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Review

Apoptosis inducing lead compounds isolated from marine organisms of potential relevance in cancer treatment

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ABSTRACT

Apoptosis is a critical defense mechanism against the formation and progression of cancer and exhibits distinct morphological and biochemical traits. Targeting apoptotic pathways becomes an intriguing strategy for the development of chemotherapeutic agents particularly if the process is selective to cancer cells. Marine natural products have become important sources in the discovery of antitumour drugs, especially when recent technological and methodological advances have increased the scope of investigations of marine organisms. A high number of individual compounds from diverse organisms have induced apoptosis in several tumour cell lines *via* a number of mechanisms. Here, we review the effects of selected marine natural products and their synthetic derivatives on apoptosis signalling pathways in association with their pharmacological properties. Providing an outlook into the future, we also examine the factors that contribute to new discoveries and the difficulties associated with translating marine-derived compounds into clinical trials.

Contents

Abbreviations: MNPs, marine natural product; FDA, Food And Drug Administration; TNFR, tumour necrosis factor receptor; TRAIL, TNF-related apoptosis inducing ligand; FasL, Fas ligand; FADD, Fas-associated protein with death domain; RIP1, receptor-interacting protein 1; ROS, reactive oxygen species; Apaf-1, apoptotic protease activating factor 1; Bid, BH3 interacting-domain; Bak, BCL2 antagonist/killer; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Bcl-XL, B-cell lymphoma-extra large; Mcl-1, myeloid cell leukaemia sequence 1; Bim, B-cell lymphoma 2 interacting mediator; Puma, p53 upregulated modulator of apoptosis; PI3K, phosphatidylinositol 3 kinase; AKT, serine/threonine-specific protein kinase; ET-743, Ecteinascidin-743; STS, soft tissue sarcoma; NER, nucleotide excision repair; Smac, second mitochondria-derived activator of caspases; DIABLO, IAP binding protein with low pI; PARP, poly ADP ribose polymerase; GTP, guanosine-5′-triphosphate; NCI, National Cancer Institute; MAPKs, mitogen-activated protein kinases; JNK, Jun N-terminal kinases; VEGF, vascular endothelial growth factor; KF, Kahalalide F; Her2/neu, human epidermal growth factor receptor 2; NSCLC, non-small-cell lung carcinoma; PC-3, prostate cancer-3 cell line; SKBR3, breast cancer cell line; ERBB3, receptor tyrosine-protein kinase erbB-3 enzyme; COX-2, cyclooxygenase-2; c-Myc, c-myelocytomatosis; cflip, FADD-like IL-1β-converting enzyme-inhibitory protein; TRAF1, TNF receptor-associated factor 1; IAP, inhibitors of apoptosis; Endo G, endonuclease G; HtrA2/Omi, mitochondrial serine protease; XIAP, X-linked inhibitor of apoptosis protein; MMP-9, matrix metallopeptidase 9; ICAM-1, intercellular adhesion molecule 1; MCF-7, breast cancer cell line; AIF, apoptosis inducing factor; NMR, nuclear magnetic resonance.

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1. Introduction

Cancer is a disease of worldwide importance. Its incidence in the developed countries is rising and its mortality occupies second rank in the order of cause for death. About 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008 worldwide [1]. According to Dr. Lee Jong-Wook, former Director General of the WHO, "*by the year 2020, cancer could kill more than 10.3 million people per year unless action is taken in both the field of prevention and treatment*." Cancer results from a multi-stage and multi-mechanism process consisting of initiation, promotion, and progression phases. Epigenetic changes and in particular aberrant promoter hypermethylation associated with inappropriate gene silencing contribute significantly to the initiation and progression of human cancer [2]. Currently available anticancer treatments such as ionising radiation, hyperthermia, alkylating agents, DNA topoisomerase inhibitors and platinum compounds induce DNA damage indiscriminately killing both normal and rapidly proliferating cancer cell. Since these drugs are not specifically selective, cancer patients suffer from adverse side effects including nausea, anaemia, fatigue, hair loss and infections [3].

These cytotoxic anticancer treatments are currently being supplemented by targeted therapies to improve treatment efficacy and reduce side effects. Targeted therapies include apoptosis-inducers, angiogenesis inhibitors, signal-transduction inhibitors, monoclonal antibodies and gene therapy [4]. Apoptosis-inducers, in particular chemopreventive agents can be used to induce cell death *via* genes and proteins that control apoptosis, since tumour-specific alterations in apoptotic programmes provide opportunities to target cell death in a selective manner [5]. Chemoprevention as first defined by Sporn in 1976, entails the use of specific natural products or synthetic chemical agents to reverse, suppress, or prevent premalignancy before the development of invasive cancer [6]. This approach is particularly of interest since it is based on the concepts of multifocal field carcinogenesis and multistep carcinogenesis [7]. Several epidemiological and experimental studies have reported the apoptotic effect of natural products including sulphoraphane [8,9], resveratrol [10,11], genistein [12,13], cucurmin [14,15], and caffeic acid phenyl ester [16,17] in the containment of carcinogenesis. Hence, since natural products can modulate apoptosis pathways in human cancers, these compounds may provide novel opportunities for cancer drug development.

Over the last two decades, thousands of bioactive natural products have been isolated from nature for biomedical research. Most of these natural compounds, especially those from edible and natural sources such as fruits, vegetables and plants, have long been an attractive source of drug molecules [18] mainly because of their easy availability and also folk traditions have described beneficial effects from their uses [19]. Even today natural compounds remain a high-output source of promising chemotherapeutic or chemopreventive agents with substantial numbers of anticancer agents used in the clinic being either natural or derived from natural products from various sources such as plants, animals and microorganisms. Indeed, almost 60% of drugs approved for cancer therapy are of natural origin: vincristine (VCR), irinotecan etoposide, taxanes and campthotecins are all examples of plant derived anticancer agents while actinomycin D, mitomycin C, bleomycin, doxorubicin and L-asparaginase are drugs isolated from microbial sources [20]. However, in contrast to the above described terrestrial sources of anticancer agents, a still almost completely unexplored potential source of bioactive natural agents is represented by the sea [19]. Although oceans have attracted the attention of researchers since the 1950s with the discovery of the *Cryptotheca crypta* sponge-derived nucleosides spongothymidine and spongouridine, the technical difficulties of collecting marine organisms together with the poor knowledge of their habitat have posed relevant obstacles. Nevertheless, the implementation of scuba diving tools and the development of instruments for the isolation of natural products from marine organisms have rejuvenated research in the area of marine natural product [21–23].

1.1. Marine natural product

The world's ocean, covering 70% of the earth's surface and 95% of its tropical biosphere represents an extraordinarily rich source of chemical and biological diversity, surpassing that of the terrestrial diversity [24,25]. It is home to nearly one million multicellular (plants and animals) and one billion unicellular organisms [26]. Particularly, the marine reef is a highly crowded ecosystem where more than 1000 species are sheltered per m^2 and live side by side in fierce competition for the limited space and nutrients [25]. Living in such a highly competitive environment, marine organisms have evolved biochemical and physiological mechanisms that include the production of secondary metabolites that are involved in ecological processes such as reproduction, communication and protection against predation, infection and competition. Light, water current, and temperature represent additional growth limiting components, further fueling competition [27]. Sessile and soft bodied marine invertebrates like sponges, bryozoans, tunicates, mostly lacking morphological defense structures usually depend heavily on secondary metabolites that are toxic or objectionable to their competitors for survival [24]. These chemical adaptations generally take the form of so called "marine natural product" (MNPs) that involve such well known chemical classes as terpenoids, alkaloids, polypeptides, peptides, shikimic acid derivatives, sugars, steroids, and a multitude of mixed biogenesis metabolites [28]. In addition, and unique to the marine environment, these metabolites possess structural features which are distinct from their terrestrial counterparts due to their frequent incorporation of halogen atoms from the surrounding sea water [29]. Therefore, it is clear that the marine environment represents an important source of unknown natural compounds whose medicinal potential must be evaluated.

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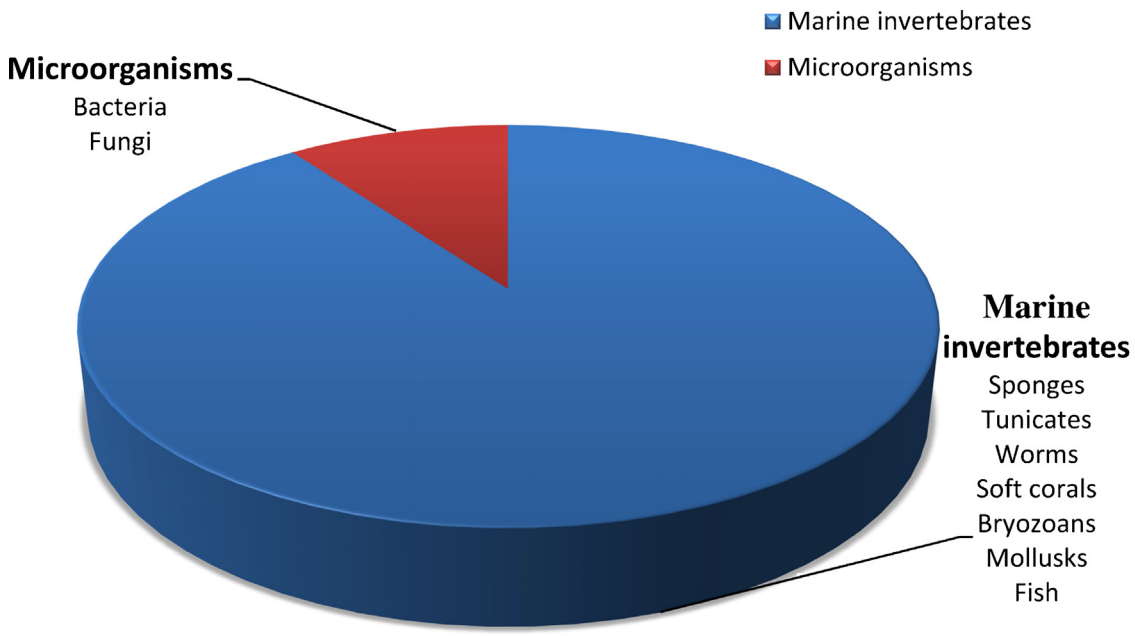

Fig. 1. Marine-derived drugs and clinical trial agents divided by their subsequently shown or predicted source organisms.

Although this "silent world" has a much richer biodiversity than that of the terrestrial areas, efforts to exploit marine organisms for drug discovery is still at a relatively early stage. Nevertheless, extensive research in the past 30–40 years on MNPs have been very productive and has led to the discovery of many potently active agents worthy of further clinical applications. A small number of marine plants, animals and microbes have already yielded more than 22,000 novel natural products (compared to 131,000 from terrestrial sources) [30]. A large proportion of these natural products have been extracted from marine invertebrates (Fig. 1) which not only produce a great number of marine natural products currently known but also show the largest diversity of natural products including alkaloids, peptides, terpenes and polyketides, and others [31].

Besides their chemical novelty, numerous *in vitro* and *in vivo* studies have demonstrated a broad spectrum of biological activities of these compounds with novel mode of actions such as cytotoxic, anti-cancer, anti-inflammatory and antimicrobial. Additionally, their low effective dosage, better selectivity against target malignant tissues and relative non-vulnerability against resistance development as compared to compounds of terrestrial origin, render them useful target molecules [32]. Interestingly, despite the technological challenges in characterising and scaling up production of bioactive compounds from marine organisms some 13 marine derived biologically active agents are presently in clinical trials, 11 with an indication for cancer, one for cognition and schizophrenia, one for Alzheimer's (along with cancer), and one for wound healing [33]. Thus, the pipeline of promising marine derived or inspired agents is very strong, and we will certainly see several of these agents entering the pharmacological market in the coming years.

Within the sphere of cancer, research has revealed promising compounds isolated from marine sources with proven anticancer activity. This includes the anticancer drug Ara-C (cytarabine) which is the first drug from the sea, in use since 1970s and which has been isolated from the sponge *C. crypta*. It is a synthetic material based on the 'lead structure' provided by an unusual nucleoside isolated from this sponge in the 1950s. The second drug from the oceans to be approved for clinical use in recent times comes from a tropical sea-squirt-the Carribean tunicate *Ecteinacidia turbinata.* This compound, Trabectedin (Yondelis/Ecteinascidin-743) was approved by the European Union in 2007 for the treatment of soft-tissue sarcoma. The latest marine drug is Halaven® (eribulin mesylate), an analogue of the sponge metabolite halichondrin B derived from the sponge *Halichondria* sp. which has been approved in November 2010 for the treatment of advanced metastatic cancer. In addition, several more compounds such as bryostatin, didehydrodidemnin, dolastatins, kahalalide, and others are currently at different stages of clinical trials. Growing evidence indicates that these drugs function by targeting signalling intermediates in apoptosis-inducing pathways, which appears to be associated with their effectiveness in modulating the process of carcinogenesis [19,23,33].

2. Apoptosis pathways as therapeutic targets in cancer therapy

Apoptosis as a form of programmed cell death is one of the major mechanisms of cell death in response to cancer therapies [34]. It is also a naturally occurring and evolutionarily conserved process by which no longer useful cells are directed to their deaths [35]. Apoptosis plays an indispensable role and is a fundamental process in development, physiology and homeostasis [36–39]. Its deregulation however, can lead to diverse pathological conditions such as cancer initiation, promotion and progression or results in treatment failures [40,41]. As apoptosis does not usually trigger inflammatory or immune response, it is a preferable way of cancer cell death during cancer treatments. As such, modulation of apoptotic pathways and selective induction of apoptosis by natural products are likely to be a promising approach for cancer therapy [42–45,30,46].

Apoptosis is mediated by the activation of different caspases, a family of enzymes that act as death effector molecules in various forms of cell death [34,47]. In mammals, there are two major signalling systems that result in the activation of caspases, *i.e.* the extrinsic death receptor pathway and the intrinsic mitochondrial pathway (Fig. 2) [48–51]. Increasing evidence indicates that these two pathways are not isolated systems but, instead, there are many crosstalks between them.

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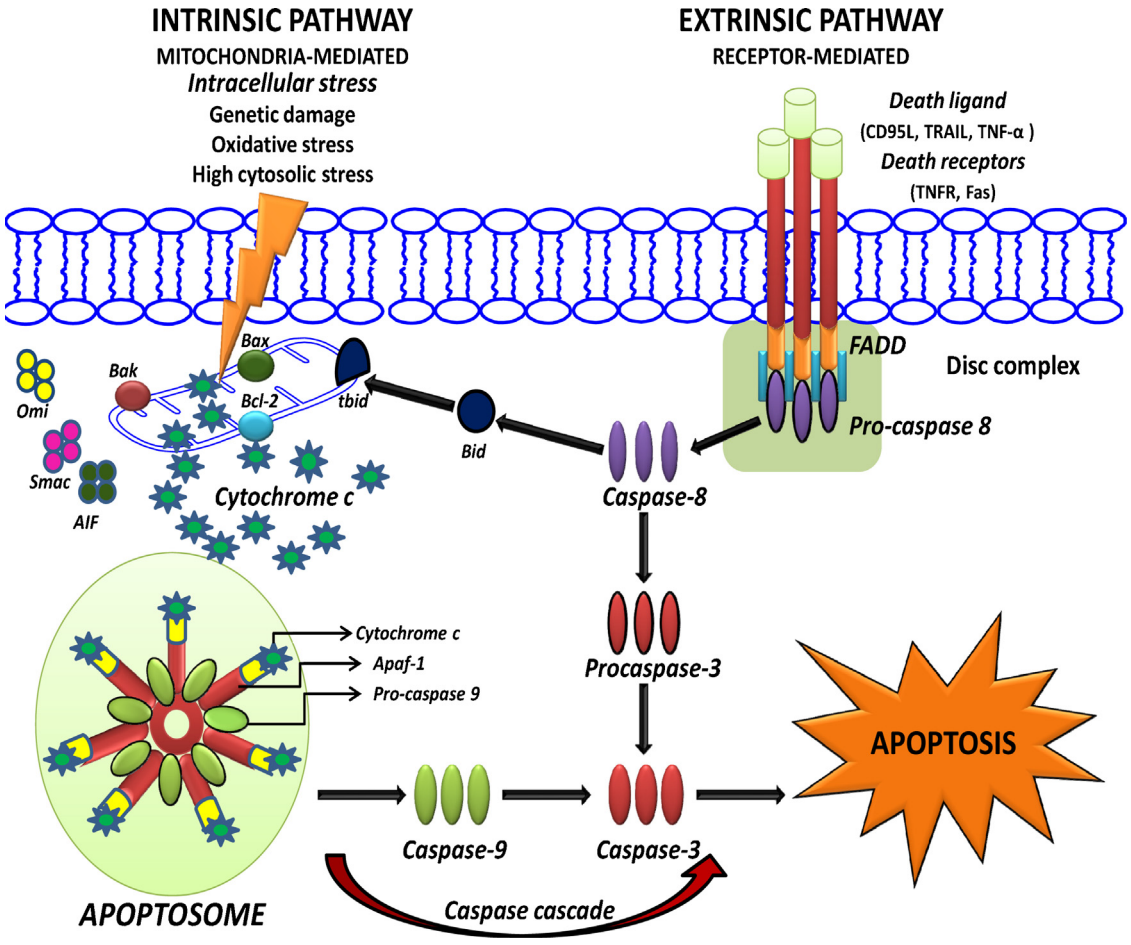

Fig. 2. Extrinsic and intrinsic pathways involved in apoptosis. Apoptotic cell death can be induced through the extrinsic (also called receptor-mediated) or the intrinsic (also called mitochondria-mediated) signalling pathways. The extrinsic pathway involves ligation of death receptors with their ligands resulting in a sequential activation of caspase-8, and -3, which cleaves target proteins leading to apoptosis. This pathway is negatively controlled by the anti-apoptotic proteins c-FLIP and XIAP that inhibit activation of caspase-8 and caspase-3, respectively. Intrinsic death stimuli, *e.g.* ROS, DNA-damaging reagents, or Ca2+ mobilisation directly or indirectly activate the mitochondrial pathway by inducing release of cytochrome c and formation of the apoptosome, composed of Apaf-1 and caspase-9. Caspase-9 is activated at the apoptosome and, in turn, activates pro-caspase-3. This death pathway is largely controlled by the proapoptotic (*e.g.* Bax, Bak, Bid and Smac/DIABLO) and anti-apoptotic (*e.g.* Bcl-2, Bcl-xL, Mcl-1 and XIAP) proteins. Caspase-8 may also induce cleavage of Bid, which induces the translocation of Bax and/or Bak to the mitochondrial membrane and amplifies the mitochondrial apoptosis pathway.

2.1. Extrinsic pathway

The extrinsic pathway of apoptosis is activated at the cell surface when a specific ligand binds to its corresponding cell surface death receptor. Death receptors (*e.g.* tumour necrosis factor receptor [TNFR], TNF-related apoptosis inducing ligand [TRAIL] receptor, and Fas) belong to the TNFR superfamily. After ligand binding (*e.g.* TNF, TRAIL, and FasL, respectively), death receptors cluster in the plasma membrane and promote the recruitment of adapter proteins. Caspase 8 is an apical caspase in the death receptor pathway. The zymogen of caspase 8 can interact with the adapter proteins (*e.g.* FADD and RIP1) to generate the active form of caspase 8. After activation, caspase 8 can trigger the activation of downstream effector caspases such as caspase 3 which cleaves target proteins leading to apoptosis [52,53].

2.2. Intrinsic pathway

Intrinsic death stimuli, *e.g.* reactive oxygen species (ROS), DNA-damaging reagents, and Ca^{2+} mobilising stimuli directly or indirectly activate the mitochondrial pathway resulting in the release of cytochrome c and the formation of the apoptosome complex consisting of cytochrome c, Apaf-1 and caspase-9 [54–56]. Caspase-9 is activated at the apoptosome and in turn activates caspase-3 [57]. Between the receptor and the mitochondrial signalling pathways the pro-apoptotic protein Bid serves as a crosstalker upon cleavage by activated caspase-8 by inducing the translocation of the pro-apoptotic proteins Bax and/or Bak to stimulate mitochondrial outer membrane permeabilisation. Thus, Bid links the receptor to the mitochondrial pathway and can initiate a mitochondrial amplification loop upon its caspase-mediated proteolytic processing [58].

There are various intervention points that control cell death pathways, since inappropriate induction of apoptosis may have detrimental effects on the cell's survival [59]. For example, pro and anti-apoptotic proteins of the Bcl-2 family play an important role in cell viability more specifically in the regulation of the mitochondrial pathway. The anti-apoptotic Bcl-2 family members comprise, *e.g.* Bcl-2, Bcl-XL and Mcl-1, while the multidomain proteins Bax and Bak and BH3 domain-only proteins such as Bid, Bim, Noxa and Puma belong to the pro-apoptotic molecules [60]. The ratio of anti-apoptotic *versus* pro-apoptotic Bcl-2 family proteins rather than the expression of one single family member is considered to control apoptosis sensitivity. These anti apoptotic control points that prevent accidental cell death under physiological conditions are often deregulated in cancers and may confer drug resistance

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Table 1
The odyssey of marine pharmaceuticals in the field of cancer research: A current pipeline perspective.

Compound name	Natural product derivative	Collected source organism	Class of agent	Molecular target	Clinical status	References
Cytarabine	Derivative (arabinosyl cytosine)	*Tethya crypta* (sponge)	Nucleoside	DNA polymerase	FDA Approved	[23,33]
Trabectedin (ET-743)	Natural Product (Ecteinascidin-743)	*Ecteinascidia turbinata* (tunicate/possible bacterial source)	Alkaloid	Tubulin	FDA Approved (EU registered only)	[33,67,79]
Eribulin mesylate (E7389)	Derivative (halichondrin B)	*Halichondria okadai* (sponge)	Macrolide	Tubulin	FDA Approved	[33,83,85]
Soblidotin	Derivative (dolastatin 10)	*Dolabella auricularia*/*Symploca* sp. (mollusc/cyanobacterium)	Peptide	Tubulin	Phase III	[93,97]
Plitidepsin (Aplidine)	Natural product (dehydrodidemnin B)	*Tridemnum solidum* (tunicate/possible bacterial or cyanobacterial source)	Cyclic depsipeptide	Rac1 and JNK activation	Phase II	[102,107,101]
Cemadotin	Derivative (dolastatin 15)	*Dolabella auricularia*/*Symploca* sp. (mollusc/cyanobacterium)	Linear peptide	Tubulin	Phase II (discontinued)	[29]
Plinabulin (NPI 2358)	Derivative (halamide)	*Aspergillus* sp. cultured from alga *Halimeda lacrimosa* (fungus)	Diketo-piperazine	Tubulin and JNK stress protein	Phase II	[23,33]
Elisidepsin	Derivative Kahalalide F	*Elysia rufescens*/*Bryopsis* sp. (mollusc/green alga)	Depsipeptide	Lysosomes/erb Pathway	Phase II	[33,110,117]
Zalypsis (PM00104)	Derivative (jorumycin)	*Joruna funebris* (nudibranch)	Alkaloid	DNA binding	Phase II	[23,33]
Marizomib (NPI 0052)	Natural Product (salinosporamide-A)	*Salinospora* sp. (bacterium)	Bicyclic γ-lactam-β lactone	20S Proteosome	Phase I	[137,138]
Hemiasterlin (HTI-286)	Derivative (hemiasterlin)	*Hemiasterella minor* (sponge)	Tripeptide	Tubulin	Phase I	[131,134,136]
Bryostatin 1	Natural product	*Bugula neritina* (bryozoan)	Polyketide	Protein kinase C	Phase I	[121,124]
Spongistatin 1	Natural product	*Spirasterella* sp. (sponge)	Macrolide	Tubulin	Preclinical	[148,151]
Disco-dermolide	Natural product	*Discodermia dissoluta* (sponge)	Polyketide lactone	Tubulin	Preclinical (in drug combination therapy)	[153,156]
Ascididemnin B	Natural product	*Didemnum* sp. (ascidian)	Aromatic alkaloid	Caspase 2/mitochondria	Preclinical	[29,46]
Lamellarin D	Natural product	*Lamellaria* sp. (mollusc and various soft coral)	Pyrrole alkaloid	Topoisomerase/mitochondria	Preclinical	[29]

[61]. Therefore, inhibition of Bcl-2 or induction of Bax becomes a good strategy for triggering an apoptotic process [62]. Moreover, since caspases are involved in both intrinsic and extrinsic apoptosis pathways, identification of caspase activators becomes another approach for the discovery of novel anticancer agents [63,64]. The phosphatidylinositol 3-kinase/threonine-specific protein kinase (PI3K/AKT) signalling pathway plays a critical role in many cellular functions and blocking the PI3K/AKT signal transduction network could also be an effective strategy for targeted anticancer therapy [65]. Hence by modulating and improving physiological functions, bioactive marine natural products may provide new therapeutic applications for the prevention and/or treatment of cancer.

In short, apoptosis is a very complicated process and involves a huge number of signalling molecules, and failure of apoptosis activation is one of the major impediments to the treatment of cancer. Therefore, a good strategy for the development of new anticancer agents is to identify or develop such agents that can target multiple apoptosis-regulating genes. Over the last two decades thousands of marine natural products exhibiting anticancer activity through multiple mechanisms have been isolated, and those having an effect *via* apoptosis induction are of particular interest to us. As numerous comprehensive overviews on compounds from marine origin exist, our focus will be only on those MNPs or their synthetic derivatives that are either already in clinical trials or are promising experimental drugs reported to affect apoptotic pathways in tumour cells. In this review we therefore describe the ten most promising apoptosis inducing marine agents in order of their clinical activities rather than by source or chemical class, and in order to aid the reader, we have shown in Table 1 their relevant marine sources, class of agent, molecular target and trial level achieved to date.

2.3. Ecteinascidin-743 (ET-743)

Ecteinascidin-743 (ET-743), a marine natural product isolated from the Carribean tunicate *Ecteinascidia turbinata* illustrates a significant milestone in the development of marine derived drugs. Almost 40 years after its discovery and 17 years after the publication of its structure, it became the first marine-derived anticancer agent approved in the European Union for patients with soft tissue sarcoma (STS) and patients with relapsed platinum-sensitive ovarian cancer [66,67]. The potent cytotoxicity of the tunicate extracts

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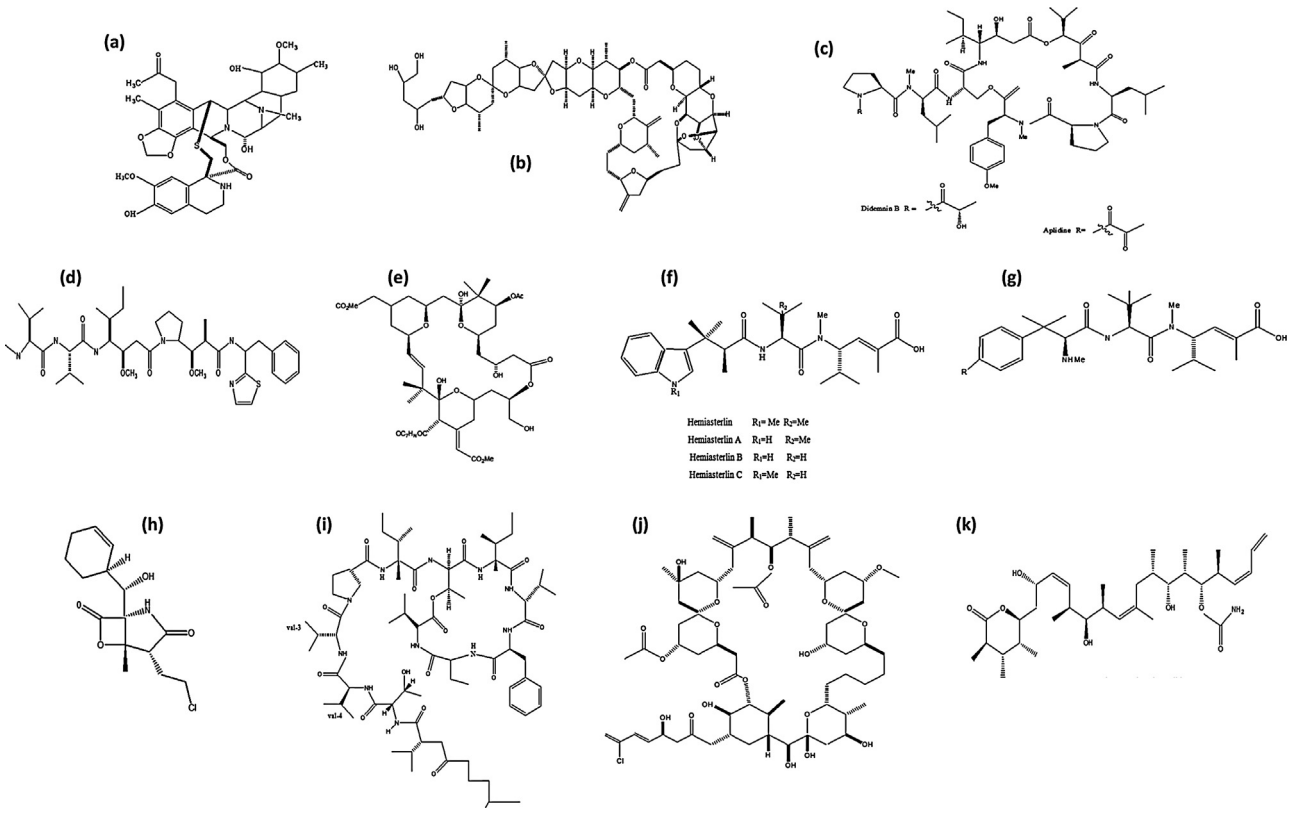

Fig. 3. Chemical structures of some anti-cancer compounds isolated from marine organisms: (a) Ecteinascidin-749, (b) halichondrin B, (c) didemnin B, (d) dolastatin 10 (e) bryostatin 1, (f) hemiasterlins, (g) HTI-286, (h) salinosporamide A (i) Kahalalide F, (j) spongistatin 1, and (k) discodermolide.

was first reported by Sigel et al. in 1969. However, the purification of the active compound was not completed until 1990 [68]. ET-743 comprises three tetrahydroisoquinoline rings connected by a thioether bridge (Fig. 3a). To compensate for the scarcity of availability from the natural source, the compound is obtained by chemical synthesis starting from safracin B cyano which is produced in bulk through fermentation of the marine bacterium *Pseudomonas fluorescens* [69].

Preclinical studies of ET 743 have shown high *in vitro* cytotoxic activity at low doses (0.1–1 ng/ml) against a number of tumour cell lines including melanoma, non-small cell lung, ovarian, renal, prostate, and breast cancers [70,71]. The cytotoxic compound has also shown strong *in vivo* antitumour effects in various mice models bearing P388 lymphoma, B16 melanoma, M5076 ovarian sarcoma, Lewis and LX-1 human lung carcinoma and MX-1 human mammary carcinoma xenografts [68,70]. Moreover, ET 743 combination treatments with doxorubicin [72], paclitaxel [73], or irinotecan [74] against sarcomas revealed synergistic effects between the drugs.

Although many objective responses have been observed in clinical tumours, the precise details of the mechanism of action of ET-743 have been difficult to pinpoint. Early studies have shown that ET-743 binds reversibly to the minor groove of DNA [75] thereby altering the normal function of DNA repair and transcription processes, resulting in arrest of proliferation, differentiation, and cell death. ET743 is a selective transcription inhibitor, which has the unique characteristic of poisoning transcription-coupled nucleotide excision repair (NER) system [76–78]. However, in contrast to other bulky adducts such as those caused by other alkylating agents like cisplatin in the case of ET-743, the NER kills the cell rather than repairing the damage caused by the adducts [76]. Moreover, cell cycle studies on tumour cells reveal that ET-743 arrests at G2/M and the apoptotic response is independent of p53 [79].

Nevertheless, though the compound has been described as a strong apoptotic agent, information on its apoptotic mechanism is not well defined in cancer cells. A recent report describes ET-743 as a strong apoptotic agent that induced apoptosis by activating the extrinsic and intrinsic pathways in breast cancer cell lines in a time and dose dependent manner. The apoptotic pathway involved selective activation of death receptor pathway molecules such as TRAIL-R1/DR4, TRAIL-R2/DR5, FAS/TNFRSF6, TNF RI/TNFRSF1A, and FADD and mitochondrial pathway related pro-apoptotic including proteins Bax, Bad, Cytochrome c, Smac/DIABLO, and Cleaved Caspase-3. Moreover, the expression levels of anti-apoptotic proteins Bcl-2 and Bcl-XL were also reduced significantly [80].

Based on its interesting *in vitro* and *in vivo* results, ET-743 has been approved by the European Commission in 2007 and developed by PharmaMar under the trade name Yondelis and generic name Trabectedin for the treatment of STS and ovarian cancer [33]. Concluded Phase I and Phase II studies revealed that ET 743 has remarkable antitumour activity against solid tumours in particular breast cancer and renal carcinoma and STS [81]. Regarding its safety profile, the most frequent adverse event appears to be neutropenia which is reversible and transaminase elevations which were also transient. No mucosis, alopecia, neurotoxicity, cardiotoxicity or cumulative toxicities have however been observed [81]. Currently ET-743 is in a variety of Phase II trials in the United States and Europe against other cancer conditions such as breast, lung, prostate and paediatric cancer and Phase III trials for first-line therapy in STS [33]. ET-743 combination drug therapy has been recommended for further investigation in humans [81,82].

2.4. Halichondrin B

The halichondrins are a family of polyether macrolides that were originally isolated in small quantities and reported by Uemura and

coworkers in 1985 from the rare Japanese sponge *Halichondria okadai* [83]. Subsequently, a number of sponges from the Pacific and Indian Oceans were also reported to contain one or more of these macrolides including *Axinella* sp. from the Western Pacific, *Phakellia carteri* from the Western Indian Ocean and from a deep water *Lyssodendoryx* sp. off the east coast of South Island, New Zealand [22] which suggests that this skeletal type maybe constructed by an associated microorganism.

Many studies have reported the cell growth inhibitory activity of halichondrins at nanomolar concentrations (1×10^{-9} M) [84]. Among the Halichondrin family, halichondrin B (Fig. 3b) was identified as the most potent congener which exhibited high *in vitro* IC_{50} value of 0.3 nM against L1210 Leukaemia and remarkable *in vivo* activities against various human solid tumour xenografts, including LOX melanoma, KM20L colon, FEMX melanoma and OVCAR-3 ovarian tumours. Following the discovery of potent anticancer activity of halichondrin B, it was tested and compared with other known antimitotic and anticancer agents using the United States (US) National Cancer Institute's (NCI) 60 cell line screen [85,86]. While antiproliferative patterns of halichondrin B were found similar to those of other antitubulin drugs, its biochemical mechanism interaction with tubulin was unique [85]. Differential cytotoxicity data indicated that halichondrin B binds tubulin at the vinca peptide binding site [85,87]. Induction of mitotic arrest by halichondrin B leads to multisite phosphorylation and inactivation of anti-apoptotic protein Bcl-2, cytochrome c release from mitochondria, proteolytic activation of caspase-3 and -9, and cleavage of the caspase-3 substrate poly(ADP-ribose) polymerase (PARP) eventually stimulating cell death *via* apoptosis [88].

Although the extraordinary potency of halichondrin B generated considerable interest for development, limited availability of the natural product severely restricted such efforts. However, the existence of a synthetic route for halichondrin B and knowledge that its activity resides in the macrocyclic lactone C1–C38 moiety have permitted development of its structurally simplified synthetic analogue the eribulin mesylate (E7389) that retain the exceptional activity of the parent [89]. Like halichondrin B, eribulin mesylate also inhibits microtubule dynamics *via* a novel mechanism of action which is thought to involve binding to a unique binding site on tubulin [87], resulting in the suppression of microtubule polymerisation without effects on depolymerisation together with sequestration of tubulin into non-functional aggregates [90]. By inhibiting mitotic spindle formation, eribulin causes irreversible mitotic block which ultimately leads to cell cycle arrest in the G2-M Phase and apoptosis [87,88,90]. Based on the novel mechanism of action of eribulin which is distinct from other known classes of tubulin targeted agents and its encouraging preclinical activity, it was hypothesised that eribulin may have efficacy in patients with malignancies that are resistant to other tubulin-targeted agents together with a more favourable tolerability profile.

Eribulin was presented to NCI Drug development group in 1998 and entered Phase I clinical trials in 2002. On November 15, 2010, U.S. Food and Drug Administration approved eribulin for treatment of patients with metastatic breast cancer (MBC) who have previously received an anthracycline and a taxane in either the adjuvant or metastatic setting and at least two chemotherapeutic regimens for the treatment of metastatic disease [91]. Preliminary safety data showed that the major toxicity issue was neutropaenia and leukopaenia [92]. Currently eribulin is in phase II trials for non-small lung cancer, pancreatic, prostate, head and neck cancer and bladder and ovarian and related gynaecological tumours and phase III clinical trial as second line therapy for the treatment of advance breast cancer. The trials are taking place across the world under the sponsorship of Eisai [33].

2.5. Dolastatin 10

The dolastatins are a series of remarkable cytotoxic compounds isolated from the Indian Ocean seahare, *Dolabella auricularia*. The impetus for investigations of *D. auricularia* as a source of new anticancer compounds derived from the initial discovery by Pettit's group in 1972 of potent cytotoxic extracts of this seahare [93]. However, it was later determined that dolastatins were actually produced by the marine cyanobacteria that were consumed by the seahare [94,95]. Since then, many dolastatins with anticancer effects have been isolated. The most important of these is the dolastatin 10, the structure of which is depicted in Fig. 3d. The latter is a pentapeptide with four of the residues being structurally unique (dolavaline, dolaisoleucine, dolaproline, and dolaphenine, in addition to valine) [96].

Interestingly at the time of its discovery, dolastatin 10 was the most potent antiproliferative agent known with an $ED_{50} = 4.6 \times 10^{-5}$ μg/ml against murine PS leukaemia cells [97] Subsequently the peptide demonstrated remarkable *in vitro* activity against various human cancer cell lines including melanoma, colorectal cancer, sarcoma and ovarian cancer cells [98]. Further work revealed that dolastatin 10 is a potent antimitotic agent that exhibits non competitive inhibition at the Vinca alkaloid binding site on tubulin thus affecting microtubule assembly and tubulin-dependent GTP hydrolysis. A reason for the high antimitotic activity is its prolonged cellular retention that facilitates tubulin-binding [97]. Dolastatin 10 has also been shown to induce apoptosis in various tumour cell lines including breast cancer, lung cancer, leukemias or lymphomas. The mechanism of apoptosis induction is likely induced by an upregulation of the pro-apoptotic molecule Bax and the concurrent downregulation of the anti-apoptotic molecule Bcl-2 [88]. Due to their growth inhibition and apoptotic effects on tumour cell lines, dolastatins entered Phases I and II clinical trials which unfortunately failed because of their less effectiveness against prostate cancer. Nevertheless, dolastatin 10 offered a logical starting point for further clinical studies and synthetic drug design ultimately leading to the analogue Soblidotin (TZT-1027) [98].

Soblidotin was designed with the goal of maintaining the potent antitumour activity while reducing the toxicity of the parent compound [99]. It only differs from dolastatin 10 in replacement of the terminal dolaphenine amino-acid residue with a simple phenylamine group [100]. Nevertheless, both Soblidotin and dolastatin 10 share the same mechanism of activity and bind near the vinca peptide site on tubulin and inhibit tubulin polymerisation with equal potency [101]. Intravenous injection of the Soblidotin in mice resulted in significant inhibition of P388 leukaemia growth and the diminution of three solid tumour cell lines (B16 melanoma, colon26 adenocarcinoma and M5076 sarcoma). Additionally TZT-1027 was effective in two human xenografts model, MX-1 breast carcinoma and LX-1 lung carcinoma [98].

Soblidotin entered phase I clinical trials in 2002 in Japan, Europe and the USA under the auspices of Teikoku Hormone, Daiichi Pharmaceuticals. The compound is currently in three different phases of clinical trials (phase I, II, III) with different companies using it as "warhead" linked *via* modified peptides to specific monoclonal antibodies [33].

2.6. Didemnin B and Aplidine

Ecteinascidin-743 was not the only lead anticancer agent found from marine tunicates. Didemnin B (Fig. 3c), a cyclic antiproliferative depsipeptide has also been isolated from the tunicate *Tridemnum solidum* collected in tropical Mediterranean waters. The compound was first isolated by Rinehart group at the University of Illinois in 1978 and displayed excellent antiviral activity

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and subsequent cytotoxic activity against a variety of solid tumour models [102]. Early *in vitro* testing of didemnin B showed that the compound was active against colorectal [103], lymphatic [104] and prostate [105] cancer at nanomolar concentrations and later against Erhlich's carcinoma in mice models.

Didemnin B was advanced into preclinical and clinical trials (phase I and phase II) under the auspices of the NCI in the early 1980s as the first defined chemical compound directly from a marine source to go into clinical trials for any major human disease. Despite many different treatment protocols and testing against many types of cancer, the compound turned out to be too toxic for use and trials were officially terminated in the middle 1990s by NCI [106].

Even though this compound did not make it to Phase III trials and to the market, the experience gained from these trials led to the development of Plitidepsin (dehydrodidemnin B), a simple analogue of didemnin B. Plitidepsin was first reported in a 1991 patent by Rinehart and is obtained from the Mediterranean tunicate *A. albicans* [107]. The peptide exhibits similar levels of antitumour activity to didemnin B in cultured tumour cells [108] but differs from the natural product only in replacement of N-lactyl side chain with a pyruvyl group (Fig. 3c). Mechanistically, the compound interferes with the synthesis of DNA and proteins and induces cell cycle arrest. It induces apoptosis by induction of oxidative stress [107] which triggers the pro-apoptotic receptor Fas (CD-95) and induces mitochondrion-mediated apoptosis [95,97]. Plitidepsin also activates p 38 mitogen activated protein kinases (MAPKs) and JNK [98,99] and inhibits secretion of vascular endothelial growth factor (VEGF) [100].

Preclinical studies with different tumour types both *in vitro* and *in vivo* were the basis for selection of Plitidepsin and design of the phase I and phase II trials. Clinically Plitidepsin has demonstrated efficacy in two different phase II clinical trials in relapsing and refractory multiple myeloma and T cell lymphoma [101]. The encouraging results gathered from these clinical trials support further clinical research particularly in combination with other active agents. The main toxicity found with most schedules included muscular toxicity, transient increase of transaminases, fatigue, diarrhoea, and cutaneous rash [109]. Plitidepsin is currently being developed by PharmaMar under the tradename Aplidin [33].

2.7. Kahalalide F

Kahalalide F (KF) is a novel marine derived cyclic peptide with potential antineoplastic activity belonging to the Kahalalide family of compounds. It was initially isolated by the Scheur group in 1993 from the seasonal collection of the sacoglossan (sea slug) *Elysia rufescens* [110]. KF is a C_{75} cyclic tridecapeptide (Fig. 3(i)) that contains several unusual amino-acid residues including the rare dehydroaminobutyric acid which is only found in a few peptides including the antiobiotics cypemycin [111] and hassallidin A [112]. *E. rufescens* is an herbivorous Hawaiian opisthobranch that feeds on the alga *Bryopsis* sp. which is the true source of KF [110,113]. The synthesis of the original structure of the KF with essentially the same activity as the natural product was accomplished a few years following its discovery and was licensed by the University of Hawaii to PharmaMar [114].

KF has shown cytotoxicity in a variety of tumour cell lines derived from breast, NSCL or hepatic cancer cell lines [115,116]. However, its mechanism of action has not been fully elucidated. A preclinical study of KF showed that several caspase dependent apoptosis markers like caspase 3, PARP or cytochrome c release were negative after incubation with KF. Furthermore, there was no change in cytotoxicity of Bcl-2 or Her2/neu overexpressing cells [115]. Hence, this leads to the presumption that KF induce oncolytic rather than apoptotic cell death. Despite the independence of apoptotic markers, the mitochondria and lysosomes seem to be involved in KF induced cell death because incubation of PC3 cells with KF leads to a rapid loss of membrane potential and to an altered plasma membrane permeabilisation of lysosomes [117]. Furthermore a disruption of the cytoplasmic structure was found in SKBR3 cell which was shown by an extensive vesiculation of cytoplasmic organelles, dilation of the endoplasmic reticulum elements and cytoskeletal degradation. In addition a necrosis like process was observed in several human KF-sensitive breasts, vulval, NSCLC and hepatic and colon carcinoma cell lines in which downregulation of the ERBB3 protein and inhibition of the phosphatidylinositol 3 kinase (PI3K)-AKT signalling pathway were identified as determinants of its cytotoxicity [115,118].

Elisidepsin, one of the most potent analogues of KF has been selected for clinical development based on its *in vivo* activity in xenografted human tumours, as well as an acceptable non-clinical toxicology profile [119]. Elisidepsin is currently in phase II clinical development with preliminary evidence of antitumour activity and a favourable therapeutic index for solid tumours including melanoma, NSCLC and hepatocellular carcinoma [120] and is being developed by Pharmamar [33].

2.8. Bryostatin 1

The Bryostatins comprise a group of 20 novel macrocyclic lactones originally isolated from the marine Bryozoan *Bugula neritina* [121]. Bryostatin 1 is one of the most cytotoxic agents and most abundant of this group [99]. In 1968, a collaborative programme between Pettit and co-workers at the Arizona State University and National Cancer Institute found that extracts of *B. neritina* collected from Gulf of Mexico exhibited remarkable activity against murine P388 lymphocytic leukaemia cells [100]. However, isolation and identification of the active component posed serious problems as a result of dealing with vanishingly small quantity of the potent agent. Following significant amount of work, the structure of bryostatin 1 was finally solved in 1982 by crystallographic and spectroscopic analyses [101]. The compound is a 26 membered macrocyclic lactone with 11 stereocentres and a unique polyacetate carbon backbone (Fig. 3e) which has not been previously encountered in natural products. Although there are many synthetic derivatives of bryostatin 1 until now the complete synthesis of bryostatin 1 has not been accomplished and the production is still carried out by cultivation of *B. neritina* [109].

However, the low abundance of the compound precluded supplies of enough compounds for early preclinical and clinical studies as anticancer drug. Subsequent research by the NCI eventually gave pure quality bryostatin 1 from a 13 tonne collection of *B. neritina* from Californian waters [122].

Bryostatin 1 is known to exhibit a range of properties including immunostimulating and anticancer properties [123]. It binds to protein kinase C with high affinity, induce cytokine release and expand tumour specific lymphocyte population [124]. Bryostatin 1 may also be used in tandem with other cytotoxic agents in cancer treatment, since single agent therapies in phase II studies have shown minimal activity and protein kinase C activation can promote chemoresistance [125]. They potentiate the pro-apoptotic effects when used in combination with chemotherapeutic agents. For example, bryostatin 1 when used in combination with chemotherapeutic agent such as paclitaxel, it synergistically increases the paclitaxel-induced upregulation of caspases even in Bcl-xL overexpressing cells [126]. Therefore, it is an interesting agent in combination therapy.

With its promising anticancer activity, bryostatin 1 has been investigated either alone or in combination with other

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chemotherapeutic agents in numerous clinical trials (phase I and phase II) for myeloid leukaemia, melanoma, lymphocytic leukaemia, non-Hodgkin's lymphoma and NSCL, metastatic myeloma, relapsed lymphoma, and chronic lymphocytic leukaemia [127–129] and other refractory malignancies [130]. A review of bryostatin 1 provides key information on the history of clinical development of the drug [118,131]. The major dose limiting toxicity in all cases is myalgia which explains a direct toxic effect of bryostatin. Currently Bryostatin 1 is in two phase 1 clinical trials and is being assesses as an anti-Alzheimer's drug [33].

2.9. Hemiasterlin

Originally identified as natural products from the marine sponge *Hemiasterella minor* by Kashman and co workers in 1994 [131] and subsequently from other unrelated sponges such as *Cymbastella* sp., *Siphonochalina* sp., and *Auletta* sp. [132], hemiasterlins comprise a small family of naturally occurring tripeptides containing three highly modified amino acids (Fig. 3f) [131].

All hemiasterlins have shown cytotoxicity in the nanomolar range (concentration 1×10^{-9} M) against a variety of cultured human and murine cell lines. The related isomers hemiasterlin A and B, as reported by Anderson and coworkers are the most potent members in the hemiasterlin group [132]. Their potent cytotoxicities are due to induction of mitotic arrest in metaphase with similar dynamics to those of known tubulin binders such as paclitaxel or vinblastine [133]. The antimitotic activity of hemiasterlin is mediated *via* a tubulin-based mechanism that leads to tumour cell apoptosis. It binds to the *Vinca*-peptide site in tubulin and induces G (2)-M arrest, caspase-3 activation and poly ADP ribose polymerase cleavage, which are typical biochemical markers of apoptosis [134].

Extremely limited quantities of hemiasterlins hampered development of this series of compounds, particularly in animal models where limited testing has been reported [132]. However, synthetic methods for producing hemiasterlin and its analogues [133] have allowed the evaluation of many related compounds for antitumour properties and a better understanding of the binding site of peptide-like molecules with tubulin. The optimal analogue was considered to be HTI-286 (Fig. 3g) that retains potency in cellular models resistant to several chemotherapeutics, including taxanes and *Vinca* alkaloids [132]. Preclinical studies has shown that HTI-286 causes tumour regression and growth inhibition of human xenografts in mice. Even cell lines expressing P-glycoprotein or resistant to paclitaxel were shown to be sensitive to HTI-286 inhibition, but required higher doses than non-resistant cell lines [135].

An open-label phase I clinical trial was completed in patients with advanced solid tumours; however there were no objective responses and common toxicities observed included neutropaenia, nausea, alopecia and pain [136]. Phase II trials have been halted. Nevertheless, there is still interest in view of recent results including high antitumour activity in androgen-dependent and androgen-independent mouse models of refractory prostate cancer and in newly established *in vitro* taxane resistant prostate PC-3 cell lines [136].

2.10. Salinosporamide A

Salinosporamide A is a MNP that has been recently discovered during the fermentation of *Salinispora tropica*, a new sea water actinomycete found in marine sediments in Bahamas [137]. The compound (Fig. 3h) which has an unusual bicyclic ß-lactone γ-lactam structure was found to be a potent inhibitor of all the three proteolytic activities of the mammalian 20S proteasomal trypsin-, chymotrypsin- and caspase-like subunits and shows cytotoxic effects in tumour cells [138].

A limited number of studies have revealed that the compound can induce apoptosis of multiple myeloma cells [139], colon cancer cells [140], chronic lymphocytic leukaemia [125,141], acute myeloid leukaemia and acute myeloid leukaemia [142]. In animal model systems this proteosome was found to suppress the growth of multiple myeloma [143] and colon cancer [144]. Mechanistically the compound exhibits potent and selective inhibition of the proteosome, a multicatalytic enzyme complex that is responsible for non-lysosomal protein degradation in cells and represents a validated target for the treatment of cancer. Proteosome inhibition occurs *via* a novel mechanism involving acylation of the N-terminal catalytic Thr1O$^{\gamma}$ residue followed by displacement of chloride resulting in prolonged proteosome inhibition *in vitro* and *in vivo* [143–145]. It has been reported to potentiate apoptosis by activating caspase-8 and -9 molecules and downregulate gene products that mediate cell proliferation (cyclin D1, cyclooxygenase-2 [COX-2], and c-Myc), cell survival (Bcl-2, Bcl-xL, cFLIP, TRAF1, IAP1, IAP2, and survivin), invasion (matrix metallopro-teinase-9 [MMP-9] and ICAM-1), and angiogenesis (vascular endothelial growth factor) [146]. Further studies also clearly demonstrated the potential for using salinosporamide A in combination with other biologics or chemotherapeutics [129].

These findings provided the basis for Nereus Pharmaceuticals to initiate several concurrent phase I clinical trials in patients with multiple myeloma, lymphomas, leukemias and solid tumours. In an important demonstration of industrial marine microbial, clinical supplies of salinosporamide A drug substances are being manufactured through a robust saline fermentation process *S. tropica* strain NPS21184 [147]. The anticancer compound is currently in phase I clinical trials in relapsed/refractory multiple myeloma and is being developed under the trade name Marizomib (NPI-0052) by Nereus Pharmaceuticals Inc. [33].

2.11. Spongistatin 1

The spongistatins comprise an important family of architecturally complex marine macrolides that display extraordinarily antitumour activities against a variety of human cancer cell lines. These unique natural products were isolated from the marine sponges *Spirasterella* sp. and *Hyrtios* sp. by the group of Pettit in 1993 while the absolute structure remained unknown until confirmation by synthesis in 1997 [148]. The spongistatin family includes 9 macrolides (Fig. 3j) all of which possess remarkable growth inhibition properties against the US NCI's panel of sixty human cancer cell lines. Spongistatin 1 is recognised as the most cytotoxic member of the spongistatin family and has an average IC_{50} value of $2.5–3.5 \times 10^{-11}$ M against a subset of highly chemoresistant tumour types [148,149].

Spongistatin 1 shows interesting apoptotic features in various tumour cells. In leukemic cell lines it triggers cascade dependent apoptosis through the release of cytochrome c, Smac/Diablo and Omi/HtrA2 from the mitochondria into the cytosol. Spongistatin 1 leads to the degradation of the anti-apoptotic X-linked inhibitor of apoptosis protein (XIAP) and thus might be a promising drug for the treatment of chemoresistance due to overexpression of XIAP [150]. Moreover spongistatin 1 induces apoptosis more efficiently in human primary leukemic cells of children suffering acute leukaemia at low nanomolar concentrations than clinically applied conventional drugs used in micromolar concentrations [150].

Besides leukemic cells, spongistatin showed promising apoptotic potential in mammary cancer cells including the treatment-resistant cell line MCF-7 lacking caspase 3. Spongistatin-1 -induced cell death mainly caspase independent, involves the pro-apoptotic proteins AIF and endonuclease G. Both proteins translocate from mitochondria to the nucleus and contribute to spongistatin-1

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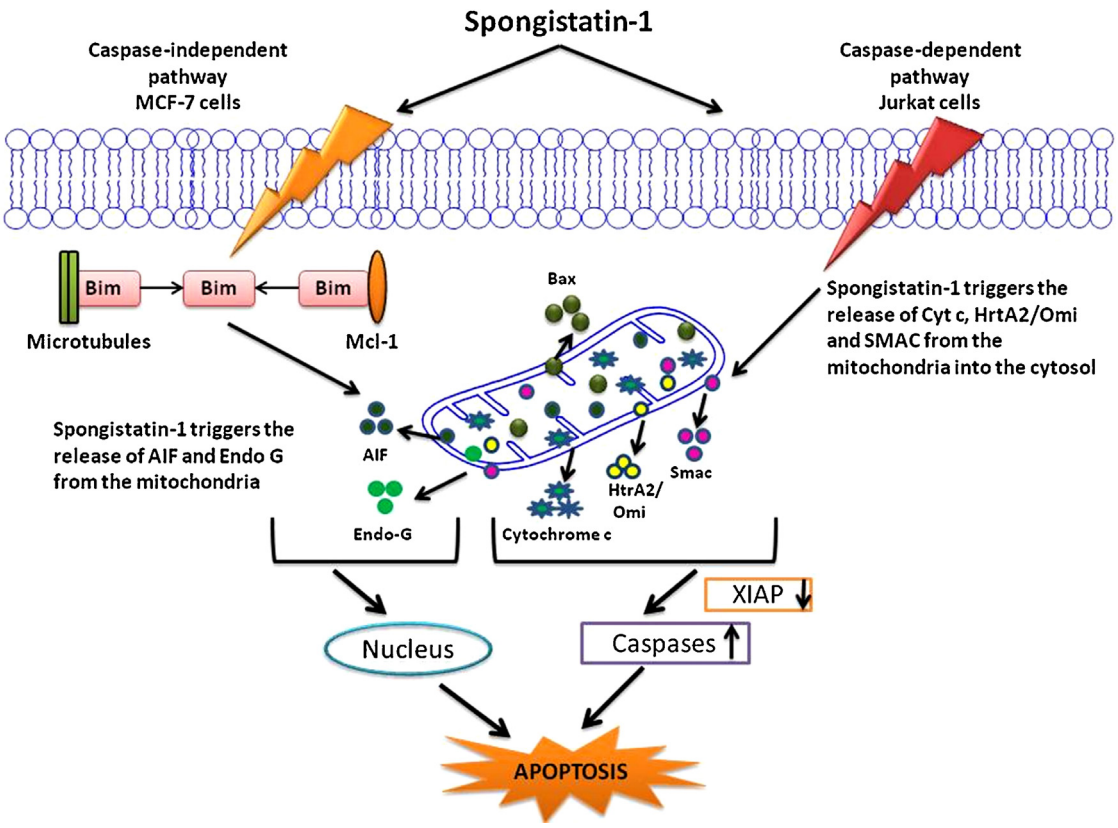

Fig. 4. Proposed apoptotic pathway for spongistatin 1 in MCF-7 mammary tumour cells. *Abbreviations*: Bim, B-cell lymphoma 2 interacting mediator; Mcl-1, myeloid cell leukaemia sequence 1; AIF, apoptosis inducing factor; Endo G, endonuclease G; HtrA2/Omi, mitochondrial serine protease; SMAC, second mitochondria-derived activator of caspases; XIAP, X-linked inhibitor of apoptosis protein.

mediated apoptosis as shown *via* gene silencing. Second, spongistatin acts as a tubulin depolymerising agent and is able to free the pro apoptotic Bcl-2 family member Bim from its sequestration both by microtubular complex and by the anti-apoptotic protein Mcl-1 (Fig. 4) [151]. Silencing of Bim by siRNA leads to a diminished translocation of AIF and endonuclease G to the nucleus and subsequently reduces rate of apoptosis. By using spongistatin as a chemical tool, Bim has been suggested to be an important factor upstream of mitochondria by executing a central role in the caspase-independent apoptotic signalling pathway induced by spongistatin 1 [151]. These different apoptotic features indicate that the apoptosis signalling is cell line specific. Subsequently, spongistatin 1was shown to be a potent antitumour and antimetastatic agent *in vitro* and *in vivo* against invasive pancreatic cancer cells. Mechanistically the compound affects highly invasive pancreatic tumour cells by not only inhibiting their invasion and migration but also by inducing anoikis in these cells. Bcl-2 seems to be a major target for spongistatin1 in these processes [152]. These findings thus substantiate the notion that spongistatin 1 represents an important class of tubulin agents whose anticancer therapeutic potential warrants clinical investigation.

2.12. Discodermolide

Discodermolide is a highly functionalised, marine sponge derived polyketide lactone (Fig. 3k), first isolated in 1990 and characterised by Gunasekara et al. at the Harbour Branch Oceanographic Institution from the deep water sponge *Discodermia dissoluta* [153]. Crude extracts of the sponge showed strong potency against the murine P388 lymphocytic leukaemia cell line and bioassay guided fractionation resulted in the purification of discodermolide. The structure of discodermolide was first elucidated by analysis of nuclear magnetic resonance (NMR) and mass-spectrometric data. The initial reports of discodermolide's strong *in vitro* antiproliferative and *in vitro* and *in vivo* immunosuppressive activities created a wide interest among the synthetic organic community. The absolute chemistry was subsequently defined by total synthesis conducted by the Schreiber group [154,155].

Discodermolide acts as an immunosuppressant and induces G2/M Phase cell cycle arrest and apoptosis in various tumour cell lines. Investigations into the mechanism of cycle-arrest by discodermolide competitively bind to tubulin and stabilise microtubules with another tubulin binding agent, paclitaxel. Fortunately discodermolides bind with a higher affinity for tubulin and have effects even in paclitaxel-resistant cell lines. Despite their competitive behaviour, discodermolides and paclitaxel show synergistic effects [156]. This contradictionary effect may be caused by overlapping rather than identical binding sites. Although both compounds bind to the taxane binding pocket, taxol interacts with the M-loop and discodermolide orients itself away in the direction of S1–S2 loop. Furthermore the two compounds seem to show a complementary stabilising effect on microtubules [157].

Discodermolide treatment causes a late activation of caspase 3 and caspase 8 as well as a cleavage of PARP in NSLCS cells. Treatment with discodermolide also leads to an efflux of cytochrome c from the mitochondria. Despite these findings, neither overexpression of Bcl-2 nor FADD-negative cells or inhibition of caspases could prevent cells from undergoing apoptosis [158]. However, although discodermolide has shown more potent effects in tumour cells than paclitaxel and has also shown promising effects in murine models, the pharmaceutical company Novartis has withdrawn it from phase I trials due to cytotoxicity problems [159]. However potentials remain for its use in combination drug therapy.

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3. Future prospects of marine natural products as anti-cancer drugs

The above examples illustrate the intense excitement which surrounds the past decade's achievement in the area of cancer research from the marine biosphere. A substantial number of marine-derived agents with apoptotic properties are progressing through the development process, primarily with indications in cancer. However, it is also interesting to note that these marine compounds also display antitumour activity *via* multiple targets. For example dolastatin 10 not only induces apoptosis associating with a decrease in BCl-2 level and an increase in p53 expression in the lymphoma cell lines, but also inhibits microtubule formation assembly and tubulin dependent GTP hydrolysis [160]. Plitidepsin's mechanism of action involves several pathways, including cell cycle arrest, inhibition of protein synthesis and anti-angiogenic activity [161]. On the other hand, except apoptosis, necrosis and autophagy also play an important role in cancer cell death and some of the marine natural products also induce cell death *via* necrosis and cell death. For example, Kahalalide not only induce cell death but also increases autophagosome content resulting in rapid loss of cell integrity and necrotic cell death in SKBR3 cell lines [118]. However, since we have elected to focus mainly on apoptotic pathway, the details on necrosis and autophagy induced by some marine natural products are not discussed.

The last decade has seen an evolving strategy for the screening and discovery of new anticancer leads from the marine environment and this is proving effective. From former times of evaluation of crude extracts by *in vivo* screens to current evaluation of peak or pre-fractionated libraries in mechanism-based assays, the level of sophistication and success has improved considerably [162]. As a result, it can be anticipated that marine natural products will become a more significant part of the pipeline for developing new therapeutics in the future. However, as with all new emerging technologies, many challenges await the field of marine natural product biotechnology before it can reach its full potential of providing practical approaches to supplying complex marine organic molecules for clinical evaluation and development. Current limitations that are actively being addressed in academia include the supply problem. Perhaps this is the most important factor challenging the development of products from the sea. Indeed, in order to obtain 1 g of ET-743, close to 1 metric tonne (wet weight) of *E. Turbinata* has to be collected and extracted. For Halichondrin B, it does seem to be more difficult to obtain enough amounts: 1 metric tonne (wet weight) of the sponge (*Lissodendoryx* sp.) yield 300 mg of mixture of two halichondrins [163]. Consequently, the application in clinical is extremely difficult because of the low productivity of these compounds. Clearly, natural exploitation of these compounds cannot be reasonably envisaged without causing the destruction of marine ecosystems.

Synthesis of marine bioactive compounds remains therefore the alternate method. However, due to the high complexity of certain structures their synthesis, in many cases, is not feasible. One successful example of the synthetic production of a marine-product drug in unlimited quantities is the conus toxin ziconotide, because of its peptide nature [164]. Another alternative to exploit natural products is marine culture. The aquaculture of the source organisms, including sponges, tunicates, bryozoans, with the aim of securing a steady supply of drug product, has progressed notably in cancer applications. For example, by culturing *B. neritica*, American groups have obtained large amounts of bryostatin 1 and the latter was produced at reasonable cost [165]. However, in most cases the biomass generated is still far short of those required should a marine-based drug finally enter the market [166].

Furthermore, a long-standing and perplexing question in marine natural products chemistry has been the identification of the metabolite producing organism or potentially metabolite biotransforming organism, in systems involving an invertebrate host and symbiotic microorganisms [167]. Many studies have successfully provided data to support the involvement of microorganisms including actinomycetes, cyanobacteria, microalgae such as dinoflagellates, and others in the biosynthesis of natural products isolated from invertebrates. For instance, the structural study on ET-743 has led to the identification of a congener named safracin B from *P. fluorescens*. Also Symbostatin 1, a similar skeleton to dolastatin 10 was isolated from the green alga *Symploca hydnoides* [163]. In a sense, this is quite fortunate, for the biosynthetic pathways that code for natural compounds in prokaryotes are better understood and more amenable for study than those in eukaryotes. Progress in understanding how these biosynthetic pathways operate at the genetic and biochemical levels is opening new doors for harnessing this potential, and the future looks very optimistic for realising tangible benefits from such efforts, such as new designer molecules with improved biological and pharmaceutical properties.

4. Conclusion

It is clear that the marine ecosystem offers a huge potential in the naturally based pharmacopoeia of this century. However, an unfavourable balance between discovery and the very small number of candidates incorporated for clinical evaluation exists. So it appears that a better and more pragmatic approach is urgently warranted in order to translate innovative discoveries into active clinical therapeutics. The available data demonstrate that the marine ecosystem is not only productive to discover anticancer entities but is also a tool to identify new cellular targets for therapeutic intervention.

Nevertheless, efforts being made in different studies involving marine derived therapeutic research are continuously opening new avenues. Also the in depth characterisation of the molecular pharmacodynamic changes induced by bioactive marine compounds in human cancer cells can also generate information to understand the genetic basis behind the response/resistance to these compounds projecting the possibility to developed customised therapies according to a given molecular signature. With this in mind, important therapeutic contributions coming from the marine ecosystem are expected to emerge in the near future.

Conflict of interest statement

None declared.

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