available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Review

Marine pharmacology in 2003–2004: Anti-tumour and cytotoxic compounds

Alejandro M.S. Mayer[a,*], *Kirk R. Gustafson*[b]

[a]*Department of Pharmacology, Chicago College of Osteopathic Medicine, Midwestern University, Prabhu Hall 108, 555 31st Street, Downers Grove, IL 60515, USA*

[b]*Molecular Targets Development Program, Center for Cancer Research, NCI-Frederick, Building 1052, Room 121, Frederick, MA 21702, USA*

ARTICLE INFO

Article history:
Received 23 March 2006
Received in revised form 9 May 2006
Accepted 10 May 2006
Available online 9 August 2006

Keywords:
Marine
Anti-tumour
Cytotoxic
Anti-cancer
Anti-neoplastic
Agents
Preclinical
Clinical
Pharmacology
Review
Global

ABSTRACT

During 2003 and 2004, marine pharmacology research directed towards the discovery and development of novel anti-tumour agents was published in 163 peer-reviewed articles. The purpose of this review is to present a structured assessment of the anti-tumour and cytotoxic properties of 150 marine natural products, many of which are novel compounds that belong to diverse structural classes, including polyketides, terpenes, steroids and peptides. The organisms yielding these bioactive marine compounds include invertebrate animals, algae, fungi and bacteria. Anti-tumour pharmacological studies were conducted with 31 structurally defined marine natural products in a number of experimental and clinical models that further defined their mechanisms of action. Particularly potent *in vitro* cytotoxicity data generated with murine and human tumour cell lines was reported for 119 novel marine chemicals with as yet undetermined mechanisms of action. Noteworthy is the fact that marine anti-cancer research was sustained by a global collaborative effort, involving researchers from Australia, Austria, Canada, China, Egypt, France, Germany, Italy, Japan, Mexico, the Netherlands, New Zealand, Papua New Guinea, the Philippines, South Africa, South Korea, Spain, Switzerland, Taiwan, Thailand and the United States of America (USA). Finally, this 2003–2004 overview of the marine pharmacology literature highlights the fact that the discovery of novel marine anti-tumour agents continued at the same pace as during 1998–2002.

1. Introduction

The purpose of this article is to review the 2003–2004 research literature in the field of marine anti-tumour pharmacology, using a format similar to the one used in our previous four reports, which covered 1998–2002.[1–4] The pharmacology of marine compounds with anthelminthic, anti-bacterial, anti-coagulant, anti-diabetic, anti-fungal, anti-inflammatory, anti-malarial, anti-platelet, anti-protozoal, anti-tuberculosis and anti-viral activities; affecting the cardiovascular and nervous systems, and other miscellaneous mechanisms of action have been reviewed elsewhere.[5–8]

Consistent with our previous reviews, only those articles reporting on anti-tumour pharmacology or cytotoxicity of marine compounds with established chemical structures (Figs. 1 and 2) were included in the present review, and are presented in alphabetical order in Tables 1 or 2. The literature reporting novel information on the preclinical and/or clinical pharmacology of marine chemicals with previously *determined* mechanisms of action has been summarised in Table 1 and is

* *Corresponding author:* Tel.: +1 630 515 6951; fax: +1 630 515 6295.
E-mail address: amayer@midwestern.edu (A.M.S. Mayer).

0959-8049/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2006.05.019

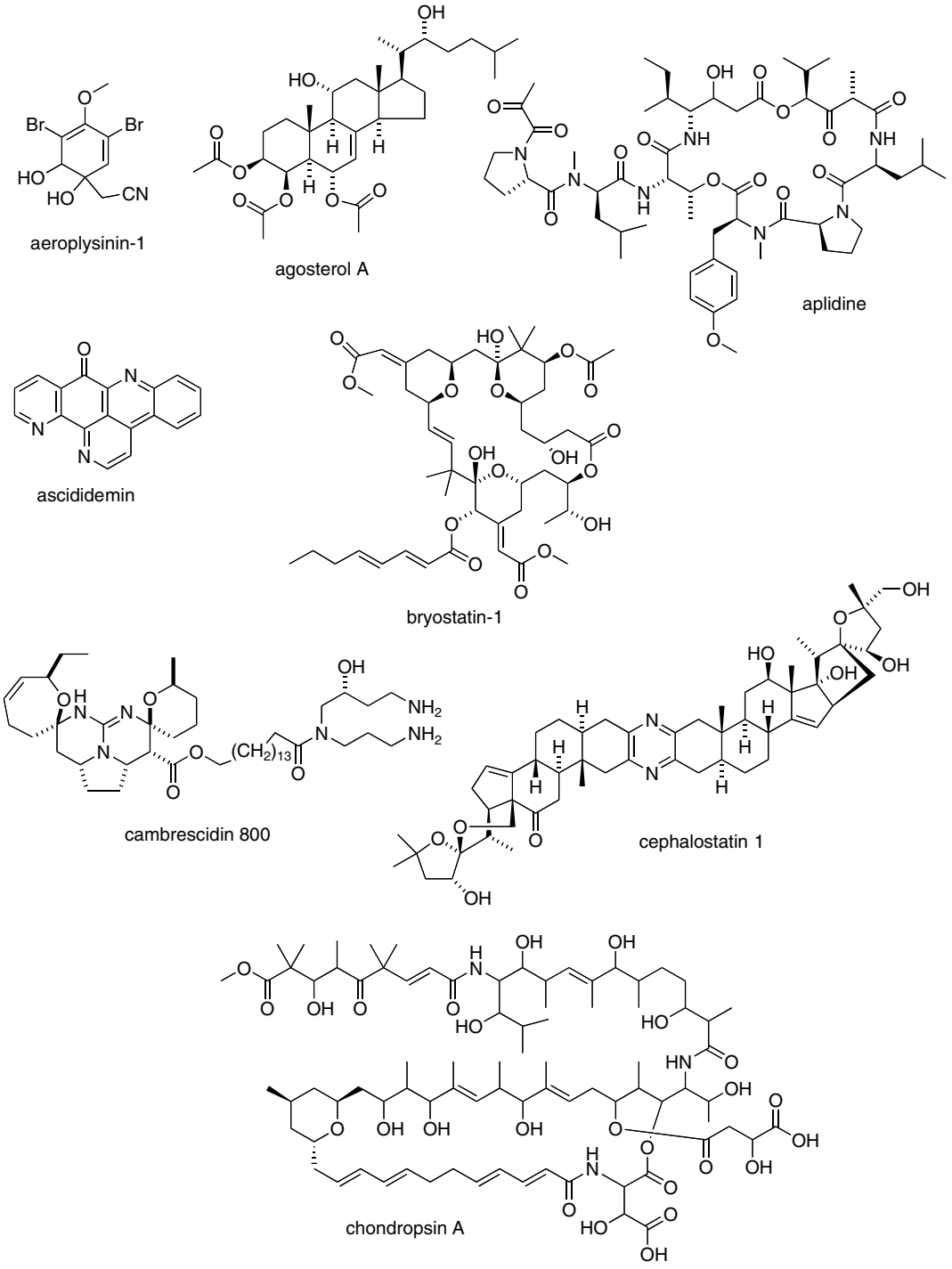
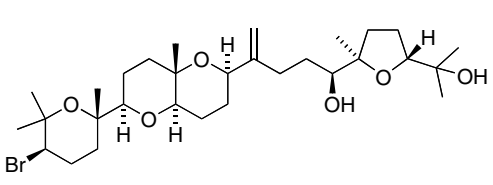


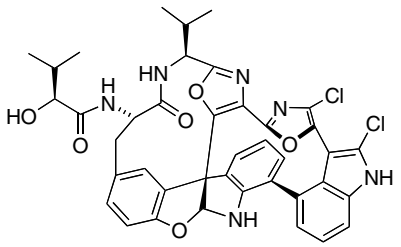
Fig. 1 – Structures of marine natural products reported in 2003 and 2004 with established mechanisms of action.

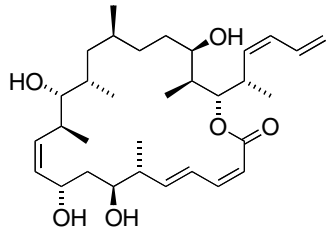
discussed briefly in the text of this review. On the other hand, reports on novel marine chemicals which demonstrated significant cytotoxicity but with as yet *undetermined* mechanisms of action are grouped in Table 2. With few exceptions, studies on the preclinical anti-tumour pharmacology of synthetic analogues of marine metabolites as well as reports on research with marine extracts or as yet structurally *uncharacterised* marine chemicals are not included in this review, although several promising studies were published during 2003–2004.[9–13]

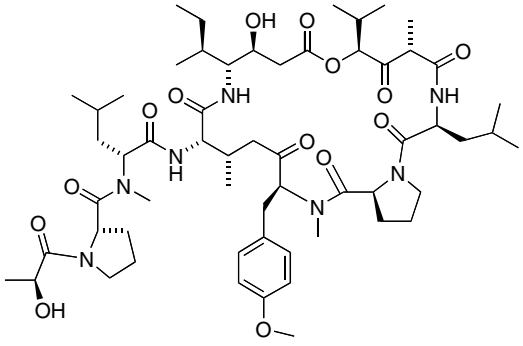
2. 2003–2004: anti-tumour pharmacology of marine natural products with *established* mechanisms of action

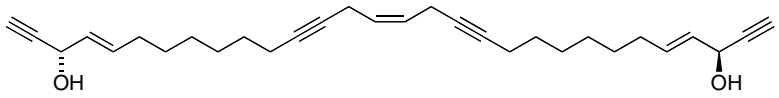
Table 1 summarises novel mechanism of action research from preclinical studies of 31 marine compounds (selected structures are shown in Fig. 1). Reports on clinical trials with some of these marine compounds are excluded from Table 1, but discussed in this section of the article.

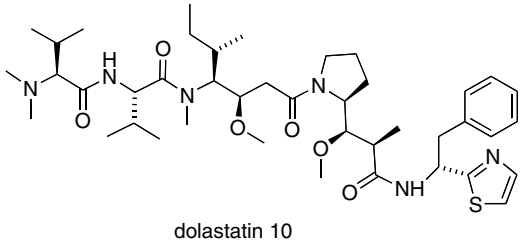












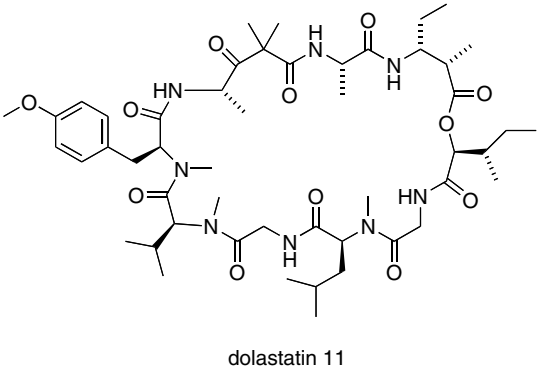
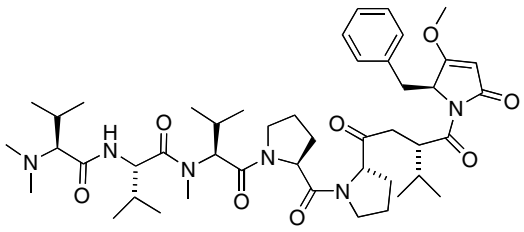


Fig. 1 – *continued*

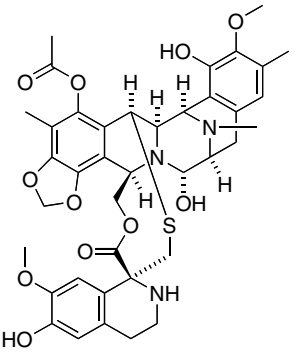
New information was published during 2003–2004 on the preclinical and clinical pharmacology of the following marine compounds that we have reviewed previously:[1–3] aeroplysinin-1, agosterol A, aplidine, ascididemin, bryostatin-1, dehydrothyrsiferol, didemnin B, dideoxypetrosynol A , dolastatins, ecteinascidin-743, halichondrin B, hemiasterlin, kahalalide F, motuporamines and peloruside.

One study extended the preclinical pharmacology of **aeroplysinin-1**, a compound we reviewed previously. Gonzalez-Iriarte and colleagues[14] developed a modification of the chorioallantoic membrane assay using quail embryos to investigate the anti-angiogenic properties of this marine compound. With this novel assay they demonstrated that the pro-apoptotic properties of aeroplysinin-1 are strong, in particular with proliferating endothelial cells.

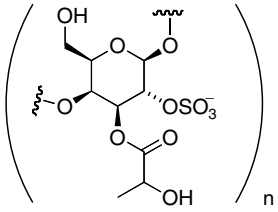
Two studies were published during 2003–2004 on the preclinical pharmacology of **agosterol A**, a polyhydroxylated sterol acetate isolated from the marine sponge *Spongia* sp. Mitsuo and colleagues[15] working with several human epidermoid carcinoma KB cell sub-lines, showed that [I^{125}]-azido agosterol A photolabelled P-glycoprotein (PGP) with high affinity in the absence of glutathione by binding strongly to the N-terminal fragment. The authors suggested that this new photolabelling probe will enable further investigation of the specific residues on P-glycoprotein that are required for agosterol A binding, as well as enhance the therapeutic



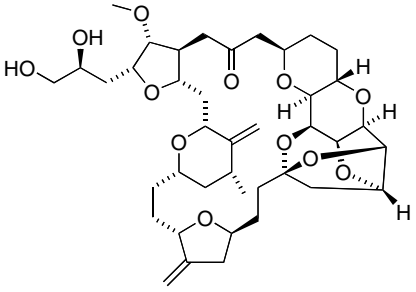
dolastatin 15



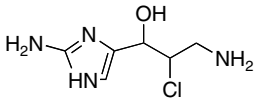
ecteinascidin-743



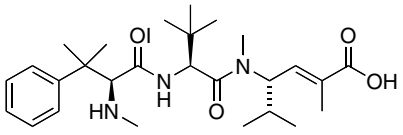
GA3P
a derivatized 1-4 linked
β-D-galactose oligomer



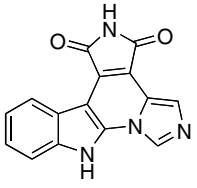
halichondrin B
analogue E7389



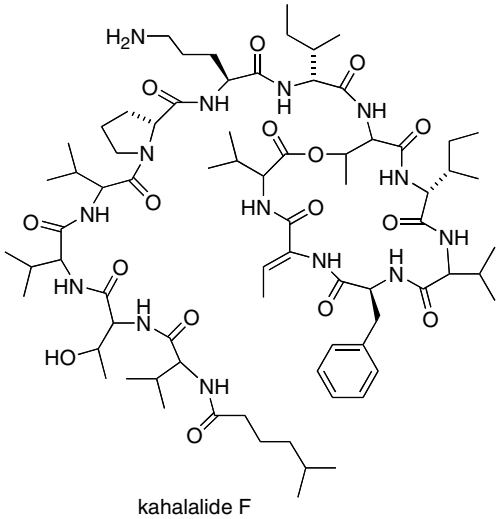
girolline



hemiasterlin
analogue HTI-286



isogranulatimide



kahalalide F

Fig. 1 – *continued*

activity of this molecule in reversing multidrug resistance. Furthermore, Ren and colleagues[16] in a detailed mechanistic study further characterised the glutathione-dependent [I^{125}]-azido agosterol A photolabelling site on the C-terminal half of the 190 kDa human membrane multidrug resistance protein 1 (MRP1), a frequently overexpressed transporter in non-P-glycoprotein-mediated multidrug resistance in tumour cells. Their studies demonstrated that binding of azido agosterol-A on MRP1 occurs in residues within the transmembrane helix (TM) 14–17, with the charged amino acid Arg^{1202} proximate to TM helix 16 as a critical determinant of this process.

Eight preclinical studies contributed during 2003–2004 to the further characterisation of the cellular and molecular pharmacology of the cyclic depsipeptide **aplidine**, also known as aplidin or dehydrodidemnin B, which was previously isolated from the marine tunicate *Aplidium albicans*. While investigating the MOLT-4 human leukaemia cell line, Broggini and colleagues[17] demonstrated that aplidine's cytotoxic activity was the consequence of inhibition of the vascular endothelial

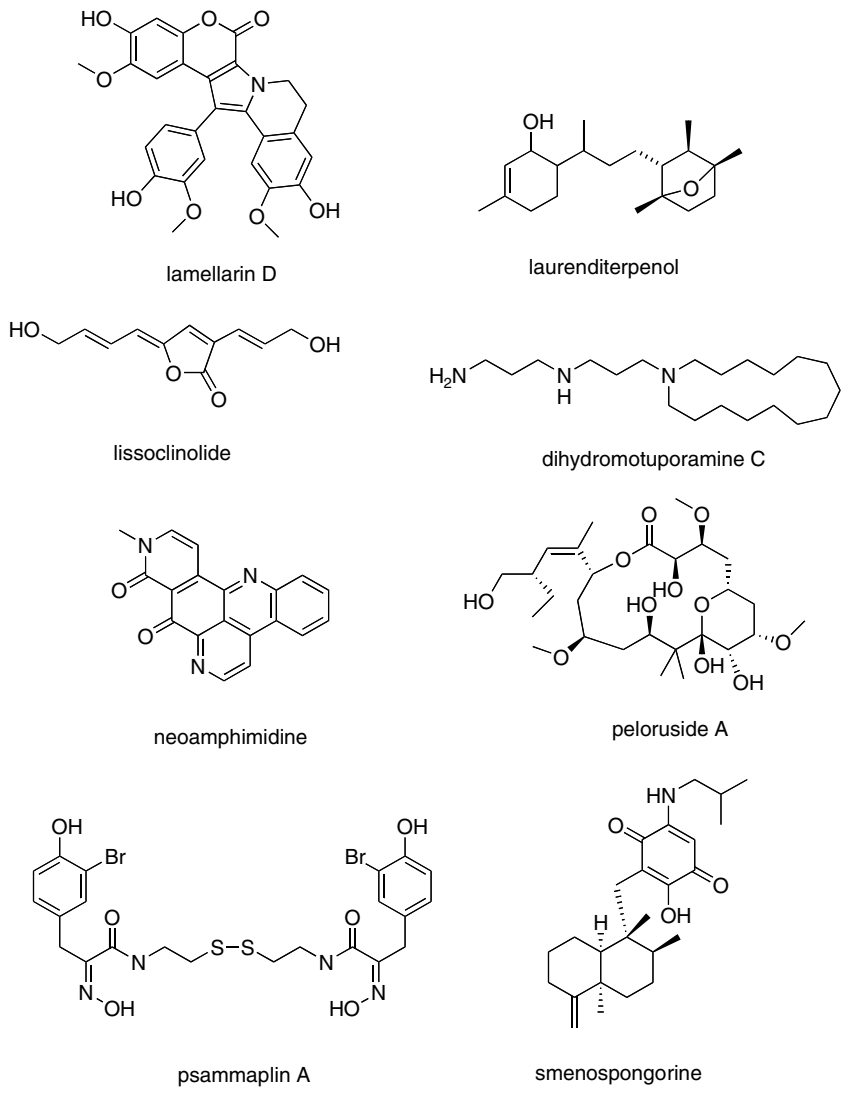


Fig. 1 – *continued*

growth factor (VEGF)/VEGF receptor-1 autocrine loop by direct inhibition of VEGF secretion by the tumour cells. VEGF is an important mediator of angiogenesis and, although the detailed mechanism by which aplidine inhibits VEGF is currently unknown, the authors noted that VEGF inhibition by an anti-cancer agent had 'never been described before'. Erba and colleagues[18] reported that aplidine had a potent anti-leukaemia effect against human acute lymphoblastic leukaemia cell lines, as well as freshly isolated leukaemia bone marrow samples from 14 patients aged 1–13 years. Aplidine-induced cell death was related to induction of apoptosis with concomitant G_1 arrest and G_2 blockage. Losada and colleagues[19] discovered that C-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinases (p38 MAPK) and concomitant mitochondrial apoptosis was 'slight and transient' in an aplidine-resistant sub-line of human HeLa adenocarcinoma cells they established. These observations suggested that continued activation of these signal transduction enzymes was required for aplidine-triggered apoptosis. Cuadrado and colleagues[20] working with human breast and renal cancer cells, also observed that, at nM concentrations, aplidine induced apoptosis and there was sustained activation of the serine/threonine kinases JNK and p38 MAPK. A possible mechanism might involve aplidine's induction of oxidative stress, leading to a reduction of glutathione levels and activation of the Src tyrosine kinase. Gajate and colleagues[21] noted that aplidine was an extremely rapid and potent apoptosis inducer in leukaemic cells by triggering the Fas/CD95 cell death receptor and concomitant mitochondrial-mediated apoptotic signalling pathways. Because of the rapid and potent apoptosis of leukaemic cells with a concomitant sparing of normal cells in short incubations with aplidine, the authors proposed that the marine natural product might be useful in 'purging approaches to leukaemia treatment'. Taraboletti and colleagues[22] found that aplidine blocked angiogenesis in several *in vivo* models, while 'at concentrations achievable in patients' plasma' several endothelial cell functions related to angiogenesis were inhibited. Gomez and colleagues[23] using long-term competitive repopulation assays performed in mice determined that doses of aplidine that produced a

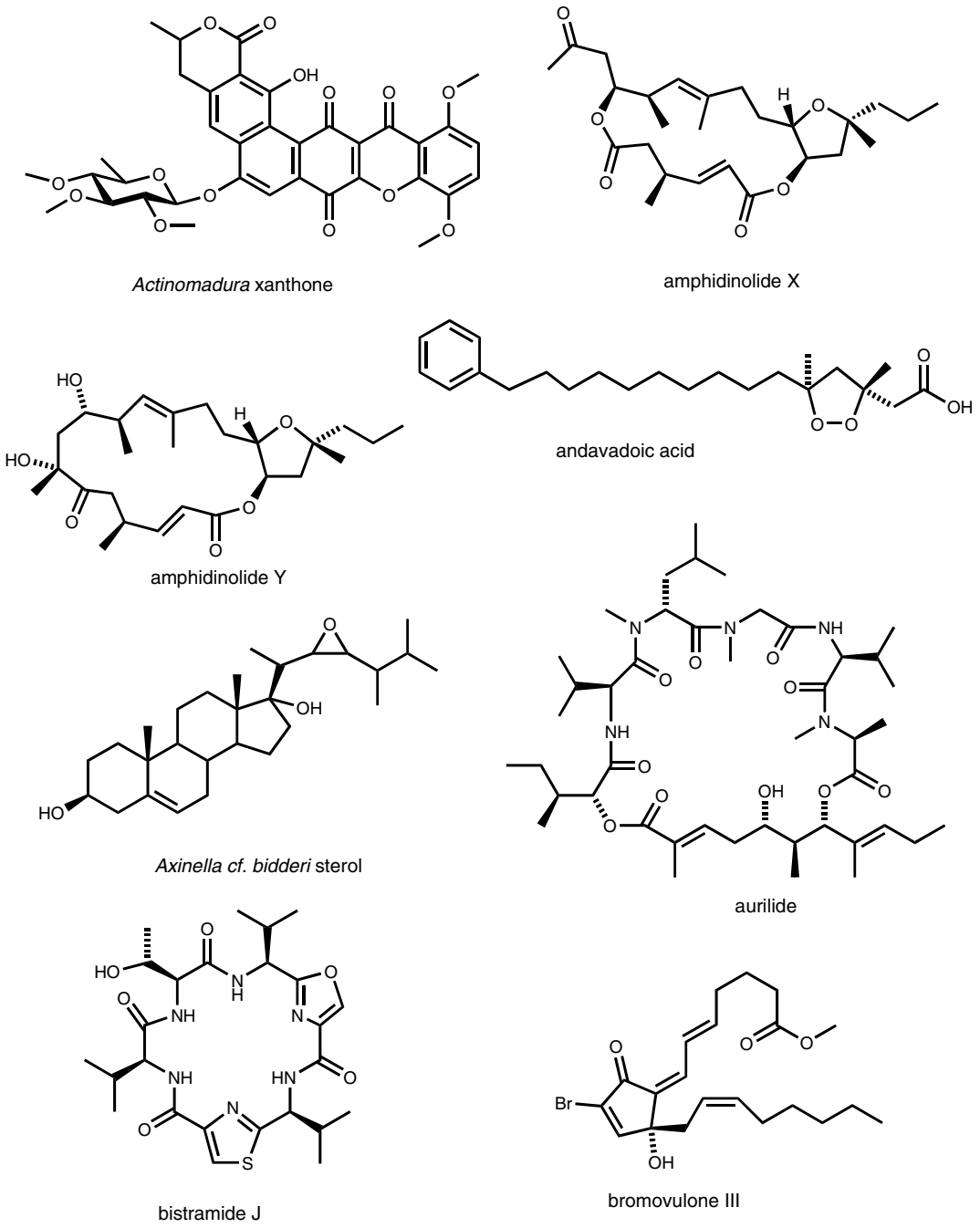


Fig. 2 – Structures of new marine natural products reported in 2003 and 2004 with undetermined mechanisms of action.

reduction of myeloid progenitors did not appear to affect haematopoietic stem cells. Confirmation of these observations with human haematopoietic stem cell remains to be investigated.

Two preclinical studies contributed during 2003–2004 to the further characterisation of the anti-neoplastic pharmacology of **ascididemin**, a pyridoacridine alkaloid isolated from the marine sponge *Amphimedon* sp. Matsumoto and colleagues[24] reported experimental results that suggested direct iminoquinone reduction and reactive oxygen species generation as the probable mechanism responsible for ascididemin cytotoxicity. Dirsch and colleagues[25] investigated the signalling pathways involved in ascididemin-triggered apoptosis in human leukaemia Jurkat T cells. Ascididemin was shown to trigger a mechanism that involved C-Jun N-terminal protein kinase and activation of caspase-2 to induce mitochondrial dysfunction and subsequent apoptotic cell death.

Several studies published during 2003–2004 extended the pharmacology of **bryostatin-1**, a macrocyclic lactone derived from the marine bryozoan, *Bugula neritina*, which has continued to receive considerable attention in view of its demonstrated anti-neoplastic activity *in vitro* and *in vivo*.[1,3,4] Three preclinical studies contributed new information on the molecular pharmacology of bryostatin-1 at both the cellular and

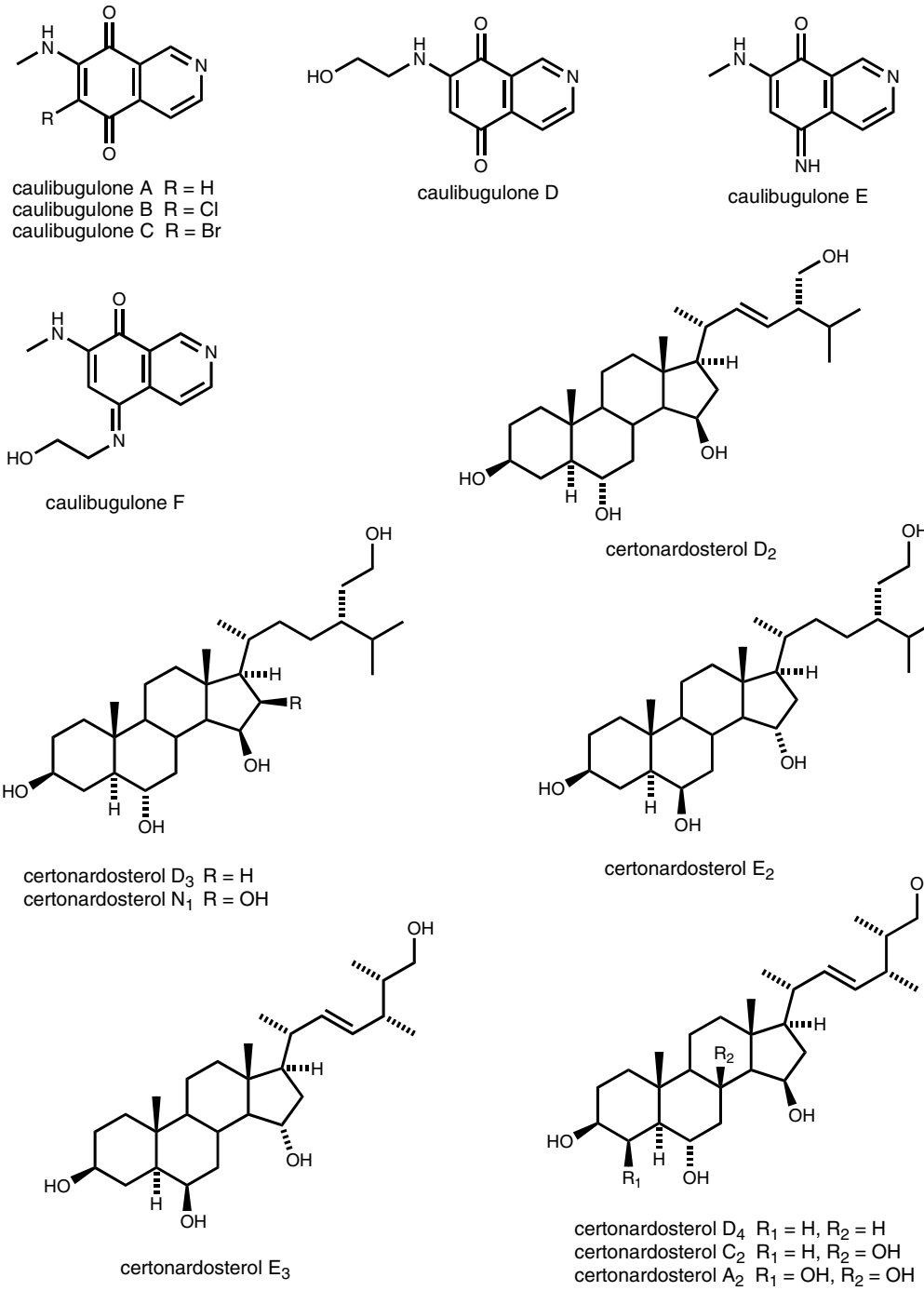


Fig. 2 – *continued*

molecular level. Ali and colleagues[26] discovered that bryostatin-1 potentiated the anti-proliferative and apoptotic effects of gemcitabine in human breast cancer cell lines through a protein kinase C-dependent process, although the exact molecular mechanisms remain undetermined. The authors proposed that bryostatin-1 plus gemcitabine might become a valuable new combined therapy with possible selectivity for gemcitabine-sensitive cancers. With the purpose of determining the molecular nature of severe myalgias, which are a common dose-limiting side-effect of bryostatin treatment, De Lorenzo and colleagues[27] assessed the induction of cyclo-oxygenase-2 (COX-2) in squamous carcinoma and lung adenocarcinoma cell lines. The observation that bryostatin-1-induced COX-2 mRNA, COX-2 protein and prostaglandin synthesis in the nM range via a protein kinase C, mitogen-activated protein kinase, activator protein-1 pathway suggest that addition of selective COX-2 inhibitors might increase the anti-tumour efficacy of bryostatin-1 as an anti-tumour agent. Wang and colleagues[28] characterised the effects of bryostatin-1 on 1-β-D-arabinofuranosylcytosine(ara-C)-induced apoptosis of

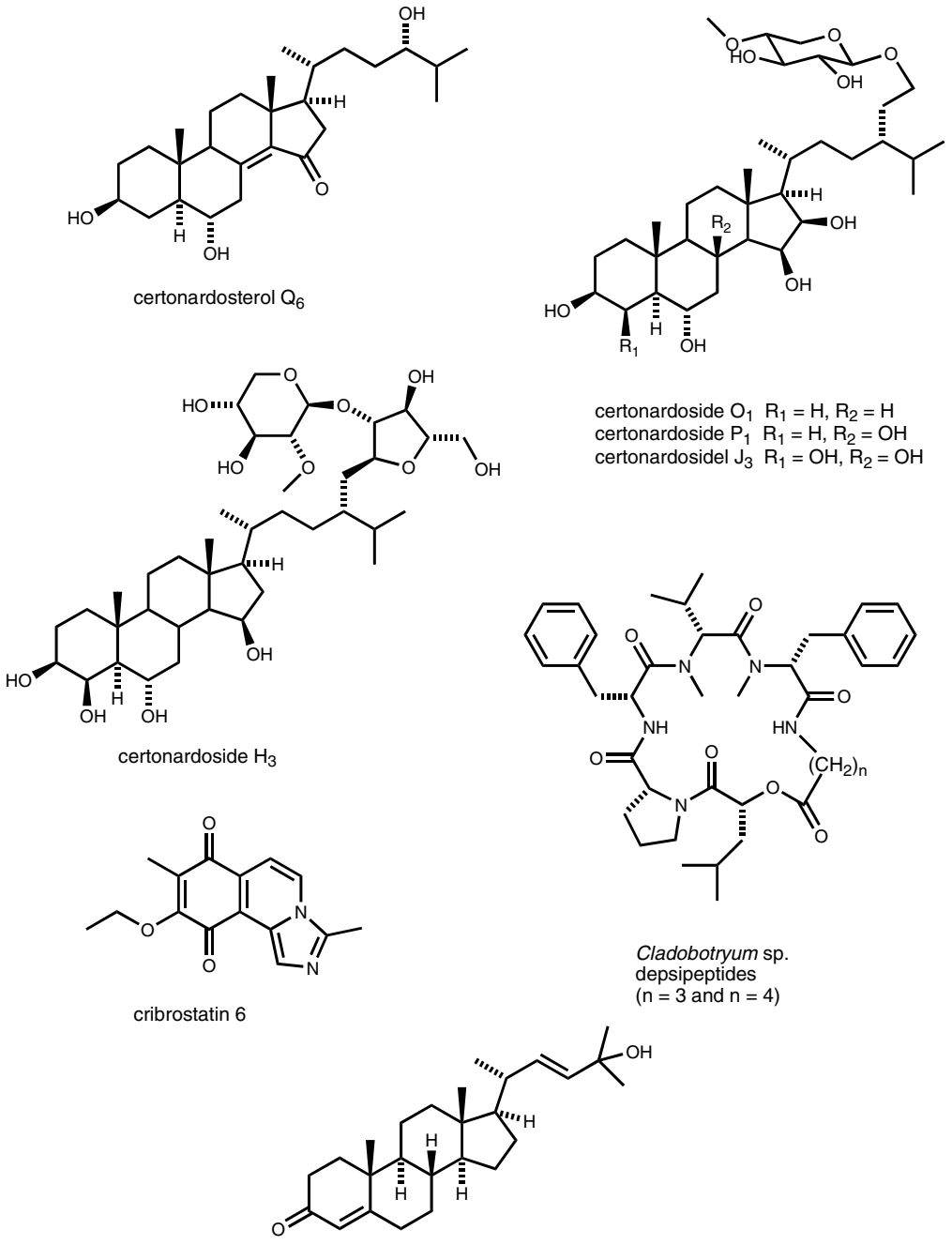


Fig. 2 – *continued*

human myeloid leukaemia cell lines. The investigation demonstrated that potentiation of ara-C apoptosis resulted from 'protein kinase C-dependent release of tumour necrosis factor α' and concomitant activation of the extrinsic apoptotic cascade.

One study was reported during 2003 on the preclinical pharmacology of **dehydrothyrsiferol** (DT), a polyether triterpenoid isolated from a Canary island collection of the red alga *Laurencia viridis sp. nov.* Pec and colleagues[29] studied the biochemical nature of the cytotoxic effect of DT on human oestrogen receptor$^+$ (ER$^+$) and oestrogen receptor$^-$ (ER$^-$) breast cancer cell lines. Although they were able to exclude the possibility that DT functions as a mitosis inhibitor, they noted that induction of apoptosis 'was induced more efficiently and with distinct cell cycle-related patterns in the more aggressive ER$^-$ cells' while being less complete in ER$^+$ breast cancer cell lines.

One study completed during 2003–2004 extended the pharmacology of the **didemnin** cyclic depsipeptides, which are produced by different ascidians of the family *Didemnidae*. Marco and colleagues[30] in a detailed mechanistic study evaluated the structural basis for the binding of the didemnins to human elongation factor eEF1A and the rationale for the

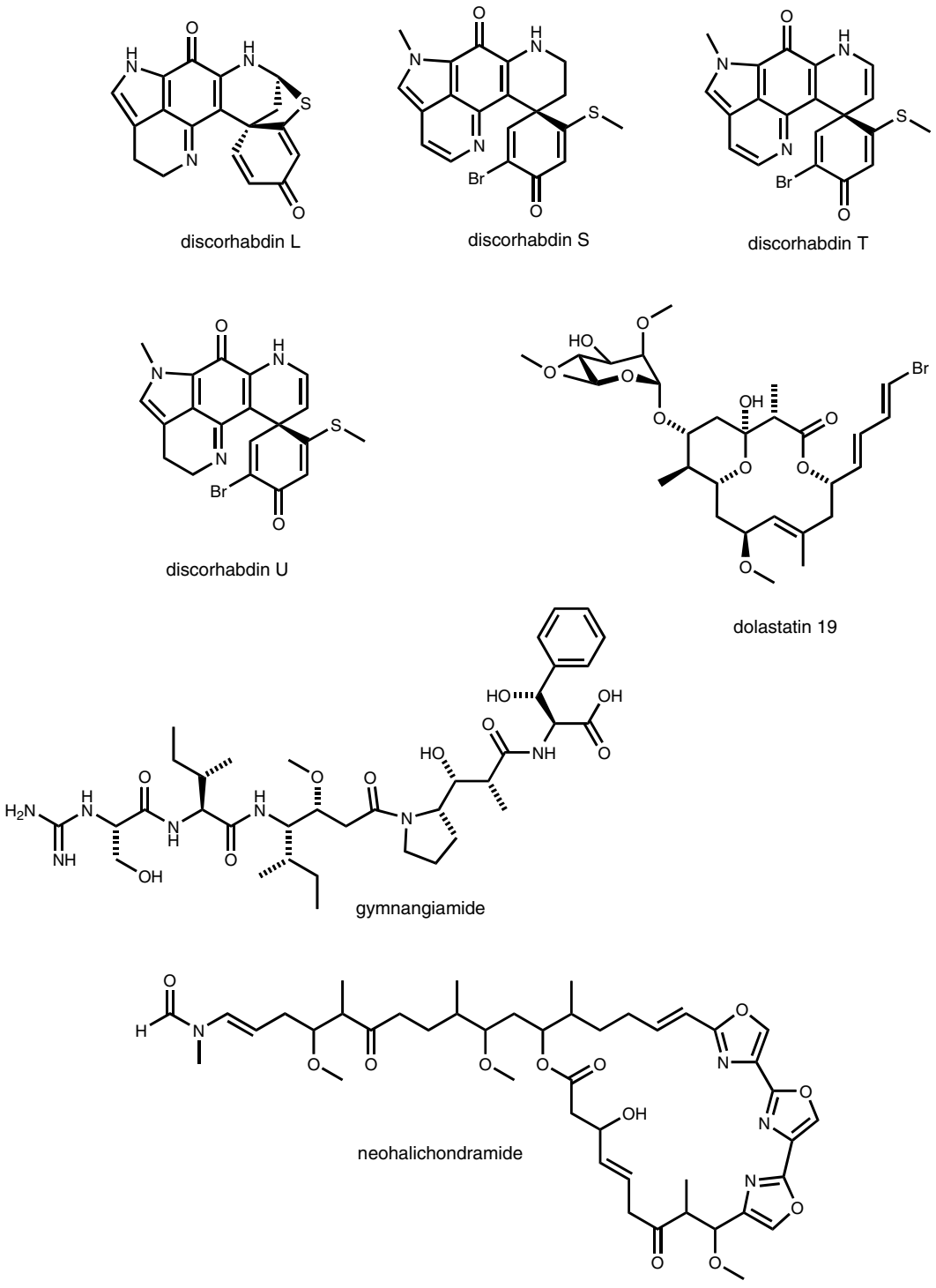


Fig. 2 – *continued*

potent anti-tumour activity. Their model suggests that an eEF1A-didemnin complex that binds to the ribosomal A-site would 'get stuck', leading to translational arrest, thus clearly demonstrating the importance of inhibition of the protein synthesis machinery in tumour cells as an important strategy for anti-cancer drug design.

Choi and colleagues[31] extended the preclinical pharmacology of **dideoxypetrosynol A**, a polyacetylene from the marine sponge *Petrosia sp*. While investigating the anti-proliferative action on human skin melanoma cells they noted both growth inhibition and apoptosis. Apoptosis appeared to be mediated by an increase in Bax expression and activation of caspases, thus suggesting induction of a mitochondrial-signalling pathway.

Three studies were published during 2003–2004 on the preclinical pharmacology of the **dolastatins**, a family of

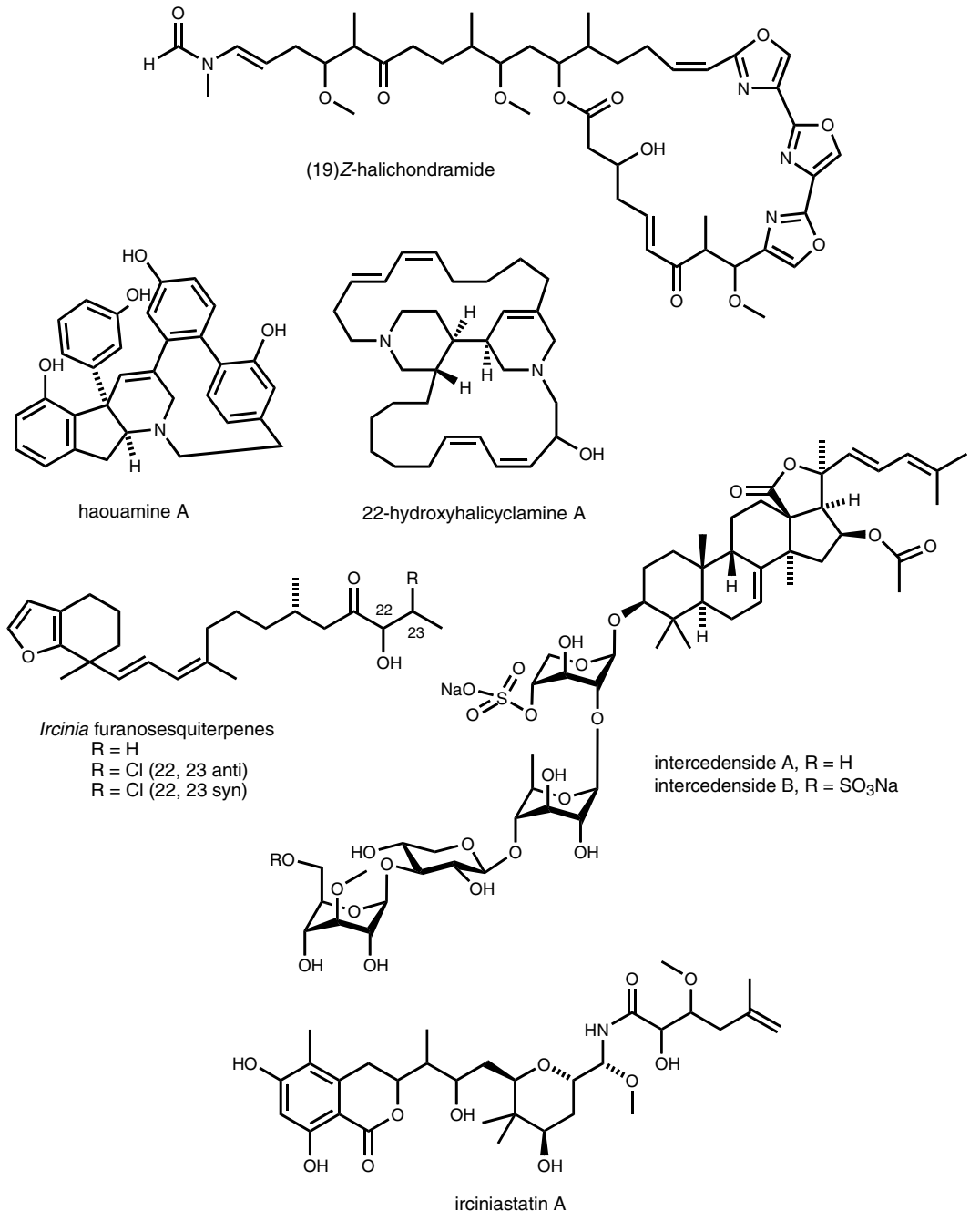


Fig. 2 – *continued*

modified peptides originally isolated from the marine mollusc *Dolabella auricularia* that induce actin assembly *in vivo*. Oda and colleagues[32] investigated the molecular mechanism of F-actin stabilisation by dolastatin 11 using X-ray fibre diffraction diagrams. This detailed investigation which revealed that dolastatin 11 localises in the gap region between the two long-pitch strands of F-actin provides a molecular mechanism to explain the observed stabilisation of microfilaments by dolastatin 11. Using the Hummel-Dreyer chromatographic method, Cruz-Monserrate and colleagues[33] demonstrated that dolastatin 15 binds with relatively low binding affinity (apparent K_d of ≅30 μM) to the vinca domain of αβ-tubulin heterodimer, the subunit protein of microtubules that is the intracellular target of several anti-mitotic peptides and depsipeptides. The investigators suggest that based on their studies the 'vinca domain', and particularly the 'peptide site' is possibly a 'large binding pocket on the surface of β-tubulin' that could perhaps enable binding of different complex natural product ligands in putatively overlapping domains. Bai and colleagues[34] explored the potential of the direct photo-affinity labelling technique to determine the dolastatin 10-binding site on tubulin. Their studies demonstrated that binding of [^{3}H]-dolastatin 10 to the β-tubulin peptide spanned amino acid residues 2–31, with probable

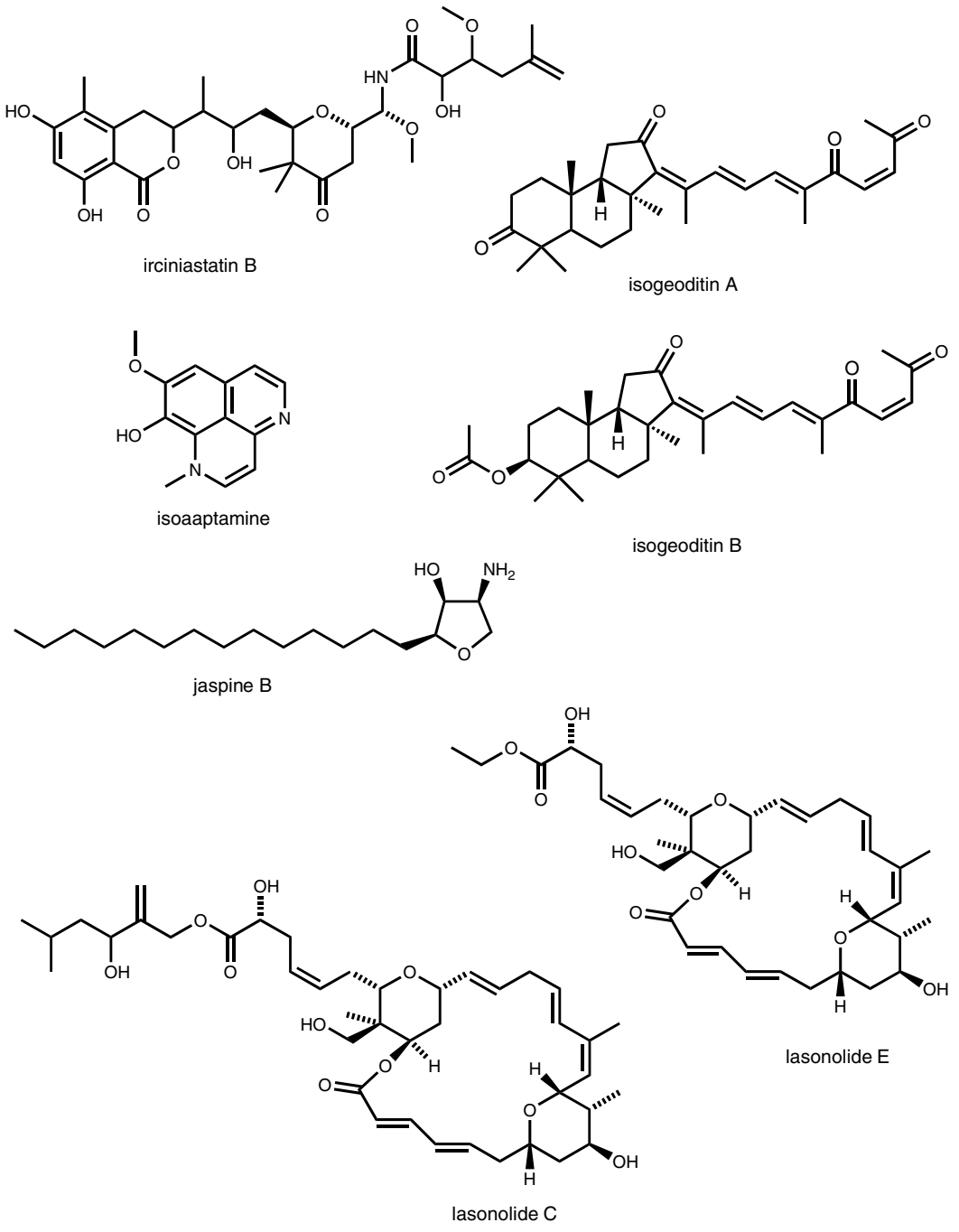


Fig. 2 – *continued*

covalent bond formation 'between the sulphur atom of Cys-12 and the thiazole ring of dolastatin 10'.

Research on the tetrahydroisoquinoline alkaloid **ecteinascidin-743** (ET-743), an anti-tumour agent originating from the Caribbean tunicate *Ecteinascidia turbinata*, continued at an active pace during 2003–2004. Seven preclinical and 5 clinical articles extended the pharmacology of ET-743 during 2003–2004.

Biroccio and colleagues[35] contributed additional insight into the molecular pharmacology of ET-743 by examining the impact of telomerase function on the sensitivity of human melanoma cells to ET-743. The studies demonstrated that reconstitution of telomere dysfunction in cell lines with reduced human telomerase reverse transcriptase expression, telomerase activity and telomere shortening, improved 'the functional status of telomeres' and decreased the sensitivity to ET-743 as a result of recovery from drug-induced G_2/M block and apoptosis.

Preclinical cellular pharmacology of ET-743 involved several studies during 2003–2004. D'Incalci and colleagues[36] reported on the effects of the combination of ET-743 and cisplatin in human cancer cell lines growing *in vitro* and in

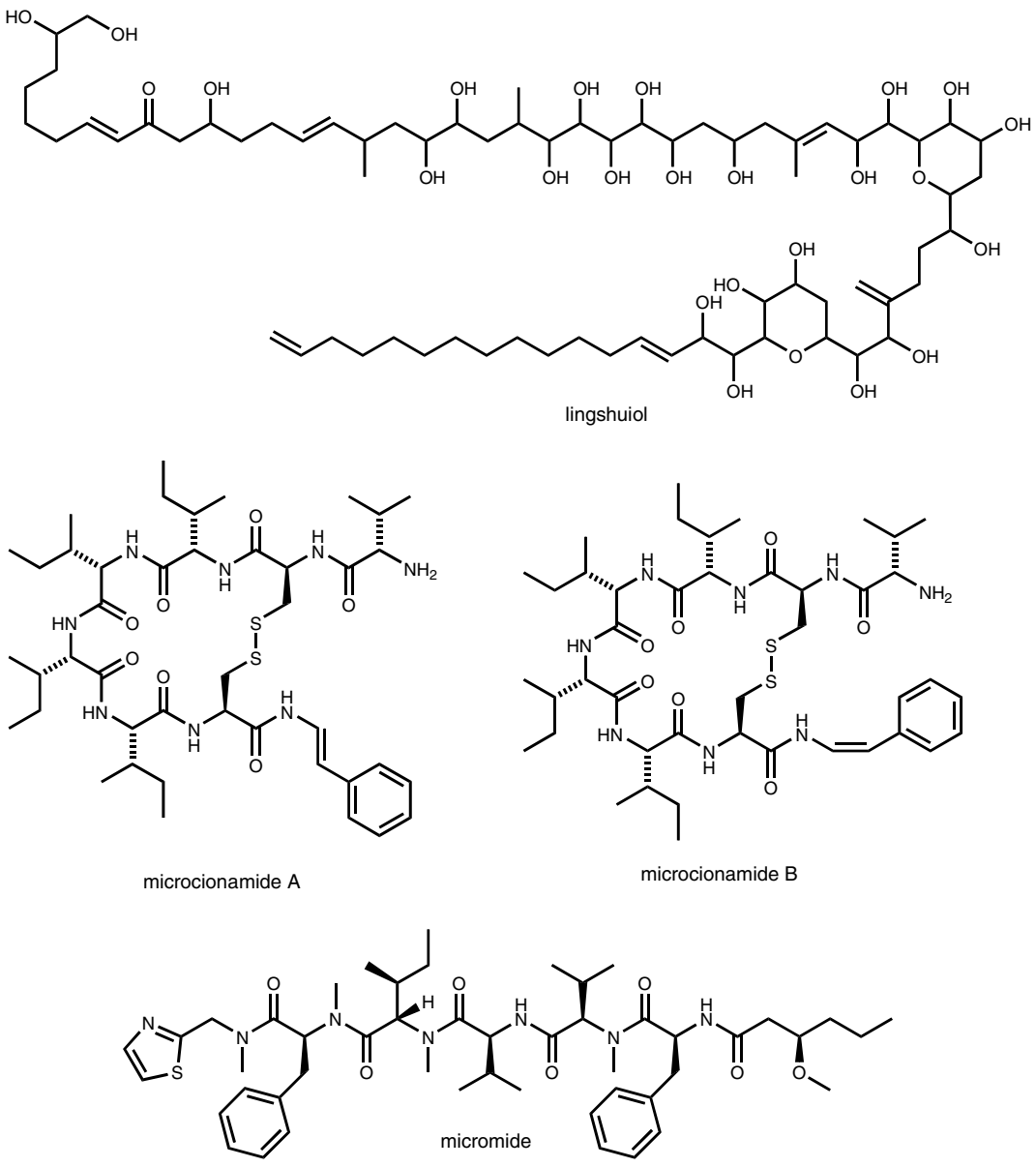


Fig. 2 – *continued*

xenografts derived from different human tumours in nude mice. The results demonstrated that the combination of ET-743 and cisplatin was synergistic both *in vitro* and *in vivo* and that both agents could be combined at the maximum tolerated dose perhaps as a result of a lack of overlapping toxicities. The investigators concluded that the results 'provide a strong rationale to undertake investigations on this combination at the clinical level'. Simoens and colleagues[37] determined the *in vitro* interaction of ET-743 and radiation, and its relation to the cell cycle in four human tumour cell lines. Pre-treatment with ET-743 during 24 h prior to radiation resulted in a moderate increase in radiosensitising properties in 3 out of the 4 cell lines used in the study. Although the investigators determined that the radiosensitivity appeared to be due to a G2/M block, they concluded that further investigation would be necessary to confirm the role of 'cell-cycle effects caused by ET-743' in the mechanism of radiosensitisation which was observed to be cell line-dependent. Shao and colleagues[38] working with a human chondrosarcoma cell line assessed the transcriptional and cellular alterations resulting from resistance to ET-743. They reported that the cell morphology and migratory ability of the ET-743-resistant cell line variant was reduced, and concomitantly there were marked rearrangements of the cytoskeleton architecture which correlated with a decrease of type I collagen α1 chain mRNA in the ET-743-resistance sarcoma cell line.

Three studies extended the preclinical *in vivo* pharmacology of ET-743. Meco and colleagues[39] investigated the cytotoxic and anti-tumour effects of the combination of ET-743 and doxorubicin in both nude mice that received a human rhabdomyosarcoma or C3H mice injected with a murine fibrosarcoma. The combination of ET-743 and doxorubicin pro-

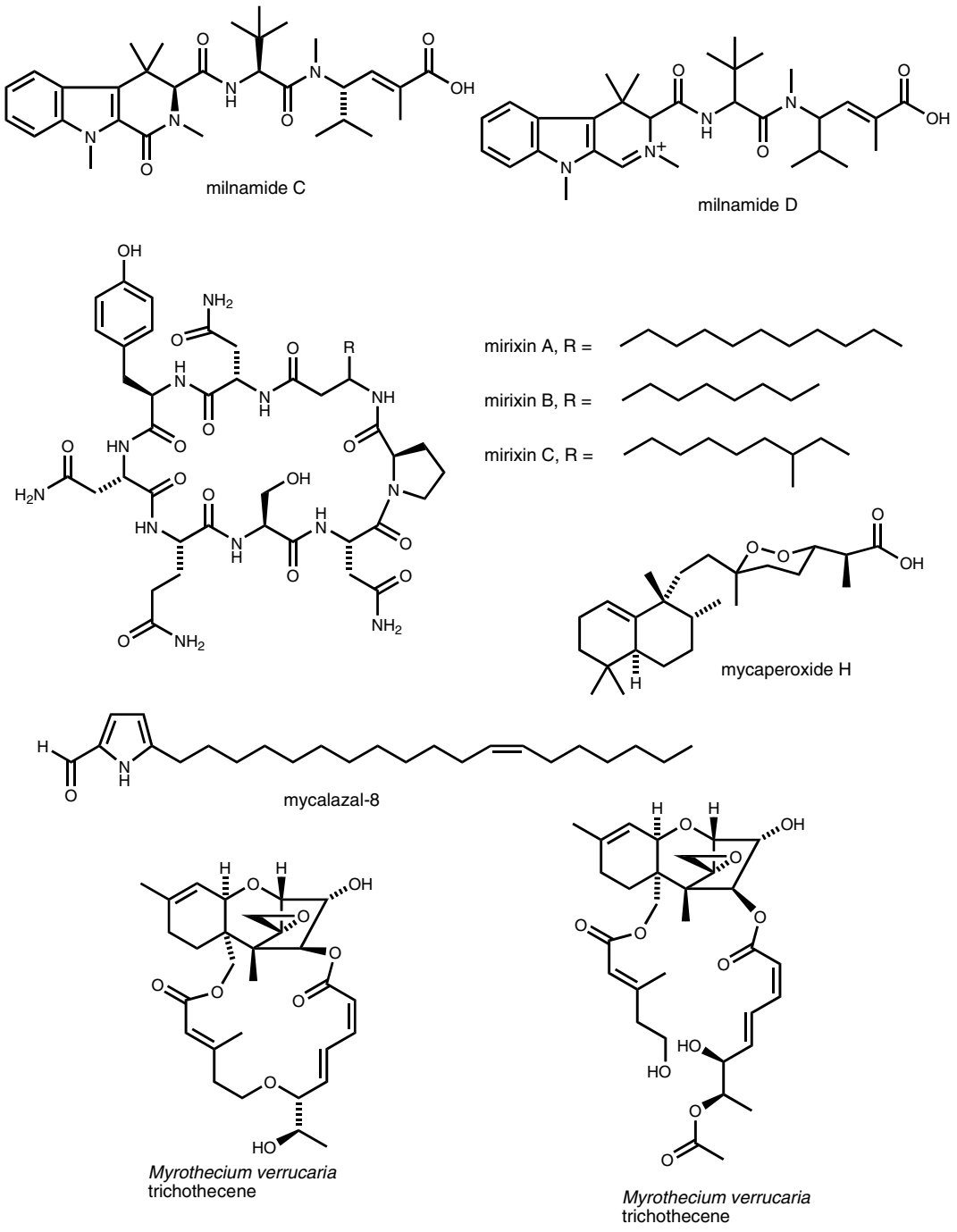


Fig. 2 – *continued*

duced a significant anti-tumour effect on both human tumours as well as doxorubicin-resistant mouse fibrosarcoma. The authors concluded that synergy of the two drugs 'could be effective for tumours displaying low sensitivity to either ET-743 or doxorubicin'. Two reports focused on the efforts to study strategies to ameliorate the hepatotoxicity of ET-743. Donald and colleagues[40] reported that pre-treatment with high-dose dexamethasone ameliorated or abrogated the biochemical, histopathological and gene expression changes induced by ET-743 in rat liver. Interestingly, dexamethasone did not compromise the anti-tumour efficacy of ET-743 in murine tumour models used in this study. In a subsequent study designed to reduce ET-743 hepatotoxicity, Donald and colleagues[41] investigated indole-3-carbinol (IC), the aglycone of glucobrassicin which constitutes a microconstituent of cruciferous vegetables (e.g. broccoli, Brussels sprouts) and that is a potent inducer of cytochrome P450 enzymes, as a putative agent to protect against ET-743-induced hepatotoxicity. The results of this study demonstrated that dietary IC counteracted the unwanted effects of ET-743 in the liver while not interfering with the anti-tumour effect in a model of mammary carcinoma, and thus hinting 'at the feasibility of a novel

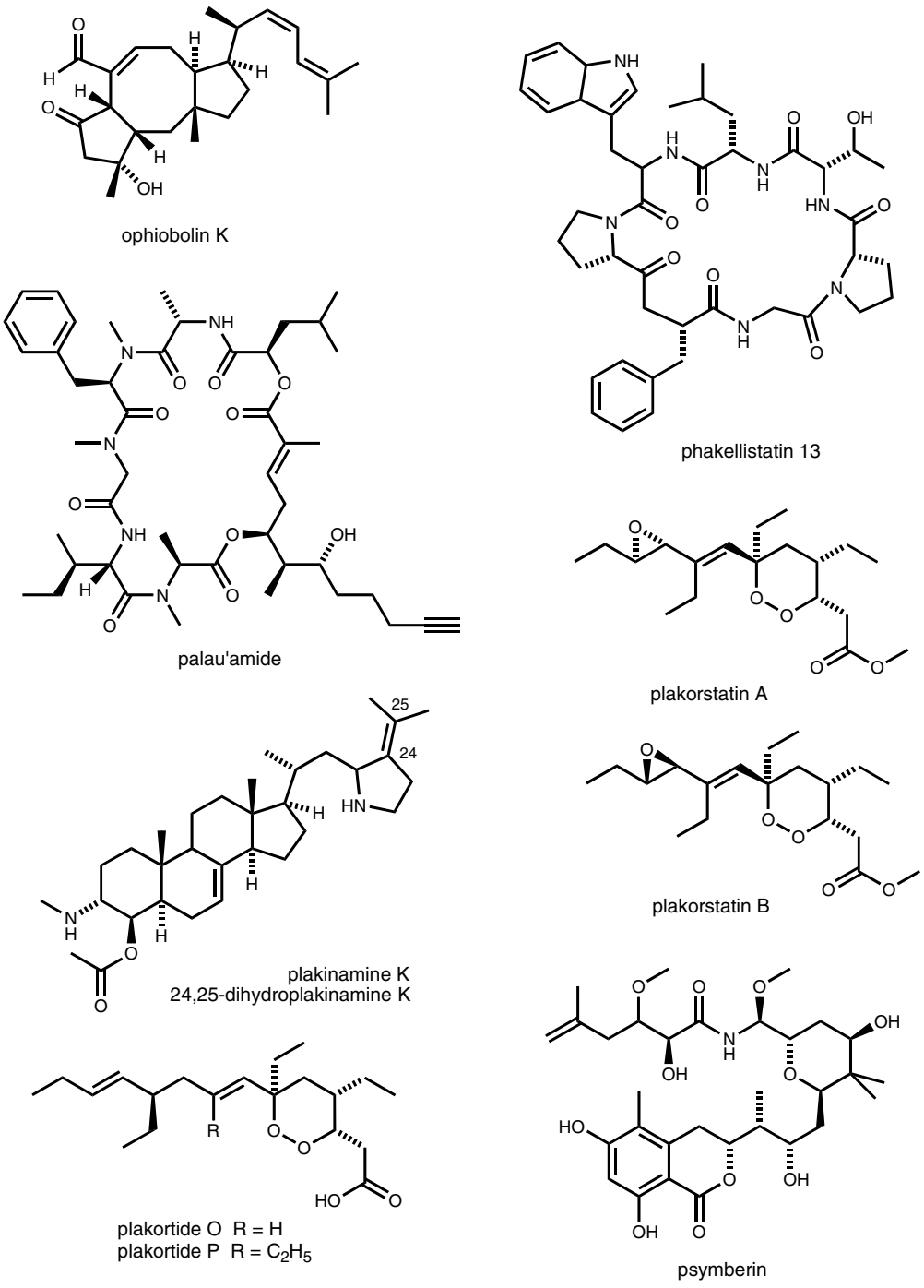


Fig. 2 – *continued*

pharmacological strategy to ameliorate the hepatotoxicity of ET-743 in humans'.

One phase I and four phase II trials extended the clinical pharmacology of ET-743 during 2003–2004. Twelves and colleagues[42] completed a phase I dose escalation and pharmacokinetic study with ET-743 in 72 adult patients with metastatic or advanced solid tumours. This study demonstrated efficacy of ET-743 in patients with soft-tissue sarcoma and that it can be administered safely to patients by 1- and 3-h i.v. infusions. Laverdiere and colleagues[43] contributed the results of a phase II study with ET-743 as salvage therapy for 25 patients with recurrent osteosarcoma, a drug-resistant disease with a dismal prognosis with standard chemotherapeutic agents. Although 3 patients (12%) achieved minor responses and ET-743 was observed to be well tolerated, it had limited anti-tumour activity when used as a single agent in heavily pre-treated osteosarcoma patients. The 16 authors suggested that 'trials in less pre-treated patients or ET-743 in combination with cisplatin or doxorubicin should be considered'. Blay and colleagues[170] published the results of a phase II study

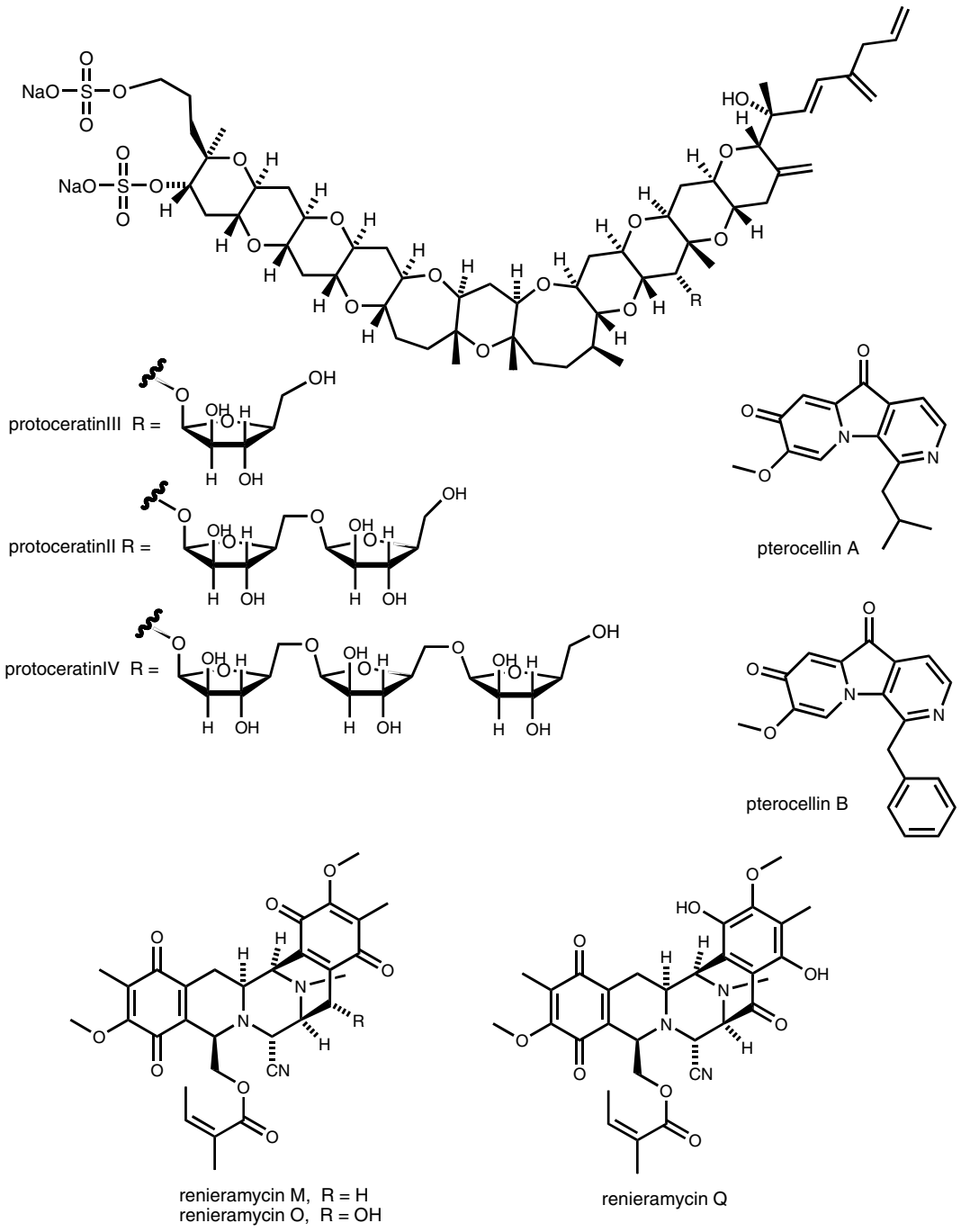


Fig. 2 – *continued*

with ET-743 in 28 patients with gastrointestinal stromal tumours (GIST), a type of tumour for which there were few alternative therapeutic options prior to the imatinib era. Although the treatment with ET-743 was well tolerated, with only 33% of the patients achieving stable disease as a best response, it was concluded that ET-743 at the dose and schedule used was 'not an effective treatment for advanced GIST'. Garcia-Carbonero and colleagues[44] completed a phase II and pharmacokinetic study with ET-743 in 36 patients with progressive sarcomas of soft tissues refractory to chemotherapy. Although ET-743 evidenced acceptable safety and tolerability in this study, objective responses to ET-743 were observed in only 3 patients, with 1 complete response and 2 partial responses. These results led the investigators to propose that ET-743 was a promising new agent for the management of several subtypes of soft tissue sarcoma that annually account for approximately 1% of adult neoplastic disease in the United States of America (USA). Yovine and colleagues[45] reported a phase II study of ET-743 evaluating efficacy, safety and pharmacokinetics of a 24-h ET-743 infusion regimen in 54 pre-treated patients with advanced soft tissue sarcoma. While the toxicities observed in this study 'were manageable',

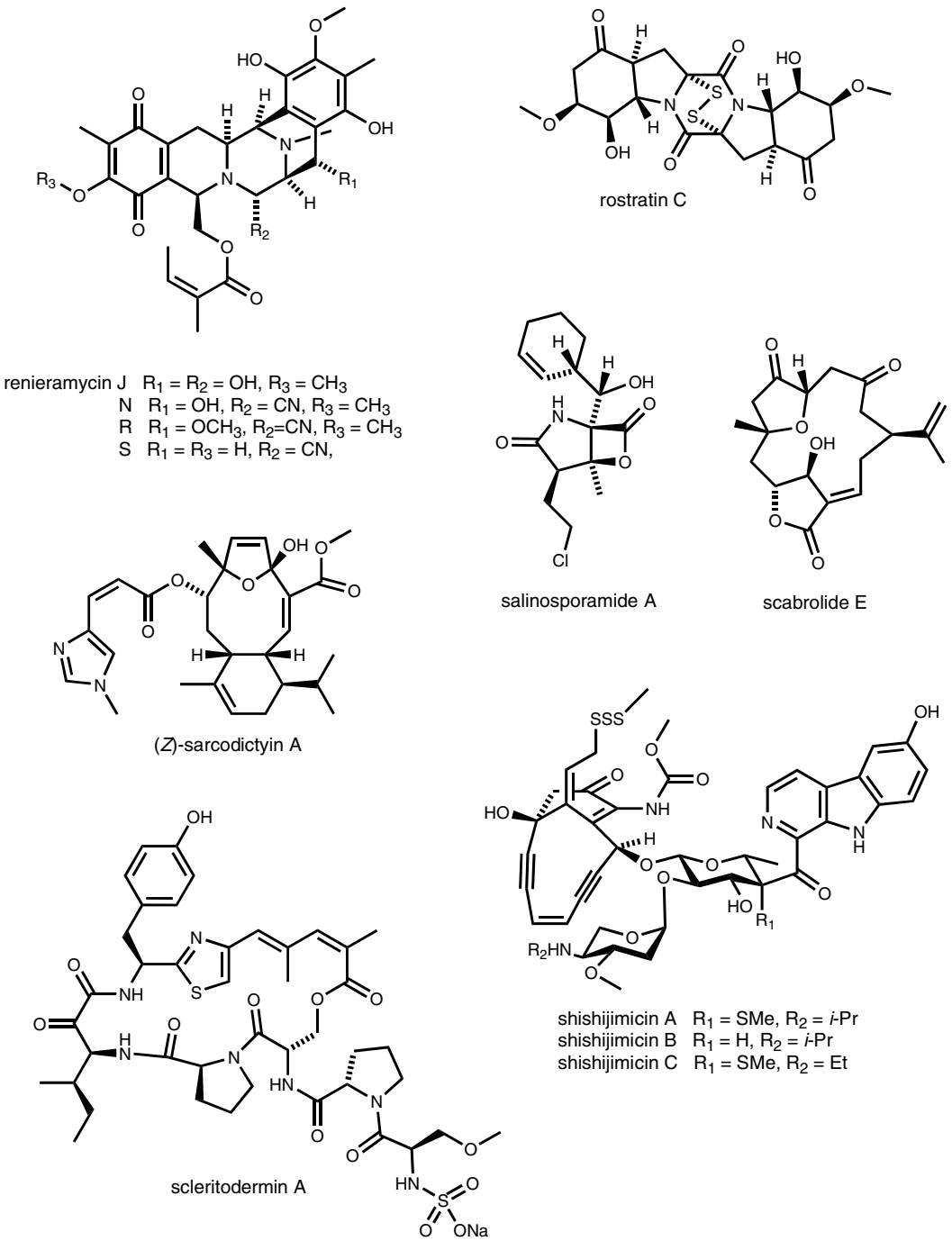
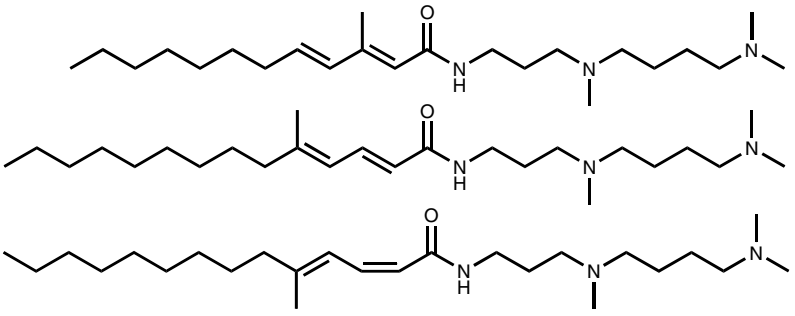


Fig. 2 – *continued*

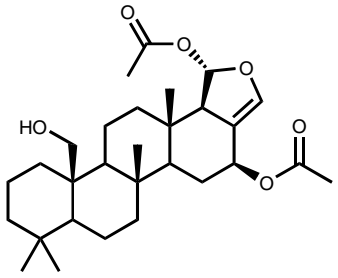
interestingly the rate of disease control of 38.8% at 3 months and 24.1% at 6 months, provided rather encouraging evidence for an anti-tumour effect of ET-743 in this patient population of 'highly pre-treated, progressing, advanced, metastatic, and resistant or refractory sarcoma patients'.

Kuznetsov and colleagues[46] extended the pharmacology of **halichondrin B**, a large polyether macrolide found in a variety of marine sponges, with a macrocyclic ketone analogue E7389. Investigating human histiocytic lymphoma and prostate cancer cell lines they noted that several morphological and biochemical correlates of apoptosis were clearly observed after prolonged mitotic blockage in the G_2-M phase of the cell cycle with $\geqslant$10 nM E7389, providing a putative mechanistic basis for the significant *in vivo* anti-cancer efficacy of this analogue of parental halichondrin B.

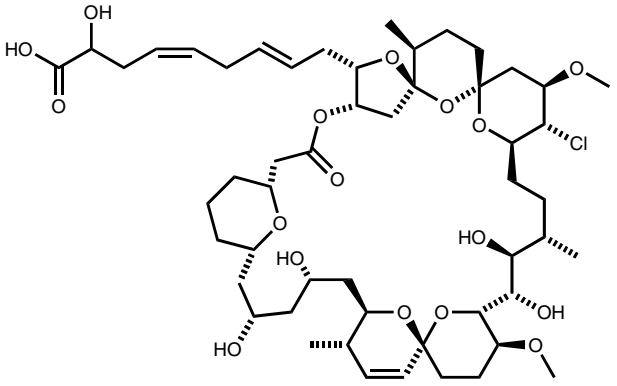
With the purpose of contributing to the development of novel anti-microtubule agents that may overcome resistance and have improved pharmacological profiles, Loganzo and colleagues[47] investigated the pharmacology of a synthetic analogue of **hemiasterlin**, a tripeptide containing three highly modified amino acids isolated from marine sponges. Development of the synthetic hemiasterlin analogue HTI-286 allowed



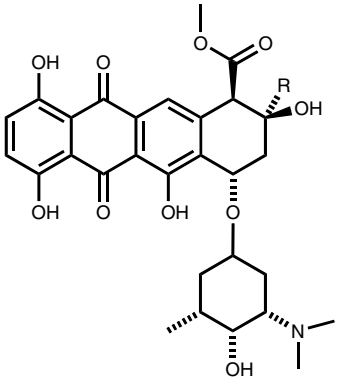
Sinularia sp.
acylspermidines



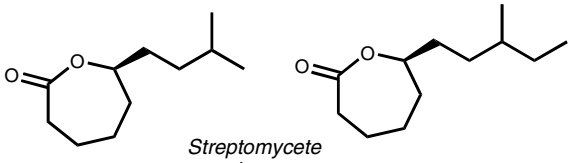
Smenospongia sesterterpene



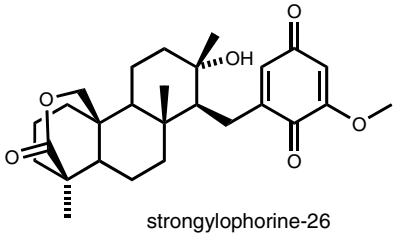
spirastrellolide A



Streptomycete
anthracyclins
R = CH_3, CH_2CH_3, or CH_2COCH_3



Streptomycete
capraloctones



strongylophorine-26

Fig. 2 – *continued*

for extensive *in vitro* studies with 18 human tumour cell lines which demonstrated the analogue potently inhibited proliferation in the nM range, depolymerised microtubules and overcame 'P-glycoprotein-mediated resistance to paclitaxel or vincristine or both in xenograft models and most cell lines that express the protein'. Clinical trials with HTI-286 will be required to determine its clinical utility in cancer treatment.

Suarez and colleagues[48] extended the preclinical pharmacology of **kahalalide F**, a naturally occurring depsipeptide isolated from the Hawaiian herbivorous marine mollusc *Elysia rufescen* and currently under clinical investigation. Kahalalide F resulted in potent loss of mitochondrial membrane potential, lysosomal integrity, as well as cytotoxicity against human prostate and breast cancer cell lines at an $IC_{50} < 0.3$ μM. There was concomitant rapid and severe cytoplasmatic swelling and vacuolisation, but with no caspase activity or alteration of nuclear structure. The investigators concluded that kahalalide F induced cell death by a non-apoptotic mode of action termed oncosis, a process involving a 'progression of cellular events leading to necrotic cell death'.

Novel preclinical pharmacology of the anti-invasion and anti-angiogenic alkaloids **motuporamines**, isolated from the

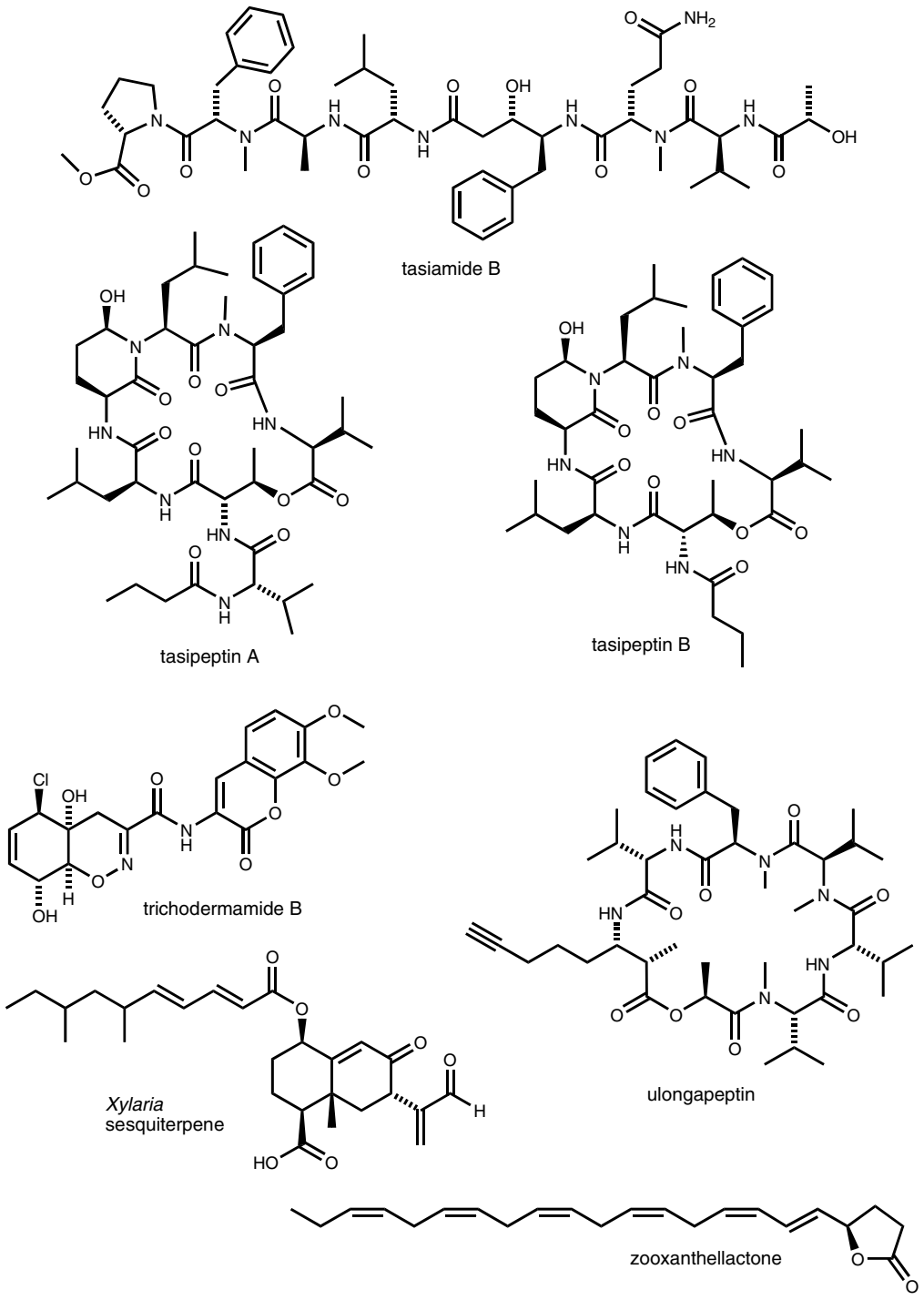


Fig. 2 – *continued*

sponge *Xestospongia exigua* was reported by McHardy and colleagues.[49] Dihydromotuporamine C, an analogue resulting from a comparative structure-activity study, lacks the double-bond in the non-polar group and caused inhibition of tumour cell invasion with concomitant increase in the number and thickness of actin-containing stress fibres, large focal adhesion complexes and activation of the small GTP-binding protein Rho. The authors suggested that activation of Rho by dihydromotuporamine C was a 'critical component of its anti-invasive properties *in vitro*, and presumably, its anti-cancer activity *in vivo*'.

Preclinical anti-tumour research continued during 2003–2004 with the macrolide **peloruside A**, which is currently available both synthetically as well as from the aquacultured New Zealand marine sponge *Mycale hentscheli*. Gaitanos and colleagues[50] established that peluroside A, a microtubule-stabilising agent, directly induced tubulin polymerisation in the absence of microtubule-associated proteins by targeting a site

Table 1 – 2003–2004: anti-tumour pharmacology of marine natural products with established mechanisms of action

Compound	Organism	Chemistry	Experimental or clinical model[a]	Mechanism of action[b]	Country[c]	References
Aeroplysinin-1	Sponge	Alkaloid	Quail chorioallantoic membrane assay	Induction of apoptosis on proliferating endothelial cells	SPA	[14]
Agosterol A	Sponge	Steroid	HU epidermoid carcinoma cell sub-lines	[I^{125}]-azido agosterol A photolabelled PGP N-terminal fragment with high affinity in absence of glutathione	JAPN	[15]
			MRP1-transfected pig kidney cells	[I^{125}]-azido agosterol A binding to MRP1 in TM 14-17, with Arg^{1202} proximate to TM helix 16 as critical determinant	JAPN	[16]
Aplidine	Ascidian	Depsipeptide	HU adenocarcinoma & colon carcinoma cell lines	Induction of resistance and concomitant lack of MAP Kinase activation and apoptosis	SPA	[19]
			HUVECs, HU ovarian carcinoma & angiogenesis assay	Inhibition of angiogenesis by affecting endothelial cells directly	ITA, USA	[22]
			HU leukaemia cell lines and bone marrow cells	Induction of apoptosis with concomitant G_1 arrest and G_2 blockage	ITA, SPA, USA	[18]
			HU leukaemia cell line	Inhibition of vascular endothelial growth factor (VEGF)/VEGF receptor-1 autocrine loop	ITA, USA	[17]
			HU breast and renal cancer cell lines	Sustained activation of epidermal growth factor receptor; tyrosine and serine-threonine kinases; induction of glutathione depletion	SPA	[20]
			HU leukaemia & breast cell lines and bone marrow aspirates	Rapid Fas/CD95 receptor-induction of mitochondrial apoptosis	SPA	[21]
			HU squamous carcinoma and lung adenocarcinoma cell lines	Induction of COX-2 mRNA, protein and prostaglandin biosynthesis	USA	[27]
Ascididemin	Ascidian	Alkaloid	Assessment of DNA cleavage and intercalation	Direct iminoquinone reduction and reactive oxygen species generation	USA, NZEL	[25]
			HU leukaemia cell line	Apoptosis by activation of JNK and caspase-2 upstream of mitochondria	GER	[24]
Bryostatin-1	Bryozoan	Macrolide	HU leukaemia cell lines	Potentiation of ara-C induced apoptosis by PKC-dependent release of TNF-α	USA	[28]
Cambrescidin 800	Sponge	Alkaloid	HU leukaemia cell line	Induction of eythroid differentiation and cell cycle arrest	JAPN	[52]
Cephalostatin	Worm	Steroid	HU leukaemia cell line	Induction of Smac/DIABLO release, apoptosis and increased mitochondrial matrix density	GER, USA	[53]
Chondropsin A	Sponge	Macrolide	NCI 60-tumour cell line panel	*In vitro* inhibition of V-ATPase enzymes	USA	[54]

(*continued on next page*)

Table 1 – *continued*

Compound	Organism	Chemistry	Experimental or clinical model[a]	Mechanism of action[b]	Country[c]	References
Dehydrothrysiferol	Alga	Triterpene	HU breast cancer cell lines	Enhanced apoptosis induction in estrogen receptor negative breast cancer cells	AUST, SPA	[29]
Diazonamide A	Ascidian	Peptide	HU breast, prostate and lung tumour cell lines	Disruption of mitosis and cellular microtubules with inhibition of GTP hydrolysis	USA	[55]
Dictyostatin-1	Sponge	Polyketide	HU lung, breast and uterine cell lines	Induction of tubulin polymerisation and active in P-glycoprotein-expressing cells	USA	[56]
Didemnin B	Ascidian	Depsipeptide	Molecular dynamics simulations	Binding to human elongation factor eEF1A and protein translation inhibition	SPA	[30]
Dideoxypetrosynol A	Sponge	Fatty acid	HU skin melanoma cells	Induction of apoptosis via mitochondrial signalling pathway	S. KOR	[31]
Dolastatin 10	Mollusc	Peptide	Direct photo-affinity labelling	Binds to amino-terminal peptide of β-tubulin containing cysteine 12	USA	[34]
Dolastatin 11	Mollusc	Peptide	X-ray fibre diffraction analysis	F-actin stabilisation by connection between two long-pitch strands	GER, JAPN, USA	[32]
Dolastatin 15	Synthetic	Peptide	Hummel-Dreyer chromatography	Low affinity binding (Kd $\cong$ 30 μM) with β-tubulin suggesting overlapping binding domains.	USA	[33]
Ecteinascidin-743	Ascidian	Isoquinoline alkaloid	HU melanoma cell lines	Telomere dysfunction increases susceptibility to ET-743	ITA	[35]
GA3 polysaccharide	Alga	Polysaccharide	HU tumour panel	Inhibition of topoisomerase I and II	JAPN	[58]
Girolline	Sponge	Alkaloid	HU epithelial, lung and amnion tumour cell lines	Induction of G_2/M cell cycle arrest and p53 proteasome recruitment	JAPN	[59]
Halichondrin B analogues	Sponge/Synthetic	Macrolide derivative	HU histiocytic lymphoma & prostate tumour cell lines	Induction of mitotic blockage and apoptosis	JAPN, USA	[46]
Hemiasterlin analogue	Sponge/synthetic	Tripeptide	18 HU tumour cell lines and *in vivo* human tumour xenografts	Induction of microtubule depolymerisation; low P-glycoprotein resistance *in vitro* and *in vivo*	CAN, USA	[47]

Isogranulatimide and analogues	Ascidian/synthetic	Alkaloid	G_2 checkpoint and protein kinase assays	Inhibition of protein kinase Chk1 leading to G_2 checkpoint inhibition	CAN, USA	[59]
Kahalalide F	Mollusc	Depsipeptide	HU prostate and breast cancer cell lines	Potent cytotoxicity and induction of necrosis	SPA	[48]
Lamellarin D	Mollusc	Alkaloid	HU and MU tumour cell lines	Potent inhibition of topoisomerase I; less efficient than camptothecin in stabilizing topoisomerase I- DNA complexes	FRA, SPA	[60]
Laurenditerpenol	Alga	Diterpene	Breast tumour cell-based reporter assay	Inhibition of transcription factor hypoxia-inducible factor-1 activation	USA	[61]
Lissoclinolide	Ascidian	Fatty acid	NCI 60 tumour cell line panel	G_2/M cell cycle arrest	USA	[62]
Dihydromotuporamine C	Sponge	Alkaloid	HU breast carcinoma and fibroblast cell lines	Remodelling of stress fibres and focal adhesions, activation of Rho and increased Na^+-H^+ exchange	CAN	[49]
Neoamphimedine	Sponge	Alkaloid	HU tumour cell lines	Induction of topoisomerase IIα-mediated catenation of DNA	PHIL, USA	[63]
Peloruside A	Sponge	Macrolide	HA and HU tumour cell lines	Tubulin binding site different from paclitaxel	NZEL, SPA	[50]
			HU ras-transformed tumour cell line	Induction of enhanced cytotoxicity and apopotosis in *ras*-transformed cells	NZEL	[51]
Psammaplin A	Sponge	Alkaloid	HU & MU tumour cell lines	Inhibition of aminopeptidase N and suppression of angiogenesis *in vitro*	S. KOR	[64]
			MU cell line	Inhibition of topoisomerase I, replication protein A & DNA polymerase α-primase complex	S. KOR	[65]
Smenospongorine	Sponge	Sesquiterpene	HU leukaemia cell line	Induced differentiation, haemoglobin production, glycophorin A and p21 expression	JAPN	[66]

a Experimental or clinical model: HU, human; MU, murine.
b Mechanism of action.
c Country: AUS, Australia; AUST, Austria; CAN, Canada; FRA, France; GER, Germany; ITA, Italy; JAPN, Japan; NZEL, New Zealand; PHIL, Philippines; S.KOR, South Korea; SPA, Spain.

Table 2 – 2003–2004: anti-tumour pharmacology of marine natural products with undetermined mechanism of action

Compound	Organism	Chemistry	Preclinical tumour cell line model[a]	50% growth inhibition or cytotoxicity	Country[b]	References
Actinomadura sp. Xanthone	Bacterium	Xanthone	HU & MU	0.001 μM	SPA	[104,105]
Amphidinolide X	Alga	Macrolide	HU & MU	0.6–7.5 μg/ml	JAPN	[106]
Amphidinolide Y	Alga	Macrolide	HU & MU	0.8–8 μg/ml	JAPN	[107]
Andavadoic acid	Sponge	Fatty acid	HU & MU	0.1–0.7 μM	SPA, FRA	[108]
Aurilide	Sea hare	Depsipeptide	NCI 60-cell line panel	0.011 μg/ml	JPAN	[109]
Axinella cf. bidderi sterol	Sponge	Steroid	HU & MU	0.60 μg/ml	SPA, FRA	[110]
Bistratamide J	Ascidian	Peptide	HU	1 μg/ml	USA	[111]
Bromovulone III	Octocoral	Prostanoid	HU	0.5 μg/ml	S. KOR	[112]
Caulibugulones A–F	Bryozoan	Quinone	MU	0.03–1.67 μg/ml	USA	[113]
Certonardosterol	Seastar	Sterol	HU	0.01–>1 μg/ml	S. KOR	[114]
Certonardoside	Seastar	Sterol	HU	0.26–>1 μg/ml	S. KOR	[114]
Certonardoa semiregularis sterol	Starfish	Steroid	HU	0.12–0.48 μg/ml	S. KOR	[115]
Cladobotryum sp. cyclodepsipeptide	Fungus	Depsipeptide	MU	0.14 μM	NZEL	[116]
Cribrostatin 6	Sponge	Quinone	HU & MU	0.29–>1 μg/ml	USA	[117]
Dasystenella acanthina steroid	Octocoral	Steroid	HU	0.9 μg/ml	SPA	[118]
13-*Epi*-9-deacetoxyxenicin	Soft Coral	Diterpene	MU	0.1 μg/ml	AUS	[119]
Dehydrocyclostellettamine D	Sponge	Alkaloid	HU & MU	0.6–4.3 μg/ml	NETH, JAPN	[70]
Dihydroflabellatene A & B	Sea pen	Diterpene	HU	14.5–90.3 nM	SPA	[120]
Discodermolide analogues	Sponge	Polyketide (synthetic)	HU & MU	0.0024–7.65 μM	USA, SWI	[121]
Discorhabdins C, D analogues	Sponge	Alkaloid	HU	0.119–0.232 μM	N.ZEL, S.AFR, USA	[122]
Discorhabdins I & L	Sponge	Alkaloid	HU	0.12–0.35 μM	SPA	[123]
Discorhabdins S, T & U	Sponge	Alkaloid	HU & MU	0.069–5 μM	USA	[124]
Dolastatin 19	Sea Hare	Macrolide	HU	0.72–0.76 μg/ml	USA	[125]
Gymnangiamide	Hydroid	Peptide	HU	0.46–11 μg/ml	USA	[126]
Halichondramides	Sponge	Macrolides	HU	0.38–0.90 μg/ml	S. KOR, USA	[127]
Haouamine A	Ascidian	alkaloid	HU	0.1 μg/ml	SPA	[128]
22-hydroxyhalicyclamine A	Sponge	Alkaloid	MU	0.45 μg/ml	JAPN, NETH	[88]
Intercedenside A & B	Sea cucumber	Triterpene glycoside	HU	0.61–4.0 μg/ml	CHI, USA	[129]
Ircinia sp. furanosesterterpenes	Sponge	Sesterterpene	HU	<0.1 μg/ml	JAPN	[130]
Irciniastatins A & B	Sponge	Polyketide	HU & MU	0.0001–0.0041 μg/ml	AUS, USA	[131]
Isogeoditin A & B	Sponge	Triterpene	HU	0.07–3.7 μg/ml	CHI, NETH, GER	[132]
Jaspine B	Sponge	Alkaloid	HU	0.24 μM	FRA	[133]
Lasonolide C & E	Sponge	Macrolide	HU	0.13–0.57 μg/ml	USA	[134]
Lingshuiol	Alga	Polyketide	HU	0.21–0.23 μM	CHI	[135]
Microcionamides A & B	Sponge	Peptide	HU	0.098–0.177 μM	PHIL, USA	[136]
Micromide	Bacterium	Alkaloids	HU	0.26 μM	USA	[137]

Milnamide C	Sponge	Peptide	HU	0.32 μg/ml	USA	[72]
Milnamide D	Sponge	Peptide	HU	0.067 μM	USA	[138]
Mixirin A,B,C	Bacterium	Peptide	HU	0.68–1.6 μg/ml	CHI	[139]
Mycalazal-6	Sponge	Alkaloid	HU & MU	0.2–4.5 μg/ml	MEX, SPA	[140]
Mycaperoxide H	Sponge	Sesterterpene	HU	0.8 μg/ml	THAIL, JAPN	[141]
Myrothecium verrucaria Trichothecenes	Fungus	Macrolide	HU 60-cell line panel	0.001–9.8 μM	USA	[142]
Ophiobolin K	Fungus	Sesterterpene	HU & MU	0.27–0.65 μM	JAPN	[143]
Palau'amide	Bacterium	Peptide	HU	0.013 μM	USA	[144]
Phakellistatin 13	Sponge	Peptide	HU	0. 01 μg/ml	CHI	[145]
Plakinamine K Dihydroplakinamine K	Sponge	Steroid alkaloid	HU	1.4 μM	USA	[146]
Plakorstatins 1 & 2	Sponge	Polyketide	HU & MU	0.91–>10 μg/ml	USA	[147]
Plakortide O & P	Sponge	Polyketide	NCI 60-cell line panel	0.01–11.1 μM	USA	[148]
Protoceratin II–IV	Alga	Polyether glycoside	HU	0.0005 μM	USA	[149]
Psymberin	Sponge	Polyketide	HU 60-cell line panel	0.0025–25 μM	USA	[150]
Pterocellins A & B	Bryozoan	Alkaloid	HU & MU	0.3–0.5 μg/ml MU 0.03–1.4 μM HU	NZEL	[151]
Renieramycin J	Sponge	Alkaloid	HU & MU	0.053–0.012 μM	JAPN, NETH	[152]
Renieramycin M, N	Sponge	Alkaloid	HU	0.0056–0.019 μM	JAPN, THAI	[153]
Renieramycin O, Q, R,S	Sponge	Alkaloid	HU	15–59 nM	THAIL, JAPN	[154]
Rostratin C	Fungus	Alkaloid	HU	0.76 μg/ml	USA	[155]
Salinosporamide A	Bacterium	Alkaloid	NCI 60-cell line panel	< 0.020 μM	USA	[71]
(Z)-sarcodictyin A	Soft coral	Diterpene	HU & MU	0.09 μg/ml	JAPN	[156]
Scabrolide E	Soft coral	Diterpene	HU	0.5–0.7 μg/ml	EGPT, TAIW	[157]
Scleritodermin A	Sponge	Peptide	HU	0.67–1.9 μg/ml	USA	[68]
Shishijimicins A–C	Ascidian	Alkaloid	HU & MU	0.47–34 pg/ml	JAPN	[158]
Synularia acylspermidines	Soft coral	Fatty acid	HU	0.017 μg/ml	JAPN	[159]
Smenospongia sp. Sesterterpene	Sponge	Sesterterpene	HU	0.02 μg/ml	S. KOR	[160]
Spirastrellolide A	Sponge	Macrolide	HU	0.1 μg/ml	CAN, NETH	[161]
Streptomycete sp. capralactones	Fungus	Fatty acid	HU	0.11–2.7 μg/ml	GER	[162]
Streptomycete-derived anthracycline	Bacterium	Quinone	MU	0.4–0.06 μg/ml	NZEL	[163]
Strongylophorine-26	Sponge	Diterpene	HU	1 μg/ml	CAN, PAPUA, NETH	[67]
Tasiamide B	Bacterium	Peptide	HU	0.8 μM	USA	[164]
Tasipeptins A & B	Bacterium	Depsipeptide	HU	0.82–0.93 μM	USA	[165]
Trichodermamide B	Fungus	Peptide	HU	0.32 μg/ml	USA	[166]
Ulongapeptin	Bacterium	Depsipeptide	HU	0.63 μM	USA	[167]
Xylaria sesquiterpene	Fungus	Sesquiterpene	HU	0.9 μg/ml	USA	[168]
Zooxanthellactone	Alga	Fatty acid	HU	0.23–0.27 μM	JAPN	[169]

a HU, human; MU, murine.

b Country: AUS, Australia; CAN, Canada; CHI, China; EGPT, Egypt; FRA, France; GER, Germany; JAPN, Japan; MEX, Mexico; NETH, Netherlands; NZEL, New Zealand; PAPUA, Papua New Guinea; PHIL, Philippines; S. AFR, South Africa; S. KOR, South Korea; SPA, Spain; SWI, Switzerland; THAIL, Thailand; TAIW, Taiwan.

on tubulin that may be the same that binds laulimalide, but that is clearly different from the paclitaxel-binding site. The authors concluded that 'these results establish a new perspective in tumour chemotherapy because peloruside and laulimalide may prove more effective than other microtubule-stabilising drugs against tumour cells'. Miller and colleagues[51] determined that peloruside A was more cytotoxic to *ras* oncogene-transformed cells than non-transformed cells, blocking the cells in G_2/M phase of the cell cycle, and ultimately causing apoptosis.[51] Thus, peloruside A contributes to the search for novel and selective agents that enhance tumour cell apoptosis, one of the major mechanisms explored in anti-cancer research.

Table 1 also lists several marine natural products which were *not* previously reviewed:[2–4] cambrescidin 800, cephalostatin 1, chondropsin A, diazonamide A, dictyostatin-1, girolline, GA3P polysaccharide, isogranulatimide, lamellarin D, laurenditerpenol, lissoclinolide, neoamphimedine, psammaplin A and smenospongorine.

Aoki and colleagues[52] reported on the differentiation of K562 chronic myelogenous leukaemia cells exposed to **crambescidin 800**, a pentacyclic guanidine alkaloid isolated from the marine sponge *Crambe crambe*. The *in vitro* studies demonstrated that crambescidin 800 induced differentiation of K562 cells into erythroblasts while concomitantly increasing haemoglobin production and arresting the cell cycle at the S-phase.

Dirsch and colleagues[53] showed that **cephalostatin 1**, a bissteroidal marine natural product isolated from the marine worm *Cephalodiscus gilchristi*, induced apoptosis in human leukaemia Jurkat cells. The mechanism involved selective triggering release of Smac/DIABLO (second mitochondria-derived activator of caspases/direct IAP-binding protein with a low isoelectric point) and concomitant appearance of mitochondria with an increased matrix density.

Bowman and colleagues[54] communicated that the sponge metabolite **chondropsin A** and other members of this family of macrolide lactams, potently inhibited mammalian V-ATPase enzymes which are implicated in a variety of cancerous processes including proliferation, tumour invasion, and drug resistance. Chondropsin macrolides produced a distinctive pattern of selective cytotoxicity in the NCI 60 tumour cell line panel that is characteristic of other known V-ATPase inhibitors.

Cruz-Monserrate and colleagues[55] extended the molecular pharmacology of the peptide **diazonamide A**, originally isolated from the marine ascidian *Diazona angulata*. Diazonamide A and a synthetic oxygenated analogue potently inhibited microtubule assembly with concomitant inhibition of tubulin-dependent GTP hydrolysis. The investigation was unable to determine whether diazonamide A and the analogue had a 'unique binding site on tubulin differing from the vinka alkaloid and dolastatin 10 biding sites' or if this marine peptide bound weakly to unpolymerised tubulin yet bound 'strongly to microtubule ends'.

Isbrucker and colleagues[56] investigated the molecular pharmacology of the highly cytotoxic macrolide polyketide **dictyostatin-1**, originally derived from a Republic of Maldives marine sponge from the genus *Spongia sp.* Dictyostatin-1 arrested human lung adenocarcinoma cells in the G_2/M phase of the cell cycle at concentrations as low as 10 nM. Furthermore, dictyostatin-1 was observed to induce a rapid polymerisation of purified bovine brain tubulin *in vitro* and, interestingly, to be highly cytotoxic towards two paclitaxel-resistant human cancer cell lines expressing active P-glycoprotein. Further investigation of this compound will determine whether dictyostatin-1 and paclitaxel are ligands to the same binding site on tubulin.

Tsukamoto and colleagues[57] contributed a preclinical pharmacological study on **girolline**, a 2-aminoimidazole derivative originally isolated from the marine sponge *Peudaxinyssa cantharella*. Girolline exhibited G_2/M cell cycle arrest and induced accumulation of polyubiquitinated p53 in lung, human amnion and epithelial tumour cell lines in a concentration dependent manner. While the mechanisms of p53-dependent G_2 arrest have not been elucidated, the authors concluded that girolline was a novel-type inhibitor against the ubiquitin-dependent proteolytic pathway that warranted further mechanistic studies.

Umemura and colleagues[58] continued studies on the extracellular acidic polysaccharide **GA3P, a D-galactan sulphate** associated with L-(+)-lactic acid produced by the marine microalga *Gymnodinium sp.* GA3P was shown to be a potent inhibitor of topoisomerases I and II, a process that did not involve accumulation of DNA-topoisomerase I/II cleavable complexes, suggesting that this polysaccharide is a catalytic inhibitor with dual activity and high affinity. Furthermore, GA3P exhibited significant *in vitro* cytotoxicity against 39 human tumours (range 0.67–11 μg/ml).

With the purpose of continuing the development of compounds that inhibit the G_2 checkpoint as potentially valuable agents for enhancing the effectiveness of DNA-damaging agents in tumours with mutated p53, Jian and colleagues[59] published a detailed study on the molecular pharmacology of the marine alkaloid **isogranulatimide**, originally isolated from the Brazilian ascidian *Didemnum granulatum*. Using natural and synthetic isogranulatimide analogues the investigators demonstrated that the imide and basic nitrogen at position 14 or 15 in the imidazole ring were requirements for G_2 checkpoint inhibition, and that concomitant inhibition of the DNA damage response Chk1 protein kinase (IC_{50} = 0.1 μM) played an important role in the process. By X-ray crystallography the authors determined the structural elements required for isogranulatimide activity as a Chk1 kinase inhibitor, and concluded that this agent may be a 'promising candidate for modulating checkpoint responses in tumours'.

With the purpose of contributing to the search for non-camptothecin topoisomerase I poisons, Facompre and colleagues[60] found that the hexacyclic marine alkaloid **lamellarin D**, isolated from the mollusc *Lamellaria* sp. was a potent inhibitor of DNA topoisomerase I. The pharmacological properties of lamellarin D and LAM-501, a synthetic lamellarin derivative, were compared with those of camptothecin, from which topotecan and irinotecan have been derived for treatment of metastatic ovarian and colon cancers. The results of this investigation collectively identify lamellarin D as low-affinity DNA intercalator yet a potent inhibitor of the DNA/cleavage activity of topoisomerase I, which interacts differently with the topoisomerase I-DNA interface than camptothecin, and

which 'should be considered as a new pharmacophore for topoisomerase I targeting '.

The development of novel marine agents to target hypoxic tumour cells' induction of the transcription factor hypoxia-inducible factor-1 (HIF-1) gene expression that is associated with poor prognosis and treatment resistance was investigated by Mohammed and colleagues.[61] Using a human breast tumour cell-based reporter assay and bioassay-guided fractionation these investigators isolated a novel diterpene **laurenditerpenol** in the red alga *Laurencia intricata* which inhibited HIF-1 (IC_{50} = 0.4 μM) probably as a result of blocking the induction of nuclear HIF-1α protein. The investigators noted that this was the first report of a marine diterpene that 'selectively and potently inhibits physiological hypoxia-induced HIF-1 activation in tumour cells'.

Richardson and Ireland[62] continued the characterisation of the anti-tumour activity of the small non-nitrogenous lactone **lissoclinolide** isolated from the marine ascidian *Lissoclinum patella*. Lissoclinolide was able to particularly inhibit growth of cell lines in the NCI colon tumour panel. While the ultimate molecular target of lissoclinolide remains undetermined, most notable was the observation that 2.4 μM lissoclinolide strongly arrested the G_2/M phase of the cell cycle in both p53 competent and null human colon carcinoma HCT 116 cell lines after 24- or 48-h exposure.

Marshall and colleagues[63] extended the molecular pharmacology of **neoamphimedine**, a pyridoacridine isomer of amphimedine which was isolated from the Philippine marine sponge *Xestospongia sp*. Low concentrations of neoamphimedine induced catenation of plasmid DNA in the presence of active topoisomerase IIα (top2), which correlated with DNA aggregation. Interestingly, neoamphimedine but not amphimedine, showed potent anti-tumour activity in athymic mice bearing human KB tumours, which was equivalent to etoposide, thus suggesting that this marine compound has 'a novel top2-mediated mechanism of toxicity and anticancer potential'.

Two papers extended the pharmacology of the marine bromotyrosine derivative **psammaplin A**, isolated from a two-sponge association between, *Poecillastra* sp. and *Jaspis* sp. Shim and colleagues[64] showed that psammaplin A inhibited aminopeptidase N (APN) (IC_{50} = 18 μM) in a non-competitive manner, a finding of considerable interest because APN is an enzyme that is crucial for angiogenesis, a process involved in both in tumour cell growth and metastasis. The authors concluded that psammaplin A's inhibition of APN activity suggests a potentially novel approach to prevent angiogenesis-related diseases. Furthermore, Jian and colleagues[65] noted that psammaplin A inhibited SV40 DNA replication *in vitro* by inhibiting the DNA polymerase α-primase complex.

With the purpose of contributing to the search for new differentiation-inducing agents for haematopoietic cancer, Aoki and colleagues[66] investigated the marine sesquiterpene aminoquinone **smenospongine** isolated from the Indonesian marine sponge *Dactylospongia elegans*. Smenospongine increased haemoglobin production in human chronic myelogenous leukaemia (CML) cells, with concomitant increased expression of glycophorin A, a marker for erythroid differentiation. Furthermore the marine compound induced cell cycle arrest at G_1 phase probably due to increased expression of p21, while also inhibiting Crkl phosphorylation, a substrate of the Bcr-Abl tyrosine kinase known to be involved in CML pathogenesis. The authors conclude that smenospongine 'is expected to be a promising candidate for treatment of CML'.

3. 2003–2004: anti-tumour pharmacology of marine natural products with *undetermined* mechanisms of action

Table 2 encompasses 119 novel marine natural products published during 2003–2004 that demonstrated particularly potent activity in cytotoxicity assays (IC_{50} of < = 1.0 μg/ml) and structures are shown in Fig. 2. The preclinical pharmacology completed with these marine compounds consisted mainly of *in vitro* and/or *in vivo* cytotoxicity testing with panels of either human or murine tumour cell lines. In a few reports cytotoxicity studies were more extensive and included the National Cancer Institute (NCI) 60-tumour cell line screen. It is clear that additional pharmacological testing will be required to help determine if the potent cytotoxicity observed with these marine chemicals resulted from a pharmacological rather than a simple toxic effect on the tumour cells used in these investigations. Although contrasting with the extensive preclinical and clinical investigation completed with the marine compounds presented in Table 1, mechanism of action research was reported for several of the marine compounds listed in Table 2: inhibition of Matrigel invasion by human breast carcinoma MDA-231 cells by strongylophorine-26;[67] induction of apoptosis by scleritodermin A[68] and ritterazine B;[69] inhibition of histone deacetylase enzyme by dehydrocyclostellettamine D;[70] inhibition of proteasomal chymotrypsin-like proteolytic activity by salinosporamide A [71] and microtubule depolymerisation by milnamide C.[72]

Although less potent than the marine natural products included in Table 2, 30 additional reports were published during 2003–2004 describing novel structurally characterised molecules with cytotoxic activity (IC_{50}) mostly in the >1–4.0 μg/ml range.[73–102] Although only the cytotoxicity against selected murine or human cancer cells was determined *in vitro* in the majority of these reports, mechanistic work was reported in a few studies, e.g. induction of erythroid differentiation in human leukaemia by 5-*epi*-smenospongorine.[101]

4. Conclusion

Anti-tumour marine pharmacology research in 2003–2004 consisted of a combination of preclinical research focused on the molecular and cellular pharmacology of marine cytotoxic agents, as well as clinical studies with a limited number of marine compounds, i.e. bryostatin 1, cryptophycins, dolastatins and ecteinascidin-743. Although during 2003–2004 no new marine natural product was approved for cancer patient treatment by the US Food and Drug Administration, the present 2003–2004 overview of the anti-tumour and cytotoxic pharmacology of marine chemicals demonstrates that more than 54 years after the discovery by Bergman and colleagues[103] of spongothymidine and spongouridine, global research aimed at the discovery of novel and clinically useful anti-tumour agents derived from marine organisms continues at a remarkably active pace.

Conflict of interest statement

None declared.

Acknowledgements

This publication was made possible by grant number 1 R15 ES12654-01 (to A.M.S.M) from the National Institute of Environmental Health Sciences, NIH. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the NIEHS, NIH. This work was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. The excellent support for literature searches and article retrieval by library staff members as well as medical and pharmacy students of Midwestern University is most gratefully acknowledged. The authors specially thank Mrs Victoria Sears and Ms Mary Hall for excellent secretarial assistance in the preparation of this manuscript.

REFERENCES

1. Mayer AMS. Marine pharmacology in 1998: antitumor and cytotoxic compounds. *The Pharmacologist* 1999;**41**:159–64.
2. Mayer AMS, Lehmann VKB. Marine pharmacology in 1999: antitumor and cytotoxic compounds. *Anticancer Research* 2001;**21**:2489–500.
3. Mayer AMS, Gustafson KR. Marine pharmacology in 2000: antitumor and cytotoxic compounds. *Int J Cancer* 2003;**105**:291–9.
4. Mayer AMS, Gustafson KR. Marine pharmacology in 2001–2: antitumour and cytotoxic compounds. *Eur J Cancer* 2004;**40**:2676–704.
5. Mayer AMS, Lehmann VKB. Marine pharmacology in 1998: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, anthelmintic, antiplatelet, antiprotozoal, and antiviral activities; with actions on the cardiovascular, endocrine, immune, and nervous systems; and other miscellaneous mechanisms of action. *The Pharmacologist* 2000;**42**:62–9.
6. Mayer AMS, Hamann MT. Marine pharmacology in 1999: compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, anthelmintic, anti-inflammatory, antiplatelet, antiprotozoal and antiviral activities;affecting the cardiovascular, endocrine, immune, and nervous systems; and other miscellaneous mechanisms of action. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 2002;**132**:315–39.
7. Mayer AMS, Hamann MT. Marine pharmacology in 2000: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antituberculosis, and antiviral activities; affecting the cardiovascular, immune, and nervous systems and other miscellaneous mechanisms of action. *Mar Biotechnol (NY)* 2004;**6**:37–52.
8. Mayer AMS, Hamann MT. Marine pharmacology in 2001–2002: marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. *Comp Biochem Physiol C Toxicol Pharmacol* 2005;**140**:265–86.
9. Jimenez PC, Fortier SC, Lotufo TMC, et al. Biological activity in extracts of ascidians (Tunicata, Ascidiacea) from the northeastern Brazilian coast. *J Exp Marine Biol* 2003;**287**:93–101.
10. Brown JW, Cappell S, Perez-Stable C, Fishman LM. Extracts from two marine sponges lower cyclin B1 levels, cause a G2/M cell cycle block and trigger apoptosis in SW-13 human adrenal carcinoma cells. *Toxicon* 2004;**43**:841–6.
11. McFadden DW, Riggs DR, Jackson BJ, Vona-Davis L. Keyhole limpet hemocyanin, a novel immune stimulant with promising anticancer activity in Barrett's esophageal adenocarcinoma. *Am J Surg* 2003;**186**:552–5.
12. Iijima R, Kisugi J, Yamazaki M. L-amino acid oxidase activity of an antineoplastic factor of a marine mollusk and its relationship to cytotoxicity. *Dev Comp Immunol* 2003;**27**:505–12.
13. Gingras D, Boivin D, Deckers C, Gendron S, Barthomeuf C, Beliveau R. Neovastat – a novel antiangiogenic drug for cancer therapy. *Anticancer Drugs* 2003;**14**:91–6.
14. Gonzalez-Iriarte M, Carmona R, Perez-Pomares JM, et al. A modified chorioallantoic membrane assay allows for specific detection of endothelial apoptosis induced by antiangiogenic substances. *Angiogenesis* 2003;**6**:251–4.
15. Mitsuo M, Noguchi T, Nakajima Y, et al. Binding site(s) on P-glycoprotein for a newly synthesized photoaffinity analog of agosterol A. *Oncol Res* 2003;**14**:39–48.
16. Ren XQ, Furukawa T, Aoki S, et al. Localization of the GSH-dependent photolabelling site of an agosterol A analog on human MRP1. *Br J Pharmacol* 2003;**138**:1553–61.
17. Broggini M, Marchini SV, Galliera E, et al. Aplidine, a new anticancer agent of marine origin, inhibits vascular endothelial growth factor (VEGF) secretion and blocks VEGF-VEGFR-1 (*flt-1*) autocrine loop in human leukemia cells MOLT-4. *Leukemia* 2003;**17**:52–9.
18. Erba E, Serafini M, Gaipa G, et al. Effect of aplidin in acute lymphoblastic leukaemia cells. *Br J Cancer* 2003;**89**:763–73.
19. Losada A, Lopez-Oliva JM, Sanchez-Puelles JM, Garcia-Fernandez LF. Establishment and characterisation of a human carcinoma cell line with acquired resistance to aplidin™. *Br J Cancer* 2004;**91**:1405–13.
20. Cuadrado A, Garcia-Fernandez LF, Gonzalez L, et al. Aplidin induces apoptosis in human cancer cells via glutathione depletion and sustained activation of the epidermal growth factor receptor, Src, JNK, and p38 MAPK. *J Biol Chem* 2003;**278**:241–50.
21. Gajate C, An F, Mollinedo F. Rapid and selective apoptosis in human leukemic cells induced by aplidine through a Fas/CD95- and mitochondrial-mediated mechanism. *Clin Cancer Res* 2003;**9**:1535–45.
22. Taraboletti G, Poli M, Dossi R, et al. Antiangiogenic activity of aplidine, a new agent of marine origin. *Br J Cancer* 2004;**90**:2418–24.
23. Gomez SG, Bueren JA, Faircloth GT, Jimeno J, Albella B. In vitro toxicity of three new antitumoral drugs (trabectedin, aplidin, and kahalalide F) on hematopoietic progenitors and stem cells. *Exp Hematol* 2003;**31**:1104–11.
24. Matsumoto SS, Biggs J, Copp BR, Holden JA, Barrows LR. Mechanism of ascididemin-induced cytotoxicity. *Chem Res Toxicol* 2003;**16**:113–22.
25. Dirsch VM, Kirschke SO, Estermeier M, Steffan B, Vollmar AM. Apoptosis signaling triggered by the marine alkaloid ascididemin is routed via caspase-2 and JNK to mitochondria. *Oncogene* 2004;**23**:1586–93.
26. Ali S, Aranha O, Li Y, Pettit GR, Sarkar FH, Philip PA. Sensitization of human breast cancer cells to gemcitabine by

the protein kinase C modulator bryostatin 1. *Cancer Chemother Pharmacol* 2003;**52**:235–46.
27. De Lorenzo MS, Yamaguchi K, Subbaramaiah K, Dannenberg AJ. Bryostatin-1 stimulates the transcription of cyclooxygenase-2: evidence for an activator protein-1-dependent mechanism. *Clin Cancer Res* 2003;**9**:5036–43.
28. Wang S, Wang Z, Grant S. Bryostatin 1 and UCN-01 potentiate 1-beta-D-arabinofuranosylcytosine-induced apoptosis in human myeloid leukemia cells through disparate mechanisms. *Mol Pharmacol* 2003;**63**:232–42.
29. Pec MK, Aguirre A, Moser-Thier K, et al. Induction of apoptosis in estrogen dependent and independent breast cancer cells by the marine terpenoid dehydrothyrsiferol. *Biochem Pharmacol* 2003;**65**:1451–61.
30. Marco E, Martin-Santamaria S, Cuevas C, Gago F. Structural basis for the binding of didemnins to human elongation factor eEF1A and rationale for the potent antitumor activity of these marine natural products. *J Med Chem* 2004;**47**:4439–52.
31. Choi HJ, Bae SJ, Kim ND, Jung JH, Choi YH. Induction of apoptosis by dideoxypetrosynol A, a polyacetylene from the sponge *Petrosia* sp., in human skin melanoma cells. *Int J Mol Med* 2004;**14**:1091–6.
32. Oda T, Crane ZD, Dicus CW, Sufi BA, Bates RB. Dolastatin 11 connects two long-pitch strands in F-actin to stabilize microfilaments. *J Mol Biol* 2003;**328**:319–24.
33. Cruz-Monserrate Z, Mullaney JT, Harran PG, Pettit GR, Hamel E. Dolastatin 15 binds in the vinca domain of tubulin as demonstrated by Hummel-Dreyer chromatography. *Eur J Biochem* 2003;**270**:3822–8.
34. Bai R, Covell DG, Taylor GF, et al. Direct photoaffinity labeling by dolastatin 10 of the amino-terminal peptide of beta-tubulin containing cysteine 12. *J Biol Chem* 2004;**279**:30731–40.
35. Biroccio A, Gabellini C, Amodei S, et al. Telomere dysfunction increases cisplatin and ecteinascidin-743 sensitivity of melanoma cells. *Mol Pharmacol* 2003;**63**:632–8.
36. D'Incalci M, Colombo T, Ubezio P, et al. The combination of yondelis and cisplatin is synergistic against human tumor xenografts. *Eur J Cancer* 2003;**39**:1920–6.
37. Simoens C, Korst AE, De Pooter CM, et al. *In vitro* interaction between ecteinascidin 743 (ET-743) and radiation, in relation to its cell cycle effects. *Br J Cancer* 2003;**89**:2305–11.
38. Shao L, Kasanov J, Hornicek FJ, Morii T, Fondren G, Weissbach L. Ecteinascidin-743 drug resistance in sarcoma cells: transcriptional and cellular alterations. *Biochem Pharmacol* 2003;**66**:2381–95.
39. Meco D, Colombo T, Ubezio P, et al. Effective combination of ET-743 and doxorubicin in sarcoma: preclinical studies. *Cancer Chemother Pharmacol* 2003;**52**:131–8.
40. Donald S, Verschoyle RD, Greaves P, et al. Complete protection by high-dose dexamethasone against the hepatotoxicity of the novel antitumor drug Yondelis (ET-743) in the rat. *Cancer Res* 2003;**63**:5902–8.
41. Donald S, Verschoyle RD, Greaves P, et al. Dietary agent indole-3-carbinol protects female rats against the hepatotoxicity of the antitumor drug ET-743 (trabectidin) without compromising efficacy in a rat mammary carcinoma. *Int J Cancer* 2004;**111**:961–7.
42. Twelves C, Hoekman K, Bowman A, et al. Phase I and pharmacokinetic study of Yondelis (Ecteinascidin-743; ET-743) administered as an infusion over 1 h or 3 h every 21 days in patients with solid tumours. *Eur J Cancer* 2003;**39**:1842–51.
43. Laverdiere C, Kolb EA, Supko JG, et al. Phase II study of ecteinascidin 743 in heavily pretreated patients with recurrent osteosarcoma. *Cancer* 2003;**98**:832–40.
44. Garcia-Carbonero R, Supko JG, Manola J, et al. Phase II and pharmacokinetic study of ecteinascidin 743 in patients with progressive sarcomas of soft tissues refractory to chemotherapy. *J Clin Oncol* 2004;**22**:1480–90.
45. Yovine A, Riofrio M, Blay JY, et al. Phase II study of ecteinascidin-743 in advanced pretreated soft tissue sarcoma patients. *J Clin Oncol* 2004;**22**:890–9.
46. Kuznetsov G, Towle MJ, Cheng H, et al. Induction of morphological and biochemical apoptosis following prolonged mitotic blockage by halichondrin B macrocyclic ketone analog E7389. *Cancer Res* 2004;**64**:5760–6.
47. Loganzo F, Discafani CM, Annable T, et al. HTI-286, a synthetic analogue of the tripeptide hemiasterlin, is a potent antimicrotubule agent that circumvents P-glycoprotein-mediated resistance in vitro and in vivo. *Cancer Res* 2003;**63**:1838–45.
48. Suarez Y, Gonzalez L, Cuadrado A, Berciano M, Lafarga M, Munoz A. Kahalalide F, a new marine-derived compound, induces oncosis in human prostate and breast cancer cells. *Mol Cancer Ther* 2003;**2**:863–72.
49. McHardy LM, Sinotte R, Troussard A, et al. The tumor invasion inhibitor dihydromotuporamine C activates RHO, remodels stress fibers and focal adhesions, and stimulates sodium-proton exchange. *Cancer Res* 2004;**64**:1468–74.
50. Gaitanos TN, Buey RM, Diaz JF, et al. Peloruside A does not bind to the taxoid site on beta-tubulin and retains its activity in multidrug-resistant cell lines. *Cancer Res* 2004;**64**:5063–7.
51. Miller JH, Rouwe B, Gaitanos TN, et al. Peloruside A enhances apoptosis in H-*ras*-transformed cells and is cytotoxic to proliferating T cells. *Apoptosis* 2004;**9**:785–96.
52. Aoki S, Kong D, Matsui K, Kobayashi M. Erythroid differentiation in K562 chronic myelogenous cells induced by crambescidin 800, a pentacyclic guanidine alkaloid. *Anticancer Res* 2004;**24**:2325–30.
53. Dirsch VM, Muller IM, Eichhorst ST, et al. Cephalostatin 1 selectively triggers the release of Smac/DIABLO and subsequent apoptosis that is characterized by an increased density of the mitochondrial matrix. *Cancer Res* 2003;**63**:8869–76.
54. Bowman EJ, Gustafson KR, Bowman BJ, Boyd MR. Identification of a new chondropsin class of antitumor compound that selectively inhibits V-ATPases. *J Biol Chem* 2003;**278**:44147–52.
55. Cruz-Monserrate Z, Vervoort HC, Bai R, et al. Diazonamide A and a synthetic structural analog: disruptive effects on mitosis and cellular microtubules and analysis of their interactions with tubulin. *Mol Pharmacol* 2003;**63**:1273–80.
56. Isbrucker RA, Cummins J, Pomponi SA, Longley RE, Wright AE. Tubulin polymerizing activity of dictyostatin-1, a polyketide of marine sponge origin. *Biochem Pharmacol* 2003;**66**:75–82.
57. Tsukamoto S, Yamashita K, Tane K, et al. Girolline, an antitumor compound isolated from a sponge, induces G2/M cell cycle arrest and accumulation of polyubiquitinated p53. *Biol Pharm Bull* 2004;**27**:699–701.
58. Umemura K, Yanase K, Suzuki M, Okutani K, Yamori T, Andoh T. Inhibition of DNA topoisomerases I and II, and growth inhibition of human cancer cell lines by a marine microalgal polysaccharide. *Biochem Pharmacol* 2003;**66**:481–7.
59. Jiang X, Zhao B, Britton R, et al. Inhibition of Chk1 by the G_2 DNA damage checkpoint inhibitor isogranulatimide. *Mol Cancer Ther* 2004;**3**:1221–7.
60. Facompre M, Tardy C, Bal-Mahieu C, et al. Lamellarin D: a novel potent inhibitor of topoisomerase I. *Cancer Res* 2003;**63**:7392–9.
61. Mohammed KA, Hossain CF, Zhang L, Bruick RK, Zhou YD, Nagle DG. Laurenditerpenol, a new diterpene from the tropical marine alga *Laurencia intricata* that potently inhibits

HIF-1 mediated hypoxic signaling in breast tumor cells. *J Nat Prod* 2004;**67**:2002–7.
62. Richardson AD, Ireland CM. A profile of the in vitro antitumor activity of lissoclinolide. *Toxicol Appl Pharmacol* 2004;**195**:55–61.
63. Marshall KM, Matsumoto SS, Holden JA, et al. The anti-neoplastic and novel topoisomerase II-mediated cytotoxicity of neoamphimedine, a marine pyridoacridine. *Biochem Pharmacol* 2003;**66**:447–58.
64. Shim JS, Lee HS, Shin J, Kwon HJ. Psammaplin A, a marine natural product, inhibits aminopeptidase N and suppresses angiogenesis in vitro. *Cancer Lett* 2004;**203**:163–9.
65. Jiang Y, Ahn EY, Ryu SH, et al. Cytotoxicity of psammaplin A from a two-sponge association may correlate with the inhibition of DNA replication. *BMC Cancer* 2004;**4**:70.
66. Aoki S, Kong D, Matsui K, Kobayashi M. Smenospongine, a spongean sesquiterpene aminoquinone, induces erythroid differentiation in K562 cells. *Anticancer Drugs* 2004;**15**:363–9.
67. Warabi K, McHardy LM, Matainaho L, et al. Strongylophorine-26, a new meroditerpenoid isolated from the marine sponge *Petrosia* (Strongylophora) *corticata* that exhibits anti-invasion activity. *J Nat Prod* 2004;**67**:1387–9.
68. Schmidt EW, Raventos-Suarez C, Bifano M, Menendez AT, Fairchild CR, Faulkner DJ. Scleritodermin A, a cytotoxic cyclic peptide from the lithistid sponge *Scleritoderma nodosum*. *J Nat Prod* 2004;**67**:475–8.
69. Komiya T, Fusetani N, Matsunaga S, et al. Ritterazine B, a new cytotoxic natural compound, induces apoptosis in cancer cells. *Cancer Chemother Pharmacol* 2003;**51**:202–8.
70. Oku N, Nagai K, Shindoh N, et al. Three new cyclostellettamines, which inhibit histone deacetylase, from a marine sponge of the genus *Xestospongia*. *Bioorg Med Chem Lett* 2004;**14**:2617–20.
71. Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W. Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinospora*. *Angew Chem Int Ed Engl* 2003;**42**:355–7.
72. Sonnenschein RN, Farias JJ, Tenney K, et al. A further study of the cytotoxic constituents of a milnamide-producing sponge. *Org Lett* 2004;**6**:779–82.
73. Aiello A, Fattorusso E, Luciano P, et al. Conicaquinones A and B, two novel cytotoxic terpene quinones from the Mediterranean ascidian *Aplidium conicum*. *Eur J Org Chem* 2003:898–900.
74. Ahmed AF, Shiue RT, Wang GH, Dai CF, Kuo YH, Sheu JH. Five novel norcembranoids from *Sinularia leptoclados* and *S. parva*. *Tetrahedron* 2003;**59**:7337–44.
75. Bringmann G, Lang G, Steffens S, Gunther E, Schaumann K. Evariquinone, isoemericellin, and stromemycin from a sponge derived strain of the fungus *Emericella variecolor*. *Phytochemistry* 2003;**63**:437–43.
76. Calcul L, Longeon A, Al Mourabit A, Guyot M, Bourguet-Kondracki ML. Novel alkaloids of the aaptamine class from an Indonesian marine sponge of the genus *Xestopongia*. *Tetrahedron* 2003;**59**:6539–44.
77. Chill L, Aknin M, Kashman Y. Barrenazine A and B; two new cytotoxic alkaloids from an unidentified tunicate. *Org Lett* 2003;**5**:2433–5.
78. Davis RA, Sandoval IT, Concepcion GP, da Rocha RM, Ireland CM. Lissoclinotoxins E and F, novel cytotoxic alkaloids from a Philippine didemnid ascidian. *Tetrahedron* 2003:2855–9.
79. Gross H, Kehraus S, Nett M, Konig GM, Beil W, Wright AD. New cytotoxic cembrane based diterpenes from the soft corals *Sarcophyton cherbonnieri* and *Nephthea* sp.. *Org Biomol Chem* 2003;**1**:944–9.
80. Issa HH, Tanaka J, Rachmat R, Higa T. Floresolides, new metacyclophane hydroquinone lactones from an ascidian, *Aplidium* sp.. *Tetrahedron Lett* 2003;**44**:1243–5.
81. Shen YC, Lu CH, Chakraborty R, Kuo YH. Isolation of sesquiterpenoids from sponge *Dysidea avara* and chemical modification of avarol as potential antitumor agents. *Nat Prod Res* 2003;**17**:83–9.
82. Sheu JH, Wang GH, Duh CY, Soong K. Pachyclavulariolides M-R, six novel diterpenoids from a Taiwanese soft coral *Pachyclavularia violacea*. *J Nat Prod* 2003;**66**:662–6.
83. Taglialatela-Scafati O, Craig KS, Reberioux D, Roberge M, Andersen RJ. Briarane, erythrane, and aquariane diterpenoids from the Caribbean gorgonian *Erythropodium caribaeorum*. *Eur J Org Chem* 2003;**18**:3515–23.
84. Wang W, Li F, Hong J, et al. Four new saponins from the starfish *Certonardoa semiregularis*. *Chem Pharm Bull (Tokyo)* 2003;**51**:435–9.
85. Usami Y, Yamaguchi J, Numata A. Gliocladins A-C and glioperazine; Cytotoxic dioxo- or trioxopiperazine metabolites from a *Gliocladium* sp. separated from a sea hare. *Heterocycles* 2004;**63**:1123–9.
86. Tanaka C, Yamamoto Y, Otsuka M, et al. Briarane diterpenes from two species of octocorals, *Ellisella* sp. and *Pteroeides* sp.. *J Nat Prod* 2004;**67**:1368–73.
87. Masuno MN, Pawlik JR, Molinski TF. Phorbasterones A-D, cytotoxic nor-ring A steroids from the sponge *Phorbas amaranthus*. *J Nat Prod* 2004;**67**:731–3.
88. Matsunaga S, Miyata Y, van Soest RW, Fusetani N. Tetradehydrohalicyclamine A and 22-hydroxyhalicyclamine A, new cytotoxic bis-piperidine alkaloids from a marine sponge *Amphimedon* sp.. *J Nat Prod* 2004;**67**:1758–60.
89. Mansoor TA, Hong J, Lee CO, et al. New cytotoxic metabolites from a marine sponge *Homaxinella* sp.. *J Nat Prod* 2004;**67**:721–4.
90. Iguchi K, Fukaya T, Yasumoto A, Watanabe K. New marine sesquiterpenoids and diterpenoids from the Okinawan soft coral *Clavularia koellikeri*. *J Nat Prod* 2004;**67**:577–83.
91. Gunasekera SP, Isbrucker RA, Longley RE, Wright AE, Pomponi SA, Reed JK. Plakolide A, a new gamma-lactone from the marine sponge *Plakortis* sp.. *J Nat Prod* 2004;**67**:110–1.
92. El Gamal AA, Wang SK, Dai CF, Duh CY. New nardosinanes and 19-oxygenated ergosterols from the soft coral *Nephthea armata* collected in Taiwan. *J Nat Prod* 2004;**67**:1455–8.
93. Youssef DT. Tasnemoxides A-C, new cytotoxic cyclic norsesterterpene peroxides from the Red Sea sponge *Diacarnus erythraenus*. *J Nat Prod* 2004;**67**:112–4.
94. Bowden BF, McCool BJ, Willis RH. Lihouidine, a novel spiro polycyclic aromatic alkaloid from the marine sponge *Suberea* n. sp. (Aplysinellidae, Verongida). *J Org Chem* 2004;**69**:7791–3.
95. Li X, Choi HD, Kang JS, Lee CO, Son BW. New polyoxygenated farnesylcyclohexenones, deacetoxyyanuthone A and its hydro derivative from the marine-derived fungus *Penicillium* sp.. *J Nat Prod* 2003;**66**:1499–500.
96. Maskey RP, Li F, Qin S, Fiebig HH, Laatsch H. Chandrananimycins A approximately C: production of novel anticancer antibiotics from a marine *Actinomadura* sp. isolate M048 by variation of medium composition and growth conditions. *J Antibiot (Tokyo)* 2003;**56**:622–9.
97. Tan LT, Cheng XC, Jensen PR, Fenical W. Scytalidamides A and B, new cytotoxic cyclic heptapeptides from a marine fungus of the genus *Scytalidium*. *J Org Chem* 2003;**68**:8767–73.
98. Fisch KM, Bohm V, Wright AD, Konig GM. Antioxidative meroterpenoids from the brown alga *Cystoseira crinita*. *J Nat Prod* 2003;**66**:968–75.
99. Suzuki M, Watanabe K, Fujiwara S, et al. Isolation of peridinin-related norcarotenoids with cell growth-inhibitory activity from the cultured dinoflagellate of *Symbiodinium* sp.,

a symbiont of the Okinawan soft coral *Clavularia viridis*, and analysis of fatty acids of the dinoflagellate. *Chem Pharm Bull (Tokyo)* 2003;**51**:724–7.
100. Pettit GR, Tan R. Antineoplastic agents 390. Isolation and structure of phakellistatin 12 from a Chuuk archipelago marine sponge. *Bioorg Med Chem Lett* 2003;**13**:685–8.
101. Aoki S, Kong D, Matsui K, Rachmat R, Kobayashi M. Sesquiterpene aminoquinones, from a marine sponge, induce erythroid differentiation in human chronic myelogenous leukemia, K562 cells. *Chem Pharm Bull (Tokyo)* 2004;**52**:935–7.
102. Mitchell SS, Nicholson B, Teisan S, Lam KS, Potts BC. Aureoverticillactam, a novel 22-atom macrocyclic lactam from the marine actinomycete *Streptomyces aureoverticillatus*. *J Nat Prod* 2004;**67**:1400–2.
103. Bergmann W, Feeney RJ. Contributions to the study of marine products. XXXII. The nucleosides of sponges. *J Org Chem* 1951;**16**:981–7.
104. Malet-Cascon L, Romero F, Espliego-Vazquez F, Gravalos D, Fernandez-Puentes JL. IB-00208, a new cytotoxic polycyclic xanthone produced by a marine-derived *Actinomadura*. I. Isolation of the strain, taxonomy and biological activites. *J Antibiot (Tokyo)* 2003;**56**:219–25.
105. Rodriguez JC, Fernandez Puentes JL, Baz JP, Canedo LM. IB-00208, a new cytotoxic polycyclic xanthone produced by a marine-derived *Actinomadura*. II. Isolation, physico-chemical properties and structure determination. *J Antibiot (Tokyo)* 2003;**56**:318–21.
106. Tsuda M, Izui N, Shimbo K, et al. Amphidinolide X, a novel 16-membered macrodiolide from dinoflagellate *Amphidinium* sp.. *J Org Chem* 2003;**68**:5339–45.
107. Tsuda M, Izui N, Shimbo K, et al. Amphidinolide Y, a novel 17-membered macrolide from dinoflagellate *Amphidinium* sp.: plausible biogenetic precursor of amphidinolide X. *J Org Chem* 2003;**68**:9109–12.
108. Rudi A, Afanii R, Gravalos LG, et al. Three new cyclic peroxides from the marine sponge *Plakortis aff simplex*. *J Nat Prod* 2003;**66**:682–5.
109. Suenaga K, Mutou T, Shibata T, et al. Aurilide, a cytotoxic depsipeptide from the sea hare *Dolabella auricularia*: isolation, structure determination, synthesis, and biological activity. 2004;**60**:8509–27..
110. Funel C, Berrue F, Roussakis C, Fernandez RR, Amade P. New cytotoxic steroids from the Indian Ocean sponge *Axinella* cf. *bidderi*. *J Nat Prod* 2004;**67**:491–4.
111. Perez LJ, Faulkner DJ. Bistratamides E-J, modified cyclic hexapeptides from the Philippines ascidian *Lissoclinum bistratum*. *J Nat Prod* 2003;**66**:247–50.
112. Shen YC, Cheng YB, Lin YC, Guh JH, Teng CM, Ko CL. New prostanoids with cytotoxic activity from Taiwanese octocoral *Clavularia viridis*. *J Nat Prod* 2004;**67**:542–6.
113. Milanowski DJ, Gustafson KR, Kelley JA, McMahon JB. Caulibugulones A-F, novel cytotoxic isoquinoline quinones and iminoquinones from the marine bryozoan *Caulibugula intermis*. *J Nat Prod* 2004;**67**:70–3.
114. Wang W, Hong J, Lee CO, Im KS, Choi JS, Jung JH. Cytotoxic sterols and saponins from the starfish *Certonardoa semiregularis*. *J Nat Prod* 2004;**67**:584–91.
115. Wang W, Jang H, Hong J, et al. Additional cytotoxic sterols and saponins from the starfish *Certonardoa semiregularis*. *J Nat Prod* 2004;**67**:1654–60.
116. Feng Y, Blunt JW, Cole AL, Cannon JF, Robinson WT, Munro MH. Two novel cytotoxic cyclodepsipeptides from a mycoparasitic *Cladobotryum* sp.. *J Org Chem* 2003;**68**:2002–5.
117. Pettit GR, Collins JC, Knight JC, et al. Antineoplastic agents. 485. Isolation and structure of cribrostatin 6, a dark blue cancer cell growth inhibitor from the marine sponge *Cribrochalina* sp.. *J Nat Prod* 2003;**66**:544–7.
118. Mellado GG, Zubia E, Ortega MJ, Lopez-Gonzalez PJ. New polyoxygenated steroids from the Antarctic octocoral *Dasystenella acanthina*. *Steroids* 2004;**69**:291–9.
119. Bowden BF, Cusack BJ, Dangel A. 13-Epi-9-deacetoxyxenicin, a cytotoxic diterpene from the soft coral *Asterospicularia laurae* (Alcyonacea). *Marine Drugs* 2003;**1**:18–26.
120. Reyes F, Arda A, Martin R, et al. New cytotoxic cembranes from the sea pen *Gyrophyllum sibogae*. *J Nat Prod* 2004;**67**:1190–2.
121. Gunasekera SP, Mickel SJ, Daeffler R, et al. Synthetic analogues of the microtubule-stabilizing agent (+)-discodermolide: preparation and biological activity. *J Nat Prod* 2004;**67**:749–56.
122. Antunes EM, Beukes DR, Kelly M, et al. Cytotoxic pyrroloiminoquinones from four new species of South African latrunculid sponges. *J Nat Prod* 2004;**67**:1268–76.
123. Reyes F, Martin R, Rueda A, et al. Discorhabdins I and L, cytotoxic alkaloids from the sponge *Latrunculia brevis*. *J Nat Prod* 2004;**67**:463–5.
124. Gunasekera SP, Zuleta IA, Longley RE, Wright AE, Pomponi SA. Discorhabdins S, T, and U, new cytotoxic pyrroloiminoquinones from a deep-water Caribbean sponge of the genus *Batzella*. *J Nat Prod* 2003;**66**:1615–7.
125. Pettit GR, Xu JP, Doubek DL, Chapuis JC, Schmidt JM. Antineoplastic Agents. 510. Isolation and structure of dolastatin 19 from the Gulf of California sea hare *Dolabella auricularia*. *J Nat Prod* 2004;**67**:1252–5.
126. Milanowski DJ, Gustafson KR, Rashid MA, Pannell LK, McMahon JB, Boyd MR. Gymnangiamide, a cytotoxic pentapeptide from the marine hydroid *Gymnangium regae*. *J Org Chem* 2004;**69**:3036–42.
127. Shin J, Lee HS, Kim JY, Shin HJ, Ahn JW, Paul VJ. New macrolides from the sponge *Chondrosia corticata*. *J Nat Prod* 2004;**67**:1889–92.
128. Garrido L, Zubia E, Ortega MJ, Salva J. Haouamines A and B: a new class of alkaloids from the ascidian *Aplidium haouarianum*. *J Org Chem* 2003;**68**:293–9.
129. Zou ZR, Yi YH, Wu HM, Wu JH, Liaw CC, Lee KH. Intercedensides A-C, three new cytotoxic triterpene glycosides from the sea cucumber *Mensamaria intercedens* Lampert. *J Nat Prod* 2003;**66**:1055–60.
130. Issa HH, Tanaka J, Higa T. New cytotoxic furanosesterterpenes from an Okinawan marine sponge, *Ircinia* sp.. *J Nat Prod* 2003;**66**:251–4.
131. Pettit GR, Xu JP, Chapuis JC, et al. Antineoplastic agents. 520. Isolation and structure of irciniastatins A and B from the Indo-Pacific marine sponge *Ircinia ramosa*. *J Med Chem* 2004;**47**:1149–52.
132. Lv F, Deng Z, Li J, et al. Isomalabaricane-type compounds from the marine sponge *Rhabdastrella aff. distincta*. *J Nat Prod* 2004;**67**:2033–6.
133. Ledroit V, Debitus C, Lavaud C, Massiot G. Jaspines A and B: two new cytotoxic sphingosine derivatives from the marine sponge *Jaspis* sp.. *Tetrahedron Lett* 2003;**44**:225–8.
134. Wright AE, Chen Y, Winder PL, Pitts TP, Pomponi SA, Longley RE. Lasonolides C-g, five new lasonolide compounds from the sponge *Forcepia* sp.. *J Nat Prod* 2004;**67**:1351–5.
135. Huang XC, Zhao D, Guo YW, et al. Lingshuiol, a novel polyhydroxyl compound with strongly cytotoxic activity from the marine dinoflagellate *Amphidinium* sp.. *Bioorg Med Chem Lett* 2004;**14**:3117–20.
136. Davis RA, Mangalindan GC, Bojo ZP, et al. Microcionamides A and B, bioactive peptides from the Philippine sponge *Clathria (Thalysias) abietina*. *J Org Chem* 2004;**69**:4170–6.
137. Williams PG, Yoshida WY, Moore RE, Paul VJ. Micromide and guamamide: cytotoxic alkaloids from a species of the marine cyanobacterium *Symploca*. *J Nat Prod* 2004;**67**:49–53.

138. Chevallier C, Richardson AD, Edler MC, Hamel E, Harper MK, Ireland CM. A new cytotoxic and tubulin-interactive milnamide derivative from a marine sponge *Cymbastela* sp. *Org Lett* 2003;**5**:3737–9.
139. Zhang HL, Hua HM, Pei YH, Yao XS. Three new cytotoxic cyclic acylpeptides from marine *Bacillus* sp. *Chem Pharm Bull (Tokyo)* 2004;**52**:1029–30.
140. Ortega MJ, Zubia E, Sanchez MC, Salva J, Carballo JL. Structure and cytotoxicity of new metabolites from the sponge *Mycale cecilia*. *Tetrahedron* 2004;**60**:2517–24.
141. Phuwapraisirisan P, Matsunaga S, Fusetani N, Chaitanawisuti N, Kritsanapuntu S, Menasveta P. Mycaperoxide H, a New cytotoxic norsesterterpene peroxide from a thai marine sponge *Mycale* sp. *J Nat Prod* 2003;**66**:1039–40.
142. Amagata T, Rath C, Rigot JF, et al. Structures and cytotoxic properties of trichoverroids and their macrolide analogues produced by saltwater culture of *Myrothecium verrucaria*. *J Med Chem* 2003;**46**:4342–50.
143. Wei H, Itoh T, Kinoshita M, Nakai Y, Kurotaki M, Kobayashi M. Cytotoxic sesterterpenes, 6-epi-ophiobolin G and 6-epi-ophiobolin N, from marine derived fungus *Emericella variecolor* GF10. *Tetrahedron* 2004;**60**(28):6015–9.
144. Williams PG, Yoshida WY, Quon MK, Moore RE, Paul VJ. The structure of Palau'amide, a potent cytotoxin from a species of the marine cyanobacterium *Lyngbya*. *J Nat Prod* 2003;**66**:1545–9.
145. Li WL, Yi YH, Wu HM, et al. Isolation and structure of the cytotoxic cycloheptapeptide phakellistatin 13. *J Nat Prod* 2003;**66**:146–8.
146. Ridley CP, Faulkner DJ. New cytotoxic steroidal alkaloids from the Philippine sponge *Corticium niger*. *J Nat Prod* 2003;**66**:1536–9.
147. Pettit GR, Nogawa T, Knight JC, Doubek DL, Hooper JN. Antineoplastic agents. 535. Isolation and structure of plakorstatins 1 and 2 from the Indo-Pacific sponge *Plakortis nigra*. *J Nat Prod* 2004;**67**:1611–3.
148. Del Sol JM, Garzon SP, Rodriguez AD. Plakortides M and N, Bioactive Polyketide Endoperoxides from the Caribbean Marine Sponge *Plakortis halichondrioides*. *J Nat Prod* 2003;**66**:1404.
149. Konishi M, Yang X, Li B, Fairchild CR, Shimizu Y. Highly cytotoxic metabolites from the culture supernatant of the temperate dinoflagellate *Protoceratium* cf. *reticulatum*. *J Nat Prod* 2004;**67**:1309–13.
150. Cichewicz RH, Valeriote FA, Crews P. Psymberin, a potent sponge-derived cytotoxin from *Psammocinia* distantly related to the pederin family. *Org Lett* 2004;**6**:1951–4.
151. Yao B, Prinsep MR, Nicholson BK, Gordon DP. The pterocellins, novel bioactive alkaloids from the marine bryozoan *Pterocella vesiculosa*. *J Nat Prod* 2003;**66**:1074–7.
152. Oku N, Matsunaga S, van Soest RW, Fusetani N. Renieramycin J, a highly cytotoxic tetrahydroisoquinoline alkaloid, from a marine sponge *Neopetrosia* sp. *J Nat Prod* 2003;**66**:1136–9.
153. Suwanborirux K, Amnuoypol S, Plubrukarn A, et al. Chemistry of renieramycins. Part 3.(1) isolation and structure of stabilized renieramycin type derivatives possessing antitumor activity from Thai sponge *Xestospongia* species, pretreated with potassium cyanide. *J Nat Prod* 2003;**66**:1441–6.
154. Amnuoypol S, Suwanborirux K, Pummangura S, Kubo A, Tanaka C, Saito N. Chemistry of renieramycins. Part 5. Structure elucidation of renieramycin-type derivatives O, Q, R, and S from thai marine sponge *Xestospongia* species pretreated with potassium cyanide. *J Nat Prod* 2004;**67**:1023–8.
155. Tan RX, Jensen PR, Williams PG, Fenical W. Isolation and structure assignments of rostratins A-D, cytotoxic disulfides produced by the marine-derived fungus *Exserohilum rostratum*. *J Nat Prod* 2004;**67**:1374–82.
156. Nakao Y, Yoshida S, Matsunaga S, Fusetani N. (Z)-sarcodictyin A, a new highly cytotoxic diterpenoid from the soft coral *Bellonella albiflora*. *J Nat Prod* 2003;**66**:524–7.
157. Ahmed AF, Su JH, Kuo YH, Sheu JH. Scabrolides E-G, three new norditerpenoids from the soft coral *Sinularia scabra*. *J Nat Prod* 2004;**67**:2079–82.
158. Oku N, Matsunaga S, Fusetani N. Shishijimicins A-C, novel enediyne antitumor antibiotics from the ascidian *Didemnum proliferum*. *J Am Chem Soc* 2003;**125**:2044–5.
159. Ojika M, Islam MK, Shintani T, Zhang Y, Okamoto T, Sakagami Y. Three new cytotoxic acylspermidines from the soft coral, *Sinularia* sp. *Biosci Biotechnol Biochem* 2003;**67**:1410–2.
160. Rho JR, Lee HS, Shin HJ, et al. New sesterterpenes from the sponge *Smenospongia* sp. *J Nat Prod* 2004;**67**:1748–51.
161. Williams DE, Roberge M, Van Soest R, Andersen RJ. Spirastrellolide A, an antimitotic macrolide isolated from the Caribbean marine sponge *Spirastrella coccinea*. *J Am Chem Soc* 2003;**125**:5296–7.
162. Stritzke K, Schulz S, Laatsch H, Helmke E, Beil W. Novel caprolactones from a marine streptomycete. *J Nat Prod* 2004;**67**:395–401.
163. Phipps RK, Blunt JW, Cole ALJ, Munro MHG. Anthracycline derivatives from a marine-derived New Zealand Streptomycete. *Arkivoc* 2004;**X**:94–100.
164. Williams PG, Yoshida WY, Moore RE, Paul VJ. The isolation and structure elucidation of Tasiamide B, a 4-amino-3-hydroxy-5-phenylpentanoic acid containing peptide from the marine Cyanobacterium *Symploca* sp. *J Nat Prod* 2003;**66**:1006–9.
165. Williams PG, Yoshida WY, Moore RE, Paul VJ. Tasipeptins A and B: new cytotoxic depsipeptides from the marine cyanobacterium *Symploca* sp. *J Nat Prod* 2003;**66**:620–4.
166. Garo E, Starks CM, Jensen PR, Fenical W, Lobkovsky E, Clardy J. Trichodermamides A and B, cytotoxic modified dipeptides from the marine-derived fungus *Trichoderma virens*. *J Nat Prod* 2003;**66**:423–6.
167. Williams PG, Yoshida WY, Quon MK, Moore RE, Paul VJ. Ulongapeptin, a cytotoxic cyclic depsipeptide from a Palauan marine cyanobacterium *Lyngbya* sp. *J Nat Prod* 2003;**66**:651–4.
168. McDonald LA, Barbieri LR, Bernan VS, Janso J, Lassota P, Carter GT. 07H239-A, a new cytotoxic eremophilane sesquiterpene from the marine-derived Xylariaceous fungus LL-07H239. *J Nat Prod* 2004;**67**:1565–7.
169. Onodera K, Fukatsu T, Kawai N, et al. Zooxanthellactone, a novel gamma-lactone-type oxylipine from dinoflagellates of *Symbiodinium* sp.: structure, distribution, and biological activity. *Biosci Biotechnol Biochem* 2004;**68**:848–52.
170. Blay JY, Le Cesne A, Verweij J, et al. A phase II study of ET-743/trabectedin ('Yondelis') for patients with advanced gastrointestinal stromal tumours. *Eur J Cancer* 2004;**40**:1327–31.