

# Marine Natural Products and Related Compounds in Clinical and Advanced Preclinical Trials<sup>†</sup>

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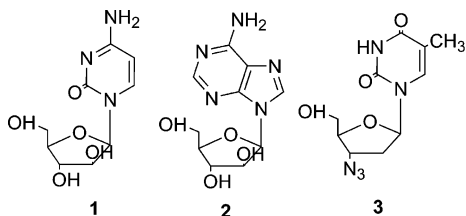
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The marine environment has proven to be a very rich source of extremely potent compounds that have demonstrated significant activities in antitumor, antiinflammatory, analgesia, immunomodulation, allergy, and anti-viral assays. Although the case can and has been made that the nucleosides such as Ara-A and Ara-C are derived from knowledge gained from investigations of bioactive marine nucleosides, no drug directly from marine sources (whether isolated or by total synthesis) has yet made it to the commercial sector in any disease. However, as shown in this review, there are now significant numbers of very interesting molecules that have come from marine sources, or have been synthesized as a result of knowledge gained from a prototypical compound, that are either in or approaching Phase II/III clinical trials in cancer, analgesia, allergy, and cognitive diseases. A substantial number of other potential agents are following in their wake in preclinical trials in these and in other diseases.

## Introduction

The initial discoveries from the marine environment that led to the belief that true marine-derived drugs would not be overly long in reaching the market can be traced to the reports of Bergmann on the discovery and subsequent identification of spongthymidine and spongouridine in the early 1950s from the Caribbean sponge *Tethya crypta*.<sup>1–3</sup> These reports actually led to a complete reversal of the then current dogma, which prior to these discoveries was “that for a nucleoside to have biological activity, it had to have ribose or deoxyribose as the sugar, but that the base could comprise a multiplicity of heterocycles and even carbocycles”. The subsequent explosion of compounds is described with the relevant citations by Suckling<sup>4</sup> and Newman et al.,<sup>5</sup> and these discoveries led to the identification of a close analogue, cytosine arabinoside, as a potent antileukemic agent; this compound (**1**) subsequently was commercialized by Upjohn (now Pharmacia) as Ara-C. Other closely related compounds such as adenine arabinoside (Ara-A) (**2**), an antiviral compound synthesized and commercialized by Burroughs Wellcome (now Glaxo Smith-Kline) and later found in the Mediterranean gorgonian *Eunicella cavolini*, and even azidothymidine (AZT) (**3**) can be traced back to this initial discovery of the “other than ribose-substituted bioactive nucleosides”.



The advent of scuba techniques approximately 60 years ago and their subsequent utilization by natural products chemists and biologists working closely with them led to questions such as, Why are certain marine invertebrates not prey for organisms higher up the evolutionary tree?

<sup>†</sup> Dedicated to the late Dr. D. John Faulkner (Scripps) and the late Dr. Paul J. Scheuer (Hawaii) for their pioneering work on bioactive marine natural products.

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Why do fish not eat particular algae? Why do two sponges grow and expand until they touch, but do not grow over each other? One possibility was that the organisms have some form of chemical communication or defense that enables an individual organism to establish a particular niche and thrive there. One has to realize that these marine invertebrates and marine plants, with very few exceptions, are sessile and require a “foot-hold” on a nonmoving, fixed substrate (rock or coral) that permits them to feed by filtration of the seawater flowing in and around them.

Initial attempts at determining the chemistries of marine organisms were simply extensions of tried and true phytochemical techniques. Thus, easily accessible organisms (generally sponges and encrusting organisms such as ascidians) were collected by hand using snorkel or simple scuba systems, and then their chemical components were extracted and identified. Any biological activity was found as an afterthought in these initial experiments (though as shown above, active compounds could be found by these techniques that would ultimately be useful as treatments for human diseases).

A corollary to the more systematic searching for marine-derived products was that very sensitive analytical tools had to be used, as in general, the amounts of bioactive materials that could be recovered were exceedingly small. There are examples given later in detail, but levels of 1 mg of compound per 3 kg of organism were not uncommon. Thus high-field NMR (originally 200 MHz and then up through 600–800 MHz), mass spectrometry that involved MS–MS techniques, and chromatographic methods of all types were used. It should be emphasized that HPLC, the use of which is effectively a *sine qua non* in modern isolation methods, was not generally available until the late 1970s, and thus isolations often required large amounts of materials due to the level of sophistication of the techniques available.

In retrospect, this is one of the major reasons that the field evolved slowly. Discovery of a given compound was easy in relative terms, but development, which required large amounts, was, and still is, not simple, as will be shown in examples later in the review.

Although Paul Scheuer at the University of Hawaii was the first (marine) natural products chemist to systematically explore the chemistry of marine invertebrates, from his original work in Hawaii in the 1950s until his death

**Table 1.** Status of Marine-Derived Natural Products in Clinical and Preclinical Trials

name	source	status (disease)	comment
didemnin B dolastatin 10	<i>Trididemnum solidum</i> <i>Dolabella auricularia</i> (marine microbe derived; cyanophyte)	Phase II (cancer) Phase I/II (cancer)	dropped middle 90s many derivatives made synthetically; no positive effects in Phase II trials; no further trials known
giroline bengamide derivative	<i>Pseudaxinyssa cantharella</i> <i>Jaspis</i> sp.	Phase I (cancer) Phase I (cancer)	discontinued due to hypertension licensed to Novartis, Met-AP1 inhibitor, withdrawn 2002
cryptophycins (also arenastatin A)	<i>Nostoc</i> sp. & <i>Dysidea</i> <i>arenaria</i>	Phase I (cancer)	from a terrestrial cyanophyte, but also from a sponge as arenastatin A; synthetic derivative licensed to Lilly by Univ. Hawaii, but withdrawn 2002
bryostatin 1	<i>Bugula neritina</i>	Phase II (cancer)	now in combination therapy trials; licensed to GPC Biotech by Arizona State Univ.; may be produced by bacterial symbiont
TZT-1027 cematodin	synthetic dolastatin synthetic derivative of dolastatin 15	Phase II (cancer) Phase I/II (cancer)	also known as auristatin PE and soblidotin some positive effects on melanoma pts in Phase II; dichotomy on fate
ILX 651, synthatodin	synthetic derivative of dolastatin 15	Phase I/II (cancer)	in Phase II for melanoma, breast, NSCLC
ecteinascidin 743	<i>Ecteinascidia turbinata</i>	Phase II/III (cancer) in 2003	licensed to Ortho Biotech (J&J); produced by partial synthesis from microbial metabolite dehydrodidemnin B, made by total synthesis
aplidine E7389	<i>Aplidium albicans</i> <i>Lissodendoryx</i> sp	Phase II (cancer) Phase I (cancer)	Eisai's synthetic halichondrin B derivative licensed to Novartis by Harbor Branch Oceanographic Institution
discodermolide	<i>Discodermia dissoluta</i>	Phase I (cancer)	licensed to Novartis by Harbor Branch Oceanographic Institution
kahalalide F	<i>Eysia rufescens</i> / <i>Bryopsis</i> sp.	Phase II (cancer)	licensed to PharmaMar by Univ. Hawaii; revision of structure
ES-285 (spisulosine)	<i>Spisula polynyma</i>	Phase I (cancer)	<i>Rho</i> -GTP inhibitor
HTI-286 (hemiassterlin derivative)	<i>Cymbastella</i> sp	Phase II (cancer)	synthetic derivative made by Univ. British Columbia; licensed to Wyeth
KRN-7000	<i>Agelas mauritanus</i>	Phase I (cancer)	an agelasphin derivative
squalamine	<i>Squalus acanthias</i>	Phase II (cancer)	antiangiogenic activity as well
Æ-941 (Neovastat)	shark	Phase II/III (cancer)	defined mixture of <500 kDa from cartilage; antiangiogenic activity as well
NVP-LAQ824	Synthetic	Phase I (cancer)	derived from psammaplin, trichostatin, and trapoxin structures
Laulimalide Curacin A	<i>Cacospongia mycofijiensis</i> <i>Lyngbya majuscula</i>	preclinical (cancer) preclinical (cancer)	synthesized by a variety of investigators synthesized, more soluble combi-chem derivatives being evaluated
vitilevuamide	<i>Didemnum cucliferum</i> & <i>Polysyncraton lithostrotum</i>	preclinical (cancer)	
diazonamide eleutherobin	<i>Diazona angulata</i> <i>Eleutherobia</i> sp.	preclinical (cancer) preclinical (cancer)	synthesized and new structure elucidated synthesized and derivatives made by combi- chem; can be produced by aquaculture
sarcodictyin	<i>Sarcodictyon roseum</i>	preclinical (cancer) (derivatives)	combi-chem synthesis performed around structure
peloruside A salicylhalimides A	<i>Mycale hentscheli</i> <i>Haliclona</i> sp.	preclinical (cancer) preclinical (cancer)	first marine Vo-ATPase inhibitor; similar materials from microbes, synthesized
thiocoraline ascididemnin	<i>Micromonospora marina</i>	preclinical (cancer) preclinical (cancer)	DNA polymerase $\alpha$ inhibitor reductive DNA-cleaving agents
variolins dictyodendrins	<i>Kirkpatrickia variolosa</i> <i>Dictyodendrilla</i> <i>verongiformis</i>	preclinical (cancer) preclinical (cancer)	Cdk inhibitors telomerase inhibitors
GTS-21 (aka DMBX)		Phase I (Alzheimer's)	modification of a worm toxin; licensed to Taiho by Univ. Florida
manoalide IPL-576,092 (aka HMR-4011A)	<i>Luffariaella variabilis</i> <i>Petrosia contignata</i>	Phase II (antipsoriatic) Phase II (antiasthmatic)	discontinued due to formulation problems derivative of contignasterol; licensed to Aventis
IPL-512,602 IPL-550,260	derivative of 576092 derivative of 576092	Phase II (antiasthmatic) Phase I (antiasthmatic)	with Aventis with Aventis
ziconotide (aka Prialt)	<i>Conus magus</i>	Phase III (neuropathic pain)	licensed by Elan to Warner Lambert
CGX-1160	<i>Conus geographus</i>	Phase I (pain)	contulakin G
CGX-1007	<i>Conus geographus</i>	Phase I (pain & epilepsy)	conantokin G; discontinued
AMM336	<i>Conus catus</i>	preclinical (pain)	$\omega$ -conotoxin CVID
$\chi$ -conotoxin	<i>Conus</i> sp.	preclinical (pain)	conotoxin MR1A/B
CGX-1063	Thr10-contulakin G	preclinical (pain)	modified toxin
ACV1	<i>Conus victoriae</i>	preclinical (pain)	$\alpha$ -conotoxin Vc1.1

early in 2003, initially investigating marine toxin structures, the work of Rinehart at the University of Illinois at Champaign-Urbana and of Pettit at Arizona State University led the way in the discovery of biologically active molecules (i.e., potential human use pharmaceutical agents)

from the marine environment. Both of these research groups were funded by the U.S. government but in somewhat different ways in the beginning. Pettit was part of an antineoplastic drug discovery effort whereby organisms were collected and extracted by NCI-funded groups, and

**Table 2.** Phase I and Phase II Combination Studies with Bryostatatin 1

year	phase	schedule	dose range	tumor type(s)	# pts	CR	PR	SD	side effects	reference
2001	I	24 h infusion & bolus of vincristine, dose escalation of bryostatatin, 1–5 cycles	12.5–62.5 $\mu\text{g}\cdot\text{M}^2$ bryostatatin; 1.4 $\text{mg}\cdot\text{M}^2$ of vincristine	B-cell cancer	25	1	2	4	myalgia; neuropathy	Dowalti et al. <sup>324</sup>
2002	I	24 h infusion, days 1 & 11, AraC on days 2, 3, 9, 10, bryostatatin dose escalation, fixed AraC, 1–6 cycles	12.5–50 $\mu\text{g}\cdot\text{M}^2$ bryostatatin; 1–3 $\text{mg}\cdot\text{M}^2$ AraC	leukemia	23	5	1 <sup>a</sup>	0	myalgia; neutropenia	Cragg et al. <sup>325</sup>
2002	I	24 h infusion, fludarabine for days 2–6, repeat at 28d, or reverse addition order, 6–9+ cycles	16–50 $\mu\text{g}\cdot\text{M}^2$ bryostatatin; 12.5–25 $\text{mg}\cdot\text{M}^2$ FAra	CLL; NHL	53	<i>b</i>	<i>b</i>	<i>b</i>	neutropenia	Roberts et al. <sup>326</sup>
2003	II	1 h infusion of paclitaxel on 1, 8, & 15d; 24 h infusion of bryostatatin on 2, 9, 16d, repeated on 28d cycle, 1–4 cycles	40–50 $\mu\text{g}\cdot\text{M}^2$ bryostatatin; 90 $\text{mg}\cdot\text{M}^2$ paclitaxel	NSCLC	11	0	2 <sup>a</sup>	5	myalgia	Winegarden et al. <sup>327</sup>

<sup>a</sup> Some question as to response level. <sup>b</sup> 23 “nondefined objective responses”.

the extracts tested by NCI contractors for their ability to inhibit the growth of tumors in mice. The active principles were then isolated by NCI-funded groups by following the bioactivity in mice. This was probably the first large-scale application in the marine area of what has come to be known as “bioactivity-driven isolations”. Rinehart, however, was funded by a number of U.S. government agencies but initially used his MS–MS and NMR capabilities to determine the potential structures of the bioactive agents that he found in organisms collected predominately in the Caribbean during NSF-funded expeditions.

There have been a number of recent reviews covering aspects of this area, either not in as much detail or from a clinical or preclinical aspect. The reader should consult them for comparative purposes;<sup>6–13</sup> any reviews that are specific to a class of agents will be cited under the agents themselves.

We will discuss agents by clinical activities rather than by source or chemical class, and in order to aid the reader, we have shown in Table 1 all of the sources, diseases, trial level achieved at date of review submission, and a short comment where necessary on the compounds that we discuss in the review. The order is by type of pharmacological activity and then clinical and/or preclinical results for each activity, with those that have been discontinued listed first in each disease.

### Introduction to Agents that Entered Antitumor Clinical Trials

The significant number of compounds from marine sources that have been entered into antitumor preclinical and clinical trials since the early 1980s is due to two serendipitous findings. The first is that the agents elaborated by marine organisms must be affected by the dilution effects of seawater; thus any “chemical warfare” agent must be extremely potent, as it has to overcome dilution en route to its target. This process may be considered as analogous to the role of phytoalexins in the plant kingdom, or similar to the emission of pheromones by insects, though the purpose in the latter case is to attract rather than repel! The other is that the U.S. National Cancer Institute (NCI) has funded, either directly or indirectly, most of the search for agents active against cancer, irrespective of the source. Thus, one has the systems in place for collection, bioactivity determinations, and subsequent testing in animals and humans, with the aim of finding new and potent treatments for cancers.

Because of the extremely long time frame involved in such processes (for example, paclitaxel (Taxol), took over 20 years from structural determination and reporting until FDA

approval in the early 1990s), the compounds that will be discussed fall into two approximate time frames: those from the initial collection programs (which aided the didemnin B discovery *vide infra*) and those that are further back in the current system that have been discovered as a result of the modified NCI screens utilizing the 60 cell line (or functional equivalent) screen that has been in use from the early 1990s. Some of the agents whose mechanisms of action (MOA) were discovered as a result of the latter screening system are now either just entering or about to enter clinical trials.

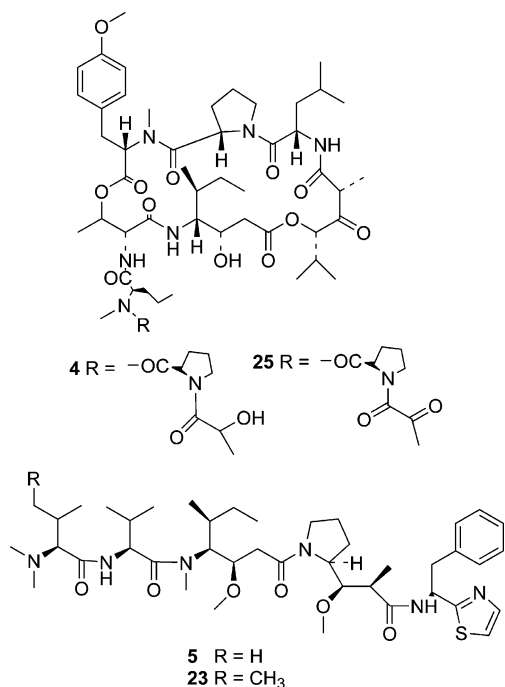
### Agents Now Withdrawn from Antitumor Clinical Trials

**Didemnin B.** This compound (**4**) was isolated by Rinehart’s group from extracts made of the tunicate *Trididemnum solidum*<sup>14</sup> that demonstrated excellent antiviral activity and subsequent cytotoxic activity against P388 and L1210 murine leukemia cell lines. Didemnin B was advanced into preclinical and clinical trials (Phases I and II; see Table 3 in Nuijen et al. for a discussion of these trials<sup>13</sup>) under the auspices of the NCI in the very early 1980s as the first defined chemical compound *directly* from a marine source to go into clinical trials for any major human disease. Despite many different treatment protocols and testing against many types of cancer, the compound turned out to be too toxic for use, and trials were officially terminated in the middle 1990s by NCI.

Even though this compound did not make it to Phase III trials and then to market, the experience gained from these efforts was immensely helpful in aiding the trials of other natural product-derived agents/compounds. Thus Rinehart’s group developed methods of large-scale isolation and purification and, as would become essential much later in time, total syntheses that permitted significant structure–activity relationships to be derived.<sup>15</sup> This work permitted materials to be provided to others so that basic biochemical studies could be performed, leading to the identification of a potential MOA for this compound, with the binding to elongation factor 1- $\alpha$  (ef1- $\alpha$ ) being reported by Crews et al. in the middle 1990s.<sup>16</sup> Subsequent reports from Crews’ group showed that didemnin B binds noncompetitively to palmitoyl protein thioesterase,<sup>17</sup> and the following year, Johnson and Lawen reported that rapamycin inhibited the didemnin-induced apoptosis of human HL-60 cells, perhaps by binding to the FK-506 binding protein(s).<sup>18</sup> Inferentially, from this latter result, didemnin B might bind to or modulate the FK-binding proteins as part of its immunomodulatory process and thus lead to cell death via apoptosis.

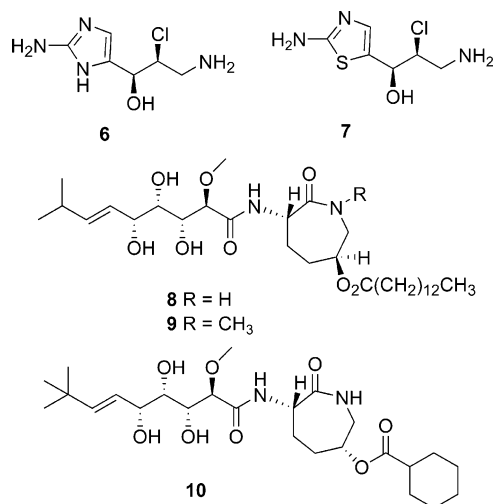
In 2002, Vera and Joullie<sup>19</sup> published an excellent review of didemnins as cell probes and targets for syntheses and also made some reasonable arguments that the dosing schedules used in the early clinical trials may well have been nonoptimal for demonstrating activity as a cytotoxin rather than as an immunosuppressive/modulator. It will be interesting to compare the dosing schedules and responses for didemnin B and aplidine (Aplidin; PharmaMar, vide infra) in man once the latter are fully reported in the literature.

Although didemnin B was not successful, a very close chemical relative is currently in clinical trials (cf. aplidine below), and in 2000 Rinehart published an overview of these compounds as part of a discussion of antitumor compounds from tunicates, which the reader may consult for further details.<sup>20</sup>



**Dolastatin 10.** The dolastatins are a series of cytotoxic peptides that were originally isolated in very low yield from the Indian Ocean mollusk *Dolabella auricularia* by Pettit's group as part of its work on marine invertebrates.<sup>21–25</sup> Due to the potency and mechanism of action of dolastatin 10 (5), a linear depsipeptide that was shown to be a tubulin interactive agent binding close to the *vinca* domain at a site where other peptidic agents bound,<sup>26,27</sup> the compound entered Phase I clinical trials in the 1990s under the auspices of the NCI. Since the natural abundance was so low, Pettit and others developed synthetic methods that provided enough material under current Good Manufacturing Practice (cGMP) conditions to allow clinical trials to commence.<sup>25</sup>

Dolastatin 10 progressed to Phase II trials as a single agent, but although tolerated at the doses used, which were high enough to give the expected levels in vivo to inhibit cell growth, it did not demonstrate significant antitumor activity in a Phase II trial against prostate cancer in man.<sup>28</sup> Similarly, no significant activity was seen in a Phase II trial against metastatic melanoma, even though again, levels high enough to affect cells were demonstrated.<sup>29</sup> There are other dolastatins and molecules related to them that are still in clinical and preclinical trials; they will be covered in later sections.



**Girolline (Girodazole).** This very simple compound, a substituted imidazole (6), was reported from the sponge *Pseudaxinyssa cantharella*<sup>30</sup> and was shown by workers at Rhone-Poulenc Rorer to be an inhibitor of protein synthesis, acting preferentially on the termination step in eukaryotic protein synthesis, in contrast to other known protein synthesis inhibitors such as emitine, homoharringtonine, anguidine, and bruceantin, which generally act at either the initiation or elongation steps.<sup>30</sup> Girolline proceeded to Phase I clinical trials in man, but the trials were stopped due to significant hypertensive effects seen in treated patients.

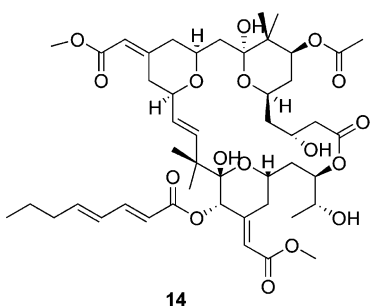
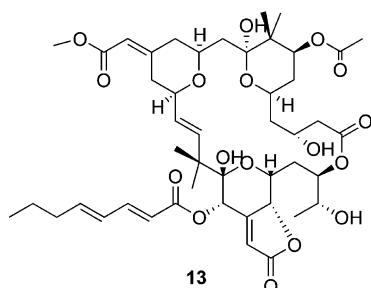
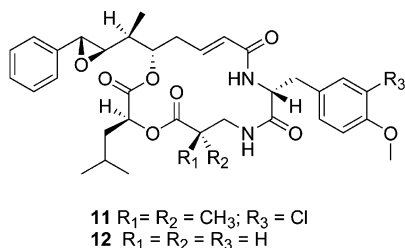
In 2002, Schiavi et al.<sup>31</sup> reported on the synthesis of one of the two possible thiazole derivatives of girolline, 5-deazathiogirolline (7), hoping that this simple substitution might alter the human toxicity characteristics. Although protected intermediates in the synthetic scheme were about 10% as active in girolline in comparable systems, the final deprotected product (the thiazole derivative) was effectively inert.

**Bengamide Derivatives.** Bengamides A (8) and B (9) were first reported in 1986 as antihelminthic compounds (together with some antibiotic and cytotoxic activities) by Crews' group at the University of California, Santa Cruz.<sup>32</sup> The number of bengamide analogues isolable from the same sponge was extended to bengamide G, with details being reported on their isolation and absolute stereochemistry in two more papers from the same group.<sup>33,34</sup> In a subsequent paper with workers from Novartis, the number of compounds in the group was extended, and their antitumor activities were reported.<sup>35</sup>

The bengamides were evaluated by Novartis (initially by Ciba-Geigy), as Ciba-Geigy was the then current industrial partner of the UCSC group in an NCI-funded National Cooperative Natural Products Drug Discovery Group (NC-NPDDG). As a result of their intrinsic activities, a synthetic program was put in place that developed a derivative of bengamide A (10) as a clinical candidate. This derivative was shown to be an inhibitor of methionine aminopeptidases and subsequently entered Phase I clinical trials in 2000, but was withdrawn in the middle of 2002.

**Cryptophycins.** These compounds were reported from two blue-green algae, initially by a group from Merck in 1990 using a *Nostoc* species (ATCC 53789) originally isolated from a lichen on a Scottish Island; they reported only the antifungal activity, finally deciding not to proceed with development, as it was too toxic. Moore's group at the University of Hawaii then identified the same compound<sup>36</sup> from a nonmarine cyanophyte, *Nostoc* sp. strain GSV-224,

and in addition, almost contemporaneously, a similar molecule was reported by Kobayashi et al. from an Okinawan sponge (see below). The University of Hawaii and Wayne State University licensed the natural cryptophycins and synthetic derivatives to the Lilly Company for advanced preclinical and clinical development. This led to the selection of cryptophycin 52 (LY355703) (**11**) as a Phase I clinical candidate in the middle 1990s, with a single publication<sup>37</sup> in late 2002 giving the Phase I and pharmacological results from a variety of schedules, with an intermittent schedule being chosen for Phase II studies.



The routes, both chemical and pharmacological, leading to the choice of this particular derivative were described by Shih and Teicher<sup>38</sup> of the Lilly Research Laboratories. The compound progressed toward Phase II trials, but in 2002, cryptophycin 52 was withdrawn from trial (personal communication, Dr. R. Moore).

Although the original cryptophycins came from terrestrial cyanophytes and the clinical candidate came from semisynthetic modifications of the natural product, in 1994 Kobayashi et al. reported that an acetone extract of the Okinawan sponge *Dysidea arenaria* had potent cytotoxicity,<sup>39</sup> and on purification, the compound arenastatin A (**12**) subsequently turned out to be identical to cryptophycin 24 (**12**) reported by Moore's group in 1995.<sup>40,41</sup> A later report from the Japanese group<sup>42,43</sup> demonstrated that arenastatin A and synthetic analogues also are tubulin interactive agents similar in activity to the other cryptophycins reported by Moore et al.

#### Agents Currently in Clinical Trials as Antitumor Agents

**Bryostatins.** In 1968, NCI commissioned a large-scale (for those days) collection of the bryozoan *Bugula neritina* by Jack Rudloe of the Gulf Specimen Company off the west

coast of Florida that was sent to Pettit's group for chemical workup. The aqueous 2-propanol extract was subsequently tested by NCI for its intrinsic activity as an antitumor agent in the then current P388 and L1210 murine leukemia *in vivo* models. Subsequently, the extract was found to be inactive against L1210 but to give a 68% increase in life span using P388 at the same concentration.<sup>44</sup> Following significant amounts of work by Pettit and his group, including more collections on a larger scale, significant problems with isolation as a result of dealing with vanishingly small quantities of a very potent agent, and problems related to assay reproducibility, the compound was purified and identified as bryostatin 3 (**13**), one of a series of closely related compounds that now number 20.<sup>44-49</sup>

Subsequent work by Pettit's group identified two other geographic areas where significant (in relative terms) quantities of bryostatin 1 (**14**) could be isolated from *B. neritina* colonies. What is important, however, is that although a number of reports have been made about other taxa producing bryostatins, in almost all cases, on careful examination, the putative producing organism actually contains *B. neritina*. However, as a result of prodigious efforts on the part of Pettit and collaborators and workers at NCI-Frederick, by 1990 there was enough cGMP-grade material to commence systematic clinical trials, though prior to this time frame, small quantities of bryostatin 1 had been supplied to a variety of collaborators so that basic biochemical studies and initial clinical trials in the U.K. could be performed.

From these studies, which are summarized in recent reviews by a number of authors,<sup>48-50</sup> it was shown that bryostatins bind to the same receptors as the tumor-promoting phorbol esters, the protein kinase C (PKC) isozymes, but have little or no tumor promoter activity. A recent paper from Hale's group<sup>51</sup> where they made a modified analogue has shown that the binding site for this compound and, by inference, the bryostatins is almost certainly at the cysteine-rich domain 2 (CRD2) in human PKC- $\alpha$ . As a result of this binding, the PKC isozymes in various tumor cells are significantly down-regulated, leading to inhibition of growth, alteration of differentiation, and/or death.

To date, bryostatin 1 has been in more than 80 human clinical trials, with more than 20 being completed at both the Phase I and Phase II levels. There have been some responses to the compound as a single agent with effects ranging from complete remission (CR), to partial remission (PR), to stable disease (SD). However, the use as a single agent is probably not the optimal usage for this compound. More detailed reports of the clinical development are given in the recent reviews by Pettit<sup>50</sup> and by Clamp and Jayson.<sup>52</sup> However, when bryostatin is combined with another cytotoxin, such as the *vinca* alkaloids or nucleosides, and the carcinomas are leukemic in nature, then the response rates, even in Phase I trials, begin to demonstrate that such mixed treatments may well be worth further investigation (cf. Table 2 for details/citations).

Thus a combination with high levels of AraC and low levels of bryostatin in patients with leukemias, in a population that included patients who had failed high-dose AraC (HiDaC) therapy, five of 23 patients presented with complete responses in a recent Phase I trial. Similarly, patients with chronic lymphocytic leukemia (CLL) and non-Hodgkins lymphoma (NHL) treated with fludarabine and bryostatin were reported to show close to 50% "objective responses" in the trial report. With non-small cell lung cancer (NSCLC) and paclitaxel/bryostatin, seven of 11

patients in a Phase II trial demonstrated positive responses (PR/SD) but no CRs.

Currently (01/2004), there are four Phase I and five Phase II trials underway (data from the NCI clinical trials web site <http://clinicaltrials.gov>), and in every case, these are combination studies with biologicals such as interleukin 2 or granulocyte macrophage-colony stimulating factor (GM-CSF), nucleoside derivatives such as gemcitabine, cladribine, or AraC, or other cytotoxic agents such as paclitaxel, vincristine, or cisplatin. These combinations are being tested against leukemias and lymphomas and ovarian and prostate carcinomas. Hopefully, results similar to those demonstrated in Table 2 will be reported in due course.

In all of the clinical trials so far reported the major cause of dose-limiting toxicity (DLT) appears to be myalgia, but in almost all cases reported this was treatable by standard supportive therapies and patients continued on trial. Details as to the protocols for all trials and the results reported are given in two articles currently in press.<sup>53,54</sup>

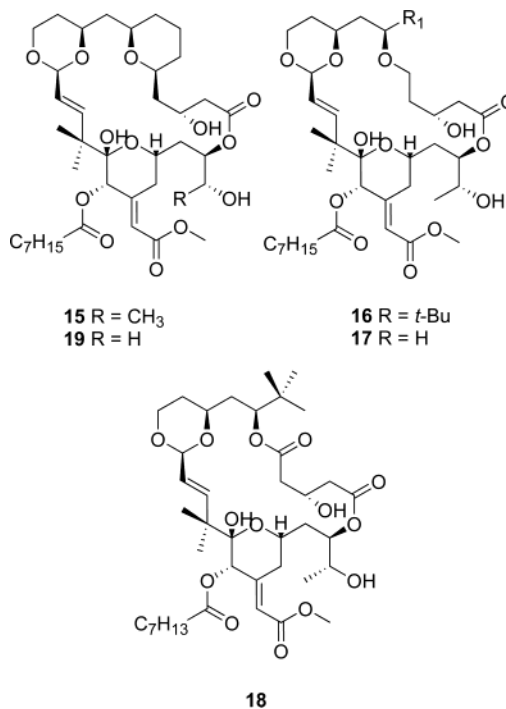
A very interesting "side effect" of the use of bryostatin as a clinical candidate was the early realization that wild collections would not suffice to produce enough of the material for use as a clinical entity. For example, to obtain enough material for the initial clinical trials under NCI auspices, it was necessary to begin with 13 metric tonnes<sup>55</sup> of wild-collected *B. neritina* and then process the material using large-scale chromatographic techniques in order to produce 18 g of cGMP bryostatin 1. Subsequently, NCI funded in-sea and on-land aquaculture (total NCI expenses above \$1M) in order to establish the parameters necessary to produce bryostatin 1 in sufficient quantities at a "reasonable" cost if it progressed through the development pipeline. The processes involved and the successful results have recently been reviewed by Mendola,<sup>56</sup> and this review should be consulted for specific information as to methods, economics, etc.

Since the publication of the first structure by Pettit in 1982, these molecules have been the target of many synthetic chemistry groups. Many partial syntheses have been published where specific portions of the molecule have been made, but to date, only three of the 20 reported bryostatins have been synthesized. The first was the enantioselective total synthesis of bryostatin 7 in 1990 by Masamune et al.,<sup>57</sup> the second by Evans et al. on the enantiomeric total synthesis of bryostatin 2 in 1999,<sup>58</sup> and the third, the synthesis of bryostatin 3, by the group of Nishiyama and Yamamura<sup>59</sup> in 2000. In addition to these papers, three excellent review articles have been published covering information available through 2002, on the syntheses of these three and other partial bryostatin structures including bryostatin 1, and should be consulted for specific details of reaction schema and comparisons of routes.<sup>48,49,60</sup>

From inspection of the three reviews referred to above it can be stated that the total synthesis of bryostatin 1 is not the process that one would wish to utilize to produce this agent. However, if one could synthesize a simpler analogue with comparable activity, then chemical production of such an agent might well be a viable option.

In 1986, Wender et al. analyzed the potential binding site of the phorbol esters on PKC as a guide to the design of simpler analogues of these agents.<sup>61</sup> In 1988, this work was expanded by modeling bryostatin 1 onto the same binding site as a result of the initial results indicating that bryostatin 1 interacted with PKC.<sup>62</sup> Subsequently, the modeling work was refined to produce three analogues that would maintain the putative binding sites at the oxygen

atoms at C<sub>1</sub> (ketone), C<sub>19</sub> (hydroxyl), and C<sub>26</sub> (hydroxyl) in the original molecule. These requirements gave rise to structures (15–17) that maintained the recognition features but removed a significant amount of the peripheral substituents. These molecules demonstrated nanomolar binding constants when measured in displacement assays of tritiated phorbol esters, with the figures being in the same general range as bryostatin 1, and two compounds (15, 16) had activities in in vitro cell line assays close to those demonstrated by bryostatin 1 itself.<sup>63–66</sup> Following on from these examples, modifications were made to the base structure (15) to introduce a second lactone (18) that had an 8 nM binding affinity and also inhibited P388 with an ED<sub>50</sub> of 113 nM.<sup>67</sup> Concomitantly, modifications were made to the base analogue (15) where different fatty acid esters were made (structures not shown). These, too, exhibited binding affinities for PKC isozymes in the 7–232 nM range depending upon the fatty acid used.<sup>68</sup>



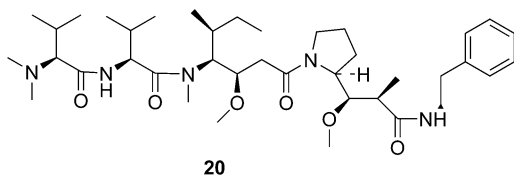
To show the versatility of the base structure, recently Wender published a simple modification where by removal of a methyl group in the C<sub>26</sub> side chain in compound 15 to produce compound 19, the binding affinity to PKC was increased to the picomolar level,<sup>69</sup> and the compound demonstrated greater potency than bryostatin 1 in in vitro cell line assays. Finally, at the end of 2003, he published improved syntheses of the molecules, which could permit further rapid improvements of the model compound(s) with the potential for much greater overall yields.<sup>70,71</sup>

One very interesting question arising from the search for bryostatin sources was, why is the nominal producing organism so ubiquitous, but the number of *B. neritina* colonies that actually produce detectable bryostatin 1–3 levels so low and geographically spread? One possible answer to this question came from the work of Haygood and her collaborators at the Scripps Institution of Oceanography. Haygood showed that the bryozoan is actually the host to a symbiotic microorganism that may well be the actual producer of the compound; in an elegant series of experiments, she and her colleagues demonstrated by use of molecular probes the presence of a putative type I polyketide synthase (PKS) gene fragment in the microbial

flora of colonies that produced bryostatin but that was absent in the corresponding flora of nonproducers.<sup>72</sup> In addition, Davidson and Haygood demonstrated that there are subdivisions within *B. neritina* samples taken from the same sites but at different depths. Thus, at depths greater than 9 m (the D or deep type), bryostatins 1–3 and minor components are found (these are also known as producers of chemotype O for “octa-2,4-dienoic chain”), whereas, at less than 9 m (S or shallow type), only the minor derivatives are seen (chemotype M). The symbiotic microbes (*Candidatus Endobugula sertula*) isolated from each type differ in their mitochondrial carboxylase I (CO I) sequences by 8%, giving rise to the possibility that the bryozoans are also different taxonomically.<sup>73</sup>

There were reports at the Society for Industrial Microbiology (SIM) meetings in 2002 and 2003 that demonstrated that Haygood and collaborators were pursuing the possibility of transferring this particular PKS fragment to other, more amenable microbes in order to further investigate the possibility of producing bryostatin by fermentation. At the recent 6th International Marine Biotechnology Conference (IMBC) in Chiba, Japan, Haygood<sup>74</sup> reported on the current status of the PKS search, suggesting that this system resembled that reported by Piel<sup>75,76</sup> for the *Paederus* beetle's pseudomonas symbiont PKS that produces pederine, in that there are no acyl-transferase (AT) domains in the clusters, unlike the usual PKS system, but that an AT domain was found in another, more remote, area of the overall PKS system. Further work is ongoing utilizing such “remote” AT domains from another organism.

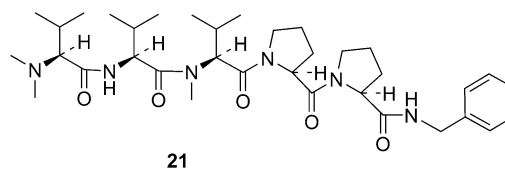
It will be very interesting to follow these results if Haygood is successful, as cultivation of the organism, or a surrogate with the bryostatin PKS system expressed, would potentially solve any supply problems if bryostatin becomes a commercial drug.



**Dolastatin Derivative, TZT-1027 (Auristatin PE or Soblidotin).** As a result of the synthetic processes alluded to earlier, many derivatives of the dolastatins have been synthesized with TZT-1027 (**20**), now in Phase I clinical trials in Europe, Japan, and the United States under the auspices of either Teikoku Hormone, the originator, or the licensee, Daiichi Pharmaceuticals. This compound is also known as Auristatin PE and Soblidotin, and an initial report on Phase I studies was given in abstract form<sup>77</sup> at the American Society for Clinical Oncology (ASCO) in 2002. Recently, a further report from investigators at Teikoku Hormone indicated that in nude mice the transfected vascular endothelial growth factor (VEGF)-secreting human lung cell line SBC-3/VEGF and also the mock transfected cell line were effectively totally inhibited as either early or advanced stage xenografts at levels of 1 or 2 mg·kg<sup>-1</sup>, conditions under which only vincristine was similarly active and combretastatin was not, even at 500 mg·kg<sup>-1</sup>. What was of significant interest in addition to these results was that TZT-1027 also exhibited a potent antivasular effect at these levels, thus suggesting that a dual mechanism might well be possible with this agent.<sup>78</sup>

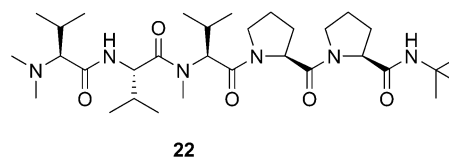
Very recently, a multinational group of investigators demonstrated the potential for a directed delivery of this compound to prostate cancer cells, by using the up-

regulation of the adhesion molecule, E-selectin, that is found in the epithelium of prostate carcinomas and demonstrated that a monoclonal antibody directed to this protein with auristatin linked via a cathepsin B-labile linker gave more than 85% inhibition of growth of prostate carcinoma cell lines in mouse models.<sup>79</sup>



**Dolastatin Derivative, Cematodin (LU-103793).** Another derivative of dolastatin 15 known as Cematodin (**21**) (and also as LU-103793) was placed into Phase I clinical trials by BASF Pharma under their Knoll division for treatment of breast and other cancers. The results from six trials have been reported at the Phase I level with dose-limiting toxicities being neutropenia or cardiotoxicity. A number of these trials used a very rapid bolus (5 min iv), and others used a longer time frame, even up to 5 days of continuous infusion; from them, the investigators' recommended ranges for Phase II studies were at the 2.5–10 mg·M<sup>2</sup> dose levels.<sup>80–85</sup> The compound progressed into Phase II studies against malignant melanoma, metastatic breast cancer, and non-small-cell lung cancer, and reports demonstrated no objective responses in any of the trials<sup>86–88</sup> although stable disease was seen in both the melanoma and breast cancer trials and there was a subjective increase in a quality of life measure in the lung trial.

Currently, there is some dichotomy in the literature as to whether work is actively continuing with this compound; thus Amador et al. report Phase II trials still ongoing as of June 2003 in breast, ovarian, lung, prostate, and colon carcinomas,<sup>12</sup> whereas it is now currently (01/2004) listed as discontinued in the Prous Ensemble database.



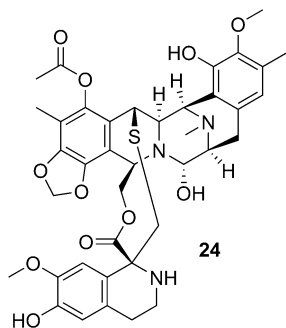
**Dolastatin Derivative, ILX651 (Synthadotin).** There have been six scientific reports in the last two years on the Phase I studies with this agent (**22**), all as presentations at ASCO meetings,<sup>89–92</sup> the American Association for Cancer Research (AACR) meeting,<sup>93</sup> or the joint US–European (AACR-NCI-EORTC) molecular targets meeting.<sup>94</sup> ILX651 is an orally active third generation dolastatin 15 derivative that was licensed by Ilex from BASF Pharma, and two reports<sup>95,96</sup> in 2003 indicated that Ilex Oncology is initiating Phase II studies in melanoma, breast, and non-small-cell lung cancers, as there were responses in these tumors in Phase I patients.

From a nonclinical perspective, dolastatin 15 has proven to be a useful “bioprobe” in tubulin interaction studies. Thus, by using tritium-labeled dolastatin 15, Hamel's group at NCI<sup>97</sup> recently reported that the *vinca* domain in tubulin may well be composed of a series of overlapping domains rather than being a single entity, as different levels/types of competition were found when selected tubulin interactive agents were used to investigate the binding characteristics of the labeled probe.

**Source(s) of the Dolastatins.** Similarly to the situation with the bryostatins, there was always a potential question

with the dolastatins as to whether they were microbial in origin, as peptides with unusual amino acids have been well documented in the literature as coming from the *Cyanophyta*. In the past few years, this supposition has been shown to be fact. Thus, in 1998, workers at the Universities of Guam and Hawaii reported<sup>98</sup> the isolation and purification of simplostatins 1 (**23**) from the marine cyanobacterium *Simploca hynoides*. This molecule differed from dolastatin 10 by the addition of a methyl group on the first *N*-dimethylated amino acid. Subsequently, in 2001, the same groups reported the direct isolation of dolastatin 10 from another marine cyanobacterium that was known to be grazed on by *D. auricularia*.<sup>99</sup> Dolastatin 10 was in fact isolated from the nudibranch following feeding of the cyanophyte, thus confirming the original hypothesis (personal communication, Dr. V. J. Paul).

Very recently, the MOA of simplostatins 1 was evaluated both in vitro and in vivo, and it was shown to be similar to dolastatin 10 but to be somewhat more toxic to mice at comparable doses.<sup>100</sup> In addition, two further examples of dolastatin-like peptides isolated from different collections of the ubiquitous cyanophyte *Lyngbya majuscula* have recently been reported in the literature, viz., dolastatin 16 from a Madagascan collection by Nogle and Gerwick<sup>101</sup> and homodolastatin 16 from a Kenyan collection by Davies-Coleman et al.,<sup>102</sup> further evidence for the microbial source of these peptidic cytotoxins.



**Ecteinascidin 743.** Antitumor activity from the ascidian *Ecteinascidia turbinata* had been reported as early as 1969 by Sigel et al.,<sup>103</sup> but it was not until 1990 that the structures of the active components were published simultaneously by Rinehart et al.<sup>104</sup> and Wright et al.<sup>105</sup>

The structure of the most stable member of the series, known as Et743 from the absorption maximum, is shown (**24**). The base structure, without the exocyclic isoquinoline group, is a well-known chemotype<sup>106</sup> originally reported from microbes, where the compound classes are saframycins, naphthyridinomycins, safracins, and quinocarcins. Similar molecules were reported from marine mollusks, i.e., jorumycin from the nudibranch *Jorunna funebris*<sup>107</sup> and from sponges, the renieramycins, with the latest variation, renieramycin J, being recently reported by Oku et al.<sup>108</sup> However, with Et743, the exocyclic substituent was novel, as was the bridging sulfur.

The yield from natural sources was very low, and in order to provide enough material to perform basic studies as to the MOA and in vitro and in vivo animal studies, significant amounts of the ascidian had to be collected from areas around the Caribbean. The compound was synthesized by Corey<sup>109</sup> in a chemical "tour de force", and as a result of his synthetic approach, his group also made a version where the exocyclic ring was a phthalimido substituent. This compound, phthalascidin, demonstrated significant activity in the same test systems used initially

with Et743.<sup>110</sup> Subsequently, he improved the synthetic schema and developed a refined process that produced both Et743 and phthalascidin at much higher yields.<sup>111</sup> Other synthetic chemistry groups have continued work on the basic compound, but as yet, none of their compounds have had any biological activity reported in the literature.<sup>112,113</sup>

The natural compound was licensed by the University of Illinois to the Spanish Company PharmaMar for subsequent development. Following very large-scale wild collections and aquaculture on both land and in-sea in efforts to obtain enough source material for further preclinical and clinical workup, PharmaMar chemists performed an elegant semisynthesis from the marine *Pseudomonas fluorescens* metabolite cyanosafracin B that provided cGMP grade Et743 from a 21-step synthetic process on a scale large enough to provide enough material for clinical trials. This was feasible despite a low overall yield of 1.4% because the starting material could be obtained on a large scale by fermentation. The original paper<sup>114</sup> together with a relatively recent review article,<sup>115</sup> both from the PharmaMar group, should be consulted for further details as to synthetic strategies, etc., employed for production of this compound.

A number of reports have been published in the literature over the past few years giving possibilities as to the MOA(s) of Et743 when tumor cells are treated in vitro. A significant problem with some of the reports is that the concentration(s) used in the experiments are orders of magnitude greater than those that demonstrate activity in vitro. These levels are in the low nanomolar to high picomolar range, and thus care should be taken when evaluating published work on the MOA of this compound.

At physiologically relevant concentrations the MOAs of Et743 have been shown to include the following: effects on the Transcription-coupled Nucleotide Excision Repair process (TC-NER)<sup>116,117</sup> and interaction between the Et743 DNA adduct and DNA transcription factors, in particular the NF-Y factor.<sup>118</sup> In the recent review on Et743 by a Dutch group,<sup>119</sup> further details as to other possible mechanisms are given in their Table 1; the references that they cite should be consulted for in-depth information and discussion for other potential MOAs ascribed to Et743. As addenda to the results given in the paper above, there were two presentations at the AACR-NCI-EORTC molecular targets meeting in November 2003 reporting gene expression profiles on sarcoma lines using the "Oncochip", a 6700 gene array of genes prevalent in cancer cell proliferation. The first, using cells from treated sarcoma patients,<sup>120</sup> reported that when the IC<sub>50</sub> values for Et743 were <100 nM, early changes (within 6 h) in genes related to apoptosis, cell cycle, transcription factors, growth factors/receptors, and binding to nucleic acids were demonstrable. In contrast, with cells showing IC<sub>50</sub> values ≥100 nM (nominally resistant to Et743), there was a marked delay in critical regulatory gene changes. In the other presentation<sup>121</sup> Martinez et al. reported that using human chondrosarcoma lines, there was a 5.5% difference between the sensitive and an isogenic resistant line, particularly in the cyclin D1/D3, GRO1, and NF-κB pathways. As alluded to earlier, although there are a number of other mechanisms postulated, on careful inspection, these are usually shown to occur at concentrations of drug well above (i.e. > ~250 nM) those that are physiologically relevant.<sup>106</sup>

The compound was placed into human clinical trials while these mechanisms were being worked out, and by 2002 it had been in over a 1000 patients in Phase I and Phase II trials<sup>8</sup> covering a variety of cancers. Results from



the European Phase I and pharmacokinetic trials were recently reported by Twelves et al.,<sup>122</sup> and details of the human pharmacokinetics (PK) and activities against bone tumor cells *in vitro* were also published recently.<sup>123</sup> In 2001, Et743 was licensed to Johnson and Johnson (Ortho Biotech) under the brand name Yondelis, with the generic name of trabectedin. Two recent full reports on the Phase II trials have been published<sup>119,124</sup> giving details of toxicities and response levels in sarcomas and other carcinomas with both pretreated and naive patients, and at the November 2003 AACR-NCI-EORTC molecular targets symposium, there were a further series of reports showing objective responses in long-term follow-up studies in sarcoma in Phase II studies,<sup>125</sup> preliminary results from a combination study of Et743 and doxorubicin in untreated sarcoma and non-anthracycline-treated breast cancer patients where PR and SD were observed,<sup>126</sup> and the potential for the use of paclitaxel and ET-743 where a PR has been observed in a Phase I study.<sup>127</sup> The article by the Dutch group gives in-depth discussions of most of the so-far reported trial results,<sup>119</sup> and for further information on other aspects, the review by D'Incalci and Jimeno should also be consulted.<sup>128</sup>

As a result of these earlier trials, Et743 was preregistered in the EU and granted orphan drug status for sarcoma by the European Commission's Committee for Orphan Medicinal Products (COMP) of the European Agency for the Evaluation of Medicinal Products (EMA). However, in late July 2003, the EU's Committee for Proprietary Medicinal Products (CPMP) recommended, on a majority vote, that marketing authorization for advanced soft tissue sarcoma not be granted for the EU. This decision was appealed in September 2003 by PharmaMar,<sup>129</sup> but in December 2003 the appeal was denied.<sup>130</sup> The compound was granted orphan drug status for ovarian cancer by the CPMP during the appeal process on sarcoma referred to above.<sup>131</sup>

Further evidence as to the possibilities of combination studies has been reported by D'Incalci et al.,<sup>132</sup> where work with mice demonstrated that there was synergy against the Et743-resistant/cisplatin-partially resistant ovarian cell line HOC 8 when cisplatin was added to the treatment protocol. Both drugs were used at their maximum tolerated dose (MTD) levels, thus demonstrating that although synergy occurred with activity, there was no cross/synergistic toxicity shown.

One of the predominant toxicities exhibited by Et743 in preclinical studies was hepatotoxicity, particularly in the female rat, and similar effects had been seen in human patients but could be controlled by dose-reduction. However, in a recent publication, Donald et al.<sup>133</sup> demonstrated that pretreatment with high-dose dexamethosone gave complete protection against hepatotoxicity in this animal. Thus such a treatment in humans may well be a method of controlling this Et743-related toxic side effect.

Currently Et743 is in a variety of Phase II trials in the United States and Europe for the treatment of sarcoma<sup>134,135</sup> and is listed as being in Phase III in Europe in the Prous Ensemble database at time of writing.

**Aplidine.** This compound, formally dehydrodidemnin B (**25**), was first reported in a patent application in 1989, with a U.K. patent issued<sup>136</sup> in 1990 and then referred to in the paper<sup>15</sup> from Rinehart's group in 1996 on the structure-activity relationships among the didemnins. The antitumor potential was first reported by PharmaMar scientists<sup>137,138</sup> in 1996, and the total synthesis was reported in a patent application<sup>139</sup> in 2000 and the patent was issued in 2002.

The compound, generic name "aplidine or dehydrodidemnin B" and with a trade name of Aplidin, was placed

into Phase I clinical trials in 1999 under the auspices of PharmaMar in Canada, Spain, France, and the U.K. for treatment of both solid tumors and non-Hodgkin's lymphoma. A summary of five of the trial results is given in Table 2 of Amador et al.,<sup>12</sup> which should be consulted for specific dosage details, and the actual abstracts from the three ASCO meetings may be consulted for further information.<sup>140-144</sup> These were successfully completed with over 200 patients and demonstrated that a dosage of up to 5 mg·M<sup>2</sup> was well tolerated in either a 3 or 24 h infusion every other week.<sup>135</sup> The DLT was muscle pain that was responsive to either dose limitation or addition of carnitine. Interestingly, in the presence of carnitine, the maximum tolerated dose could be increased by 40% to 7 mg·M<sup>2</sup>. Phase II clinical trials are now underway in Europe comparing the two dosage regimens in renal and colon carcinomas, together with an outpatient regimen, and very recently (07/2003), the European Commission's COMP/EMA awarded orphan drug status<sup>145</sup> for acute lymphoblastic leukemia (ALL) to aplidine. Other Phase II trials are also ongoing in Europe and Canada covering renal, head and neck, and medullary thyroid, but no patient details have yet been published.

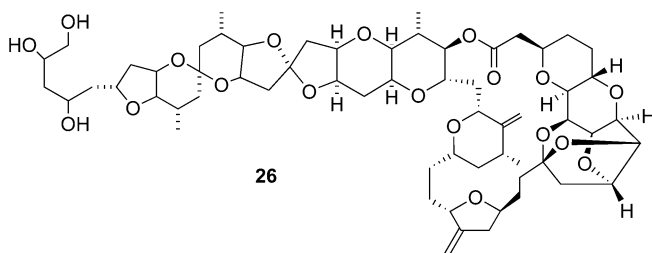
The precise MOA of this agent is not yet known, but it appears to block VEGF secretion and blocks the corresponding VEGF-VEGF-Receptor-1 (also known as *flt-1*) autocrine loop in leukemic cells.<sup>146</sup> In addition, effects on the kinases *JNK*, *src*, and p38-MAPK, possibly mediated via glutathione depletion, were recently reported,<sup>147</sup> with the end result being induction of the apoptotic cascade in MDA-MB-231 breast cancer cells at levels of 5 nM, below the blood levels achievable in man. In these experiments, general caspase inhibitors decreased apoptotic efficacy by ~50%, thus implicating at least two different mechanisms of apoptosis, one via caspases, the other not involving caspase activation. Of significant interest are the recent reports by Straight et al. on the effects of aplidine on ARO-81 anaplastic thyroid cancer cells<sup>148</sup> and of Bravo et al. on human thyrocytes from different pathologies.<sup>149</sup> In the first case, induction of apoptosis was observed together with a reduced or absent expression of angiogenic genes, and in the second case, a low but constant apoptotic rate was established that caused over 90% reduction in cell numbers within 72 h at 100 nM aplidine. Thus in these cell types, aplidine had both a cytotoxic and an antiangiogenic effect.

In leukemic cells obtained from pediatric patients, aplidine demonstrated little cross-resistance with other cytotoxic drugs, and in particular, bone marrow cells from normal patients were 2-7 times more resistant to aplidine than the cells from leukemia patients, indicating that studies with other cytotoxins could be justified in cancer patients. The original paper should be consulted for specific sets of drug combinations/level of interactions.<sup>150</sup> Further evidence for a lack of myelosuppression by aplidine, Et743, and kahalide F, compounds currently in Phase II, II/III, and II, respectively, has been reported by the PharmaMar group using a murine competitive repopulating model as the test system, but these findings will have to be confirmed in human patients/bone marrow cells as well.<sup>151</sup>

What is very interesting, both chemically and pharmacologically, is that the removal of two hydrogen atoms, i.e., conversion of the lactyl side chain to a pyruvyl side chain, appears to significantly alter the toxicity profile, as this is the only formal change in the molecule when compared to didemnin B, although the comments on dosage regimens under didemnin B (*vide supra*) from Vera and Joullie<sup>19</sup> should be taken into account when such comparisons are

made in the future. Similarly, the resemblance to didemnin B is emphasized by the recent work of Cardenas et al., who reported<sup>152</sup> that in DMSO solution aplidine, like didemnin B, does not exhibit a formal  $\beta$ -turn in its side chain in approximately 20% of its solution conformers, thus suggesting that the presence of such a turn is not required for biological activity. As the authors point out, there may well be other, as yet unrecognized minor conformers that are responsible for some/all of the biological activities demonstrated.

**Halichondrin B (and Derivatives).** Halichondrin B (**26**) is one of a series of compounds originally isolated and reported<sup>153,154</sup> by Uemura et al. in 1985 from the Japanese sponge *Halichondria okadai*. Subsequently, a number of sponges from other areas of the Pacific and Indian Oceans were reported to contain one or more of these macrolides, including *Axinella* sp. from the Western Pacific,<sup>155</sup> *Phakellia carteri* from the Eastern Indian Ocean,<sup>156</sup> and from a deep water *Lissodendoryx* sp. off the East Coast of South Island, New Zealand.<sup>157</sup>



Although there was enough halichondrin B available for some initial experiments and to determine that the possible mechanism of action was as a tubulin interactive agent, affecting tubulin depolymerization at a site close to, but distinct from, the *vinca* site,<sup>158–160</sup> and to show initial *in vivo* activity,<sup>161</sup> there was not enough material for further development work. In 1992, NCI issued a request for groups that could provide a variety of scarce natural products from natural sources, and a consortium from New Zealand composed of the University of Canterbury (who had discovered that a deep water *Lissodendoryx* sp. produced the halichondrins at approximately 1 mg·kg<sup>-1</sup> wet weight) and the National Institute for Water and Atmospheric Research (NIWA) was successful in convincing the NCI to fund a large-scale recovery and isolation program as a joint venture with them and the New Zealand Government.

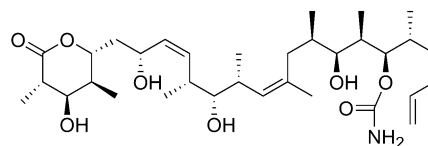
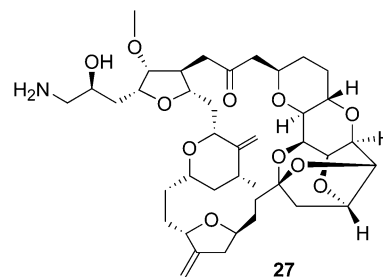
Following an environmental assessment of the potential collection area paid for by the Developmental Therapeutics Program (DTP) of the NCI, the NZ Government gave permission to collect 1 metric tonne from the Kaikoura shelf at a depth of 100 m and greater by trawling. Following extensive workup, these samples produced 300 mg of halichondrin B, but what was just as important, were the experiments conducted by NIWA scientists (also partially funded by DTP/NCI) that demonstrated that the deep-water *Lissodendoryx* could be successfully aquacultured in water as shallow as 10 m and still produce the halichondrin complex at levels roughly comparable with those found from wild collections.

Concomitantly with the start of this large-scale wild collection program, Kishi's group at Harvard, also funded by the DTP/NCI, reported that they had successfully synthesized both halichondrin B and norhalichondrin B.<sup>162</sup> Working with the U.S. division of the Japanese pharmaceutical company Eisai, Kishi's synthetic schemes were utilized to synthesize a large number of variants of hali-

chondrin B, particularly smaller molecules that maintained the biological activity but were intrinsically more chemically stable, due to the substitution of a ketone for the ester linkage in the macrolide ring. Two of these agents were subsequently evaluated by DTP in conjunction with the Eisai Research Institute in the United States, and one of the compounds, originally ER-086526 (NSC 707389) and now renumbered E7389 (**27**), entered Phase I clinical trials in 2002 in conjunction with the NCI.

At the 2003 ASCO meeting, there were two presentations on E7389, one showing pharmacokinetics of this agent in man<sup>163</sup> in the current Phase I trial demonstrating that levels above those required for cytotoxicity *in vitro* were achievable for up to 72 h at doses below the DLT of 0.5 mg·M<sup>2</sup>, and the other demonstrating that this agent exhibits p53-independent anticancer activity versus non-small cell lung cancer (NSCLC) *in vitro* at the 0.5 pM level,<sup>164</sup> orders of magnitude below the 1500 pM levels achievable in man. Thus NSCLC may well be a worthwhile target for this agent.

Details of the biology and chemistry of this compound and other compounds in the series have recently been published by both the Harvard<sup>165</sup> and Eisai scientists,<sup>166,167</sup> thus demonstrating the power of current synthetic chemistry when applied to a very potent marine-derived natural product. Using the synthetic techniques described, enough cGMP material, produced by total synthesis, was provided to the NCI for the initial clinical trials.



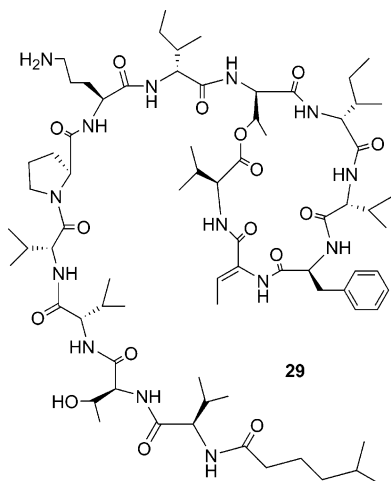
**Discodermolide.** This polyhydroxylated lactone (**28**) was reported by the Harbor Branch group in 1990 following isolation from the Caribbean sponge *Discodermia dissoluta*, originally collected at a depth of 33 m off the Bahamas,<sup>168</sup> with a revision to the stereochemistry being published the following year.<sup>169</sup> Originally, the compound was judged to be a new immunosuppressive and an incidental cytotoxin.<sup>170–172</sup> However, in 1996, it was reported that discodermolide bound to microtubules more potently than Taxol, a discovery that confirmed in silico studies at the University of Pittsburgh.<sup>173</sup> Concomitantly with these reports, a variety of chemical synthetic groups had seen discodermolide as a good candidate for total synthesis. Thus the initial report from Harbor Branch (which as noted above was later corrected) led to synthesis of the (–)-isomer by Schreiber's group<sup>174</sup> and others, and then in the late 1990s–2003, Marshall and Johns,<sup>174</sup> Halstead,<sup>175</sup> Smith et al.,<sup>176</sup> and Paterson et al.<sup>177</sup> all reported syntheses that would produce varied isomers in good yield. Recently, Paterson and Florence have published an excellent re-

view<sup>178</sup> of the various synthetic schema in use, and finally, in the middle of 2003, the Novartis group published their formal synthesis of the (+)-isomer.<sup>179</sup> These various methods have demonstrated that kilogram amounts are now achievable by total synthesis. In addition, as another potential method of preparation, in 2001, NCI awarded Kosan a Phase I SBIR grant to attempt to produce (+)-discodermolide by genetic engineering techniques.

In the interim, Harbor Branch licensed the compound to Novartis as a preclinical candidate, and it is now in Phase I clinical trials as a potential treatment against solid tumors. Recently, at the 2003 ASCO meeting, the first formal report of a Phase I trial of the compound was presented. No objective responses have yet been seen, but stable disease in ~20% of the patients (who all had advanced solid malignancies) was reported, and aside from one patient, the DLT had not yet been reached.<sup>180</sup> Further work in nonhuman experiments was also presented at the same meeting, with McDaid et al. reporting<sup>181</sup> that discodermolide and paclitaxel, although formally similar in their MOAs, give synergistic responses in vitro and in vivo in mouse models with ovarian or NSCLC xenografts; thus this combination may well be worth using in human trials.

The Harbor Branch group is still discovering more derivatives of the natural product and recently published the structures and initial in vitro activity of five new analogues from sponges in the genus *Discodermia* but not of the same species.<sup>182</sup> Finally, a relatively recent paper from Horwitz' group<sup>183</sup> demonstrates how discodermolide and Taxol may well fit into the same site on tubulin.

**Kahalalide F.** This cyclic depsipeptide was isolated from the Sacoglossan mollusk *Elysia rufescens* following grazing by the mollusk on a green macroalga, *Bryopsis* sp. Following isolation and identification, it was discovered that the depsipeptide also occurs in the alga, but on a wet weight basis, the mollusk concentrates the depsipeptides significantly. Thus from 216 g of the animal, 2.1 g of kahalalide F (**29**) was recovered, compared to the 5 mg recovered from 3 kg of the alga collected at the same site.<sup>184,185</sup>



The compound was licensed to PharmaMar by the University of Hawaii in the 1990s, and it entered preclinical testing. Its actual MOA had not yet been fully determined, but it was known to target lysosomes,<sup>186</sup> thus suggesting selectivity for tumor cells with high lysosomal activity such as prostate tumors. The compound was synthesized in a very efficient manner using solid phase peptide techniques by a group in the Chemistry Department at the University of Barcelona<sup>187</sup> and entered Phase

I clinical trials in Europe in December 2000 for the treatment of androgen-independent prostate cancer.

Recently, questions were raised about the stereochemistries given in the original structure by Hamann as confirmed by Goetz et al. in 1999,<sup>188</sup> as when that stereoisomer was synthesized by the Spanish group, it was inactive and exhibited different chromatographic and spectroscopic properties.<sup>189</sup> Bonnard et al. reinvestigated the stereochemical assignments of the natural product and confirmed that Valine 3 should be D-Val and Valine 4 should be L-Val, rather than the reverse.<sup>189</sup> The group at Barcelona had synthesized this stereoisomer, and it had biological and chemical properties identical to that of the natural compound; this material was then used for clinical work.

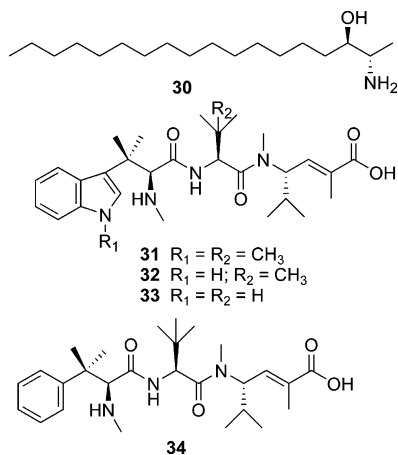
The compound has now entered Phase II trials predominantly as a treatment for prostate cancers, and a second report on a Phase I trial on androgen-resistant prostate cancer patients was made by Rademaker-Lakhai et al. at the 2003 Meeting of the Dutch Society for Clinical Pharmacology and Biopharmacy,<sup>190</sup> where results showed a diminution in prostate specific antigen (PSA) levels in a number of patients. An earlier report<sup>191</sup> on the same trial was given in 2002. Concomitantly with this trial, there was a report on clinical benefit shown in a Phase I trial on solid tumors other than prostate,<sup>192</sup> and then in the middle of 2003, it was announced<sup>193</sup> that PharmaMar was commencing Phase II studies in liver carcinoma as a result of clinical benefits demonstrated in Phase I trials in advanced solid tumors.

Although the specific MOA(s) of this compound are still not fully delineated, it has a specificity for the lysosomal compartment in cells, and recently, Suarez et al.<sup>194</sup> demonstrated that kahalalide F induces cell death via "oncotic" (the progression of cellular processes leading to necrotic cell death) possibly initiated by lysosomal membrane depolarization in both prostate and breast cancer cell lines. As reported by Gomez et al.,<sup>151</sup> this compound does not appear to affect hematopoietic progenitors or stem cells in a murine model at up to 10  $\mu$ M concentrations, well above those achievable in patients.

**Spisulosine.** In 1999, workers from PharmaMar reported on the initial studies with a molecule known as ES-285 or spisulosine (**30**), isolated from the marine clam *Spisula polynyma*. This initial report at a conference on molecular targets was followed rapidly by a full paper in 2000 that demonstrated that this compound causes a loss of actin stress fibers, which may well be due to its resemblance to lysophosphatidic acid (LPA) and hence an interaction with the LPA receptor, which is known to modulate the levels of the *Rho* proteins.<sup>195</sup> The compound demonstrated a wide in vitro therapeutic index when tumor cells were compared to normal cell lines, with a 50–100 fold difference in IC<sub>50</sub> values,<sup>196</sup> and appears to interact with the endothelial cell differentiation gene (EDG) receptors as originally postulated by Cuadros et al.<sup>195</sup> and now confirmed in two recent presentations.<sup>197,198</sup> This molecule is currently in Phase I trials against solid tumors in Europe under the aegis of PharmaMar.

**HTI-286 (Hemiasterlin Derivative).** Hemiasterlin (**31**) was originally reported by Kashman's group<sup>199</sup> from the South African sponge *Hemiasterella minor*, an organism that also contained jaspamide and geodiamolide TA. This report was quickly followed by the report of a group of cytotoxic peptides isolated by Andersen's group at the University of British Columbia (UBC) from a Papua New Guinea sponge that was originally classified as *Pseudoax-*

*inyssa* sp. (now revised to a *Cymbastela* sp.). This particular sponge produced a number of peptides, including geodi-amolides A–F, hemiasterlin as described by Kashman, two novel hemiasterlins, A (**32**) and B (**33**), and other geodi-amolides and criamides.<sup>200</sup>



In 1997, following testing of the hemiasterlin and the A and B derivatives in experiments to determine their MOAs, it was discovered that these agents interact with tubulin to produce microtubule depolymerization in a manner similar to that reported for nocodazole and vinblastine.<sup>201</sup> Further investigations by Hamel's group using hemiasterlin isolated at NCI<sup>202</sup> indicated that this peptide, together with cryptophycin 1 and dolastatin 10, inhibited tubulin assembly and probably bound at what is being called the "peptide binding site".<sup>203</sup>

Subsequently, Andersen commenced a synthetic program in order to produce the original hemiasterlin using a scheme that would permit variations on the overall structure in order to determine SAR requirements.<sup>204</sup> In that report, Andersen makes the very telling point that one should always confirm the biological activity of naturally occurring peptides by testing their synthetic counterparts in the same assay, pointing out the problems that Pettit reported with the biological activity of the natural stylopeptide 1 versus the inactive synthetic stylopeptide 1, which were identical by all physicochemical measurements.<sup>205</sup>

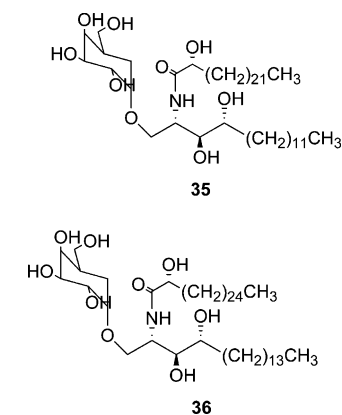
The hemiasterlins, including the analogues made by Andersen's group, which included HTI-286 (**34**), known by Andersen's group<sup>206</sup> as Synthetic Peptide Analogue (SPA) 110, were licensed by UBC to Wyeth for development as part of the NCNPDDG, of which Andersen was a component. Significant amounts of synthetic work were performed by Wyeth around these structures, as reported<sup>207</sup> at the 2002 AACR meeting, but, as also reported<sup>208</sup> at the same meeting, the original agent was still superior and is currently in Phase I clinical trials and scheduled to enter Phase II shortly.

A full paper giving details of the in vitro and in vivo animal data was recently published by Loganzo et al.,<sup>209</sup> and a presentation at the 2003 AACR meeting gave some very interesting data on HTI286–dolastatin 10 hybrids<sup>210</sup> where the tubulin binding site appeared to be similar for both the dolastatins and HTI286. The hybrids were also much more active than dolastatin 10 in cells that express the P-glycoprotein efflux pump.

There is a very nice example of source country collaboration and benefit-sharing in this particular case, as UBC has already made a payment to Papua New Guinea as part of a collection agreement that allows for flow-back of benefit

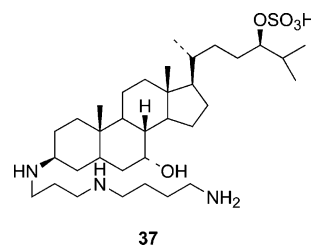
to the source country as required by the Convention on Biodiversity (CBD) and also the NCI's own Letter of Collection (LOC).

**KRN-7000.** In 1993, workers at the Kirin Brewery in conjunction with Higa at the University of the Ryukyus reported the first isolation of  $\alpha$ -galactosylceramides from natural sources. The agelasphins (**35**) were obtained from the marine sponge *Agelas mauritianus*, and very interestingly, demonstrated antitumor and potential immunostimulatory activities.<sup>211,212</sup> Following these results, and continued proof that these molecules were potent in vivo active agents against the murine B16 melanoma, various derivatives were made,<sup>213,214</sup> culminating in the production of KRN-7000 (**36**). This compound entered Phase I clinical trials in both Asia and Europe in 2001 for cancer immunotherapy.



Both reports on the PK<sup>215</sup> and effects on Natural Killer T-Cell (NKT-cells) populations in patients<sup>216</sup> have now been reported from the same Phase I trial. No significant adverse effects were seen, and biological effects were observed in the few patients with high levels of NKT cells. Since no objective antitumor responses were reported from this trial, it was felt that a preselection of patients with high natural NKT cells might give objective responses in other trials. Although not in humans, a very interesting recent finding was the report of primary tumorigenesis inhibition<sup>217</sup> in mouse models; this could imply that the agent might be efficacious in inhibition of carcinogenesis in man.

**Squalamine.** This compound, isolated from the common dogfish shark, *Squalus acanthias*, collected off the New England coast, was originally reported in 1993 by Zasloff's group at NIH and collaborators from the University of Pennsylvania<sup>218</sup> and was shown to be a fairly simple aminosterol (**37**) with broad spectrum antibiotic activity.



The compound was licensed to Maganin Pharmaceuticals (now Genaera) for development and has progressed into Phase II clinical trials for nonresponding solid tumors as part of a combination with standard agents and as primary treatment for advanced ovarian cancer. Early on in its development it was reported that squalamine exhibited

significant effects in an antiangiogenesis assay,<sup>219</sup> and therefore this fact was taken into account during its preclinical and clinical development.

There are recent reports of a Phase I/II trial demonstrating safety at a dose of 192 mg·M<sup>2</sup>·day<sup>-1</sup> where no objective responses were found in the patients, all of whom had refractory solid carcinomas of varying types,<sup>220</sup> and in another Phase I trial, up to 500 mg·M<sup>2</sup>·day<sup>-1</sup> was feasible if infused over 5 days; again, no objective responses were seen.<sup>221</sup> In contrast, in a Phase I/IIA trial in patients with advanced NSCLC, there were demonstrated PRs in 12 (28%) of patients with 8 (19%) more having SD. The responses were probably due to the combination therapies (squalamine plus carboplatin or paclitaxel), as squalamine as a single agent does not have a growth effect on tumor cells in vitro and appears to have its greatest effect on newly emerging vessels.<sup>222</sup>

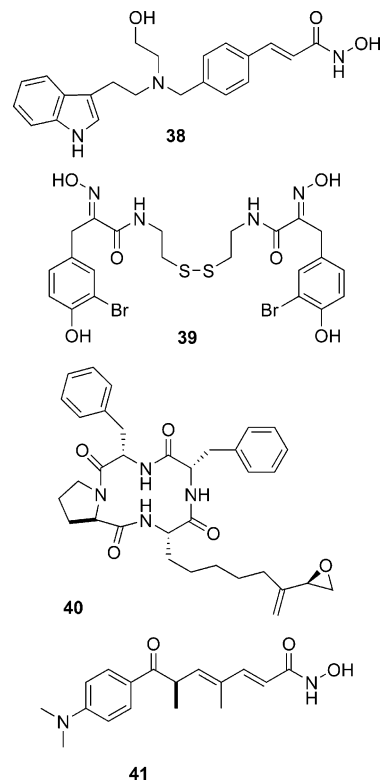
Aside from its antiangiogenic effects in treatment of cancer, squalamine has another very interesting property. It decreases the chorioidal neovascularization in a laser injury rat model that resembles age-related macular degeneration (AMD) in man.<sup>223</sup> Although there are no peer-reviewed reports in the literature as yet, there have been reports in the trade press that squalamine is demonstrating improvements in vision in AMD patients in a Phase I/II trial of AMD in man.<sup>224</sup>

**Æ-941 (Neovastat).** This is not a true compound, but is probably best thought of as a "defined mixture" in that it is a standardized liquid extract comprising the <500 kDa fraction from shark cartilage. This material is made under cGMP conditions from taxonomically identified shark species harvested under sustainable conditions and has quality controls that permitted both the FDA and its Canadian equivalent to give approval for clinical trials. The methods of preparation, etc., have been published in reasonable detail by Sorbera et al.,<sup>225</sup> and the first formal report of angiostatic and antitumor activity was given at the 1997 AACR meeting,<sup>226</sup> with a more complete study in 2002 demonstrating that Æ-941 specifically induces activation of caspases in endothelial cells.<sup>227</sup>

The preparation has been in many Phase II/III clinical trials in Canada, Europe, and the United States, with initial details of the pivotal studies in Phase III renal carcinomas being given in two recent reviews, one published in early 2003 by Gingras et al.<sup>228</sup> and the other in late 2003 by Bukowski<sup>229</sup> from the Cleveland Clinic. Currently, it appears that Æterna (the company producing the material) is concentrating on the renal carcinoma trials referred to earlier and to the NSCLC clinical trials in conjunction with NCI.

**NVP-LAQ824.** This compound (**38**), which is made by total synthesis, was derived from work with both natural products and synthetic derivatives. The marine natural product psammaphin A (**39**), which had originally been identified by the groups of Schmitz<sup>230</sup> at the University of Oklahoma and Crews<sup>231</sup> at the University of California, Santa Cruz, in 1987, was screened, together with congeners and the microbial products trapoxin B (**40**) and trichostatin A (**41**), for their activity as histone deacetylase (HDAC) inhibitors by Novartis (then Ciba-Giegy) as part of an NCNPDDG involving these groups. In 2001, the announcement was made in an abstract at the AACR meeting that the psammaphins were extremely potent HDAC inhibitors.

The synthetic path from psammaphins, trapoxin, and trichostatin structures to the compound now known as NVP-LAQ824 was described in three papers<sup>232–234</sup> in 2003, and these, in particular the review by Remiszewski,<sup>234</sup>



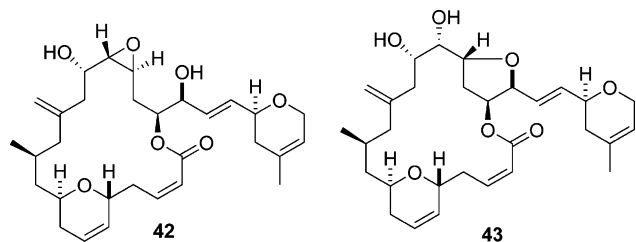
should be consulted for the chemical rationales that led from these natural products to the current clinical candidate. In cells from human multiple myeloma patients, this agent demonstrated significant activity in in vitro experiments, inducing apoptotic signaling and also exhibiting proteasome inhibition. Currently, the material is in a Phase I trial against hematologic malignancies<sup>235</sup> at the Dana-Farber Cancer Institute.

### Selected Antitumor Compounds from Marine Sources in Preclinical Status

This section is not meant to be exhaustive in nature, but is designed to show the vast differences in chemical structures that have mechanisms of action in common and, in some specific cases, the chemistry that has been performed around the basic structure(s), thus demonstrating the value of natural products as "scaffolds" upon which to perform combinatorially directed syntheses with the aim of "improving upon Mother Nature". Further examples, not just in cancer, have been given in a series of articles in the October 13, 2003, issue of *Chemical and Engineering News* by Rouhi,<sup>236–238</sup> and these, together with the very interesting review by Tietze et al.<sup>239</sup> on the potential for natural product hybrids to be scaffolds and/or drug candidates, should be consulted by the interested reader.

**Tubulin Interactive Agents.** Due to the discovery of the mechanism of action of paclitaxel, and the potential that other agents with a similar MOA might have, a large number of groups began a systematic study of marine natural products using some form of tubulin interactive assay as their major bioactivity screen. These screens ranged from assays using tubulin directly and measuring inhibition and/or activation of assembly, to whole cell screens of the types published by the groups led by Barrows<sup>240</sup> in 1996, Mooberry<sup>241</sup> in 1998, and Roberge<sup>242</sup> in 2000. Using these assay systems, a wide variety of marine-derived compounds have now been identified as having activity against microtubules. In some cases, the possible binding sites have been identified; in others, no

data are yet published. Such compounds include, but are not limited to, the following:



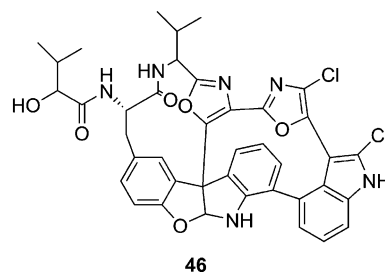
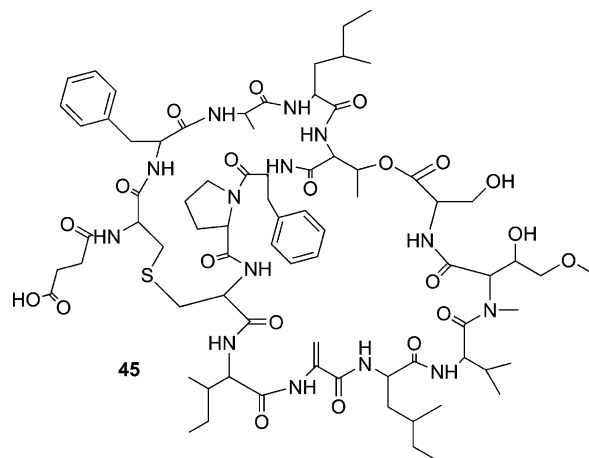
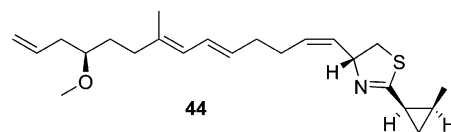
**Laulimalide (42)** and **Isolaulimalide (43)** from the Pacific Ocean sponge *Cacospongia mycofijiensis* by Mooberry et al.<sup>241</sup> These compounds are also known as fijianolides B and A, respectively, and have been reported in other sponge genera, including *Hyatella*, *Fasciospongia*, and *Dactylospongia*, and also from a chromodorid nudibranch grazing on the sponge. Although this agent is a microtubule-stabilizing agent, Hamel<sup>27</sup> indicated that it might bind at a site different from the taxanes, although it is possible that it might also be binding to unpolymerized tubulin or to aberrant polymeric tubulin. Furthermore, in late 2003, Mooberry et al.<sup>243</sup> reported that this agent, like other microtubule stabilizers, has an additional mechanism independent of mitotic arrest whereby G<sub>1</sub> aneuploid cells are formed due to aberrant mitotic events at 5–7 nM, concentrations approximately 30% of those required for mitotic accumulation. A large number (over 10) of synthetic routes to laulimalide have been published, plus many more that give methods of synthesis of “subassemblies” of the overall molecule, and for a thorough discussion of the results of these endeavors, the reader should consult the excellent synthetic paper from Miltzer’s group<sup>244</sup> and the very recent review by Miltzer and Ohler.<sup>245</sup>

**Curacin A (44)** from the cyanobacterium *L. majuscula* by Gerwick et al.<sup>246</sup> This compound is exquisitely potent but is effectively insoluble in any formulation and thus has not been reported to produce activity in in vivo animal models. However, in a series of combinatorial experiments, Wipf at the University of Pittsburgh has been able to perform chemistry around the basic structure and now has more soluble variants that are undergoing evaluation.<sup>247</sup>

**Vitilevuamide (45)** from the ascidians *Didemnum cuculiferum* and *Polysyncrator lithostrotum* by Ireland’s group.<sup>248</sup> This compound should be compared with those given in the recent review of bioactive peptides by Janin.<sup>249</sup>

**Diazonamide (46)** from the ascidian *Diazona angulata* by Fenical’s group.<sup>250</sup> This compound languished for a significant amount of time due to supply problems, though finally another supply of organism was found that enabled further biological evaluations to be performed. Formal syntheses were published using the structure as published by Fenical’s group; however, the original structure was questioned, and Harran’s group published syntheses of the original structure, an oxo analogue, and then their revised structure (cf. Burgett et al. and references therein<sup>251</sup>), with a second formal synthesis by Nicolaou’s group being reported<sup>252</sup> very recently. The details of the interactions of these compounds with tubulin, demonstrating that the revised structure is biologically identical with isolated diazonamide A, are given by Cruz-Monserrate et al.<sup>253</sup>

**Eleutherobin (47)** from the Australian octacoral *Eleutherobia* sp., originally isolated by Fenical’s group,<sup>254</sup> then reisolated from the Caribbean octacoral *Erythropodium caribaeorum* by Andersen et al.<sup>255</sup> and recently reported from whole organism aquaculture by Andersen et al.<sup>256</sup>

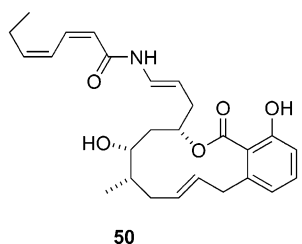
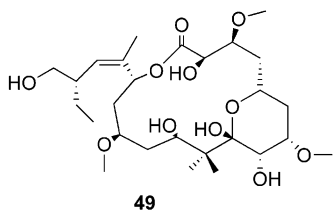
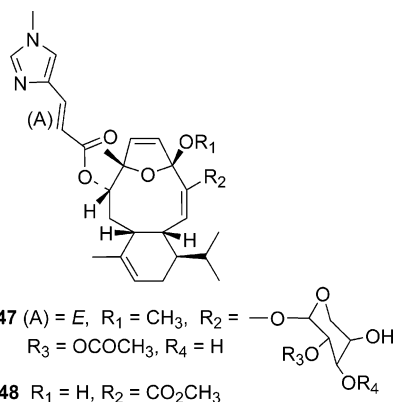


**Sarcodictyins (48)** from the Mediterranean corals *Sarcodictyon roseum* and *Eleutherobia aurea* by Pietra’s group.<sup>257,258</sup> These were originally reported as compounds without biological activity, and then their activity versus tubulin was reported<sup>259</sup> by a group from Pharmacia-Upjohn at the 1997 AACR meeting.

What is of great interest in the case of these compounds and the eleutherobins are the combinatorial chemistry syntheses that Nicolaou’s group reported in a series of papers in the late 1990s, which permitted formation of hybrid molecules of the two base structures. These results are discussed in more detail by Newman et al. and by Kingston and Newman; the interested reader should consult those publications<sup>5,260</sup> for further information.

**Peloruside A (49)** by Northcote’s group from the New Zealand marine sponge *Mycale hentscheli*.<sup>261</sup> The biological activity of this compound demonstrating induction of apoptosis following a G<sub>2</sub>-M arrest was recently reported by Hood et al., and as they point out, its relatively simple structure may lend itself well to synthetic modifications.<sup>262</sup> As noted by Ghosh and Kim<sup>263</sup> in their paper reporting their enantioselective synthesis of the C<sub>1</sub>–C<sub>9</sub> segment, it also has structural similarities to the epothilones. Their synthesis may significantly aid in the production of enough material to further evaluate the full potential of this compound.

**Vo-ATPases, Salicylhalimide A.** ATPase enzymes occur throughout eukaryotes, and their prime function is to pump hydrogen ions from one side of a membrane to the other. These particular ATPases perform this function within vacuoles in the cell and are dependent upon ATP for the necessary energy to perform the function. In 1997, Boyd’s group at the NCI reported on the discovery and isolation of two closely related very cytotoxic novel macrolide structures, salicylhalimides A (50) and B from the



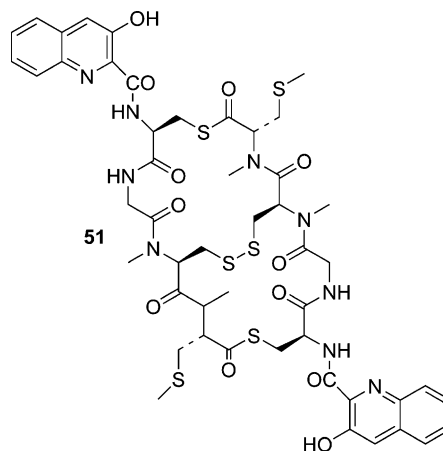
Western Australian marine sponge *Haliclona* sp., with GI<sub>50</sub> values below 1 nM for sensitive melanoma lines in the NCI 60 cell line screen.<sup>264</sup> These agents were “COMPARE-negative” as far as the NCI’s standard agent database was concerned, but did show patterns similar to the bafilomycin and concanamycin derivatives, compounds known to exhibit Vo-ATPase inhibitory activity but to be too toxic for human use, though bafilomycins have been used for plant protection.

Subsequent work from the same laboratory expanded the range of the structures to include another marine-derived product, the lobatamides, and both were shown to be specific for the higher eukaryotic Vo-ATPases but not the fungal equivalents. Subsequently, similar molecules have been isolated from bacteria and fungi.<sup>265,266</sup> Although syntheses have been published, to date, no formal in vivo assays of the original compounds have been performed due to a lack of the natural source from Western Australia; it is hoped that this paucity of source material will be solved in the near future through collaborations with Australian scientists in Australia, thus permitting a formal evaluation to occur under defined conditions.

This overall class of compounds is of interest not only as antitumor agents but also in bone resorption; thus they may have utility in osteoporosis. Two recent reviews should be consulted for further information: the first by two of the original discoverers,<sup>265</sup> and the second, giving fuller details of synthetic schemes by Yet.<sup>266</sup>

**Inhibitor of DNA Polymerase  $\alpha$ , Thiocoraline.** Although there are many hundreds of reports of microbial products from terrestrial sources, and now a number of compounds from marine microbes that are active biologically against tumor and other cells,<sup>267</sup> other than the compounds from cyanophytes, very few have moved into advanced preclinical trials. One that has is the thiopeptide thiocoraline (**51**), originally reported from a

marine actinomycete, *Micromonospora marina*, collected off the Mozambique coast.<sup>268,269</sup> This compound demonstrated activity against a variety of subpanels in the NCI’s 60 cell line screen, including breast, colon, renal, and melanoma, and was reported by PharmaMar scientists to have in vivo activity. Subsequently, they demonstrated that the probable MOA was inhibition of DNA polymerase  $\alpha$  activity by using a primer extension assay where inhibitory concentrations mimicked those required for cell inhibition. However, further work must be performed before this is confirmed as the actual MOA of this compound.



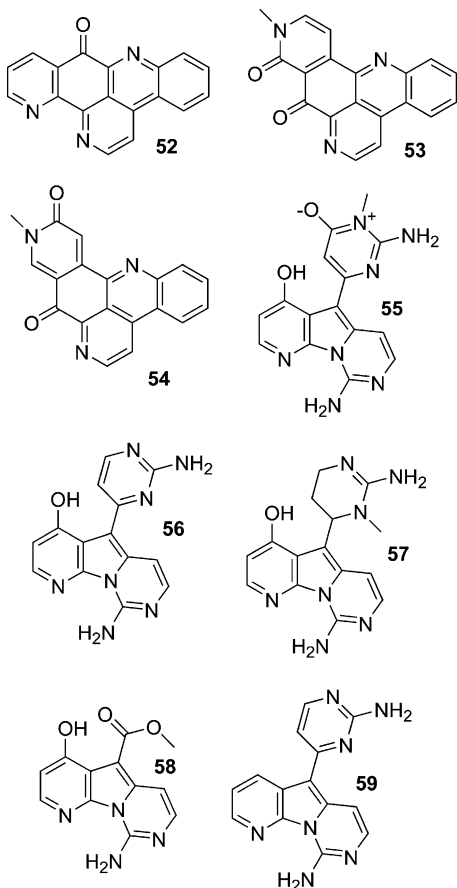
#### Reductive DNA-Cleaving Agents, Ascidiemnin.

These agents and their manifold structures are the subject of an excellent review by Delfourne and Bastide,<sup>270</sup> which built on previous 1993 and 1999 reviews (cf. references in Delfourne and Bastide). Recently, in addition to this review, specific agents either have had their mechanisms elucidated or are in the process of being modified to produce more active agents. Thus the work by Delfourne et al. around the ascidiemnin structure (**52**) has led to semi-synthetic compounds that exhibit submicromolar activity against some of a panel of 12 human tumor cell lines. Further iterations on the structures are underway.

As an example of the novel mechanism of a natural product of this class, the recent paper by Marshall et al.<sup>271</sup> has demonstrated that neoamphimedine (**53**) but not its regioisomer amphimedine (**54**) is active in in vitro and in vivo experiments at a level comparable to etoposide and appears to interact with topoisomerase II but does not stabilize cleavable complexes, unlike all other currently used topoisomerase II inhibitors.

**Potential Cyclin-Dependent Kinase (Cdk) Inhibitors. Variolins.** In 1994, the New Zealand group of Blunt and Munro reported<sup>272,273</sup> the isolation of a series of compounds, the variolins (**55–58**), from the Antarctic sponge *Kirkpatrickia variolosa*. These compounds had a ring system, a pyrido[3',2':4,5]pyrrolo[1,2-c]pyrimidine, that had not been described from terrestrial or marine sources prior to their publication. Variolin B (**56**) was the most active, with activities reported against the murine P388 leukemia and *Herpes simplex* type I virus. The compound was licensed to PharmaMar for further development, and over the next few years, a variety of investigators published syntheses, with the first total synthesis by Anderson and Morris in 2001, being followed by a full paper in late 2003 from a group led by Alvarez<sup>274</sup> at the University of Barcelona covering in detail the total syntheses of variolin B and deoxyvariolin B (**59**).

What is significant about these compounds is that in addition to demonstrating nanomolar level activity against

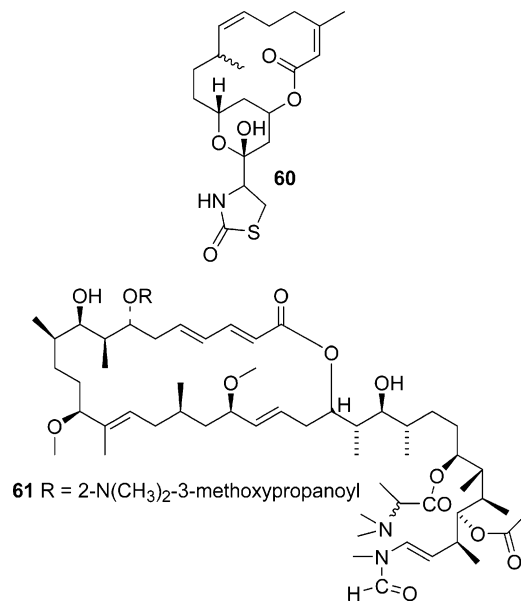


a variety of standard cell lines *in vitro*, such activity appears to be independent of p53 status,<sup>275</sup> and in Jurkat leukemia cells, but not in colon or breast carcinoma lines, very rapid apoptosis was observed in 4–6 h of exposure. Further experiments using flow cytometry indicated that the apoptosis may well be caspase 3-mediated. Additionally, no DNA strand breakage was observed in treated colon, breast, or Jurkat lines, and in *in vitro* enzyme assays at 100–1000 nM, the kinase activity of at least three different Cdk/cyclin complexes was inhibited.<sup>276</sup> These data are suggestive of these agents being novel Cdk inhibitors. Currently they are in preclinical development with PharmaMar.

**Actin-Active Agents.** Actin, an essential component of the cell's cytoskeleton, can in some ways be considered the "other component" to tubulin in the maintenance of cell shape, and now, following the publication of the recent work by Gachet et al.<sup>277</sup> (cf. the commentary by Nakaseko and Yanagida<sup>278</sup>), the role(s) of actin and its interplay with tubulin/microtubules in cell division is beginning to emerge, using, as the probe, latrunculin B (**60**), which was isolated from a marine sponge.

The marine environment has produced a significant number of extremely potent cytotoxic agents that have been shown to interact with G or F actin, or both. The initial agents from marine sources (there had been prior work with the fungal metabolites, the cytochalasins, and the plant metabolites, the cucurbitacins) were the latrunculins, and since the original report<sup>279</sup> from Kashman's group in 1980, a number of very different structural motifs, almost all from marine sources, have been shown to interact with actin.

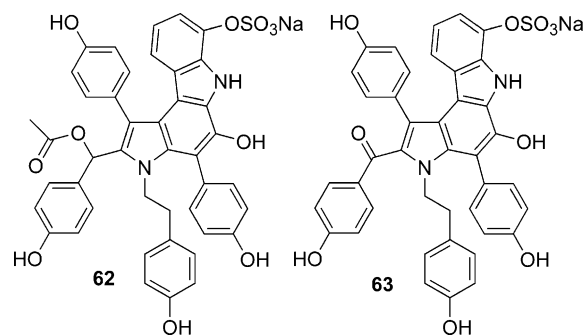
In a number of cases, very significant chemistry has been performed around the basic structures in order to determine the structure–activity relationships, as exemplified



by Yamada's group from Nagoya University and his collaborators on modifications around the basic aplyronine A (**61**) structure.<sup>280,281</sup> Recently, Yeung and Paterson<sup>282</sup> published an excellent review detailing the successful syntheses of swinholide A, scytophycin C, aplyronine A, mycalolide A, and a diastereomer of ulapualide A, all highly cytotoxic and all acting on various forms of actin.

However, despite very significant amounts of work on the isolation, identification, synthesis, and pharmacology of agents such as those referred to above, and also including derivatives of jaspamide/jasplakinolide, to date, no successful demonstration of realistic *in vivo* activity in animals has been reported. DTP/NCI scientists and their collaborators spent considerable amounts of time and money trying to demonstrate *in vivo* activity in mice with jaspamide and cucurbitacins and were not able to demonstrate a realistic therapeutic index.<sup>283</sup>

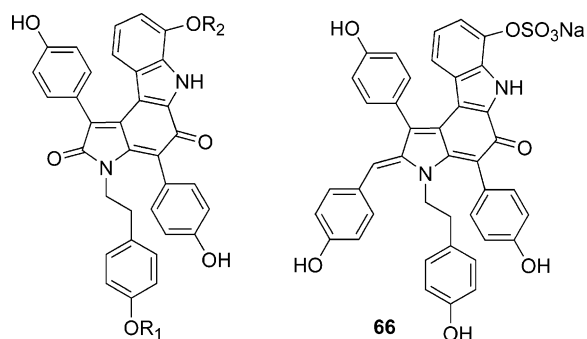
If it proves possible to synthesize a molecule that can be delivered via a targeting strategy (monoclonal antibodies, carrier peptides, polymeric linkers, etc.), then some of these exquisitely potent actin interactive agents may yet become viable drug candidates, but to date, none have been reported to have progressed beyond basic biological testing.



**Telomerase Inhibitors, Dictyodendrins.** Although synthetic compounds<sup>284</sup> and some natural products<sup>285</sup> have been reported to inhibit telomerases both *in vitro* and *in vivo*, no metabolite from a marine organism had been reported to have such an activity. Recently, Warabi et al. reported<sup>286</sup> the isolation of five new alkaloids, dictyodendrins A–E (**62–66**), from the Japanese sponge *Dictyodendrilla verongiformis* that demonstrated 100% inhibition of human telomerase at 50  $\mu\text{g}\cdot\text{mL}^{-1}$ , and, in what appeared



to be quite significant from an SAR aspect, the presence of a free hydroxyl rather than a sulfate ester apparently abolished all telomerase inhibitory activity.

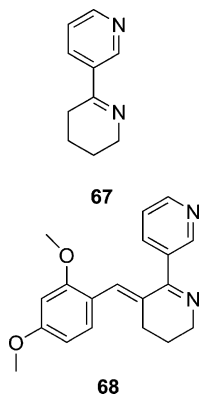


64  $R_1 = H; R_2 = SO_3Na$

65  $R_1 = R_2 = SO_3Na$

### Agents with Other Pharmacologic Activities

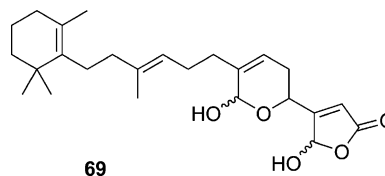
**Anti-Alzheimer's Activity. GTS-21.** In 1971, Kem et al. reported<sup>287</sup> the isolation of hoplonemertine toxin, a compound that subsequently became known as anabaseine (67). A variety of synthetic analogues of the basic structure were made by Kem's group, and one, DMXBA, which is also known by another acronym, GTS-21 (68), has been shown to have cytoprotective and memory-enhancing effects, perhaps due to the ability to displace the binding of nicotinic ligands and to affect the function of the  $\alpha 4\beta$  and  $\alpha 7$  subtypes of this receptor,<sup>288</sup> with the  $\alpha 7$  subtype in particular being thought to be important in the control of  $\beta$ -amyloid-mediated neurotoxicity.<sup>289</sup>



Following pharmacokinetic trials reported in 1998 by Kem's group,<sup>290</sup> DMXBA was licensed to the Japanese company Taiho by the University of Florida for clinical trials as a potential anti-Alzheimer's agent. It is currently in Phase I trials in both Europe and the United States under the auspices of Taiho for this indication. To date, there has been only one report from human trials; a paper published in 2003 that covered work up to about 18 months earlier demonstrated that normal healthy volunteers could tolerate treatment at up to 450 mg·day<sup>-1</sup>, and when compared to a placebo, significant positive cognitive responses were observed.<sup>291</sup> As reported from studies in rats by Kem et al., the major metabolite in the human trial was also the 4-hydroxy derivative, which is also pharmacologically active.<sup>292</sup>

**Antiinflammatory Compounds, Manoalide.** The quintessential marine-sourced compound that exhibited activity as an antiinflammatory agent was the sponge metabolite manoalide (69). This compound was originally

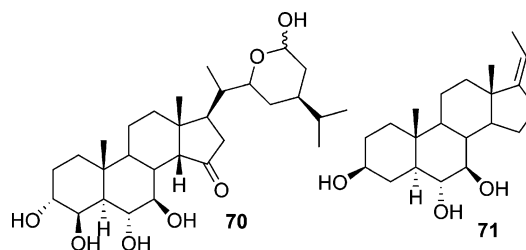
reported by Scheuer's group<sup>293</sup> following isolation from the marine sponge *Luffariella variabilis*, but this was a report of the compound as an antibiotic agent. The groups of Jacobs<sup>294-297</sup> and Dennis<sup>298,299</sup> independently established that this compound was a potent inhibitor of the enzyme phospholipase A<sub>2</sub>, which is intimately involved in the initial step of the inflammatory response.



Manoalide was subsequently isolated by Faulkner's group<sup>300</sup> while performing a thorough search for metabolites from a series of *Luffariella variabilis* organisms collected at various parts of the Palauan Islands. They demonstrated that manoalide could be obtained in fair yield from this sponge, and therefore it was considered to be a good candidate for drug development. The original compound was licensed to Allergan and placed into clinical trials as a topical antipsoriatic with a company code name of AGN-190093. It advanced to Phase II, but work on the natural product stopped, as sufficient quantities of the compound would not pass through the skin using the formulations developed for the trials. Another related compound (made by synthesis) was considered as a replacement, but no published results are currently available.

There are at least 14 derivatives listed in the Ensemble database at the time of writing that are structurally derived from the basic manoalide structure in that they have the pyrofuranone moiety as part of their structures. That scientists apart from those at Allergan and Lilly are still interested in these structures can be seen by the publication in 2000 by Scettri's group<sup>301</sup> of novel syntheses of this particular subunit of manoalide and the closely related cacospongiolides.

**Antiinflammatory Compounds, IPL-576092.** In 1992, Burgoyne and Andersen reported the isolation of contignasterol (70) from the marine sponge *Petrosia contignata* (Thiele),<sup>302</sup> with the absolute configuration being reported 10 years later.<sup>303</sup> Pharmacologic testing subsequently demonstrated that this compound, and, later, a series of chemically modified derivatives, could inhibit the release of histamine from rat mast cells<sup>304</sup> and also from the lung tissue of guinea pigs.<sup>305</sup> These findings and others (cf. Coulson and O'Donnell,<sup>306</sup> Shen and Burgoyne<sup>307</sup>) led to the introduction of IPL-576092 (71) into clinical trials as an



antiasthma agent by Inflazyme in conjunction with Aventis Pharma under the code number HMR-4011A. Inflazyme has reported on their web site that this compound successfully completed a Phase II "Allergen Challenge" trial in April 2002 as a novel oral therapy for asthma and that it is now in clinical trials for inflammatory diseases of the skin and eye. Aside from a repeat of this information on

DailyDrugNews.com, no formal publication appears to have yet been published.

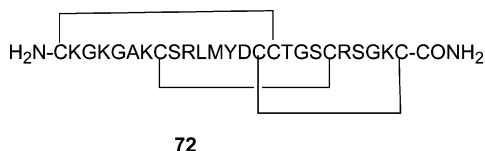
There are currently two further derivatives of the IPL576 series in clinical trials. One derivative, IPL512,602, has replaced IPL576,092 as their lead compound and entered Phase II trials in the United States in 2003. The other, IPL550,260, is currently in Phase I trials also against inflammatory processes. The structures of these last two compounds have not yet been published in the general literature, but it is reported that they are further derivatives of contignasterol (references in the Ensemble database that refer to the website <http://DailyDrugNews.com>).

#### Antiinflammatory Compounds, Pseudopterrosins.

These compounds, part of a complex mixture reported by Fenical's group<sup>308,309</sup> in 1986 from the Caribbean gorgonian *Pseudopterogorgia elisabethae*, have the distinction of being the first commercialized human use marine natural product. As a partially purified defined mixture, they are a constituent of the cosmetic "antiwrinkle cream" sold by Estee Lauder under the brand name "Resilience".

However, once the mechanisms were further delineated by Mayer et al.,<sup>310</sup> a simpler modification of the pseudopterrosins may have entered Phase I clinical trials as an antiinflammatory agent, though the structure and company were not listed in the reference to the trial,<sup>8</sup> nor can any other record be found using conventional search strategies.

**Analgesia and Other Activities, Conus Toxins.** The extremely complex mixtures of short (usually 10–35 residue) peptide toxins elaborated by the snails of the genus *Conus* have turned out to be a treasure trove of pharmacologically active materials, initially in the area of analgesia but now in inflammation and neurochemistry as well. In addition, the separated peptides have led to fundamental biochemical studies in voltage-gated channels of all types. A recent paper<sup>311</sup> by Olivera and collaborators (the person who has probably done more to realize the potential of these peptides than any other) should be consulted as a guide to the potential for these peptides in a variety of pharmacologic areas.



72

**Analgesia, the *Conus* Toxin Ziconotide.** This 25-residue peptide with three interlocking cystinyl bridges (72) was originally isolated by Olivera's group from *Conus magus* and was known as MVIIA toxin. It demonstrated a potent activity against voltage-gated  $\text{Ca}^{2+}$  channels, and because of its novel binding characteristics, Olivera coined the phrase "Janus Ligand" for this and other peptidic agents, as they appear to have both a "docking face" and a "locking face" at the receptor level. The peptide demonstrated significant effects as an analgesic and was licensed to Neurex Inc., who then proceeded to synthesize over 200 variations on the structure, deciding in the end that the original structure was optimal. The compound name went from MVIIA to SNX-111 to ziconotide when it entered clinical trials for neuropathic pain in the 1990s under Neurex.

Neurex was then purchased by Elan Pharmaceuticals, and an approvable letter was given to Elan by the FDA in early 2000. However, due to questions as to side effects, ziconotide (now with the trade name of Prialt due to a licensing arrangement with Warner Lambert, now Pfizer) is in further Phase III clinical trials to determine the effects

of intrathecal ziconotide in intractable pain. The first report of these trials (requested by the FDA after the previous approvable letter) has just been published.<sup>312</sup> The conclusions were that intrathecal ziconotide provided clinically and statistically significant analgesia in pain from cancer and AIDS. Elan is now planning to file an amendment to its NDA in the near future and hopes to launch in early 2005 in the United States. An application was also submitted in the middle of 2003 for approval in the EU.

**Analgesia, Other *Conus* Toxins.** The potential for toxins to become drugs is high, and the recent review by Lewis and Garcia<sup>313</sup> should be consulted for detailed information on these agents from all sources, not just marine. In the specific case of other *Conus*-derived peptides, one, CGX-1007 (conantokin G), which was in Phase I trials for neuropathic pain and intractable epilepsy, is now discontinued according to the Proux Ensemble database, and CGX-1160 (contulakin G from *Conus geographus*, a neurotensin agonist) is reported to be in Phase I clinical trials sponsored by Cognetix. In addition to these two, there are at least four other toxins in preclinical studies; these are the following.

**CGX-1063:** Thr10-contulakin G, modified from the natural product by Cognetix.

**ACVI:**  $\alpha$ -conotoxin Vc1.1 from *Conus victoriae* for neuropathic pain, in preclinical development by the University of Melbourne.

**AMM336:**  $\omega$ -conotoxin CVID from *Conus catus* for severe morphine-resistant pain.

$\chi$ -conotoxin MR1A/B for neuropathic pain.

For readers who wish to keep up to date on the potential of metabolites from the *Conus* species, there is an excellent web site that is devoted to this genus, with links to both scientific papers and to ephemera, at the URL <http://grimwade.biochem.unimelb.edu.au/cone/main.html>.

**Antimicrobials, Antimalarials, and Anti-HIV Agents.** Predominately due to the sources of past funding, the majority of the pharmacological activities that have been reported for marine metabolites have been in the anticancer arena. However, there are now some significant reports of activities from a particular class of metabolites, the manzamines, as potential drugs or leads to drugs that might be effective as antimalarial, anti-TB, and other infective agents. As examples, Hamann's group at the University of Mississippi, in conjunction with Hill's group at the Center of Marine Biotechnology (COMB) at Maryland, have been investigating the chemistry and microbiology of deep-water sponges collected in Indonesia and have recently published a series of papers<sup>314–316</sup> and reviews<sup>317–319</sup> covering such activities with the metabolites isolated from the sponges and also (vide infra) of metabolites obtained by fermentation of the commensal microbes. These should be consulted for specific agents/techniques, but the list of activities and their potential as leads in these areas is very significant, particularly as new antibiotics are desperately needed for such infections.

Although the psammaplins were used by Novartis to generate the novel HDAC inhibitor referred to earlier, the base structure was also recently utilized by Nicolaou et al. as the starting point for a total synthesis of psammaplins A and then modified by use of a combinatorial scrambling strategy to produce a library of 3828 members, six of which demonstrated minimum inhibitory concentration (MIC) levels in methicillin-resistant/intermediate vancomycin-resistant strains of *Staphylococcus aureus* at  $<1 \mu\text{g}\cdot\text{mL}^{-1}$ ,

thus demonstrating the power of modern combinatorial techniques when applied to a base active structure from nature.<sup>320</sup>

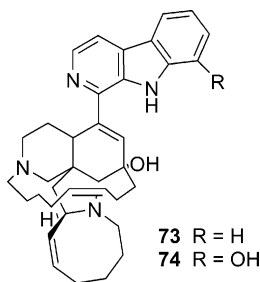
### Are Microbes the Actual Source of Many Marine-Derived Metabolites?

The continuing question of whether symbiotic (and/or commensal) microbes are the actual producers of cytotoxic and other metabolites is being investigated by a variety of groups, and as information evolves, the probability increases that a very significant number of marine-sourced, polyketide-derived compounds and non-ribosomally produced peptides are of microbial origin. A recent review by Janin<sup>249</sup> shows the structures of a large number of such molecules.

As can be seen by inspection of the structure of Et743, it bears a close similarity to the microbial metabolites saframycin B, naphthridinomycin, and safracin and to invertebrate metabolites of the renieriamycin/jorumycin class. There are many other agents from marine sources such as the mycalamides and onnamides that have structures or partial structures similar to molecules isolated from terrestrial or other marine phyla, and it was posited from such circumstantial evidence that a significant number of ostensibly marine-derived compounds, particularly from the *Porifera*, were in fact derived from commensal and/or symbiotic microbes.

A recent and relevant example is the one given by Piel on the production of pederine by the pseudomonad symbiont from *Paederus* beetles referred to earlier. In a presentation at the 2003 SIM meeting, Piel gave further information that demonstrated the formation of a very close structural relative of the marine sponge cytotoxins, the onnamides, by this terrestrially derived PKS system.<sup>321</sup>

However, it was not until the autumn of 2003 that there was other than noncircumstantial evidence of production, when Hill (University of Maryland) and Hamann (University of Mississippi) reported at the 6th IMBC on their work with a purified commensal *Micromonospora* sp. isolated from a deep-water Indonesian sponge. They demonstrated that the microbe, when grown in a laboratory setting, under certain culture conditions with specific media, but not with others, produced manzamine A (**73**) and 8-hydroxymanzamine A (**74**), compounds that were isolated from the sponge itself following wild collection.<sup>322,323</sup>



### Conclusion

As has been demonstrated in this review, the potential for marine natural products as sources and/or leads to drugs that cover a very wide range of pharmacological effects (i.e., cancer, anti-infectives, analgesia, Alzheimer's disease, inflammation, immunomodulation) is only now being realized. It is probable that within the next two years at least one marine-derived novel agent will enter commerce as an anticancer or analgesia drug following governmental approval.

Perhaps the most important current discovery, however, is the proof with manzamine A that, as suspected by many investigators over the years, a commensal microbe isolated from the invertebrate and cultured in a laboratory setting is the actual producer of the metabolite.

In closing, the vast repertoire of structures that have so far been identified from marine invertebrates frequently have no comparable equivalent in terrestrial organisms. This dichotomy will only increase as more and more investigators use marine-derived agents as bioprobes, scaffolds for synthesis, and drug leads and/or candidates. The work by (predominately) young investigators on the genetic control of biosynthesis in the commensal and/or symbiotic microbes associated with these invertebrates, or on the microbes isolated from shallow and deep sediments, will only increase the numbers of structural types available for further work. The surface has hardly been scratched as yet!

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