

Drugs and Drug Candidates from Marine Sources: An Assessment of the Current “State of Play”

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Key words

- marine-sourced drugs
- clinical trials
- cancer
- HIV

Abstract

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The potential of the marine environment to produce candidate compounds (structures) as leads to, or even direct drugs from, has been actively discussed for the last 50 or so years. Over this time frame, several compounds have led to drugs, usually in the area of cancer (due to funding sour-

ces). This review is designed to show where there have been successes, but also to show that in a number of disease areas, there are structures originally isolated from marine invertebrates and free-living microbes that have potential, but will need to be “adopted” by pharmaceutical houses in order to maximize their potential.

Introduction

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The oceans of the world cover roughly 71% of the planet with a median depth of > 3000 m and a mean depth of 3800 m. Of this enormous expanse, less than 5% of the deep sea has been explored in any way and less than 0.01% of the deep sea floor has been sampled in detail [1]. What is also of significance is that of the 36 animal phyla identified taxonomically, 34 are found in the marine environment [2] and, of these, approximately half are only found in marine environments. Of the remainder, approximate half are both marine and terrestrial (though predominately marine) and only one, the phylum *Onychophora* (the velvet worms), is exclusively terrestrial.

Although organisms had been recovered from relatively shallow depths in the 1800s and had been seen for many centuries on coral reefs and beaches, not until the Challenger expedition in the 1872–1876 time frame, and then the Galathea expedition in 1950–1952 where live animals from the Philippines Trench at 10190 m [3] were recovered, was it realized that with the possible exception of the anoxic zones in the Black Sea, living animals could be found at all depths. The reason for using “possible” as a modifier in the sentence above is that the foramaniferan tube worms found at “black smokers” are in anoxic environments and utilize microbes of the sulfur cycle to survive and grow.

Who first decided to investigate the marine environment as a source of medicaments is unknown, but both Japanese and Chinese herbals contained various mixtures that were used as part of traditional medicine, and certainly the toxic properties of marine-derived products were well known centuries ago, with perhaps the best known example being the puffer fish (*Fugu*) in Japanese cuisine (though see the comments on tetrodotoxin later in this review). From a historical perspective, “Tyrian Purple”, a dyestuff from a Mediterranean mollusk used in Roman times, actually has significant activity in some cancers and was used as a part of traditional Chinese medicine (TCM) for the treatment of leukemia. It is only recently that it was realized that the active chemical compounds (marine sourced and plant sourced, respectively) were of the same basic chemical class and may well be of microbial origin in both cases. This article will deal with the potential of the marine (aquatic) environment and its organisms, as leads to agents that have been approved for use against diseases of man (and in one case, for use in agriculture), by the relevant agencies of governments worldwide. These will be drugs, not food supplements or functional foods, etc. We will discuss those that are approved, are in clinical trials, and show where the molecules came from, directly, modified by synthesis, or even total synthesis based on a marine-sourced compound. In one case, a compound that had been in clinical trials

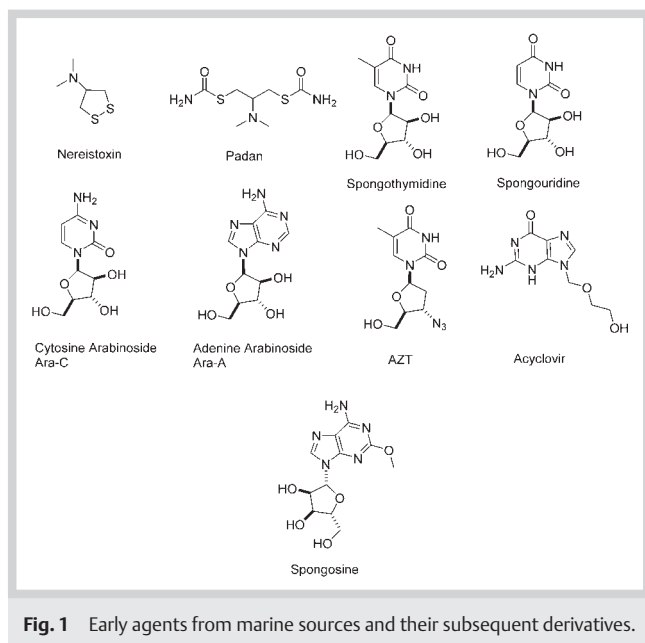
received August 20, 2015
revised Nov. 30, 2015
accepted January 5, 2016

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DOI <http://dx.doi.org/10.1055/s-0042-101353>
Published online February 18, 2016
Planta Med 2016; 82: 775–789
© Georg Thieme Verlag KG
Stuttgart · New York ·
ISSN 0032-0943

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as an antitumor agent has now gained a new “lease on life” as a potential treatment for other unrelated diseases.

What will, however, become obvious as the stories unfold is that the original organism from which the compound was isolated (usually a marine invertebrate) may or may not be the actual producer of the metabolite(s) of interest. Where the true producer is known, it will be identified, but in a significant number of cases, the evidence is still circumstantial, though in other cases, the organism is directly identifiable.

The Early Record

Though found in both marine and aquatic environments ranging from the Sea of Japan to Lake Victoria in Central Africa, one can argue that one of the first generally used “marine” invertebrate-sourced secondary metabolites was the toxin known as nereistoxin (● Fig. 1) from the flatworm *Lumbrineris brevicirra* (also named *Lumbriconeris heteropoda*). This worm had probably been used for centuries by fishermen to stun fish within the lake, thus permitting them to harvest their catches rather easily. It might almost be called a “chemical equivalent” of the dynamite fishing system used by fishermen in countries such as the Philippines and other areas of SE Asia. In addition, Japanese fishermen had known for years that the flatworm had anti-insecticidal properties, as it killed carnivorous insects when they landed on it.

In 1934, Nitta isolated the active principle in the flatworm, with a structure being proposed by Okaichi and Hashimoto in 1962 and confirmed by total synthesis as reported by Hagiwara et al. in 1965 [4]. This compound was shown to be an acetylcholine antagonist and over the next few years, insecticides were developed from the base structure, with a close analogue, Padan® (● Fig. 1), being marketed by Takeda in 1967.

There had been sporadic reports from at least two millennia ago of pharmacologically active agents being isolated and identified from marine organisms; but, it was not until the middle of the 20th Century that any form of systematic investigation occurred. Thus, in the early 1960s to early 1970s, academic groups in the

USA, and pharmaceutical company-linked organizations such as the Roche Institute for Marine Pharmacology in Australia, reported their findings in a variety of formats. The best source for information on these and earlier studies is probably the 1976 review by Ruggieri, which should be consulted by interested readers [5].

Arabinose-containing nucleosides

One may argue, and the authors (in a news interview in the early 1990s) and another have done so [6, 7], that the seminal discoveries, and thus the impetus for the investigation of marine biodiversity and the subsequent vision of marine-derived drugs on the market, can be traced to the identification by Bergmann in the early 1950s of the arabinose-containing bioactive nucleosides spongothymidine and spongouridine (● Fig. 1) from the Caribbean sponge *Tethya crypta* [8–10]. These discoveries overthrew the then current dogma; a nucleoside had to have either ribose or deoxyribose as the sugar moiety in order to demonstrate biological activity.

Thus, cytosine arabinoside (Ara-C, cytarabine, ● Fig. 1), a very well-known and prescribed antitumor agent, though not originally found in such a marine environment as “Ara-C”, can trace its chemical lineage back to the discovery of bioactive nucleosides that contained arabinose rather than ribose or deoxyribose. Though we were not the first to recognize the importance of such substitutions, since, as mentioned earlier, Suckling, in a review in a journal that was not well known [6], reported on the chemistry involved in the syntheses of these and other such arabinose-linked nucleosides with common or uncommon bases, we were perhaps the first to formally link the discoveries of the marine-sourced natural arabinoses by the Bergmann group to the “design” of this agent. So, Ara-C can be legitimately considered to be a marine-derived agent, since without arabinose in place of deoxyribose or ribose, it would simply have been a normal component of nucleic acids.

As of the 15th of August 2015, there are 828 trials listed in the NIH (National Institutes of Health, Bethesda, Maryland, USA) clinical trials database, with 262 of them being open studies that are recruiting and covering a large number of cancers, ranging from Phase IV down to Phase I. In the corresponding European database, 127 clinical trials covering the same phases, but with some overlap, are listed, and in the World Health Organization database, just looking at Australasia, there are 17 open trials not duplicated in the other two databases. As with other well-known approved drugs, cytarabine is still in use, more than 40 years after its initial approval, with an interesting recent paper questioning the use of high-dose cytarabine therapy during remission in adults with acute myeloid leukemia [11].

Other closely related compounds such as adenine arabinoside (Ara-A; ● Fig. 1), an antiviral compound synthesized and commercialized by Burroughs Wellcome (now Glaxo SmithKline), were based upon the arabinose substitution, but in contrast to Ara-C, this compound was later found in the Mediterranean gorgonian *Eunicella cavolini* [12], and thus could be considered a natural product, even though it was synthesized *de novo* before its discovery in the gorgonian. Similar reasoning related to unusual sugars and bioactivity led to investigations on the minimum size of sugar rings and the effect of other substitutions on the sugar ring(s); thus azidothymidine (AZT; ● Fig. 1) and even acyclovir (● Fig. 1) can be traced back to this initial discovery.

Very recently, a group of investigators at the Scripps Institution of Oceanography reported that a strain of the Gram-negative bacte-

rium, *Vibrio harveyi*, which was isolated from the sponge *Tectithya crypta* (the current name for the sponge that Bergmann used in the 1950s), produced spongosine and spongothymidine (● Fig. 1) amongst other nucleosides on fermentation [13]. Whether the microbe is the only producer has not been conclusively proven, there may be interplay between the microbe and the sponge host, but it is extremely suggestive as other related materials were obtained from other strains of the microbe, and a putative spg biosynthetic cluster was identified in the microbe's DNA.

History

By the middle 1960s, the terrestrial “plant world” had been reasonably well explored for novel anticancer drug candidates and reported on by a number of groups, with agents such as the *Vinca* alkaloids first being reported as potential antitumor agents in the late 1950s. Similarly, the terrestrial microbial world had been explored in a somewhat systematic fashion, from roughly the late 1940s, with success in both antibiotic and antitumor areas. A number of agents from microbial sources, or successors utilizing their pharmacophores, which are still in current clinical use, were first identified in the late 1950s to early 1960s, mainly in concert with industry. Good examples being the anthracyclines in cancer, penicillins, and cephalosporins and their thousands of “chemical offspring”, all with a beta-lactam ring as antimicrobials, or the antimicrobial carbohydrate-based aminoglycosides, some of which have gained a “new lease on life” as molecular biology tools rather than antibiotics.

Although there had been a number of reports of biologically active agents from marine (and freshwater) environments (*cf* discussion on arabinosides above), predominately by academic researchers who received funding from either the National Institutes of Health (NIH) component institutes or the National Science Foundation (NSF) for basic research, no systematic explorations had been performed of marine environments as sources of structural or direct leads to medicaments.

Starting in the early 1960s, the National Cancer Institute (NCI), the largest institute within the US government's NIH, expanded its horizons beyond the testing of synthetic compounds as experimental antitumor agents and began large-scale studies of natural products. Plant materials were collected and recollected when required (and if feasible) in conjunction with the United States Department of Agriculture (USDA). Microbial products were usually provided by pharmaceutical companies and small numbers of marine invertebrates were also obtained by purchase from commercial collectors.

These materials were then extracted by predominately academic groups, the extracts tested initially in whole animal models (mouse and rat tumors) and later in fast-growing mouse leukemia cell lines (P388 and L1210) by contractor laboratories to the US government, with “active materials” then being isolated and identified by the academic collaborators. At the same time, academic investigators, including those who were also acting as NCI collaborators/contractors, were also expanding their investigator-initiated grant applications (known as R01 grants) towards the marine environment, with the aim of collecting and utilizing marine invertebrates as sources of drug leads (marine biodiscovery).

Approved Marine-Derived Antitumor Agents and (Chemically) Related Compounds

As implied by the title of this section, if there are related compounds that entered clinical trials they will be discussed after the approved agent with notes as to their current status. Examples as discussed below include the MAb-linked agents involving dolastatin derivatives and molecules related to ET743.

Dolastatin-derived approved agent (Adcetris®) and related antibody-drug conjugates

One of the two antitumor compounds that came from what might be considered the earliest systematic investigations of the marine environment for such agents, as distinct from finding activities for isolated compounds, was dolastatin 10 (● Fig. 2), one of a series of linear and cyclic peptides isolated from the herbivorous sea hare *Dolabella auricularia*. The extensive isolation/synthesis programs and then clinical trials in man over the next 20 plus years have been given in detail by two of the early coinvestigators and should be consulted for details if desired [14, 15]. Subsequently, the dolastatins were shown to be produced not by the animal but by a cyanobacterium of the genus *Symploca* [16], though very recently this organism was renamed into the new genus *Caldora* and species *penicillata* [17].

None of the dolastatins, including dolastatin 10, progressed beyond Phase II clinical trials as the isolated compound, which was initially synthesized due to the very low levels found in the animal. A variety of later chemical variations also entered clinical trials. These included auristatin PE (trials under the names of soblidotin, TZT-1027, or YHI-201), cemadotin, and synthadotin (also known as tasidotin or ILX-651), but none proceeded beyond Phase I or early II levels.

However, due to the very high potency of two variations on auristatin, compounds later known as monomethylauristatin E [MMAE or vedotin (● Fig. 2)] and its phenylalanine variant, monomethylauristatin F [MMAF (● Fig. 2)], Seattle Genetics were able to link specific monoclonal antibodies to both of these “warheads”, leading in 2011 to the approval of the antibody drug conjugate (ADC) Adcetris® (● Fig. 2). The vast amount of work required, first in optimizing vedotin-based warheads and then developing the linkers that couple the antibody to the compounds, was described by Doronina et al. in 2006 [18]. Some of these were designed to release the warhead (vedotin) by simple hydrolysis of a linker bond, whereas others require the enzymatic digestion of the antibody, releasing the warhead plus appendages.

Adcetris®, also known as “cAC10-vcMMAE”, consists of a human/mouse monoclonal antibody linked to 8 valine-citrulline-MMAE molecules where the MAb was directed against the CD30 epitope expressed in leukemic cells. The agent was approved by the FDA in 2011 and subsequently approval was given in the EU late in 2012 and launched in the UK in early 2013, all for CD30 positive leukemias. Full explanations of the methodologies used and the utility of this agent against a variety of lymphomas were published in the 2010–2013 time frame and should be consulted by the interested reader [19–22].

In late 2013, the authors listed the extensive number of MMAE and MMAF antibody conjugates that were in clinical trials [23], and on checking the records approximately 20 months later, as expected, “trial attrition” has occurred, with the numbers now reduced to six MMAE-MAb compounds (four in Phase II and two in Phase I) with one MMAF-MAb in Phase I. In late 2014, Smaglo et al. published an excellent review on ADCs discussing others in

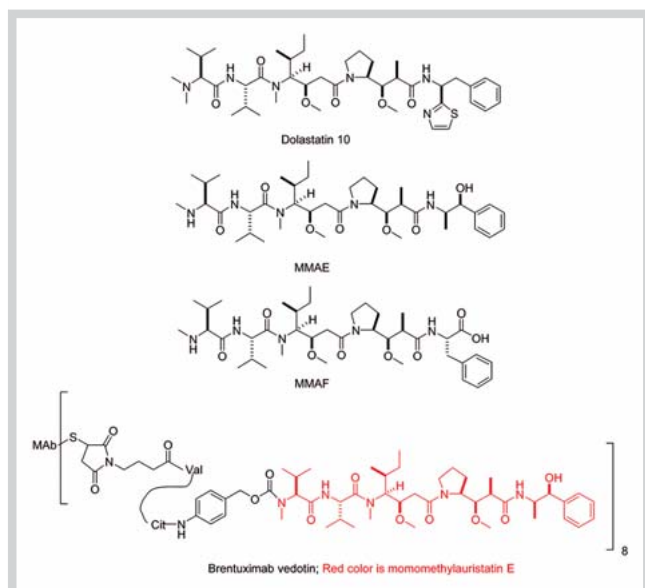


Fig. 2 Dolastatin-derived approved agent (Adcetris®) and related antibody-drug. (Color figure available online only.)

addition to the MMAE/F series, which should be consulted for general and specific studies [24].

Currently (August 9th, 2015), the following agents utilizing MMAE/F as warheads are still in “active and recruiting” clinical trials and their status will be discussed in brief in the following section. Thus, as mentioned above, a considerable number of the molecules listed in the early 2014 Marine Drugs review [23] are not included as there are currently no trials falling under these criteria listed in any of the governmental databases. Since in the majority of cases results are published well after the trial(s) have finished, no update(s) is/are feasible at this stage as to why compounds were dropped from clinical trials.

Glebatumumab vedotin (Phase II)

In this combination, MMAE is linked to a fully human monoclonal antibody CR011 (an anti-CG56972) via the valine-citrulline dipeptide linker as in Adcetris®. It was initially targeted against patients with unresectable melanomas at stage III or IV who had failed one cytotoxic chemotherapy regimen, and was then expanded to include metastatic breast cancer as well. The combination has had a variety of names during its evolution including CDX-011, CR-011, and CR011-vcMMAE, so searching for data can be a trifle challenging.

The initial report of the use of this combination was given by investigators from CuraGen in 2006 [25], followed by a report of xenograft activity in 2007 from the same group [26]. The potential of the monoclonal's target in triple negative breast cancer was described in 2010 by Rose et al. [27], and the corresponding details in melanoma were described in 2012 by a group from the People's Republic of China [28]. Currently, three completed studies at the Phase I/II levels are reported in the NIH clinical trials database with a recent discussion on the management of metastatic breast cancer with this agent by Vaklavas and Forero [29]. There are reports of clinical activity in breast cancer patients [30, 31], and a report of clinical efficacy in advanced melanoma [32]. There is some dichotomy as to the clinical trial status of this compound, but using the clinical trials database from the NIH

and the Celldex website (<http://www.celldex.com/pipeline/pipeline-overview.php>), as of August 11th 2015, the compound is listed as being at Phase II with a new indication showing recurrent or refractory osteosarcoma (NCT02487979).

Pinatuzumab vedotin; DCDT-2980 S (Phase II)

This ADC from Genentech is a humanized IgG1 antibody directed against the CD22 epitope expressed in leukemias. The warhead is linked in a similar manner to that used in Adcetris®, and releases monomethylauristatin E on protease cleavage. A modified safety protocol was used in the development of this agent, as the CD22 epitope is not expressed in rodents, so trials for safety were performed in cynomolgus monkeys, and demonstrated adequate safety in primates plus efficacy in xenografts [33]. Currently, there is one Phase II trial recruiting in the NIH clinical database (NCT01691898) and one duplicative record in the EU equivalent (www.clinicaltrialsregister.eu/ctr-search/search?query; EudraCT number 2011-004377-84). This trial is in non-Hodgkin's lymphoma, not solid tumors.

Polatuzumab vedotin; DCDS-4501 A (Phase II)

This is also a Genentech/Roche product, and is an ADC with monomethylauristatin E linked to an anti-CD79b monoclonal. It is currently in the same Phase II trial as DCDT-2980 S (NCT01691898) as an alternative treatment against non-Hodgkin's lymphoma, and is also in an ongoing Phase II trial (NCT02257567) against various lymphomas in a dose escalation study. In addition, there is a Phase I trial against B-cell non-Hodgkin's lymphoma (NCT01992653). As with the Roche agent above (EudraCT number 2011-004377-84), no other trials are listed in the EU database.

Lifastuzumab vedotin; DNIB-0600Aor RG-7599 (Phase II/I)

This ADC is a humanized IgG1 monoclonal antibody directed against the NaPi2b epitope linked to valine-citrulline-monomethylauristatin E. There are three clinical trials shown on the NIH site. NCT01995188 is a Phase I trial against ovarian cancer, NCT01363947 is another Phase I trial directed against non-squamous non-small cell lung cancer and ovarian cancer, and the third, NCT01991210, is a Phase II trial against ovarian cancer, epithelial, tumors, peritoneal neoplasms, and malignant fallopian tube cancer. Two reports in abstract form have been published to date, one from the 2013 American Society for Clinical Oncology (ASCO) meeting [34], and one from the 2014 European Society for Medical Oncology (ESMO) meeting [35].

DSTP-3086 S

(RG-7450; thio-antiSTEAP1-MC-vc-PAB-MMAE; Phase I)

This is a Genentech (Roche) ADC using an antibody against a humanized anti-STEAP1 IgG1 antibody, which was modified via determination/modification of reactive thiols according to the patent application by Bhakta and Junutula [36], and then coupled to the standard valine-citrulline-linked MMAE. The antibody is directed against the six-transmembrane epithelial antigen of prostate 1, hence the STEAP1 acronym, and was initially evaluated as the basic ADC with the vc-MMAE with an unmodified MAb and then the thio-modified antibody. The decision was made to proceed with the thio-modified version from the PK determinations [37, 38]. Currently, there is one Phase I study recruiting patients with metastatic castration-resistant prostate cancer (NCT01283373) with preliminary reports showing some clinical responses given at the 2013 and 2014 ASCO Meetings [39, 40].

HuMab-TF-011-vcMMAE (HuMax-TF-ADC; TF-011-MMAE; Phase I)

TF-011-vcMMAE is an ADC under clinical development by Genmab and Seattle Genetics for the treatment of solid tumors. It is composed of a human tissue factor (TF) specific antibody (TF-011), linked to valine-citrulline-MMAE. This ADC is currently in a Phase I safety study against ovarian, cervical, endometrial, bladder, prostate, esophageal, and non-small cell lung cancers under trial number NCT02001623. The NIH website shows trials in the US and the EU (UK and Denmark, but there is no current record in the EudraCT database).

GSK2857916 (J6M0-mcMMAF; Phase I)

Currently, this ADC is the only MMAF-linked combination in clinical trials, and unlike the MMAE-based ADCs, this ADC uses the protease stable maleimidocaproyl (mc) linker technology as described by Doronina et al. in 2006 [18] rather than the more common protease cleavable valine-citrulline version.

As of the time of this writing, this ADC is in one clinical trial (NCT02064387), an open-label, dose escalation with sites in the USA, Canada, and the UK. The patients desired for this trial will have one of the following: relapsed/refractory multiple myeloma or other advanced hematologic malignancies that express B-cell maturation antigen (BCMA). This antigen is a member of the tumor necrosis factor receptor super-family (TNFRSF17), which is known to be selectively induced during plasma cell differentiation and is effectively absent on naive and memory B cells.

ET743 [EMA-Approved drug (Yondelis®)] and Related Compounds in Clinical Trials

This compound could be considered the “poster child” for marine-derived antitumor agents. The reason is that currently it is the only molecule in use as an antitumor agent, identical to a compound originally isolated from *Ecteinascidia turbinata*. The evolution of this agent from the discovery, preclinical, and clinical development followed a tortuous path. It utilized materials from massive direct collections of the “producing” tunicate in the Caribbean and Mediterranean seas, in-sea and on land aquaculture, and finally, semi-synthesis from a precursor molecule, cyanosafracin B, obtained by fermentation of a marine-derived microorganism.

The initial reports of bioactivity in this organism in 1970 were first reported by Sigel et al. [41], which was then followed by the initial identification of the “ecteinascidin series” by Holt in his PhD thesis in 1986 [42]. Further work by the Rinehart group at the University of Illinois (Urbana Champaign, Illinois, USA) and the Wright group at Harbor Branch Oceanographic Institution (Fort Pierce, Florida, USA) led to the simultaneous publication in 1990 of the structure of ET743 and associated molecules [43,44]. ET743 was licensed to the Spanish company PharmaMar following the demise of the US biotech company SeaPharm in 1988, and the subsequent semisynthetic work on this compound was well described by the investigators at PharmaMar in 2009 [45], and as part of a discussion on the development of marine-sourced drug entities in 2012 [46]. Both of these reports demonstrated the power of semi-synthesis and optimization of processes to obtain active drug principles.

The molecule was approved in the European Union (EU) in 2007 for treatment of advanced soft tissue sarcoma (STS). Currently, it is approved in over 70 countries as monotherapy for STS and in a

number of countries for treatment of recurrent platinum-sensitive ovarian cancer when coupled to liposomal doxorubicin. In 2009, the corresponding US FDA application for the STS treatment was withdrawn. However, in February 2015, it was accepted for priority review for STS in the USA following submission of a New Drug Application (NDA) in late November 2014.

The mechanism of action (and there may well be others) is related to the excision repair complex as described by Soares et al. in 2007 [47]. In 2014, D’Incalci et al. published an excellent short review on the current state of knowledge of the mechanism(s) of action, and they stated in that review that “trabectedin not only inhibits the growth of cancer cells but also affects the tumour microenvironment (TME) by limiting the numbers of macrophages and by inhibiting the production of macrophage products that eventually promote tumour growth” [48].

The commonalities and differences in the pharmacological response of trabectedin and its close relatives, zalypsis® and lurbinectedin (● Fig. 3; *vide infra*), were discussed in 2013 with respect to their experimental effect upon the Fanconi anemia pathway. Martinez et al. [49] demonstrated that these three related agents inhibited the Fanconi anemia pathway in the cell lines tested, increasing their sensitivity to mitomycin C. This is in contrast to mitomycin C by itself, which in the same cell lines always activated that pathway. The authors suggested that as a result of these findings, these three agents might be useful clinically in “Fanconizing” cancer cells in order to gain sensitivity against other antitumor drugs.

The same year, Romano et al. [50,51] reported that in *in vitro* and *in vivo* models, no relationship was found between the *in vitro* cytotoxic potency and *in vivo* antitumor activity in syngeneic mouse models, suggesting that there might well be a host response in these models. Also, the pharmacokinetics differ, even between the quite similar trabectedin and lurbinectedin in humans, and as expected due to the differences in structure, Zalypsis® has been shown to differ in pharmacokinetics in humans [52].

As of August 12th, 2015, there were 20 open studies found for ET743 in the NIH clinical trials database, five at Phase III, eight at Phase II, one at Phase I, and six that were not given a Phase designation, with cancer types covering breast, prostate, soft tissue sarcoma, and osteosarcoma, plus general carcinomas. Searching the corresponding EU Clinical Trials Register (database), 26 trials were listed from 2006 to 2015 with eight being Phase II trials not found on the NIH site. These listings demonstrate that once a compound has been approved for treatment of one type of cancer, it will be placed in clinical trials for many others, either individually or as part of a new drug regimen.

A discussion of the evidence and probabilities of ET743 and its congeners being produced by as yet uncultured microbes associated with the source tunicate was published by Giddings and Newman in 2013 [53]. That this is in fact the case was confirmed by the very recent publication by the Sherman group at the University of Michigan identifying the microbe genetically [54]. This is the second paper using isolation and genetic analysis of an as yet uncultured microbe to prove the presence of the biosynthetic cluster(s) of the secondary metabolites found in the marine invertebrate in the microbe, with the first being the seminal paper by the Piel group published in Nature in early 2014 [55].

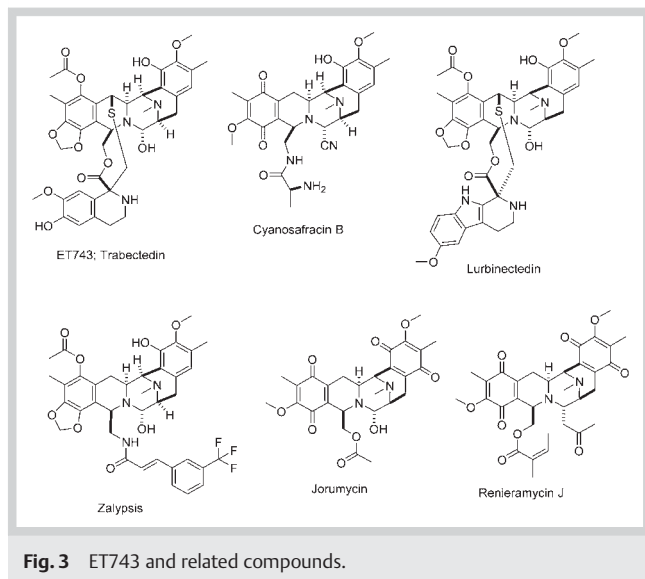


Fig. 3 ET743 and related compounds.

Lurbectedin (PM01183; Phases I–III)

This compound is another variation on the basic structure of the dimeric isoquinoline alkaloids such as ET743, but with a tetrahydro- β -carboline moiety instead of the tetrahydroisoquinoline present in ring C, and like ET743, it binds in the DNA minor groove [56]. The compound had different pharmacokinetics in patients to ET743 and attenuated nuclear excision repair (NER). It also had synergy with platinum-based agents *in vitro*, which suggested a potential treatment regimen, as it also demonstrated activity against platinum-resistant cell lines [57]. As of August 12th, 2015, there are 10 open clinical trials on the NIH site, one at Phase III, four at Phase II, and five at Phase I, plus one Phase II (in Spain) on the EU site that is not duplicated on the NIH site.

PM-10450 (Zalypsis®; Phase II)

This compound is yet another variation on the basic structure of the dimeric isoquinoline alkaloids, and was derived from the structure of jorumycin, a compound isolated from the skin and mucin of the nudibranch *Joruna funebris* [58], and renieramycin J (● Fig. 3) from a species of the marine sponge *Netropsia* sp. [59]. Zalypsis® was synthesized by workers at PharmaMar using techniques similar to those described for the ET743 synthesis from safracin B [60]. The molecular pharmacology of this agent was described by Leal et al. in 2009 and, surprisingly, though it has a close resemblance to ET743, it does not activate the DNA damage checkpoint response [61].

There is a dichotomy between the PharmaMar web site and the EudraCT (EU) web site as to status of the compound. On the PharmaMar site (www.PharmaMar.com; accessed August 12th, 2015), this compound is not listed as being in any trials. However, on the EU site, there is still a listing under 2009–016054–40 of an ongoing Phase II trial in Spain. No open trials, however, are shown on the NIH site, so it is feasible that this compound is being dropped.

Eribulin, (Halaven®; E-7389), a halichondrin B derivative

This agent is a wholly synthetic molecule modeled on a little over half of the structure of the naturally occurring antitubulin compound halichondrin B (● Fig. 4), specifically the ring plus a small “carbon tail”. This molecule came from a tour-de-force based

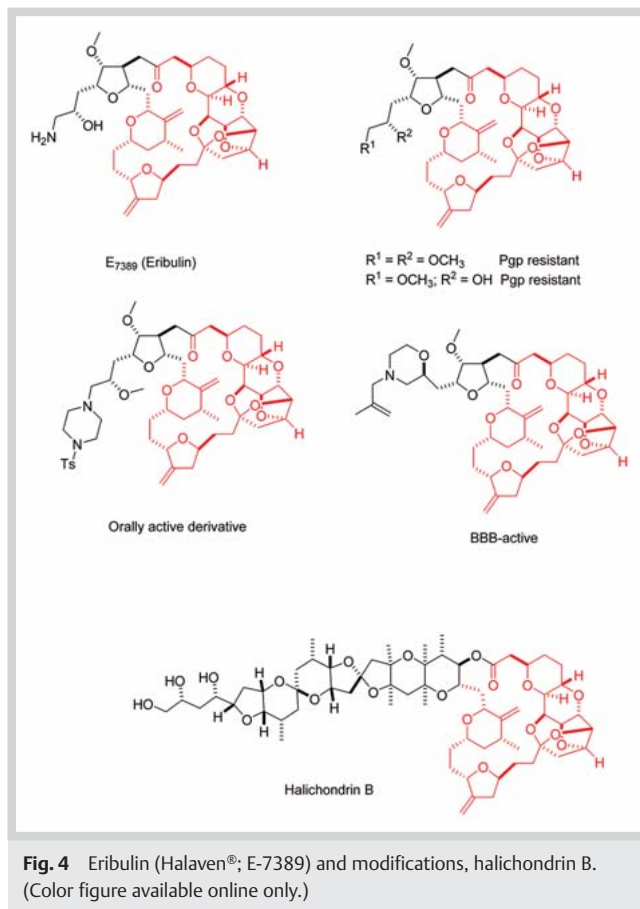


Fig. 4 Eribulin (Halaven®; E-7389) and modifications, halichondrin B. (Color figure available online only.)

upon the synthetic method for halichondrin B first reported by Kishi's group in 1992 [62]. During this synthesis and the investigation by scientists at the Eisai Research Institute (ERI) in Woburn, Massachusetts of the synthetic intermediates, Kishi and ERI scientists realized that the active part of the molecule resided in the macrocyclic ring (approx MW of 600) and not in the “tail” (the remaining 400+ of the overall 1000+ MW). Chemists at the ERI working very closely with the Kishi group synthesized over 200 molecules and, in conjunction with the Developmental Therapeutics Program (DTP) at NCI, they chose the modified truncated macrocyclic ketone (eribulin, E-7389; ● Fig. 4) as the candidate compound when compared in *in vitro* and *in vivo* studies to pure halichondrin B (obtained by DTP in conjunction with New Zealand scientists). Much fuller details of the synthetic and base biological information were published by the leaders of the synthetic studies in 2005 [63]. This review article should be read by the interested reader for fuller details of the evolution of this compound through early 2005.

Eribulin, as with the “natural product parent”, is a tubulin interactive agent with very potent activity at the nanomolar level in *in vitro* studies. In 2005, Jordan et al. [64] demonstrated that suppression of microtubule growth was the primary antimetabolic mechanism of eribulin, with differential effects due to the concentration when studied in MCF7 cells. At low concentrations, eribulin potently inhibited microtubule dynamics, resulting in a prolonged arrest of mitosis and inducing apoptosis, whereas at tenfold the IC₅₀ value (or higher concentrations), it induced depolymerization [64,65]. Later work demonstrated eribulin's interaction with centromere dynamics [66].

That the actual interaction of eribulin with tubulin may be even more complex than was originally thought is shown by the data reported by Jordan's group in a 2010 paper [67]. Using ^3H -eribulin, they found a very high affinity site ($K_d 400 \pm 200 \text{ nM}$) on 25% of the tubulin mass (which might be the $\alpha\beta_{III}$ tubulin dimer), with a stoichiometry of 0.26 ± 0.12 moles of eribulin per tubulin dimer. Another high affinity site ($K_d 3.5 \pm 0.6 \mu\text{M}$; stoichiometry of 14.7 ± 1.3 eribulin:tubulin dimer) was also identified together with a low affinity site ($K_d \sim 46 \pm 28 \mu\text{M}$; stoichiometry of 1.3 ± 0.4 saturable sites per tubulin dimer).

The binding isotherm from these results is highly complex, which might well be due to the competition for eribulin between soluble tubulin and microtubules, though there is photo- and electron-micrographic evidence in the paper, and in earlier ones from the same group, for binding to the plus end of microtubules and preferentially to β -tubulin. As mentioned in the paper [67], further experimentation will be required to explain these complex results.

In 2007, a review by Wang et al. [68] gave extensive coverage of the patent routes to eribulin, with some duplication of the work presented in the 2005 review by Yu et al. [63]. Then, in 2009, three publications from the Kishi group detailed improved methods for the synthesis of eribulin. Though these papers were written from an academic perspective, they considered the relative costs of production [69–71]. Following on the synthetic stories, two excellent reviews were written in early 2009 by the Phillips' group at the University of Colorado, covering the published syntheses by many groups of halichondrin B, norhalichondrin B, and eribulin [72,73]. These papers were then followed by three articles in 2013 that covered the process chemistry involved in producing multi hundred gram quantities under cGMP conditions [74–76], and the overall story by the leader of the initial chemical synthesis [77].

In 2011, three papers were published by the ERI group demonstrating that with only a relatively minor change to the "tail" of the molecule (● Fig. 4), significant pharmacological effects could be demonstrated. If the diol that is the eribulin precursor was changed to the dimethoxy substituent, or the terminal amino group was changed to a methoxy group, the compounds now had a much lower propensity for inducing P-glycoprotein susceptibility. Both compounds were potent *in vivo* and had a reduction of approximately 30-fold in terms of being substrates for P-glycoprotein compared to eribulin [78].

The ERI chemists then demonstrated that by substituting a substituted 1,4-diazacyclohexanyl group for the terminal amino group in eribulin and changing the sidechain hydroxyl group to a methoxyl, the compound (● Fig. 4) demonstrated oral activity in a subcutaneous LOX melanoma model and maintained a low susceptibility to P-glycoprotein induction [79]. Since there are very few treatments for brain tumors, the group then modified the base eribulin molecule by ring closure at the "tail" to give a morpholino derivative (● Fig. 4). This molecule was subtly different from the orally active compound referred to above, and demonstrated intravenous *in vivo* activity in an orthotopic murine model of a human glioblastoma [80].

Although it took 25 years from the original report in 1985 on halichondrin B until late 2010 to approve eribulin, the interplay of academic, industrial, and government laboratories in three continents led to this novel agent, the most complex drug molecule yet produced by total synthesis. What is of great importance is that without the structure of the halichondrin B from a Japanese sponge and the subsequent purification of halichondrin B by NZ

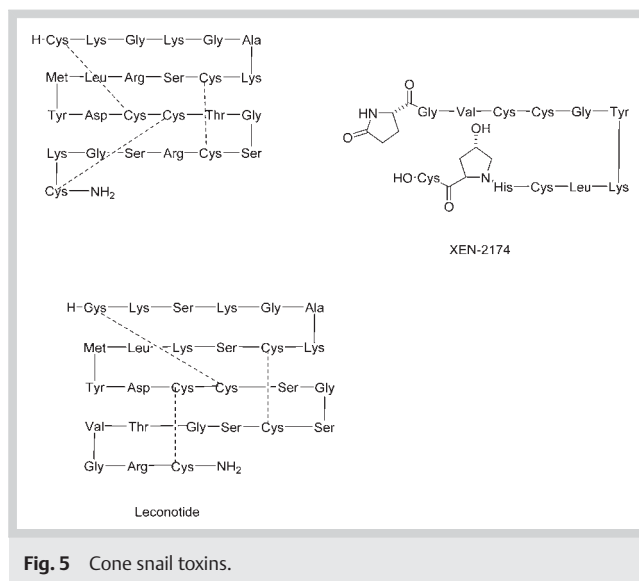


Fig. 5 Cone snail toxins.

scientists and the NCI, eribulin would not have been approved, nor would the very interesting and novel agents referred to in the preceding paragraph have been synthesized.

Ziconotide: Cone Snail Toxins as Pain Treatment

The fundamental work by Olivera et al. on the peptidic neurotoxins from fish-hunting cone snails led over the next 20 or so years to a massive amount of information on the sources, the utility in both the snail, and the potential for use or leads to a drug of use in man [81]. When it was realized by Olivera and his colleagues that these peptidic toxins were very specific as to which of the cation-gating channels they inhibited and/or activated, then the study of these potential neurological agents began in earnest [82].

As a result of Olivera's work at the University of Utah, a small company (Neurex) was set up to evaluate the 25-mer cyclic peptide, now known as ziconotide (● Fig. 5), that was one of the many peptidic toxins found in *Conus magus*. Close to 200 modifications of the tricyclic peptide (three CYS-CYS linkages being the major structural motif) were synthesized and evaluated for their pharmaceutical potential but in the end, the natural product was the choice, even though made synthetically [83].

This compound (ziconotide or Prialt®; ● Fig. 5) subsequently became the first marine-derived product to gain approval as a drug on its approval by the FDA on the 28th of December 2004, though it passed through a number of companies *en route*, followed rapidly by approval in the EU by the EMEA two months later. Ziconotide, though effective in its limited application, is not a drug that is taken easily as it has to be delivered via an intrathecal injection route.

XEN-2174 (Phase II?)

This compound is a very slight modification of the naturally occurring χ -conotoxin Mr1A. That compound was originally isolated from *C. marmoratus* and then optimized by medicinal chemistry [84]. Unlike the other conotoxins, either approved or in various levels of testing, this particular agent is a 13-residue peptide and is a noncompetitive inhibitor of the neuronal norepinephrine transporter (NET) [85].

The source company, Xenome, is still listed on various sites as having this compound in Phase II trials against cancer pain, and in an abrogated trial at Phase II countering pain from bunion surgery in Bulgaria under the EudraCT protocol 010-019 109-40-BG. This latter trial was terminated by the FDA, but no data has yet been published. However, a search of the internet undertaken in August 10th 2015, shows that the web site “www.xenome.com” is up for sale, thus the current status of this compound is unknown, but fuller details of the development of this compound and of Prialt® were published (February 2015) in a book chapter that can be consulted for further information [86]. Due to the uncertainty as to the actual status commented on above, we have used the “Phase II?” heading for this section.

Leconotide (ω -Conotoxin CVID; Phase I)

This molecule, a 27 residue peptide with three internal CYS-CYS bonds, similar in overall structure to ziconotide, had reached Phase I trials sponsored by Relevare Pharmaceuticals (Australia; previous name was CNSBio) for treatment of pain related to cancer. It is a calcium channel blocker and was originally identified by researchers at the University of Queensland. Although initial experiments used the intrathecal route (as with ziconotide) [87], the protocol used systemic administration [88]. The company involved has now liquidated, so just as with XEN-2174, the current status is unknown.

Recently, Craik and coworkers in Australia have demonstrated that by taking structural cues from plant cyclotides, they can alter the peptide's (including cone snail toxins) pharmacological characteristics, producing oral analgesic activity in animal tests, thus opening up other methods of delivery, avoiding the very difficult current intrathecal route [89,90].

As mentioned above, a recent book has chapters written by the experts in this field and we recommend that interested readers consult the chapters written in conjunction with Olivera [91] and Alewood [92] for much more background information on these very interesting natural products and derivatives.

Unapproved Marine-Sourced Compounds in Current Clinical Trials

There are a number of marine-derived agents that have been in trials up through Phase II but for one reason or another, have not been continued. Some were for toxicity, some for lack of efficacy, but these are not discussed any further. However, in at least one case, that of a hemiamsterlin derivative HTI-286 or taltobulin, the compound, though headed for Phase II, was discontinued for “business reasons” by Wyeth. The history of that particular compound was presented by Andersen et al. in 2012 [93].

However, the compounds discussed in this section are those that are not “descended from or related to” those that are currently approved, but are all in clinical trials at the time of this writing, or in some cases, the trial(s) has/have been completed, but no details have yet been published. They are divided by the disease(s) for which they are under development.

Analgesia: tetrodotoxin (Phase II/III)

Aside from the conotoxins, perhaps the most unusual agent at this stage is a very well-known “marine toxin”, the highly substituted guanidine-derivative tetrodotoxin (● Fig. 6) [94–96]. WEX Pharmaceuticals has placed the compound into two Phase III trials in the USA as an agent (Tectin®) against inadequately con-

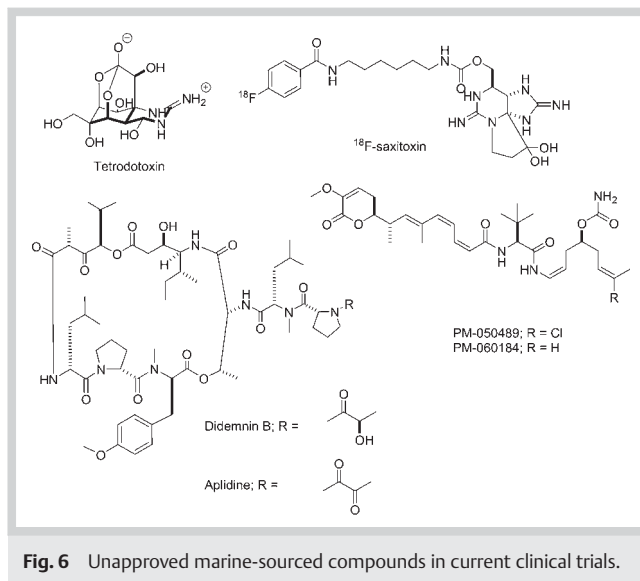


Fig. 6 Unapproved marine-sourced compounds in current clinical trials.

trolled pain related to cancer (NCT00725114; 00726011). One (NCT01655823), again in the USA, was terminated under the same company at Phase II (against neuropathic pain resulting from chemotherapy-induced peripheral neuropathy), because the interim results were such that they wished to move directly to a Phase III trial for the same indication.

Although there was debate in years gone by over the actual source of this agent, there is now little doubt that it is produced by a commensal microbe, though which one(s) is/are still open for debate [97]. The synthesis of the compound and other derivatives has been published from a variety of chemists, with an excellent review by Nishikawa and Isoke in 2013 [98], giving the highlights of their methodologies and covering some of the early history of this class of toxins. This paper should be read in conjunction with the more comprehensive review on the biology and chemistry of tetrodotoxin by Bane et al., where they even mention the 25 plus variations on the basic structure so far reported [99].

PET-Imaging ^{18}F -Saxitoxin

For comparison, another algal/cyanophyte/bacterial product closely related to tetrodotoxin is being considered for *in vivo* PET-MR imaging of voltage-gated sodium channels; in this case, ^{18}F -saxitoxin (● Fig. 6) [100]. Saxitoxin has been well described with an excellent review in 2014, so this agent may well end up not only as a curiosity but as a valuable agent from marine sources, though whether a formal clinical trial will occur is not certain [101].

Antitumor Compounds

Aplidine (Plitidepsin, Phase II/III)

Aplidine, which is formally dehydrodidemnin B (● Fig. 6), is a very close chemical relative of the first direct-from-the-sea antitumor compound didemnin B (● Fig. 6). The latter compound went to Phase II trials, but was terminated due to toxicity problems, possibly due to the method(s) of delivery, as suggested by Vera and Jouille [102]. Aplidine was originally isolated from *Aplidium albicans* and first reported in a patent application in 1989,

with a UK patent issued in 1990. It was formally identified in the chemical literature in a paper from Rinehart's group in 1996 covering the structure-activity relationships amongst the didemnins [103]. The earlier work on aplidine, its entry into Phase I and II trials, and the preferred method of synthesis was described in detail through late 2004 by PharmaMar scientists [104], with further information through late 2011 by PharmaMar scientists in the second edition in 2012 of the 2005 book [46].

Currently, this agent is listed in four clinical trials by cross-referencing against the three clinical databases, one at Phase III, two at Phase II, and one Phase I. In the EudraCT clinical trials database, one Phase III trial (2009–016 138–29) is listed in multiple myeloma. This is also listed in the NCI clinical trials database as NCT01102426, a Phase III trial with or without dexamethasone against resistant multiple myeloma, the ADMYRE trial.

There is a difference in the NIH and the EU databases with respect to a Phase II trial against liposarcoma, where the trial (2009–010980–18) in the EU database is listed as ongoing in France, the NIH trial (NCT01876043) is listed as being terminated in early 2015, which agrees with the WHO data. However, since the WHO listing is populated by the NIH data, there is still some confusion as to the status.

The EU database still has an open Phase II trial in Spain under 2004–001 117–34 against relapsed or refractory non-Hodgkin's lymphoma, whereas the NIH database shows that the trial (NCT00884286) has been completed. There is one Phase I trial (NCT02100657) recruiting at the Phase I level for multiple myeloma that commenced in the middle of 2014. This is not shown in the EU database, but is in the WHO one, though probably populated from the NIH database.

The precise MOA of this agent in a tumor environment is not fully described, but in 2009, Munoz-Alonso et al. described some of the then known cellular interactions [105], and in 2014, scientists working with PharmaMar described the further interactions with elements of the immune system [106]. This paper should be consulted for further specific interactions. In abstracts presented at the EORTC conference in November 2014, PharmaMar scientists and colleagues reported that aplidin bound directly to the eukaryotic elongation factor 1A2 (eEF1A2), a target that is over-expressed in multiple myeloma (MM) and acute lymphoblastic leukemia (ALL) [107, 108]. For more information on these pathways, the two recent reviews from the Pelletier group at McGill University should be consulted [109, 110].

PM050489/PM060184 (Phase I)

This agent, a polyketide and its chloro derivative (PM050489), was isolated from the Madagascan sponge *Lithoplocamia lithistoides*, with the extract demonstrating antimetabolic activity and the isolated agents, tubulin-binding activities. Due to the low level of these agents in the sponge, syntheses were described by the PharmaMar group [111] in 2013. This initial paper was followed the same year with evidence for binding at or close to the vinca domain [112]. In 2014, further evidence of activity of the des-chloro derivative (PM060184, ● Fig. 6) against P-glycoprotein-expressing tumors was published [113].

Currently, PM060184 (● Fig. 6) is in one listed Phase I trial in the NIH database (NCT01299636) that commenced in San Antonio, Texas in 2011, with the same trial listed in the WHO database and no listings in the EU database. It is listed in Phase I in the PharmaMar pipeline (accessed August 8th, 2015) and at the 2014 EORTC Meeting, there was an interesting report of *in vivo* activity in mice at low levels of this agent [114].

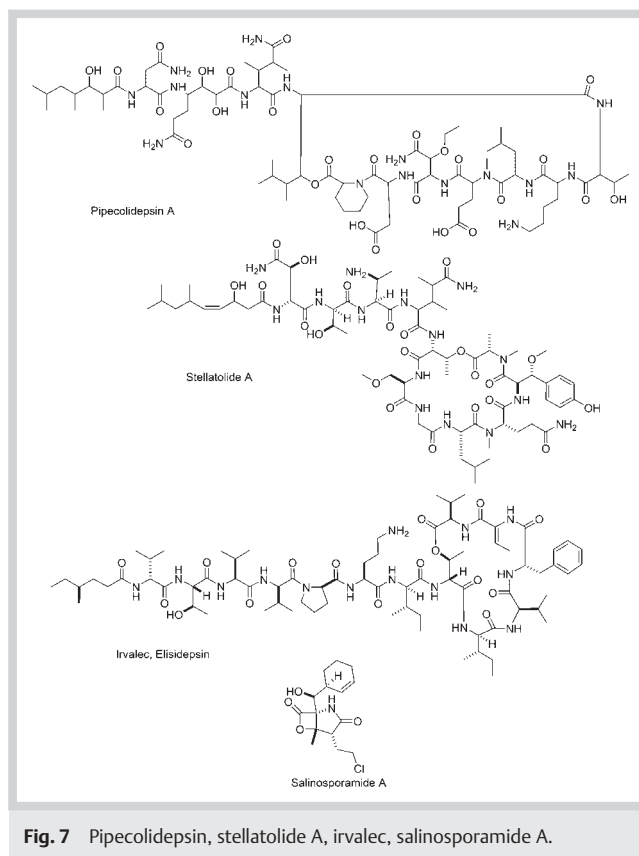


Fig. 7 Pipecolidepsin, stellatolide A, irvalec, salinosporamide A.

Also at the EORTC 2014 meeting, there was a very interesting report from PharmaMar showing that the chloro-derivative (PM050489) could be linked to trastuzumab by reduction of the antibody with a phosphine reagent, coupling to a maleimido derivative of PM050489, followed by purification by size exclusion chromatography to yield the resulting conjugate (MI130004) [114]. This ADC showed *in vitro* activity when tested against cell lines with selectivity towards cell lines with high HER2 expression (IC₅₀s 0.282 and 0.182 nM against HCC-1954 and SK-BR-3 cell lines, respectively), whilst HER2 null cells were unaffected in the range of concentrations tested (up to 300 nM). *In vivo*, MI130004 also demonstrated efficiency in causing an outstanding tumor reduction, with complete regressions in most of the animals that received the treatment [115].

Pipecolidepsin, stellatolide A, and irvalec

The PharmaMar group recently presented data at the 2014 EORTC meeting that demonstrated activity in these three modified marine-derived compounds (● Fig. 7) [51, 116, 117]. Irvalec was pulled from clinical trials by PharmaMar at the Phase II level, so it is interesting that they are now reporting on it and the other two [118].

Marizomib® (Salinosporamide A; NPI-0052; Phase II/I)

The story of this compound from its discovery from the marine actinomycete *Salinispora tropica* and its identification as a proteasome inhibitor has been covered extensively in the scientific literature. These reports include a workup to give the cGMP product from the first marine medium-based large-scale fermentation through synthesis by a variety of chemists both in academia and companies, and identification of the biosynthetic cluster in

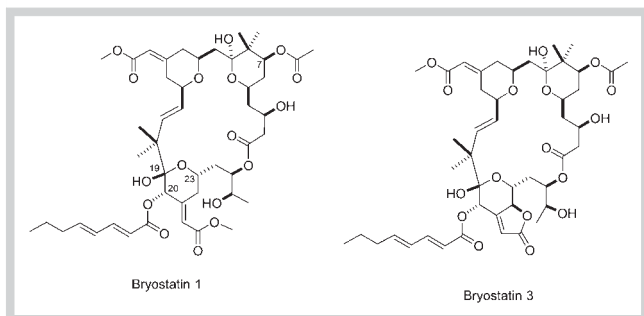


Fig. 8 Alzheimer's disease and antiviral compounds.

the producing organism(s) [119–122]. These were followed in 2014 by an excellent article by Jensen et al. giving up to date information on the genetic analyses of the producing organism and others from the same genus [123]. Then later the same year, there was a report of the first discovery of the gene cluster for salinosporamide A in *Salinispora arenicola* (rather than *S. tropica*) collected in temperate waters by Goo et al. [124].

Nereus Pharmaceuticals, the company set up to develop this compound and others from Jensen/Fenical laboratories, liquidated two or so years ago and all IP, etc., were transferred to Triphase Research and Development Corporation. Currently, in the NIH database, there are two Phase I trials “recruiting” (NCT02330562 and 02 103 335), both commencing in 2014, plus a Phase I/II trial in multiple myeloma listed as “active but not recruiting” (NCT00461045). This latter trial started in 2007 under Nereus and from 2013, was listed under Triphase. This data is in the clinical trials archive listing, accessible from the regular NIH clinical trials web site.

Alzheimer's Disease and Antiviral Compounds

Bryostatin 1 (Phase II, Alzheimer's Disease)

The isolation of the first of this 20-membered (now 21 plus *vide infra*) class of macrolidic cytotoxins from the fouling invertebrate *Bugula neritina* over 30 years ago led to massive collections of the nominal producing organism and to very elegant syntheses of various components. The initial discovery of bryostatin 3 (● Fig. 8) was indirectly reported in 1970, the subsequent developments leading to the report of the isolation and X-ray structure of bryostatin 1 (● Fig. 8) in 1982, together with the multi-year program that culminated in the isolation and purification of 18 bryostatins, have been well documented [125].

All of the known bryostatins possess a 20-membered macrolactone ring with three remotely substituted pyran rings linked by a methylene bridge and an (*E*)-disubstituted alkene; all have geminal dimethyls at C₈ and C₁₈, and a four carbon side chain (carbons 4–1) from the A ring to the lactone oxygen, with another four carbon chain (carbons 24–27) on the other side of the lactone oxygen to the C ring. Most have an exocyclic methyl enoate in their B and C rings, though bryostatin 3, in particular, has a butenolide rather than the C ring methyl enoate, and bryostatins 16 and 17 have glycals in place of the regular C₁₉ and C₂₀ hydroxyl moieties [125, 126].

Work reported from the Peoples' Republic of China in 1998 [127] and in 2004 [128] gave the structure for bryostatin 19 purified from a South China Sea collection of *Bugula neritina*. Then, in

the same year, this report was followed by the publication by Lopanik et al. reporting the isolation of bryostatin 20 from an Atlantic-sourced *B. neritina* [129]. Comparison with the structures of the other 18 bryostatins shows that these are closely related to bryostatin 3 in terms of their basic ring components. Very recently, the Chinese group published the structure of bryostatin 21 and also four derivatives of known bryostatins [130], so there are now 21 to 25 naturally occurring bryostatins. The substituted bryostatins 22–25 could be artifacts of the isolation techniques used, so until further confirmation is obtained, we will use 21 bryostatins to date.

Bryostatin 1 has been through well over 80 Phase I and Phase II clinical trials, with or without the addition of a cytotoxic agent in the protocols, but in no case has the compound progressed. In some of the earlier trials, the mode of delivery (a bolus at or close to the maximum tolerated dose or MTD) may have been the major problem, as myalgia was a serious side effect.

In 2011, the Keck group reported the first complete total synthesis of bryostatin 1 [131], which was rapidly followed by a paper from Manaviyar and Hale with details of a shorter route to the same compound [132]. Later the same year, Trost et al. published papers on the ring-expanded versions of bryostatins obtained by total synthesis, so the synthetic story of this class of macrocycles has not yet finished [133, 134].

It is now almost certain that bryostatins are produced by an as yet uncultured microbe found initially in the larvae of the bryozoan. Current information can be obtained by inspection of the review by the Haygood group published in 2010 [135].

What is of current importance, however, is the report by Neurotrope that the Phase IIa study (NCT02221947) of bryostatin 1 in Alzheimer's disease, which was terminated and replaced by the trial listed below, demonstrated that bryostatin 1 could be delivered safely to patients. The follow-on Phase II trial (NCT02431468) is listed as “not yet recruiting”, effective the middle of April 2015 with Neurotrope Inc. (and is still listed the same way in late November 2015); this is in contrast to the earlier trial listed for the same indication but with no information posted since 2008 (NCT00606164) under a different sponsor.

Bryostatin 1 and “Latent” HIV

A number of simplified bryostatin analogues (often called “bryologs”) have been synthesized using methods such as function-oriented synthesis. This technique was employed by Wender and other workers to develop simplified analogues with comparable or much improved activities, in some cases orders of magnitude in *in vitro* assays [136–138]. Further information was given in a review by Newman [139] and in a recent 2013 paper by the Keck group [140]. What is also of significant importance is the recent report by the Wender group of *in vitro* anti-HIV activity for some of their newer analogues [141].

In June 2015, Aphios Inc. in Massachusetts announced that they had enrolled patients for a Phase I/II study of bryostatin 1 in a trial that is designed to “release” the HIV virions from their latent sites in patients. This is a well-known phenomenon of a number of viruses including HIV, and is the basis of the anti-HIV treatment known as HAART (highly active antiretroviral therapy). These trials are being conducted in Spain, with a Phase I record under NCT02269605. Bryostatin 1 was produced using Aphios' now patented supercritical fluid technique, which was used, though not under cGMP conditions, as a comparator many years ago in bryostatin 1 purification processes [142].

Though bryologs have now yet been tried, if these trials are successful, then it is feasible that one or more of the Wender or Keck compounds could be a candidate for trials against HIV or other viruses that exhibit latency, such as the herpes simplex complex.

Griffithsin (Microbial Virucide Headed for Clinical Trials)

This 121-residue peptidic agent was first reported in 2005 following its isolation from the red alga *Griffithsia* sp. Due to source constraints, it was subsequently produced by transfer of the DNA sequence corresponding to the peptide via a plasmid into, and subsequent expression in, *Escherichia coli* [143]. The material, either natural or recombinant, was ultimately shown to bind to specific mannose-rich regions of HIV viral proteins [144]. In 2009, it was reported that the compound could function as a topical microbicide with potential as an intra-vaginal agent to protect against HIV transmission [145]. Since that publication, work has continued with this compound by demonstrating large-scale production in plants [145], further efficacy [146], and toxicity studies in rodent models [147].

Currently, the Population Council is developing a griffithsin-containing microbicide to prevent the transmission of HIV and other sexually transmitted infections (<http://www.popcouncil.org/research/developing-and-testing-a-griffithsin-non-arv-microbicide>). That web site has the following statement: "In 2013, the Council was awarded a cooperative agreement from PEPFAR, and administered by USAID, to investigate griffithsin (GRFT) as a vaginal microbicide. GRFT is a naturally occurring protein isolated from algae that has potent activity against HIV and other STIs. GRFT for drug development is produced in tobacco plants, and initial pre-clinical testing has shown it to be safe and non-irritating in vitro (in cells and tissue) and in vivo (in animals). Council researchers are developing a GRFT-containing microbicide gel for pre-clinical and clinical studies. Results from these studies will inform the development of other delivery systems, including an intravaginal ring to provide protection immediately and up to 90 days." (accessed August 17th, 2015).

In Conclusion

In this review, we initially concentrated on materials that are approved as drugs by the relevant authorities. In addition, we have discussed compounds that are chemically related to those approved drugs, and then compounds that are in clinical trials mainly against cancer, but also Alzheimer's disease and HIV. The emphasis on cancer is simply due to the fact that the major funding agency for marine-derived compounds for many years was the NIH/NCI, and to some extent, this carried over in other countries.

There are a large number of very interesting compounds that have been isolated, usually via activity-driven isolation processes from single-celled organisms, in particular the cyanobacteria. There are some excellent current reviews covering compounds from these photosynthetic bacteria that have bioactivities ranging from antitumor to neurological diseases, but none have yet made it to clinical trials [148–150]. What is noticeable, however, is that no antimicrobial compounds from marine sources have yet made it beyond the discovery phase into what is known in the pharmaceutical industry as "the preclinical phase". This may well be a function of the current lack of antimicrobial research in the major pharmaceutical companies (which used to be a major source of natural product-based compounds), coupled to a lack

of funding in this area for both discovery and, particularly, development. However, a very recent review by Ng et al. demonstrates that there are definitely possibilities in these disease areas for marine-sourced compounds [151].

Finally, we consider that the potential for marine-based compounds in all disease areas is immense. When one realizes that currently there are approximately 25 000 defined chemical compounds reported to date from marine sources, and there are seven approved drugs [(including the omega-3-acid ethyl esters and vidarabine not discussed in this article [152], and one may also check the web site of Professor A. M. S. Mayer in the USA (<http://marinepharmacology.midwestern.edu/clinPipeline.htm>) for these sources (direct or modified structures)], then a success ratio of 1 in 3500 is orders of magnitude better than those from synthetic molecules.

Conflict of Interest



Both authors declare that there are no conflicts of interest with the content of this manuscript.

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