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Review

Marine pharmacology in 2001–2: antitumour and cytotoxic compounds

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Abstract

During 2001 and 2002, marine antitumour pharmacology research aimed at the discovery of novel antitumour agents was published in 175 peer-reviewed articles. The purpose of this paper is to present a structured Review of the antitumour and cytotoxic properties of 97 marine natural products, many of them novel compounds that belong to diverse structural classes, including polyketides, terpenes, steroids, and peptides. The organisms yielding these bioactive compounds comprise a taxonomically diverse group of marine invertebrate animals, algae, fungi and bacteria. Antitumour pharmacological studies were conducted with 30 structurally characterised natural marine products in a number of experimental and clinical models which further defined their mechanisms of action. Particularly potent *in vitro* cytotoxicity data generated with murine and human tumour cell lines was reported for 67 novel marine chemicals with as yet undetermined mechanisms of action. Noteworthy, is the fact that marine anticancer research was sustained by a collaborative effort, involving researchers from Australia, Brazil, Canada, Denmark, Egypt, France, Germany, Italy, Japan, Netherlands, New Zealand, the Phillipines, Russia, Singapore, South Korea, Thailand, Taiwan, Turkey, Spain, Switzerland, Taiwan, Thailand, Turkey, and the United States. Finally, this 2001–2 overview of the marine pharmacology literature highlights the fact that the discovery of novel marine antitumour agents has continued at the same pace as during 1998, 1999 and 2000.

Keywords: Marine; Antitumour; Cytotoxicity; Anticancer; Antineoplastic; Agents; Preclinical; Clinical; Pharmacology; Review

1. Introduction

The purpose of this article is to review the research literature published during 2001–2 in the field of marine antitumour pharmacology using a format similar to the one used in our previous three reports, which covered the peer-reviewed literature published during 1998, 1999 and 2000 [1–3]. Consistent with our previous Reviews, only those articles reporting on antitumour 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 Table 1 or Table 2. The literature reporting novel information on the preclinical and/or clinical pharmacology of marine chemicals with previously *determined* mechanisms of action has been presented in Table 1 and is further discussed in the text of this Review. By contrast, reports on novel marine chemicals which demonstrated significant cytotoxicity, but with as yet *undetermined* mechanisms of action are grouped in Table 2. With a few

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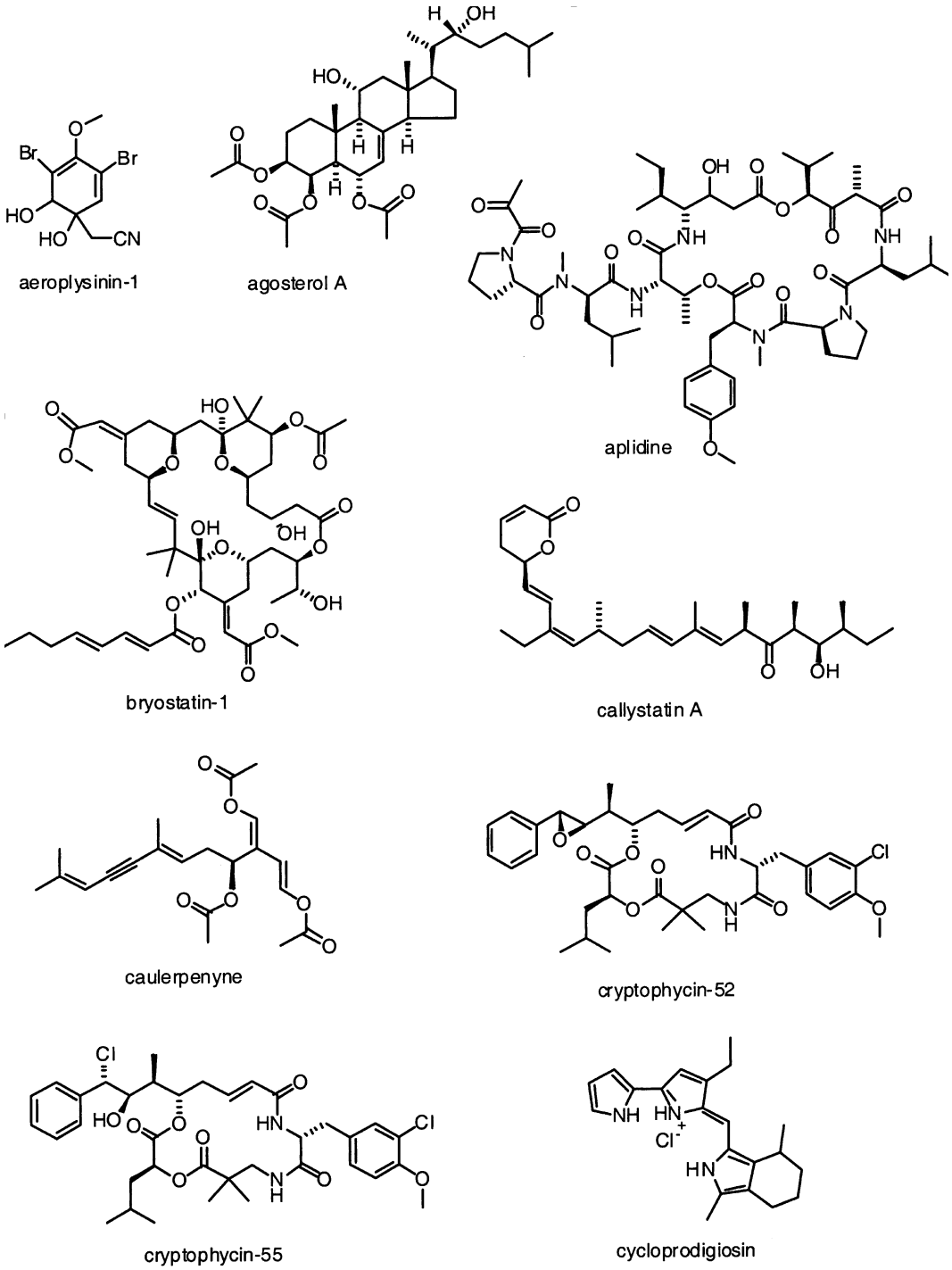


Fig. 1. Structures of marine natural products reported in 2001 and 2002 with established mechanisms of action.

exceptions, studies on the preclinical antitumour pharmacology of synthetic analogues of marine metabolites, as well as reports on research with marine extracts or as yet structurally *uncharacterised* marine chemicals have been excluded from the present Review, although several studies were published during 2001–2 [4,5].

2. 2001–2 Antitumour pharmacology of marine natural products with *established* mechanisms of action

Table 1 summarises research on the drugs with novel mechanism of actions from preclinical studies of 30 marine compounds (selected structures are shown in Fig. 1). Reports on clinical trials with some of these marine

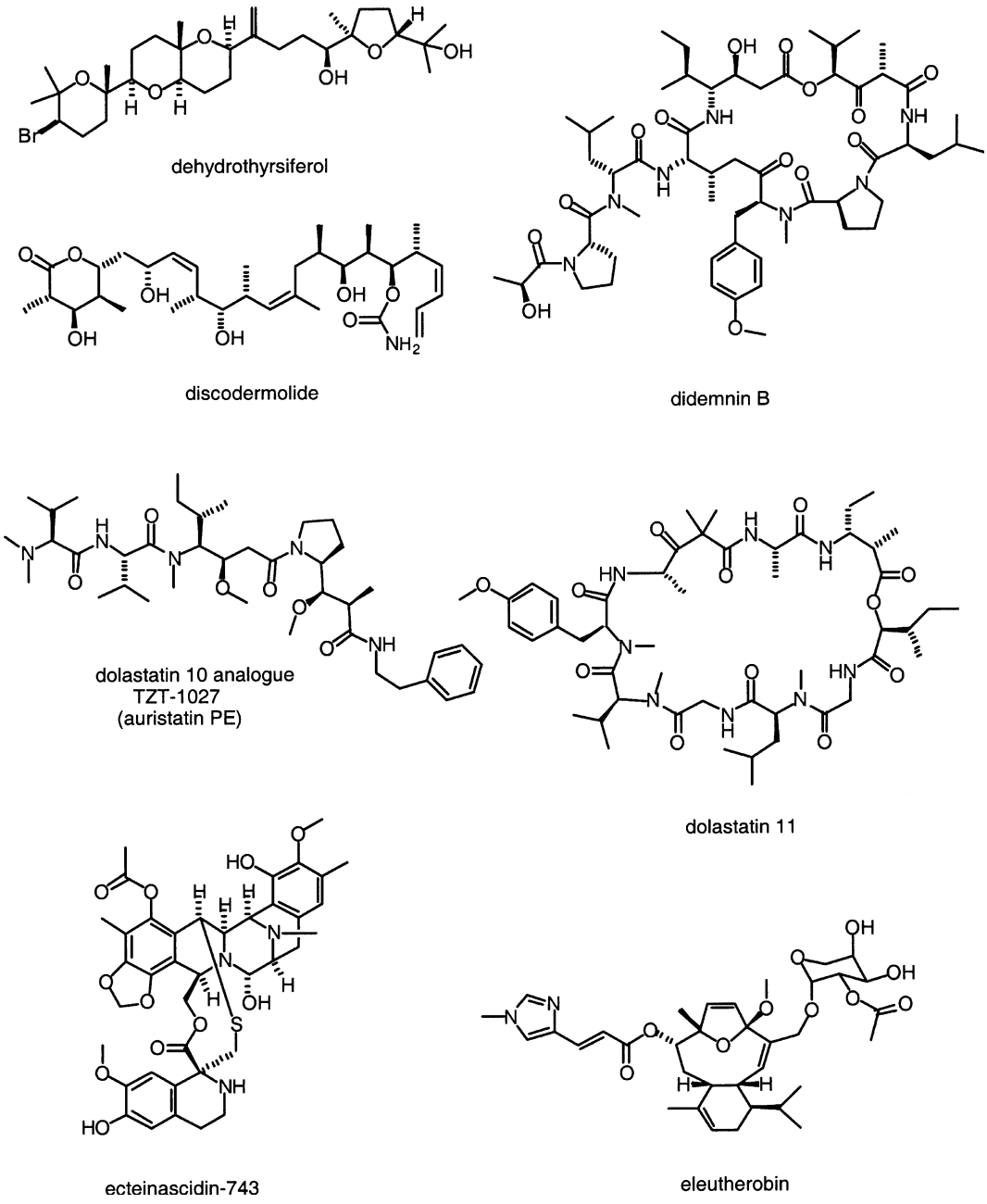


Fig. 1 (*continued*)

compounds are excluded from Table 1, but discussed in this section of the article.

New information was published during 2001–2 on the preclinical and clinical pharmacology of the following marine compounds which were reviewed previously in [1–3]: agosterol A, aplidine, bryostatin-1, cryptophycins, dehydrothyrsiferol, didemnin B, discodermolide, dolastatins, ecteinascidin-743, eleutherobin, fascaplysin, halichondrin B, jaspamide and peloruside.

Four studies were published during 2001–2 on the preclinical pharmacology of **agosterol A**, a polyhydroxylated sterol acetate isolated from the marine sponge *Spongia* sp.. Aoki and colleagues [6], working with the human epidermoid carcinoma KB cell line, determined that agosterol A potently inhibited the efflux of anticancer agents by interaction with P-glycoprotein and multiple drug-resistant associated protein 1 (MRP1), thus reversing multidrug resistance. Futhermore, Chen and colleagues [7], in a detailed mechanistic study with human epidermoid carcinoma cells transfected with MRP1 cDNA, noted that the multidrug resistance reversing activity of this marine steroid appeared to be due to inhibition of MRP1 as a result of the concomitant reduction of intracellular glutathione. While

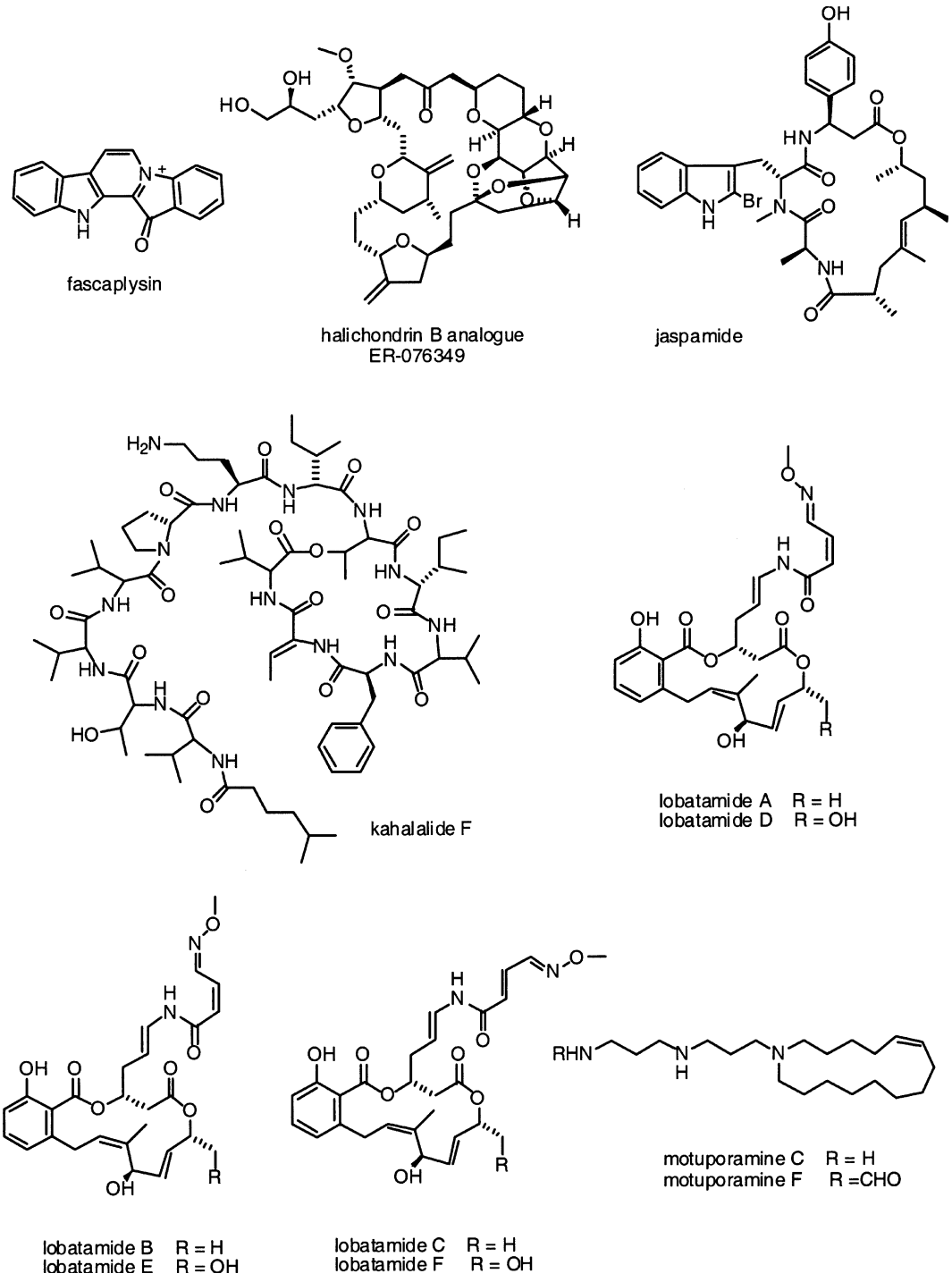
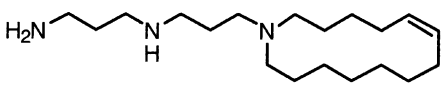


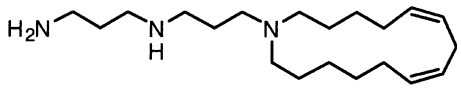
Fig. 1 (*continued*)

structure–activity studies demonstrated that all functional groups were important for agosterol A's reversal of multidrug resistance, Ren and colleagues [8] found that the acetoxyl groups appeared to play a critical role in reversing P-glycoprotein-mediated drug resistance. The hydroxyl groups on agosterol A, as well as glutathione, may be important for the binding of the marine drug to the C-terminal half of MRP1 and the reversal of MRP1-mediated drug resistance. More recently, this group of researchers demonstrated that a positively charged amino acid proximal to the C-terminus of the TM helix 17 of MRP1 is indispensable for glutathione-dependent binding of agosterol A [9].

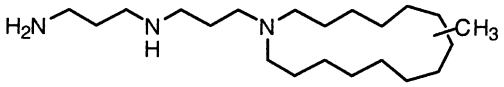
Five studies were completed during 2001–2 on the preclinical pharmacology of the cyclic depsipeptide **aplidine** (dehydrodidemnin B), isolated from the marine



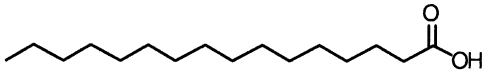
motuporamine D



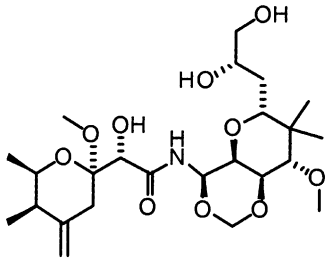
motuporamine E



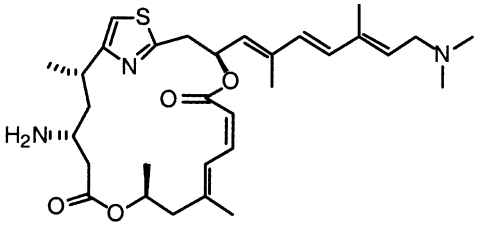
motuporamines G, H, I



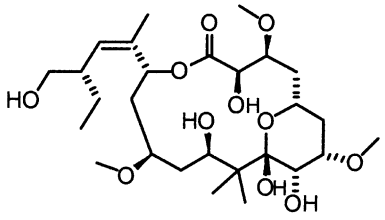
palmitic acid



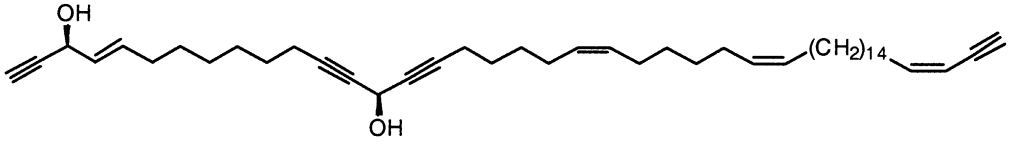
mycalamide A



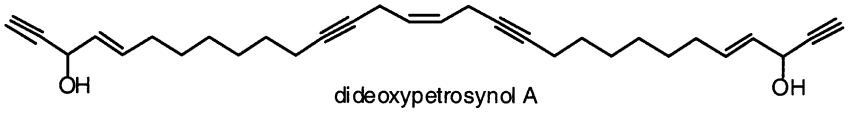
pateamine



peloruside A



3*S*,14*S*-petrocortyne



dideoxypetrosynol A

Fig. 1 (*continued*)

tunicate *Aplidium albicans*. Two preclinical studies investigated the haematotoxicity of aplidine: Gomez and colleagues [10] reported that, regardless of the origin of the haematopoietic progenitor cells (human cord blood or bone marrow), the concentration of aplidine causing 50% inhibition (IC_{50}): 0.15–2.25 μM was one to three orders of magnitude lower than that observed in previous studies with tumoural cell lines. Furthermore, in a study designed to evaluate the capacity of *in vitro* haematopoietic cultures to predict the toxicity of aplidine, Albella and colleagues [11] observed that the IC_{50} values for aplidine were similar to those reported for doxorubicin. Taken together, these observations suggest that no clinically relevant haematotoxicity is likely to result if aplidine is used at the recommended clinical doses in Phase I trials. However, as discussed by Dr. D'Incalci, while the use of these haematotoxicity assays provides important information for the planning of Phase I clinical trials, at the present time these *in vitro* studies "*cannot replace toxicological tests perfomed in vivo in different animal species*" [12]. Two studies contributed to the molecular pharmacology

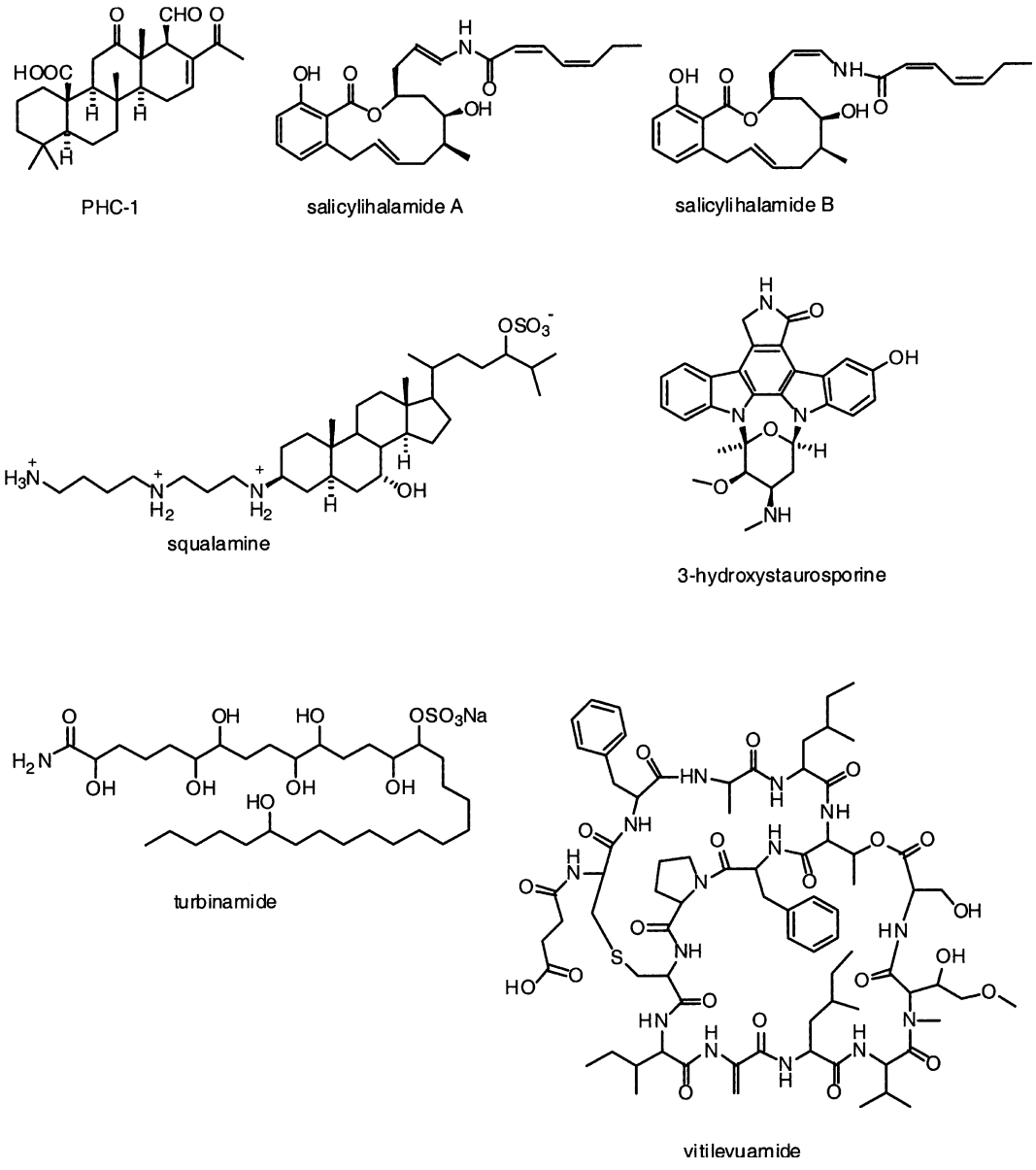
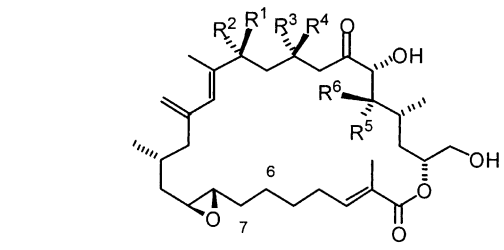


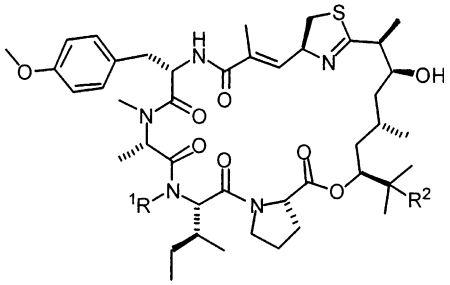
Fig. 1 (*continued*)

of aplidine: Erba and colleagues [13] discovered cell cycle perturbations and apoptosis in human Molt-4 leukaemia cells treated with aplidine at concentrations (20 nM) which are achieved clinically in patients. Aplidine was observed to induce not only G1 arrest, but also a G2 blockage probably related to activation of cell cycle checkpoints. Marchini and colleagues [14] characterised the time-dependent effect of aplidine on the human Molt-4 leukaemic cell line using cDNA microarray technology. Aplidine was reported to actively modulate the expression profile of several genes, among them ETR-103, a zinc finger transcription factor and potential regulator of 30 genes, thus providing a potentially new approach to elucidate the mechanism of action of this marine compound.

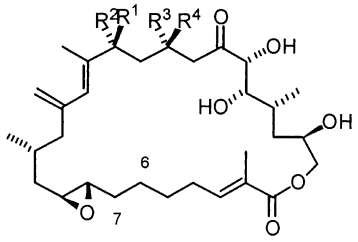
Seventeen studies published during 2001–2 extended the preclinical and clinical pharmacology of **bryostatin-1**, a macrocyclic lactone derived from the marine bryozoan, *Bugula neritina*, which has received considerable attention over the past few years in view of its considerable antineoplastic activity, *in vitro* and *in vivo* (1-3). Seven preclinical studies contributed novel information on the molecular pharmacology of bryostatin-1, at both the cellular and molecular level. Curiel and colleagues [15] noted that bryostatin-1 and interleukin-2 synergised to induce interferon-γ gene expression in freshly isolated human peripheral blood T cells, at both the transcriptional and post-transcriptional levels, through a p38 mitogen-activated kinase-dependent process. The authors proposed that bryostatin-1 plus interleukin-2



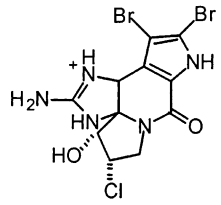
amphidinolide H2 $R^2 = CH_3$, R^4, $R^5 = OH$, R^1, R^3, $R^6 = H$, $^{2\ 6,7}$
amphidinolide H3 $R^1 = CH_3$, R^3, $R^6 = OH$, R^2, R^4, $R^5 = H$, $^{2\ 6,7}$
amphidinolide H4 $R^1 = CH_3$, R^3, $R^5 = OH$, R^2, R^4, $R^6 = H$, 6,7-dihydro
amphidinolide H5 $R^2 = CH_3$, R^4, $R^5 = OH$, R^1, R^3, $R^6 = H$, 6,7-dihydro



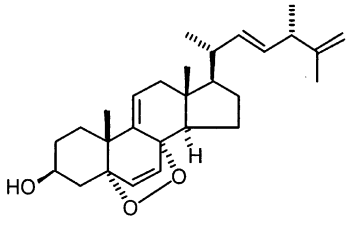
apratoxin A R^1, $R^2 = CH_3$
apratoxin B $R^1 = H$, $R^2 = CH_3$
apratoxin C $R^1 = CH_3$, $R^2 = H$



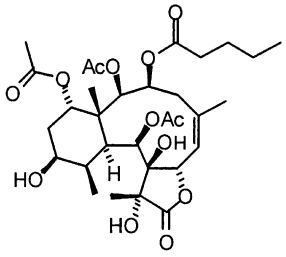
amphidinolide G2 $R^2 = CH_3$, $R^4 = OH$, R^1, $R^3 = H$, $^{2\ 6,7}$
amphidinolide G3 $R^1 = CH_3$, $R^3 = OH$, R^2, $R^4 = H$, 6,7-dihydro



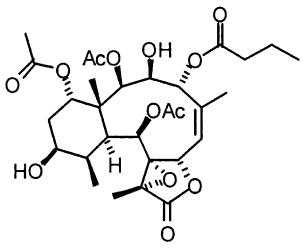
Axinella brevistyla alkaloid



Axinyssa sp. sterol



briaexcavatolide L



briaexcavatolide P

Fig. 2. Structures of new marine natural products with cytotoxic or antitumour activity reported in 2001 and 2002.

might become a valuable new combined therapy for cancer treatment where T cell responses to tumour cells are impaired. With the purpose of developing a murine tumour model which could contribute to human cancer therapy, Chin and colleagues [16] investigated the adoptive transfer of bryostatin-1 and ionomycin activated T cells for the treatment of early and late stage mammary tumours. The investigators reported that $CD8^+$ T-lymphocytes were responsible for inducing tumour regression in the short-term *ex vivo* expansion protocol they developed which they propose "*overcomes important obstacles to the practicality of adoptive immunotherapy in human clinical trials*". In studies designed to better understand the molecular mechanisms of bryostatin-1-induced B-cell differentiation of a pre-B human acute lymphoblastic leukaemia (ALL) cell line, Wall and colleagues [17] assessed extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signalling. These biochemical and pharmacological studies revealed that ERK/MAPK signalling was important for bryostatin-1 induced differentiation of the human ALL line. Perturbations of the MAPK signalling cascade were also observed by Vrana and colleagues [18] while studying the synergistic induction of apoptosis in human leukaemia cells (U937) exposed to bryostatin-1 and the proteasome inhibitor lactacystin. This particular study supports current evidence for the presence of molecular cross-talk between the actions of proteasome inhibitors and the activation of signal transduction pathways, in particular the PKC/Raf/MEK cascade in leukaemia cells. Using an established human monocytic leukaemia cell line for their studies aimed at determining the underlying mechanisms that confer resistance to apoptosis, Lin and colleagues [19] demonstrated that

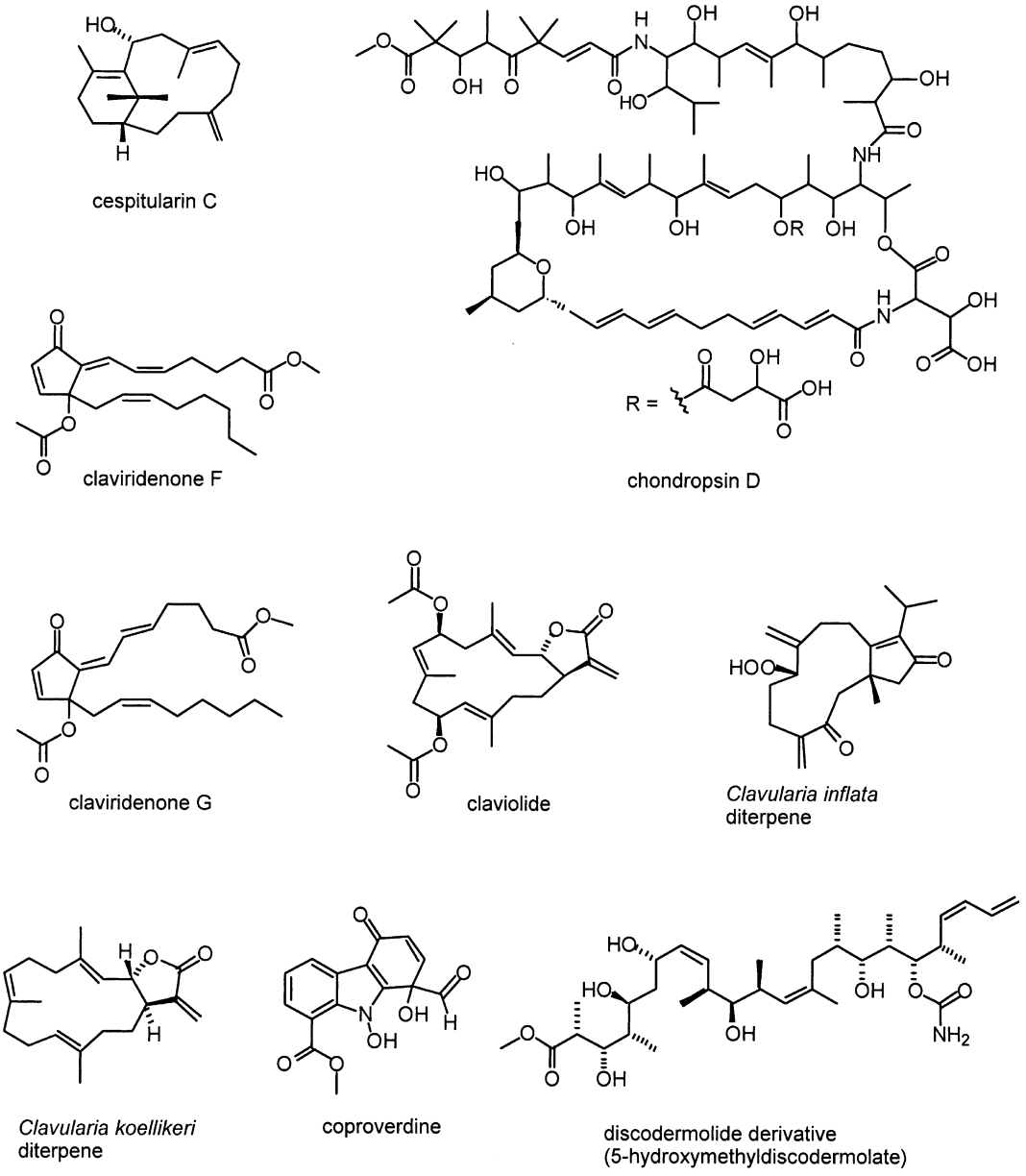


Fig. 2 (*continued*)

the MEK/MAPK pathway was involved in bryostatin-1 induced monocytic differentiation and that monocytic differentiation was correlated with the upregulation of the X-linked inhibitor of apoptosis protein. Roddie and colleagues [20] reported that primary acute myeloid leukaemia blasts, isolated from several patients which were resistant to cytokine-induced differentiation, overcame the differentiation block when treated with bryostatin-1 *in vitro*. The authors proposed that bryostatin-1 promotes differentiation by interfering with protein kinase C activity or inhibition of cell cycle progression. Wang and colleagues [21] showed that bryostatin-1 increased 1-β-D-arabinofuranosylcytosine-induced cytochrome C release and apoptosis in human leukaemia cells ectopically expressing the anti-apoptotic protein Bcl-X_L, probably by interfering with the ability of this protein to block the release of cytochrome C.

The clinical pharmacology of bryostatin-1 involved 3 Phase I and 7 Phase II clinical trials during 2001–2. Cragg and colleagues [22] completed a Phase I trial and correlative laboratory studies of bryostatin-1 and high-dose 1-β-D-arabinofuranosylcytosine in patients with refractory acute leukaemia. The fact that several complete remissions were achieved suggested this regimen has activity in acute leukaemia and "*warrants additional investigation*". Marshall and colleagues [23] reported a Phase I study of bryostatin-1 in patients with advanced malignancies to determine the safety

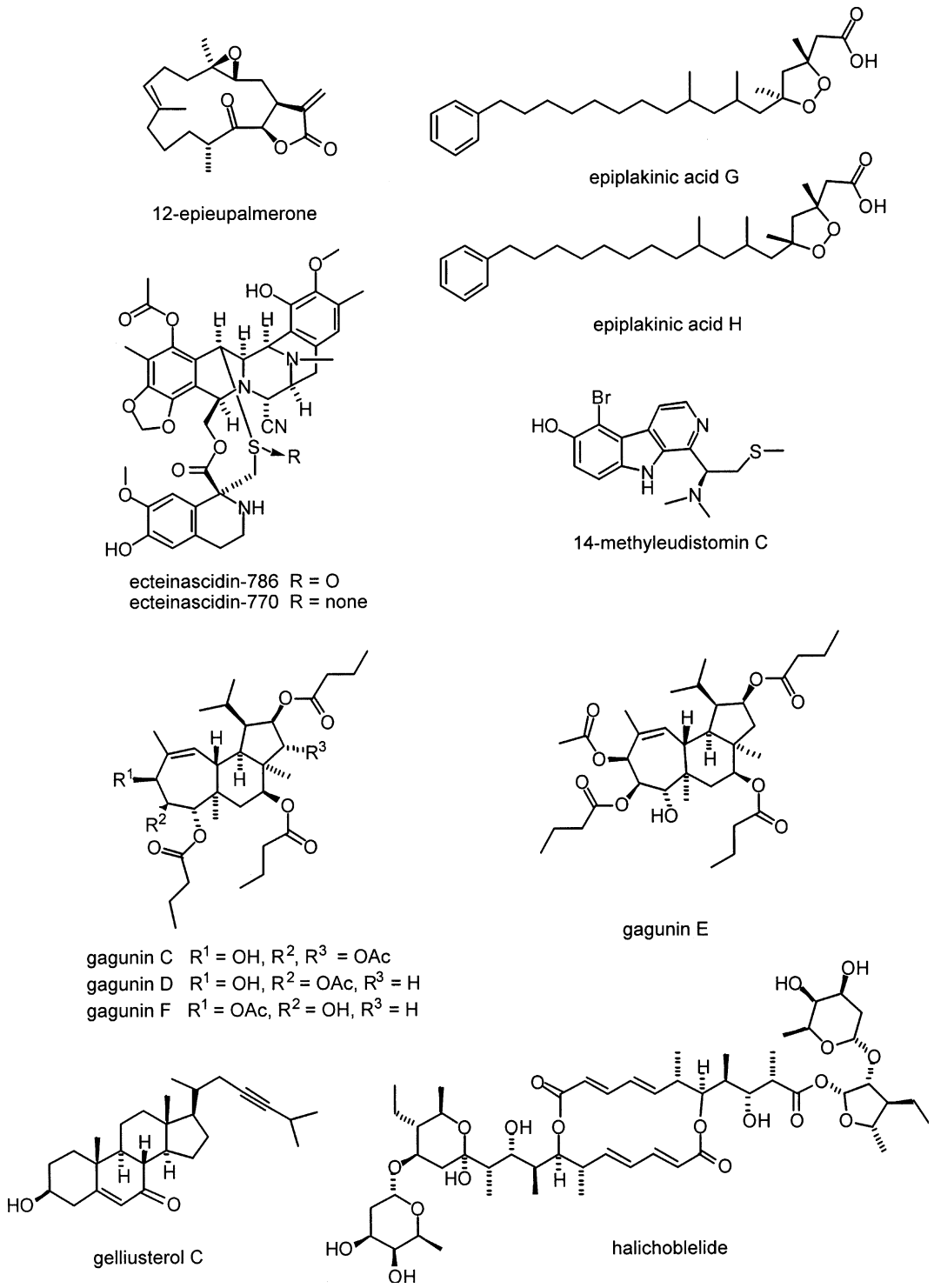


Fig. 2 (*continued*)

and recommended dose of prolonged infusion. Their study suggested that bryostatin-1 can be safely administered over prolonged infusion schedules, with toxicities limited to myalgias and fatigue which were dose-related. Roberts and colleagues [24] published the results of a Phase I study of bryostatin-1 with fludarabine, noting that a 24-h infusion of bryostatin-1, either before or after a 5-day course of fludarabine, appeared to be well tolerated in patients with chronic lymphocytic leukaemia and indolent non-Hodgkin's lymphoma.

With the purpose of finding new anticancer drugs for metastatic soft tissue sarcoma and head and neck can-

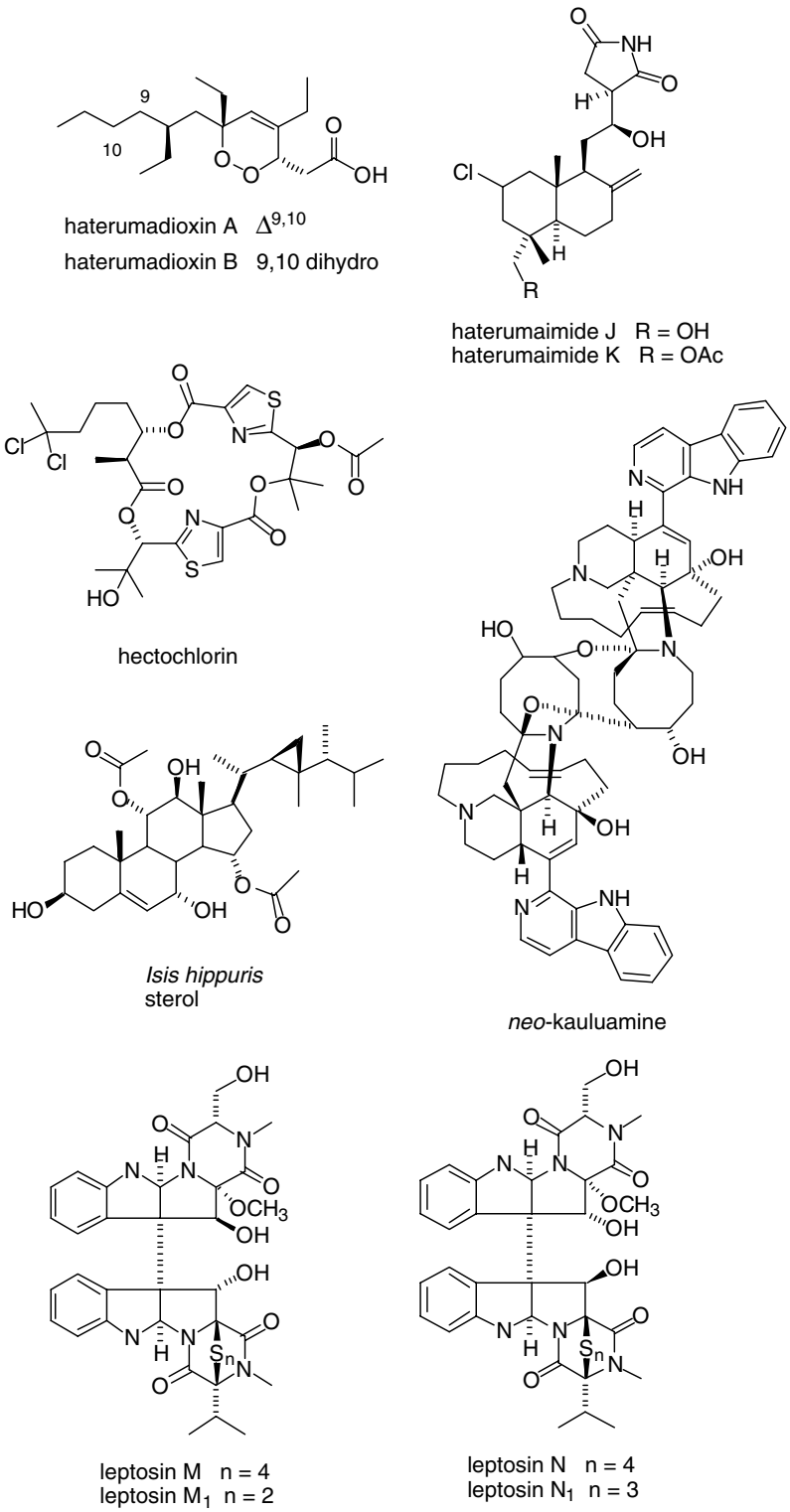
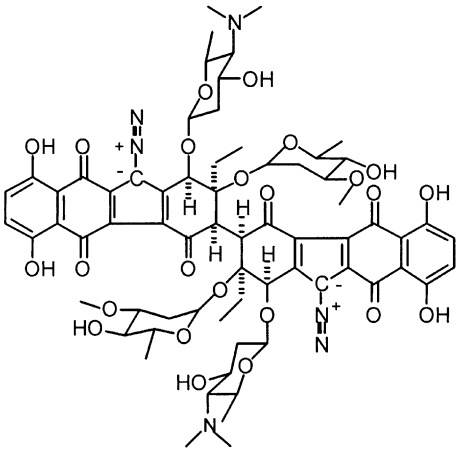
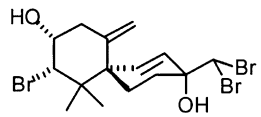


Fig. 2 (*continued*)

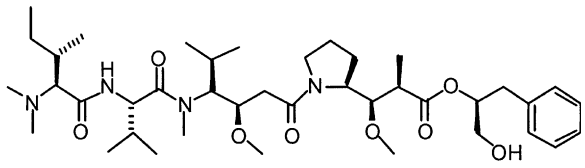
cer, Brockstein and colleagues [25] conducted a multi-institutional Phase II trial of bryostatin-1 in 24 patients. Although no patients showed a significant response to bryostatin-1, 6 patients had evidence of disease stabilisation, with non-haematological toxicity limited to myalgia and hyponatraemia. Pfister and colleagues [26] also reported a Phase II trial of bryostatin-1 in 15 patients with metastatic or recurrent squamous cell carcinoma of the head and neck. Because of a lack of objective responses in these patients, the authors concluded that



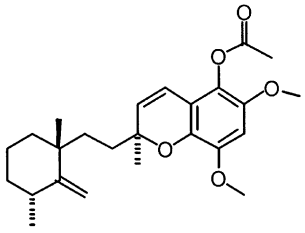
lomaiviticin A



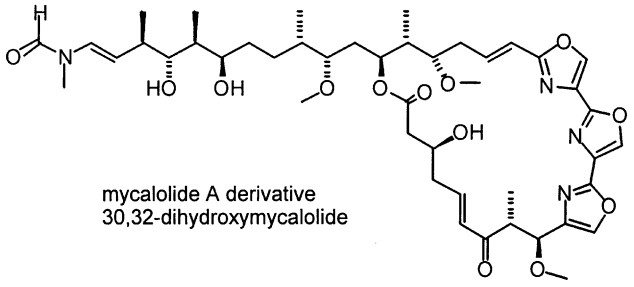
ma'iliohydrin



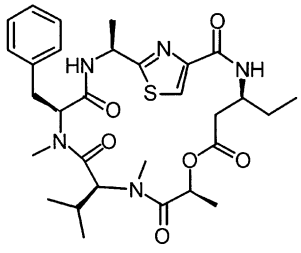
malevamide D



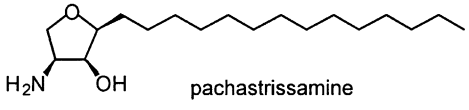
metachromin B acetate



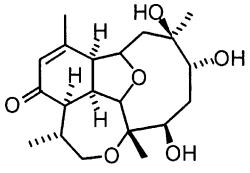
mycalolide A derivative
30,32-dihydroxymycalolide



obyanamide



pachastrissamine



pachylavulariaenone G

Fig. 2 (*continued*)

bryostatin-1 was not recommended for use as a single agent for the treatment of this type of cancer. Bedekian and colleagues [27] completed a Phase II evaluation of bryostatin-1 in 49 patients with metastatic melanoma, a type of tumour for which only one drug, namely dacarbazine, has been approved by the United States (US) Food and Drug Administration over the past three decades. Although one patient had a partial response lasting over 7 months, most patients demonstrated limited clinical tumour regression.Tozer and colleagues [28] also reported a randomised Phase II study of two schedules of bryostatin-1 in thirty-two patients with advanced malignant melanoma. Although 25 μg/m^2 bryostatin-1 given as a 24 h continuous infusion weekly was better tolerated than a higher dose given as a 72 h continuous infusion every 2 weeks, this study also concluded that bryostatin-1 had little efficacy in the treatment of metastatic melanoma. Blackhall and colleagues [29] finalised a Phase II trial of bryostatin-1 in 17 patients with progressive non-Hodgkin's lymphoma of the indolent type.

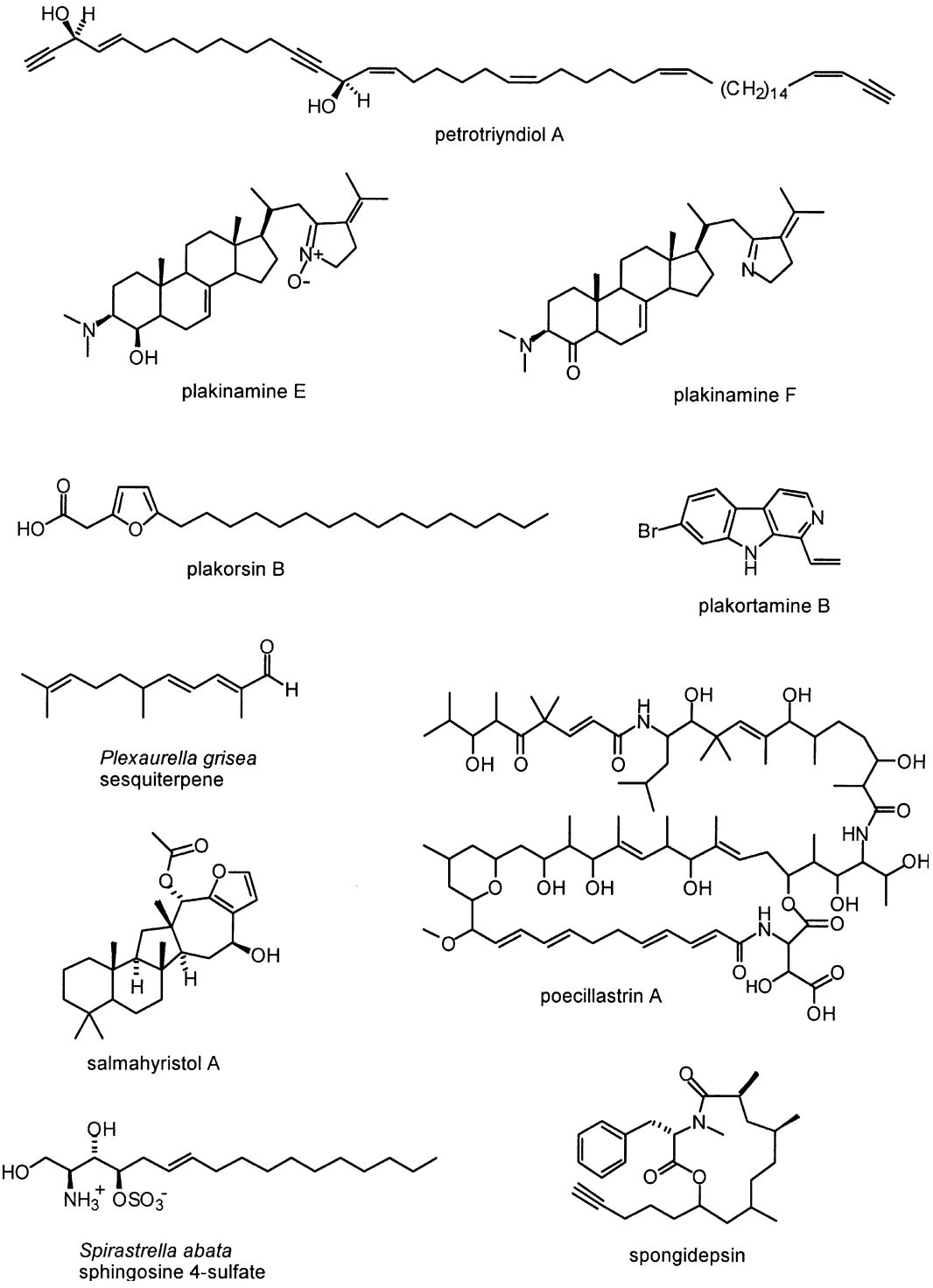


Fig. 2 (*continued*)

Although stable disease was attained in one patient for 9 months, this study failed to show significant benefit in the other patients, with phlebitis a significant toxicity in this study. In an effort to find novel treatments for multiple myeloma, a cancer for which there has been little improvement in 5-year survival rates since the mid-1970's, Varteasian and colleagues [30] completed a Phase II trial with bryostatin-1 in 9 patients with relapsed multiple myeloma. Although the administration of bryostatin-1 by 72-h infusion every 2 weeks was well tolerated, with myalgias constituting the primary toxicity, there were no responses in the 9 patients. Zonder and colleagues [31] reported on a Phase II trial of bryostatin-1 in the treatment of 28 patients with advanced

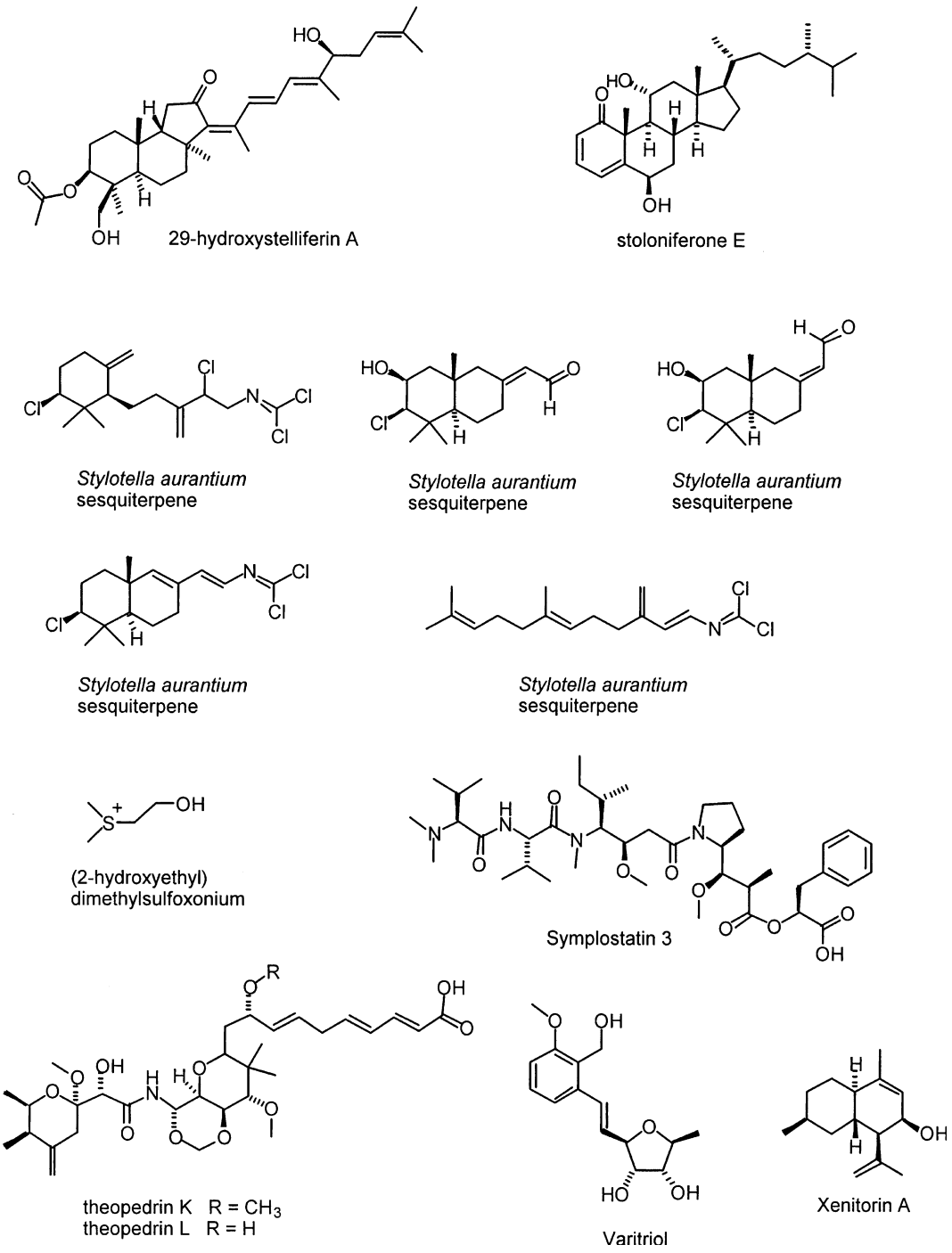


Fig. 2 (*continued*)

colorectal cancer, a tumour for which current chemotherapy is relatively ineffective. While myalgia was noted as the most common toxicity, bryostatin-1 failed to demonstrate any clinically meaningful activity when used as a single agent. Thus, most of the clinical studies with bryostatin-1 reported during this period appeared to have arrived at a similar conclusion, namely that while bryostatin-1 may have no significant activity when used as a single agent, its clinical utility may lie in its use in combination therapy. This remains to be shown in present ongoing clinical studies.

During 2001–2, two studies contributed to the preclinical and clinical pharmacology of the **cryptophycins**, a family of antimitotic depsipeptides isolated from a marine cyanobacterium of the genus *Nostoc*, which bind with high affinity to microtubule ends at the vinca binding domain. In a preclinical study with human non-small-cell lung carcinoma cells in culture, Lu and

Table 1
2001–2 Antitumour pharmacology of marine natural products with *established* mechanisms of action

Compound	Organism	Chemistry	Experimental or clinical model[a]	Mechanism of action[b]	Country[c]	[Ref.]
aeroplysinin-1	Sponge	Alkaloid	Bovine and HU endothelial cells; matrigel plug assays	Inhibition of angiogenesis	SPA	[71]
agosterol A	Sponge	Steroid	HU epidermoid carcinoma cell line	Multidrug resistance reversal by P-glycoprotein and MRP1 inhibition	JAPN	[6]
			HU transfected epidermoid carcinoma cell line	Direct inhibition of MRP1-mediated drug transport; reduction of intracellular glutathione levels	JAPN, USA	[7]
			HU transfected epidermoid carcinoma cell line	Glutathione-dependent binding to C-terminal half of human MRP1	JAPN	[8]
			Pig and insect cell transfected cell lines	Binding to amino acids 1223-1295, proximal to C-terminus of TM helix 17 of MRP1	JAPN	[9]
aplidine	Tunicate	Depsipeptide	HU bone marrow and cord blood cells growth inhibition	Haematotoxicity 1-3 orders of magnitude lower than against tumour cell lines	SPA	[10]
			HU bone marrow progenitor cells growth inhibition	Low haematotoxicity, similar to doxorubicin	SPA, USA	[11]
			HU leukaemia cell line	G1 arrest and G2 blockage	ITA, SPA, USA	[13]
			HU leukaemia cell line	Modulation of growth factors, signal transduction and transcription factors by cDNA array study	ITA, USA	[14]
bryostatin-1	Bryozoan	Macrolide	HU blood T cells	Synergy with interleukin 2 to induce interferon-γ	USA	[15]
			MU tumour cell lines	Synergy with ionomycin-activated T cells mediated tumour regression	USA	[16]
			HU acute lymphoblastic leukaemia cell line	MAPK is required for differentiation of pre-B-ALL	USA	[17]
			HU monoblastic leukaemia cell line	Apoptosis induced by synergy with proteasome inhibitor involving PKC/MAPK disregulation	USA	[18]
			HU monocytic leukaemia cell line	Induction of X-linked inhibitor of apoptosis protein via activation of MEK/MAPK pathway	USA	[19]
			HU monocytic leukaemia cell line	Increases cytochrome C release and apoptosis	USA	[21]

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Table 1 (*continued*)

Compound	Organism	Chemistry	Experimental or clinical model[a]	Mechanism of action[b]	Country[c]	[Ref.]
callystatin A	Sponge	Polyketide	HU tumour cell line	Cytotoxicity dependent on β-hydroxyketone portion of molecule	JAPN	[72]
caulerpenyne	Alga	Sesquiterpene	HU neuroblastoma cell line	Inhibition of microtubule assembly and tubulin aggregation	FRA	[73]
cryptophycins	Bacterium	Depsipeptide	HU tumour cell lines	Hyperphosphorylation of Bcl-2 and cell cycle arrest	USA	[32]
cycloprodigiosin hydrochloride	Bacterium	Alkaloid	HU tumour cell line	Inhibition of nuclear factor-κB	JAPN	[74]
			HU colon tumour cell lines	Inhibition of apoptosis and cytosolic acidification	JAPN	[75]
			dehydrothyrsiferol	Alga	Triterpene	HU tumour cell lines
No inhibition of MRP1-dependent drug efflux	SPA	[34]				
didemnin B	Tunicate	Depsipeptide	HU tumour and peripheral blood monuclear cells	Induction of apoptosis	AUS	[35]
			HU tumour cell lines	Induction of apoptosis is caspase-dependent process	AUS	[36]
discodermolide	Sponge	Polyketide	HU tumour cell lines	No apparent role for apoptosis in cytotoxicity	NETH	[37]
			HU tumour cell line	Acetylation of C-7 hydroxyl increased cytotoxicity; C-17 hydroxyl key role in pharmacophore stereochemistry	USA	[38]
dolastatin 11	Mollusc	Depsipeptide	MU normal kidney cell line	Hyperassembly of purified F-actin	USA	[41]
dolastatin 10 analogue TZT-1027 (auristatin PE)	Synthetic	Depsipeptide	Rabbits and mice	Lack of peripheral neurotoxicity *in vivo*	JAPN	[42]
ecteinascidin-743	Tunicate	Isoquinoline alkaloid	HU and hamster tumour cell lines	Decreased activity in NER-deficient cell lines	ITA,USA	[46]
			HU and hamster tumour cell lines	Increased sensistivity of cells in G1 phase	ITA, USA	[47]
			HU tumour cell lines	Lethal DNA breaks and antiproliferative activity dependent on transcription coupled-NER activity	USA, JAPN	[48]

			HU tumour cell lines	Inhibition of activated but not constitutive transcription	USA	[51]
			NMR-based molecular dynamics	Complex with DNA resembles RNA–DNA hybrid and zinc finger-induced DNA distortions	SPA	[52]
			HU tumour cell line panel	Changes in DNA damage response, transcription and signal transduction genes	JAPN, USA	[53]
			HU tumour cell lines	Effective cytotoxicity in P-gp/MDR1-overexpressing cells	JAPN, USA	[54]
eleutherobin analogues	Coral	Diterpene glycoside	Human breast carcinoma cell line	Eleutherobin pharmacophore requires 2′,3′ double bond for tubulin binding activity	CAN	[65]
fascaplysin	Sponge	Alkaloid	Calorimetry, spectroscopy and circular dichroism	Intercalation into DNA	SWI	[66]
halichondrin B analogues	Sponge/Synthetic	Macrolide derivative	*In vitro* and *in vivo* HU tumour cell lines	Tubulin depolymerisation	JAPN, USA	[67]
jaspamide	Sponge	Depsipeptide	HU promyelocytic leukaemia cell line	Induction of apoptosis and CD10/neutral endopeptidase expression	JAPN	[68]
kahalalide F	Mollusk	Depsipeptide	Preclinical toxicity studies in rat	Fractionation of maximum tolerated dose reduces drug-induced toxicity	USA	[77]
lobatamides A–F	Tunicate	Macrolide	HU tumour cell lines	Inhibition of mammalian V-ATPase	USA, ITA, JAPN	[83]
motuporamines C-I	Sponge	Alkaloid	HU breast and prostate carcinoma and glioma cell lines	Inhibition of tumour cell invasion and angiogenesis	CAN	[84]
mycalamide A	Sponge	Alkaloid	Pig, MU and HU cell lines	Induction of apoptosis	NZEL	[86]
palmitic acid	Alga	Fatty acid	HU leukaemia cell lines	Induction of apoptosis and cell cycle arrest	JAPN	[87]
pateamine	Sponge	Alkaloid	Pig, MU and HU cell lines	Induction of apoptosis	NZEL	[86]
peloruside A	Sponge	Macrolide	MU and HU cell lines	Lack of PKC inhibition	NZEL	[69]
			HU tumour cell line	Microtubule-stabilising agent	NZEL	[70]
Petrosia sp. polyacetylenes	Sponge	Fatty acid	HU tumour cell lines	Initiation stage of DNA replication and topoisomerase I inhibition	S.KOR	[88]
Phyllospongia chondrodes sesterterpene (PHC-1)	Sponge	Sesterterpene	HU chronic myelogenous leukaemia cell line	Expression of glycophorin A, enucleation and erythroid differentiation	JAPN	[89]
salicylihalamide A	Sponge	Macrolide	HU tumour cell lines	Inhibition of mammalian V-ATPase	USA, ITA, JAPN	[83]

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Table 1 (*continued*)

Compound	Organism	Chemistry	Experimental or clinical model[a]	Mechanism of action[b]	Country[c]	[Ref.]
squalamine	Synthetic	Aminosterol	HU tumour cell lines	Disorganisation of F-actin stress fibres and reduction of VE-cadherin	USA	[90]
staurosporine	Ascidian	Alkaloid	HU leukaemia cell lines	RNA and DNA synthesis inhibition	AUS, GER	[91]
turbinamide	Ascidian	Fatty acid	MU tumour cell lines	Induction of apoptosis	ITA	[92]
vitilevuamide	Ascidian	Cyclic peptide	HU tumour cell lines	Inhibition of tubulin polymerisation	USA	[93]

MAPK, mitogen-activated protein kinase; PKC, protein kinase C; B-ALL, B-acute lymphoblastic leukaemia; NMR, nuclear magnetic resonance.

[a] HU, human; MU, murine.

[b] Pgp, P-glycoprotein; MRP, multi-drug resistance protein; NER, nucleotide excision repair.

[c] AUS, Australia; CAN, Canada; FRA, France; GER, Germany; ITA, Italy; JAPN, Japan; NETH, The Netherlands; NZEL, New Zealand; S.KOR, South Korea; SPA, Spain; SWI, Switzerland.

colleagues [32] observed that cryptophycin-52 and -55, synthetic cryptophycins produced by total chemical synthesis at Lilly Research Laboratories, induced hyperphosphorylation of the anti-apoptotic protein Bcl-2, cell cycle arrest and growth inhibition. The authors concluded that signal transduction pathways triggered by Bcl-2 phosphorylation may be involved in cryptophycin-induced lethality. Sessa and colleagues [33] published the results of a Phase I and pharmacokinetic clinical study designed to evaluate the administration of cryptophycin-52 to adult patients with advanced solid tumours that had not responded to prior anticancer therapy. The results of this study demonstrated that administration of cryptophycin-52 once every 3 weeks at the dose recommended for Phase II trials was associated with a unique toxicity profile consisting of neuropathy and myalgias, with noticeable interpatient variability.

One study was reported during 2002 on the preclinical pharmacology of **dehydrothyrsiferol**, a polyether triterpenoid isolated from the Canarian red alga *Laurencia viridis sp. nov*. [34]. The investigators noted that dehydrothyrsiferol was not a substrate for multidrug resistance-associated protein 1-dependent drug transport, a mechanism of major relevance in clinical resistance to anticancer drugs.

Two preclinical studies completed during 2001–2 extended the pharmacology of the **didemnin** depsipeptides. In one investigation, Baker and colleagues [35] demonstrated that didemnin B induced apoptosis in a large number of transformed tumour cell lines. Furthermore, because proliferating but not resting peripheral blood mononuclear cells were noted to undergo apoptosis, the authors suggested that didemnin B may have potential as a chemotherapeutic agent for the treatment of leukaemia. In another contribution, Grubb and colleagues [36] investigated the mechanism for rapid induction of apoptosis triggered by didemnin B in cultures of the human promyelocytic leukaemia cell line, HL60. Interestingly, they noted that changes in mitochondrial membrane potential, as well as mitochondrial cytochrome C release, were dependent on caspase activation, genomic DNA fragmentation and apoptotic body formation.

Four articles were published during 2001–2 on the preclinical pharmacology of **discodermolide**, a marine cytotoxic microtubule-stabilising compound with the same mechanism of action as the taxanes, an important class of chemotherapeutic drugs of which paclitaxel was the first to be introduced into the clinic in 1993. While investigating the cellular and molecular events underlying the cytotoxicity of discodermolide, Bröker and colleagues [37] noted that despite several apoptotic features detected at relatively late time-points after drug exposure, activation of apoptotic pathways played a negligible role in mediating the cytotoxic effects of discodermolide in non-small cell lung cancer cells. These

Table 2
2001–2 Antitumour 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]	[Ref.]
amphidinolide H2-H5, G2 and G3	Dinoflagellate	Macrolide	HU and MU	0.02–1.3 μg/mL	JAPN	[134]
apratoxin A, B, C	Bacterium	Depsipeptide	HU	0.36–21.3 nM	USA	[135,136]
Axinella brevistyla alkaloid	Sponge	Alkaloid	MU	0.66 μg/mL	NETH, JAPN	[137]
Axinyssa sterol	Sponge	Sterol	39 HU tumour panel	0.22–2.16 μg/mL	JAPN	[138]
briaexcavatolide L	Soft coral	Diterpene	HU and MU	0.5 μg/mL	TAIW	[139]
briaexcavatolide P	Soft coral	Diterpene	HU and MU	0.9–4.8 μg/mL	TAIW	[140]
cespitularin C	Soft coral	Diterpene	HU and MU	0.01 to >1 μg/mL	TAIW	[141]
chondropsin D	Sponge	Macrolide	HU	0.010–0.250 μg/mL	USA	[142]
claviridenone F and G	Soft coral	Prostanoid	HU and MU	0.52 pg/mL–1.22 μg/mL	TAIW	[143]
claviolide	Soft coral	Diterpene	HU and MU	0.38–0.84 μg/mL	TAIW	[143]
Clavularia inflata diterpene	Soft coral	Diterpene	HU and MU	0.052–0.56 μg/mL	TAIW	[144]
Clavularia koellikeri diterpene	Soft coral	Diterpene	39 HU tumour panel	0.66 to >1 μg/mL	JAPN	[94]
coproverdine	Ascidian	Alkaloid	HU and MU	0.3–1.6 μM	NZEL	[145]
discodermolide analogues	Sponge	Polyketide	HU and MU	13.5–170 nM	USA	[39]
ecteinascidins 770 and 786	Tunicate	Alkaloid	HU and MU	2.5–150 nM	THAIL, JAPN	[146]
epieupalmerone	Soft coral	Diterpene	HU	0.01–0.90 μg/mL	USA	[147]
epiplakinic acid G and H	Sponge	Fatty Acid	HU	0.16–0.39 μM	AUS, USA	[148]
14-methyleudistomin C	Ascidian	Alkaloid	HU	0.41–0.98 μg/mL	USA	[149]
gagunins C-F	Sponge	Diterpene	HU	0.03–0.71 μg/mL	S.KOR	[150]
gelliusterol C	Sponge	Sterol	HU and MU	0.5 to >1 μg/mL	NZEL, USA	[151]
halichoblelide	Bacterium	Macrolide	HU and MU	0.63 μg/mL	JAPN	[152]
haterumadioxin A and B	Sponge	Polyketide	HU and MU	0.005–0.011 μg/mL	JAPN	[95]
haterumaimides J and K	Ascidian	Diterpene	MU	0.23–0.45 ng/mL	JAPN	[153]
hectochlorin	Bacterium	Lipopeptide	HU	0.02–0.3 μM	USA	[97]
Isis hippuris sterol	Soft coral	Sterol	HU	<1 μg/mL	JAPN, USA	[154]
neo-kauluamine	Sponge	Alkaloid	HU	1 μg/mL	NZEL, SING, USA	[155]
leptosins M-N1	Bacterium	Alkaloid	HU and MU	0.18–1.40 μg/mL	JAPN	[98]
lomaiviticin A	Bacterium	Alkaloid	HU	0.01–98 ng/mL	USA	[156]
ma'iliohydrin	Alga	Sesquiterpene	HU	10 nM	USA	[157]
malevamide D	Bacterium	Peptide	HU and MU	0.3–0.7 nM	USA	[158]
metachromins	Sponge	Sequiterpenes	HU	0.22–1.32 μg/mL	TAIW	[159]
mycalolide	Sponge	Alkaloid	HU	2.6 ng/mL	NETH, JAPN	[160]
obyanamide	Bacterium	Depsipeptide	HU	0.58 μg/mL	USA	[161]
pachastrissamine	Sponge	Amino alcohol	HU and MU	0.01 μg/mL	SPA, JAPN	[162]
pachyclavulariaenone G	Soft coral	Diterpene	HU and MU	0.2–3.2 μg/mL	TAIW	[127]
petrotriyndiol A	Sponge	Fatty Acid	HU	0.6 to >1 μg/mL	S.KOR	[96]
plakinamines E and F	Sponge	Steroidal alkaloid	HU	0.2–1.3 μg/mL	USA	[163]
plakorsin B	Sponge	Fatty acid	HU	0.28–3.43 μg/mL	TAIW	[164]
plakortamine B	Sponge	Alkaloid	HU	0.62 μM	AUS, USA	[148]
Plexaurella grisea sesquiterpene	Soft coral	Sequiterpene	MU	0.5 μg/mL	SPA	[165]
poecillastrin A	Sponge	Polyketide	NCI 60-cell-line panel	<25 nM to >10 μM	USA	[166]
salmahyrtisol A	Sponge	Sesterterpene	HU and MU	1 μg/mL	EGY, NZEL, USA	[167]
sphingosine 4-sulfate	Sponge	Fatty Acid	HU	0.3 μg/mL	S.KOR	[168]
spongidepsin	Sponge	Macrolide	MU and HU	0.42–0.66 μg/mL	FRA, ITA	[169]
29-hydroxystelliferin A	Sponge	Triterpene	HU	0.11–1.62 μg/mL	USA	[170]
stoloniferone E	Soft coral	Steroid	HU and MU	0.12 ng/mL–6.4 μg/mL	TAIW	[143]
Stylotella aurantium sesquiterpenes	Sponge	Sequiterpene	HU and MU	0.1–1 μg/mL	JAPN	[171]
(2-hydroxyethyl) dimethylsulfoxonium	Sponge	Sulfoxonium salt	MU	0.15 μg/mL	JAPN	[172]
symplostatin 3	Bacterium	Peptide	HU and MU	3.9–10.3 nM	USA	[173]
theopederins K and L	Sponge	Polyketide	HU and MU	0.1–7.3 nM	USA	[174]

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Table 1 (*continued*)

Compound	Organism	Chemistry	Preclinical tumour cell line model[a]	50% growth inhibition or cytotoxicity	Country[b]	[Ref.]
varitriol	Fungus	Polyketide	NCI 60-cell-line panel	0.16–95 nM	DEN, SPA	[175]
xenitorin A	Soft coral	Sesquiterpene	HU and MU	0.79 μg/mL	TAIW	[176]

[a] HU, human, MU, murine.

[b] AUS, Australia; DEN, Denmark; EGY, Egyptl; FRA, France; JAPN, Japan; ITA, Italy; NETH, Netherlands; NZEL, New Zealand; SING, Singapore; S.KOR, South Korea; SPA, Spain; TAIW, Taiwan; THAIL, Thailand; NCI, National Cancer Institute.

findings suggest that alternative, caspase-independent forms of cell death may be responsible for the cytototoxic effects of discodermolide. Isbrucker and colleagues [38] completed a structure–activity relationship study of discodermolide and its semisynthetic acetylated analogues on microtubule function and cytotoxicity. While observing that the C-17 hydroxyl group had an important role in maintaining the favourable stereochemistry of the pharmacophore, acetylation of discodermolide at the C-7 hydroxyl group potentiated cytotoxicity to human lung adenocarcinoma cells, probably imparting a "*secondary cytotoxic quality*" which the authors suggest may be related to a more extensive bundling of microtubules than that caused by the parent compound. Current interest in the discodermolides appears to be high: during 2001–2, five new discodermolide analogues from the marine sponge *Discodermia* species were characterised [39] and 12 semisynthetic analogues were prepared [40]. Both the natural and semisynthetic analogues showed a significant variation in cytotoxicity, confirming the importance of the C-7 through C-19 molecular fragment for biological activity against human and murine tumour lines.

Although no anti-actin compound currently has a role in cancer chemotherapy, 5 studies were published during 2001–2 on the preclinical and clinical pharmacology of the **dolastatins**, a family of peptide/depsipeptides originally isolated from the marine mollusc *Dolabella auricularia* that induce actin assembly *in vivo*. Two preclinical studies contributed novel information on the mechanism of action of the dolastatins. Following the successful synthesis of the pentapeptide, dolastatin 11, Bai and colleagues [41] determined that both cytokinesis arrest in normal rat kidney cells, as well as a 3-fold increase in cytotoxicity, resulted from hyperassembly of the cellular F-actin filament network. Furthemore, this study suggested that, although as yet undetermined, dolastatin 11 may bind to a "*different site on the actin polymer than the other peptides*".

To evaluate the neurotoxic potential of TZT-1027, a synthetic dolastatin-10 derivative, Ogawa and colleagues [42], carried out a neurotoxicity screening study in rabbits and mice. Interestingly, their results suggested that, "*TZT-1027 had no or at least a very low neurotoxic potential*", in marked contrast to vincristine and paclitaxel, both anti-microtubule agents that caused peripheral neurotoxicity in control animal studies. With the goal of developing new therapies for pancreatic cancer, the fourth leading cause of cancer-related deaths in the US, Mohammad and colleagues [43] conducted a preclinical evaluation of the combination of bryostatin-1 with auristatin PE, a structural analogue of dolastatin-10. Although the number of mice used in this study was low, the drug combination had an enhanced antitumour effect in a human pancreatic xenograft model, suggesting that further experimentation might lead to the development of a novel clinical treatment for carcinomas of the pancreas. Two Phase II trials with dolastatin-10 were completed during 2001–2. Margolin and colleagues [44] reported on a Phase II clinical trial of dolastatin-10 in 12 patients with advanced melanoma who had received no prior chemotherapy. Although the elimination of dolastatin-10 from plasma was much longer than previously reported and the drug was well tolerated in this Phase II trial, no patient in this study experienced an objective response. Saad and colleagues [45] completed a Phase II study of dolastatin-10 as first-line treatment for advanced colorectal cancer in 14 patients with no prior chemotherapy for metastatic disease. Although dolastatin-10 was well tolerated in the study, the drug lacked clinically significant antineoplastic activity at the dose and schedule used, thus leading the investigators to conclude that "*further evaluation of dolastatin-10 in colorectal cancer appears to be unwarranted*".

Research on the tetrahydroisoquinoline alkaloid **ecteinascidin-743 (ET-743)**, an antitumour agent originating from the Caribbean tunicate *Ecteinascidia turbinata*, continued at a fast pace during 2001–2. Thirteen preclinical and five clinical articles extended the pharmacology of ET-743 during 2001–2002. New information on the mechanism of action of ET-743 on DNA-repair mechanisms was contributed by several articles. Damia and colleagues [46], while investigating the cytotoxic activity of ET-743 in cell systems with well-defined deficiencies in their DNA-repair mechanisms, observed that ET-743 had decreased activity in nucleotide excision repair (NER)-deficient cell lines, as well as in cells with active DNA-dependent protein kinase, suggesting "*a unique mechanism of interaction with DNA*". While carefully characterising the effect of ET-743 on a number of human and hamster cell lines, Erba and colleagues [47] also noted that NER-proficient cells had less resistance to ET-743 and that the G1 phase of the cell cycle was particularly hypersensitive. The

biological significance of this observation is currently under investigation by these researchers because it would appear it has "*never been described before for other DNA-interacting agents*". While studying the transcription-interfering properties of ET-743,Takebayashi and colleagues [48] discovered that the antiproliferative activity of ET-743 was dependent on the transcription-coupled pathway of NER, an interaction that induced lethal DNA strand-breaks. In an additional contribution from this laboratory, ET-743 was shown to induce protein-linked single-strand DNA breaks in human colon carcinoma [49]. These DNA breaks were observed to persist after drug removal and to be strongly suppressed at 0 °C. Finally, as the result of an extensive molecular pharmacology study with ET-743, Zewail-Foote concluded that both inefficiency of incisions of ET-743-DNA adducts by NER nucleases, as well as the unique structural feature of the ET-743-DNA adducts, could possibly provide an explanation for the repair-dependent toxicities of this marine-derived anticancer agent to cells [50].

Other reports during 2001–2002 contributed additional insights into the molecular pharmacology of ET-743. Although it was initially hypothesised that ET-743 targeted a single transcription factor, experimental observations by Friedman and colleagues [51] led them to re-assess the mechanism by which ET-743 blocks transcription. The investigators proposed that ET-743 was "*a novel, potent, and general inhibitor of activated, but not uninduced transcription*". Using nuclear magnetic resonance (NMR)-based molecular dynamics simulations, Marco and colleagues [52] determined that the ET-743-DNA complex resembled both an RNA–DNA hybrid and mimicked zinc finger-induced DNA structural distortions. These investigators also noted that the ET-743-DNA complex, adopted A-DNA characteristics in the strand complementary to the one alkylated by ET-743, a conformation reminiscent of that found in DNA-RNA hybrids. Using a panel of 36 human cancer cell lines, flow cytometry and oligonucleotide microarrays, Martinez and colleagues [53] compared the antitumour activity and gene expression-based profiling of ET-743 and phthalascidin, a synthetic analogue of ET-743. Results of the activity patterns, as well as the array-based gene expression experiments, were highly similar for ET-743 and phthalascidin, confirming earlier findings that suggested both compounds have similar pharmacological properties. Kanzaki and colleagues [54] reported a study designed to further investigate the relationship between P-glycoprotein/MDR1 and the activity of ET-743. Because ET-743 was observed to potentiate the activity of doxorubicin and vincristine by downregulating P-gp/MDR1, these researchers concluded that combination of ET-743 and agents that are substrates for P-gp/MDR1 "*may be valuable in the clinic*".

Preclinical cellular pharmacology of ET-743 involved several studies during 2001–2002. Li and colleagues [55] compared the cytotoxicity of ET-743 with that of methotrexate, doxorubicin, etoposide and paclitaxel on several human soft tissue sarcoma cell lines which expressed p53. They observed that ET-743 was more potent than all of these clinically used anticancer drugs, with cytotoxicity in the pM range resulting in S/G_2 block, though interestingly, with no alteration in the expression of Pg-P and the anti-apoptotic bcl-2 proteins. With the purpose of determining how to better use ET-743 clinically, Takahashi and colleagues [56] assessed the *in vitro* cytotoxicity resulting from combining ET-743 with doxorubicin, trimetrexate and paclitaxel on two soft tissue sarcoma cell lines. Although synergy was evident for all drug combinations, the sequence of ET-743 followed by doxorubicin treatment was the most effective cytotoxic regimen against both cell lines, suggesting that it may be "*the most favourable regimen for future clinical trials*" for soft tissue sarcoma. Scotlandi and colleagues [57] reported on the effectiveness of ET-743 against a panel of drug-sensitive and -resistant human osteosarcoma and Ewing's sarcoma cell lines. The potent activity of ET-743 observed against drug-sensitive and drug-resistant bone tumours at concentrations achievable in patients (from pM to nM range), and in particular against Ewing's sarcoma cells, encouraged the authors to propose "*inclusion of this drug in the treatment of patients with bone tumours*".

Two studies extended the preclinical *in vivo* pharmacology of ET-743. In an effort to address the hepatotoxicity of ET-743 and help design novel therapeutic rescue strategies, Donald and colleagues [58] completed a detailed analysis of ET-743-induced changes in rat liver pathology, biochemistry and accompanying gene expression profiles. Their results suggested that ET-743 toxicity to rat liver was the consequence of biliary rather than hepatocellular damage, long duration of altered liver pathology and enhanced liver cell proliferation involving an increase in cell cycle *Cdc2a* and *Ccnd1* gene expression. With the specific aim of determining the role of cytochrome P-450 metabolism in gender-dependent hepatotoxicity, Reid and colleagues [59] characterised rat and human hepatic microsomal metabolism and ET-743 pharmacokinetics and biliary excretion in male and female rats. They reported that the predominant P-450s that catalyse ET-743 are those of the CYP3A subfamily in both rats and humans, with gender-dependent metabolism observed for rats, but not in humans. Thus, the researchers concluded that it is unlikely that there will be "*gender-dependent ET-743 pharmacokinetics or toxicity in cancer patients*".

Four Phase I and one Phase II trials extended the clinical pharmacology of ET-743 during 2001–2002. Ryan and colleagues [60] completed a Phase I study with ET-743 in 21 adult patients with refractory solid

tumours. Although this study demonstrated that doses of ET-743 administered as 72-h continuous intravenous (i.v.) infusions were tolerated, there were no objective responses to the therapy in the patients, with the exception of one with epitheliod mesothelioma. Taama and colleagues [61] published the results of a Phase I study with ET-743, administered as a 24-h continuous infusion in 52 patients with treatment-refractory solid tumours. Antitumour activity was limited: three patients achieved partial responses (breast cancer, osteosarcoma and liposarcoma) and 4 patients with progressing soft tissue sarcoma achieved clinically meaningful disease stabilisations lasting >3 months. Van Kesteren and colleagues [62] reported a Phase I study with ET-743 administered as a 1- and 3-h infusion in 72 patients with confirmed diagnosis of solid tumour not amenable to established forms of treatment. The investigation showed that ET-743 was well tolerated with delayed toxicities being pancytopenia and fatigue. Villalona-Calero completed a Phase I study of ET-743 on a daily 1-h i.v. infusion during 5 days every three weeks in 42 patients with solid malignancies [63]. Antitumour activity was recorded in three patients with leiomyosarcoma, primary peritoneal and ovarian carcinomas, with neutropenia being the main haematological toxicity noted in this study. Ryan and colleagues [64] contributed results from the first Phase II trial and pharmacokinetic study of ET-743 in 20 patients with advanced gastrointestinal stromal tumours. The investigators observed that there was no significant antitumour activity in this patient population underscoring the chemorefractory nature of this malignancy, perhaps as a the combined result of MDR and enhanced systemic clearance by "*an unclear mechanism*".

Novel preclinical research with the microtubule-stabilising diterpene **eleutherobin**, originally isolated from the soft coral *Eleutherobia* sp. from western Australia, was reported during 2001–2. In a brief communication, Britton and colleagues [65] completed the first detailed investigation of synthetic transformations of eleutherobin which revealed new features of its antimitotic pharmacophore. Because in a cell-based antimitotic assay 2′,3′-dihydroeleutherobin was 1,000-fold less active than eleutherobin, the 2′,3′, double bond in eleutherobin would appear to be a stringent requirement for its ability to promote the polymerisation of purified bovine tubulin [65].

Hormann and colleagues [66] extended the preclinical pharmacology of **fascaplysin**, an alkaloid originally isolated from the Fijian sponge *Fascaplysinopsis* sp. Using a combination of calorimetric and spectroscopic methods, the binding of fascaplysin to DNA was carefully investigated. These studies revealed that the binding mode and affinity constants of fascaplysin were comparable to those of typical DNA intercalating agents, thus suggesting that some of fascaplysin's pharmacological activity might be attributable to its interference with the cell's genetic material.

Towle and colleagues [67] extended the pharmacology of **halichondrin B**, a large polyether macrolide found in a variety of marine sponges, for which limited availability severely restricted research efforts in recent years. Two fully synthetic macrocyclic ketone analogues of halichondrin B had sub-nM growth inhibitory activities *in vitro* against numerous human cancer cell lines as well as marked *in vivo* activities against four human xenografts (breast, colon, melanoma and ovarian cancer). The observation that these synthetic compounds induced G_2-M cell cycle arrest and disruption of mitotic spindles, consistent with the tubulin-based antimitotic mechanism of halichondrin, led these researchers to propose the development of "*halichondrin B-based agents as highly effective, novel anticancer drugs*".

The preclinical pharmacology of the marine cyclodepsipeptide **jaspamide**, isolated from a number of sponges including *Jaspis johnstoni* and *Hemiastrella minor*, was extended by Cioca and colleagues [68]. These investigators determined that the mechanism of jaspamide-induced CD10/neutral endopeptidase expression and apoptosis in the human promyelocytic leukaemia HL-60 cell line appeared to involve a caspase-independent pathway of cell death.

Preclinical antitumour research with the macrolide **peloruside A**, isolated from the marine sponge *Mycale hentscheli* from New Zealand, was reported during 2001–2. In view of the structural similarity of particular regions of peluroside to the protein kinase C (PKC)-binding pharmacophore of bryostatin, Hood and colleagues [69] compared the effects of peloruside A and bryostatin, on PKC-dependent pathways in murine and human cell lines. Peluroside A, though a potent inhibitor of cell proliferation demonstrated a unique mode of action independent of PKC. More recently, Hood and colleagues [70] established that peluroside A was a novel microtubule-stabilising agent with potentially unique properties. The observation that peluroside A altered microtubule dynamics in a manner similar to that reported for paclitaxel by inducing tubulin polymerisation *in situ* and in cell-free systems, and also caused cells to arrest in the G_2-M phase of the cell cycle, suggested that peluroside A may represent a novel agent with "*major importance for clinical treatment of solid tumours*".

Table 1 lists several marine natural products which were not previously reviewed (1-3) : aeroplysinin-1, callystatin A, caulerpenyne, cycloprodigiosin, kahalalide F, lobotamides A–F, motuporamines, mycalamide A, palmitic acid, *Phyllospongia chondrodes* sesterterpenes, *Petrosia* sp. polyacetylenes, salicylihalamide A, squalamine, spirulan, turbinamide and vitilevuamide.

Rodriguez-Nieto and colleagues [71] reported on the anti-angiogenic activity of **aeroplysinin-1**, a brominated

tyrosine derivative isolated from the marine sponge *Aplisina aerophoba*. The *in vitro* studies demonstrated that aeroplysinin-1 inhibited several essential steps of the angiogenic process, perhaps by inhibition of receptor tyrosine kinases, suggesting that this compound might become a potential candidate for the inhibition of angiogenesis *in vivo*.

Murakami and colleagues [72] completed a structure–activity relationship study with the cytotoxic polyketide **callystatin A**, a marine natural product isolated from the sponge *Callyspongia truncata*. Investigation of the structural requirements for potent cytotoxicity determined that each of the four asymmetric centres in the β-hydroxyketone portion of the callystatin A molecule was involved in rigid and stable binding through strong lipophilic interactions, rather than hydrogen bonding to the amino acid residues of the receptor molecule.

Barbier and colleagues [73] investigated the antiproliferative activity of **caulerpenyne**, a sesquiterpenoid isolated from the tropical marine alga *Caulerpa taxifolia* previously shown to be cytotoxic to several cell lines. Caulerpenyne inhibited proliferation of a human neuroblastoma cell line (IC_{50} = 10 μM) while concomitantly affecting both the microtubule network organisation and neurite presence. *In vitro*, caulerpenyne inhibited microtubule assembly and induced polymerisation of purified tubulin in a time- and concentration-dependent manner. The molecular pharmacology of caulerpenyne remains of great interest because the toxin appears to be a multifunctional agent that also interacts with DNA synthesis and signal transduction pathways.

Three papers extended the molecular pharmacology of **cycloprodigiosin hydrochloride**, a red pigment produced by the marine bacterium *Pseudoalteromonas denitrificans*, previously shown to induce apoptotic cell death in various cancerous cell lines [74]. Kamata and colleagues noted that cycloprodigiosin hydrochloride enhanced the apoptotic process induced by tumour necrosis factor-α in HeLa cells by suppressing the transcriptional activity of trancription nuclear factor κB (NF-κB), thus providing one possible explanation for the immunosuppressant and antitumour effects of this marine molecule. Yamamoto and colleagues [75] reported that cycloprodigiosin hydrochloride inhibited the growth of two human colon cancer cell lines *in vitro* at the sub-μM range by inducing acidification of the cytosol and apoptosis. Because of the high potency of cycloprodigiosin hydrochloride on colon cancer cells and its low toxicity to normal cells, the authors proposed that this marine natural product might be useful for the treatment of colon cancer. Interestingly, in a more recent study, Yamamoto and colleagues [76] observed that cycloprodigiosin hydrochloride and epirubicin had synergistic effects, suppressing growth of a human breast cancer cell line *in vitro*, as well as xenografted tumour cells in nude mice. In both experimental models, accelerated apoptosis appeared to be the mechanism responsible for the antitumour activity.

Several studies extended the *in vivo* preclinical pharmacology of **kahalalide F**, a cyclic depsipeptide isolated from the marine mollusk *Elysia rufescens* shown to have significant activity against androgen-independent prostate tumours. Brown and colleagues [77] reported preclinical toxicity studies with kahalalide F designed to determine both acute and multiple dose toxicities in the rat when administering the drug i.v., the intended route of clinical administration. The findings demonstrated that fractionation of a maximum tolerated dose of kahalalide F reduced drug-induced toxicity which was mainly renal, and would appear "*a viable option for the clinical evaluation of kahalalide F for the treatment of cancer*". Furthermore, several drug development studies were reported: kahalalide F was shown to be metabolically stable [78], to cause no haemolysis [79], and to remain chemically stable for at least five days irrespective of the temperature (ambient vs. refrigerated), infusion container material and kahalalide F concentration used [80]. A stable lyophilised parenteral formulation of kahalalide F for Phase I clinical trials in the US and Europe [81] has now been developed [82].

Boyd and colleagues [83] reported that a series of novel benzolactone enamides, **lobatamides A–F** from the marine tunicate *Aplidium lobatum* and **salicylihalamide A** isolated from the marine sponge *Haliclona* sp., selectively inhibited mammalian vacuolar-type (H+)-ATPases. Because plasma membrane V-ATPases appear to be involved in angiogenesis, cellular proliferation, tumour metastasis, apoptosis and programmed cell death, this enzyme system has received considerable attention as a potential molecular target for cancer therapeutics. Interestingly, both salicylihalamide A (IC_{50} = 0.40–0.62 nM) and lobatamides A–F (IC_{50} = 0.68–14 nM) show unprecedented selectivity for mammalian versus non-mammalian V-ATPases.

Roskelley and colleagues [84] reported on the **motuporamines**, alkaloids isolated from the marine sponge *Xestospongia exigua*. Motuporamine C was reported to interfere with migration of human breast carcinoma, prostate carcinoma and glioma cells in culture and inhibited angiogenesis in both an *in vitro* sprouting assay and an *in vivo* chick chorioallantoic membrane assay. These pharmacological properties and the fact that motuporamine C showed low toxicity *in vitro* and appears simple to synthesise, suggests that this marine alkaloid may become an attractive drug candidate. Williams and colleagues [85] identified new motuporamines D, E, F and a mixture of G, H, and I that was observed to have anti-invasion and anti-angiogenic activities.

Hood and colleagues [86] extended the preclinical pharmacology of the cytotoxic metabolites **mycalamide A** and **pateamine** isolated from the marine sponge

Mycale sp. in New Zealand. Using DNA laddering, annexin-V staining and morphological analysis, both compounds were observed to induce cell death by apoptosis in several murine and human tumour cell lines. Interestingly, increased susceptibility to apoptosis was noted in cell lines transformed with the *ras* or *bcr-abl* oncogenes.

Harada and colleagues [87] characterised the *in vitro* and *in vivo* antitumour activity of **palmitic acid** isolated from the red alga *Amphiroa zonata*. Surprinsingly, palmitic acid showed selective toxicity and induced apoptosis in leukaemic cell lines, while concomitantly demonstrating very low cytotoxicity to a normal cell line used as a control in these studies.

Novel molecular findings with **polyacetylenes** isolated from the marine sponge *Petrosia* sp. were reported by Kim and colleagues [88]. Dideoxypetrosynol A and 3*S*,14*S*-petrocortyne A appeared to exhibit selective toxicity towards the non-small cell lung cancer subpanel used in this investigation, with concomitant inhibition of the initiation stage of DNA replication and DNA cleavage by topoisomerase I.

As a direct result of a research programme designed to identify novel differentiation-inducing substances for cancer cells, Aoki and colleagues [89] isolated several novel scalarane-type sesterterpenes from the marine sponge *Phyllospongia chondrodes*. Interestingly, the sesterterpene PHC-1 induced erythroid terminal differentiation in the human chronic myelogenous leukaemia cell line, K562, an all-*trans*-retinoic acid-non responsive leukaemia, as demonstrated by the observation of both enucleation and cell cycle arrest at the G1 phase in the tumour cells.

Williams and colleagues [90] reported on pharmacological studies with **squalamine**, a marine aminosterol that appears to be an angiogenesis inhibitor and that currently is undergoing Phase II clinical trials in cancer patients. The investigators noted that the use of **squalamine** for the treatment of human tumour xenografts in nu/nu mice enhanced platinum-based chemotherapies. Furthermore, the observation that squalamine induced both disorganisation of F-actin stress fibres and a reduction of endothelial cadherin (VE-cadherin) led the investigators to conclude that squalamine interfered with "*endothelial cell movement and cell-cell communication necessary for growth of new blood vessels*" in the human xenografts.

Schupp and colleagues [91] characterised the antiproliferative effects of new **staurosporine** derivatives, indolocarbazole alkaloids isolated from a marine ascidian *Eudistoma toealensis* and its predatory flatworm *Pseudoceros* sp. They determined that while the staurosporines tested inhibited the cellular proliferation of twelve human leukaemia cell lines, the cell lines differed in their sensitivity towards the individual staurosporine derivatives. Furthermore, structure–activity relationships demonstrated that hydroxylation of staurosporine at position 3 of the indolocarbazole moiety increased the antiproliferative activity, as well as the RNA and DNA synthesis inhibition of the staurosporine analogues. The authors postulated that the differences in efficacy of the staurosporine derivatives in modulating growth may result from differences in their ability to inhibit certain kinases involved in cell growth and tumour promotion.

The molecular pharmacology of **turbinamide**, isolated from the marine ascidian *Sydnium turbinatum*, was carried out by Esposito and colleagues [92]. The results of studies of cell viability, membrane lipoperoxidation, DNA fragmentation and apoptosis demonstrated that the selective toxicity of turbinamide to C6 rat glioma cells was due to apoptosis, suggesting that "*turbinamide may be useful in the therapy of glioma*".

Edler and colleagues [93] described novel information on the mechanism of inhibition of tubulin polymerisation by **vitilevuamide**, a bicyclic 13 amino acid peptide isolated from two marine ascidians, *Didemnum cuculiferum* and *Polysyncranton lithostrotum*. Vitilevuamide was shown to inhibit polymerisation of purified tubulin *in vitro* with an $IC_{50} = 2$ μM, to exhibit non-competitive inhibition of vinblastine binding to tubulin and to weakly affect guanidine triphosphate (GTP) binding. This work provides pharmacological evidence that suggests that vitilevuamide "*inhibits tubulin polymerisation via an interaction at a unique site*" problably distinct from colchicine, the vinca alkaloids and dolastatin-10.

3. 2001–2 Antitumour pharmacology of marine natural products with *undetermined* mechanisms of action

Table 2, encompasses novel marine natural products published during 2001–2 that demonstrated particularly potent activity in cytotoxicity assays (IC_{50} of $\leqslant 1.0$ μg/mL) and selected 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 and Compare programme analysis. 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, it should be highlighted that mechanism of action research has been initiated with a few of the marine compounds shown in Table 2: a novel pattern of differential growth inhibition as shown by Compare programme analysis

was reported for both a new cembrane diterpenoid from the soft coral *Clavularia koellikeri* [94], as well as the new cytotoxic endoperoxides haterumadioxins A and B from the *sponge Plakortis lita* [95]; significant inhibition of Simean Virus 40 (SV40) DNA replication was noted for cytotoxic polyacetylenes from the marine sponge *Petrosia species* [96]; stimulation of actin assembly was shown for hectochlorin, a novel lipopeptide from the cyanobacteria *Lyngbya majuscula* [97], while inhibition of tyrosine protein kinases was determined for leptosin M, a chemical isolated from the marine fungus *Leptosphaeria* sp. [98].

Although less potent than the marine natural products included in Table 2, 30 additional reports were published during 2001–2 describing novel structurally characterised molecules with cytotoxic activity (IC_{50}), mostly in the 1–4.0 μg/mL range [99–128]. Although only the cytotoxicity against selected murine or human cancer cells was determined *in vitro* in most of these reports, mechanistic work was reported in a few studies: reversal of multidrug resistance in human carcinoma cell lines overexpressing P-glycoprotein was observed with brianthein A, a novel briarane-type diterpene [109]; putative inhibition of the Ras signalling pathway by Ras-converting enzyme inhibition was shown to occur with terpenoids from the marine sponge *Hippospongia* sp. [107]; induction of apoptosis was determined with orostanal, a novel sterol from the sponge *Stelletta hiwasaensis* [115], and putative selective cytotoxicity against cyclin-dependent kinase inhibitory protein p21-deficient tumour cell lines was described with the both stellettins, marine terpenes isolated from the sponge *Rhabdastrella globostellata* [123] and the sebastianines, alkaloids from the ascidian *Cystodytes dellechiajei* [124]. It appears likely that if further structure–activity relationship studies are completed with these marine compounds, novel analogs may yet reveal increased potency and potentially important antitumour properties.

4. Conclusions

This Review highlights the fact that antitumour marine pharmacology research in 2001–2 remained a combination of preclinical research focused on the molecular and cellular pharmacology of marine cytotoxic agents, as well as clinical studies with a relatively small number of marine compounds, i.e. bryostatin 1, cryptophycins, dolastatins, and ecteinascidin-743. Although the Review has mainly focused on recent developments in the preclinical pharmacology of marine cytotoxic agents, it should be noted that concomitant to the mechanistic characterisation of these marine natural products, the issues of supply, formulation, and manufacturing are extremely important for the successful development of novel pharmaceutical agents. This issue has been highlighted by the current contributions to new drug development by three leading companies focused on developing novel pharmaceuticals from marine sources [129–132].

Even though during 2001–2 no new marine natural product was approved for cancer patient treatment by the US Food and Drug Administration, the present 2001–2 antitumour and cytotoxic overview demonstrates that more than 50 years after the discovery by Bergman and Feeney [133] of spongothymidine and spongouridine, research efforts aimed at the discovery of novel and clinically useful antitumour agents derived from marine organisms continue to be both sustained and global in nature.

Conflict of interest statement

None declared.

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