II clinical studies in 40 patients with either refractory or advanced solid tumors. IV doses from 0.25 to 2 mg/m^2 on 1, 7 and 15 days at 28-day cycle were able to stabilize tumor growth. Participants reported side effect such as mainly neutropenia (6%) and to a lower degree hypoglycemia, hypokalemia and fatigue. The maximum tolerated dose for E7389 was 1.4 mg/m^2/week. Currently, the phase II and phase III studies for E7389 are conducted mainly in Europe, Japan and the United States, showing satisfactory results against breast and lung cancer. If E7389 is approved by the FDA, this drug could be used as a third-line treatment for patients with breast cancer for whom previous treatments with anthracyclines, taxanes and capacitabine have been ineffective [118].

Kahalalide F (K-F)

K-F is a cyclic depsipeptide (**23**) isolated from the sea slug *Elysia rufescens*, which is found in the Hawaiian shores. *E. rufescens* is a herbivore mollusk that feeds on green algae, especially *Bryopsis* sp. The concentration of K-F in these algae is around 1.7x10^{-4}%. However, the concentration of K-F in the slug tissue is close to 0.97%, w/w [107]. K-F initially showed selective activity against A-549 (IC_{50}= 2.5 μg/mL), HT-29 (IC_{50}= 0.25 μg/mL) and LOVO (IC_{50}= <1.0 μg/mL) tumor cell lines. Further *in vitro* studies showed activity against the type II herpes virus having an IC_{50} of 0.5 μg/mL and a 96% reduction of the infection. Additionally, at a concentration of 50 μg/mL, K-F is active against the fungi *Aspergillus oryzae* (19 mm), *Penicillium notatum* (26 mm), *Trichophyton mentagrophytes* (34 mm) and *Candida albicans* (16 mm) using 6 mm sensidiscs [119]. The mechanism of action of K-F is not completely understood. Cytological studies have showed physiological changes in the lysosomes. Furthermore, ATP is depleted causing cellular depolarization, oncosis and cell turgor, especially in the tumor cell lines located in the vulve, prostate, breast, colon and liver [120].

Phase I studies were conducted in 38 patients with advanced solid tumors. They received an IV infusion of K-F for 1 hour at the regimen of 266 μg/m^2/week. The maximum tolerated dose was 800 μg/m^2/week and the recommended dose for the phase II studies was 650 μg/m^2/week. The transaminases level of those patients increased by 75%, indicating mild hepatoxicity [121]. Phase II studies of K-F were conducted by Algarra and collaborators in 2009. Results showed no statistically significant results within the therapeutic range. As a consequence, this molecule was rejected as a therapeutic agent for the treatment of malignant cutaneous melanoma [122].

Currently, a K-F synthetic analogue PM02734 (Elisidepsin, Irvalec®, PharmaMar, **24**) is being evaluated as a new therapeutic alternative. PM02734 is a novel cyclic peptide belonging to the K- F family of compounds. The isopentenyl side chain in K-F is replaced by a 2-methyl butyl chain in PM02734. Pharmamar, in June 2010, reported that Irvalec is on course for recruitment of patients for three phase I trials. The first phase I study is a 3-hour infusion of the single agent; the second is Irvalec in combination with erlotinib (Tarceva), and the third is Irvalec in combination with either carboplatin or gemcitabine. Pharmamar also reported the completion of the protocol (IMAGE) Phase Ib/II for Irvalec using two treatment regimens: either 24 hours every two weeks or 3 hours weekly. These treatment regimens will be evaluated in patients with unrespectable tumors, advanced or metastatic esophageal, gastric or gastroesophageal cancers. The study is being conducting in 10 centers in the UK, France and Spain [123]. Other reports of Irvalec have shown promising results in patients with small cell lung cancer, when administered at a single-agent dose of 2 mg (flat dose) given IV as a 30 minute infusion every 3 weeks [124].

Bryostatin 1 (B-1)

This macrocyclic lactone was isolated in 1968 from the bryozoan *Bugula neritina*, which is found in the Gulf of Mexico. Researchers from Arizona State University and NCI isolated bryostatin 1 (B-1). B-1 (**25**) presented a strong biological activity against P338 lymphocytic leukemia cells having an ED_{50} of 0.89 μg/ml and a yield of ~2x10^{-6}% [125].

Pharmacological studies of B-1 were initially relegated due to the low yield obtained after the tedious extraction process. However, the process of obtaining the amount required for phase I and II studies was staggering. Approximately 28,000 pounds of *B. neritina* were extracted with 10,000 gallons of isopropanol/methanol to obtain 10 g of pure B-1 [126]. The demand for the organism was achieved by researchers from CalBioMarine Technologies. They employed aquaculture technology to grow the organism in large amounts preventing the depletion of the ecosystem [127]. Computational studies contributed to the search for less complex structural analogs and determined the pharmacophore core of B-1. As a result, the oxygen in the C1, C19 and C26 positions of B-1 are critical for the interaction of protein kinase-C (PKC) with B-1. These studies have also been carried out on structural derivatives of phorbol-diterpene, which are isolated from croton oil [128].

B-1 binds strongly to protein kinase-C, altering cell differentiation and hence, causing cell death. B-1 acts on

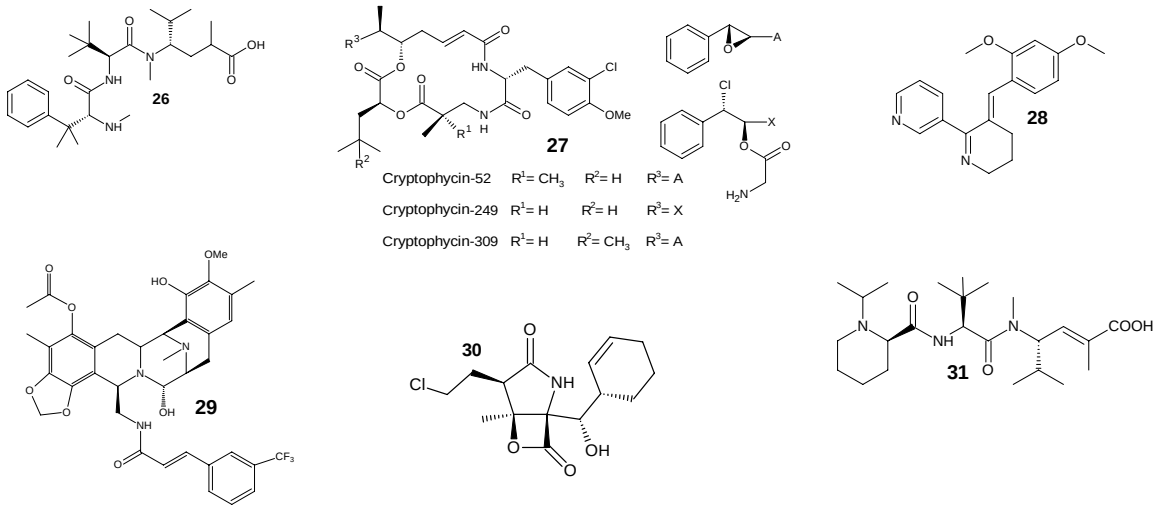


Figure 7: Marine organism-derived drug candidates under pipeline perspectives.

Table 1: Marine natural products in current pipeline perspectives.

Compound & (manufacturer)	Source (specie)	Pharmacological activity	Status	References
Hemiasterlin	Sponge. *(Hemiasterella minor)*	Anticarcinogenic	Phase I/II	[133] [134] [135]
HTI‑286 (**26**) (Taltobulin, Wyeth Inc.)	Synthetic analogue			
Cryptophycins (**27**) Cryptophycin-52 (LY355703) Cryptophycin-309 Cryptophycin-249 (Eli Lilly)	Cyanobacterium. *(Nostoc* spp*)* Synthetic analogue	Anticarcinogenic	Phase I/II	[136]
Anabaseine GTS-21 (**28**) (DMXBA, Athenagen Inc)	Marine worm. *(Amphiporus lactifloreus)* Synthetic analogue	Alzheimer's disease	Phase II	[137] [138]
Jorumicina PM00104 (**29**) (Zalypsis®, PharmaMar)	Nudibranch *(Jorunna funebris)* Synthetic analogue	Anticarcinogenic	Phase II	Started May 2009 [139]
Salinosporamide A NPI-0052 (**30**) (Marizomib, Nereus Pharmaceuticals)	Actinomycete (*Salinispora tropica*) Fermentation product	Anticarcinogenic	Phase I	[140]
Hemiasterlin (E7974, Eisai Inc.) (**31**)	Sponge (*Hemiasterella minor* and other sponges) Synthetic analogue	Anticarcinogenic	Phase I	[141]

the same active site as phorbol esters (CRD1 and CRD2 of PKCδ). The mechanism of action involves phosphorylation of PKC, translocation to the cell membrane and subsequent proteolysis. B-1 has also shown other biological activities such as stimulation of cell differentiation and synergy with other anticancer drugs such as Ara-C, taxol, D-10, vincristine, doxorubicin and prednisolone [129].

In 2001 Phase I studies were conducted in 25 patients at a dose from 12.5 to 62.5 μg/m^2. Patients received an IV infusion of B-1 and vincristine for 24 hours. The most common side effects included myalgias and neuropathy. Further studies were developed in 53 patients with lymphocytic leukemia and non-Hodgkin's lymphoma. The maximum tolerated dose for patients was 180 μg/m^2. This dose was determined by the onset of myalgia symptoms [107]. Other reported side effects included photophobia, eye pain, fatigue, phlebitis, nausea, anemia, fever and flu symptoms.

Phase II studies were developed in a group of 15 patients with lung cancer. Patients received a B-1 dose ranging from 25 to 35 μg/m^2/week by IV infusion for 1 hour every 4 weeks. Since four patients developed severe myalgia reactions they were removed from the study. The life expectancy of the 11 remaining patients increased on average by 31 weeks [130]. Other studies evaluated B-1 as a monotherapy against melanoma, renal, ovarian and colorectal carcinomas, but showed poor activity against these tumors. Currently, the clinical studies of B-1 are focused on the anticancer activity against B-cells and non-Hodgkin's lymphomas in combination with vincristine, cladribine, and some interleukins [131].

GPC Biotech is currently conducting clinical studies of B-1 in combination with temsirolimus against solid tumors. B-1 is also being used in combination with paclitaxel and vincristine against pancreatic cancer and non-Hodgkin's lymphomas. GPC Biotech has also initiated phase II studies to evaluate the activity of B-1 in Alzheimer's disease [132].

Conclusions: In Figure 2, the statistics show two main groups according to the source for these bioactive molecules. The first one corresponds to the marine sponges (56.2%), corals (11%), tunicates (7.4%), mollusks (5.6%), ascidians (5.2%), sea cucumbers (3.0%), bryozoans (1.8%), gorgonides (1.7%) and fish (1.4%). The second group only represents the 6.4% and considers mussels, anemones, ciliates, crinoids, mussels, sea slugs, worms, sea urchins and starfish. The prokaryotic kingdom accounted for 9.2% of the reports, as illustrated in Figure 3, and included bacteria that have a symbiotic relationship with animals. The vegetable kingdom was mainly represented by seaweeds (98.9%), but only contributed to 8.8% of the literature reports. The fungal and protist kingdoms contributed to 5.8% and 1.1%, respectively. Most of the species showed some biological activity in the test evaluated. That might be reflected in the new and novel therapeutic options for the treatment of human diseases in the near future. These results prove the pharmacological potential of compounds isolated from marine organisms. It is estimated that nearly 40% of marine organisms have been studied, but there is still a lot of work to be done.

As has been demonstrated in this review, marine natural products have been new sources and/or leads to drugs that cover a very wide range of pharmacological activities (cancer, analgesia, Alzheimer's disease, etc). In Table 1 are listed other marine natural products in the current pipeline for commercial use. Nowadays, some marine natural product drugs are being evaluated against a wide range of cancers, and this could allow approval of these drugs for wider therapeutic use. It is probable that within the next ten years at least two marine-derived novel agents will enter commercial use as new drugs following governmental approval.

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