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Review

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

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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.

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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 anthelmintic, 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 ner-

vous 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

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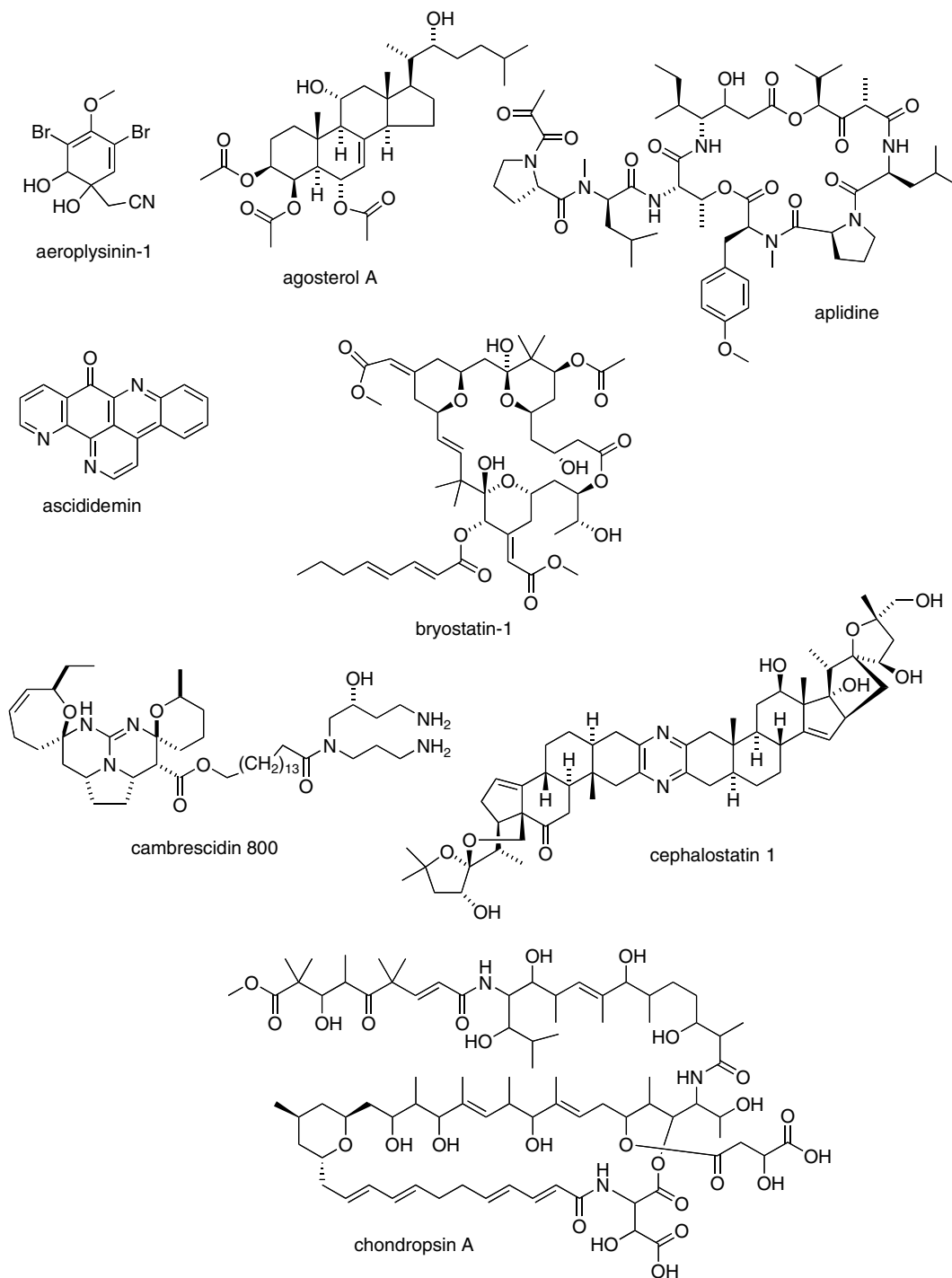


Fig. 1 – Structures of marine natural products reported in 2003 and 2004 with established mechanisms of action.

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.

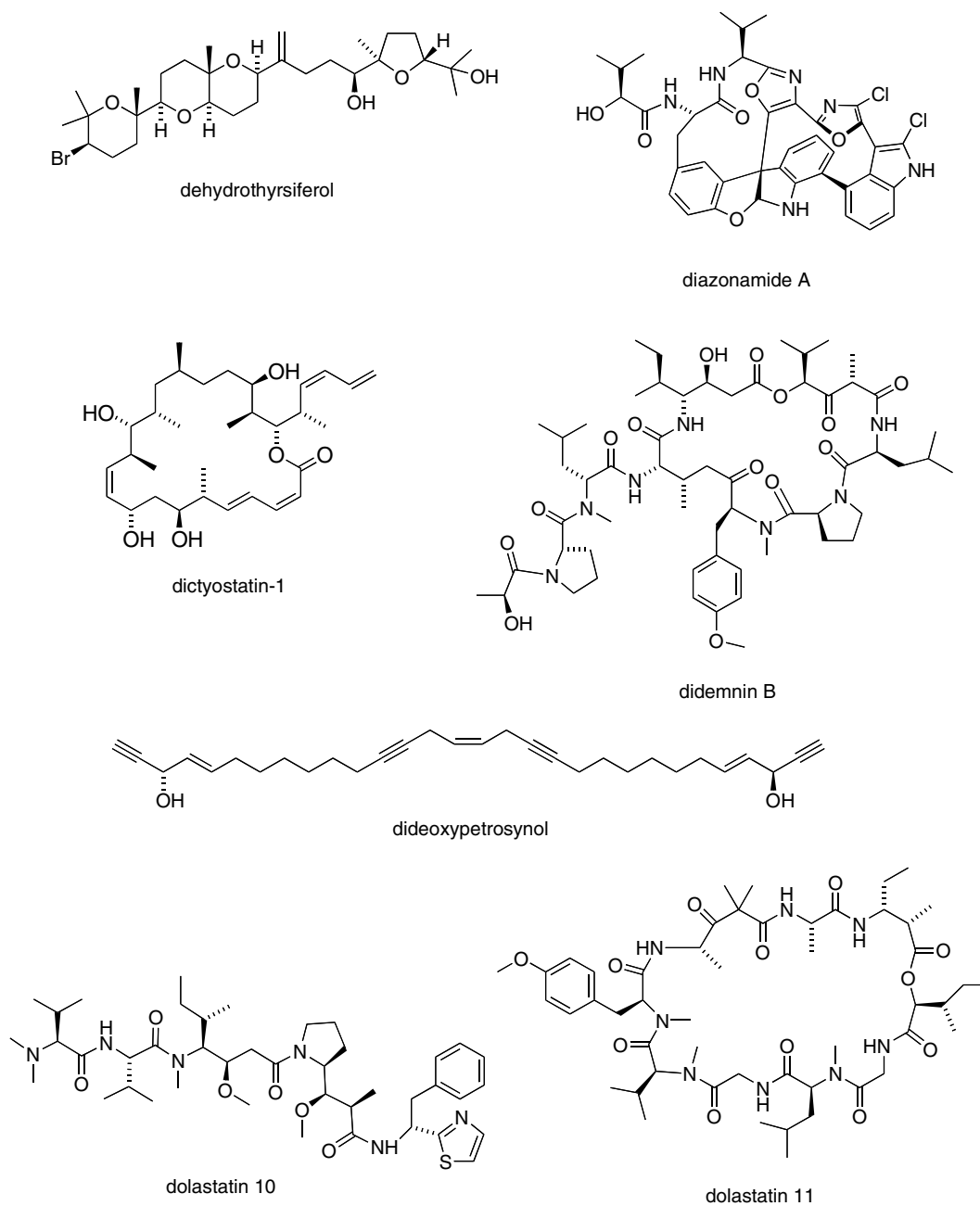


Fig. 1 – continued

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, dehydrothysiferol, didemnin B, dideoxypetrosynol A, dolastatins, ecteinascidin-743, halichondrin B, hemiasterlin, kahalide F, motuporamines and peloruside.

One study extended the preclinical pharmacology of **aeroplysinin-1**, a compound we reviewed previously. Gonzalez-Iriarte and colleagues¹⁴ 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 colleagues¹⁵ working with several human epidermoid carcinoma KB cell sub-lines, showed that [¹²⁵I]-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

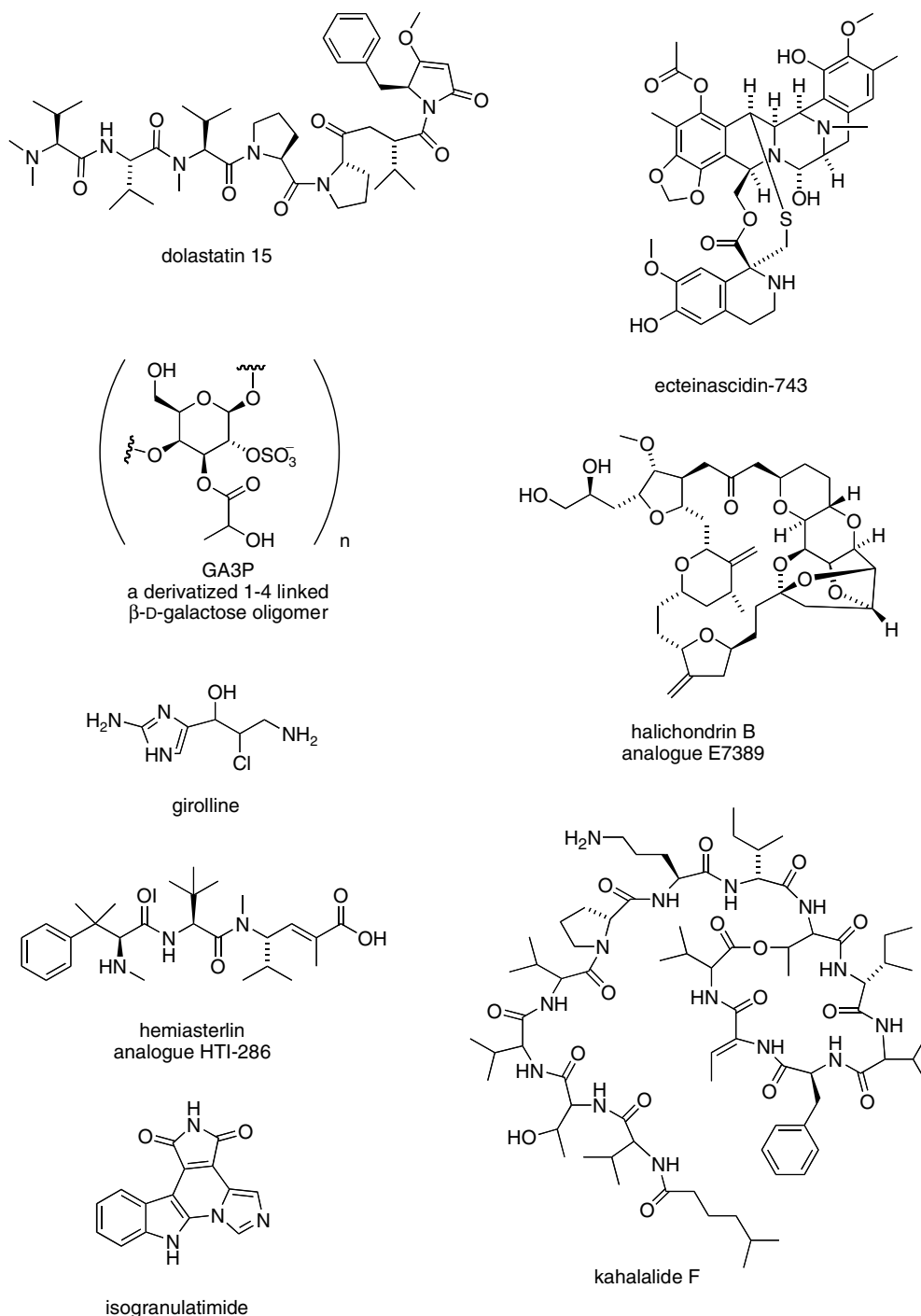


Fig. 1 – continued

activity of this molecule in reversing multidrug resistance. Furthermore, Ren and colleagues¹⁶ in a detailed mechanistic study further characterised the glutathione-dependent [¹²⁵I]-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 Arg¹²⁰²

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 apolidin or dehydrididemin B, which was previously isolated from the marine tunicate *Aplidium albicans*. While investigating the MOLT-4 human leukaemia cell line, Broggin and colleagues¹⁷ demonstrated that apolidine's cytotoxic activity was the consequence of inhibition of the vascular endothelial

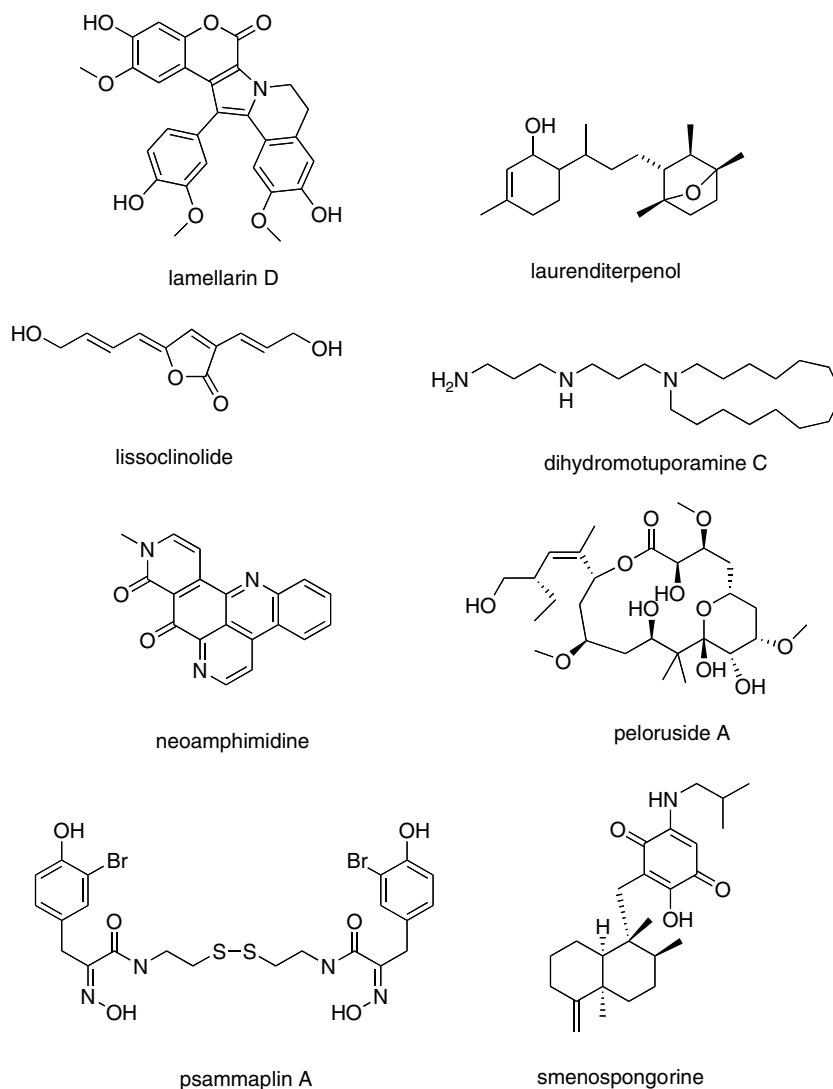


Fig. 1 – continued

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 colleagues¹⁸ 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 G₁ arrest and G₂ blockage. Losada and colleagues¹⁹ 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 colleagues²⁰ 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 colleagues²¹ 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 colleagues²² 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 colleagues²³ using long-term competitive repopulation assays performed in mice determined that doses of aplidine that produced a

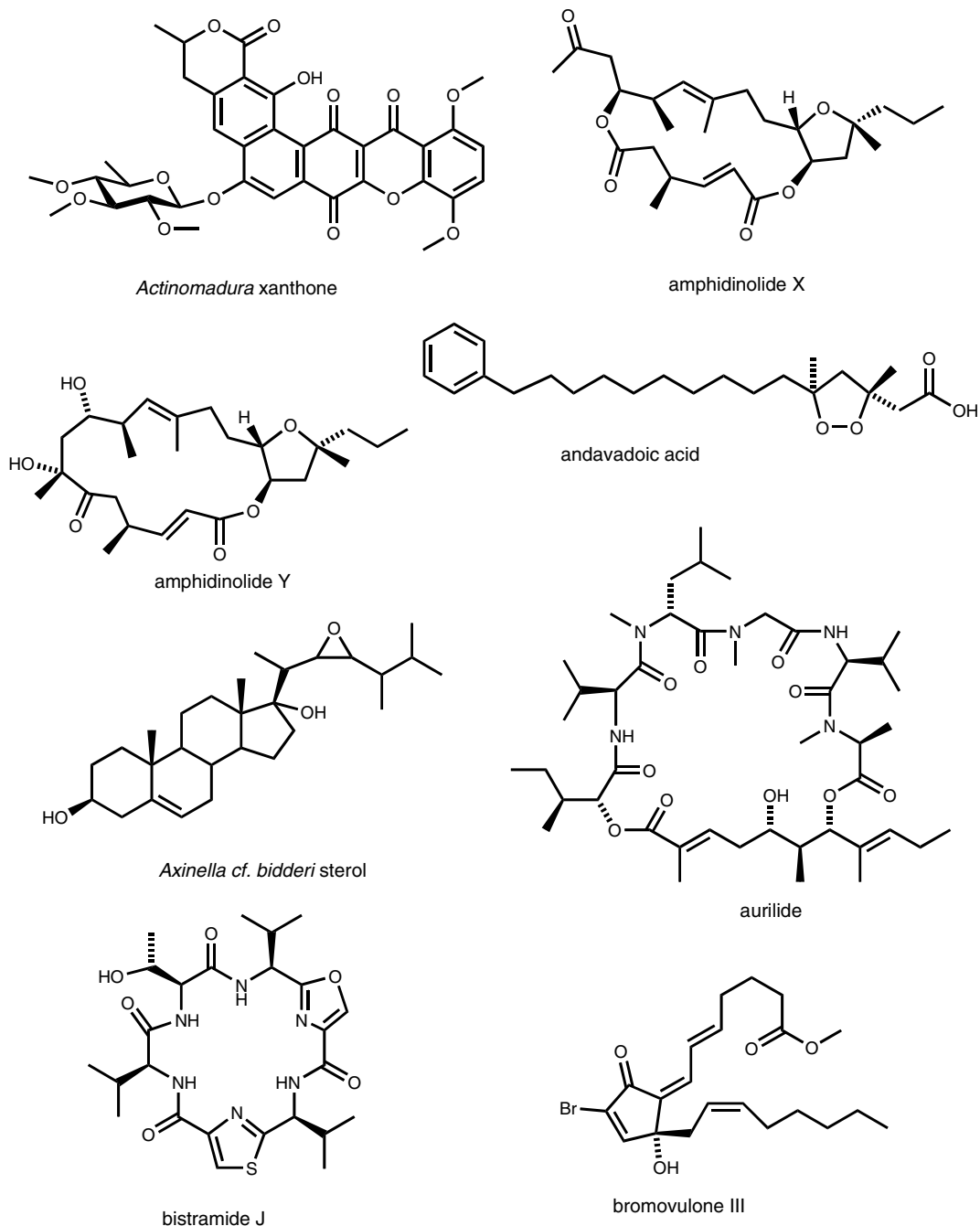


Fig. 2 – Structures of new marine natural products reported in 2003 and 2004 with undetermined mechanisms of action.

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 colleagues²⁴ reported experimental results that suggested direct iminoquinone reduction and reactive oxygen species generation as the probable mechanism responsible for ascididemin cytotoxicity. Dirsch and colleagues²⁵ investigated the signal-

ling 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

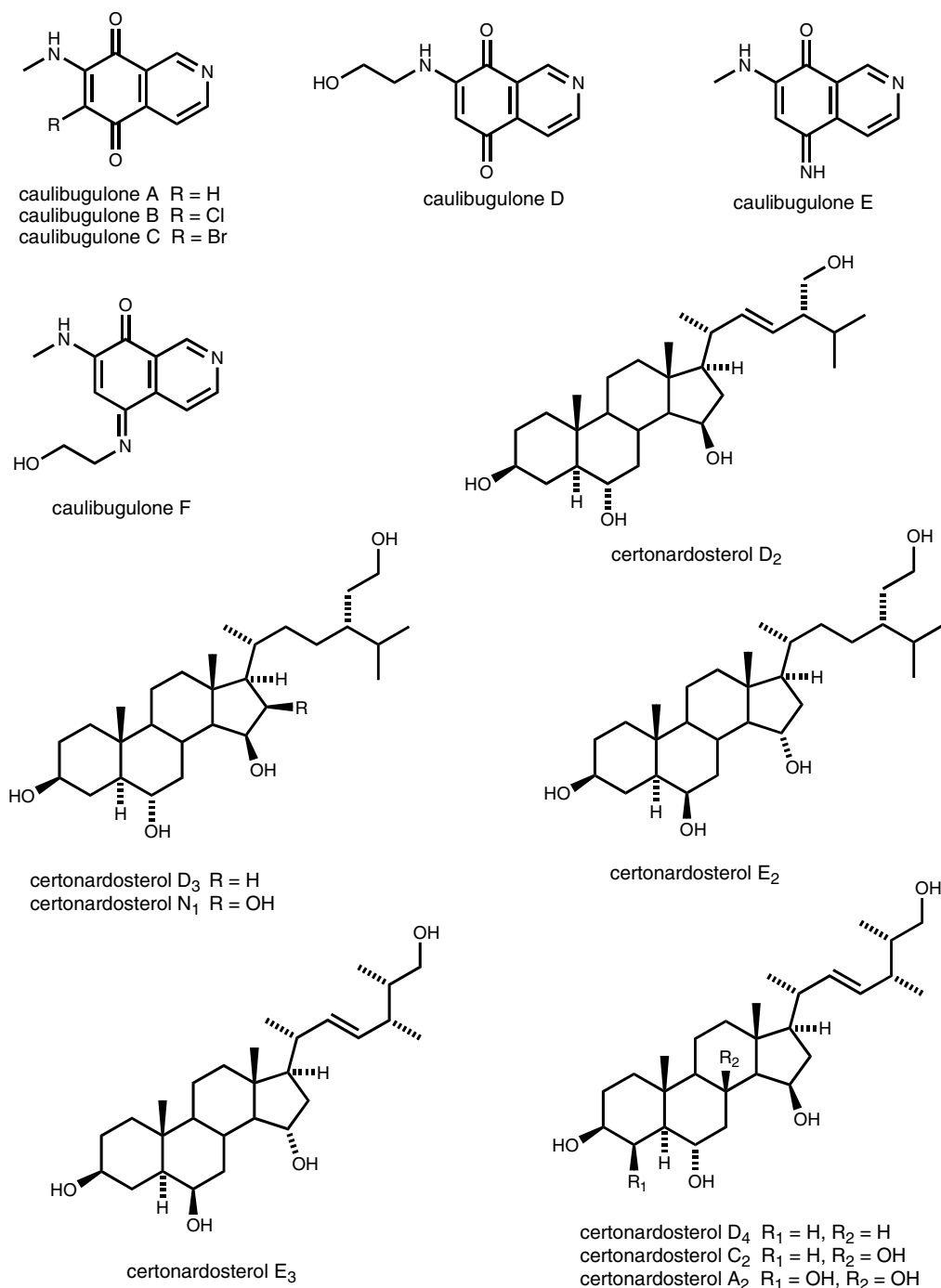


Fig. 2 – continued

molecular level. Ali and colleagues²⁶ 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 colleagues²⁷ 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 colleagues²⁸ characterised the effects of bryostatin-1 on 1-β-D-arabinofuranosylcytosine(ara-C)-induced apoptosis of

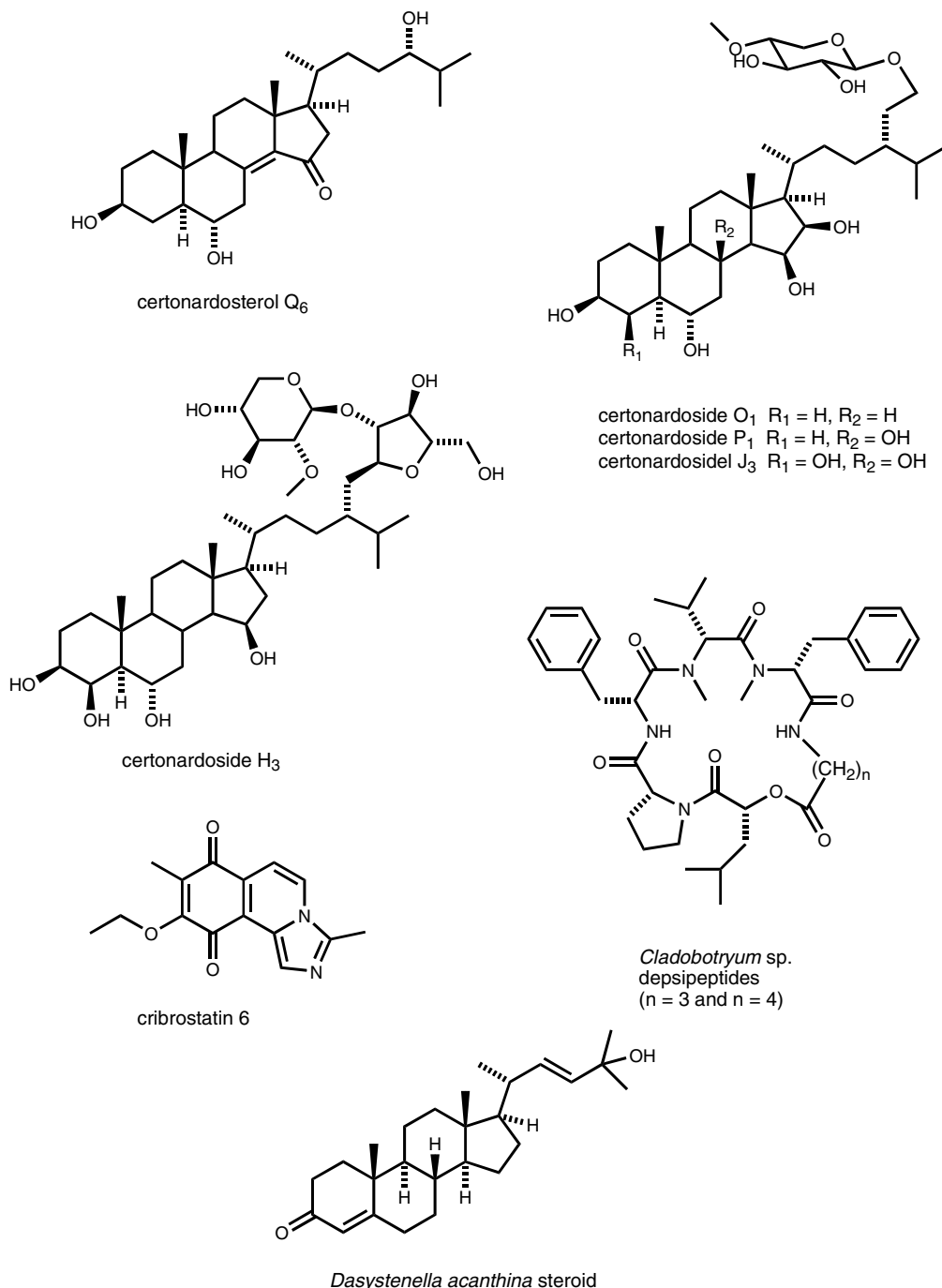


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human myeloid leukaemia cell lines. The investigation demonstrated that potentiation of ara-C apoptosis resulted from 'protein kinase C-dependent release of tumour necrosis factor α ' and concomitant activation of the extrinsic apoptotic cascade.

One study was reported during 2003 on the preclinical pharmacology of **dehydrothysiferol** (DT), a polyether triterpenoid isolated from a Canary island collection of the red alga *Laurencia viridis* sp. nov. Pec and colleagues²⁹ 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 colleagues³⁰ 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

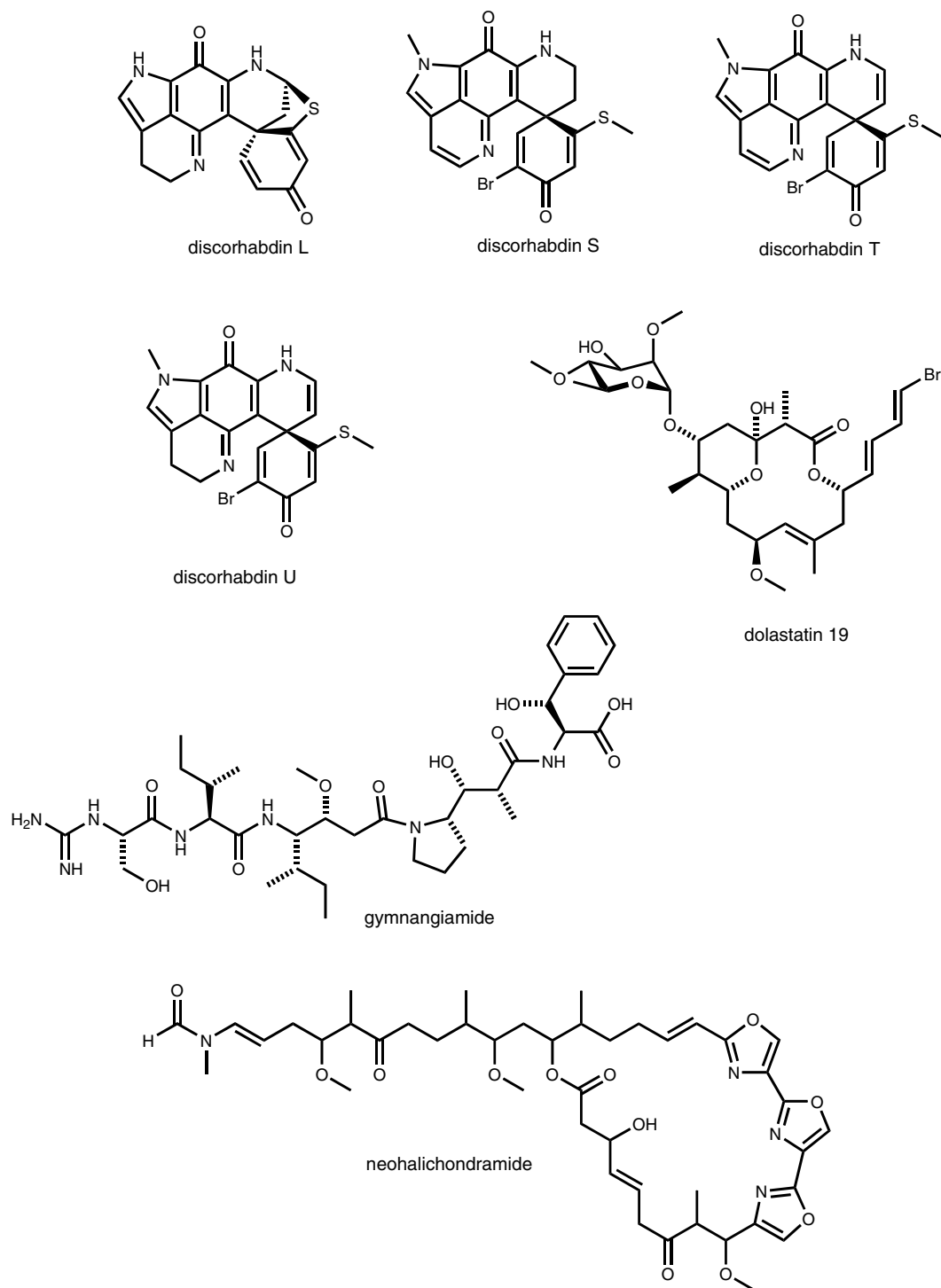


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potent anti-tumour activity. Their model suggests that an eEF1A-didemnin complex that binds to the ribosomal A-site would 'get stuck', leading to translational arrest, thus clearly demonstrating the importance of inhibition of the protein synthesis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

Choi and colleagues³¹ 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

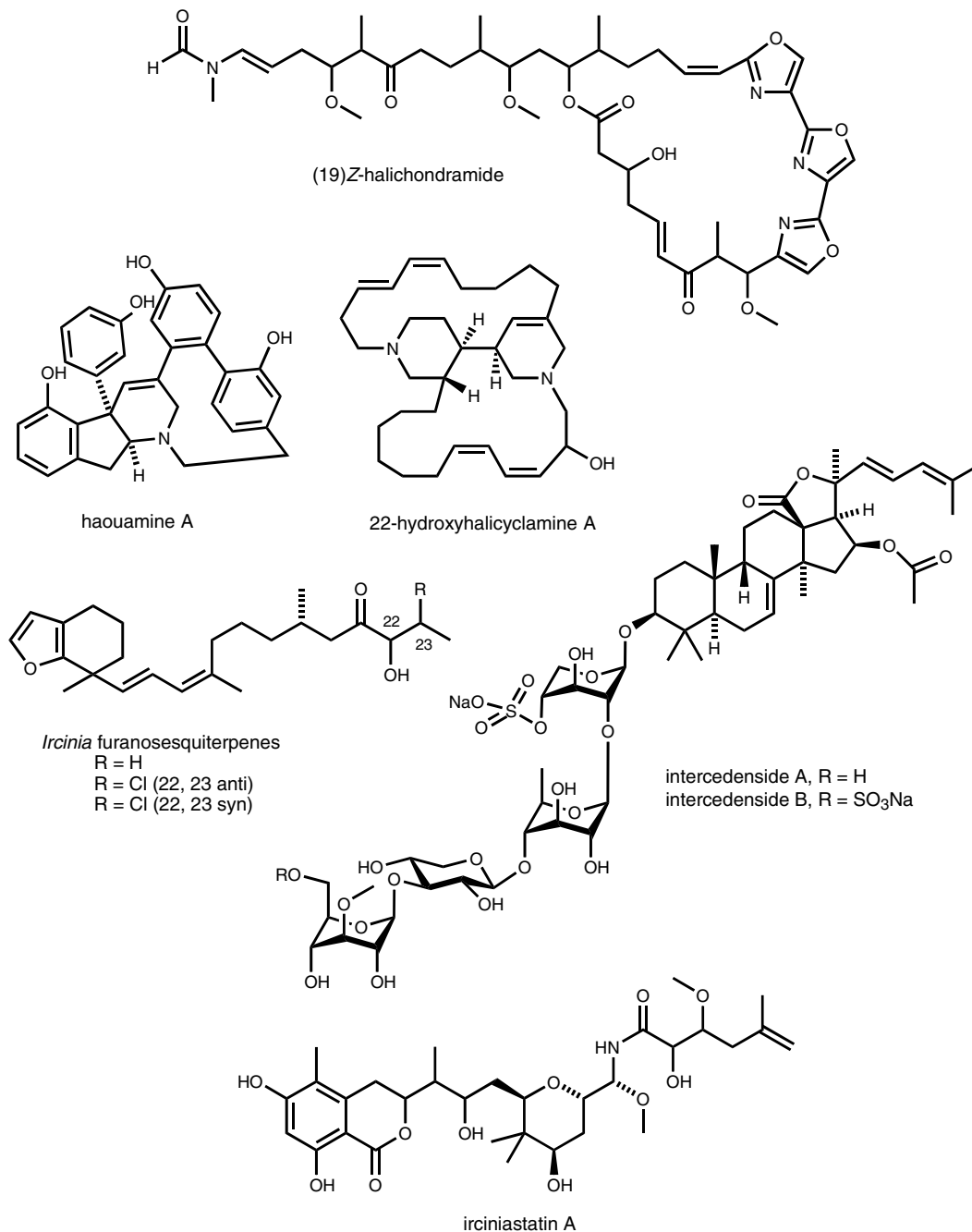


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modified peptides originally isolated from the marine mollusc *Dolabella auricularia* that induce actin assembly *in vivo*. Oda and colleagues³² investigated the molecular mechanism of F-actin stabilisation by dolastatin 11 using X-ray fibre diffraction 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 colleagues³³ demonstrated that dolastatin 15 binds with relatively low binding affinity (apparent K_d of $\approx 30 \mu\text{M}$) to the vinca domain

of $\alpha\beta$ -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 colleagues³⁴ 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 [³H]-dolastatin 10 to the β -tubulin peptide spanned amino acid residues 2-31, with probable

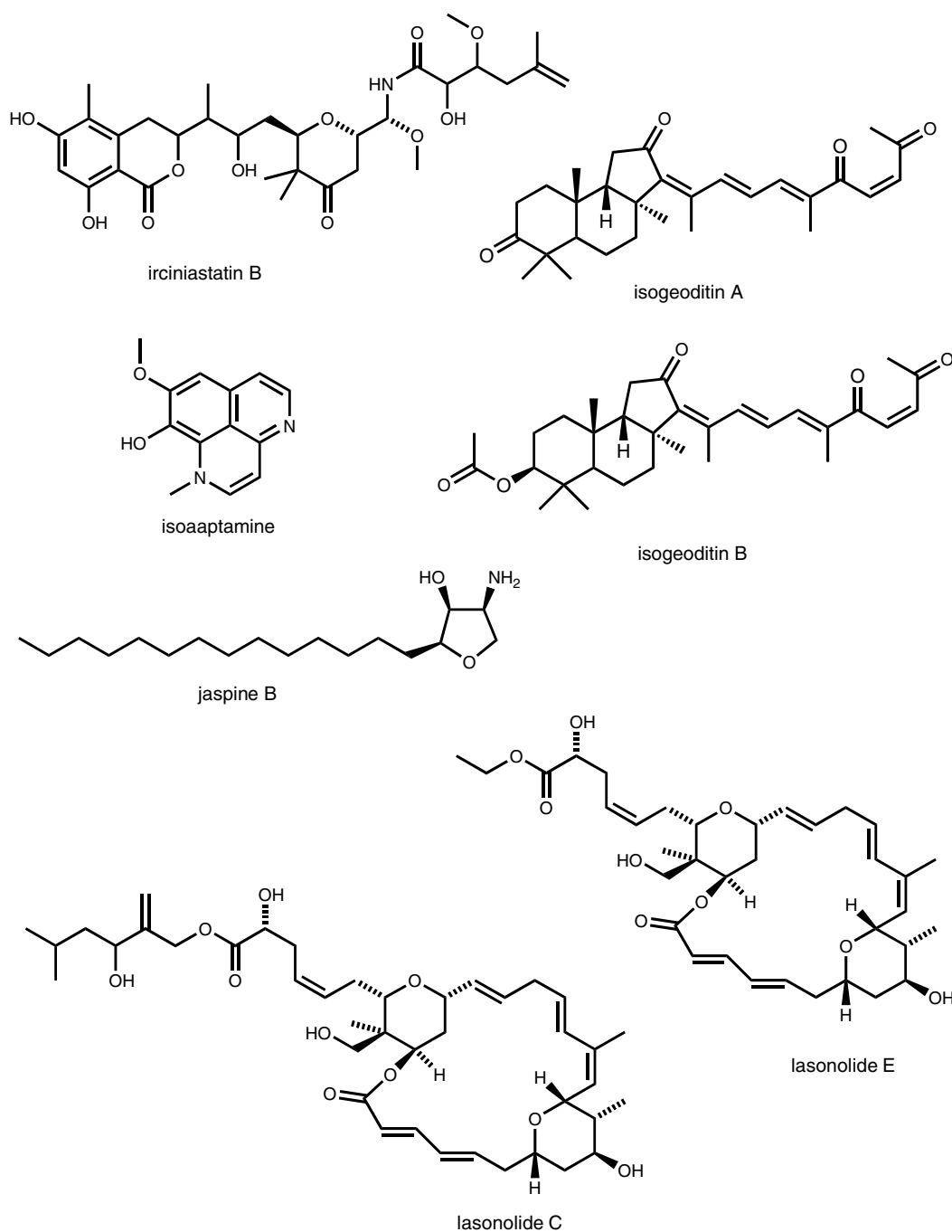


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covalent bond formation 'between the sulphur atom of Cys-12 and the thiazole ring of dolastatin 10'.

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 colleagues³⁵ contributed additional insight into the molecular pharmacology of ET-743 by examining the impact of telomerase function on the sensitivity of hu-

man 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 colleagues³⁶ reported on the effects of the combination of ET-743 and cisplatin in human cancer cell lines growing *in vitro* and in

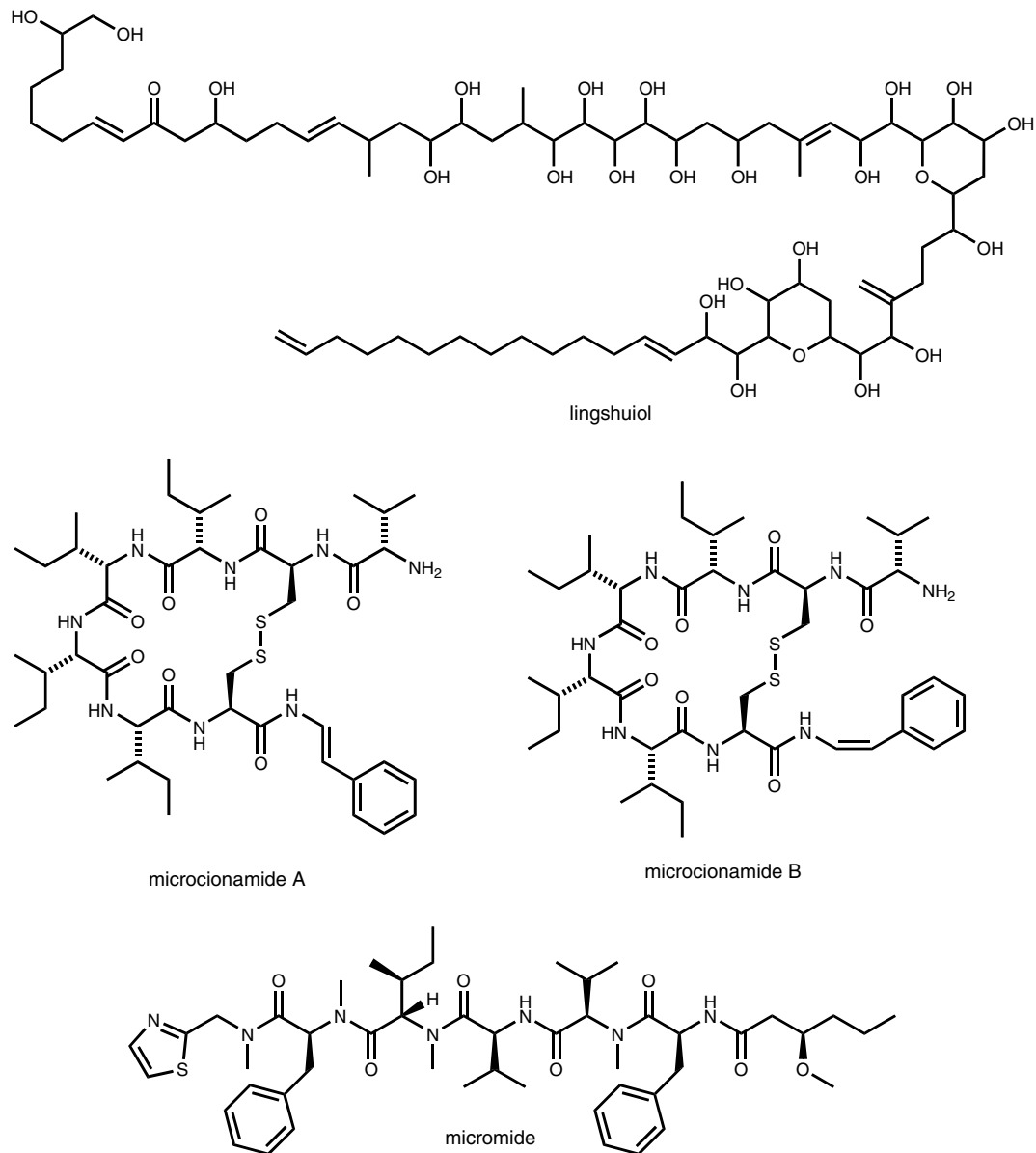


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xenografts derived from different human tumours in nude mice. The results demonstrated that the combination of ET-743 and cisplatin was synergistic both *in vitro* and *in vivo* and that both agents could be combined at the maximum tolerated dose perhaps 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 colleagues³⁷ 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 colleagues³⁸ 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 $\alpha 1$ chain mRNA in the ET-743-resistance sarcoma cell line.

Three studies extended the preclinical *in vivo* pharmacology of ET-743. Meco and colleagues³⁹ 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-

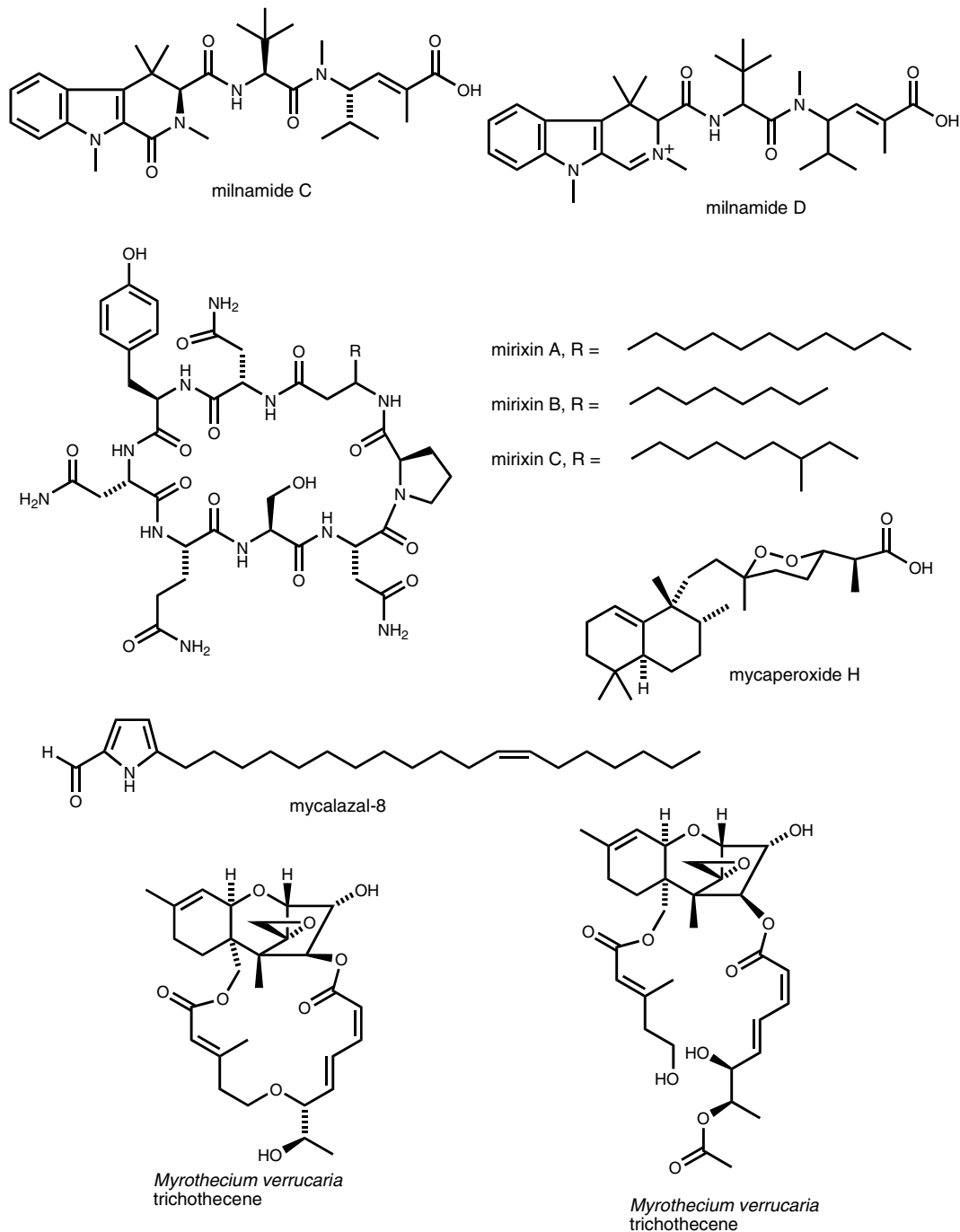


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duced a significant anti-tumour effect on both human tumours as well as doxorubicin-resistant mouse fibrosarcoma. The authors concluded that synergy of the two drugs 'could be effective for tumours displaying low sensitivity to either ET-743 or doxorubicin'. Two reports focused on the efforts to study strategies to ameliorate the hepatotoxicity of ET-743. Donald and colleagues⁴⁰ 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 colleagues⁴¹ 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

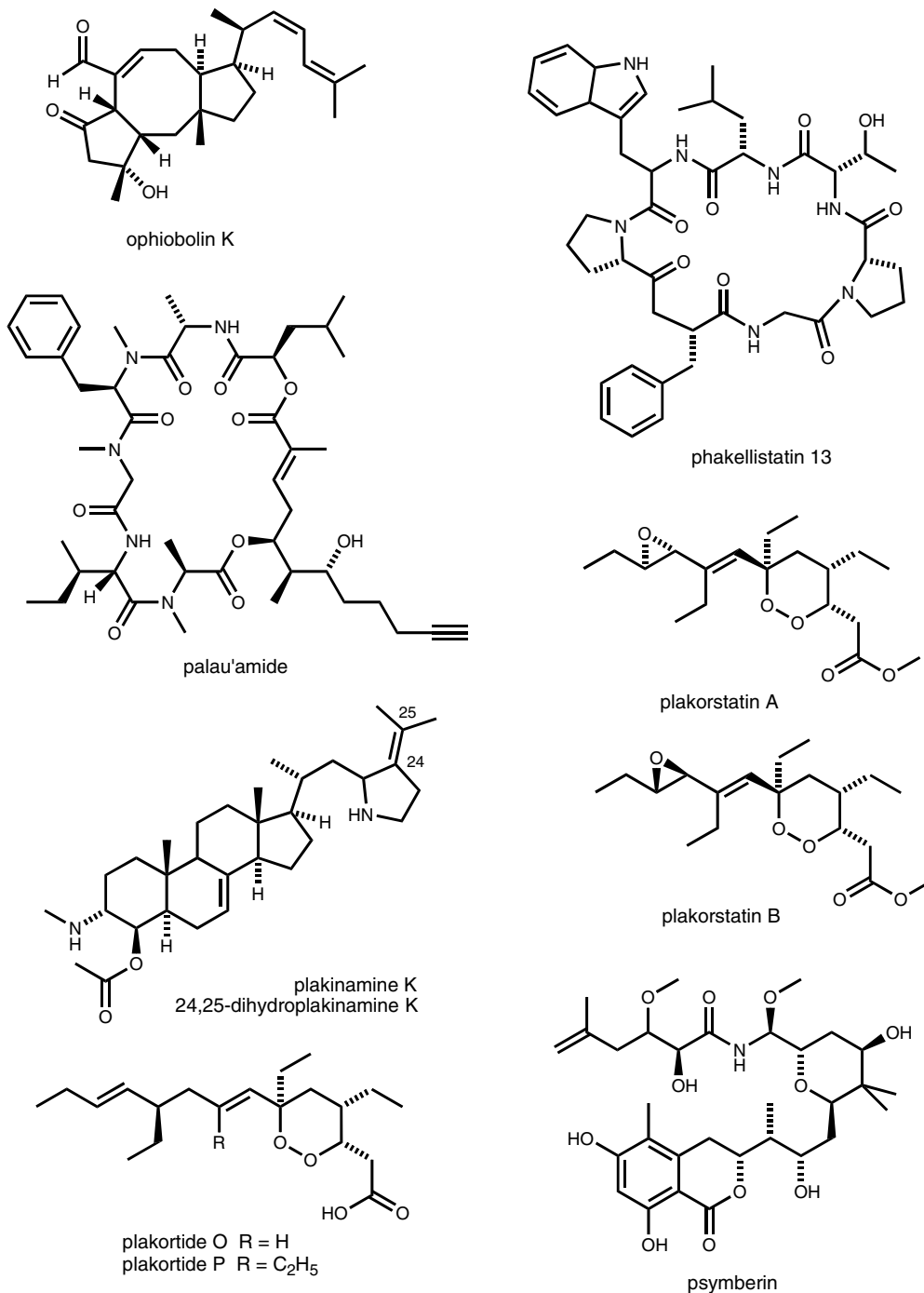


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pharmacological strategy to ameliorate the hepatotoxicity of ET-743 in humans'.

One phase I and four phase II trials extended the clinical pharmacology of ET-743 during 2003–2004. Twelves and colleagues⁴² 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 colleagues⁴³ 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 colleagues¹⁷⁰ published the results of a phase II study

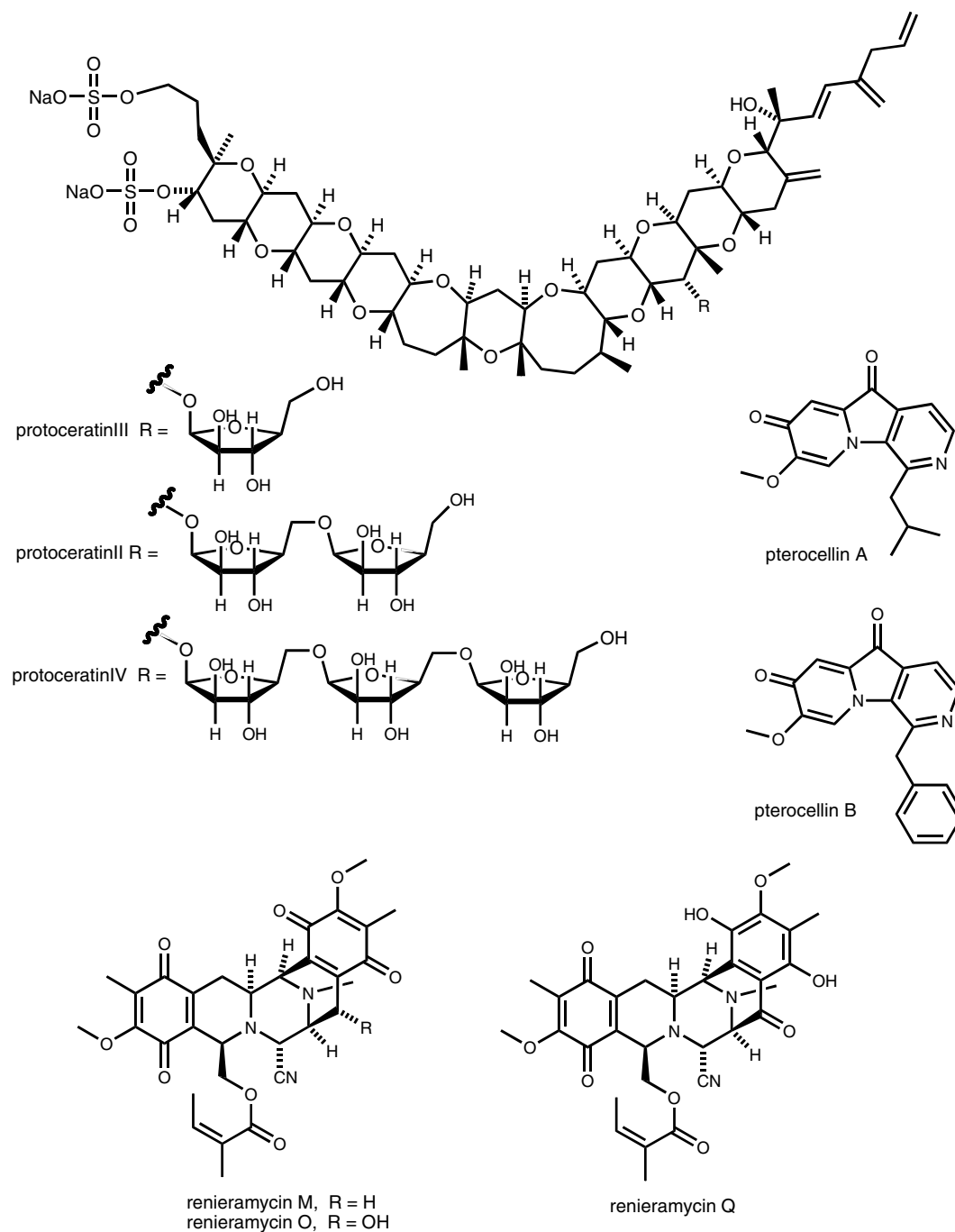


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with ET-743 in 28 patients with gastrointestinal stromal tumours (GIST), a type of tumour for which there were few alternative therapeutic options prior to the imatinib era. Although the treatment with ET-743 was well tolerated, with only 33% of the 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 colleagues⁴⁴ 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 colleagues⁴⁵ 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',

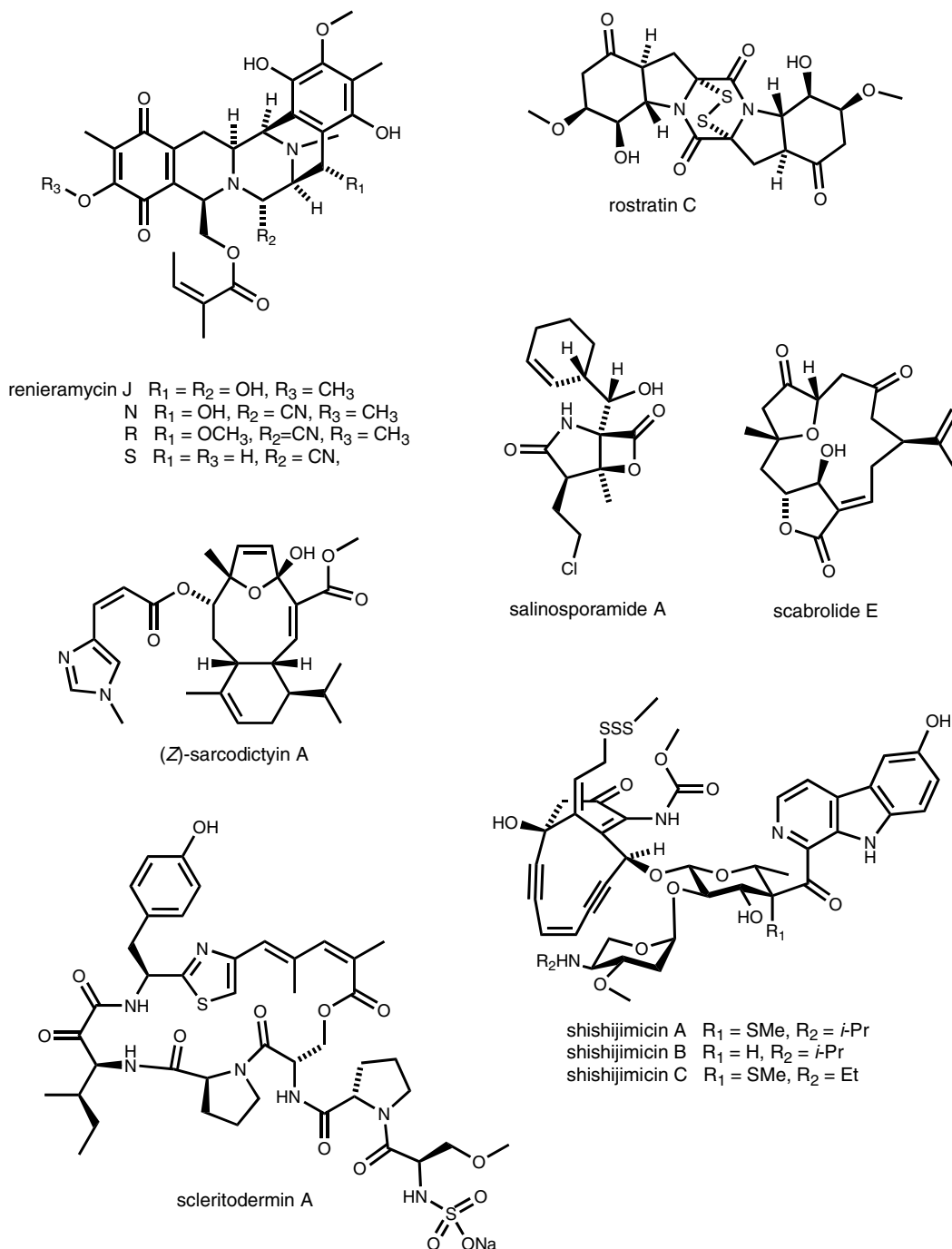


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interestingly the rate of disease control of 38.8% at 3 months and 24.1% at 6 months, provided rather encouraging evidence for an anti-tumour effect of ET-743 in this patient population of 'highly pre-treated, progressing, advanced, metastatic, and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

Kuznetsov and colleagues⁴⁶ 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 $G_2\text{-M}$ phase of the cell cycle with ≥ 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 colleagues⁴⁷ 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

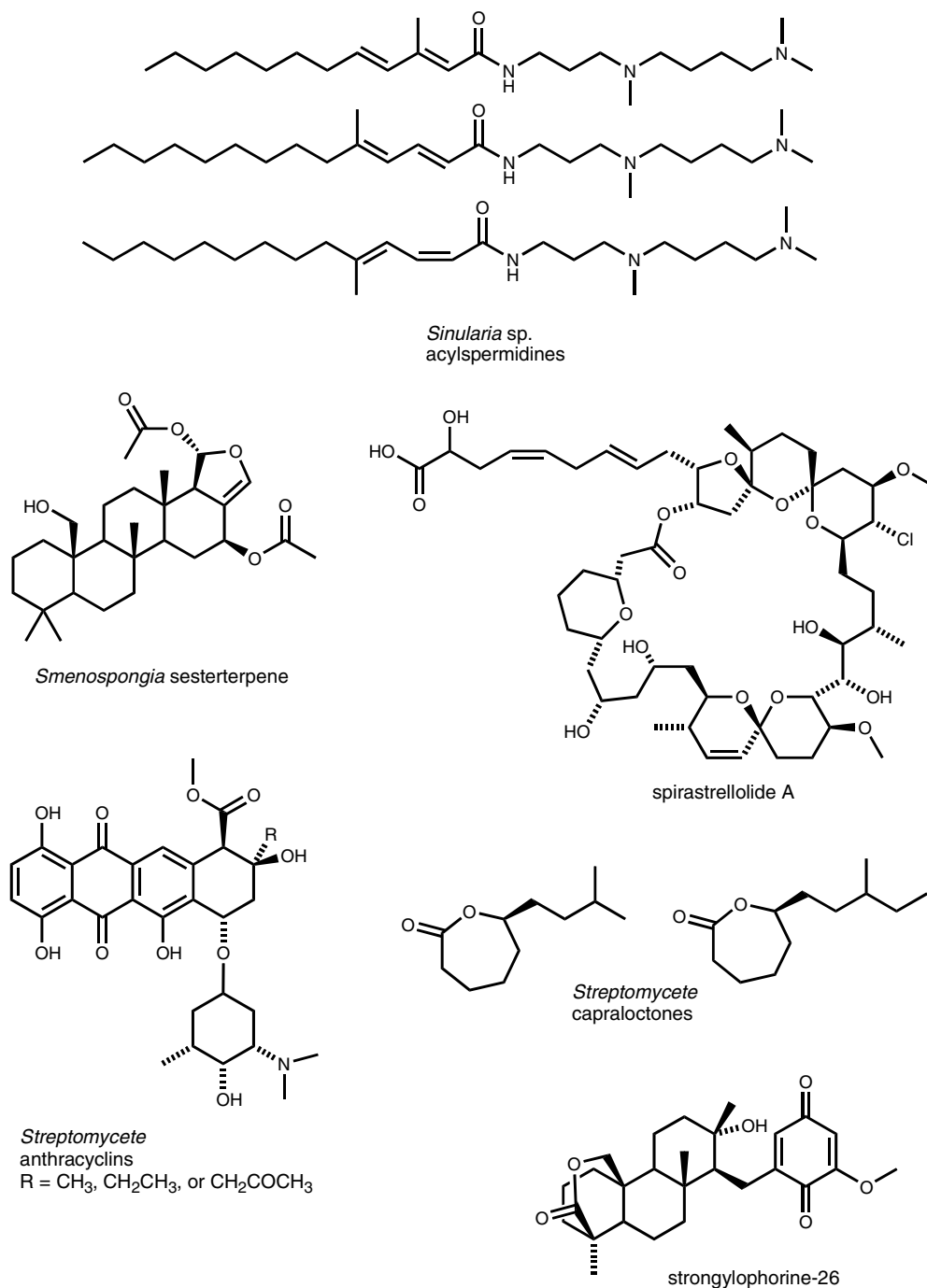


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for extensive *in vitro* studies with 18 human tumour cell lines which demonstrated the analogue potently inhibited proliferation in the nM range, depolymerised microtubules and overcame 'P-glycoprotein-mediated resistance to paclitaxel or vincristine 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 colleagues⁴⁸ extended the preclinical pharmacology of **kahalalide F**, a naturally occurring depsipeptide isolated from the Hawaiian herbivorous marine mollusc *Elysia rufescens* 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 $IC_{50} < 0.3 \mu M$. There was concomitant rapid and severe cytoplasmic 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

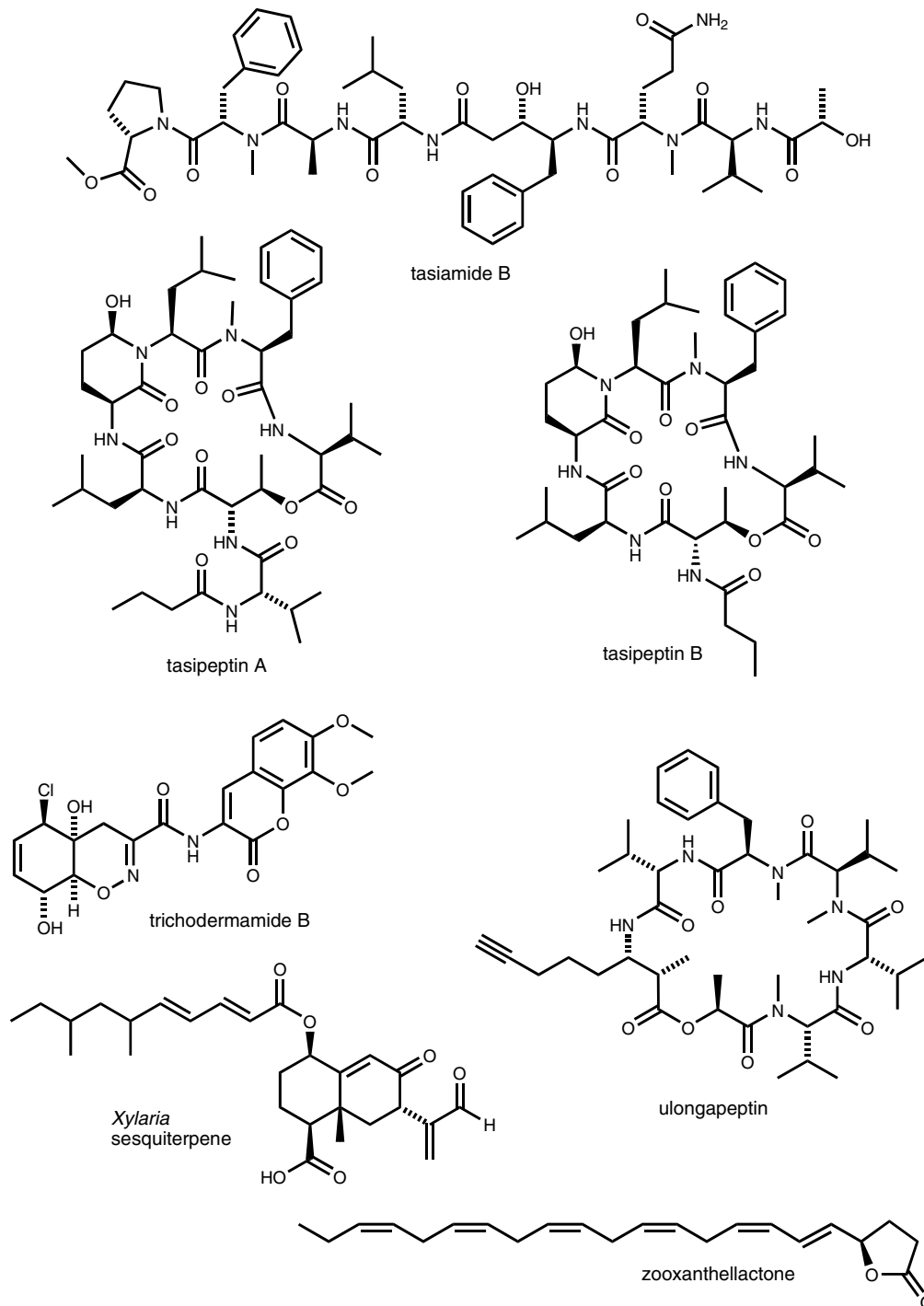


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sponge *Xestospongia exigua* was reported by McHardy and colleagues.⁴⁹ Dihydromotuporamine C, an analogue resulting from a comparative structure-activity study, lacks the double-bond in the non-polar group and caused inhibition of tumour cell invasion 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 colleagues⁵⁰ established that peloruside 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
Aerophysinin-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	[¹²⁵ I]-azido agosterol A photolabelled PGP N-terminal fragment with high affinity in absence of glutathione	JAPN	[15]
			MRP1-transfected pig kidney cells	[¹²⁵ I]-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]
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	Potential of ara-C induced apoptosis by PKC-dependent release of TNF- α	USA	[28]
Cambrescidin 800	Sponge	Alkaloid	HU leukaemia cell line	Induction of erythroid 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	<i>In vitro</i> 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
Dehydrothryserol	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 ($K_d \approx 30 \mu\text{M}$) 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 <i>in vivo</i> human tumour xenografts	Induction of microtubule depolymerisation; low P-glycoprotein resistance <i>in vitro</i> and <i>in vivo</i>	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	Depsideptide	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 II α -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 apoptosis in ras-transformed cells	NZEL	[51]
Psammaplin A	Sponge	Alkaloid	HU & MU tumour cell lines	Inhibition of aminopeptidase N and suppression of angiogenesis <i>in vitro</i>	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
<i>Actinomadura</i> sp. Xanthone	Bacterium	Xanthone	HU & MU	0.001 μ M	SPA	[104,105]
Amphidinolide X	Alga	Macrolide	HU & MU	0.6–7.5 μ g/ml	JAPN	[106]
Amphidinolide Y	Alga	Macrolide	HU & MU	0.8–8 μ g/ml	JAPN	[107]
Andavadoic acid	Sponge	Fatty acid	HU & MU	0.1–0.7 μ M	SPA, FRA	[108]
Aurilide	Sea hare	Depsipeptide	NCI 60-cell line panel	0.011 μ g/ml	JPAN	[109]
<i>Axinella</i> cf. <i>bidderi</i> sterol	Sponge	Steroid	HU & MU	0.60 μ g/ml	SPA, FRA	[110]
Bistratamide J	Ascidian	Peptide	HU	1 μ g/ml	USA	[111]
Bromovulone III	Octocoral	Prostanoid	HU	0.5 μ g/ml	S. KOR	[112]
Caulibugulones A–F	Bryozoan	Quinone	MU	0.03–1.67 μ g/ml	USA	[113]
Certonardosterol	Seastar	Steroid	HU	0.01–>1 μ g/ml	S. KOR	[114]
Certonardoside	Seastar	Steroid	HU	0.26–>1 μ g/ml	S. KOR	[114]
<i>Certonardoa semiregularis</i> sterol	Starfish	Steroid	HU	0.12–0.48 μ g/ml	S. KOR	[115]
<i>Cladobotryum</i> sp. cyclodepsipeptide	Fungus	Depsipeptide	MU	0.14 μ M	NZEL	[116]
Cribrostatin 6	Sponge	Quinone	HU & MU	0.29–>1 μ g/ml	USA	[117]
<i>Dasystemella acanthina</i> steroid	Octocoral	Steroid	HU	0.9 μ g/ml	SPA	[118]
13-Epi-9-deacetoxyxenicin	Soft Coral	Diterpene	MU	0.1 μ g/ml	AUS	[119]
Dehydrocyclostelletamine D	Sponge	Alkaloid	HU & MU	0.6–4.3 μ g/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 μ M	USA, SWI	[121]
Discorhabdins C, D analogues	Sponge	Alkaloid	HU	0.119–0.232 μ M	N.ZEL, S.AFR, USA	[122]
Discorhabdins I & L	Sponge	Alkaloid	HU	0.12–0.35 μ M	SPA	[123]
Discorhabdins S, T & U	Sponge	Alkaloid	HU & MU	0.069–5 μ M	USA	[124]
Dolastatin 19	Sea Hare	Macrolide	HU	0.72–0.76 μ g/ml	USA	[125]
Gymnangiamide	Hydroid	Peptide	HU	0.46–11 μ g/ml	USA	[126]
Halichondramides	Sponge	Macrolides	HU	0.38–0.90 μ g/ml	S. KOR, USA	[127]
Haouamine A	Ascidian	alkaloid	HU	0.1 μ g/ml	SPA	[128]
22-hydroxyhalicyclamine A	Sponge	Alkaloid	MU	0.45 μ g/ml	JAPN, NETH	[88]
Intercedenside A & B	Sea cucumber	Triterpene glycoside	HU	0.61–4.0 μ g/ml	CHI, USA	[129]
<i>Ircinia</i> sp. furanosesterterpenes	Sponge	Sesterterpene	HU	<0.1 μ g/ml	JAPN	[130]
Irciniastatins A & B	Sponge	Polyketide	HU & MU	0.0001–0.0041 μ g/ml	AUS, USA	[131]
Isogeoditin A & B	Sponge	Triterpene	HU	0.07–3.7 μ g/ml	CHI, NETH, GER	[132]
Jaspine B	Sponge	Alkaloid	HU	0.24 μ M	FRA	[133]
Lasonolide C & E	Sponge	Macrolide	HU	0.13–0.57 μ g/ml	USA	[134]
Lingshuiol	Alga	Polyketide	HU	0.21–0.23 μ M	CHI	[135]
Microcionamides A & B	Sponge	Peptide	HU	0.098–0.177 μ M	PHIL, USA	[136]
Micromide	Bacterium	Alkaloids	HU	0.26 μ M	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]
<i>Myrothecium verrucaria</i> 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]
<i>Synularia</i> acylspermidines	Soft coral	Fatty acid	HU	0.017 µg/ml	JAPN	[159]
<i>Smenospongia</i> sp. Sesterterpene	Sponge	Sesterterpene	HU	0.02 µg/ml	S. KOR	[160]
Spirastrelloide A	Sponge	Macrolide	HU	0.1 µg/ml	CAN, NETH	[161]
<i>Streptomyces</i> sp. capralactones	Fungus	Fatty acid	HU	0.11–2.7 µg/ml	GER	[162]
<i>Streptomyces</i> -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]
<i>Xylaria</i> 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 colleagues⁵¹ determined that peloruside A was more cytotoxic to *ras* oncogene-transformed cells than non-transformed cells, blocking the cells in G₂/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, psammoplin A and smenospongoringe.

Aoki and colleagues⁵² 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 colleagues⁵³ showed that **cephalostatin 1**, a bis-steroidal 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 colleagues⁵⁴ 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 colleagues⁵⁵ 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 binding sites' or if this marine peptide bound weakly to unpolymerised tubulin yet bound 'strongly to microtubule ends'.

Isbrucker and colleagues⁵⁶ 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 G₂/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 colleagues⁵⁷ contributed a preclinical pharmacological study on **girolline**, a 2-aminoimidazole derivative originally isolated from the marine sponge *Peudaxinyssa cantharella*. Girolline exhibited G₂/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 G₂ 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 colleagues⁵⁸ 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 G₂ checkpoint as potentially valuable agents for enhancing the effectiveness of DNA-damaging agents in tumours with mutated p53, Jian and colleagues⁵⁹ 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 G₂ checkpoint inhibition, and that concomitant inhibition of the DNA damage response Chk1 protein kinase (IC₅₀ = 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 colleagues⁶⁰ 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 ($IC_{50} = 0.4 \mu\text{M}$) probably as a result of blocking the induction of nuclear HIF-1 α 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 Ireland⁶² 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 $2.4 \mu\text{M}$ lissoclinolide strongly arrested the G₂/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 colleagues⁶³ 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 II α (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 anti-cancer potential'.

Two papers extended the pharmacology of the marine bromotyrosine derivative **psammaphin A**, isolated from a two-sponge association between, *Poecillastra* sp. and *Jaspis* sp. Shim and colleagues⁶⁴ showed that psammaphin A inhibited aminopeptidase N (APN) ($IC_{50} = 18 \mu\text{M}$) 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 psammaphin A's inhibition of APN activity suggests a potentially novel approach to prevent angiogenesis-related diseases. Furthermore, Jian and colleagues⁶⁵ noted that psammaphin 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 colleagues⁶⁶ 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 G₁ 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 $\leq 1.0 \mu\text{g/ml}$) 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 stronglyphorine-26;⁶⁷ induction of apoptosis by scleritodermin A⁶⁸ and ritterazine B,⁶⁹ inhibition of histone deacetylase enzyme by dehydrocyclostelletamine D;⁷⁰ inhibition of proteasomal chymotrypsin-like proteolytic activity by salinosporamide A⁷¹ 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 $>1\text{--}4.0 \mu\text{g/ml}$ 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*-smenospongine.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 colleagues¹⁰³ 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.

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