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MINI REVIEW

MARINE PHARMACOLOGY IN 2000: ANTITUMOR AND CYTOTOXIC COMPOUNDS

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During 2000, marine antitumor pharmacology research aimed at the discovery of novel antitumor agents was published in 85 peer-reviewed articles. The purpose of this article is to present a structured review of the antitumor and cytotoxic properties of 143 marine natural products, many of them novel compounds that belong to diverse structural classes, including polyketides, terpenes, steroids and peptides. The organisms yielding these bioactive compounds comprised a taxonomically diverse group of marine invertebrate animals, algae, fungi and bacteria. Antitumor pharmacological studies were conducted with 19 marine natural products in a number of experimental and clinical models that defined or further characterized their mechanisms of action. Potentially promising *in vitro* cytotoxicity data generated with murine and human tumor cell lines were reported for 124 novel marine chemicals with as yet undetermined mechanisms of action. Noteworthy is the fact that marine anticancer research clearly remains a multinational effort, involving researchers from Austria, Australia, Brazil, Canada, England, France, Germany, Greece, Indonesia, Italy, Japan, New Zealand, Russia, Spain, South Korea, Switzerland, Taiwan, the Netherlands and the United States. Finally, this 2000 overview of the marine pharmacology literature highlights the fact that the discovery of novel marine antitumor agents continued at the same high level of research activity as during 1998 and 1999.

Key words: *marine; antitumor; cytotoxic; cancer; pharmacology; review*

The purpose of this article is to review the research literature published during 2000 in the field of marine antitumor pharmacology using a format similar to the one used in our previous 2 reports, which reviewed the marine antitumor pharmacology research during 1998 and 1999.[1,2] Consistent with our previous 2 articles, only those reporting on the antitumor pharmacology or cytotoxicity data of marine compounds with established chemical structures (Figs. 1 and 2) are included in the present review and are presented in alphabetical order in Tables I and II. Research articles reporting on novel preclinical and/or clinical antitumor pharmacological research of marine chemicals with determined mechanisms of action have been presented in Table I and are discussed with a focus on the new information on the mechanism of action. On the other hand, reports on the cytotoxicity of marine chemicals with undetermined mechanisms of action are grouped in Table II. Publications on the preclinical antitumor or cytotoxic activity of synthetic analogues of marine metabolites as well as that of marine extracts or as yet structurally uncharacterized marine compounds have been excluded from the present review, although several promising studies were reported during 2000.[3–5]

2000 ANTITUMOR PHARMACOLOGY OF MARINE NATURAL PRODUCTS WITH DETERMINED MECHANISMS OF ACTION

Table I provides a summary of the main conclusions of 23 studies that reported research involving 19 different marine compounds (selected structures are illustrated in Fig. 1). Reports on clinical trials are not included in Table I but are discussed in the text of the article.

New information became available during 2000 on the pharmacology of Bryostatin-1, Cryptophycins, Didemnin B, Discodermolide, Dolastin 10, Ecteinascidin-743 and Eleutherobin, marine compounds also included in both our 1998 and 1999 reviews.[1,2]

Five studies were published during 2000 on the preclinical and clinical pharmacology of Bryostatin-1. Cartee *et al.*[6] investigated whether chronic exposure to the macrolide Bryostatin-1 could circumvent the resistance to ionizing radiation-induced apoptosis conferred by the overexpression of the antiapoptotic gene Bcl-2 in human histiocytic lymphoma U937 cells. Their findings suggest that Bryostatin-1 sensitizes Bcl-2 overexpressing human leukemia cells to ionizing radiation-mediated antiproliferative effects through a mechanism that does not appear to involve the induction of apoptosis. With the purpose of contributing to clinical trials with the combination of Bryostatin-1 and paclitaxel, Koutcher *et al.*[7] determined the effect of the sequential use of both agents on tumor growth, mitotic entry and blood flow using a tumor-bearing mouse model. Their studies determined that there is a definite sequence dependence of this combination, as prior treatment with Bryostatin-1 inhibited tumor responses to paclitaxel. Furthermore, the authors concluded that when phase 1 clinical trials are started, the "sequence of paclitaxel followed by bryostatin will be critical in the clinical trial design."[7] Mohammad *et al.*[8] studied the antitumor effects of the cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) regimen alone or in combination with Bryostatin-1 on a xenograft model for resistant diffuse large cell lymphoma (DLCL) in mice with severe combined immune deficiency. The *in vitro* and *in vivo* results reported documented that combining Bryostatin-1 with the CHOP regimen enhanced the effect against DLCL, thus suggesting further clinical investigation of this novel marine agent for the treatment of lymphomas. Two clinical reports were published during 2000. Pagliaro *et al.*[9] reported the results of a single-institution phase 2 trial of Bryostatin-1 in patients with metastatic renal cell carcinoma, a type of cancer for which there is currently a poor prognosis and no optimal systemic therapy available. Although 2 out of a total of 30 patients evi-

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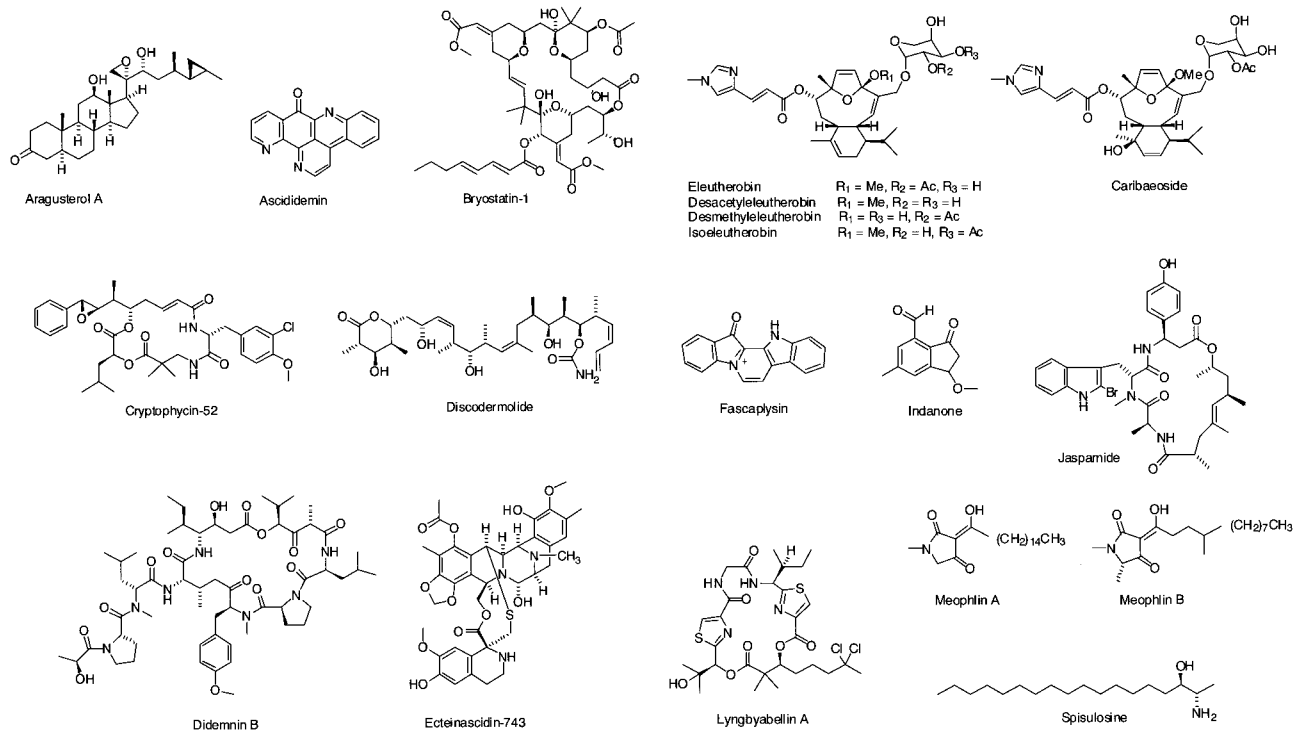

FIGURE 1 – Structures of marine natural products reported in 2000 with established mechanisms of action.

denced significant tumor regression, the median survival for the patients was 13.1 months, which differed little from overall median survival for patients with metastatic renal cell carcinoma, currently 1 year. Thus, the study demonstrated that "there is minimal, if any, clinically relevant single-agent activity of Bryostatin-1" at the dose and schedule used.[9] Varterasian *et al.*[10] reported on a phase 2 trial of Bryostatin-1 in 25 patients with relapsed low-grade non-Hodgkin's lymphoma (LG-NHL) or chronic lymphocytic leukemia (CLL). Bryostatin-1 alone resulted in 1 complete remission of a patient with LG-NHL and 2 partial remissions of patients with CLL. The authors concluded that Bryostatin-1 "resulted in a modest although definite activity as a single agent."[10]

During 2000, 3 preclinical studies were reported with the marine depsipetides Cryptophycins, a family of novel antimitotic compounds. Panda *et al.*[11] reported their research with the antitumor compound Cryptophycin-52, a novel member of the cryptophycin family, currently produced by total chemical synthesis, which suppresses microtubule dynamics. Cryptophycin-52 at low picomolar concentrations was shown to inhibit cancer cell proliferation by stabilizing spindle microtubules, binding tightly and noncovalently to a single high-affinity site on tubulin, while also inducing a conformational change in the tubulin molecule. Lilly Research Laboratories reported 2 preclinical animal studies with Cryptophycins.[12,13] The investigators undertook extensive research with Cryptophycin-52 and -55, noting these agents can be added to other anticancer agents simultaneously or sequentially, thus leading to additive to greater-than-additive tumor responses in several human tumor xenograft models (*e.g.*, colon and ovarian carcinoma) and "warrant further investigation."[11]

Three studies were reported during 2000 on the preclinical and clinical pharmacology of the Didemnin depsipeptides.[14] This contrasts with the 5 pharmacological studies with Didemnin B and Aplidine published during 1999.[1] In a preclinical study, Ahuja *et al.*[14] determined that the Didemnins were moderately potent inhibitors of protein synthesis *in vitro* and that the intact depsipeptide ring was required for this activity. Interestingly, a correlation was noted between inhibition of protein synthesis in cell lysates and in human adenocarcinoma MCF-7 cells, suggesting that protein synthesis may be inhibited by the binding of Didemnins to ribosome-EF-1α complexes in both cell types. Kucuk *et al.*[15] reported on a phase 2 trial of Didemnin B in previously treated non-Hodgin's lymphoma patients who had failed initial therapy and had a poor prognosis. Unfortunately, Didemnin B appeared to have only modest activity in the 29 patients who received the marine antitumor agent, while also concomitantly showing evidence of considerable toxicity to this patient population. In an extensive pharmaceutical formulation study with dehydrodidemnin B or Aplidine, Nuijen *et al.*[16] completed a series of studies that resulted in the development of a freeze-dried formulation that appeared to be a stable parenteral pharmaceutical dosage that will contribute significantly to phase 1 clinical testing of Aplidine.

During 2000, one article was published discussing research with the marine polyketide Discodermolide. Martello *et al.*[17] reported on a schedule-independent synergistic combination of Taxol and Discodermolide, in 4 different human carcinoma cell lines, which was surprising in view of the fact that the mechanism of action of both drugs has been proposed to be similar. The authors suggest that one possibility to explain this synergy is that Discodermolide has additional targets that could cause an increase in apoptosis, a mechanism unrelated to the tubulin-binding properties of Discodermolide. The authors conclude by suggesting that Taxol and Discodermolide may constitute a promising chemotherapeutic combination "that merits exploration."[17]

A phase 1 clinical study with the pentapeptide Dolastin 10 isolated from the marine mollusk *Dolabella auricularia* was reported during 2000. Madden *et al.*[18] determined the maximum tolerated dose, clinical pharmacokinetics and metabolism of Dolastin 10 as well as hematological and nonhematological toxicity and antitumor activity in 22 patients with pathologically advanced solid tumors using a single rapid i.v. infusion repeated every 22 days. Although no anticancer response was observed in the patients, the investigators concluded their extensive study by suggesting that "further study of escalated Dolastin 10 dosing with

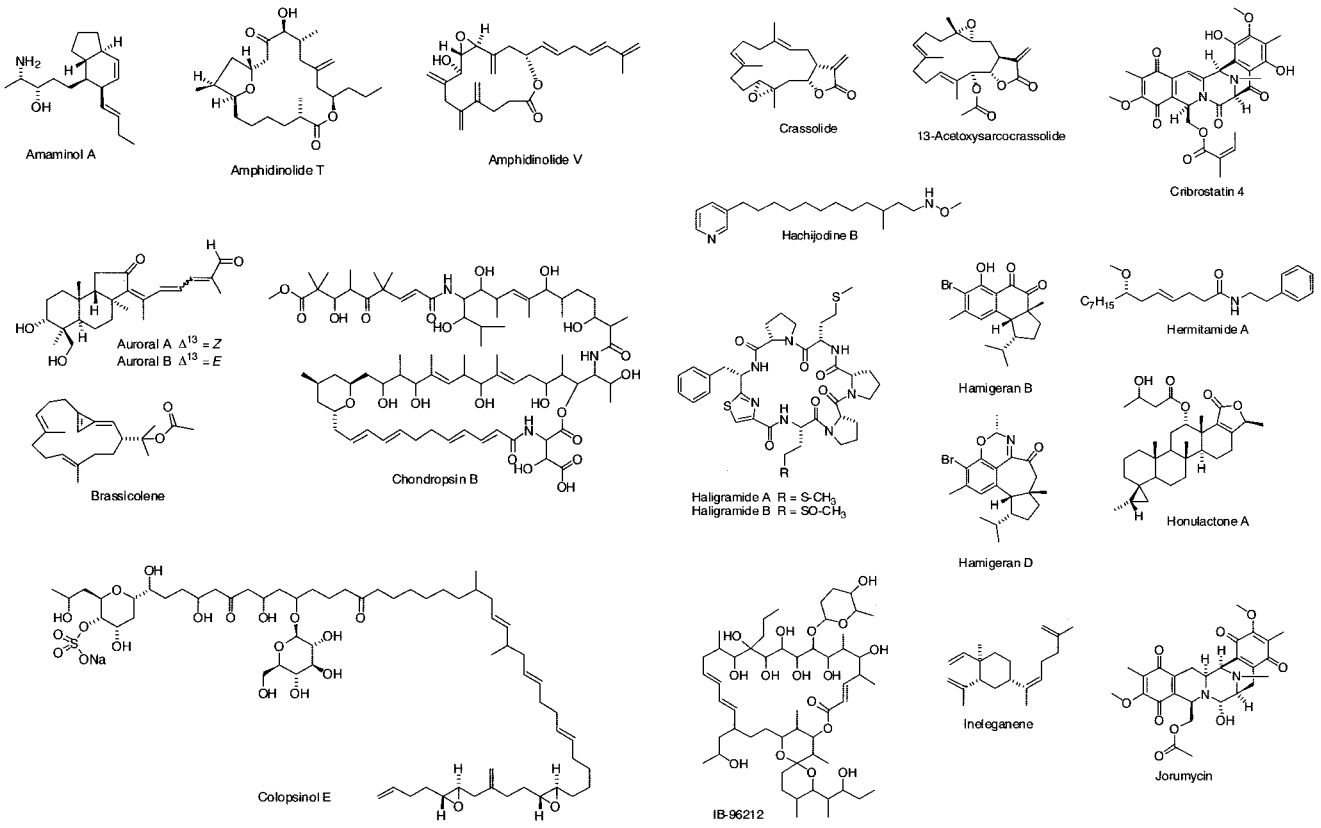

FIGURE 2 – Selected structures of new marine compounds with cytotoxic or antitumor activity reported in 2000.

cytokine support is warranted" to minimize the myelosuppresive effects of this marine natural product.[18]

Research with the isoquinoline alkaloid Ecteinascidin-743, an antitumor agent originating from the Caribbean tunicate *Ecteinascidia turbinata* continued to be very active during 2000. In addition to 3 reports published in 1998[2] and 4 articles in 1999,[1] 3 preclinical and 1 clinical article were published during 2000. Jin *et al.*[19] reported that physiologically relevant nM concentrations of Ecteinascidin-743 blocked the activation of the human P glycoprotein gene (MDR1) promoter by multiple stress inducers (*e.g.*, UV irradiation) in MDR1-transfected human colon carcinoma cells. The investigators thus suggested that Ecteinascidin-743 might become the "prototype for a distinct class of transcription-targeted chemotherapeutic agents" and perhaps become adjuvants for the treatment of multidrug resistant tumors.[19] In an accompanying article, Minuzzo *et al.*[20] investigated the interference of transcriptional activation by Ecteinascidin-743 in transfected mouse NIH 3T3 fibroblasts, and they observed that Ecteinascidin behaved as a promoter-specific, transcription-interfering inhibitor. The effect was very rapid, a 1-min preincubation being sufficient, and it was observed at pharmacological concentrations (nM) that correlated with those observed in the plasma of patients undergoing clinical trials. Garcia-Nieto *et al.*[21,22] in 2 separate publications, using unrestrained molecular dynamics simulations, studied complexes of Ecteinascidin-743 with 2 DNA target sequences in aqueous solutions. They demonstrated that drug binding causes the widening of the minor groove, a bending toward the major groove, and perhaps increased DNA binding specificity through putative protein-DNA interactions, thus providing further insight into the mechanism of anticancer activity of this compound. Van Kestern *et al.*[23] studied the pharmacokinetics and pharmacodynamics of Ecteinascidin-743 in a phase 1 dose-finding study designed to identify the maximum tolerated dose and dose-limiting toxicities (DLT). Although considerable interpatient variability was observed at all dose levels evaluated, this phase 1 trial indicated that Ecteinascidin-743 administered as a 24-hr intravenous infusion every 3 weeks is well tolerated at a dose of 1,500 $\mu g/m^2$ with thrombocytopenia and neutropenia as the DLTs.

Antitumor research with the diterpene Eleutherobin, originally isolated from the soft coral *Eleutherobia sp.* from western Australia, also continued during 2000. The single article reviewed in 1998[2] was followed by 2 articles in 1999[1] and 2 more in 2000 describing the isolation of the marine natural product from the Caribbean octocoral *Erythropodium caribaeorum*. Cinel *et al.*[24] studied Eleutherobin and 6 new antimitotic diterpenes and encountered sufficient structural variations in the tubulin-binding regions in these marine natural products to test a recently proposed pharmacophore model for microtubule stabilizing compounds. One of these compounds, Caribaeoside, showed evidence of a significant decrease in the antimitotic activity (IC_{50} = 20 μM) relative to Eleutherobin (IC_{50} = 100 nM), thus demonstrating the importance of the B region of the Eleutherobin pharmacophore for tubulin binding and providing initial support for Ojima's pharmacophore model presented in our previous review.[1] Roberge *et al.*[25] in an accompanying article used a novel cell-based assay for antimitotic compounds to characterize 6 new Eleutherobin analogues, whose antimitotic activity ranged from 20 nM to 20 μM. The authors propose that this cell-based antimitotic assay will greatly facilitate the discovery, development and pharmacological characterization of novel antimitotic agents because it selects for both "activity against a particular target" as well as other desirable properties and potentially novel mechanisms of action that may lead to mitotic arrest.[25]

Table I lists 9 additional marine natural products, with determined mechanisms of action, for which no reports were published during 1998 and 1999:[1,2] Aragusterol, Ascididemnin, Fascaplysin,

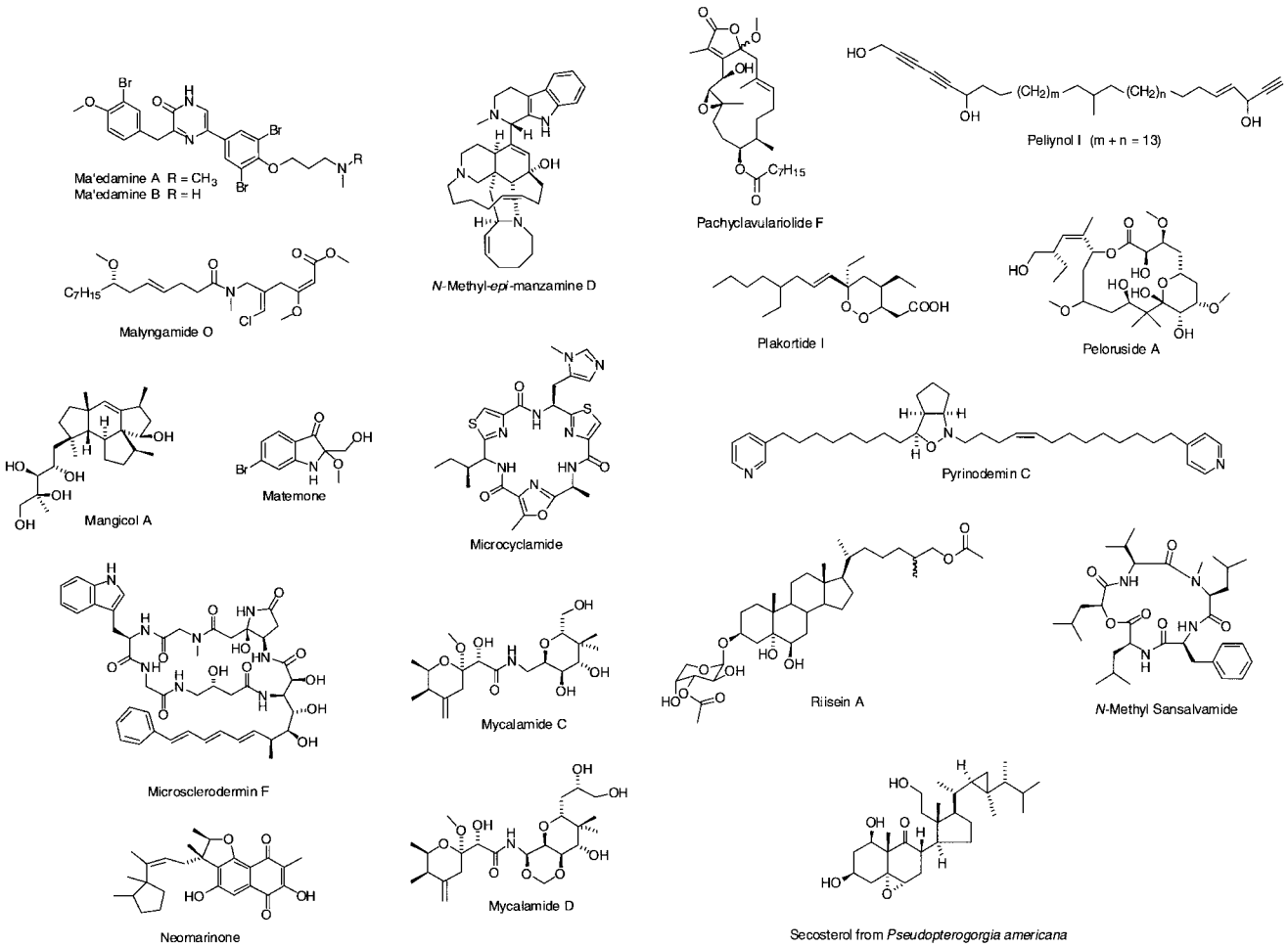

FIGURE 2 – CONTINUED

an Indanone from *Lyngbya majuscula*, Lyngbyabellin A, Jaspamide, Melophlins A and B and Spisulosine.

In an extensive and detailed study, Fukuoka *et al.*[26] carefully characterized the growth-inhibitory activity of Aragusterol A, a steroid isolated from the Okinawan marine sponge *Xestospongia sp.*, against a panel of 14 human cancer cell lines *in vitro*, observing a broad-spectrum growth-inhibitory activity. Interestingly, when Aragusterol A was administered *in vivo* to mice bearing intraperitoneally implanted murine tumors, a similar broad spectrum and high degree of antitumor activity was observed. The investigators concluded that Aragusterol A targeted the G_1 phase of the cell cycle by downregulating cyclin-dependent kinases and G_1 cyclins involved in G_1/S transition, thus blocking the entry of human tumor cells into the S-phase.

Dassonneville *et al.*[27] extended current knowledge on the mechanism of action of Ascididemin, a DNA-intercalating alkaloid isolated from the Mediterranean ascidian *Cystodytes dellechiajei* by focusing their extremely detailed molecular investigation on the effect of this marine natural product on the catalytic activities of topoisomerases I and II, enzymes known to regulate DNA topology. Although the investigators demonstrated that Ascididemin was a poison for purified topoisomerases, studies with several cell lines led them to conclude that neither toposoisomerase I nor II could be considered potential cellular targets for this marine natural product. Interestingly, Ascididemin was shown to be a potent inducer of apoptosis in both human and murine leukemia cells. Matsumoto *et al.*[28] in a short communication provided interesting evidence that Ascididemin can cause oxidative damage to DNA via a thiol-dependent conversion of oxygen to DNA-cleaving oxygen radicals, a process that may lead to concomitant DNA fragmentation. Brief but novel information on the structural features in the Ascididemin molecule required for DNA-cleaving activity as well as indications of probable intracellular damage, as evidenced by flow cytometry, were also provided by the authors.

Soni *et al.*[29] discovered a potential new use for the marine natural product Fascaplysin, an alkaloid derived from the sponge *Fascaplysinopsis sp*. While screening for inhibitors of cyclin-dependent protein kinases, key enzymes involved in the mammalian cell cycle, they observed that Fascaplysin selectively inhibited Cdk4 kinase *in vitro* with an $IC_{50} = 0.35\ \mu M$. Molecular modeling studies showed that Fascaplysin binds to the ATP binding pocket of Cdk4 by "interacting through a bidentate hydrogen bond/acceptor pair."[29] The fact that Fascaplysin caused G_1 arrest not only in normal human fibroblasts but also in both human colon carcinoma and osteogenic sarcoma cell lines makes this marine chemical an interesting candidate for further study of cellular processes regulated by Cdk4 kinase in mammalian cells. Using new gene transcription-based high-throughput assays for inhibitors of activation of the vascular endothelial growth factor gene (VEGF), an angiogenic factor that is produced by tumor cells, Nagle *et al.*[30] discovered a new indanone in extracts of the marine cyanobacterium *Lyngbya majuscula*. Although the indanone inhibited VEGF expression ($IC_{50} = 25\ \mu M$) in transfected human hepatocellular carcinoma Hep3B cells, it appears unlikely that further *in vivo*

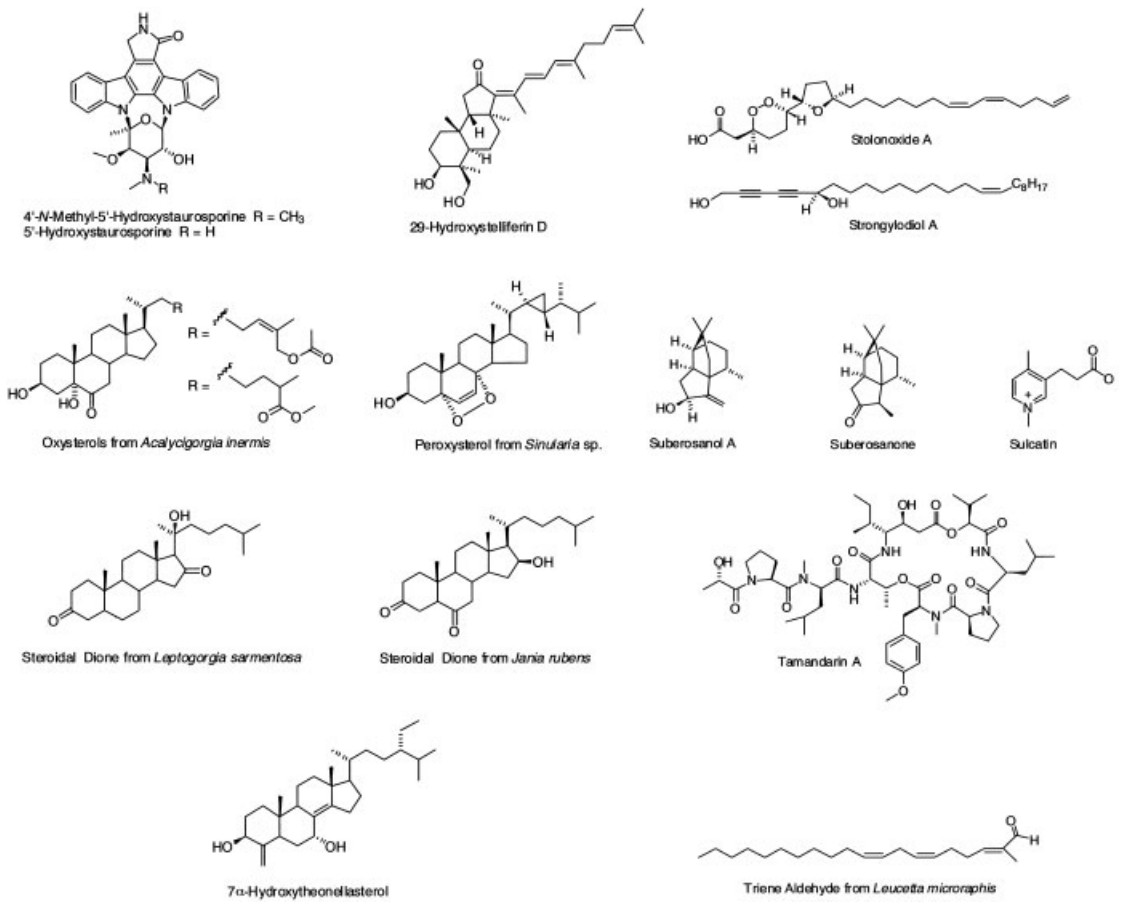

FIGURE 2 – CONTINUED

evaluation will be pursued because it did not inhibit Hep3B cell growth.

Luesch *et al*.[31] reported on the isolation, structure determination and biological activity of a novel depsipeptide Lyngbyabellin A from the marine cyanobacterium *Lyngbya majuscula*. Although Lyngbyabellin A was observed to be cytotoxic to human nasopharyngeal and colon carcinoma cell lines (IC_{50} ranging from 0.03 to 0.5 μg/mL, respectively) and to disrupt specifically cellular microfilament networks in smooth muscle cells at concentrations of 0.01–5 μg/mL, it lacked *in vivo* activity when tested at sublethal doses against murine colon or mammary adenocarcinomas.

As part of their project to investigate novel drugs to treat chemotherapy-insensitive tumors, Nakazawa *et al*.[32] extended the pharmacology of Jaspamide, a depsipeptide isolated from sponges of the genus *Jaspis* and *Hemiastrella* using the HL-60 human promyelocytic leukemia cell line. Experimentation with cytogenetic and flow cytometric analysis of DNA revealed that nanomolar concentrations of Jaspamide induced inhibition of cell proliferation and an increase in polynuclear cells. Although the mechanism of polyploidy induction by Jaspamide remains undetermined at this time, this is a significant investigation because it contributes to the current knowledge of mechanisms involved in the molecular control of polyploidy, deemed essential for the understanding of cancer progression.

Aoki *et al*.[33] isolated 2 novel tetramic acids, Melophlins A and B from the sponge *Melophlus sarassinorum*, which reversed the ras-transformed phenotype of NIH3T3 fibroblasts at 5 μg/mL. Cell cycle analysis by flow cytometry demonstrated that Melopholins A and B arrested ras-transformed fibroblasts in the G_1 phase of the cell cycle, thus suggesting that because Melophlins reverse the transformed phenotype, these moderately cytotoxic marine agents may have potential as a new type of anticancer agent.

Cuadros *et al*.[34] reported on the effects of Spisulosine, an alkyl amino alcohol isolated form the clam *Spisula polynyma*, on the growth and morphology of several cell lines. A striking observation was that Spisulosine promoted disassembly of actin stress fibers, whose formation is regulated by the small GTP-binding protein Rho. Thus, although the authors acknowledged that the specific molecular target of Spisulosine was as yet undetermined, they speculated that perhaps this marine chemical could act as an antagonist of the lysophosphatidic acid receptor or perhaps also play a role as an antagonist for sphingosine-1 receptor. Furthermore because Spisulosine "abolishes Rho activity [it] could have an obvious role as antitumoral agent."[34]

2000 ANTITUMOR PHARMACOLOGY OF MARINE NATURAL PRODUCTS WITH UNDETERMINED MECHANISMS OF ACTION

Table II encompasses 124 marine natural products that demonstrated activity in cytotoxicity assays and selected structures are shown in Figure 2. In contrast with the extensive preclinical and clinical investigation completed with the marine compounds presented in Table I, no detailed mechanism of action research was performed with most of the marine compounds shown in Table II, with the notable exception of 2 interesting articles. Zhou *et al*.[35] studied 2 novel Manzamine alkaloids in a novel antiangiogenesis assay, while Hirano *et al*.[36] showed that Ma'edamine A had inhibitory activity against the *c-erbB*-2 kinase *in vitro*. In all the other studies listed in Table II, the marine compounds were tested in cytotoxicity assays that most

TABLE I – 2000 ANTITUMOR PHARMACOLOGY OF MARINE NATURAL PRODUCTS WITH DETERMINED MECHANISMS OF ACTION

Compound	Organism	Chemistry	Experimental or clinical model	Mechanism of action	Country	Reference
Aragusterol A	Sponge	Steroid	Human and murine cancer cell panel and *in vivo* assays	Targets the G_1/S cell cycle phase	Japan	26
Asciddemin	Tunicate	Alkaloid	Human and murine leukemia cell lines	Induction of apoptosis; no effect on topoisomerase I and II	France	27
Asciddemin	Tunicate	Alkaloid	Murine leukemia cell line	Reductive DNA cleavage by reactive oxygen species	United States, New Zealand	28
Bryostatin-1	Bryozooa	Macrolide	Human lymphoma cell line	Sensitizes cells to radiation-mediated antiproliferation	United States	6
Bryostatin-1	Bryozooa	Macrolide	Murine *in vivo* tumor model	Paclitaxel-Bryostatin combination is sequence-dependent	United States	7
Bryostatin-1	Bryozooa	Macrolide	Murine *in vivo* xenograft model for diffuse large cell lymphoma	Bryostatin enhances CHOP regimen for diffuse large cell lymphoma	United States	8
Cryptophycins	Bacteria	Depsipeptide	Bovine brain tubulin	Tight noncovalent binding to a tubulin high-affinity site	United States	11
Cryptophycins	Bacteria	Depsipeptide	Murine *in vivo* xenograft models	Effective in combination with doxorubicin, paclitaxel and 5-fluorouracil	United States	12
Cryptophycins	Bacteria	Depsipeptide	Murine *in vivo* xenograft models	Effective in a number of clinical combination regimens	United States	13
Didemnin B	Tunicate	Depsipeptide	Rabbit reticulocyte lysate and human adenocarcinoma cell line	Intact depsipeptide ring required for protein synthesis inhibition	United States	14
Discodermolide	Sponge	Polyketide	Human and murine tumor cell lines	Apoptosis as a potential mechanism of synergy with paclitaxel	United States	17
Ecteinascidin-743	Tunicate	Isoquinoline alkaloid	Human colon carcinoma cell line	Inhibition of human P glycoprotein gene (MDR1) transcription	United States	19
Ecteinascidin-743	Tunicate	Isoquinoline	Transfected NIH 3T3 fibroblasts	Promoter-specific transcription interference	Italy, United States	20
Ecteinascidin-743	Tunicate	Isoquinoline	Molecular dynamics	Minor groove widening and bending toward major groove and putative protein-DNA interactions	Spain	21,22
Eleutherobin analogues	Coral	Diterpene glycoside	Human breast carcinoma cell line	Eleutherobin pharmacophore B region necessary for tubulin binding	Canada, Brazil, The Netherlands	24
Eleutherobin analogues	Coral	Diterpene	Human breast carcinoma cell line	Enhanced antimitotic activity	Canada	25
Fascaplysin	Sponge	Alkaloid	Human colon carcinoma and osteogenic sarcoma cell lines and normal fibroblasts	Cyclin-dependent kinase 4 inhibition	Switzerland	29
Indanone from *Lyngbya majuscula*	Bacteria	Polyketide	Human hepatocellular carcinoma cell line	VEGF expression inhibition	United States	30
Jaspamide	Sponge	Depsipeptide	Human promyelocytic leukemia cell line	Induction of polyploidization	Japan	32
Lyngbyabellin A	Bacteria	Depsipeptide	Human nasopharyngeal and colon carcinoma cell line	Disruption of cellular microfilaments	United States	31
Melophlins A and B	Sponge	Tetramic acids	Human c-H-ras–transformed NIH3T3 cell line	Reversal of transformed phenotype to normal	Japan, Indonesia	33
Spisulosine	Clam	Alkyl amino alcohol	Monkey fibroblast cell lines	Disassembly of actin stress fibers	Spain, United States	34

commonly consisted of panels of either human or murine tumor cell lines. In a few reports, cytotoxicity studies were very extensive and included the National Cancer Institute 60-tumor cell line screen.[37–42] A number of these novel compounds showed significant cytotoxic activity, defined as an IC_{50} of $\leq$ 4.0 μg/mL, and would appear as possible candidates for additional mechanism-of-action studies. This would help determine if the reported cytotoxicity was the result of a pharmacologic

TABLE II – 2000 ANTITUMOR PHARMACOLOGY OF MARINE NATURAL PRODUCTS WITH UNDETERMINED MECHANISM OF ACTION

Compound	Organism	Chemistry	Experimental or clinical tumor model	Growth Inhibition or cytotoxicity[1]	Country	Reference
Alkanes and Alkenes	Tunicate	Alkyl sulfate	Murine tumor cell lines	12–500 μg/mL	Italy	44
Amaminols A and B	Tunicate	Alkyl amine	Murine tumor cell lines	2.1 μg/mL	Japan	45
Amphidinolide T	Dinoflagellate	Macrolide	Murine tumor cell line	18 μg/mL	Japan	46
Amphidinolide V	Dinoflagellate	Macrolide	Human and murine tumor cell line	3.2–7 μg/mL	Japan	47
Aurorals	Sponge	Sesterterpene	Human tumor cell lines	0.2–8 μg/mL	France	48
Brassicolene	Coral	Diterpene	Human and murine tumor cell lines	0.9–3.6 μg/mL	Taiwan	49
Calcigeroside B, C_1 and C_2	Sea cucumber	Triterpene glycoside	Human and murine tumor cell lines	5 μg/mL	Russia, Spain	50
Chondropsins A and B	Sponge	Macrolide	NCI 60 tumor cell line screen	2.4×10^{-8} M	United States	37
Colopsinol E	Dinoflagellate	Alkyl glycoside	Murine tumor cell line	7 μg/mL	Japan	51
Crassolide, sarco- and 13-acetoxysarco-	Coral	Diterpene	Human and murine tumor cell lines	0.16–22.4 μg/mL	Taiwan	52
Crassolide, lobo-	Coral	Diterpene	Human and murine tumor cell lines	0.012–2.99 μg/mL	Taiwan	53
Cribrostatins 3–5	Sponge	Isoquinoline alkaloid	NCI 60 tumor cell line screen	$1–10 \times 10^{-6}$ M	United States	38
Hachijodines A–G	Sponge	Alkyl pyridine	Murine tumor cell lines	1–2.2 μg/mL	Japan, The Netherlands	54
Haligramides A and B	Sponge	Peptide	Human tumor cell lines	A, 5.17–9.08 μg/mL; B, 3.89–8.82 μg/mL	United States	39
Hamigerans	Sponge	Terpene	Murine tumor cell lines	8–74.2 μM	New Zealand	55
Hermitamides A and B	Bacterium	Alkaloid	Murine tumor cell lines	2.2–5.5 μM	United States	56
Honulactones	Sponge	Sesterterpene	Murine and human cell lines	1 μg/mL	United States, New Zealand	57
IB-96212	Bacterium	Macrolide	Human and murine tumor cell lines	0.0001–1 μg/mL	Spain	58
Ineleganene	Coral	Diterpene	Human and murine tumor cell lines	0.2–3.63 μg/mL	Taiwan	59
Jorumycin	Nudibranch	Isoquinoline alkaloid	Human and murine tumor cell lines	0.0125 μg/mL	Italy, India	60
Ma'edamines A and B	Sponge	Alkaloid	Human and murine tumor cell lines and c-erbB-2 kinase	3.9–6.7 μg/mL	Japan, Australia	36
Malyngamide O	Sea hare	Alkaloid	Human and murine tumor cell lines	2 μg/mL	United States	61
Mangicols	Fungus	Sesterterpene	NCI 60 tumor cell line screen	17–36.3 μM	United States	40
Epi-Manzamine D	Sponge	Alkaloid	Human and murine tumor cell lines and yeast antiangiogenesis assay	0.1–5 μg/mL	United States	35
Matemone	Sponge	Alkaloid	Human tumor cell lines	24–30 μg/mL	France	62
Microcyclamide	Bacterium	Peptide	Murine tumor cell lines	1.2 μg/mL	Japan	63
Microsclerodermins	Sponge	Peptide	Human tumor cell line	1.1–2.4 μg/mL	United States	64
Mycalamides C and D	Sponge	Alkaloid	Murine tumor cell line	0.035–0.095 μg/mL	New Zealand, Australia	65
Mycalamide D	Sponge	Alkaloid	Human and murine tumor cell lines	0.006–0.019 μM	New Zealand	66
Neomarinone	Bacterium	Quinone	NCI 60 tumor cell line screen	8–10 μg/mL	United States	42
Pachyclavulariolide F	Coral	Diterpene	Murine tumor cell line	1 μg/mL	Canada, The Netherlands	67
Pellynol L	Sponge	Polyacetylene	Human tumor cell lines	0.08–2.23 μg/mL	United States	68
Peloruside A	Sponge	Macrolide	Murine tumor cell lines	0.010 μg/mL	New Zealand	69
Plakortides I and J	Sponge	Polyketide	Murine tumor cell lines	7–9 μg/mL	Italy	70
Pyrinodemins B–D	Sponge	Alkyl pyridine	Human and murine tumor cell lines	0.06–0.5 μg/mL	Japan	71
Riiseins A and B	Coral	Steroidal glycoside	Human tumor cell line	2.0 μg/mL	Brazil, United States	72
Sansalvamide, N-methyl	Fungus	Depsipeptide	NCI 60 tumor cell line screen	Mean, 8.3 μM	United States	41
Secosterol, epoxi	Gorgonian	Steroid	Human tumor cell lines	11–18.43 μg/mL	United States	73
Staurosporines	Bacterium	Alkaloid	Human and murine tumor cell lines	0.002–0.04 μM	Spain	74
Stelliferins, hydroxy	Sponge	Triterpene	Murine tumor cell lines	0.06–1.3 μM	Japan	75
Oxysterol	Gorgonian	Steroid	Human tumor cell line	0.9–9.7 μg/mL	Korea	76
Peroxysterols	Coral	Steroid	Human and murine tumor cell lines	0.4–10.8 μg/mL	Taiwan	77
Peroxysterol	Sponge	Steroid	Human tumor cell line	< 80 μg/mL	France	78
Steroidal Dione	Gorgonian	Steroid	Human and murine tumor cell lines	1 μg/mL	Spain	79
Steroidal Dione	Alga	Steroid	Human tumor cell line	0.5 μg/mL	France	80
Sterol, hydroxytheonella	Sponge	Steroid	Human tumor cell line	29.5 μM	United States	81
Stolonic acids A and B	Ascidian	Fatty acid	Human tumor cell lines	0.05–0.1 μg/mL	United States	82
Stolonoxides A–D	Ascidian	Fatty acid	Human and murine tumor cell lines	0.01–1 μg/mL	Spain	83
Strongylodiols A–C	Sponge	Polyacetylene	Human tumor cell lines	0.35–28 μg/mL	Japan, The Netherlands	84
Suberosanols	Gorgonian	Sesquiterpene	Murine and human tumor cell lines	5×10^{-6} to 50 μg/mL	Taiwan	77
Sulcatin	Tunicate	Alkaloid	Murine tumor cell lines	3–65 μg/mL	Italy	85
Tamandarins A and B	Tunicate	Depsipeptide	Human tumor cell line	0.99–1.79 ng/mL	United States, Brazil	86
Triene aldehyde	Sponge	Fatty Acid	Human tumor cell line	10 μg/mL	Japan	87

[1]For significant activity of pure compounds, an IC_{50} of ≤ 4.0 μg/mL is required.

rather than a toxic effect on the tumor cell used for the reported investigation.

CONCLUSION

This review highlights the fact that antitumor marine pharmacology research in 2000 remained on a combination pharmacological approach between research focused on determining the mechanism of action of cytotoxic agents, and studies with novel agents discovered to be active against specific cancer-related targets. Although our review has mainly focused on the pharmacology rather than the pharmaceutical development of marine anticancer agents, it should be noted that concomitant to the mechanistic characterization of novel marine cytotoxic or antitumor agents, the issues of supply, formulation and manufacturing are extremely important for the successful development of novel pharmaceutical agents. A review published during 2000 documents the importance of these different issues in the current development of Didemnin B, Bryostatins, Dolastatins and Ecteinascidins.[43]

Even if during 2000 no new marine natural product was approved for patient treatment by the U.S. Food and Drug Administration, the present 2000 antitumor and cytotoxic overview provides abundant evidence that 50 years after the discovery by Bergman *et al.* of Spongothymidine and Spongouridine, there continued to be a sustained and persistent multinational effort aimed at the discovery of novel and clinically useful antitumor agents derived from marine organisms.

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