ANTICANCER RESEARCH *21:* 2489-2500 (2001)

Review

Marine Pharmacology in 1999: Antitumor and Cytotoxic Compounds

ALEJANDRO M.S. MAYER[1] and VIRGINIA K. B. LEHMANN[2]

[1]Department of Pharmacology, Chicago College of Osteopathic Medicine, Midwestern University, 555 31st Street Downers Grove, Illinois 60515; [2]School of Chemical Sciences, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801, U.S.A.

Abstract. *During 1999 marine antitumor pharmacology research involved researchers in Austria, Australia, England, France, Germany, Greece, Holland, Italy, Japan, Spain, Taiwan and the United States. Thirty six papers were published in peer-reviewed journals describing the antitumor and cytotoxic properties of 30 marine natural products belonging to four structural types, namely polyketides, terpenes, nitrogen-containing compounds and polysaccharides. The organisms yielding these bioactive marine compounds comprised a diverse group of marine animals, algae, fungi and bacteria. A variety of antitumor pharmacological studies were conducted with 17 marine natural products with established mechanisms of action in a number of experimental and clinical models. Didemnin B, a tunicate-derived depsipeptide with potent antitumor effects, completed a Phase II anticancer clinical trial which resulted indeterminate in respect to activity against human melanoma due to anaphylactoid reactions. In vitro cytotoxicity data with murine and human cell lines were reported for 14 novel marine chemicals with as yet undetermined mechanisms of action. This 1999 literature overview thus highlights the fact that the multinational effort aimed at the discovery of novel marine antitumor agents remained at the same level of research activity as during 1998.*

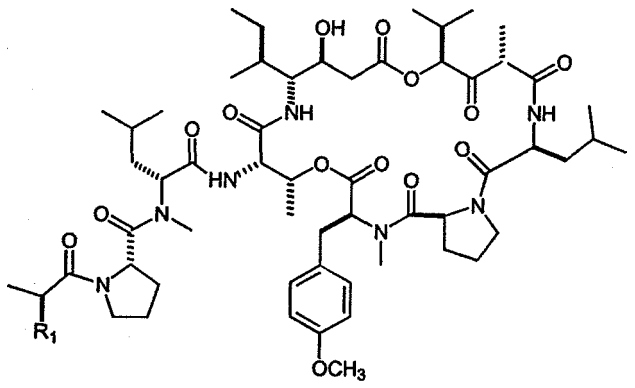
The purpose of this article is to discuss the research literature published during 1999 in the field of marine antitumor pharmacology using a similar format as the one used in our previous report that reviewed marine antitumor pharmacology research during 1998 (1). Consistent with our 1998 review, only those articles reporting on the antitumor pharmacology or cytotoxicity data of marine compounds with established chemical structures (Figure 1 and 2) were included in the present review and are presented in alphabetical order in Table I or in Table II. Research papers reporting on preclinical and/or clinical antitumor pharmacological research of marine chemicals with *determined* mechanisms of action have been presented in Table I. On the other hand, reports on the cytotoxicity of marine chemicals with *undetermined* mechanisms of action are grouped in Table II. Publications on the antitumor or cytotoxic activity of marine extracts or structurally uncharacterized marine compounds have been excluded from the present review, although several promising studies were reported during 1999 (eg. (2); (3)).

Correspondence to: Alejandro M.S.Mayer, Ph.D., Department of Pharmacology, Chicago College of Osteopathic Medicine, Midwestern University, 555 31st Street, Downers Grove, Illinois 60515, USA. Tel: (630) 515-6951, Fax: (630) 971-6414, e-mail: amayer@midwestern.edu

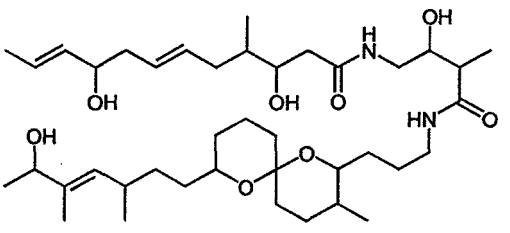
Key Words: Marine, antitumor, cancer, pharmacology, review.

2. 1999 Antitumor pharmacology of marine natural products with determined mechanisms of action

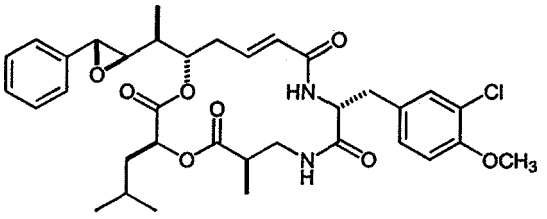
Table I summarizes the main conclusions of the 21 papers that reported research that involved the 17 marine compounds shown in Figure 1. The marine chemicals Discorhabdin P, Hemiasterlin, Isohomohalichondrin B, Makaluvamines and Secobatzellines A and B, were isolated from Porifera (sponges); Aplidine, Didemnin B, Dolastin 10, Ecteinascidin and Phthalascidin from Chordata (tunicates); Eleutherobin and Sarcodictyins A and B from Cnidaria (soft corals), 3 Phyla included in the Kingdom Animalia. Dehydrothyrsiferol and Cryptophycin 1 were isolated from blue-green algae (Kingdom Monera). Ulvan was isolated from an alga from the Phylum Chlorophyta (Kingdom Plantae), while Thiocoraline was derived from marine fungi (Kingdom Fungi). Following the chemical classification proposed by Schmitz *et al.* (4), the marine natural products shown in Figure 1 and Table I represent the following structural classes: polyketides (Isohomohalichondrin B), terpenes (Dihydrothyrsiferol, Eleutherobin and Sarcodictyins A and B), nitrogen-containing compounds (Aplidine, or Dehydrodidemnin B, Bistramide K, Cryptophycin 1, Didemnin B, Discorhabdin P, Dolastin 10, Ecteinascidin 743,



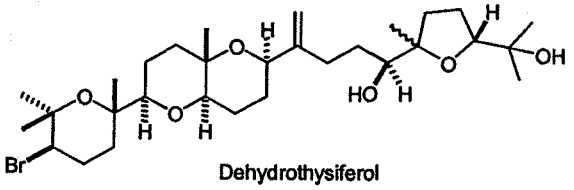
Compound	R_1
Didemnin B	OH
Aplidine	=O
(Dehydrodidemnin B)	



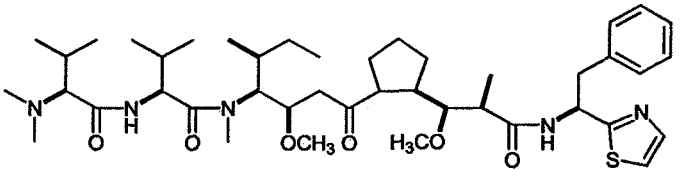
Bistramide K



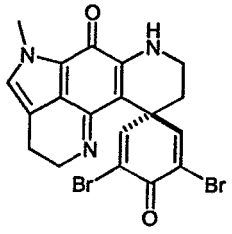
Cryptophycin 1



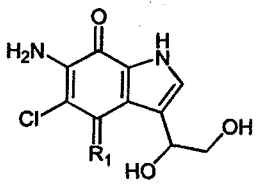
Dehydrothysiferol



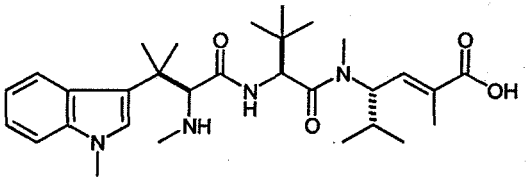
Dolastatin 10



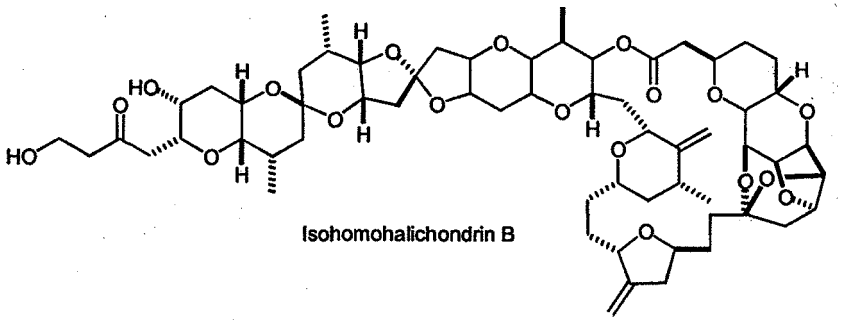
Discorhabdin P



Compound	R_1
Secobatzelline A	NH
Secobatzelline B	O

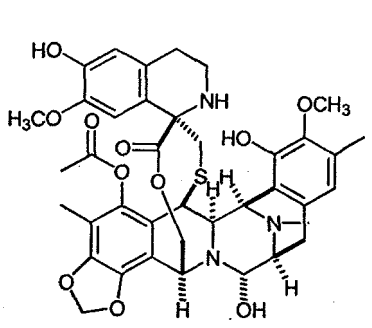


Hemiasterlin

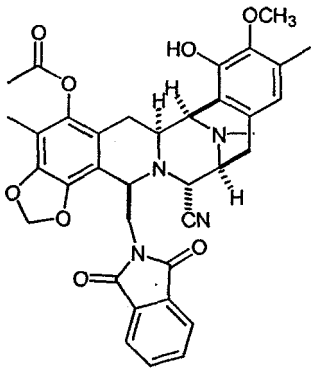


Isohomohalichondrin B

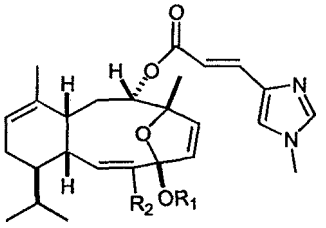
Figure 1.



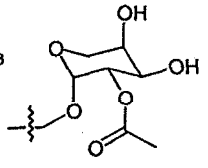
Ecteinascidin 743



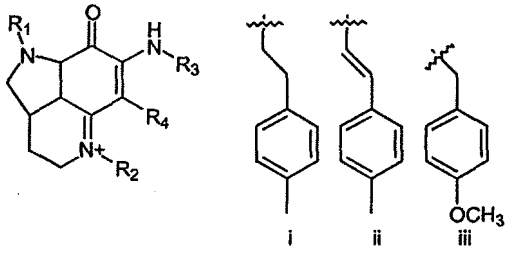
Phthalascidin



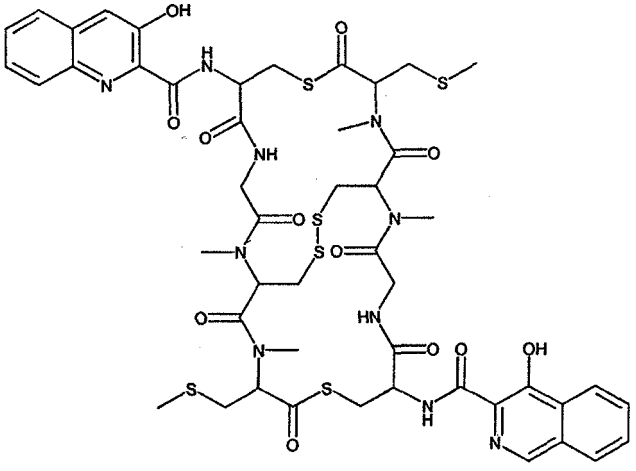
Name	R_1	R_2
Sarcodictyin A	H	COOMe
Sarocdictyin B	H	COOEt



Eleutherobin



Makaluvamine	R_1	R_2	R_3	R_4
A	CH_3	H	H	H
C	H	CH_3	H	H
D	H	H	i	H
E	CH_3	H	ii	H
H	CH_3	CH_3	H	H
I	H	H	H	H
N	H	H	H	Br
V	H	CH_3	iii	H



Thiocoraline

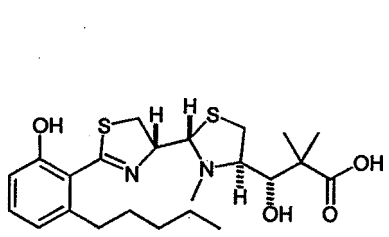
Figure 1. *continued*

Hemiasterlin, Makaluvamines, Secobatzellines A and B and Thiocoraline) and one polysaccharide (Ulvan).

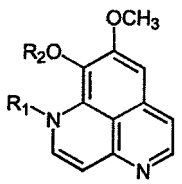
New information became available during 1999 on the pharmacology of 7 of the 17 compounds listed in Table I, also part of our 1998 anticancer review (1) namely Aplidine and Didemnin B, Dolastin 10, Dihydrothyrsiferol, Ecteinascidin 743, Eleutherobin and Sarcodictyins A and B.

Four studies reported on preclinical and clinical research with Aplidine (Dehydrodidemnin) and Didemnin B (5);(6); (7); (8). In a preclinical study, Geldof *et al.* determined that the depsipeptides Didemnin B and Aplidine as well as the macrolide Isohomohalichondrin B were more effective in the inhibition of prostate cancer cell proliferation *in vitro* than vincristine, vinorelbine or taxol. However, at the concentrations tested, these marine agents were observed to have a long lasting neurotoxic effect (5). In view of these observations, Geldof *et al.* urge "*... some caution in the clinical use of these agents because of potential neurotoxic side-effects*" (5).

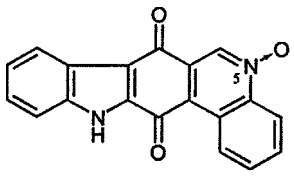
In an *in vitro* study with pharmaceutically formulated Aplidine, Nuijen et al. (6) determined that the administration of Aplidine by infusion using a polyvinyl chloride-free administration set, would not result in either hemolysis or



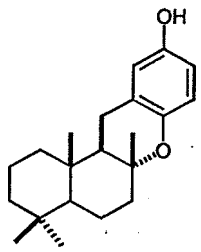
Agrochelin



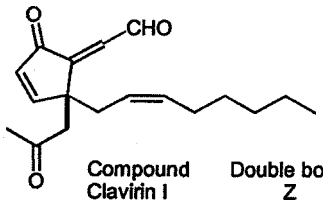
Compound	R_1	R_2
Aaptamine	CH_3	H
Isoaaptamine	H	CH_3



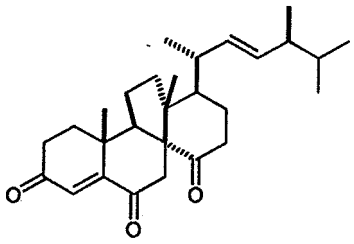
Calothrixin A
Calothrixin B = N5-deoxy



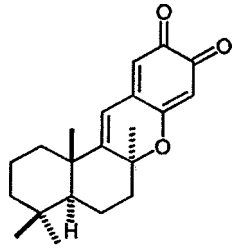
Chromazonarol



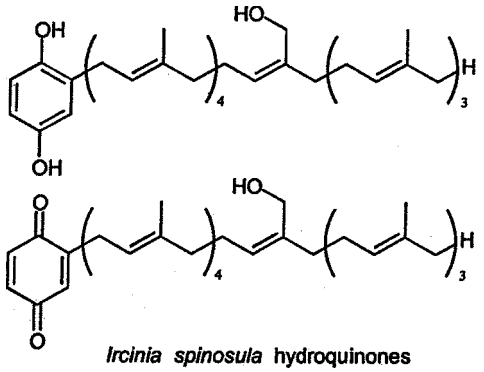
Compound	Double bond
Clavirin I	Z
Clavirin II	E



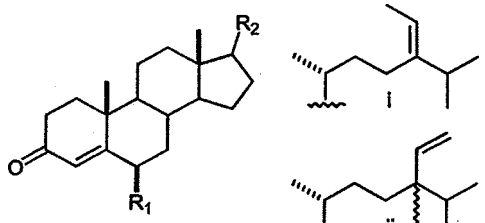
Dankasterone



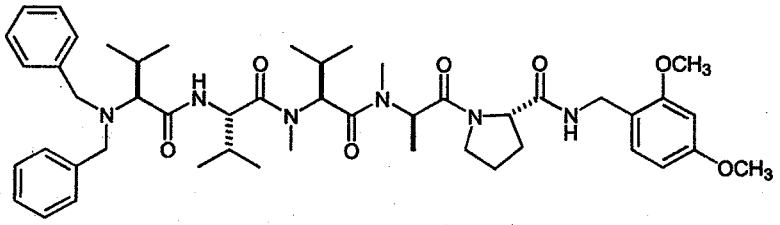
Puupehedione



Ircinia spinosula hydroquinones

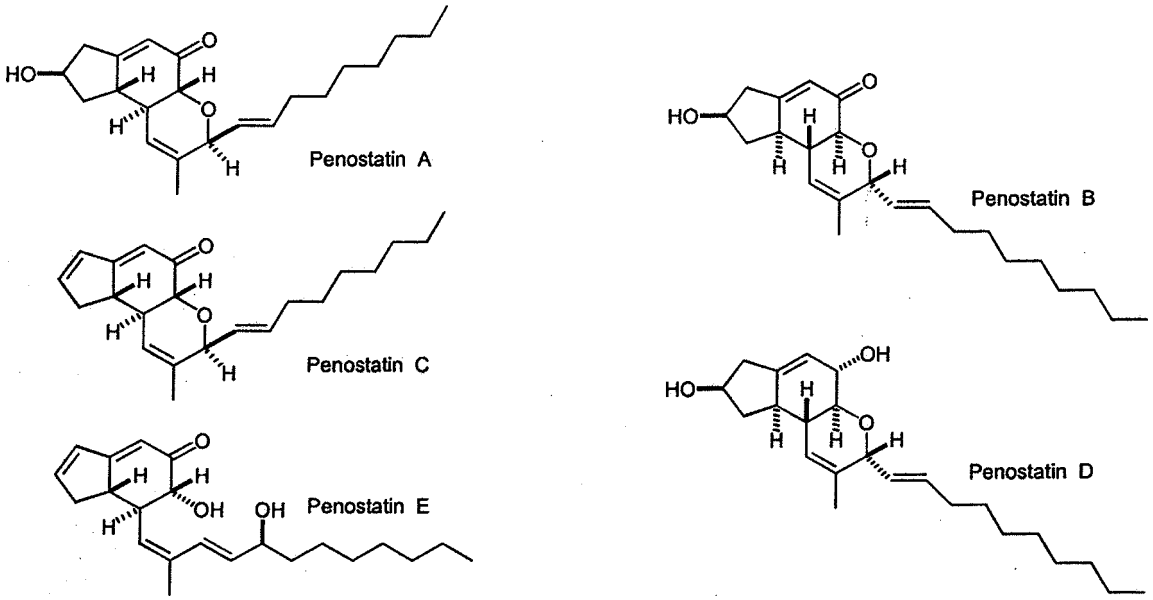


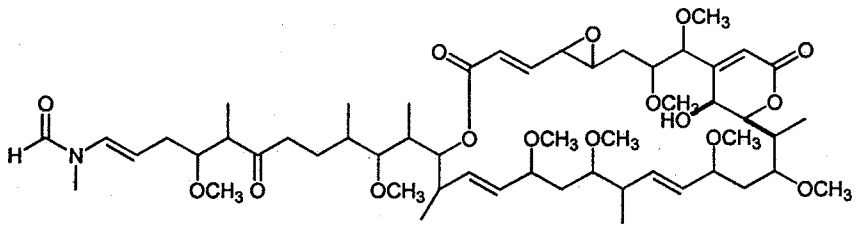
Oxygenated Fucosterols	R_1	R_2
4	H	i
5	H	ii
6	=O	i
7	=O	ii
8	OH	i
9	OH	ii



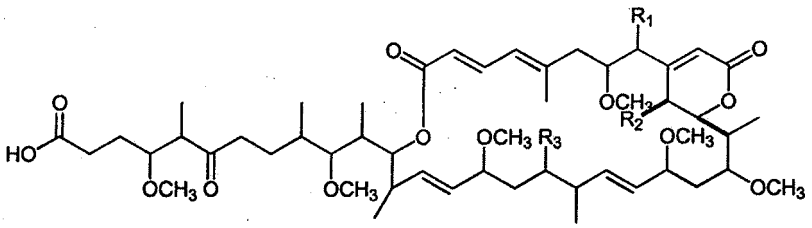
One Dolastatin 15 analog

Figure 2.





Sphinxolide E



Compound	R_1	R_2	R_3
Sphinxolide F	OCH_3	OH	OH
Sphinxolide G	H	OH	OCH_3
Reidispongiolide C	H	H	OCH_3

Figure 2. *continued*

precipitation of Aplidine upon parenteral administration. This preclinical information is viewed as important because Aplidine is currently in phase I clinical trial in Europe and Canada (6).

Hochster *et al.,* reported on the results of a phase II clinical study with Didemnin B, which was conducted on 19 patients with measurable metastatic or advanced malignant melanoma using the Response and Toxicity Criteria of the Eastern Cooperative Oncology Group (8). Hochster et al. concluded that the results were " *indeterminate with respect to the activity of Didemnin B in melanoma*" (8). Although signs of antitumoral activity were observed, a number of patients could not be fully evaluated for activity due to the occurrence of anaphylactoid reactions upon "*...the first and second drug administration...*", which the authors concluded "*... does not preclude a clinically important level of activity for Didemnin B*"

Table I. *Antitumor pharmacology of marine natural products with determined mechanisms of action.*

Compound	Organism	Chemistry	Experimental or clinical model[1]	Conclusion	Country[2]	Reference
Aplidine or Dehydrodidemnin B	Tunicate	Depsipeptide	HU prostatic and MU tumor cell line	Potent cytotoxicity and neurotoxicity observed	NETH	(5)
Aplidine or Dehydrodidemnin B	Tunicate	Depsipeptide	Preclinical *in vitro* pharmaceutical screening	I.v. infusion concentrations determined; no in vivo hemolysis or precipitation expected	SPA, NETH	(6)
Bistramide K	Tunicate	Amide	HU lung cancer cell line	Observed Induction of 2 genes, one ribosomal	FRA	(17)
Cryptophycin 1	Bacteria	Depsipeptide	MU leukemia cell line	Observed low DNA, RNA and protein synthesis	USA	(18)
Didemnin B	Tunicate	Depsipeptide	HU prostatic and MU tumor cell line	Observed potent cytotoxicity and neurotoxicity	NETH	(5)
Didemnin B	Tunicate	Depsipeptide	Phase II melanoma study	Activity against HU melanoma unclear due to anaphylactoid reactions	USA	(8)
Didemnin B	Tunicate	Depsipeptide	HU cancer cell line	Observed inhibition of mitochondrial activity	FRA	(7)
Dihydrothyrsiferol	Alga	Triterpene	HU breast tumor cell lines	No modulation of P-glycoprotein drug transport	ATRIA SPA	(14)
Discorhabdin P	Sponge	Discorhabdins	HU and MU cell lines	Observed calcineurin and caspase inhibition	USA	(19)
Dolastin 10	Tunicate	Peptide	Mice, rats and dogs	Determined maximum tolerated doses. Myelotoxicity most severe dose-limiting effect.	USA	(9)
Ecteinascidin-743	Tunicate	Isoquinoline	MU leukemia nuclear extracts	Observed binding to DNA and DNA-binding proteins	ITA,SPA, USA	(10)
Ecteinascidin-743	Tunicate	Isoquinoline	HU melanoma, non-small-cell lung and ovarian tumor xenografts	Observed effectivity against chemo-sensitive tumors.	GER, UK, NETH, SPA, USA	(11)
Ecteinascidin-743 and Phthalascidin	Tunicate and synthetic	Isoquinoline	HU and MU cell lines	Observed potent induction of DNA-protein cross-linking	USA	(12)
Ecteinascidin-743	Tunicate	Isoquinoline	Electrophoretic mobility shift assay	Determined DNA bending toward major groove	USA	(13)

continued

Table I. *(continued).*

Compound	Organism	Chemistry	Experimental or clinical model[1]	Conclusion	Country[2]	Reference
Eleutherobin	Coral	Diterpene	HU tumor cell lines and tubulin	Determined tubulin interactions and cytotoxicity	USA	(15)
Eleutherobin	Coral	Diterpene	Conformational analysis and molecular modeling	Common pharmacophore for microtubule stabilizing cytotoxic agents proposed	USA	(16)
Hemiasterlin	Sponge	Peptide	Bovine brain tubulin	Compared binding with Dolastin 10 and Cryptophycin 1	USA	(21)
Isohomohalichondrin B	Sponge	Macrolide	HU prostatic and MU tumor cell line	Observed potent cytotoxicity and neurotoxicity.	NETH	(5)
Makaluvamines	Sponge	Quinoline	HU carcinoma xenograft	Induced DNA cleavage via topoisomerase II	USA	(22)
Secobatzellines A, B	Sponge	Quinoline	HU and MU tumor cell lines	Observed calcineurin and caspase inhibition	USA	(20)
Sarcodictyins A,B	Coral	Diterpenes	HU tumor cell lines and tubulin	Determined tubulin interactions and cytotoxicity .	USA	(15)
Thiocoraline	Fungus	Depsipeptide	HU colon cell lines	Determined DNA-polymerase α inhibition	ITA,SPA, USA	(24)
Thiocoraline	Fungus	Depsipeptide	High-performance liquid chromatography	Developed a bioanalytical plasma assay for Phase I studies	NETH, SPA	(23)
Ulvan	Alga	Polysaccharide	HU colon cell line	Observed cytotoxicity and cytostaticity	FRA	(25)

(1) HU: human; MU; murine; (2) Country: ATRIA: Austria; FRA: France, GER: Germany, ITA: Italy, NETH: Netherlands, SPA:Spain.

(8). Although an explanation for these anaphylactoid reactions was unavailable at the time the paper was published, these clinical investigators suggested that Didemin B "*...should be investigated in this patient population using modern approaches to anaphylactoid reactions...*".

An interesting study by Rocchi *et al.* using quantitative microfluorometry and numerical analysis extended the molecular pharmacology of Didemnin B by determining that this marine compound can induce mitochondrial disfunction by inducing a decrease in the mitochondrial energetic state in a human lymphoblastoid cell line by a "*...direct or mediated...*" effect on these subcellular organelles (7).

In contrast to the two preclinical and one clinical study with the linear peptide Dolastin 10 that were reviewed in 1998 (1), only one study was published during 1999 by Mirsalis et al. (9). This preclinical study which evaluated the toxicity of Dolastin 10 in mice, rats and beagle dogs determined that toxicity to the bone marrow as evidenced by transient and reversible myelotoxicity was "*.. dose limiting in all three species with mice being the least sensitive....*". Furthermore, the authors concluded that "*... the preclinical toxicology study correctly predicted a safe starting dose, the dose-limiting toxicity, and the maximum tolerated dose in humans...*", as well as, "*... correlated with the degree of leukopenia that was observed in the phase I clinical trial*" (9). More importantly, the *in vitro* and *in vivo* results appear to predict human sensitivity to Dolastin 10, which is comparable to that of dogs but not mice.

Research with the isoquinoline alkaloid Ecteinascidin-743, an antitumor agent originating from the Caribbean tunicate *Ecteinascidia turbinata* continued to be very active during

Table II. *1999 Antitumor pharmacology of marine natural products with undetermined mechanism of action.*

Compound	Organism	Chemistry	Experimental or clinical tumor model[1]	Growth inhibition or cytotoxicity	Country[2]	Reference
Agrochelin	Bacteria	Alkaloid	MU and HU cell lines	IC_{50}< 1 μM.	SPA	(33)
Aaptamine and isoaaptamine	Sponge	Quinoline	MU and HU cell lines	IC_{50}=0.04-> 50 μM	TAIW	(34)
Calothrixins A and B	Bacteria	Indol	HU cell line	IC_{50}= 0.04-350 μM	AUS	(35)
Chromazonarol	Sponge	Sesquiterpene	MU and HU cell line	IC_{50}= 0.1-16 μM	SPA	(36)
Clavirins	Coral	Prostanoids	HU cell line	1 μg/mL	JAPN	(37)
Dankasterone	Fungus	Sterol	MU leukemia cell line	IC_{50}= 2.2 μg/mL.	JAPN	(38)
Dolastin 15 analogs	Synthetic	Peptide	NCI 60 HU panel	IC_{50}= 0.05- > 100 μM	TAIW.	(39)
Fucosterols	Alga	Sterol	MU and HU cell lines	IC_{50}= 0.4- > 50 μM	TAIW.	(40)
Hemiasterlin C	Sponge	Peptide	HU cell line and tubulin polymerization	IC_{50}= 0.0001-0.5 μg/mL.	USA	(41)
Ircinia spinosula hydroquinones	Sponge	Terpenes	HU cell line	IC_{50}= 9.1-17.4 μg/mL	FRA, GRE,	(42)
Penostatins A-E	Fungus	Polyketide	HU and MU cell lines	IC_{50}= 0.2-2.5 μg/mL	JAPN	(43)
Puupehedione	Sponge	Sesquiterpene	MU and HU cell line	IC_{50}= 1-2 μM	SPA	(44)
Sphinxolide E,F,G and Reidispongiolide	Sponge	Macrolides	NCI 60 HU panel	IC_{50}= 0.0007-0.3μM	ITA, FRA	(45)

(1) HU:human, MU:murine; (2) AUS: Australia, FRA: France, GRE: Greece, ITAL: Italy, JAPN: Japan, RUS: Russia, SPA: Spain, TAIW: Taiwan.

1999. The three reports included in our previous review (1) were followed during 1999 by four new reports (10); (11); (12); (13).

Bonfanti *et al.* (10) studied the ability of Ecteinascidin-743 to inhibit the binding of different transcription factors to their consensus sequences using a gel shift assay. Ecteinascidin 743 modified the interaction between some DNA binding proteins and DNA in concentrations ranging between 50-300 μM (10).

Zewail-Foote and Hurley provided a novel insight into the molecular basis of the antitumor activity of Ecteinascidin-743, by determining that this antitumor agent can bend DNA toward the major groove, a feature which appears to be unique among DNA-interactive agents that occupy the minor groove (13). Furthermore, they suggested that these observations "*...may provide a starting point to rationalize the improved clinical efficacy of this group of drugs...*".

In an effort to further explore the antitumor profile of Ecteinascidin-743 in human tumor xenografts, Hendriks *et al.* determined that even though Ecteinascidin-743 was inactive in chemo-resistant tumor xenografts, it resulted very active towards chemo-sensitive xenografts of melanoma, non-small-cell lung and ovarian cancers (11). They thus recommended the inclusion of these types of tumors in phase II clinical trials of Ecteinascidin-743.

Finally, Martinez *et al.*, reported on a study with a series of totally synthetic molecules structurally related to Ecteinascidin-743 which were designed using molecular modeling (12). Their studies characterized Phthalascidin, a novel compound that was more readily synthesized and more stable than Ecteinascidin, having very similar *in vitro* potency (IC_{50}=0.1-1 nM) and mode of action across a variety of cell types (12). Similar to Ecteinascidin-743, Phthalascidin induced DNA-protein cross-linking and topoisomerase

interaction. The characterization of Phthalascidin appears to be a significant contribution in view of the fact that supplies of Ecteinascidin-743 are inadequate for large-scale studies.

Antitumor research with Dihydrothyrsiferol, Eleutherobin and Sarcodyctins reviewed in 1998 (1) was continued during 1999. The algal terpenoid Dihydrothyrsiferol was shown by Pec et al. (14) not to modulate P-glycoprotein, a 170 kDa transport protein that confers drug resistance in human epidermoid cancer cell lines, thus suggesting a potential for the treatment of P-glycoprotein expressing cancer cells.

Hamel *et al.* (15) in a mechanistic study with Eleutherobin and Sarcodyctins A and B, two coral-derived diterpene antimitotic agents which have been successfully synthesized, demonstrated that Eleutherobin was more active than the Sarcodyctins in tubulin assembly reactions, data which correlated well with their antiproliferative activity on prostate, melanoma, breast and ovarian human cancer cell lines. In this study, perhaps the most extensive analysis of the antiproliferative effects of Sarcodyctins and Eleutherobin published so far, the authors concluded by suggesting that "*...further synthetic efforts with the Sarcodyctin class would yield compounds with activities greater than that of Paclitaxel in cells and perhaps with tubulin...*".

Ojima *et al.* (16) identified the three-dimensional pharmacophore common to Eleutherobin as well as Discodermolide, Taxol (Paclitaxel) and Epothilones A and B. The insights provided by the discovery of the common pharmacophore enabled these investigators to develop a "*...hybrid construct with demonstrated cytotoxic and tubulin-binding activity...*", which "*... succeeds in explaining the substantial structure-activity relationships of these agents...*". Furthermore the authors predicted that further improvements on their design would "*... facilitate developments of the next generation of tubulin-directed anticancer agents...*".

Table I lists 10 additional marine natural products, with determined mechanisms of actions, for which however no reports were published to our knowledge during 1998 (1): Bistramide K, Cryptophycin 1, Discorhabdin P, Hemiasterlin, Isohomohalichondrin B, Makaluvamines, Secobatzellines A and B, Thiocoraline and Ulvan.

In the only study in the current 1999 review that investigated the effect of an anticancer marine natural product at the genomic level, Siavoshian *et al.* (17) succeeded in identifying two genes which were differentially expressed after induction of *in vitro* differentiation in a non-small-cell lung cancer cell line by the amide Bistramide K. This observation is particularly noteworthy because non-small-cell lung cancers are generally refractory to chemotherapy. The two genes were cloned and partially sequenced by the investigators. While one of the genes showed strong homology to ribosomal protein L35 A, the identity of the other gene was unknown because the cDNA sequence exhibited no homology to any known sequence in the databases. The authors concluded by suggesting "*...a role of these two genes in the growth arrest induced by treatment with Bistramide K...*".

Foster *et al.* showed that the depsipeptide Cryptophycin 1, a natural product previously shown to bind to tubulin and disrupt microtubule assembly, had no effect on DNA and RNA synthesis and a minimal effect on protein synthesis (18). The investigators hypothesized that Cryptophycin 1 might inhibit a "*...specific critical protein essential to cell survival*". This intriguing possibility remains to be explored in future studies.

Gunasekera *et al.* as part of an ongoing search for new protein phosphatase inhibitors from marine organisms isolated the sulphur-containing Discorhabdin P (19) and the quinolines Secobatzellines A and B (20), marine compounds that were shown to inhibit calcineurin, an enzyme known to be involved in signal transduction and that plays a critical role in the regulation of the immune function (20). Furthermore these investigators demonstrated that these three compounds inhibited peptidase activity of CPP32, a caspase cysteine protease that plays a significant role in apoptosis and interleukin-8 secretion in rheumatoid arthritis. The authors proposed that "*...inhibitors of caspase enzymatic activities may serve to prevent the pathological damage induced by caspase-mediated apoptotic events...*".

Bai *et al.* investigated the "*... similarities and differences...*" between the effects of the sponge-derived antimitotic peptides Hemiasterlin, Dolastin 10 and Cryptophycin 1 on tubulin (21). In this very detailed investigation the researchers observed that Hemiasterlin appeared similar to other antimitotic peptides in inhibiting vinblastine binding to tubulin noncompetitively, inhibiting nucleotide exchange on tubulin, and inducing the formation of tubulin aggregates with "*... a primarily ring-like substructure*". However the authors emphasized that "*... in these aggregation reactions the three peptides* (namely Hemiasterlin, Dolastin 10 and Cryptophycin 1) *show the greatest differences in their interactions with tubulin...*" (21).

Matsumoto *et al.* further characterized the mechanism of action of the quinolines Makaluvamines, observing that "*... the makaluvamines induce dose-dependent DNA cleavage via topoisomerase II...*" *in vitro* (22) strongly suggesting that this activity might contribute to the cytotoxicity of some of these compounds. However, it was also clear from these studies that the Makaluvamines are not as topoisomerase II active as the standard compounds etoposide and mitoxantrone.

Sparidans *et al.* developed a sensitive bioanalytical assay for the depsipeptide Thiocoraline, based on reversed-phase liquid chromatography and fluorescence detection (23). This novel method should allow the quantitation of Thiocoraline in plasma samples in the 1-100 ng/ml range, a requirement for the start of phase I clinical trials.

Erba *et al.* reported on extensive studies on the mechanism of action of Thiocoraline (24). These researchers observed that Thiocoraline caused perturbations in the cell cycle, in particular G1 phase arrest in human colon cancer cell lines.

The marine compound did not inhibit DNA-topoisomerase II enzymes *in vitro* nor did it induce DNA breakage. Interestingly, Thiocoraline inhibited "... *DNA elongation by DNA polymerase* ...", most probably "... *by inhibiting DNA polymerase α activity...*".

The biological properties of Ulvan, a sulfated polysaccharide isolated from Ulva lactuca was studied by Kaeffer *et al.* (25). The isolated Ulvans (MW < 5,000), which "... *display(ed) chemical similarities with the mammalian glycosaminoglycans...*" inhibited the proliferation and differentiation program of the human carcinoma cells as well as expression of transforming growth factor-α expression.

3. 1999 Antitumor pharmacology of marine natural products with *undetermined* mechanisms of action

Table II, lists 14 marine natural products with potential antitumor activity that demonstrated activity in cytotoxicity assays. The marine natural products Aaptamine, Isoaaptamine, Chromazonarol, Hemiasterlin C, *Ircinia spinosula* hydroquinones, Puupehedione, Sphinxolide E-G and Reidispongiolide C were isolated from Porifera (sponges) while the Clavirins from Cnidaria (soft corals), all of which are included in Kingdom Animalia. Fucosterols were isolated from an alga from the Phylum Phaeophyta (Kingdom Plantae) while Penostatins A-E and Dankasterone were derived from marine fungi (Kingdom Fungi). Agrochelin and Calothrixins A and B were obtained from bacteria (Kingdom Monera). Following the chemical classification proposed by Schmitz et al. (4) these 15 marine natural products can be assigned to three chemical classes: polyketides (Clavirins, Penostatins A-E and Sphinxolide E-G and Reidispongiolide), terpenes (Chromazonarol, Dankasterone, Fucosterol, *Ircinia spinosula* hydroquinones and Puupehedione) and nitrogen-containing compounds (Agrochelin, Aaptamine and Isoaaptamine, Calothrixins A and B and Hemiasterlin C). In contrast to the compounds listed in Table I, no detailed mechanism of action studies were completed during 1999 with these marine compounds, with the exception of cytotoxicity tests against panels of human or murine tumor cell lines, which in some cases were very extensive. It remains to be determined if the reported cytotoxicity was the result of a toxic and/or a pharmacologic effect of these novel marine compounds.

4. Reviews on marine antitumor pharmacology

During 1999 a number of excellent reviews covering selected aspects of marine antitumor pharmacology were published: the patent literature (26), the discovery and development of marine antitumor agents (27); the antineoplastic dolastins (28); the National Cancer Institute's natural product drug discovery program (29); pyrroloquinoline and pyridoacridine alkaloids from marine sources (30); the rational synthesis of bryostatin analogues (31) and metabolites from tropical marine algae (32)

Kerr *et al.* reviewed the patent literature in the field of marine natural products covering the period 1996 to April 1999 (26). These researchers observed that the majority of the patents focused on antitumor agents, although some covered antiviral and antiinflammatory activities. The Phylum Porifera was the source of the majority of metabolites described in these patents.

Munro *et al.* provided an interesting and comprehensive perspective of the progress in marine anticancer research over the past 50 years (27). From the development or supply phase of marine anticancer compounds, these investigators carefully reviewed the aquaculture of the rare, deep water sponge *Lissodendoryx n. sp. 1.*, which yields the macrolides Halichondrin B and Isohomohalichondrin B. The authors proposed their research as an example of how to successfully overcome "...*perhaps the most significant hindrance to their development as drugs...*", namely "...*their limited supply...*". The investigators also extensively discussed the research effort that was required to develop sponge aquaculture in order to succeed in making these marine organisms a viable as well as a reliable option for biomass production in New Zealand, for the subsequent production of Halichondrin B and Isohomohalichondin B in gram quantities for clinical trials.

Recent developments as well as the published pharmacological literature on Dolastins 10 and 15, compounds isolated from the sea hare *Dolabella auricularia,* were extensively reviewed by Poncet (28). In particular, because the "... *efficient synthesis of natural compounds is...necessary for the supply of large quantities for in vivo bioassays and clinical trials...*", the literature on the synthesis of Dolastin 10 as well as other Dolastin metabolites was carefully reviewed. Although many aspects of the pharmacology and toxicology of the Dolastins remain to be elucidated, Poncet concluded that these marine agents "... *have already contributed, as biological probes, to improving our knowledge of several aspects of cell growth regulation...*".

Cragg and Neuman completed a comprehensive review of the discovery and development of antineoplastic agents from natural sources, which included those of marine origin (29), as part of a detailed description of the National Cancer Institute's Natural Product Drug Discovery Program. Although Halichondrin B and Dolastin 10 were included with 23 other agents in preclinical and Phase I Development under the auspices of the National Cancer Institute, they noted that up to 1999 "... *no compound from a marine source has advanced to commercial use as a chemotherapeutic agent...*"

Heterocyclic pyrroloquinoline and pyridoacridine alkaloids from marine sources were reviewed by Ding *et al.* (30). The biological and pharmacological activities of these alkaloids which include "... *in vitro and in vivo cytotoxicities against several tumor cell lines, topoisomerases I and II inhibition, DNA intercalation, stimulation of* Ca^{2+} *release from the sarcoplasmic reticulum, antifungal activities and antimicrobial activities...*"

was well presented and extensively discussed. The authors concluded that in view of the fact that there are a number of synthetic methodologies to prepare some of these compounds, there would appear to be a "... *continuing interest in the isolation, structure proof, synthesis and modification of these marine alkaloids that may provide novel potential medicinal agents*".

The low abundance of *Bugula neritina*, the source of the Bryostatins has led to several efforts to obtain them from non-natural sources. A comprehensive review on the rational design of Bryostatin analogues was accomplished by Wender *et al.* (31).

Siddhanta and Shanmugam reviewed the pharmacology of a variety of marine compounds isolated from marine algae belonging to the Class Chlorophyceae, Phylum Chlorophyta which "... *show interesting biological activities...*", suggesting to the authors that " *this algal family could be ...the source of biomedically important natural products*" . (32).

5. Conclusion

Although during 1999 no new marine natural product was approved for patient treatment by the U.S. Food and Drug Administration, the present 1999 antitumor and cytotoxic overview provides abundant evidence that 50 years after the discovery by Bergman and his co- workers of Spongothymidine and Spongouridine, there continued to be a sustained and persistent multinational effort aimed at the discovery of novel and clinical useful antitumor agents derived from marine organisms.

Acknowledgements

This publication was made possible by grant number R03-ES10138-01 (to A.M.S.M) from the National Institute of Environmental Health Sciences, National Institutes of Health. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the NIEHS, NIH. The excellent support for literature searches and article retrieval by Library staff members as well as medical and pharmacy students of Midwestern University is most gratefully acknowledged. The authors thank Mrs. Victoria Sears for excellent secretarial assistance in the preparation of this manuscript.

Reference

1 Mayer, A. M. S. Marine Pharmacology in 1998: antitumor and cytotoxic compounds. The Pharmacologist *41[4]:* 159-164. 1999.

2 Nourry M, Urvois PA, Tomasoni C, Biard JF, Verbist JF, and Roussakis C: Antiproliferative effects of a product isolated from the gorgonian Rumphella aggregata. Anticancer Res *19:* 1881-1885, 1999.

3 Koulman A, Pruijn LMC, Sandstra TSA, Woerdenbag HJ, and Pras N: The pharmaceutical exploration of cold water ascidians from the Netherlands: a possible source of new cytotoxic natural products. Journal of Biotechnology *70 (1 -3):* 85 -88 , 1999.

4 Schmitz, F J and Bowden BF and Toth SI. Antitumor and Cytotoxic Compounds from Marine Organisms. Attaway, D. H and Zaborsky, O. R. [1]: 197-308. 1993. New York and London, Plenum Press. Marine Biotechnology.

5 Geldof AA, Mastbergen SC, Henrar RE, and Faircloth GT: Cytotoxicity and neurocytotoxicity of new marine anticancer agents evaluated using in vitro assays. Cancer Chemother Pharmacol *44:* 312-318, 1999.

6 Nuijen B, Bouma M, Henrar RE, Manada C, Bult A, and Beijnen JH: Compatibility and stability of aplidine, a novel marine-derived depsipeptide antitumor agent, in infusion devices, and its hemolytic and precipitation potential upon i.v. administration. Anticancer Drugs *10:* 879-887, 1999.

7 Rocchi E, Vigo J, Viallet P, Bonnard I, Banaigs B, and Salmon JM: Multiwavelength videomicrofluorometric study of cytotoxic properties of a marine peptide, didemnin B, using adriamycin as reference compound. Anticancer Res *19:* 3559-3568, 1999.

8 Hochster H, Oratz R, Ettinger DS, and Borden E: A phase II study of Didemnin B (NSC 325319) in advanced malignant melanoma: an Eastern Cooperative Oncology Group study (PB687). Invest New Drugs *16*: 259-263, 1999.

9 Mirsalis JC, Schindler-Horvat J, Hill JR, Tomaszewski JE, Donohue SJ, and Tyson CA: Toxicity of dolastatin 10 in mice, rats and dogs and its clinical relevance. Cancer Chemother Pharmacol *44:* 395-402, 1999.

10 Bonfanti M, La Valle E, Fernandez SFJ, Faircloth G, Caretti G, Mantovani R, and D'Incalci M: Effect of ecteinascidin-743 on the interaction between DNA binding proteins and DNA. Anticancer Drug Des *14:* 179-186, 1999.

11 Hendriks HR, Fiebig HH, Giavazzi R, Langdon SP, Jimeno JM, and Faircloth GT: High antitumour activity of ET743 against human tumour xenografts from melanoma, non-small-cell lung and ovarian cancer. Ann Oncol *10:* 1233-1240, 1999.

12 Martinez EJ, Owa T, Schreiber SL, and Corey EJ: Phthalascidin, a synthetic antitumor agent with potency and mode of action comparable to ecteinascidin 743. Proc Natl Acad Sci USA *96:* 3496-3501, 1999.

13 Zewail-Foote M and Hurley LH: Ecteinascidin 743: a minor groove alkylator that bends DNA toward the major groove. J Med Chem *42:* 2493-2497, 1999.

14 Pec MK, Moser-Thier K, Fernandez JJ, Souto ML, and Kubista E: Growth inhibition by dehydrothyrsiferol - a non-Pgp modulator, derived from a marine red alga - in human breast cancer cell lines. Int J Oncol *14:* 739-743, 1999.

15 Hamel E, Sackett DL, Vourloumis D, and Nicolaou KC: The coral-derived natural products eleutherobin and sarcodictyins A and B: effects on the assembly of purified tubulin with and without microtubule-associated proteins and binding at the polymer taxoid site. Biochemistry *38:* 5490-5498, 1999.

16 Ojima I, Chakravarty S, Inoue T, Lin S, He L, Horwitz SB, Kuduk SD, and Danishefsky SJ: A common pharmacophore for cytotoxic natural products that stabilize microtubules. Proc Natl Acad Sci USA *96:* 4256-4261, 1999.

17 Siavoshian S, Jacquot C, Biard JF, Briand G, and Roussakis C: Subtractive hybridization and differential screening identified two genes differentially expressed after induction of in vitro (atypical) terminal differentiation in the NSCLC-N6 cell line by a marine substance (bistramide K). Anticancer Res *19:* 5361-5365, 1999.

18 Foster BJ, Fortuna M, Media J, Wiegand RA, and Valeriote FA: Cryptophycin 1 cellular levels and effects in vitro using L1210. Invest New Drugs *16:* 199-204, 1999.

19 Gunasekera SP, McCarthy PJ, Longley RE, Pomponi SA, Wright AE, Lobkovsky E, and Clardy J: Discorhabdin P, a new enzyme inhibitor from a deep-water Caribbean sponge of the genus Batzella. J Nat Prod *62:* 173-175, 1999.

20 Gunasekera SP, McCarthy PJ, Longley RE, Pomponi SA, and Wright AE: Secobatzellines A and B, two new enzyme inhibitors from a deep-water caribbean sponge of the genus batzella [In Process Citation]. J Nat Prod *62:* 1208-1211, 1999.

21 Bai R, Durso NA, Sackett DL, and Hamel E: Interactions of the sponge-derived antimitotic tripeptide hemiasterlin with tubulin:

comparison with dolastatin 10 and cryptophycin 1. Biochemistry *38:* 14302-14310, 1999.

22 Matsumoto SS, Haughey HM, Schmehl DM, Venables DA, Ireland CM, Holden JA, and Barrows LR: Makaluvamines vary in ability to induce dose-dependent DNA cleavage via topoisomerase II interaction. Anticancer Drugs *10:* 39-45, 1999.

23 Sparidans RW, Henrar RE, Jimeno JM, Faircloth G, Floriano P, and Beijnen JH: Bioanalysis of thiocoraline, a new marine antitumoral depsipeptide, in plasma by high-performance liquid chromatography and fluorescence detection. J Chromatogr B Biomed Sci Appl *726:* 255-260, 1999.

24 Erba E, Bergamaschi D, Ronzoni S, Faretta M, Taverna S, Bonfanti M, Catapano CV, Faircloth G, Jimeno J, and D'Incalci M: Mode of action of thiocoraline, a natural marine compound with anti- tumour activity. Br J Cancer *80:* 971-980, 1999.

25 Kaeffer B, Benard C, Lahaye M, Blottiere HM, and Cherbut C: Biological properties of ulvan, a new source of green seaweed sulfated polysaccharides, on cultured normal and cancerous colonic epithelial cells. Planta Med *65:* 527-531, 1999.

26 Kerr RG and Kerr SS: Marine natural products as therapeutic agents [Review]. Expert Opinion on Therapeutic Patents 9: 1207-1222, 1999.

27 Munro MH, Blunt JW, Dumdei EJ, Hickford SJ, Lill RE, Li S, Battershill CN, and Duckworth AR: The discovery and development of marine compounds with pharmaceutical potential. J Biotechnol *70:* 15-25, 1999.

28 Poncet J: The dolastatins, a family of promising antineoplastic agents. Curr Pharm Des *5:* 139-162, 1999.

29 Cragg GM and Newman DJ: Discovery and development of antineoplastic agents from natural sources. Cancer Invest 17: 153-163, 1999.

30 Ding Q, Chichak K, and Lown JW: Pyrroloquinoline and pyridoacridine alkaloids from marine sources. Curr Med Chem *6:* 1-27, 1999.

31 Wender PA, Hinkle KW, Koehler MF, and Lippa B: The rational design of potential chemotherapeutic agents: synthesis of bryostatin analogues. Med Res Rev *19:* 388-407, 1999.

32 Siddhanta AK and Shanmugam M: Metabolites of tropical marine algae of the family Codiaceae (Chlorophyta): chemistry and bioactivity. J Indian Chem Soc *76:* 323-334, 1999.

33 Acebal C, Canedo LM, Puentes JL, Baz JP, Romero F, de la CF, Gravalos MD, and Rodriguez P: Agrochelin, a new cytotoxic antibiotic from a marine Agrobacterium. Taxonomy, fermentation, isolation, physico-chemical properties and biological activity. J Antibiot (Tokyo) *52:* 983-987, 1999.

34 Shen YC, Lin TT, Sheu JH, and Duh CY: Structures and cytotoxicity relationship of isoaaptamine and aaptamine derivatives. J Nat Prod *62:* 1264-1267, 1999.

35 Rickards RW, Rothschild JM, Willis AC, de Chazal NM, Kirk J, Kirk K, Saliba KJ, and Smith GD: Calothrixins A and B, novel pentacyclic metabolites from Calothrix cyanobacteria with potent activity against malaria parasites and human cancer cells. Tetrahedron 55: 13513-13520, 1999.

36 Barrero AF, Alvarez-Manzaneda EJ, Herrador MM, Chahboun R, and Galera P: Synthesis and antitumoral activities of marine ent-chromazonarol and related compounds. Bioorg Med Chem Lett *9:* 2325-2328, 1999.

37 Iwashima M, Okamoto K, and Iguchi K: Clavirins, a new type of marine oxylipins with growth-inhibitory activity from the Okinawan soft coral, Clavularia viridis. Tetrahedron Letters *40:* 6455-6459, 1999.

38 Amagata T, Doi M, Tohgo M, Minoura K, and Numata A: Dankasterone, a new class of cytotoxic steroid produced by a Gymnascella species from a marine sponge. Chem Commun *14:* 1321-1322, 1999.

39 Hu MK and Huang WS: Synthesis and cytostatic properties of structure-simplified analogs of dolastatin 15. J Pept Res *54:* 460-467, 1999.

40 Sheu JH, Wang GH, Sung PJ, and Duh CY: New cytotoxic oxygenated fucosterols from the brown alga Turbinaria conoides. J Nat Prod *62:* 224-227, 1999.

41 Gamble WR, Durso NA, Fuller RW, Westergaard CK, Johnson TR, Sackett DL, Hamel E, Cardellina JH, and Boyd MR: Cytotoxic and tubulin-interactive hemiasterlins from Auletta sp. and Siphonochalina spp. sponges [In Process Citation]. Bioorg Med Chem *7:* 1611-1615, 1999.

42 Mihopoulos N, Vagias C, Chinou I, Roussakis C, Scoullos M, Harvala C, and Roussis V: Antibacterial and cytotoxic natural and synthesized hydroquinones from sponge Ircinia spinosula. Z Naturforsch [C] *54:* 417-423, 1999.

43 Iwamoto C, Minoura K, Oka T, Ohta T, Hagishita S, and Numata A: Absolute stereostructures of novel cytotoxic metabolites, penostatins A-E, from a Penicillium species separated from an Enteromorpha alga. Tetrahedron *55:* 14353-14368, 1999.

44 Barrero AF, Alvarez-Manzaneda EJ, Chahboun R, Cortes M, and Armstrong V: Synthesis and antitumor activity of puupehedione and related compounds. Tetrahedron *55:* 15181-15208, 1999.

45 Carbonelli S, Zampella A, Randazzo A, Debitus C, and Gomez-Paloma L: Sphinxolides E-G and reidispongiolide C: four new cytotoxic macrolides from the new caledonian lithistida sponges N. superstes and R. coerulea. Tetrahedron *55:* 14665-14674, 1999.

Received May 2, 2001
Accepted May 29, 2001