Natural Products from Marine Invertebrates and Microbes as Modulators of Antitumor Targets

D.J. Newman* and G.M. Cragg

Natural Products Branch, Developmental Therapeutics Program, NCI-Frederick, Frederick, MD, 21702, USA

Abstract: Over the last twenty-five to thirty years, exploration of the marine fauna and microbial flora has progressed from a random search by natural product chemists who liked to dive and wished to combine their hobby with their profession, to fully integrated programs of systemic investigation of the chemical agents elaborated by marine organisms of all phyla (as presumably defensive agents against predators) for their potential as leads to human-use drug candidates where the putative mechanisms have been identified as modulation of, and/or interaction with, potential molecular targets, rather than just exhibiting general cytotoxicity. This review is not exhaustive but is meant to cover the highlights of such agents and is arranged on a (nominal) target basis rather than by organism or chemical class.

Key Words: Marine natural products, molecular targets, cancer, structural modification.

INTRODUCTION

As mentioned by Kingston and Newman [1], effectively all secondary metabolites from any source can be considered as the products of a process of "natural combinatorial chemistry". This is either because they are the products of genes that have frequently been "shuffled" between taxa, or because they are the products of what might be described as "co-metabolism" whereby a molecule is biosynthesized by one organism and then modified by another. What may be considered in retrospect as the earliest example of this from the marine environment, would be the discovery by Bergmann *et al.* [2-4] of the compounds, spongouridine (**1**) and spongothymidine (**2**) from the Caribbean sponge, *Cryptotheca crypta*. What was significant about these materials was that they demonstrated for the first time that naturally occurring and bioactive nucleosides could be found containing sugars other than ribose or deoxyribose.

These two compounds effectively revolutionized the then current dogma as they demonstrated that biological systems would recognize the base and not pay too much attention to the sugar moiety. In fact they may be considered the prototypes of all of the modified nucleoside analogues made by chemists that have crossed the anti-viral and anti-tumor stages since then as chemists began to substitute the "regular pentoses" with acyclic entities, and with cyclic sugars bearing unusual substituents. These experiments led to a vast number of derivatives that were tested extensively as anti-viral and anti-tumor agents over the next thirty plus years. Suckling [5] showed how such structures evolved in the (then) Wellcome laboratories, leading to azidothymidine (AZT) and incidentally, to Nobel Prizes for Hitchens and Elion, though no direct mention was made of the original arabinose-containing leads from natural sources.

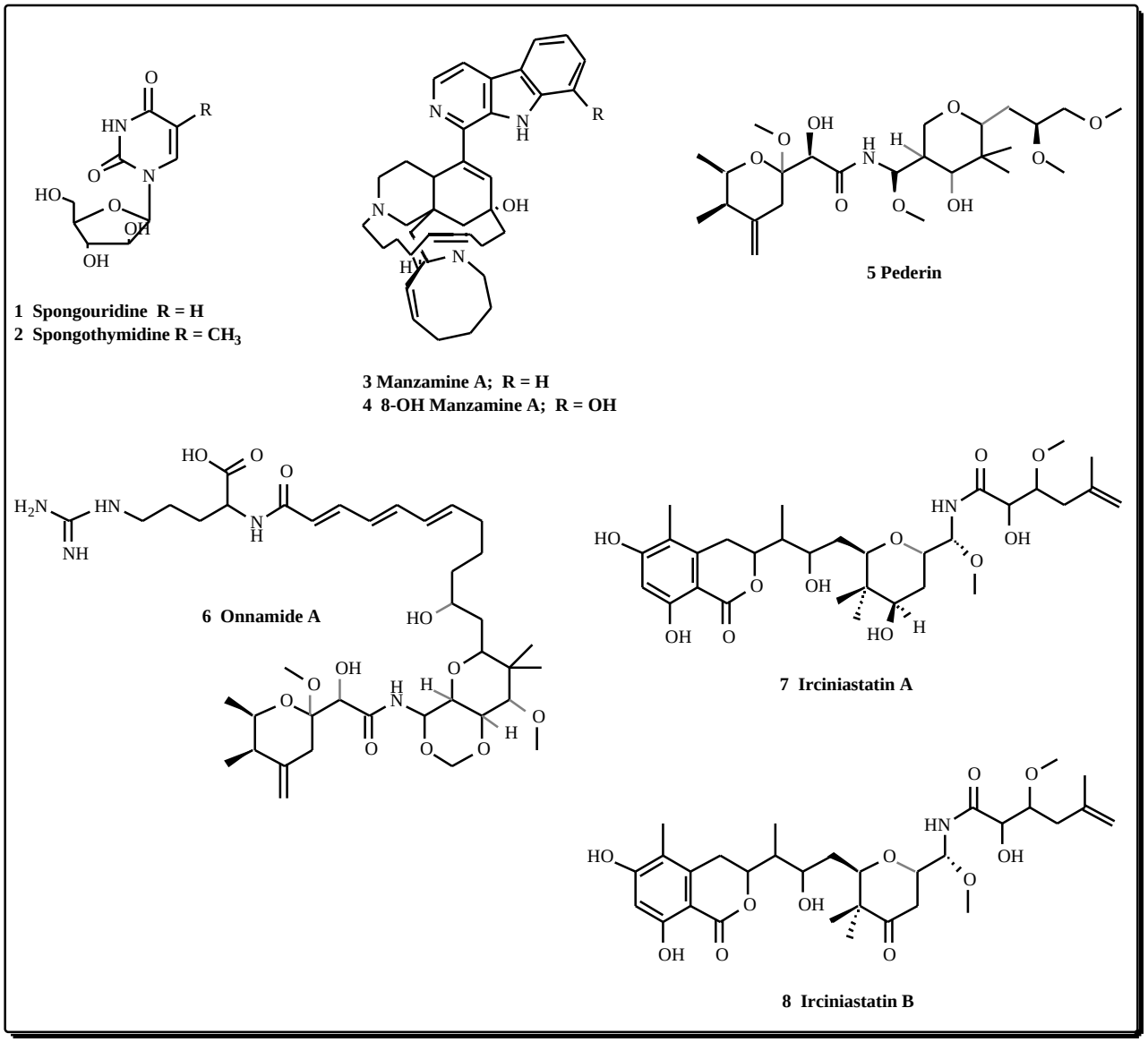
Showing that "Nature may follow chemists rather than the reverse, or conversely that it was always there but the natural products chemists were slow off the mark", arabinosyladenine (Ara-A or Vidarabine®), was synthesized in 1960 as a potential anti-tumor agent [6], was later produced by fermentation [7] of *Streptomyces griseus*, and was isolated together with spongouridine [8] from a Mediterranean gorgonian (*Eunicella cavolini*) in 1984. This example is circumstantial evidence for the involvement of microbes in marine invertebrate-sourced metabolites, but now there is very good evidence that what was frequently suggested from a comparison of marine- and terrestrial-sourced metabolite structures, is in fact the case.

Thus in the case of manzamine A [9,10] the base molecule (**3**) and the 8-hydroxy derivative (**4**) have both been isolated from a laboratory fermentation of a *Micromonospora* sp. cultured from the sponge from which manzamine A was isolated. In contrast, in the case of the pederin-based metabolites, the base structure pederin (**5**) is the product of a commensal pseudomonad isolated from the *Paederus* beetle [11-13]. In the case of the pederin analog onnamide A (**6**), it has recently been demonstrated by a metagenomic study that the producing genes can be isolated from the invertebrate, and that the pathway contains the correct genetic constructs to produce onnamide [14]. These reports effectively demonstrate that the 35 so far reported pederin-based molecules from marine invertebrates, including the irciniastatins A (**7**) and B (**8**) [15], are almost certainly products of commensal microbes and are sequestered and, in some cases modified by the host invertebrate. We should note that irciniastatin A was recently reported by the Crews' group under the name of psymberin [16], but the Pettit's group manuscript describing the same molecule was submitted earlier and thus the name irciniastatin A has precedence. A very recent review by Piel *et al.* has reported further on the marine-derived pederin family of compounds and should be consulted for further details, particularly the genetic aspects of the work [17].

Since the body of the review is arranged around projected mechanisms for the metabolites, some of the compounds referred to will be seen again. Thus in the following pages

*Adddress correspondence to this auuthor at the Natural Products Branch, Developmental Therapeutics Program, NCI-Frederick, Frederick, MD, 21702, USA; E-mails: dn22a@nih.gov, newmand@mail.nih.gov

1389-4501/06 $50.00+.00



1 Spongouridine R = H
2 Spongothymidine R = CH_3

3 Manzamine A; R = H
4 8-OH Manzamine A; R = OH

5 Pederin

6 Onnamide A

7 Irciniastatin A

8 Irciniastatin B

we will discuss the relatively recent compounds whose origins are nominally from marine invertebrates and from microbes that have been directly isolated from mainly seabed sediments. Occasionally, we will note where the producing organism is not the one from which the metabolite was nominally obtained in earlier investigations.

The list of targets is not exhaustive but does include tubulin interactive agents, proteasome inhibitors, histone deacetylase inhibitors, DNA interactive agents, protein kinase and protein phosphatase inhibitors, protein synthesis/*JNK* modulators and chemical manipulations of structures to derive other types of inhibitors. Two reviews of marine anti-tumor agents have recently been published. One that covers most if not all compounds that have been in clinical trials irrespective of mechanism [18] and the second on a smaller scale that covers some of the same ground but includes more discussion of the potential producing organisms [19].

TUBULIN INTERACTIVE AGENTS (TIAS)

The prototypical agents with tubulin as their target are the *Vinca* alkaloids and colchicines from the 1950s to 1960s and the seminal discovery in the early 1980s by Horwitz that Taxol® although a tubulin interactive agent, exhibited an entirely different mechanism of action during its interaction with tubulin, by causing the tubulin to stabilize rather than continuing to assemble and disassemble during the normal mitotic processes of the cell cycle. A very recent review by Mollinedo should also be consulted for information on mechanisms that microtubule-targeting drugs may be involved in in addition to those shown under specific agents discussed below [20]. Although all of these agents were of plant origin (though there is debate in the literature about the taxanes actually being of microbial origin [21]), it was obvious to begin to look for agents from the marine environment that might also demonstrate a similar activity. To date there are a significant number of "different" structures that in two

dimensions do not resemble taxanes but do have a similar mechanism of action. These include the tubulin stabilizers sarcodictyins / eleutherobin, discodermolide, laulimalide, dictyostatin, peloruside, and diazonamide. There are another series of marine-derived or marine-associated compounds that exhibit effects on tubulin polymerization interactions that are similar in principle to those of the vinca or colchicine alkaloids, including the dolastatins, halichondrin derivatives, the hemiasterlins and cryptophycins (though the latter are not commented on here as they were covered extensively in two recent reviews [18,19]), plus others such as vitilevuamide that that interact at an unique site also interfering with tubulin polymerization. A recent review of peptides that shows more details of the interactions of some of these agents, and also compares them with similar agents form other natural sources has recently been published by Janin and should be consulted by the interested reader [22].

These agents are discussed below with the non-paclitaxel-like agents being discussed first, since the first marine-derived tubulin interactive agents, the dolastatins, were headed for clinical trials before their probable mechanism(s) were identified, followed by tubulin stabilizers.

MOLECULES WITH *VINCA*-LIKE ACTIVITIES

The Dolastatins

The dolastatins are a series of cytotoxic peptides that were originally isolated in very low yield by Pettit's group [23-27] as part of its work on marine invertebrates from the Indian Ocean mollusk, *Dolabella auricularia.* Due to the potency and mechanism of action of dolastatin 10 (**9**), a linear depsipeptide which was shown to be a tubulin interactive agent binding close to the *vinca* domain in a site where other peptidic agents bound [28,29], 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 cGMP conditions to commence trials [23]. Dolastatin 10 progressed through to Phase II trials as a single agent, but it did not demonstrate significant antitumor activity in a Phase II trial against prostate cancer in man [30]. Similarly, no significant activity was seen in a Phase II trial against metastatic melanoma [31].

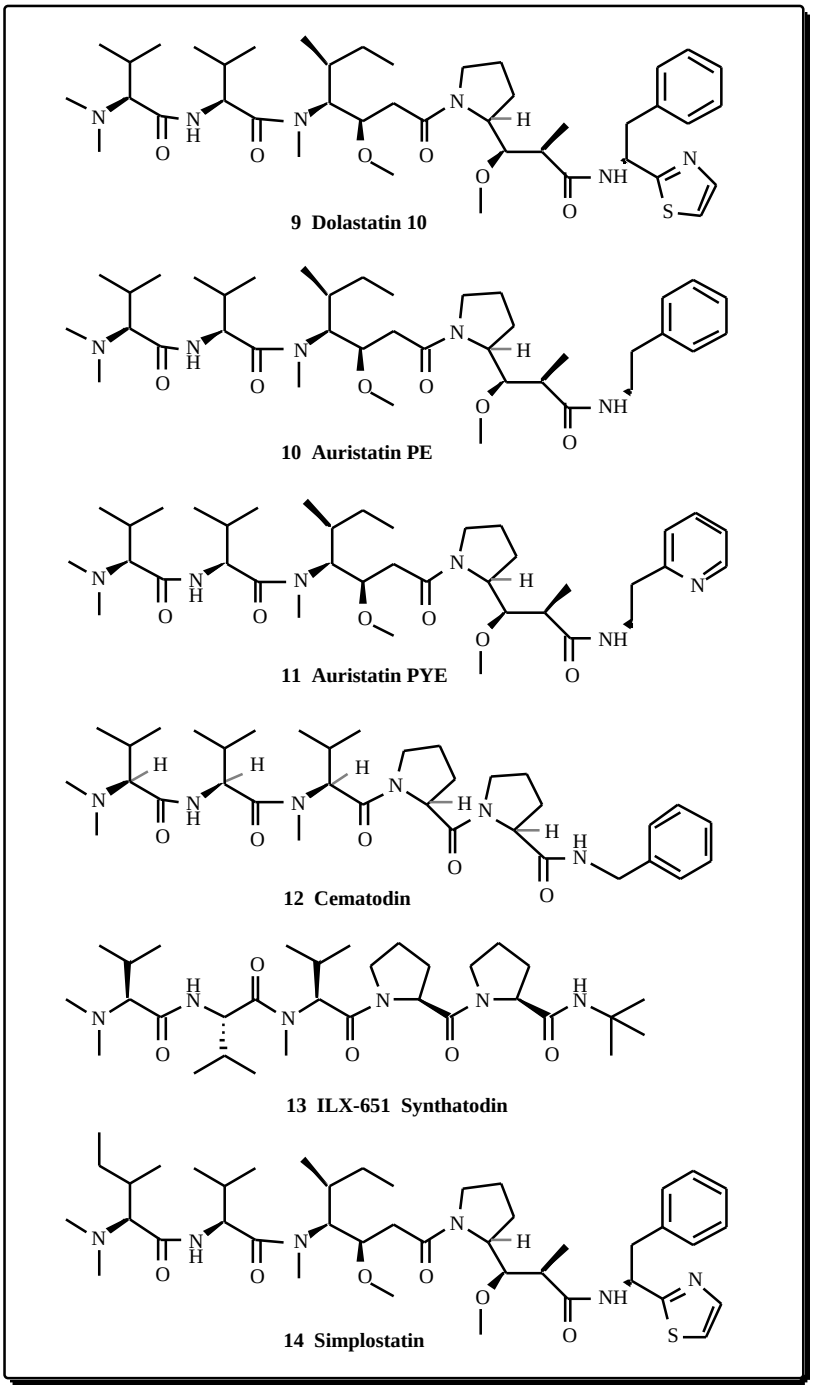
As a result of the synthetic processes alluded to earlier, many derivatives of the dolastatins have been synthesized with TZT-1027 (Auristatin PE or Soblidotin) (**10**) now in Phase II clinical trials in Europe, Japan and the USA under the auspices of either Teikoku Hormone, the originator, or the licencee, Daiichi Pharmaceuticals. Very recently, another variation of dolastatin 10, auristatin PYE (**11**) where the R_4 position was replaced by a substituted pyridyl compared to dolastatin 10, was reported to have *in vivo* efficacy in a colon adenocarcinoma model and to bind to tubulin [32]. Another derivative of dolastatin15 known as Cematodin (**12**) or LU-103793, was entered into Phase I clinical trials by the Knoll division of Abbott GMBH for treatment of breast cancer. It progressed into Phase II, but trials appear to have been discontinued.

However, an orally active third generation dolastatin 15-based molecule, known under the names of BSF-223651, LU-223651, ILX651 or Synthadotin (**13**) is now in phase II clinical trials for non-small cell lung, prostate and melanoma carcinomas following two reports [33,34] in 2003 of responses in Phase I studies in melanoma, breast and non-small-cell lung in patients. It was licensed by Ilex from BASF Pharma (and Ilex was purchased in late 2004 by Genzyme) and there have been six scientific reports in the last two years on the Phase I studies with this agent, either as presentations at ASCO meetings [35-38], the American Association for Cancer Research (AACR) meeting [39] or the joint US-European (AACR-NCI-EORTC) molecular targets meeting [40].

The pattern has continued in that the current data is also from abstracts presented at the 2004 ASCO meeting but what is very interesting is that the compound is now postulated to function in a manner different from the classical tubulin inhibitors, irrespective of whether they are the classical or the taxane variety, as the current putative mechanism for this molecule is inhibition of microtubule nucleation [41-43]. It will be very interesting to see what a full-fledged paper on this mechanism will provide in the way of evidence. This reporting *via* abstract format is still continuing with the very recent abstract by Edler *et al*. [44] stating that the active metabolite of dolastatin 15, cematodin and synthadotin, the pentapeptide N,N-dimethylvalyl-valyl-N-methylvalyl-prolyl-proline is in fact much more potent than the three "parent" compounds in its inhibition of tubulin assembly with an IC_{50} of 1 μM, almost equivalent to that of dolastatin 10 under the same assay conditions (0.8 μM), rather than the 4-10 μM levels for the other compounds.

Using a tritium-labeled dolastatin 15 as a bioprobe, Hamel's group at NCI [45] 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. The study of the interaction of radio labeled ILX651, and the "metabolite" is currently underway [44] in order to compare synthadotin etc., with the base molecule for this agent, dolastatin 15 in order to discover what "type of binding" occurs but the results will not be presented before this review is submitted.

In a similar manner to bryostatin, there was always a potential question with the dolastatins as to whether or not they were microbial in origin, as peptides with unusual amino acids have been well documented in the literature as coming from the *Cyanophyta* and the mollusk from which the peptides were isolated was known to feed on these microbes. In the last few years, this supposition has been shown to be fact as in 1998, workers at the Universities of Guam and Hawaii reported the isolation and purification of simplostatin 1 (**14**) from the marine cyanobacterium *Simploca hynoides* [46]. 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* [47]. 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). Recently, the mechanism of action of symplostatin 1 was



9 Dolastatin 10

10 Auristatin PE

11 Auristatin PYE

12 Cematodin

13 ILX-651 Synthatodin

14 Simplostatin

shown to be similar to dolastatin 10 but to be somewhat more toxic to mice at comparable doses [48].

HALICHONDRIN B AND DERIVATIVES

Halichondrin B (**15**) is one of a series of compounds originally isolated and reported [49,50] by Uemura *et al.* in 1985 from the Japanese sponge *Halichondria okadai*. Following these initial papers, other investigators reported similar materials from a number of sponges from other areas of the Pacific and Indian Oceans, including *Axinella* sp. from the Western Pacific [51], *Phakellia carteri* from the Eastern Indian Ocean [52], and particularly from a deep water *Lissodendoryx* sp. off the East Coast of South Island, New Zealand [53]. Although there was enough halichondrin B available from a variety of sources for some initial experiments and to determine that the possible mechanism of action was as an tubulin interactive agent, affecting tubulin depolymerization at a site close to, but distinct from, the *vinca* site [54-56] and to show initial *in vivo* activity [57], 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 nominally 1 $mg.Kg^{-1}$ wet weight) and the National Institute for Water and Atmospheric Research (NIWA), were 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 meters and greater by trawling. Following extensive work up, these samples produced 300 milligrams 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 meters 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 [58]. Kishi's synthetic methods were then utilized by the US division of the Japanese pharmaceutical company, Eisai, to synthesize a large number of variants of halichondrin B, particularly smaller molecules that maintained the biological activity but were intrinsically more chemically stable, due to the substitution of a methylene group for the ester oxygen in the macrolide ring, thus producing a much more stable ketone-containing ring. Following a presentation by NCI/DTP scientists at the 1998 AACR meeting on their results with the New Zealand-derived halichondrin B (which had a significantly lower toxicity in animals than the materials used in earlier studies) [59], the Eisai Research Institute and DTP evaluated two of these agents against the NZ halichondrin B. One of the compounds, originally ER-086526 (NSC 707389) and now renumbered E7389 (**16**), was approved by the then NCI's Decision Network (now Drug Development Group) in 2001 and 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 [60] in the current Phase I trial demonstrating that levels above those required for cytotoxicity *in vitro* were achievable for up to 72 hours at doses below the DLT of 0.5 $mg.M^2$; 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 [61] orders of magnitude below the 1500 pM levels achievable in man. In 2004, further evidence of possible mechanisms of E7389 were published by workers from Eisai with evidence that pointed towards induction of apoptosis at concentrations in the 10 nM level or higher where complete mitotic blocks were observed in U937 cells, together with hyperphosphorylation of Bcl-2, which may indicate a role for this pathway in the apoptotic effects of E7389.

Details of the biology and chemistry of this compound and other compounds in the series had been published by both the Harvard [62] and Eisai scientists [63], and recently some further work was reported from the Eisai group and their collaborators [64,65]. These reports amply demonstrate the power of current synthetic chemistry when applied to a very potent marine-derived natural product and by using variations on the synthetic techniques described, enough cGMP material, produced by total synthesis, was provided to the NCI for the initial clinical trials.

HEMIASTERLIN DERIVATIVES

Hemiasterlin (**17**) was originally reported by Kashman's group [66] 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 *Pseudoaxinyssa* sp. but, due to a taxonomic revision, is now a *Cymbastela* sp. This particular sponge produced a number of peptides, including geodiamolides A to F, hemiasterlin as described by Kashman, two novel hemiasterlins, A (**18**) and B (**19**), and other geodiamolides and criamides [67].

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 [68]. Further investigations by Hamel's group using hemiasterlin isolated at NCI [69] indicated that this peptide, together with cryptophycin 1 and dolastatin 10, inhibited tubulin assembly and probably bound [70] at what is being called the "peptide binding site".

In the intervening time, 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 [71]. 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 [72]

The hemiasterlins, including the analogues made by Andersen's group, which included HTI-286 (**20**) which was known by Andersen's group [73] as Synthetic Peptide Analogue (SPA) 110 and that now has the generic name of taltobulin, 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, but as reported [74] at the 2002 AACR Meeting the original agent was still superior.

Following these reports, a full paper giving details of the *in vitro* and *in vivo* animal data was published by Loganzo *et al.* in 2003 [75] and a subsequent presentation at the 2003 AACR meeting gave some very interesting data on HTI286-dolastatin 10 hybrids [76] where the tubulin binding site ap-

peared to be similar for both the dolastatins and HTI-286. The hybrids were also much more active than dolastatin 10 in cells that expressed the P-glycoprotein efflux pump. A much more detailed report on the methods used and the biological activities of HTI-286 and analogues was published in 2004 by Zask *et al*. and should be consulted for specific methodologies [77]. Further work at Wyeth indicated that a photo affinity analogue of HTI-286 cross-linked α-tubulin at a site within the 314-339 residues, distant from the colchicine site and further evidence that the binding sites of these two agents differed on tubulin was obtained by the Wyeth group by use of a stilbene derivative of HTI-286 and sedimentation centrifugation experiments [78].

From a resistance mechanism aspect, the Wyeth group recently published two papers giving information at the molecular level of potential reasons why cells become resistant to HTI-286 but are still relatively sensitive to epothilones, taxanes and colchicine. It appears that point mutations in α and or β tubulins mediate microtubule stabilization and that there may also be an ATP-binding cassette drug pump that is distinct from P-glycoprotein, ABCG2, MRP1 or MRP3 [79,80].

Currently the molecule is in Phase I trials and is scheduled for Phase II with Wyeth and 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 CBD and also the NCI's own Letter of Collection (LOC).

In the abstracts of the 2005 AACR meeting, there are three very interesting posters from the Eisai group in the USA (the same group that just reported work on laulimalide derivatives and who have been working with the halichondrin B analogue), referring to an as yet unpublished hemiasterlin analogue known as E7974. In these abstracts, they report that this compound has low nM activity against tumor cells with a potentially high therapeutic index when compared to non-dividing quiescent human fibroblasts, with high potency against taxane-resistant lines and induces caspase-3 activation and PARP cleavage on short exposure to the agent, and photo-active analogues, like the Wyeth HTI-286 equivalent, preferentially photo-labeled α-tubulin [81-83]. Once the structure is available, it will be interesting to compare the Eisai molecule(s) with those from the University of British Columbia and Wyeth.

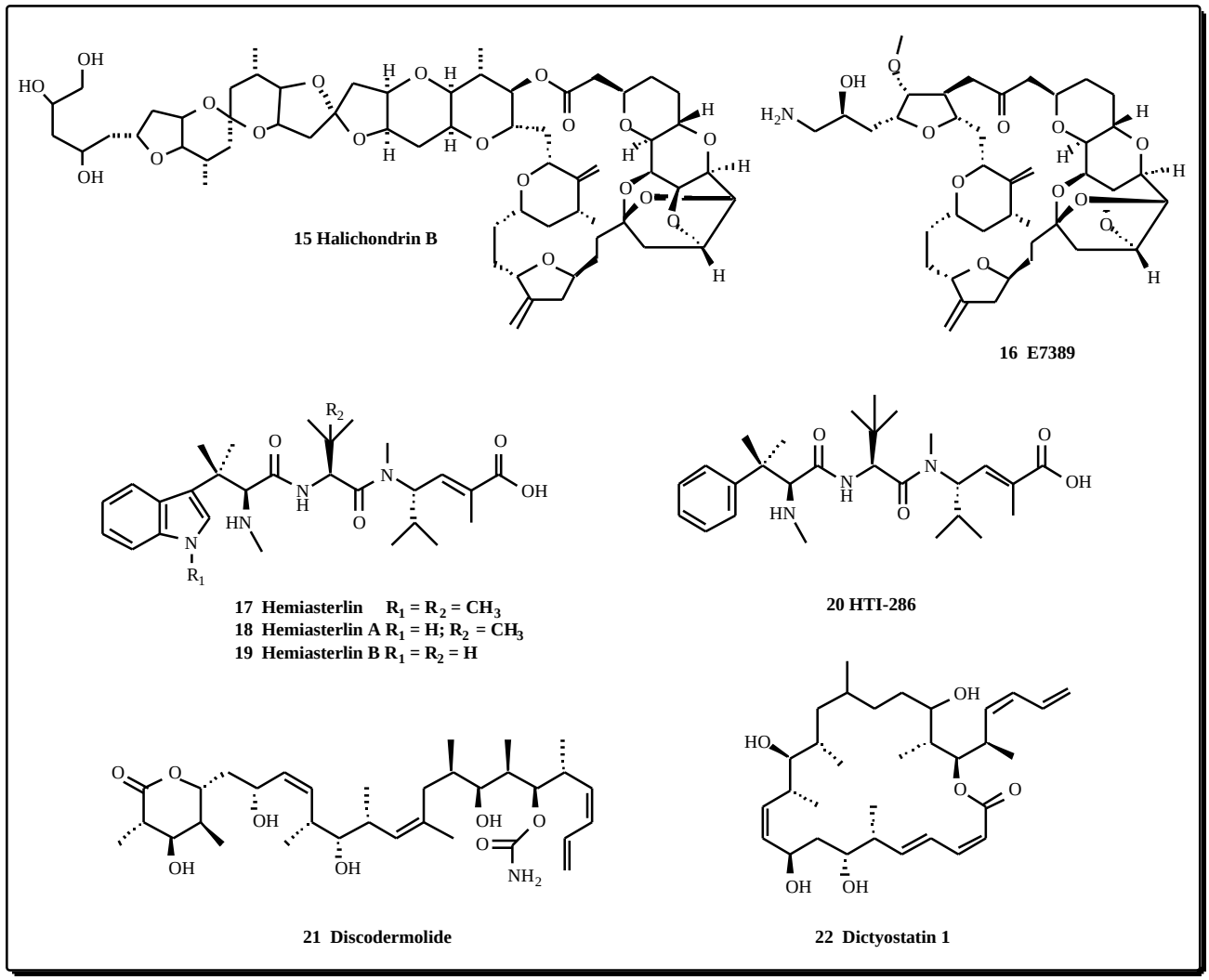
MOLECULES WITH TAXOL®-LIKE ACTIVITY

Discodermolide and Derivatives

Discodermolide (**21),** a polyhydroxylated lactone, was reported by the Harbor Branch Oceanographic Institution (HBOI) group in 1990 following isolation from the Caribbean sponge, *Discodermia dissoluta*, originally collected at a depth of 30 meters off the Bahamas [84], followed by subsequent collections at depths in excess of 100 meters using a manned submersible. The initial structure was incorrect and led to a revision to the stereochemistry being published the following year [85]. Originally, the compound was judged to be a new immunosuppressive agent and an incidental cytotoxin, but 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 [86]. The incorrect initial report from HBOI led to synthesis of the wrong (-) isomer by Schreiber's group before the revison was published [87], and this was followed by other groups synthesizing the same incorrect isomer. Subsequently other groups in the late 1990s to 2003 made the correct isomer, with Marshall and Johns [88], Halstead [89], Smith *et al.* [90], and Paterson *et al.* [91] all reporting syntheses that produced varied isomers in good yield. Kilogram amounts are now achievable by total synthesis [92] and in a veritable "tour-de-force", the process chemistry group at Novartis, to whom in the interim, HBOI had licenced the compound as a preclinical candidate, reported their cGMP synthetic route in a series of five papers in 2004 [93-97] after reporting their formal synthesis the previous year [98].

The compound is currently in Phase I clinical trials as a potential treatment against solid tumors and in addition, Kosan Inc. are working on a genetic approach to the molecule in a manner similar to their work with the epothilones. At the 2003 ASCO meeting, the first formal report of any Phase I trial of the compound was presented. No objective responses had 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 [99]. Further work in non-human experiments was also presented at the same meeting, with McDaid *et al.* reporting [100] 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. Subsequently at the 2004 ASCO meeting Mita *et al.* [101] presented further information on the pharmacokinetics with 26 patients and established that discodermolide exhibited non-linear pharmacokinetics characterized by a second peak in the terminal phase with PK characteristics suggestive of prolonged recycling of the drug between tissue and the systemic circulation and reported that minimal toxicities were seen in that particular trial.

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 [102] and a relatively recent paper from Horwitz' group [103] demonstrated how discodermolide and Taxol® may well fit into the same site on tubulin. Following up on this work that had previously demonstrated synergy between paclitaxel and discodermolide, a multinational group [104] recently reported that the compounds synergistically inhibited both microtubule dynamic stability and mitosis. Though both paclitaxel and discodermolide appear to bind to the same site on tubulin as shown by competition experiments, and demonstrate synergy with A549 cells under the conditions used, other microtubule stabilizing agents that competitively inhibit ^{3}H-paclitaxel binding such as eleutherobin and epothilones A and B, do not exhibit such synergistic behavior. The reason(s) for these differences is(are) not known, but a significant number of possibilities that may be experimentally investigated are given in the discussion of the paper and should be consulted by an interested reader and in



a very recent paper, members of the same group demonstrated that one of their postulates related to synergy (differential effects on tubulin isotypes by paclitaxel) is quite a reasonable hypothesis at this moment in time [105]. Further very recent work by members of the same group [106] has now demonstrated that paclitaxel and perhaps by inference, other agents with similar binding characteristics, exhibits a previously undescribed effect as an anti-angiogenic agent in normal endothelial cells and in an endothelial cell line (HMEC-1) at concentrations below 1 nM. Such concentrations have no observable effect on A549 cells even when these cells were incubated with hydrocortisone and epidermal growth factor. Thus very subtle changes in microtubule dynamics appear to produce manifold changes in cellular growth processes.

DICTYOSTATIN

The HBOI group recently isolated a marine macrolide, dictyostatin 1 (**22**) having some of the structural features of discodermolides, from a deep water lithistid sponge by following a tubulin interaction assay rather than relying on cytoxicity [107]. This material had previously been reported, but as a cytotoxin, from a Maldavian *Spongia* species by Pettit *et al.* [108], and hybrids of both this agent and discodermolide had also been reported from chemical synthesis by Shin *et al.* [109]. However, it was not until the deep water sponge metabolites were investigated by using a tubulin-specific assay that the underlying mechanism for the cytotoxicity was realized [107].

Following this work, two elegant syntheses by Paterson *et al.* [110] and Shin *et al.* [111] confirmed the structure and gave the absolute configurations, with both synthetic schemes confirming that the 10 stereo centers that discodermolide shares with dictyostatin were as postulated from extensive NMR and molecular modeling as reported earlier in 2004 [112]. These syntheses now open up the possibilities of production of significant quantities of this molecule and closely related ones for further investigations and the two papers should be consulted for the various proposed structures and for the diagrams that demonstrate the very close similarities between discodermolide and dictyostatin in their conformations, when the x-ray structure of the former is compared to the solution conformation of the latter.

That this compound and discodermolide have similar biological activities at the molecular level was demonstrated by Madiraju *et al.* [113] who reported that these two compounds and epothilone B exhibit very similar inhibition of binding of radiolabeled paclitaxel to microtubules and from using multiparameter high-content immunofluoresence studies, dictyostatin induced microtubule stabilization and bundling at concentrations of ~1 nM. Some SAR results also indicated that the configurations of the C19 hydroxyl, the C6 and C14 methyls and the natural *E:Z* geometry of the diene are very important for stabilization of the microtubule.

DIAZONAMIDE

In 1991, workers in Fenical's group reported the isolation of Diazonamide (**23**) from the ascidian *Diazona angulata* [114]. This compound languished for a significant amount of time due to supply problems, though finally another supply of organism was found through the involvement of the NCI's marine collection program that enabled some further biological evaluations to be performed. A number of well known synthetic chemists made attempts at synthesizing the initial structure, with formal syntheses being published. However, the original structure was questioned by Harran and this caused a reassessment of the original data, culminating in the publication of syntheses of the original structure **23**, an oxo analogue **24** and then their revised structure **25** (*cf* Burgett *et al.* and references therein [115]). This was followed by with a second formal synthesis by Nicolaou's group being reported [116] in 2003 and then recently, two more publications from Nicolaou reported two entirely different methods of synthesis of the revised structure with assignment of all chiral centers [117,118]. In these latter reports, Nicolaou's group reported that though they had made a small series of analogues, unless they had the full diazonamide architecture, no compound broke the low micromolar level barrier as a cytotoxin.

Using the molecules provided by the Harran group, Hamel and colleagues investigated the interactions with tubulin and reported that both diazonamide A and the oxygen analogue (where the dihydropyrrole ring of the revised structure had been replaced by a dihydrofuran moiety) were potent inhibitors of microtubule assembly, comparable to dolastatin 10 in their responses and roughly 6 to 20 times more potent than dolastatin 15 depending upon the specific assay. However, neither compound exhibited competition for binding sites with labeled vinblastine or dolastatin 10, nor did they stabilize labeled colchicine binding. Thus they resemble dolastatin 15 in their lack of competition for these sites but are much more potent, it is possible that they have a specific, but as yet unrecognized binding site, or they may bind at the so-called "peptide binding site", but only when this site is at the end of growing molecules. For further details and a more exhaustive discussion of the differences, the actual paper should be consulted [119].

ELEUTHEROBIN/SARCODICTYIN

In 1997, Lindel *et al.* reported the isolation and properties of the diterpenoid Eleutherobin (**26**) from the Australian alcyonacean (octacoral) *Eleutherobia* sp., demonstrating that this compound mimicked paclitaxel in its interactions with tubulin [120]. The same year, workers from the then Pharmacia-Upjohn company reported that the closely related compounds known as the Sarcodictyins (**27**), which had been reported approximately 10 years earlier from the Mediterranean corals *Sarcodictyon roseum* and *Eleutherobia aurea* by Pietra's group [121,122] but without any biological activities listed. Their activity versus tubulin was reported [123] by a group from Pharmacia-Upjohn at that year's 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, that permitted formation of hybrid molecules of the two base structures [124-129] and the final report by the Danishevsky group on their synthetic method which permitted the addition of different sugar epimers to a base tricyclic ketone [130]. Using one of these hybrid compounds (**28**) and the natural products, Hamel *et al.* described their interactions with tubulin [131] and demonstrated that the compounds interacted in a similar manner to paclitaxel and in the case of eleutherobin, competitive inhibition of labeled paclitaxel was found to occur. The hybrid compound and the sarcodictyins were much less potent as tubulin binders but did demonstrate significant activity against tumor lines *in vitro* and all of the compounds were relatively equipotent against both parental and paclitaxel-resistant cell lines, albeit at relatively high nM levels for the sarcodictyins compared to eleutherobin. The hybrid compound, which lacks the sugar moiety, was intermediate in its activity when compared to the two base natural product structures and confirmed the earlier findings of McDaid *et al.* where they reported that the arabinose enantiomer of eleutherobin was lower in activity in both binding and cytotoxicity assays [132] with the natural product. Thus, as mentioned in other discussions of a variety of tubulin-interactive agents, the absolute binding characteristics of these agents does not always correlate with their activity as cytotoxins.

Although recollection of the coral from the original area (Western Australia) was not possible for political reasons, and thus the work reported earlier was performed using the synthetic materials, including the derivation of a common pharmacophore that could accommodate paclitaxel, discodermolide, eleutherobin, the epothilones and nonataxel [133], in early 2000, Cinel *et al.,* using a specific tubulin interactive assay, discovered eleutherobin and six other related compounds [134] from the Caribbean octacoral *Erythropodium caribaeorum,* with all giving assay results that indicated paclitaxel-like activities. Approximately one year later, Andersen's group reported that eleutherobin, when isolated from the *E. caribaeorum,* was in fact an artifact of the methanolic extraction protocol as was also the eleutherobin aglycone which had previously been synthesized [135]. Further work from the same group extended the Ojima pharmacophore by demonstrating that the 2',3' olefin, when reduced, produced a compound devoid of activity in their assays, a portion of the molecule that had not been previously identified as being of import for activity [136].

Due to the problems alluded to earlier in obtaining materials from the original source area and the complexities of either synthesis or isolation from wild collections of *E. cari-*

baeorum, even in the Caribbean, the access to these agents was revolutionized in 2002, when Andersen's group reported that these materials could be isolated in comparable amounts to those found in Nature from materials grown in aquaria for the decorative seawater aquarium trade [137]. In that particular paper, work on *in vivo* models was alluded to, but no papers have been presented to date.

Even though aquacultural production is a viable method, synthetic chemists have still continued their work into novel methods to produce these agents and analogues. Thus in 2004, Chandrasekhar *et al.* reported the synthesis of a simplified oxy-analogue of eleutherobin and were able to model it onto porcine β-tubulin, and reported that the compound **29** had some cytotoxicity [138]. This work was followed in 2005 by two papers from Castoldi's group in Italy, one [139] giving a newer method of synthesis of eleutherobin involving a novel synthesis of a key intermediate in the Danishevsky synthetic scheme, and the other, a complete discussion of their work [140]. In the latter paper, they reported their syntheses of a number of C-7 modified eleutheside analogues that still retained paclitaxel-like activity and in the case of one particular molecule **30** the tubulin interactions were comparable to those of paclitaxel, but the compound was at least two orders of magnitude less cytotoxic. As they state in their discussion, such a molecule may well have utility in other pharmacologic areas and they point to the papers discussing the utility of paclitaxel-like molecules as protective agents against β-amyloid toxicity in primary neurons [141-143] and in fact, in the 2005 paper by Michaelis *et al.,* discodermolide was shown to be more potent in their assay systems than any of the four taxanes used [143].

LAULIMALIDE

Laulimalide (**31**) and isolaulimalide (**32**) were first reported as fijianolides B and A respectively by the Crews' group [144] from the Vanuatu sponge *Cacospongia mycofijiensis*, and effectively simultaneously by Corley *et al.* as laulimalides from an Indonesian *Hyatella* sp. and also from a chromodorid nudibranch, *Chromodoris lochi* grazing on the sponge [145]. The same materials were also reported from the Okinawan sponge *Fasciospongia rimosa* [146] and co-isolated with a mixture of other well-known metabolites from a *Dactylospongia* sp. by Cutignano *et al.* [147]. Though the cytotoxicity and antifungal activities of these agents had been reported by the original groups, it was not until 1999 that Mooberry *et al.* [148] reported that these compounds demonstrated paclitaxel-like activity and that this was probably the reason for the cytotoxicities reported. These compounds have also been reported in other sponge genera, including *Fasciospongia* and *Dactylospongia*.

Although a microtubule stabilizing agent, work from Hamel's group [149] indicated that this agent might well bind at a site different from the taxanes, though it is possible that it might also be binding to unpolymerized tubulin or to aberrant polymeric tubulin. Furthermore, in late 2003, Mooberry *et al.* [150] reported that this agent, like other microtubule-stabilizers, has an additional mechanism independent of mitotic arrest whereby G_1 aneuploid cells are formed due to aberrant mitotic events at 5 to 7 nM; concentrations approximately 30% of those required for mitotic accumulation.

A very interesting abstract of a poster presented at the 2005 AACR Meeting reports on yet another effect of laulimalide in particular and other agents with similar mechanisms by inference. Thus Lu *et al.* [151] reported that both laulimalide and taxotere inhibited the VEGF-induced migration of HUVECs with comparable IC_{50} values of 10 pM, whereas concentrations of 4nM were required to inhibit HUVEC proliferation. However, at a concentration of either drug of 100fM, minimal inhibition of HUVEC migration was seen. If in contrast, the two were combined at these concentrations, then HUVEC migration was inhibited by 70%. Preliminary evidence indicated that these two compounds may exhibit different effects, with taxotere inhibiting the association of the VEGF receptor with integrins whereas seemed to have a greater effect upon the phosphorylation of paxillin.

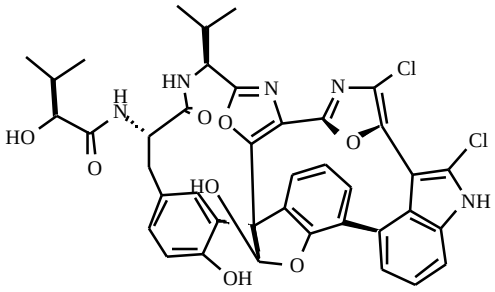
A large number (over 10) of synthetic routes to laulimalide have been published, plus many more that give methods of synthesis of "subassemblies" of the overall molecule, and for a thorough discussion of the results of these endeavors, the reader should consult the excellent synthetic paper from Multzer's group [152] and the recent review by Multzer and Ohler [153]. In addition to these, Wender's group has reported the syntheses of five laulimalide analogues with modifications in the epoxide, the C_{20} alcohol and and the C_1-C_3 enoate of the natural products that are poor substrates for Pgp and hence are effective against paclitaxel-resistant cell lines [154], following this report with an extended one the following year [155].

In addition to the groups whose work, both biological and chemical, have been shown above, two other groups have been quietly working on laulimalide analogues. The first report from the Kosan group was published as an US Patent on 30DEC03 from a filing date in early 2002 [156] and the second was from the Eisai group showing the synthetic routes and biological activities *in vitro* of 18 plus compounds [157]. The Eisai group added another modified compound to the synthetic list very recently with the publication of a route to the substituted 11-desmethyl laulimalides [158], following on from modeling predictions of Paterson *et al.* in 2004 [159]. Further work from all of these groups should prove to be very interesting in the development of laulimalides as clinical candidates.

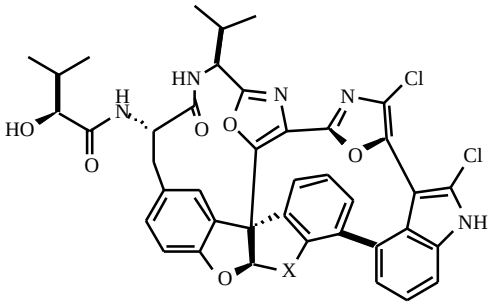
PELORUSIDE

In 2000, West and Northcote reported the isolation of the cytotoxic macrolide Peloruside A (**33**) from the New Zealand marine sponge, *Mycale hentscheli* [160]. This initial report was then followed two years later by a paper from Hood *et al.* that demonstrated that this compound was another in the series of marine-derived cytotoxins with a mechanism of action similar to that of Taxol® and in a similar fashion, it demonstrated induction of apoptosis following G_2-M arrest [161], and as they pointed out, its relatively simple structure may well lend itself to synthetic modifications.

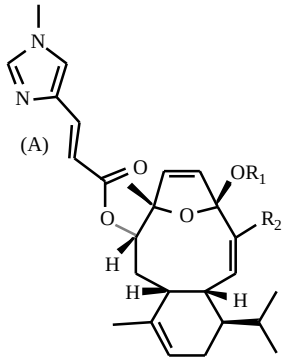
Further biological work related to its potential mechanism has now demonstrated that peloruside, like the epothilones, discodermolide and laulimalide, is a weaker substrate for the P-glycoprotein efflux pump than Taxol®



23 Original Diazonamide Structure

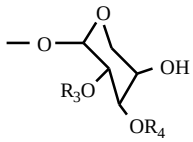


24 "OXO"-analogue, X = O
25 Revised Diazonamide Structure, X = NH



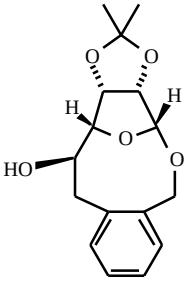
26 Eleutherobin

(A) = E, $R_1 = CH_3$, R_2 =

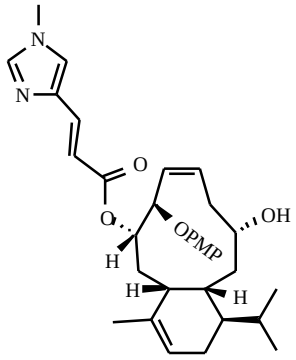


27 Sarcodictyin A, $R_1 = H$, $R_2 = CO_2CH_3$

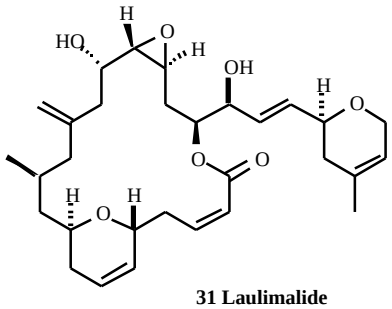
28 Compound $_1$, $R_1 = CH_3$, $R_2 = CO_2CH_3$



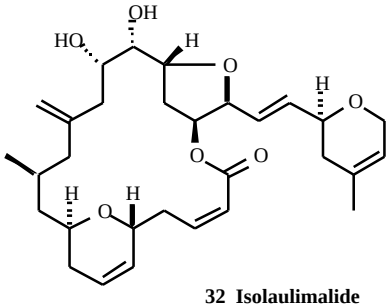
29



30



31 Laulimalide



32 Isolaulimalide

[162]. This was established by peloruside's cytotoxic activity against paclitaxel resistant cell lines and it also appears to bind at the same or a similar site to that reported for laulimalide, one significantly different to the taxoid binding site [162]. Recently a computational study of the probable binding of laulimalide and peloruside to tubulin was published by Pineda *et al*. [163]. They also found an additional preferred binding site on α-tubulin, similar results to those referred to earlier from direct experiments [163]. Further evidence of another potential mechanism related to the *ras* status of the cell lines/tumors, and hence added evidence for further development, was reported by Miller *et al*. when it was shown that peloruside enhanced apoptosis in H-*ras* transformed cells but appeared not to have any immunosuppressive effect, in contrast to the two other unrelated cytotoxins also found in the same sponge, mycalamide A and pateamine [164].

The molecule has been difficult to reisolate in any significant quantity from Nature, though there are reports of successful isolation from aquacultured sponge fragments [162] and it has been the target of a number of synthetic groups over the last few years. It has, as noted by Ghosh and Kim [165] in their paper reporting their enantioselective synthesis of the C_1-C_9 segment, structural similarities to the epothilones. That same year, Liao *et al*. reported their total synthesis and the absolute configuration of the (-)-peloruside A, thus establishing the absolute configuration of the natural isolate [166] and very recently, Jin and Taylor reported the

total synthesis of the (+), or natural epimer of the compound [167]. These syntheses, coupled to the potential for aquacultural production of the natural metabolite will significantly aid in the production of enough material to further evaluate the full potential of this compound and analogues.

INHIBITORS OF TOPOISOMERASES I AND II

Early in 2004, Cragg and Newman [168] reviewed natural product-derived agents active against these targets in an issue of *Journal of Natural Products* honoring Drs. Monroe Wall and Mansukh Wani, the co-discoverers of both Taxol® and camptothecin, and it also covered reports on new developments in the area of topoisomerase inhibitors. Although most of the new topoisomerase I inhibitors are based on the camptothecin pharmacophore, the microbial protein kinase inhibitor staurosporin (derivatives of which have been reported from marine invertebrate extracts over the years) is also a topoisomerase inhibitor and various derivatives of the basic staurosporin scaffold have activities in topoisomerase inhibition, both types I and II. Since that review was published, two important papers have been published. In the first, Denny reviewed the anticancer activity of some new topoisomerase inhibitors and covered 6 topoisomerase I, 12 topoisomerase II and 6 dual topoisomerase inhibitors [169] with most being derived from natural products. The second paper by Marshall *et al.* [170], reported the details of AK37 (**34**), a compound based on a marine-derived pyridoacridine which stabilizes the topoisomerase I cleavable complex in a manner comparable to 9-amino-camptothecin. The interested reader should also consult the recent review by Marshall and Barrows on the pyridinoacridines in general in order to see the wide variety of structures and activities in this class of natural products [171].

PROTEASOME INHIBITORS

The proteasome is a cellular multienzyme complex which is involved in the ubiquitin-proteasome pathway control of cell cycle progression, in the termination of signal transduction cascades, and in the removal of mutant, damaged, and misfolded proteins and therefore is a promising therapeutic target. The background to the proteasome is described in an excellent review by Kisselev and Goldberg [172], and the first proteasome inhibitor, the peptide boronate PS341 (Bortezomib®) (**35**) [173] has now gone into clinical use. This is a synthetic compound based upon a natural product structure [174] and the story of the compound has been described in detail by the initial inventor and will not be further described here [175].

There are however, a significant number of other compounds from Nature, and from derivatives of these compounds, that have led to a greater understanding of the intricacies of this multienzyme complex. Thus the 20S proteasome in mammals has three closely linked proteolytic activities, known from their substrates as trypsin-, chymotrypsin- and caspase-like. However, in the case of the latter two activities, their range of substrates is broader than their names would suggest, as branched chain aminoacids are cleaved quite well by the caspase-like and to a lesser extent by the chymostrypsin-like active centers. The first two do not have the classical triad of the serine proteases but have threonine as the catalytic center, and they therefore should be classified as members of the N-terminal nucleophile hydrolases; enzymes that cleave amide bonds by utilizing the side chains of their terminal serine, threonine or cysteine residues and have similar 3D structures [176].

The other major difference between the proteasome and single proteolytic enzymes is that the complex only acts as a concerted whole; individual activities are not demonstrable. In fact if the chymotrypsin-like activity is inhibited by a suitable compound (such as a peptidic aldehyde originally designed to inhibit chymotrypsin), or the specific threonine is removed by site-specific mutagenesis, then a large reduction in the rate of protein degradation is seen. However, in contrast, if the sites corresponding to the other nominal activities are modified, the overall rate of hydrolysis of proteins is not significantly changed. Due to the substrate specificity of chymotryptic sites, most inhibitors are hydrophobic whereas in the case of the other two activities, their "peptide-based" substrates / inhibitors tend to be charged. As a result, almost all of the proteasome inhibitors tend to have chymotrysin-like activities with some overlapping, but weaker, effects on the other sites.

The first report of what turned out to be a proteasome inhibitor from Nature was in 1991, when Omura *et al.* reported that the microbial metabolite, lactacystin (**36**), induced neuritogenesis in neuroblastoma cells [177]. In 1995, this original report was followed by those of Fenteany *et al.* [178] and Craiu *et al.* [179] demonstrating that radio-labeled lactacystin selectively modified the β5(X) subunit of the mammalian proteasome and irreversibly blocked the activity. In subsequent studies, Dick *et al.* demonstrated that the actual inhibitor *in vitro* was the β-lactone, *clasto*-lactacystin-β-lactone (**37**) [180] a material formed spontaneously on exposure of lactacystin to neutral aqueous media. Subsequent work from the same group [181] demonstrated that the membranes of cells were permeable to the lactone and that although rapidly hydrolyzed by water, it exists inside mammalian cells in equilibrium with lactathione, the inactive product of its reaction with glutathione. Although the β-lactone was quoted to be an irreversible inhibitor, the proteasome adduct is slowly hydrolyzed with a $t_{1/2}$ of approximately 20 hrs. Finally, in 1999, Corey and Li reported on the synthesis of the parent compound and other analogues, suggesting that the β-lactone should be named omuralide (**37**) [182].

In 2003, Fenical's group reported the isolation and structural determination of the marine bacterial metabolite, salinosporamide A (**38**) and demonstrated that it was a cytotoxic proteasome inhibitor [183], and the following year, Reddy *et al.* [184] reported a total synthesis. What is very interesting about the salinosporamide structure is that compared to omuralide, it is uniquely functionalized, with a cyclohexene ring replacing the isopropyl group found at C5 in omuralide. The significance of this substitution is that in 1999, Corey *et al.* demonstrated that the isopropyl group was essential for activity and that replacement by a phenyl ring abolished the activity [182]. Thus salinosporamide A might well interact with the 20S proteasome in a modified manner when compared to that of omuralide. Unlike omuralide, salinosporamide A appears to be the true metabolite, and not a

derivative from a precursor. However, since it is produced by fermentation, there is always the possibility that any conversion is extremely rapid during the fermentative production and thus would not be seen. Currently, this molecule is undergoing preclinical evaluation at Nereus Pharmaceuticals as a proteasome inhibitor with the expressed aim of entering clinical trials in late 2005.

There are other natural products with quite different formal structures from microbes that are very active inhibitors of this complex. Thus in 1999, Crews' group at Yale reported that the epoxyketone microbial metabolites epoxomicin (**39**) [185] and eponemycin (**40**) [186] exhibited their cytotoxic activities as a result of proteasome inhibition. Similarly to the omuralide inhibitors, epoxomicin reacted predominately with the chymotrysin-like site whilst the less potent eponomycin and its synthetic analogue, dihydroeponomycin (**41**), had roughly equal activity against both the chymotrysin-like and the caspase-like sites. In contrast to the "regular" peptidic inhibitors such as the aldehydes, boronates or vinyl sulfones, these peptidic epoxyketones reacted with both the hydroxyl and amino groups of the N-terminal threonine, forming a morpholino ring system as a result. These expoxyketones are the most selective proteasome inhibitors so far reported. When biotinylated derivatives were used as cell probes, only the proteasomal subunits were covalently modified. The reason for the high specificity may well lie in their ability, in the 2*R* configuration, to form the morpholino ring. They cannot form the comparable ring with a cysteine or serine protease since these do not have free amino functionalities adjacent to the nucleophilic group [187]. Further evidence was that on inversion at the 2 position to give the 2*S* conformer, the potency was dramatically reduced probably as a result of the steric hindrance to formation of the morpholino ring system [185,187].

HISTONES

Basic Histone Structure

Most non-biological discussions of gene structure and DNA give the impression that DNA exists as a double stranded helical structure, whereas in practice, the DNA in the human genome is packaged into chromatin, a dynamic macromolecular complex of DNA, histones and non-histone proteins. Within the overall structure, nucleosomes, which are DNA wrapped around a histone octamer (composed of an H3-H4 tetramer and two H2A-H2B dimers) form the basic repeating unit of chromatin. Within this paradigm, condensed chromatin mediates transcriptional repression whereas open chromatin permits gene transcription, although transcription occurs to some extent throughout as a result of the dynamic nature of the system, but at widely varying rates.

Extending out of the nucleosomes are the charged amino terminal "tails of the histones" and since the tail of H4 appears to interact with the H2A-H2B complex of a neighboring nucleosome, then these "tails" may well regulate higher order chromatin structural changes. These "tails", in particular those from H4, can be modified post-translationally by methylation, acetylation and phosphorylation (for further information on this model the reader should consult Fig. 1 in the excellent review by Johnstone [188]). As a result, covalent modification by acetyl transferases (HATs), histone deacetylases (HDACs) methyltransferases and kinases offers a series of mechanisms by which upstream signaling pathways may combine on a common target to regulate gene expression in positive and negative fashions. To further add to the inherent complexity but also to add another level of sophistication, the positioning of nucleosomes with respect to one another can be controlled by protein complexes that utilize ATP hydrolysis. Thus the interaction of all of these processes permits exquisite control of gene expression.

Although a significant amount of work at the protein level has been performed on the methylases, phosphorylases and acetylases, the process that has currently yielded the most promising lead compounds is inhibition of acetylation, we will discuss HDAC inhibition from the aspect of target(s), drug discovery, modification and utility and only from a marine perspective. A more thorough discussion of the other natural product sources has been given in a recent review by Kingston and Newman [189].

Histone Deacetylases

Currently, there are 18 identified HDACs in humans [188,190,191] divided into three classes based on their resemblance to known yeast transcriptional regulators. Thus Class I (yeast Rpd-3 like) is composed of HDACs 1, 2, 3, & 8; Class II (yeast Hda-1 like) is composed of HDACs 4 – 7, 9 & 10 and Class III (SIR-2 like) is composed of SIRT1-SIRT-7. There are significant differences between the classes and some subtle differences within the classes. Thus the Class I protein complexes are generally nuclear, though #3 can be found in the cytoplasm. Class II protein complexes are generally in both the nucleus and cytoplasm (shuttling) but there is a difference between sources as to the location of #6. Johnstone has it listed as a classic Class II [188] whereas Kristelett *et al*. list it as purely cytoplasmic [190]. The Class III proteins are quite different both in sequence and in the mechanism(s) of deacetylation. Thus these complexes probably use an NAD+ cofactor in a manner similar to the yeast complex, whereas the other two classes have a zinc atom at the active site. Little work has been reported on the Class III proteins other than to locate the SIRT2 protein to the nucleus [188,190].

The reasons why modification of HDACs may lead to antitumor activity are manifold, but the following particular processes may give the reader a better appreciation of the potential from such intervention.

A. HDACs are implicated in oncogenic transformation by their interaction with the cell cycle regulators *Mad/Max* and *Rb*. The *Mad/Max* heterodimers are essential components of the repression of the E-box containing growth regulatory genes in the cellular differentiation process [188,190] and the interactions are mediated by the association with the HDAC-*mSin3* complex [192]. If this repressor system is disrupted by over-expression of *Myc* or *v-Skl* (both common in tumors) then the cell cycle is unregulated and cellular progression and transformation occurs [193,194].

B. *Rb* silences genes that are active in the S phase of the cell cycle as a result of binding to their transcription factor

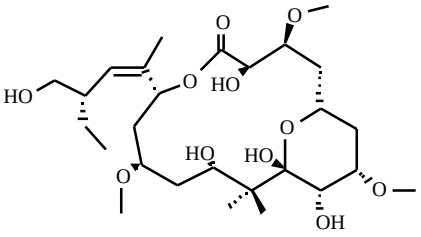
E2F. Such binding results in the active repression of the promoter sites and is apparently due to the recruitment of HDAC to *E2F*, thus converting the chromatin from a transcriptionally active, to an inactive state (*ie* hypoacetylated) [195,196]. Since in almost every human tumor the *Rb/E2F* regulatory pathway is disrupted, deletion, mutation or other inactivation of the *Rb* gene results in the loss of the *Rb*/HDAC interaction, thus favoring uncontrolled proliferation and tumor formation [195-198].

C. A third area is the involvement of Class II HDACs, in particular #4, in the physiological activation of the *ras/MAPK* signal transduction pathway which results in the constitutive nuclear localization of HDAC4. Since *ras* genes are commonly inappropriately activated in tumors and HDAC4 is known to have an important role in cellular differentiation, then the potential for a mechanism is plausible whereby mitogenic signaling and altered HDAC recruitment may be implicated in dysregulation of differentiation and oncogenesis [188,191,199].

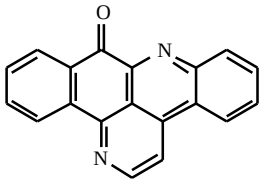
D. Finally, two relatively recent reports have shown that HDACs are involved in the promotion of angiogenesis through the suppression of the hypoxia-responsive tumor suppressor genes [200,201].

HDAC INHIBITORS (HDACIS)

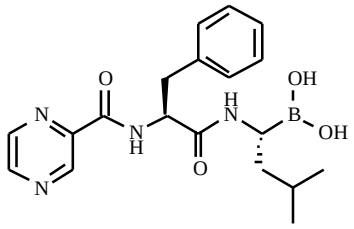
HDACIs have been described as tripartite: an enzyme binding group that is frequently aromatic; a hydrophobic spacer group; and an inhibitor group [202-204]. Such a model is well demonstrated by the natural product trichostatin A (TSA) (**42**) where the structure mimics the lysine side-chain of the substrate (the "linker"), the inhibitory end being the zinc-chelating hydroxamic acid and the aromatic enzyme binding group being the 4-dimethylaminobenzoyl group. This molecule, together with its congeners (B, C, and D) was first isolated as an antifungal agent [205], and approximately a decade later, they were found to have potent differentiation-inducing and antiproliferative activities in Friend erythroleukemia cells. This was followed by studies



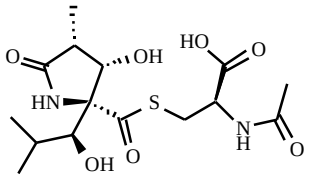
33 Peloruside A



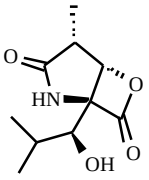
34 AK 37



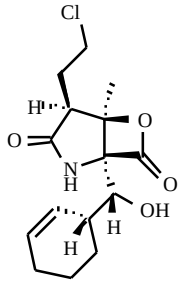
35 PS-341; Bortezomib



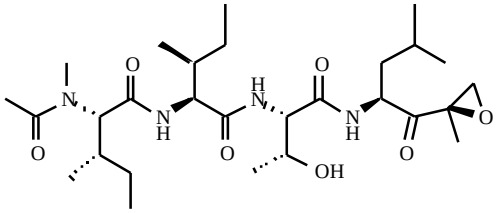
36 Lactacystin



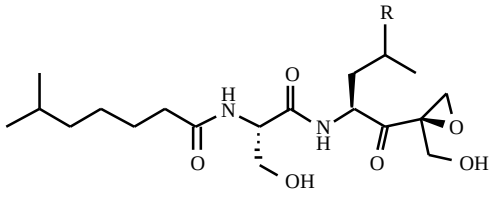
37 *clasto*-Lactacystin-b-lactone Omuralide



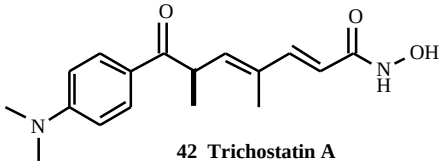
38 Salinosporamide A



39 Epoxomicin



40 Eponemycin, R = CH_2
41 Dihydroeponemycin, R = CH_3



42 Trichostatin A

in synchronous normal rat fibroblasts where G1 and G2 arrest was seen. The block at G1 was removed on removal of the TSA and normal growth was then seen. In contrast, G2-arrested cells started G1, proceeded through S, but no mitosis occurred and the cells were converted to proliferative tetraploids. TSA was subsequently shown to be a very potent (nM level) inhibitor *in vitro* and *in vivo* of the Class I and II HDACs with a slight specificity for HDAC1 & 6 compared to #4. The *S* enantiomer was inactive, and neither enantiomer had any activity against the Class III enzymes [206]. The full mechanism was not elucidated but a large series of effects were seen on signal transduction systems, including induction of apoptosis when normal and tumor cells from many sources were treated with this agent.

Although TSA and synthetic derivatives inhibit cell growth and induce apoptosis, differential display analyses revealed that only 2-10% of the genes in TSA-treated cells are significantly altered [207,208], but the basis for this selectivity is not known. Fuller details and references to the specific events seen with this compound at the molecular level can be found by inspection of tables 2 and 3 in the recent review by Vanhaecke *et al.,* which should be consulted by the interested reader [206]. Once the basic structural features of TSA and its initial activities were identified, then work began on the synthesis of compounds that were more stable and had a better water solubility.

The natural product, trapoxin (**43**), a cyclic tetrapeptide with an epoxy side chain was reported as an irreversible inhibitor of HDACs in 1993 by Kijima *et al.* [209]. These investigators found that it demonstrated some selectivity against Class I & II HDACs, inhibiting #1 and #4 but not #6, in contrast to TSA [188]. It also induced growth inhibition in a number of cell lines regardless of their p53 status [210].

The final compound in this section, NVP-LAQ824 (generic name dacinostat) (**44**), is totally synthetic but its structure is a melding by the Novartis company of chemical and biological information from three natural products and (semi-)synthetic variations. Thus this compound has in its structure the "chemical DNA" from the marine natural product psammaplin A (**45**) (which was reported by Novartis as a potent HDAC inhibitor 14 years after its first isolation by the groups of Schmitz [211] and Crews [212] in 1987), trapoxin and trichostatin A. The full story of the evolution of the compound was given in a series of three papers from the Novartis group [213-215] in 2003, and these, in particular the review by Remiszewski [215] should be consulted for the chemical rationales that led from these natural products to the current clinical candidate. Currently, the material is in a Phase I trial against hematologic malignancies at the Dana-Farber Cancer Institute according to the published literature [216] but in a footnote in the very recent review by Simmons *et al.* [19], the authors state that "recent personal communications suggest that these trials have been discontinued", though at the time of writing (03/05), the compound is still listed as "in Phase I trials" in the Prous Integrity® database.

CELL CYCLE/KINASE INHIBITORS

The Variolins

In 1994, the group of Blunt and Munro in New Zealand reported the isolation of a series of compounds, the variolins, (**46**-**49**) from the Antarctic sponge, *Kirkpatrickia varialosa* [217,218]. These compounds had previously undescribed ring system, a pyrido[3',2':4,5]pyrrolo[1,2-*c*]pyrimidine, and of the four, Variolin B (**47**) was the most active with both cytotoxic (versus murine P388 leukemia) and antiviral (versus *Herpes simplex* type I) activities. The compounds were licensed to PharmMar 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 followed by a paper in late 2003 from a group led by Alvarez [219] at the University of Barcelona covering in detail the total syntheses of variolin B and the previously undescribed analogue, deoxyvariolin B (**50**). These, when reported on by PharmaMar are known as PM-01220 and PM-01218 respectively.

What was very interesting about these compounds was that in addition to demonstrating nM level activity against a variety of standard cell lines *in vitro*, such activity appeared to be independent of p53 status and in Jurkat leukemia cells, but not in colon or breast carcinoma lines, very rapid apoptosis was observed in 4 to 6 hours of exposure in the first report at the 2003 AACR meeting [220]. Another report at the same meeting indicated that flow cytometric studies implied a caspase 3-mediated response and in addition, no DNA strand breakage was observed in treated colon, breast or Jurkat lines. In *in vitro* assays of the effect of 100 to 1000 nM concentrations of these agents on the kinase activity of at least three different Cdk/cyclin complexes, significant inhibition was observed [221], data suggestive of these agents being novel Cdk inhibitors. Then in 2004, the PharmaMar group reported that both agents had useable pharmacokinetics in mice, thus opening the way for initial *in vivo* assessment of their potential as antitumor agents [222].

Following on from these earlier reports, in the abstracts of the 2005 AACR, PharmaMar scientists and their collaborators have reported that both PM-01218 and another unidentified analogue, PM-01217 were effective against the human lung carcinoma Lx-1 without any significant toxicities [223], and that deoxyvariolin B (PM-01218) also has *in vivo* activity against a variety of leukemic lines, with significant reductions in tumor volumes at dose levels of 50% of MTD, again with no reported significant toxicities at this level [224]. Currently these molecules are in preclinical development at PharmaMar.

INDIGO AND THE INDIRUBINS

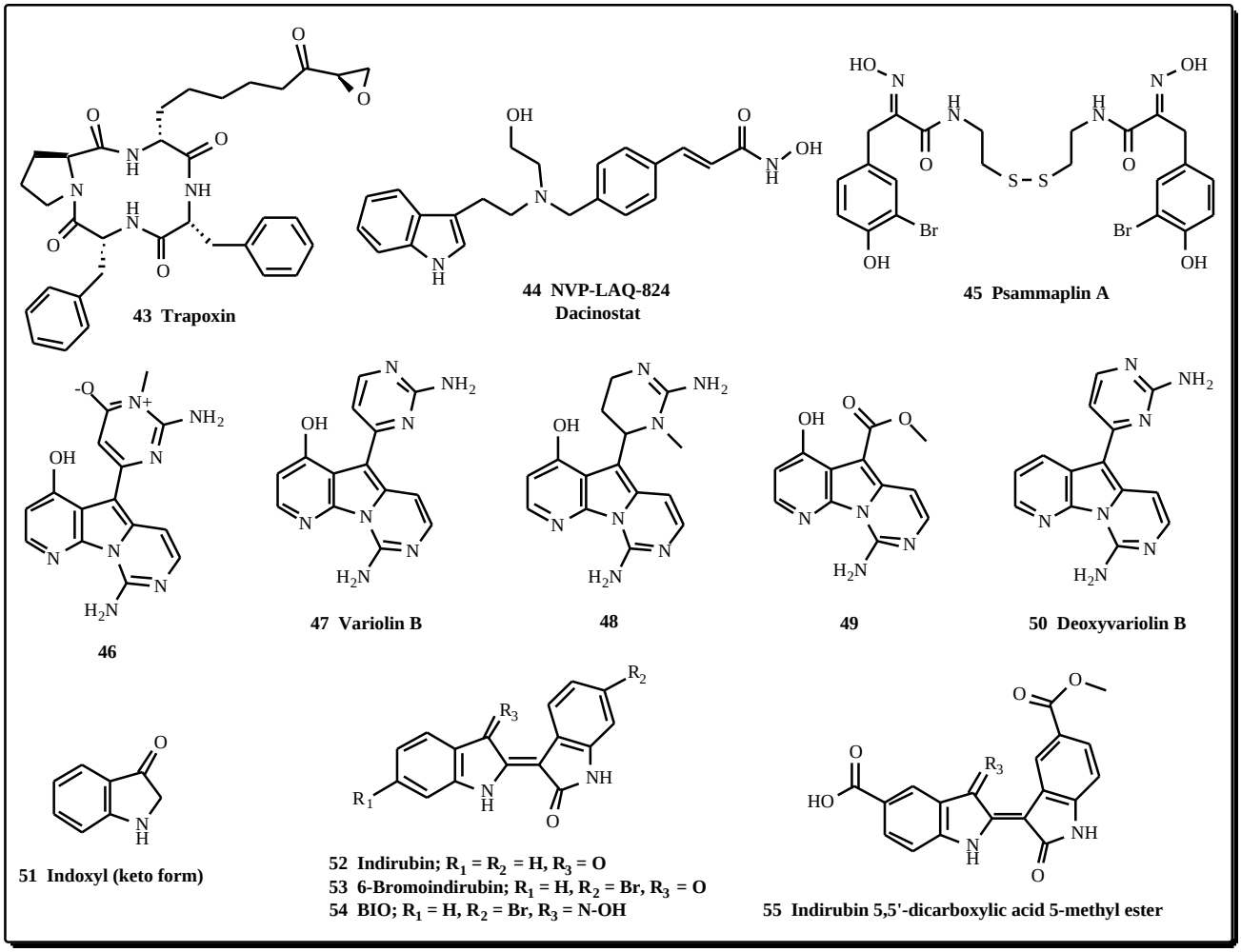
The compounds in question are all based upon the simple bicyclic heterocycle indole. The essential aminoacid tryptophan is formed from the chorismate pathway, *via* cyclization of anthranilate, and indole is formed from tryptophan catabolism. If indole is hydroxylated in the 3 position, presumably by a suitable cytochrome P_{450}, then it is tautomeric with the 3 keto analog, indoxyl (**51**). Various levels of oxidation then lead to the mixture of indigo, indirubin (**52**) and their isomers that is commonly used as the source of indigo dyestuffs for either clothing or as war-paint with the ancient Celtic "woad" being a mixture from the plant *Isatis tinctora* [225].

Although usually thought of as being plant products, indigo and the indirubins have been reported from four nomi-

nally independent sources: a variety of plants [225], a number of marine mollusks, usually belonging to the *Muricidae* family of gastropods [226], natural or recombinant bacteria [227], and from human urine [228]. However it is probable that the indirubins and indigo are the terminal oxidation products of tryptophan/indole catabolism, and maybe a method of removing an excess of toxic indole from the organism.

However, irrespective of the reason for the production, the indirubins have been identified as the major active ingredient of the Traditional Chinese Medicine recipe known as Danggui Longhui Wan, which has been used for over a century in China to treat chronic myelogenous leukemia (CML) [229,230]. What is of import from both a marine natural product and a pharmacological perspective was the recognition by Meijer's group that the indirubins as a class were both inhibitors of several Cdks and were also potent inhibitors of glycogen synthase kinase-3 (GSK-3). In 2003, Meijer *et al.* [231], described both the early work on this enzyme, and then demonstrated that simple indirubins were potent and selective inhibitors of both variants of GSK-3, with IC_{50} values of 22 and 5 nM respectively. This study included a brominated indirubin **53** and its chemically modified oxime **54** derivative; the former had not been isolated from natural sources prior to Meijer's work with the mollusk, *Hexaplex trunculus*, known from antiquity as the source of Tyrian purple. There was at least a 5-fold specificity versus Cdk1/Cyclin B and/or Cdk/p25, and significantly more specificity against a wide range of other kinases. A slightly later paper from the same group gave full details of the chemistry involved, and established structure activity relationships using X-ray crystallography and molecular modeling techniques [232].

Although not in a marine organism, Guengerich *et al.* [233] demonstrated that P_{450} enzymes could use indole and both halogenated and methylated indoles as substrates when human P_{450} 2A6 isozymes were expressed in *E. coli*, confirming earlier work from their groups [234,235], with the compounds also showing differential inhibition against Cdks and GSK-3 isozymes. Very recently, Guengerich's group published a thorough study of the potential for production of modified indigoids as protein kinase inhibitors using recombinant P_{450} human enzymes and showed that depending upon the actual indole used, nanomolar activities against GSK-3 isozymes could be obtained with a nearly 40 fold selectivity when compared to Cdk1 or 5 activities with one compound, indirubin 5,5'-dicarboxylic acid 5-methyl ester (**55**) [236]. It is often forgotten that the first example of a P_{450} enzyme was



in fact work with *Pseudomonas putida* on the hydroxylation of camphor in the early 1960s, so the production of oxygenated/hydroxylated species as a result of metabolism in lower organisms is to be expected.

By using immobilized indirubins, Meijer's group were able to conclusively identify GSK-3 as the target of these molecules in cell lyzates, to demonstrate that they had a significant effect *in vivo* on *Xenopus* development, consonant with the suggested mechanism, and to derive X-ray crystallographic evidence for the binding of the agents at the active (ATP-site) of GSK-3. They also demonstrated that the activation of the enzyme *via* phosphorylation by an as yet unidentified kinase on two specific tyrosine residues (GSK-3 is a Ser/Thr kinase) is blocked when indirubins are bound, perhaps due to a conformational shift in the bound versus free protein.

These papers were then followed by another from the same group where they demonstrated that the indirubins actually had yet another, but independent action at the cell receptor level [237]. By using the same basic suite of compounds they demonstrated that they serve as ligands for the "orphan receptor" known as the aryl hydrocarbon receptor (AhR). As yet no other natural ligands have been identified for AhR, even though, contrary to earlier beliefs, it has existed for over 450 million years. There were also slightly earlier proposals that indole-containing compounds are amongst the natural ligands of AhR as first suggested by Adachi *et al.* in 2001 [228] and further reviewed by Denison and Nagy in 2003 [238].

The main mechanism underlying the cytotoxicity of these molecules [237] appears to be kinase inhibition. As a result of a series of elegant biochemical and biological studies, including the use of cells that were *null-null* mutants for the AhR, Meijer's group was able to differentiate between the cytostatic effects of some indirubins following their activation of the AhR and kinase inhibition.

Recently the pharmacologic areas that the indirubins and their oximes have been extended to inhibition of human papilloma virus E7 effects upon the centrosome in normal human cells without any apparent effect upon the cell cycle. This inhibition and thus potential reversal of the tumorigenic effect of the E7 protein by indirubin-3'-oxime, could be abrogated by use of exogenous Cdk2/cyclin E or Cdk2/cyclin A, thus implying that Cdk2/cyclin activity was necessary for "normal" E7-related tumorigenesis but probably not for normal centrosome duplication/cell cycle progression in normal cells [239], and therefore, this is further evidence that inhibitors of Cdk2 may well have utility in tumor treatment.

Until very recently, all modeling studies with Cdk inhibitors were performed using the coordinates for CDK2 and making assumptions as to modifications at the sites in the other Cdks of interest. However, in early 2005, Mapelli *et al.* published the structure of Cdk5/p25 and demonstrated the binding of three inhibitors including indirubin-3'-oxime, thus providing further data that may be used to modify these simple compounds in order to make them more specific ligands for these very important enzyme complexes in a variety of pharmacologic areas.

Where these findings will lead to in the antitumor area is still to be seen, but what is of import in other diseases is that GSK-3 is an important target in both Alzheimer's disease and in type II diabetes, and although no reports of indole derivatives have been made in the literature related to pharmacologic intervention in these areas, their potential as leads to novel agents must be considered to be quite high. In this vein, an excellent overview of the treatment potential for inhibitors of GSK-3 has recently been published and should be consulted by the interested reader, particularly as other natural product related structures are listed in the paper as possible inhibitors in these disease states [240].

That Mother Nature still has many more molecules that are "indirubin-like" with kinase inhibitory activities is amply demonstrated by yet another paper from Meijer's group, this time in conjunction with groups in Argentina. In a recent publication, they show the biological activities of the meridianins (**56**), a group of halogenated indole derivatives that are very close to the base structures of variolin B (**47**), the psammopemmins (**57**) and discodermindol (**58**), though unlike these latter agents which were isolated from sponges, the meridianins were reported from the ascidian *Aplidium meridianum* [241]. It is of interest to note that PharmaMar are developing modifications of the variolins as candidates for antitumor therapy (*cf* section on variolins earlier).

DNA INTERACTIVE AGENTS (NON-TOPOISOMERASE INHBITORS)

Ecteinascidin 743

The antitumor activity of extracts from the ascidian *Ecteinascidia turbinata* had been reported as early as 1969 by Sigel *et al.* [242] but it was not until 1990 that the structures of the active components, a series of related alkaloids, were published simultaneously by Rinehart *et al.* [243] and Wright *et al.* [244], with the most stable member of the series being Et743 (**59**). The base structure, without the exocyclic isoquinoline group, is a well known chemotype [245] originally reported from microbes, where the compound classes are saframycins, naphthyridinomycins, safracins and quinocarcins. Similar molecules were reported from marine mollusks, *ie* jorumycin from the nudibranch *Jorunna funebris* [246] and from sponges, the renieramycins, with the latest variation, reineramycin J being recently reported by Oku *et al.* [247]. However, with Et743, the exocyclic substituent was novel as was the bridging sulfur. The work by many research groups leading up to the production of Et743 in quantities and quality good enough for human clinical trials has been reported in a number of reviews and these should be consulted for those aspects of the story [18,19].

Over the last few years, a considerable number of reports have been published in the literature 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 often orders of magnitude greater than those that demonstrate activity *in vivo.* These levels are in the low nM to high pM 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) [248,249] and interaction between the Et743 DNA adduct and DNA transcription factors, in particular the NF-Y factor [250]. In a review published in 2003 by van Kesteren *et al*. [251] other possible mechanisms were 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. Since the publication of that review, a number of reports on other potential mechanisms, including genes related to apoptosis, cell cycle, transcription factors, growth factors/receptors and cyclin D1/D3, GRO1 and NF-κB pathways have also been published with the majority being in abstract form at the moment (*cf* references in the review by Newman and Cragg [18]), though a recent paper by Dziegielewska *et al.* sheds further light on the DNA alkylation properties of Et743 in the inhibition of SV 40 DNA replication where it is at least 10 times more active in cells than saframycin (a chemical cousin) and its DNA adducts (replication intermediates) may be blocked in fork progression [252]. In the abstracts for the 2005 AACR meeting, there is one abstract by Mandola *et al*. that suggests that poly (ADP-ribose) polymerase (PARP) interactions with Et743 *in vivo* may play an important role in the cytotoxicity mechanism(s) of this agent [253]. 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 [245].

The compound was placed into human clinical trials whilst these mechanisms were being worked out, and many reports have been published in both abstract and full paper formats in the last four to five years, with the latest full papers being a report by Le Cesne *et al*. on a Phase II European trial in advanced sarcomas, where the authors considered that Et743 was a very promising drug candidate for specific soft tissue sarcomas [254] and another by Sessa *et al.* reporting a 43% objective response rate in resistant ovarian carcinomas [255]. In addition to these full reports a search of the ASCO abstracts for 2004 and of PubMed shows another three abstracts reporting preliminary results with this agent in Phase II trials, plus three more full papers covering other Phase II results. What was always one of the potential problems with Et743 were the reports of hepatotoxicity in toxicology studies in animals, particularly the rat. However, in clinical use, human hepatotoxicity appears to be controllable and in a very recent review, Beumer *et al.* have provided a thorough analysis of this problem as reported in the literature to date, indicating that human hepatoxicity appears to be controllable [256].

ZALYPSIS® (PM-00104/50)

In DailyDrugNews in January 2005, PharmaMar was reported to have placed into Phase I clinical trials, a compound that was related to jorumycin and the renieramycins, known as Zalypsis® (**60**) and very recently, an analytical method was also published [257,258]. It is quoted as being a DNA binder but does not activate the DNA damage checkpoint response and has *in vivo* activity against human xenografts covering breast, gastric, prostate and renal lines with further details to be presented at the 2005 AACR meeting [259,260]. Although there is toxicity from the agent, it is reversible and manageable in preclinical toxicology and from the structure, it appears to be derived from cyanosafracin B, the same starting material as used for the synthesis of Et743 by the PharmaMar chemistry group, but to date no methods have been published.

ASCIDIDEMNINS

These agents are reductive DNA-cleaving agents**,** and their manifold structures are the subject of an excellent review by Delfourne and Bastide [261] which built on previous 1993 and 1999 reviews (*cf* references in Delfourne and Bastide). Recently, in addition to this review, specific agents have either 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 ascididemnin structure (**61**) has led to semisynthetic compounds that exhibit submicromolar activity against some of a panel of 12 human tumor cell lines [262]. 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.* [263] has demonstrated that neoamphemidine (**62**) but not its regioisomer amphimedine (**63**), 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.

SPISULOSINE

In 1999, workers from PharmaMar reported on the initial studies with a molecule known as ES-285 or spisulosine (**64**), isolated from the marine clam, *Spisula polynyma*. This initial report (at a conference on molecular targets) was rapidly followed by a full paper in 2000 that demonstrated that the compound caused a loss of actin stress fibers, which might 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 [264]. The compound demonstrated a wide *in vitro* therapeutic index when tumor cells were compared to normal cell lines, with a 50 to 100 fold difference in IC_{50} values [265] and appeared to interact with the endothelial cell differentiation gene (EDG) receptors as originally postulated by Cuadros *et al.* [264], which was further confirmed by two presentations in 2003 [266,267]. In the 2005 AACR abstracts, further evidence is reported that suggests that the activity is related loss of actin stress fibers and overlaps with the *Rho/ROCK* signaling locus but the actual site(s) differs from these proteins. This work was performed using gene expression microarrays and fuller details will be published/presented in due course [268]. This compound is currently in Phase I trials against solid tumors in Europe under the aegis of PharmaMar.

PROTEIN FOLDS AND INHIBITORS OF KINASES AND PHOSPHATASES

A significant amount of effort has been and continues to be directed at the "fitting of structures to the ATP-binding

sites" in order to develop novel kinase inhibitors, and this approach has been quite successful in developing structures for clinical trials [269]. However, a variation on this theme has been successfully developed by Waldmann and his group at the Max Planck Institute in Dortmund, Germany over the last few years where rather than initially concentrating on the specifics of the ATP-binding site in the beginning, they used two fundamental premises in their search for kinase (and other) enzyme inhibitors.

A. They consider that biologically active natural products are viable, biologically validated starting points for library design, thus permitting the discovery of hit (or lead) compounds with an enhanced probability of success if included in high throughput screening [270,271].

B. Although there are estimates of between 100K and 450K proteins in humans, the number of topologically distinct "shapes", defined as "protein folds" is much lower, with estimates of 0.6K to 8K (Koonin *et al.* [272] and references therein).

Waldmann considered therefore, that if you could find an inhibitor of a specific "protein fold" from nature, then it could act as a prototype from which one could develop closely related structures that may inhibit proteins with similar "folds" and specificity may even be discovered. These concepts are fundamentally similar to the "privileged structure" as originally defined by Evans *et al.* [273] and utilized by Nicolaou so successfully in the case of the benzopyrans [274-276]. The Waldmann approach, however, has the added wrinkle of using protein folding patterns as the basis for subsequent screens.

That this concept was successful was shown by the superb work reported from Waldmann's group on the derivation of inhibitors of *Tie-2, IGF1R* and *VEGFR2 & 3* from the original discovery of the *Her-2/Neu* inhibitor, nakijiquinone C (**65**). This compound was first reported by Kobayashi *et al*. from a marine sponge in 1995 and was shown by them to be an inhibitor of *EGFR* (*Her-2/Neu* is a proto-oncogene from this class of receptors), *c-erbB2* and *PKC* in addition to having cytotoxic activity against L1210 and KB cell lines [277]. Using the basic structure of the sesquiterpene, Waldmann *et al.* built a library of 74 compounds and on testing against a battery of kinases (with similar protein domain folds) were able to identify 7 new inhibitors with low micromolar activity *in vitro*. Included in the 7 were one *VEGFR2* inhibitor (**66**) and four inhibitors of *Tie-2* kinase (**67-70**), a protein intimately involved in angiogenesis and for which, at the beginning of the studies, no inhibitors were known. During the work, the first natural product inhibitor of *Tie-2* kinase was reported (**71**) [278] from the plant *Acacia aulacocarpa*, and a set of four papers from another group demonstrated the activity of synthetic pyrrolo[2,3-*d*]pyrimidines as inhibitors of the same class of kinases [279-282].

The details of the models used, the chemistry leading to the nakijiquinone-based compounds, and the ribbon structures of the kinase domain of the insulin receptor with the corresponding homology domains of the as yet uncrystallized *VEGFR-2* and *Tie-2*, are given in a series of papers from Waldmann's group, with a full review being published in 2003 [283].

Not only kinase inhibitors could be identified by similar techniques, as was originally shown by the work reported earlier on dysidiolide derivatives by Waldmann's group [271] and recently extended to cover other, nominally different enzymes within the same "similarity cluster" which in this case comprised Cdc25A phosphatase, acetylcholinesterase and 11β-hydroxysteroid dehydrogenases type 1 and type 2. Thus using a 147 member library based upon dysidiolide (**72**) resulting from the postulate that the γ-hydroxybutenolide group in dysidiolide was the major determinant of phosphatase activity, they obtained a compound (**73**) 10 fold more potent (IC_{50} of 350 nM) than the parent against Cdc25A and other compounds with activities against the other two enzyme classes in the low micromolar levels. Interested readers should consult the original paper and its supporting information for fuller details of these very intriguing results [284].

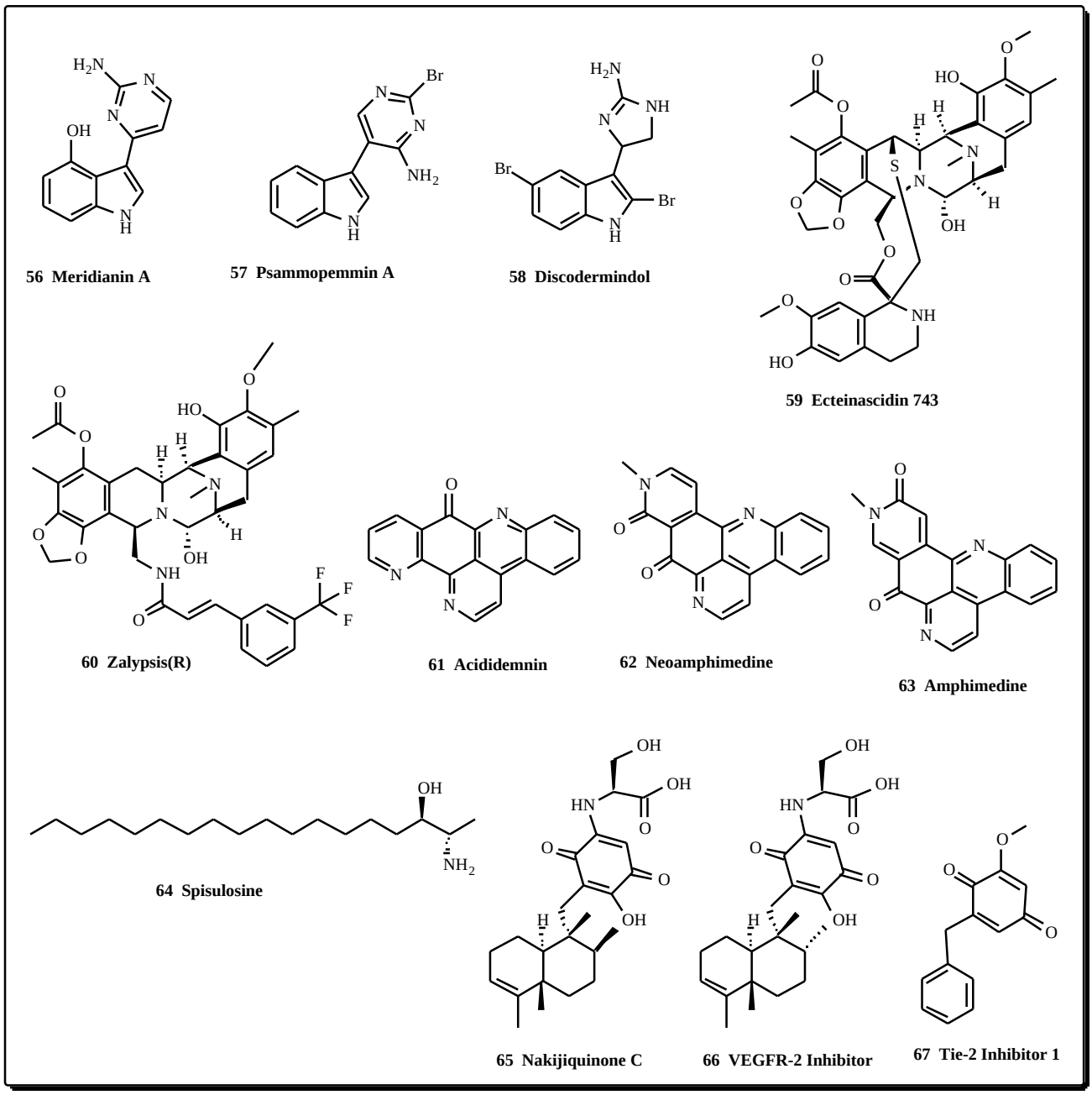
In contrast, rather than using the Waldmann group's methods, Wan *et al.* [285] used a natural product and derivatives to search by means of affinity chromatography and assays against recombinant kinases for other proteins that either bound to the ligand (natural product or modified analogue) or were inhibitors of specific enzymes. Using this technique, and hymenialdisine (HMD) (**74**), a known nanomolar-level inhibitor versus Cdks, Mek1, GSK3β and CK1, and with micromolar activity against Chk1, they modified the structure and were able to generate HMD analogues with micromolar or better activities against 11 new targets, to obtain structures with both increased selectivity compared to HMD (**75**), and with antiproliferative activities 30 fold higher that HMD (**76**).

APLIDINE, AN AGENT WITH MULTIPLE TARGETS

This compound, formally dehydrodidemnin B (**77**), was first reported in a patent application in 1989, with an UK patent issued [286] in 1990 and then referred to in the 1996 paper from Rinehart's group on structure-activity relationships amongst the didemnins [287]. In 1996, the antitumor potential was reported by PharmaMar scientists [288,289] and the total synthesis was reported in a patent application [290] 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 UK for treatment of both solid tumors and non-Hodgkin's lymphoma and published details through early 2004 are given in Newman and Cragg together with discussion as to the mechanisms of action that might be relevant [18].

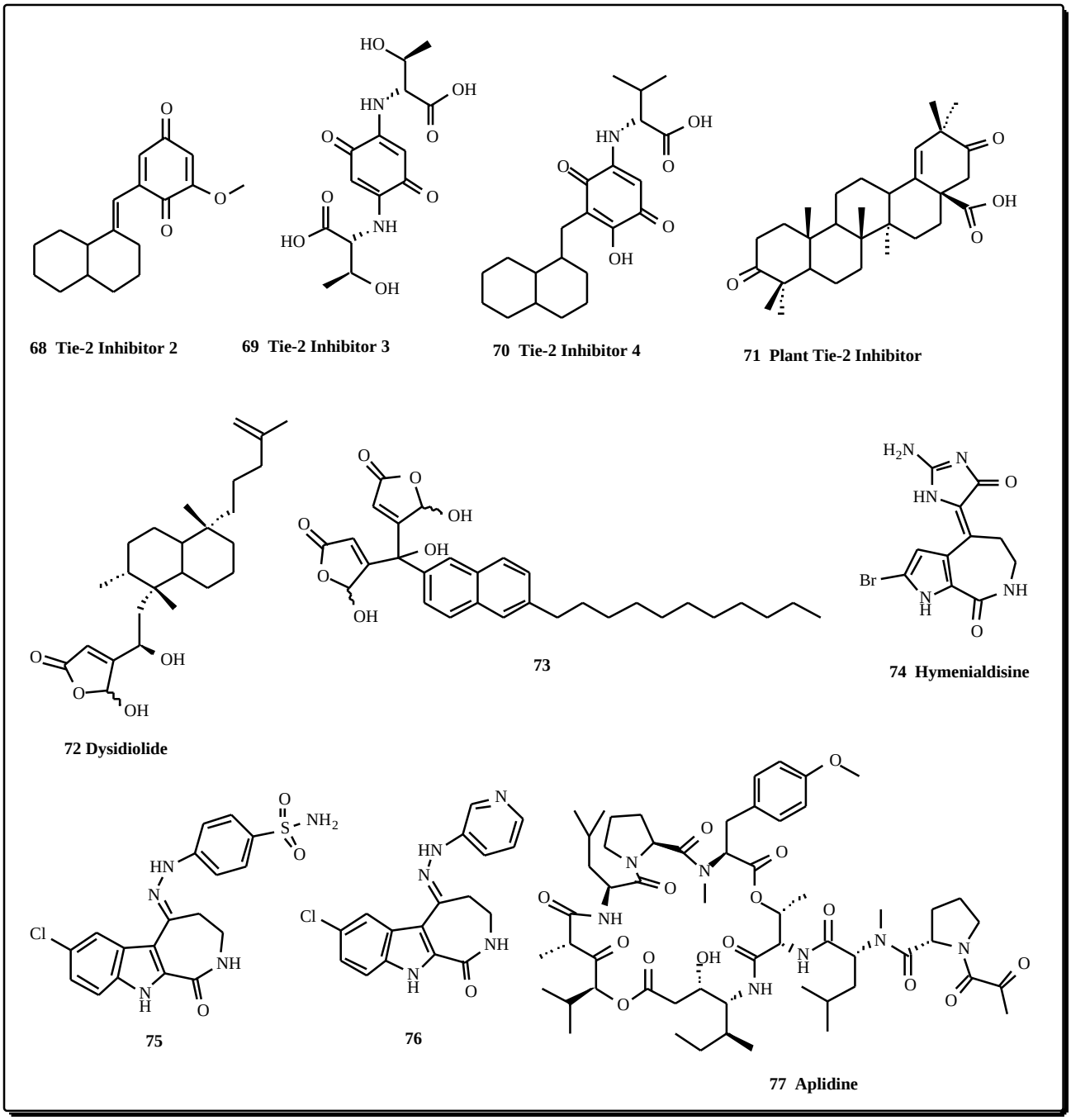
Since the latter publication, further evidence has been published confirming the inhibition of endothelial cell functions related to angiogenesis including blockade of formation of matrix metalloproteinases (MMP-2 and MMP-9) at concentrations achievable during patient treatment [291], and by use of dominant-negative kinase mutants of mouse embryo fibroblasts, Cuadrado *et al*. recently demonstrated that aplidine targets the essential kinase, *JNK* [292]. Further confirmation of this pathway being the probable primary target for aplidine, at least in its induction of apoptosis mechanism came from a recent paper from PharmaMar where investiga-



tion of the reasons for an aplidine-resistant HeLa cell line being more than 1000 fold resistant implicated the bypassing of the MAPKs activation pathway [293]. In an abstract presented at the 2005 AACR meetings, Menon *et al.* reported that in studies with human leukemic cells (K562, CCRF-CEM and SKI-DLCL) where aplidin exhibited synergy with cytarabine, aplidin-treated cells when analyzed using the U133 GeneChip from Affymetrix, suggested multiple cellular targets, including down-regulation of ribosomal 18 and 28S mRNA expression, and up-regulation of various TNF-related ligands [294].

It should be noted that the clinical trials of the very close aplidine analogue, didemnin B, were discontinued because of the toxicities observed, including significant immunosuppression. However, in contrast, evidence for a lack of myelosuppression by aplidine, Et743 and kahalide F, compounds currently in Phase II, II/III and II respectively, was reported by the PharmaMar group using a murine competitive repopulating model as the test system. However, to date, no reports have been published of confirmation in human patients' bone marrow cells [295].

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



68 Tie-2 Inhibitor 2

69 Tie-2 Inhibitor 3

70 Tie-2 Inhibitor 4

71 Plant Tie-2 Inhibitor

72 Dysidiolide

73

74 Hymenialdisine

75

76

77 Aplidine

didemnin B, though the comments on dosage regimens in the excellent review of didemnin B and analogues by Vera and Joullie [296] 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 [297] that in DMSO solution, aplidine, like didemnin B, does not exhibit a formal β-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. Though, 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.

That these latter comments may well be valid can be seen in the recent paper by Gutierrez-Rodriguez *et al.* on the modeling of aplidine and tamandarin A analogues with spirolactam β-turn mimetics which implied the possibility of a peptidylprolyl cis/trans isomerase in the MOA of aplidine. This is another potential mechanism in addition to the results described above in the discussion on *JNK* activation, and the known inhibition of protein synthesis and ornithine decarboxylase activities [298].

CONCLUSION

Though not a complete review of all antitumor active agents from marine sources, it can be seen that the marine environment, irrespective of what organism or collection of organisms actually produces an agent, is an extremely rich source of both novel chemistry and novel biology associated with these compounds. In addition, as amply demonstrated by the work of Waldmann's group and Wan's groups, these initial structures are really only the beginning of what the marine environment has to offer. When one couples these reports to the probability that the real initiator of a very large amount of the chemistry seen is in fact microbial, then all of the potential of genetic manipulation of organisms comes into play, as very recently demonstrated by the work with a genome extract from an Australian invertebrate where an international team of workers in Australia and the UK were able to express the genes producing patellamide D and ascidiacyclamide (cyclic non-ribosomal octapeptides) from a *Prochloron* species that is a commensal of the *Lissoclinum. patella* from which these metabolites were first isolated (the reference is a personal communication from Dr. Paul Long, University of London, UK, because although presented by Long *et al*. at the Society for Industrial Microbiology symposium in San Diego, CA, in November 2004, no abstract number was ever assigned).

Thus one may certainly say that "The surface has only just been scratched!"

REFERENCES

[1] Kingston, D.G.I. and Newman, D.J. (2002) *Curr. Opin. Drug Dis. Dev.*, **5**, 304-316.

[2] Bergmann, W. and Feeney, R.J. (1950) *J. Am. Chem. Soc.*, **72**, 2809-2810.

[3] Bergmann, W. and Feeney, R.J. (1951) *J. Org. Chem.*, **16**, 981-987.

[4] Bergmann, W. and Burke, D.C. (1956) *J. Org. Chem.*, **21**, 226-228.

[5] Suckling, C.J. (1991) *Sci. Prog. Edin.*, **75**, 323-360.

[6] Lee, W.W.; Benitez, A.; Goodman, L. and Baker, B.R. (1960) *J. Am. Chem. Soc.*, **82**, 2648-2649.

[7] Davis, P. US Patent 1159290 (1969)

[8] Cimino, G.; De Rosa, S. and De Stefano, S. (1984) *Experientia*, **40**, 339-400.

[9] Kasanah, N.; Rao, K.V.; Peng, J.N.; Donia, M.; Peraud, O.; Anderson, M.; Hill, R.T. and Hamann, M.T. (2004) *Abs. Ann. Mtg. Soc. Indust. Micro.*, Abs. S-48.

[10] Peraud, O.; Mohamed, N.; Montalvo, N.; Enticknap, J.J.; Hill, R.T. and Hamann, M.T. (2004) *Abs. Ann. Mtg, Soc. Indust. Micro.*, Abs. S-47.

[11] Kellner, R.L.L. (2002) *Insect Biochem. Mol. Biol.*, **32**, 389-395.

[12] Piel, J.; Hofer, I. and Hui, D. (2004) *J. Bacteriol.*, **186**, 1280-1286.

[13] Piel, J. (2004) *Nat. Prod. Rep.*, **21**, 519-538.

[14] Piel, J.; Hui, D.; Wen, G.; Butzke, D.; Platzer, M.; Fusetani, N. and Matsunaga, S. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 16222-16227.

[15] Pettit, G.R.; Xu, J.-P.; Chapuis, J.-C.; Pettit, R.K.; Tackett, L.P.; Doubek, D.L.; Hooper, J.N.A. and Schmidt, J.M. (2004) *J. Med. Chem.*, **47**, 1149-1152.

[16] Cichewicz, R.H.; Valeriote, F.A. and Crews, P. (2004) *Org. Lett.*, **6**, 1951-1954.

[17] Piel, J.; Butzke, D.; Fusetani, N.; Hui, D.; Platzer, M.; Wen, G. and Matsunaga, S. (2005) *J. Nat. Prod.*, **68**, 472-479.

[18] Newman, D.J. and Cragg, G.M. (2004) *J. Nat. Prod.*, **67**, 1216-1238.

[19] Simmons, T.L.; Andrianasolo, E.; McPhail, K.; Flatt, P. and Gerwick, W.H. (2005) *Mol. Cancer Ther.*, **4**, 333-342.

[20] Mollinedo, F. (2005) *IDrugs*, **8**, 127-143.

[21] Strobel, G.; Daisy, B.; Castillo, U. and Harper, J. (2004) *J. Nat. Prod.*, **67**, 257-268.

[22] Janin, Y.L. (2003) *Amino Acids*, **25**, 1-40.

[23] Pettit, G.R. (1997) *Fortschr. Chem. Org. Naturst.*, **70**, 1-79.

[24] Pettit, G.R.; Kamano, Y.; Herald, C.L.; Tuinman, A.A.; Boettner, F.E.; Kizu, H.; Schmidt, J.M.; Baczynkyji, L.; Tomer, K.B. and Bontems, R.J. (1987) *J. Am. Chem. Soc.*, **109**, 6883-6885.

[25] Pettit, G.R.; Kamano, Y.; Dufresne, C.; Cerny, R.L.; Herald, C.L. and Schmidt, J.M. (1989) *J. Org. Chem.*, **54**, 6005-6006.

[26] Bai, R.; Pettit, G.R. and Hamel, E. (1990) *Biochem. Pharmacol.*, **39**, 1941-1949.

[27] Bai, R.; Friedman, S.J.; Pettit, G.R. and Hamel, E. (1992) *Biochem. Pharmacol.*, **43**, 2637-2645.

[28] Bai, R.; Pettit, G.R. and Hamel, E. (1990) *J. Biol. Chem.*, **265**, 17141-17149.

[29] Hamel, E. (2002) *Biopolymers*, **66**, 142-160.

[30] Vaishampayan, H.; Glode, M.; Du, W.; Kraft, A.; Hudes, G.; Wright, J. and Hussain, M. (2000) *Clin. Canc. Res.*, **6**, 4205-4208.

[31] Margolin, K.; Longmate, J.; Synold, T.W.; Gandara, D.R.; Weber, J.; Gonzalez, R.; Johansen, M.J.; Newman, R. and Doroshaw, J.H. (2001) *Invest. New Drugs*, **19**, 335-340.

[32] Shnyder, S.D.; Cooper, P.A.; Millington, N.J.; Pettit, G.R. and Bibby, M.C. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 3421.

[33] Anon (2003) *DailyDrugNews.com (Daily Essentials)*, April 23.

[34] Anon (2003) *DailyDrugNews.com (Daily Essentials)*, October 10.

[35] Michaelson, M.D.; Rayan, D.P.; Fram, R.; Clark, J.W.; Appleman, L.J.; Kirvan, M.A.; Rattner, B.; Jennings, L.; Van Dijk, S.; Boyden, J.P.; Trang, J.; Bonate, P.; Supko, J.G. and Eder, J.P. (2002) *Proc. Am. Soc. Clin. Oncol.*, **21**, Abs. 414.

[36] Ebbinghaus, S.; Rubin, E.; Hersh, E.; Cranmer, L.; Marsh, S.; Bonate, P.; Van Dijk, S.; Fram, R. and Jekunen, A. (2003) *Proc. Am. Soc. Clin. Oncol.*, **22**, Abs. 517.

[37] Eder, J.P.; Appleman, L.J.; Kirvan-Visevatti, M.; Cunningham, C.; Ryan, D.P.; Regan, E.; Vukelja, S.; Marsh, S.; Van Dijk, S. and Jekunen, A. (2003) *Proc. Am. Soc. Clin. Oncol.*, **22**, Abs. 822.

[38] Mita, A.C.; Hammond, L.A.; Garrison, M.; McCreery, H.; Weiss, G.; Schwartz, G.H.; Jekunen, A.; Van Dijk, S. and Rowinsky, E.K. (2003) *Proc. Am. Soc. Clin. Oncol.*, **22**, Abs. 906.

[39] Ebbinghaus, S.W.; Jennings, L.; Van Dijk, S.; Dunn, H. and Fram, R. (2002) *Proc. Am. Assoc. Cancer Res.*, **43** Abs. 2743.

[40] Mita, A.C.; Hammond, L.A.; Garrison, M.; Eastham, E.; Chu, Q.; Syed, S.; Weiss, G.; Marsh, S.; Jekunen, A.; Weitman, S. and Rowinsky, E.K. (2003) *Clin. Canc. Res.*, **9** (Suppl), Abs. 264.

[41] Bonate, P.; Ebbinghaus, S.; Eder, J.P.; Mita, A.; Rubin, E.; Cunningham, C.C.; Rowinsky, E.K.; Hersh, E.; Craig, A.R. and Hammond, L.A. (2004) *J. Clin. Oncol.*, **22**, Abs. 2082.

[42] Hammond, L.A.; Ruvuna, F.; Cunningham, C.C.; Ebbinghaus, S.; Rubin, E.; Mita, A.; Hersh, E.; Eder, J.P.; Weiss, J. and Rowinsky, E.K. (2004) *J. Clin. Oncol.*, **22**, Abs. 3068.

[43] Weiss, G.R.; Mita, A.; Garrison, M.; Chu, S.-C.Q.; Syed, S.; Haider, O.; Bonate, P.; Hammond, L.A. and Rowinsky, E.K. (2004) *J. Clin. Oncol.*, **22**, 3073 (Abs).

[44] Edler, M.C.; Bai, R.; Arthaud, L.E.; Luduena, R.F.; Pettit, G.R. and Hamel, E. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 3431.

[45] Cruz-Monserrate, Z.; Mullaney, J.T.; Harran, P.G. and Pettit, G.R. (2003) *Eur. J. Biochem.*, **270**, 3822-3828.

[46] Harrigan, G.G.; Luesch, H.; Yoshida, W.Y.; Moore, R.E.; Nagle, D.E.; Paul, V.J.; Mooberry, S.L.; Corbett, T.H. and Valeriote, F.A. (1998) *J. Nat. Prod.*, **61**, 1075-1077.

[47] Luesch, H.; Moore, R.E.; Paul, V.J.; Mooberry, S.L. and Corbett, T.H. (2001) *J. Nat. Prod.*, **64**, 907-910.

[48] Mooberry, S.L.; Leal, R.M.; Tinsley, T.L.; Luesch, H.; Moore, R.E. and Corbett, T.H. (2003) *Int. J. Cancer*, **104**, 512-521.

[49] Uemura, D.; Takahashi, K.; Yamamoto, T.; Katayama, C.; Tanaka, J.; Okumura, Y. and Hirata, Y. (1985) *J. Am. Chem. Soc.*, **107**, 4796-4798.

[50] Hirata, Y. and Uemura, D. (1986) *Pure Appl. Chem.*, **58**, 701-710.

[51] Pettit, G.R.; Herald, C.L.; Boyd, M.R.; Leet, J.E.; Dufresne, C.; Doubek, D.L.; Schmidt, J.M.; Cerny, R.L.; Hooper, J.N. and Rutzler, K.C. (1991) *J. Med. Chem.*, **34**, 3339-3340.

[52] Pettit, G.R.; Tan, R.; Williams, M.D.; Doubek, D.L.; Boyd, M.R.; Schmidt, J.M.; Chapuis, J.-C.; Hamel, E.; Bai, R.; Hooper, J.N. and Tackett, L.P. (1993) *J. Org. Chem.*, **58**, 2538-2543.

[53] Gravelos, D.G.; Lake, R.; Blunt, J.W.; Munro, M.H.G. and Litaudon, M.S.P. (1993) US Patent, EP 0 572 109 A1.

[54] Bai, R.; Paull, K.D.; Herald, C.L.; Malspeis, L.; Pettit, G.R. and Hamel, E. (1991) *J. Biol. Chem.*, **266**, 15882-15889.

[55] Luduena, R.F.; Roach, M.C.; Prasad, V. and Pettit, G.R. (1993) *Biochem. Pharmacol.*, **45**, 421-427.

[56] Hamel, E. (1992) *Pharmacol. Ther.*, **55**, 31-51.

[57] Fodstad, O.; Breistol, K.; Pettit, G.R.; Shoemaker, R.H. and Boyd, M.R. (1996) *J. Exp. Ther. Oncol.*, **1**, 119-125.

[58] Aicher, T.D.; Buszek, K.R.; Fang, F.G.; Forsyth, C.J.; Jung, S.H.; Kishi, Y.; Matelich, M.C.; Scola, P.M.; Spero, D.M. and Yoon, S.K. (1992) *J. Am. Chem. Soc.*, **114**, 3162-3164.

[59] Alley, M.C.; Dykes, D.J.; Waud, W.R.; Pacula-Cox, C.M.; Munro, M.H.G.; Newman, D.J. and Sausville, E.A. (1998) *Proc. Am. Assoc. Cancer Res.*, **38**, Abs. 1545.

[60] Synold, T.W.; Lawrence, J.; Xi, B.; Colevas, A.D.; Lewis, M.D. and Doroshow, J.H. (2003) *Proc. Am. Soc. Clin. Oncol.*, **22**, Abs. 575.

[61] Kimura, T.; Synold, T.W.; Mahaffey, C.M.; Labauve, A.E.; Mack, P.C.; Lenz, H.-J.; Gandara, D.R.; Doroshow, J.H. and Gumerlock, P.H. (2003) *Proc. Am. Soc. Clin. Oncol.*, **22**, Abs. 2804.

[62] Choi, H.W.; Demeke, D.; Kang, F.A.; Kishi, Y.; Nakajima, K.; Nowak, P.; Wan, Z.K. and Xie, C.Y. (2003) *Pure Appl. Chem.*, **75**, 1-17.

[63] Towle, M.J.; Salvato, K.A.; Budrow, J.; Wels, B.F.; Kuznetsov, G.; Aalfs, K.A.; Welsh, S.; Zheng, W.; Seletsky, B.M.; Palme, M.H.; Habgood, G.J.; Singer, L.A.; DiPietro, L.V.; Wang, Y.; Chen, J.J.; Quincy, D.A.; David, A.; Yoshimatsu, K.; Kishi, Y.; Yu, M.J. and Littlefield, B.A. (2001) *Cancer Res.*, **61**, 1013-1021.

[64] Seletsky, B.M.; Wang, Y.; Hawkins, L.D.; Palme, M.H.; Habgood, G.J.; DiPietro, L.V.; Towle, M.J.; Salvato, K.A.; Wels, B.F.; Aalfs, K.K.; Kishi, Y.; Littlefield, B.A. and Yu, M.J. (2004) *Bioorg. Med. Chem. Letts.*, **14**, 5547-5550.

[65] Zheng, W.; Seletsky, B.M.; Palme, M.H.; Lydon, P.J.; Singer, L.A.; Chase, C.E.; Lemlin, C.A.; Shen, Y.; Davis, H.; Tremblay, L.; Towle, M.J.; Salvato, K.A.; Wels, B.F.; Aalfs, K.K.; Kishi, Y.; Littlefield, B.A. and Yu, M.J. (2004) *Bioorg. Med. Chem. Letts.*, **14**, 5551-5554.

[66] Talpir, R.; Benayahu, Y.; Kashman, Y.; Pannell, L. and Schleyer, M. (1994) *Tet. Lett.*, **35**, 4453-4456.

[67] Coleman, J.E.; de Silva, E.D.; Kong, F.; Andersen, R.J. and Allen, T.M. (1995) *Tetrahedron*, **51**, 10653-10662.

[68] Anderson, H.J.; Coleman, J.E.; Andersen, R.J. and Roberge, M. (1997) *Cancer Chemother. Pharmacol.*, **39**, 223-226.

[69] Gamble, W.R.; Durso, N.A.; Fuller, R.W.; Westergaard, C.K.; Johnson, T.R.; Sackett, D.L.; Hamel, E.; Cardellina II, J.H. and Boyd, M.R. (1999) *Bioorg. Med. Chem*, **7**, 1611-1615.

[70] Bai, R.; Durso, N.A.; Sackett, D.L. and Hamel, E. (1999) *Biochemistry*, **38**, 14302-14310.

[71] Andersen, R.J.; Coleman, J.E.; Piers, E. and Wallace, D.J. (1997) *Tet. Lett.*, **38**, 317-320.

[72] Pettit, G.R. and Taylor, S.R. (1996) *J. Org. Chem.*, **61**, 2322-2325.

[73] Nieman, J.A.; Coleman, J.E.; Wallace, D.J.; Piers, E.; Lim, L.Y.; Roberge, M. and Andersen, R.J. (2003) *J. Nat. Prod.*, **66**, 183-199.

[74] Loganzo, F.; Discafani, C.; Annable, T.; Beyer, C.; Musto, S.; Hardy, C.; Hernandez, R.; Baxter, M.; Ayral-Kaloustian, S.; Zask, A.; Singanallore, T.; Khafizova, G.; Asselin, A.; Poruchynsky, M.S.; Fojo, T.; Nieman, J.; Andersen, R.J. and Greenberger, L.M. (2002) *Proc. Am. Assoc. Cancer Res.*, **43**, Abs 1316.

[75] Loganzo, F.; Discafani, C.; Annable, T.; Beyer, C.; Musto, S.; Hari, M.; Tan, X.; Hardy, C.; Hernandez, R.; Baxter, M.; Singanallore, T.; Khafizova, G.; Poruchynsky, M.S.; Fojo, T.; Nieman, J.A.; Ayral-Kaloustian, S.; Zask, A.; Andersen, R.J. and Greenberger, L.M. (2003) *Cancer Res.*, **63**, 1838-1845.

[76] Zask, A.; Kaplan, J.; Beyer, C.; Discafani, C.; Musto, S. and Loganzo, F. (2003) *Clin. Canc. Res.*, **9** (Suppl), Abs. A253.

[77] Zask, A.; Birnberg, G.; Cheung, K.; Kaplan, J.; Niu, C.; Norton, E.; Suayan, R.; Yamashita, A.; Cole, D.; Tang, Z.; Krishnamurthy, G.; Williamson, R.; Khafizova, G.; Musto, S.; Hernandez, R.; Annable, T.; Yang, X.; Discafani, C.; Beyer, C.; Greenberger, L.M.; Loganzo, F. and Ayral-Kaloustian, S. (2004) *J. Med. Chem.*, **47**, 4774-4786.

[78] Lo, M.-C.; Aulabaugh, A.; Krishnamurthy, G.; Kaplan, J.; Zask, A.; Smith, R.P. and Ellestad, G. (2004) *J. Am. Chem. Soc.*, **126**, 9898-9899.

[79] Poruchynsky, M.S.; Kim, J.-H.; Nogales, E.; Annable, T.; Loganzo, F.; Greenberger, L.M.; Sackett, D.L. and Fojo, T. (2004) *Biochemistry*, **43**, 13944-13954.

[80] Loganzo, F.; Hari, M.; Annable, T.; Tan, X.; Morilla, D.B.; Musto, S.; Zask, A.; Kaplan, J.; Minnick Jr, A.A.; May, M.K.; Ayral-Kaloustian, S.; Poruchynsky, M.S.; Fojo, T. and Greenberger, L.M. (2004) *Mol. Cancer Ther.*, **3**, 1319-1327.

[81] Kowalczyk, J.J.; Schiller, S.E.R.; Spyvee, M.; Yang, H.; Seletsky, B.M.; Shaffer, C.J.; Marceau, V.; Yao, Y.; Kuznetsov, G.; Tendyke, K.; Liu, D.; Rowell, C.; Littlefield, B.A. and Suh, E.M. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 1212.

[82] Kuznetsov, G.; Tendyke, K.; Towle, M.J.; Cheng, H.; Liu, D.; Rowell, C.; Wu, J.; Kowalczyk, J.J.; Marceau, V.; Campagna, S.; Schiller, S.E.R. and Littlefield, B.A. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs 3432.

[83] Kuznetsov, G.; Towle, M.J.; Liu, J.; Cheng, H.; Tendyke, K.; Kowalczyk, J.J.; Campagna, S. and Littlefield, B.A. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 3436.

[84] Gunasekera, S.P.; Gunasekera, M.; Longley, R.E. and Schulte, G.K. (1990) *J. Org. Chem.*, **55**, 4912-4915.

[85] Gunasekera, S.P.; Gunasekera, M.; Longley, R.E. and Schulte, G.K. (1991) *J. Org. Chem.*, **56**, 1346.

[86] ter Haar, E.; Kowalski, R.J.; Hamel, E.; Lin, C.M.; Longley, R.E.; Gunasekera, S.P.; Rosenkranz, H.S. and Day, B.W. (1996) *Biochemistry*, **35**, 243-250.

[87] Nerenberg, J.B.; Hung, D.T.; Somers, P.K. and Schreiber, S.L. (1993) *J. Am. Chem. Soc.*, **115**, 12621-12622.

[88] Marshall, J.A. and Johns, B.A. (1998) *J. Org. Chem.*, **63**, 7885-7892.

[89] Halstead, D.P. Ph.D. Thesis, Harvard, Cambridge (1998).

[90] Smith III, A.B.; Kaufman, M.D.; Beauchamp, T.J.; LaMarche, M.J. and Arimoto, H. (1999) *Org. Lett.*, **1**, 1823-1826.

[91] Paterson, I.; Delgado, O.; Florence, G.J.; Lyothier, I.; Scott, J.P. and Sereinig, N. (2003) *Org. Lett.*, **5**, 35-38.

[92] Paterson, I. and Florence, G.J. (2003) *Eur. J. Org. Chem.*, 2193-2208.

[93] Mickel, S.J.; Sedelmeier, G.H.; Niederer, D.; Daeffler, R.; Osmani, A.; Schreiner, K.; Seeger-Weibel, M.; Berod, B.; Schaer, K.; Gamboni, R.; Chen, S.; Chen, W.; Jagoe, C.T.; Kinder Jr, F.R.; Loo, M.; Prasad, K.; Repic, O.; Shieh, W.-C.; Wang, R.-M.; Waykole, L.; Xu, D.D. and Xue, S. (2004) *Org. Process Res. Dev.*, **8**, 92-100.

[94] Mickel, S.J.; Sedelmeier, G.H.; Niederer, D.; Schuerch, F.; Grimler, D.; Koch, G.; Daeffler, R.; Osmani, A.; Hirni, A.; Schaer, K.; Gamboni, R.; Bach, A.; Chaudhary, A.; Chen, S.; Chen, W.; Hu, B.; Jagoe, C.T.; Kim, H.-Y.; Kinder Jr, F.R.; Liu, Y.; Lu, Y.; McKenna, J.; Prashad, M.; Ramsey, T.M.; Repic, O.; Rogers, L.; Shieh, W.-C.; Wang, R.-M. and Waykole, L. (2004) *Org. Process Res. Dev.*, **8**, 101-106.

[95] Mickel, S.J.; Sedelmeier, G.H.; Niederer, D.; Schuerch, F.; Koch, G.; Kuesters, E.; Daeffler, R.; Osmani, A.; Seeger-Weibel, M.; Schmid, E.; Hirni, A.; Schaer, K.; Gamboni, R.; Bach, A.; Chen, S.; Chen, W.; Geng, P.; Jagoe, C.T.; Kinder Jr, F.R.; Lee, G.T.; McKenna, J.; Ramsey, T.M.; Repic, O.; Rogers, L.; Shieh, W.-C.; Wang, R.-M. and Waykole, L. (2004) *Org. Process Res. Dev.*, **8**, 107-112.

[96] Mickel, S.J.; Sedelmeier, G.H.; Niederer, D.; Schuerch, F.; Seger, M.; Schreiner, K.; Daeffler, R.; Osmani, A.; Bixel, D.; Loiseleur, O.; Cercus, J.; Stettler, H.; Schaer, K.; Gamboni, R.; Bach, A.; Chen, G.-P.; Chen, W.; Geng, P.; Lee, G.T.; Loeser, E.; McKenna, J.; Kinder Jr, F.R.; Konigsberger, K.; Prasad, K.; Ramsey, T.M.; Reel, N.; Repic, O.; Rogers, L.; Shieh, W.-C.; Wang, R.-M.; Waykole, L.; Xue, S.; Florence, G. and Paterson, I. (2004) *Org. Process Res. Dev.*, **8**, 113-121.

[97] Mickel, S.J.; Niederer, D.; Daeffler, R.; Osmani, A.; Kuesters, E.; Schmid, E.; Schaer, K.; Gamboni, R.; Chen, W.C.; Loeser, E.; Kinder Jr, F.R.; Konigsberger, K.; Prasad, K.; Ramsey, T.M.; J., R.; Wang, R.M.; Florence, G.; Lyothier, I. and Paterson, I. (2004) *Org. Process Res. Dev.*, **8**, 122-130.

[98] Francavilla, C.; Chen, W. and Kinder Jr, F.R. (2003) *Org. Lett.*, **5**, 1233-1236.

[99] Mita, C.; Hammond, L.A.; Lockhart, A.C.; Shah, M.; Curtright, J.; Chen, T.; Dugan, M.; Cooper, W.; Chaffin, M.; Sharma, S.; Rowinsky, E.K. and Rothenberg, M.L. (2003) *Clin. Canc. Res.*, **9** (Suppl), Abs. A252.

[100] McDaid, H.M.; Huang, G.S.; Lopez, L.; Smith III, A.B.; Goldberg, G.L.; Perez-Soler, R. and Horwitz, S.B. (2003) *Clin. Canc. Res.*, **9** (Suppl), Abs. A249.

[101] Mita, A.; Lockhart, A.C.; Chen, T.-L.; Bochinski, K.; Curtright, J.; Cooper, W.; Hammond, L.; Rothenberg, M.; Rowinsky, E.K. and Sharma, S. (2004) *J. Clin. Oncol.*, **22**, Abs. 2025.

[102] Gunasekera, S.P.; Paul, G.K.; Longley, R.E.; Isbrucker, R.A. and Pomponi, S.A. (2002) *J. Nat. Prod.*, **65**, 1643-1648.

[103] Martello, L.A.; LaMarche, M.J.; He, L.; Beauchamp, T.J.; Smith III, A.B. and Horwitz, S.B. (2001) *Chem. Biol.*, **8**, 843-855.

[104] Honore, S.; Kamath, K.; Brauger, D.; Horwitz, S.B.; Wilson, L.; Briand, C. and Jordan, M.A. (2004) *Cancer Res.*, **64**, 4957-4964.

[105] Kamath, K.; Wilson, L.; Cabral, F. and Jordan, M.A. (2005) *J. Biol. Chem.*, **280**, 12902-12907.

[106] Pasquier, E.; Honore, S.; Pourroy, B.; Jordan, M.A.; Lehmann, M.; Briand, C. and Brauger, D. (2005) *Cancer Res.*, **65**, 2433-2440.

[107] Isbrucker, R.A.; Cummins, J.; Pomponi, S.A.; Longley, R.E. and Wright, A.E. (2003) *Biochem. Pharmacol.*, **66**, 75-82.

[108] Pettit, G.R.; Cichacz, Z.A.; Gao, F.; Boyd, M.R. and Schmidt, J.M. (1994) *Chem. Comm.*, 1111-1112.

[109] Shin, Y.; Choy, N.; Balachandran, R.; Madiraju, C.; Day, B.W. and Curran, D.P. (2002) *Org. Lett.*, **4**, 4443-4446.

[110] Paterson, I.; Britton, R.; Delgardo, O.; Meyer, A. and Poullennc, K.G. (2004) *Angew. Chem. Int. Ed.*, **43**, 4629-4633.

[111] Shin, Y.; Fournier, J.-H.; Fukui, Y.; Bruckner, A.M. and Curran, D.P. (2004) *Angew. Chem. Int. Ed.*, **43**, 4634-4637.

[112] Paterson, I.; Britton, R.; Delgado, O. and Wright, A.E. (2004) *Chem. Comm.*, 632-633.

[113] Madiraju, C.; Edler, M.C.; Raccor, B.S.; Balachandran, R.; Hamel, E.; Giuliano, K.A.; Vogt, A.; Shin, Y.; Fournier, J.-H.; Fukui, Y.; Bruckner, A.M.; Curran, D.P. and Day, B.W. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 3962.

[114] Lindquist, N.; Fenical, W.; Van Duyne, G.D. and Clardy, J. (1991) *J. Am. Chem. Soc.*, **113**, 2303-2304.

[115] Burgett, A.W.G.; Li, Q.; Wei, Q. and Harran, P.G. (2003) *Angew. Chem. Int. Ed.*, **42**, 4961-4966.

[116] Nicolaou, K.C.; Rao, P.B.; Hao, J.; Reddy, M.V.; Rassias, G.; Huang, X.; Chen, D.Y.-K. and Snyder, S.A. (2003) *Angew. Chem. Int. Ed.*, **42**, 1753-1758.

[117] Nicolaou, K.C.; Chen, D.Y.-K.; Huang, X.; Ling, T.; Bella, M. and Snyder, S.A. (2004) *J. Am. Chem. Soc.*, **126**, 12888-12896.

[118] Nicolaou, K.C.; Hao, J.; Reddy, M.V.; Rao, P.B.; Rassias, G.; Snyder, S.A.; Huang, X.; Chen, D.Y.-K.; Brenzovich, W.E.; Giuseppone, N.; Giannakakou, P. and O'Brate, A. (2004) *J. Am. Chem. Soc.*, **126**, 12897-12906.

[119] Cruz-Monserrate, Z.; Vervoort, H.C.; Bai, R.; Newman, D.J.; Howell, S.B.; Los, G.; Mullaney, J.T.; Williams, M.D.; Pettit, G.R.; Fenical, W. and Hamel, E. (2003) *Mol. Pharmacol.*, **63**, 1273-1280.

[120] Lindel, T.; Jensen, P.R.; Fenical, W.; Long, B.H.; Casazza, A.M.; Carboni, J. and Fairchild, C.R. (1997) *J. Am. Chem. Soc.*, **119**, 8744-8745.

[121] D'Ambrosio, M.; Guerriero, A. and Pietra, F. (1987) *Helv. Chim. Acta*, **70**, 2019-2027.

[122] D'Ambrosio, M.; Guerriero, A. and Pietra, F. (1988) *Helv. Chim. Acta*, **71**, 964-976.

[123] Ciomei, M.; Albanese, C.; Pastori, W.; Grandi, M.; Pietra, F.; D'Ambrosio, M.; Guerriero, A. and Battistini, C. (1997) *Proc. Am. Assoc. Cancer Res.*, **38**, Abs 30.

[124] Nicolaou, K.C.; Xu, J.-Y.; Kim, T.; Ohshima, T.; Hosokawa, S. and Pfefferkorn, J. (1997) *J. Am. Chem. Soc.*, **119**, 11353-11354.

[125] Nicolaou, K.C.; van Delft, F.; Ohshima, T.; Vourloumis, D.; Xue, J., Y.; Hosokawa, S.; Pfefferkorn, J.; Kim, S. and Li, T.H. (1997) *Angew. Chem. Int. Ed.*, **36**, 2520-2524.

[126] Nicolaou, K.C.; Xu, J.Y.; Kim, S.; Pfefferkorn, J.; Ohshima, T.; Vourloumis, D. and Hosokawa, S. (1998) *J. Am. Chem. Soc.*, **120**, 8661-8673.

[127] Nicolaou, K.C.; Winssinger, N.; Vourloumis, D.; Ohshima, T.; Kim, S.; Pfefferkorn, J.; Xu, J.-Y. and Li, T. (1998) *J. Am. Chem. Soc.*, **120**, 10814-10826.

[128] Nicolaou, K.C.; Ohshima, T.; Hosokawa, S.; van Delft, F.; Vourloumis, D.; Xu, J.Y.; Pfefferkorn, J. and Kim, S. (1998) *J. Am. Chem. Soc.*, **120**, 8674-8680.

[129] Nicolaou, K.C.; Kim, S.; Pfefferkorn, J.; Xu, J.-Y.; Ohshima, T.; Hosokawa, S.; Vourloumis, D. and Li, T. (1998) *Angew. Chem. Int. Ed.*, **37**, 1418-1421.

[130] Chen, X.-T.; Zhou, B.; Bhattacharya, S.K.; Gutteridge, C.E.; Pettus, T.R.R. and Danishefsky, S.J. (1998) *Angew. Chem. Int. Ed.*, **37**, 789-792.

[131] Hamel, E.; Sackett, D.L.; Vourloumis, D. and Nicolaou, K.C. (1999) *Biochemistry*, **38**, 5490-5498.

[132] McDaid, H.M.; Bhattacharya, S.K.; Chen, X.-T.; He, L.; Shen, H.-J.; Gutteridge, C.E.; Horwitz, S.B. and Danishefsky, S.J. (1999) *Cancer Chemother. Pharmacol.*, **44**, 131-137.

[133] Ojima, I.; Chakravarty, S.; Inoue, T.; Lin, S.; He, L.; Horwitz, S.B.; Kuduk, S.D. and Danishefsky, S.J. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 4256-4261.

[134] Cinel, B.; Roberge, M.; Behrisch, H.; van Ofwegen, L.; Castro, C.B. and Andersen, R.J. (2000) *Org. Lett.*, **2**, 257-260.

[135] Britton, R.; Roberge, M.; Berisch, H. and Andersen, R.J. (2001) *Tet. Lett.*, **42**, 2953-2956.

[136] Britton, R.; de Silva, E.D.; Bigg, C.M.; McHardy, L.M.; Roberge, M. and Andersen, R.J. (2001) *J. Am. Chem. Soc.*, **123**, 8632-8633.

[137] Taglialatela-Scafati, O.; Deo-Jangra, U.; Campbell, M.; Roberge, M. and Andersen, R.J. (2002) *Org. Lett.*, **4**, 4085-4088.

[138] Chandrasekhar, S.; Jagadeshwar, V.; Narsihmulu, C.; Sarangapani, M.; Krishna, D.R.; Vidyasagar, J.; Vijay, D. and Sastry, G.N. (2004) *Bioorg. Med. Chem. Letts.*, **14**, 3687-3689.

[139] Castoldi, D.; Caggiano, L.; Panigada, L.; Sharon, O.; Costa, A.M. and Gennari, C. (2005) *Angew. Chem. Int. Ed.*, **44**, 588-591.

[140] Castoldi, D.; Caggiano, L.; Bayon, P.; Costa, A.M.; Cappella, P.; Sharon, A. and Gennari, C. (2005) *Tetrahedron*, **61**, 2123-2139.

[141] Michaelis, M.L.; Ranciat, N.; Chen, Y.; Bechtel, M.; Ragan, R.; Hepperle, M.; Liu, Y. and Georg, G. (1998) *J. Neurochem.*, **70**, 1623-1627.

[142] Li, G.; Faibushevich, A.; Turunen, B.J.; Yoon, S.O.; Georg, G.; Michaelis, M.L. and Dobrowsky, R.T. (2003) *J. Neurochem.*, **84**, 347-362.

[143] Michaelis, M.L.; Ansar, S.; Chen, Y.; Reiff, E.R.; Seyb, K.I.; Himes, R.H.; Audus, K.L. and Georg, G. (2005) *J. Pharmacol. Exptl. Therap.*, **312**, 659-668.

[144] Quinoa, E.; Kakou, Y. and Crews, P. (1988) *J. Org. Chem.*, **53**, 3642-3644.

[145] Corley, D.G.; Herb, R.; Moore, R.E.; Scheuer, P.J. and Paul, V.P. (1988) *J. Org. Chem.*, **53**, 3644-3646.

[146] Tanaka, J.; Higa, T.; Bernadelli, G. and Jefford, C.W. (1996) *Chem. Lett.*, 255-256.

[147] Cutignano, A.; Bruno, I.; Bifulco, G.; Casapullo, A.; Debitus, C.; Gomez-Paloma, L. and Riccio, R. (2001) *Eur. J. Org. Chem.*, 775-778.

[148] Mooberry, S.L.; Tien, G.; Hernandez, A.H.; Plubrukarn, A. and Davidson, B.S. (1999) *Cancer Res.*, **59**, 653-660.

[149] Pryor, D.E.; O'Brate, A.; Bilcer, G.; Diaz, J.F.; Wang, Y.; Wang, Y.; Kabaki, M.; Jung, M.K.; Andreu, J.M.; Ghosh, A.R.; Giannakakou, P. and Hamel, E. (2002) *Biochemistry*, **41**, 9109-9115.

[150] Mooberry, S.L.; Leal, R.M.; Randall-Hlubeck, D.A. and Davidson, B.S. (2003) *Clin. Canc. Res.*, **9** (Suppl). Abs. A274.

[151] Lu, H.; Murtagh, J. and Schwartz, E.L. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 2989.

[152] Ahmed, A.; Hoegenauer, E.K.; Enev, V.S.; Hanbauer, M.; Kaehlig, H.; Ohler, E. and Mulzer, J. (2003) *J. Org. Chem.*, **68**, 3026-3042.

[153] Mulzer, J. and Ohler, E. (2003) *Chem. Rev.*, **103**, 3753-3786.

[154] Wender, P.A.; Hegde, S.G.; Hubbard, R.D.; Zhang, L. and Mooberry, S.L. (2003) *Org. Lett.*, **5**, 3507-3509.

[155] Mooberry, S.L.; Randall-Hlubeck, D.A.; Leal, R.M.; Hegde, S.G.; Hubbard, R.D.; Zhang, L. and Wender, P.A. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 8803-8808.

[156] Ashley, G. and Metcalf, B. (2003) US Patent 6,670,389 B2, 30DEC2003.

[157] Gallagher Jr., B.M.; Fang, F.G.; Johannes, C.W.; Pesant, M.; Tremblay, M.R.; Zhao, H.; Akasaka, K.; Li, X.-y.; Liu, J. and Littlefield, B.A. (2004) *Bioorg. Med. Chem. Letts.*, **14**, 575-579.

[158] Gallagher Jr., B.M.; Zhao, H.; Pesant, M. and Fang, F.G. (2005) *Tetrahedron Letts.*, **46**, 923-926.

[159] Paterson, I.; Bergmann, H.; Menche, D. and Berkessel, A. (2004) *Org. Lett.*, **6**, 1293-1295.

[160] West, L.M. and Northcote, P.T. (2000) *J. Org. Chem.*, **65**, 445-449.

[161] Hood, K.A.; West, L.M.; Rouwe, B.; Northcote, P.T.; Berridge, M.V.; Wakefield, S.J. and Miller, J.H. (2002) *Cancer Res.*, **62**, 3356-3360.

[162] Gaitanos, T.N.; Buey, R.M.; Diaz, J.F.; Northcote, P.T.; Teesdale-Spittle, P.; Andreu, J.M. and Miller, J.H. (2004) *Cancer Res.*, **64**, 5063-5067.

[163] Pineda, O.; Farras, J.; Maccari, L.; Manetti, F.; Botta, M. and Vilarrasa, J. (2004) *Bioorg. Med. Chem. Letts.*, **14**, 4825-4829.

[164] Miller, J.H.; Rouwe, B.; Gaitanos, T.N.; Hood, K.A.; Crume, K.P.; Backstrom, B.T.; La Flamme, A.C.; Berridge, M.V. and Northcote, P.T. (2004) *Apoptosis*, **9**, 785-796.
[165] Ghosh, A.K. and Kim, J.-H. (2003) *Tetrahedron Lett.*, **44**, 3967-3969.
[166] Liao, X.; Wu, J. and De Brabander, J.K. (2003) *Angew. Chem. Int. Ed.*, **42**, 1648-1652.
[167] Jin, M. and Taylor, R.E. (2005) *Org. Lett.*, **7**, 1303-1305.
[168] Cragg, G.M. and Newman, D.J. (2004) *J. Nat. Prod.*, **67**, 232-244.
[169] Denny, W.A. (2004) *Expert Opin. Emerg. Drugs*, **9**, 105-133.
[170] Marshall, K.M.; Holden, J.A.; Koller, A.; Kashman, Y.; Copp, B.R. and Barrows, L.R. (2004) *Anticancer Drugs*, **15**, 907-913.
[171] Marshall, K.M. and Barrows, L.R. (2004) *Nat. Prod. Rep.*, **21**, 731-751.
[172] Kisselev, A.F. and Goldberg, A.L. (2001) *Chem. Biol.*, **8**, 739-758.
[173] Kyle, R.A. and Rajkumar, S.V. (2004) *N. Engl. J. Med.*, **351**, 1860-1873.
[174] Newman, D.J.; Cragg, G.M. and Snader, K.M. (2003) *J. Nat. Prod.*, **66**, 1022-1037.
[175] Adams, J. (2003) *Cancer Cell*, **5**, 417-421.
[176] Brannigan, J.A.; Dodson, G.; Duggleby, H.J.; Moody, P.C.; Smith, J.L.; Tomchick, D.R. and Murzin, A.G. (1995) *Nature*, **378**, 416-419.
[177] Omura, S.; Fujimoto, T.; Otoguro, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H. and Sasaki, Y. (1991) *J. Antibiot.*, **44**, 113-116.
[178] Fenteany, G.; Standaert, R.F.; Lane, W.S.; Choi, S.; Corey, E.J. and Schreiber, S.L. (1995) *Science*, **268**, 726-731.
[179] Craiu, A.; Gaczynska, M.; Akopian, T.; Gramm, C.F.; Fenteany, G.; Goldberg, A.L. and Rock, K.L. (1997) *J. Biol. Chem.*, **272**, 13437-13445.
[180] Dick, L.R.; Cruikshank, A.A.; Grenier, L.; Melandri, F.D.; Nunes, S.L. and Stein, R.L. (1996) *J. Biol. Chem.*, **271**, 7273-7276.
[181] Dick, L.R.; Cruikshank, A.A.; Destree, A.T.; Grenier, L.; McCormack, T.A.; Melandri, F.D.; Nunes, S.L.; Palombella, V.J.; Parent, L.A.; Plamodon, L. and Stein, R.L. (1997) *J. Biol. Chem.*, **272**, 182-188.
[182] Corey, E.J. and Li, W.-D.Z. (1999) *Chem. Pharm. Bull. (Tokyo)*, **47**, 1-10.
[183] Feling, R.H.; Buchanan, G.O.; Mincer, T.J.; Kauffman, C.A.; Jensen, P.R. and Fenical, W. (2003) *Angew. Chem. Int. Ed.*, **42**, 355-357.
[184] Reddy, L.R.; Saravan, P. and Corey, E.J. (2004) *J. Am. Chem. Soc.*, **126**, 6230-6231.
[185] Meng, L.H.; Mohan, R.; Kwok, B.H.B.; Elofsson, M.; Sin, N. and Crews, C.M. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 10403-10408.
[186] Meng, L.H.; Kwok, B.H.B.; Sin, N. and Crews, C.M. (1999) *Cancer Res.*, **59**, 2798-2801.
[187] Groll, M.; Kim, K.B.; Kairies, N.; Huber, R. and Crews, C.M. (2000) *J. Am. Chem. Soc.*, **122**, 1237-1238.
[188] Johnstone, R.W. (2002) *Nat. Revs. Drug Discov.*, **1**, 287-299.
[189] Kingston, D.G.I. and Newman, D.J. (2005) *Curr. Opin. Drug Discov. Devel.*, **8**, 207-227.
[190] Kristelett, R.; Stimson, L.; Workman, P. and Aherne, W. (2004) *Expert Opin. Emerg. Drugs*, **9**, 135-154.
[191] De Ruijter, A.J.; Van Gennip, A.H.; Caron, H.N.; Kemp, S. and Van Kuilenburg, A.B. (2003) *Biochem. J.*, **370**, 737-749.
[192] Sears, R.; Ohtani, K. and Nevins, J.R. (1997) *Mol. Cell Biol.*, **17**, 5227-5235.
[193] Nomura, T.; Khan, M.M.; Kaul, S.C.; Dong, H.-D.; Wadhwa, R.; Colmenares, C.; Kohno, I. and Ishii, S. (1999) *Genes Dev.*, **13**, 412-423.
[194] Dang, C.V. (1999) *Mol. Cell Biol.*, **19**, 1-11.
[195] Magnaghi-Jaulin, L.; Groisman, R.; Naguibneva, I.; Robin, P.; Lorain, S.; Le Villain, J.P.; Troalen, F.; Trouche, D. and Harel-Bellan, A. (1998) *Nature*, **391**, 601-605.
[196] Brehm, A.; Miska, E.A.; McCance, D.J.; Reid, J.L.; Bannister, A.J. and Kouzarides, T. (1998) *Nature*, **391**, 597-601.
[197] Sellers, W.R. and Kaelin Jr., W.G. (1997) *J. Clin. Oncol.*, **15**, 3301-3312.
[198] Cress, W.D. and Seto, E. (2000) *J. Cell Phys.*, **184**, 1-16.
[199] Fischle, W.; Kiermer, V.; Dequiedt, F. and Verdin, E. (2001) *Biochem. Cell Biol.*, **79**, 337-348.
[200] Kim, M.S.; Kwon, H.J.; Lee, Y.M.; Baek, J.H.; Jang, J.-E.; Lee, S.-W.; Moon, E.-J.; Kim, H.-S.; Lee, S.-K.; Chung, H.Y.; Kim, C.W. and Kim, K.-W. (2001) *Nat. Med.*, **7**, 437-443.
[201] Deroanne, C.F.; Bonjean, K.; Servotte, S.; Devy, L.; Colige, A.; Clausse, N.; Blacher, S.; Verdin, E.; Foidart, J.-M.; V., N.B. and Castronovo, V. (2002) *Oncogene*, **21**, 427-436.
[202] Remiszewski, S.W. (2002) *Curr. Opin. Drug. Discov. Devel.*, **5**, 487-499.
[203] Yoshida, M.; Furumai, R.; Nishiyama, M.; Komatsu, Y.; Nishino, N. and Horinouchi, S. (2001) *Cancer Chemother. Pharmacol.*, **48**, S20-S26.
[204] Yoshida, M.; Matsuyama, A.; Komatsu, Y. and Nishino, N. (2003) *Curr. Med. Chem.*, **10**, 2351-2358.
[205] Tsuji, N.; Kobayashi, N.; Nagashima, K.; Wakisaka, Y. and Koizumi, K. (1976) *J. Antibiot.*, **29**, 1-6.
[206] Vanhaecke, T.; Papeleu, P.; Elaut, G. and Rogiers, V. (2004) *Curr. Med. Chem.*, **11**, 1629-1643.
[207] Van Lint, C.; Emiliani, S. and Verdin, E. (1996) *Gene Expression*, **5**, 245-253.
[208] Mariadason, J.M.; Corner, G.A. and Augenlicht, L.H. (2000) *Cancer Res.*, **60**, 4561-4572.
[209] Kijima, M.; Yoshida, M.; Sugita, K.; Horinouchi, S. and Beppu, T. (1993) *J. Biol. Chem.*, **268**, 22429-22435.
[210] Sambucetti, L.C.; Fischer, D.D.; Zabludoff, S.; Kwon, P.O.; Chamberlin, H.; Trogani, N.; Xu, H. and Cohen, D. (1999) *J. Biol. Chem.*, **274**, 34940-34947.
[211] Arabshahi, L. and Schmitz, F.J. (1987) *J. Org. Chem.*, **52**, 3584-3586.
[212] Quinoa, E. and Crews, P. (1987) *Tetrahedron Lett.*, **28**, 3229-3232.
[213] Pina, I.C.; Gautschi, J.T.; Wang, G.-Y.-S.; Sanders, M.L.; Schmitz, F.J.; France, D.; Cornell-Kennon, S.; Sambucetti, L.C.; Remiszewski, S.W.; Perez, L.B.; Bair, K.W. and Crews, P. (2003) *J. Org. Chem.*, **68**, 3866-3873.
[214] Remiszewski, S.W.; Sambucetti, L.C.; Bair, K.W.; Bontempo, J.; Cesarz, D.; Chandramouli, N.; Chen, R.; Cheung, M.; Cornell-Kennon, S.; Dean, K.; Diamantidis, G.; France, D.; Green, M.A.; Howell, K.L.; Kashi, R.; Kwon, P.; Lassota, P.; Martin, M.S.; Mou, Y.; Perez, L.B.; Sharma, S.; Smith, T.; Sorensen, E.; Taplin, F.; Trogani, N.; Versace, R.W.; Walker, H.; Weltchek-Engler, S.; Wood, A.; Wu, A. and Atadja, P. (2003) *J. Med. Chem.*, **46**, 4609-4624.
[215] Remiszewski, S.W. (2003) *Curr. Med. Chem.*, **10**, 2393-2402.
[216] Catley, L.; Weisberg, E.; Tai, Y.-T.; Atadja, P.; Remiszewski, S.W.; Hideshima, T.; Mitsiades, N.; Shringarpure, R.; LeBlanc, R.; Chauhan, D.; Munshi, N.C.; Schlossman, R.; Richardson, P.; Griffin, J. and Anderson, K.C. (2003) *Blood*, **102**, 2615-2622.
[217] Perry, N.B.; Ettouati, L.; Litaudon, M.; Blunt, J.W. and Munro, M.H.G. (1994) *Tetrahedron*, **50**, 3987-3992.
[218] Trimurtulu, G.; Faulkner, D.J.; Perry, N.B.; Ettouati, L.; Litaudon, M.; Blunt, J.W.; Munro, M.H.G. and Jameson, G.B. (1994) *Tetrahedron*, **50**, 3993-4000.
[219] Ahaidar, A.; Fernandez, D.; Danelon, G.; Cuevas, C.; Manzanares, I.; Alberico, F.; Joule, J.A. and Alvarez, M. (2003) *J. Org. Chem.*, **68**, 10020-10029.
[220] Erba, E.; Simone, M.; Jimeno, J.; Faircloth, G.T. and D'Incalci, M. (2003) *Proc. Am. Assoc. Cancer Res.*, **44**, Abs. R665.
[221] Erba, E.; Simone, M.; Damia, G.; Vikhanskaya, F.; Di Francesco, A.; Jimeno, J.; Cuevas, C.; Faircloth, G. and D'Incalci, M. (2003) *Clin. Canc. Res.*, **9** (Suppl), Abs. C78.
[222] Yin, J.; Aviles, P.; Guillen, M.J.; Ly, C.; Faircloth, G.T.; Lee, W.W.; Munt, S. and Merchante, C.C. (2004) *Proc. Am. Assoc. Cancer Res.*, **45**, Abs. 5396.
[223] Cespades, M.V.; Guillen, M.J.; Parreno, M.; Casanova, I.; Pavon, M.A.; Mangues, R.; Faircloth, G.; Cuevas, C. and Aviles, P. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 644.
[224] Cespades, M.V.; Guillen, M.J.; Parreno, M.; Casanova, I.; Pavon, M.A.; Mangues, R.; Faircloth, G.; Cuevas, C. and Aviles, P. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 640.
[225] Maugard, T.; Enaud, E.; Choisy, P. and Legoy, M.D. (2001) *Phytochem.*, **58**, 897-904.
[226] Cooksey, C.J. (2001) *Molecules*, **6**, 736-769.
[227] MacNeil, I.A.; Tiong, C.L.; Minor, C.; August, P.R.; Grossman, T.H.; Loiacono, K.A.; Lynch, B.A.; Phillips, T.; Narula, S.; Sundaramoorthi, R.; Tyler, A.; Aldredge, T.; Long, H.; Gilman, M.; Holt, D. and Osburne, M.S. (2001) *J. Mol. Microbiol. Biotechnol.*, **3**, 301-308.
[228] Adachi, J.; Mori, Y.; Matsui, S.; Takigam, H.; Fujino, J.; Kitagawa, H.C.A.; Miller III, C.H.; Kato, T.; Saeki, K. and Matsuda, T. (2001) *J. Biol. Chem.*, **276**, 31475-31478.

[229] Chen, D. and Xie, J. (1984) *Zhongcaoyao*, **15**, 534-536.
[230] Xiao, Z.; Hao, Y.; Liu, B. and Qian, L. (2002) *Leuk. Lymphoma*, **43**, 1763-1768.
[231] Meijer, L.; Skaltsounis, A.-L.; Magiatis, P.; Polychronopoulos, P.; Knockaert, M.; Leost, M.; Ryan, X.P.; Vonica, C.A.; Brivanlou, A.; Dajani, R.; Crovace, C.; Tarricone, C.; Musacchio, A.; Roe, S.M.; Pearl, L. and Greengard, P. (2003) *Chem. Biol.*, **10**, 1255-1266.
[232] Polychronopoulos, P.; Magiatis, P.; Skaltsounis, A.-L.; Myrianthopoulos, V.; Mikros, E.; Tarricone, A.; Musacchio, A.; Roe, S.M.; Pearl, L.; Leost, M.; Greengard, P. and Meijer, L. (2004) *J. Med. Chem.*, **47**, 935-946.
[233] Guengerich, F.P.; Sorrells, J.L.; Schmitt, S.; Krauser, J.A.; Aryal, P. and Meijer, L. (2004) *J. Med. Chem.*, **47**, 3236-3241.
[234] Li, Q.S.; Schwaneberg, U.; Fischer, P.M. and Schmid, R.D. (2000) *Chem. Eur. J.*, **6**, 1531-1536.
[235] Gillam, E.M.J.; Notley, L.M.; Cai, H.; DeVoss, J.J. and Guengerich, F.P. (2000) *Biochemistry*, **39**, 13817-13824.
[236] Wu, Z.-L.; Aryal, P.; Lozach, O.; Meijer, L. and Guengerich, F.P. (2005) *Chem. Biodiv.*, **2**, 51-65.
[237] Knockaert, M.; Blondel, M.; Bach, S.; Leost, M.; Elbi, C.; Hager, G.L.; Nagy, S.R.; Han, D.; Denison, M.; Ffrench, M.; Ryan, X.P.; Magiatis, P.; Polychronopoulos, P.; Greengard, P.; Skaltsounis, L. and Meijer, L. (2004) *Oncogene*, **23**, 4400-4412.
[238] Denison, M.S. and Nagy, S.R. (2003) *Annu. Rev. Pharmacol. Toxicol.*, **43**, 309-334.
[239] Duensing, S.; Duensing, A.; Lee, D.C.; Edwards, K.M.; Piboonniyom, S.-O.; Manuel, E.; Skaltsounis, A.-L.; Meijer, L. and Munger, K. (2004) *Oncogene*, **23**, 8206-8215.
[240] Cohen, P. and Goedert, M. (2004) *Nat. Rev. Drug Discov.*, **3**, 479-487.
[241] Gompel, M.; Leost, M.; De Kier Joffe, E.B.; Puricelli, L.; Franco, L.H.; Palermo, J. and Meijer, L. (2004) *Bioorg. Med. Chem. Lett.*, **14**, 1703-1707.
[242] Sigel, M.M.; Wellham, L.L.; Lichter, W.; Dudeck, L.E.; Gargus, J. and Lucas, A.H. (1969) in *Food-Drugs from the Sea Proceedings*, (Younghen Jr, H.W., Ed.), Marine Technology Society, Washington, DC, pp. 281-294.
[243] Rinehart, K.; Holt, T.G.; Fregeau, N.L.; Stroh, J.G.; Kiefer, P.A.; Sun, F.; Li, L.H. and Martin, D.G. (1990) *J. Org. Chem.*, **55**, 4512-4515.
[244] Wright, A.E.; Forleo, D.A.; Gunawardana, G.P.; Gunasekera, S.P.; Koehn, F.E. and McConnell, O.J. (1990) *J. Org. Chem.*, **55**, 4508-4512.
[245] Scott, J.D. and Williams, R.M. (2002) *Chem. Rev.*, **102**, 1669-1730.
[246] Fontana, A.; Cavaliere, P.; Wahidulla, S.; Naik, C.G. and Cimino, G. (2000) *Tetrahedron*, **56**, 7305-7308.
[247] Oku, N.; Matsunaga, S.; van Soest, R.W.M. and Fusetani, N. (2003) *J. Nat. Prod.*, **66**, 1136-1139.
[248] Zewail-Foote, M.; Li, V.-S.; Kohn, H.; Bearss, D.; Guzman, M. and Hurley, K.H. (2001) *Chem. Biol.*, **8**, 1033-1049.
[249] Takebayashi, Y.; Pourquier, P.; Zimonjic, D.B.; Nakayama, K.; Emmert, S.; Ueda, T.; Urasaki, Y.; Akiyama, S.-I.; Popescu, N.; Kraemer, K.H. and Pommier, Y. (2001) *Nat. Med.*, **7**, 961-966.
[250] Bonfanti, M.; La Valle, E.; Fernandez Sousa-Faro, J.-M.; Faircloth, G.; Caretti, G.; Mantovani, R. and D'Incalci, M. (1999) *Anti-Cancer Drug Des.*, **14**, 179-186.
[251] van Kesteren, C.; de Vooght, M.M.M.; Lopez-Lazaro, L.; Mathot, R.A.A.; Schellens, J.H.M.; Jimeno, J.M. and Beijnen, J.H. (2003) *Anti-Cancer Drugs*, **14**, 487-502.
[252] Dziegielewska, B.; Kowalski, D. and Beerman, T.A. (2004) *Biochemistry*, **43**, 14228-14237.
[253] Mandola, M.V.; Kolb, E.A. and Scotto, K.W. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 4122.
[254] Le Cesne, A.; Blay, J.Y.; Judson, I.; Van Oosterom, A.; Verweij, J.; Radford, J.; Lorigan, P.; Rodenhuis, S.; Ray-Coquard, I.; Bonvalot, S.; Collin, F.; Jimeno, J.; Di Paola, E.; Van Glabbeke, M. and Nielsen, O.S. (2005) *J. Clin. Oncol.*, **23**, 576-584.
[255] Sessa, C.; De Braud, F.; Perotti, A.; Bauer, J.; Curigliano, G.; Noberasco, C.; Zanaboni, F.; Gianni, L.; Marsoni, S.; Jimeno, J.; D'Incalci, M.; Dall'o, E. and Colombo, N. (2005) *J. Clin. Oncol.*, **23**, 1867-1874.
[256] Beumer, J.H.; Schellens, J.H.M. and Beijnen, J.H. (2005) *Pharmacol. Res.*, **51**, 391-398.
[257] Anon (2005) *DailyDrugNews.com (Daily Essentials)*, 25 JAN 05.
[258] Yin, J.; Aviles, P.; Lee, W.; Ly, C.; Guillen, M.J.; Munt, S.; Cuevas, C. and Faircloth, G. (2005) *Rapid Comm. Mass. Spec.*, **19**, 689-695.
[259] Elices, M.; Grant, W.; Harper, C.; Guillen, M.J.; Cuevas, C.; Faircloth, G. and Aviles, P. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 623.
[260] Elices, M.; Sasak, H.; Caylor, T.; Grant, W.; Guillen, M.J.; Martin, J.; Cuevas, C.; Aviles, P. and Faircloth, G. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 5882.
[261] Delfourne, E. and Bastide, J. (2003) *Med. Res. Revs.*, **23**, 234-252.
[262] Delfourne, E.; Kiss, R.; Le Corre, L.; Merza, J.; Bastide, J.; Frydman, A. and Darro, F. (2003) *Bioorg. Med. Chem.*, **11**, 4351-4356.
[263] Marshall, K.M.; Matsumoto, S.S.; Holden, J.A.; Concepcion, G.P.; Tasdemir, D.; Ireland, C.M. and Barrows, L.R. (2003) *Biochem. Pharmacol.*, **66**, 447-458.
[264] Cuadros, R.; Montejo de Garcini, E.; Wandosell, F.; Faircloth, G.; Fernandez-Sousa, J.M. and Avila, J. (2000) *Cancer Lett.*, **152**, 23-29.
[265] Alvarez-Miranda, M.; Rodriguez-Gonzalez, A.; Otero, G. and Lacal, J.C. (2003) *Clin. Canc. Res.*, **9** (Suppl), Abs. C17.
[266] Salcedo, M.; Cuevas, C.; Otero, G.; Sanchez-Puelles, J.M.; Fernandez Sousa-Faro, J.-M.; Avila, J. and Wandosell, F. (2003) *Clin. Canc. Res.*, **9** (Suppl), Abs. C24.
[267] Salcedo, M.; Cuevas, C.; Sanchez-Puelles, J.M.; Otero, G.; Fernandez Sousa-Faro, J.-M.; Avila, J. and Wandosell, F. (2003) *Proc. Am. Assoc. Cancer Res.*, **44**, Abs. 3649.
[268] Baird, R.; Clarke, P. and Workman, P. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 4111.
[269] Fischer, P.M. (2004) *Curr. Med. Chem.*, **11**, 1563-1583.
[270] Breinbauer, R.; Vetter, I.R. and Waldmann, H. (2002) *Angew. Chem. Int. Ed.*, **41**, 2878-2890.
[271] Brohm, D.; Metzger, S.; Bhargava, A.; Muller, O.; Lieb, F. and Waldmann, H. (2002) *Angew. Chem. Int. Ed.*, **41**, 307-311.
[272] Koonin, E.V.; Wolf, Y.I. and Karev, G.P. (2003) *Nature*, **420**, 218-223.
[273] Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M.; Fredinger, R.M.; Whitter, W.L.; Lundell, G.F.; Veber, D.F.; Anderson, P.S.; Chang, R.S.L.; Lotti, V.J.; Cerino, D.J.; Chen, T.B.; Kling, P.; Kunkel, K.A.; Springer, J.P. and Hirshfield, J. (1988) *J. Med. Chem.*, **31**, 2235-2246.
[274] Nicolaou, K.C.; Pfefferkorn, J.A.; Barluenga, S.; Mitchell, H.J.; Roecker, A.J. and Cao, G.-Q. (2000) *J. Am. Chem. Soc.*, **122**, 9968-9976.
[275] Nicolaou, K.C.; Pfefferkorn, J.A.; Mitchell, H.J.; Roecker, A.J.; Barluenga, S.; Cao, G.-Q.; Affleck, R.L. and Lillig, J.E. (2000) *J. Am. Chem. Soc.*, **122**, 9054-9967.
[276] Nicolaou, K.C.; Pfefferkorn, J.A.; Roecker, A.J.; Cao, G.-Q.; Barluenga, S. and Mitchell, H.J. (2000) *J. Am. Chem. Soc.*, **122**, 9939-9953.
[277] Kobayashi, J.; Madono, T. and Shigemori, H. (1995) *Tetrahedron*, **51**, 10867-10974.
[278] Zhou, B.-N.; Johnson, R.K.; Mattern, M.R.; Fisher, P.W. and Kingston, D.G.I. (2001) *Org. Lett.*, **3**, 4047-4049.
[279] Arnold, L.D.; Calderwood, D.J.; Dixon, R.W.; Johnston, D.N.; Kamens, J.S.; Munschauer, R.; Rafferty, P. and Ratnofsky, S.E. (2000) *Bioorg. Med. Chem. Lett.*, **10**, 2167-2170.
[280] Burchat, A.F.; Calderwood, D.J.; Hirst, G.C.; Holman, N.J.; Johnston, D.N.; Munschauer, R.; Rafferty, P. and Tometzki, G.B. (2000) *Bioorg. Med. Chem. Lett.*, **10**, 2171-2174.
[281] Calderwood, D.J.; Johnston, D.N.; Munschauer, R. and Rafferty, P. (2002) *Bioorg. Med. Chem. Lett.*, **12**, 1683-1686.
[282] Burchat, A.F.; Calderwood, D.J.; Friedman, M.M.; Hirst, G.C.; Li, B.; Rafferty, P.; Ritter, K. and Skinner, B.S. (2002) *Bioorg. Med. Chem. Lett.*, **12**, 1687-1690.
[283] Kissau, L.; Stahl, P.; Mazitschek, R.; Giannis, A. and Waldmann, H. (2003) *J. Med. Chem.*, **46**, 2917-2931.
[284] Koch, M.A.; Wittenberg, L.-O.; Basu, S.; Jeyaraj, D.A.; Gourzoulidou, E.; Reinecke, K.; Odermatt, A. and Waldmann, H. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 16721-16726.
[285] Wan, Y.; Hur, W.; Cho, C.Y.; Liu, Y.; Adrian, F.J.; Lozach, O.; Bach, S.; Mayer, T.; Fabbro, D.; Meijer, L. and Gray, N.S. (2004) *Chem. Biol.*, **11**, 247-259.
[286] Rinehart, K.L. and Lithgow-Bertelloni, A.M. (1990) UK Patent GB 22026, 01OCT1990.

[287] Sakai, R.; Rinehart, K.L.; Kishore, V.; Kundu, B.; Faircloth, G.; Gloer, J.B.; Carney, J.R.; Manikoshi, M.; Sun, F.; Hughes Jr, R.G.; Garcia-Gravalos, D.; Garcia de Quesada, T.; Wilson, G.R. and Heid, R.M. (1996) *J. Med. Chem.*, **39**, 2819-2834.
[288] Faircloth, G.; Rinehart, K.; Nunez de Castro, I. and Jimeno, J. (1996) *Ann. Oncol.*, **7**, 34.
[289] Urdiales, J.L.; Morata, P.; Nunez de Castro, I. and Sanchez-Jimenez, F. (1996) *Cancer Lett.*, **102**, 31-37.
[290] Cuevas, C.; Cuevas, F.; Gallego, P.; Mandez, P.; Manzanares, I.; Munt, S.; Polanco, C. and Rodriguez, I. (2002) European Patent WO 02022596, 01OCT2002
[291] Taraboletti, G.; Poli, M.; Dossi, R.; Manenti, L.; Borsotti, P.; Faircloth, G.T.; Broggini, M.; D'Incalci, M.; Ribatti, D. and Giavazzi, R. (2004) *Br. J. Cancer*, **90**, 2418-2424.
[292] Cuadrado, A.; Gonzalez, L.; Suarez, Y.; Martinez, T. and Munoz, A. (2004) *Oncogene*, **23**, 4673-4680.
[293] Losada, A.; Lopez-Oliva, J.M.; Sanchez-Puelles, J.M. and Garcia-Ferenandez, L.F. (2004) *Br. J. Cancer*, **91**, 1405-1413.
[294] Menon, L.G.; Saydam, G.; Longo, G.S.A.; Jimeno, J.M.; Bertino, J.R. and Banerjee, D. (2005) *Proc. Am. Assoc. Cancer Res.*, **46**, Abs. 5104.
[295] Gomez, S.G.; Bueren, J.A.; Faircloth, G.T.; Jimeno, J. and Albella, B. (2003) *Exp. Hematol.*, **31**, 1104-1111.
[296] Vera, M. and Joullie, M.M. (2002) *Med. Res. Revs.*, **22**, 102-145.
[297] Cardenas, F.; Caba, J.M.; Feliz, M.; Lloyd-Williams, P. and Giralt, E. (2003) *J. Org. Chem.*, **68**, 9554-9562.
[298] Gutierrez-Rodriguez, M.; Martin-Martinez, M.; Garcia-Lopez, M.T.; Herranz, R.; Cuevas, F.; Polanco, C.; Rodriguez-Campos, I.; Manzanares, I.; Cardenas, F.; Feliz, M.; Lloyd-Williams, P. and Giralt, E. (2004) *J. Med. Chem.*, **47**, 5700-5712.

Received: September 12, 2005 **Accepted: October 4, 2005**