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**REVIEW ARTICLE**

# Marine cytotoxins: callers for the various dances of death

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## ABSTRACT

Programmed cell death, which can be classified into apoptosis, autophagy, and oncosis, based on the cellular signalling pathways involved in the process, plays a critical role in cellular house-keeping. Aberrant cell death regulation leads to body malformations, as well as to numerous diseases. Here, we describe the effects of cytototoxins isolated from marine organisms on the various programmed cell death pathways, and we discuss the current and future applications of marine natural products in the fields of biomedicine and of biotechnology.

**Keywords**: *Cell death mechanisms, Marine compounds, Cancer*

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## INTRODUCTION

Programmed cell death plays a critical role in cellular housekeeping, and aberrant cell death regulation leads to body malformations and to numerous diseases, including various autoimmune diseases, cancer, stroke, myocardial infarctations, and neurodegenerative diseases (1-4). Research in the field of cell death has evolved at a very rapid pace over the last fifty years. It is now generally accepted that programmed cell death can be subdivided into three different categories: apoptosis (programmed cell death type I), autophagy (programmed cell death type II, or lysosomal cell death), and oncosis (programmed cell death type III, also referred to as necrosis) (5-8). Apoptosis, in particular, has been studied extensively (9) and autophagy has recently sparked an enormous research interest (10).

In the 1960's to early 1980's, simultaneously as renowned biologists including Richard Lockshin and John Saunders, John Kerr, Alastair Currie, and Andrew Wyllie, and Sydney Brenner, Robert Horvitz, and John Sulston initiated their investigations into cell death, research groups lead by Paul Scheuer, John Faulkner, George Pettit, and other chemists pioneered the research field of marine natural products, with the aim to identify potent anti-cancer compounds amongst the toxins produced by marine organisms as a chemical defence mechanism against their predators (7,11,12). Since then, numerous marine natural products have been identified as modulators of cell death. Here, we discuss the mechanism of action of the major marine natural products reported as inducers of apoptosis, autophagy, or oncosis.

### Programmed cell death

Programmed cell death can be classified into three different types (apoptosis, autophagy, and oncosis), based on the cellular signalling pathways involved in the process and on the morphology of the dying cell. Apoptosis is characterised by

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chromatin condensation, nuclear fragmentation and chromosomal DNA fragmentation (karyorrhexis), alterations of the cellular membrane, and cellular shrinkage. Apoptotic cells eventually break up into vesicles, which are phagocytosed, primarily by macrophages, without eliciting any inflammatory responses (3, 5-8). Autophagy is, in many respects, very closely related to apoptosis, but it differs from apoptosis by being a mainly caspase-independent process. Autophagy further distinguishes itself from apoptosis by inducing the degradation of organelles at an early stage of cell death, but by preserving the intactness of the cytoskeleton until the final phase of the dying process (5). Autophagy, which is triggered primarily by fasting and by genotoxic stress (5,13), is typically associated with the formation of autophagosomes that fuse together with lysosomes which degrade the autophagosomes' content (13). Oncosis, or "accidental cell death", most commonly results from acute cellular damage, from severe cytotoxicity, or from a failure of the ionic pumps within the plasma membrane. In contrast with apoptosis and autophagy, oncosis, as the name implies, is characterized by cellular swelling followed by cell membrane bursting and by the release of inflammatory signals (3,14,15). Although the distinctions between the three types of cell death is often very subtle, new tools assisting with the elucidation of the various cell death pathways are continuously emerging with the rapid development of cell death-related research (3,15). The cell death pathways specific to apoptosis, autophagy, or oncosis are described below.

Apoptosis, or programmed cell death type I, can be activated by an "extrinsic" pathway involving the binding of extracellular death receptor ligands to the corresponding receptors on the cell membrane or by mitochondria- or endothelium reticulum-mediated "intrinsic" pathways. The major death receptors involved in the extrinsic apoptotic pathway include the tumour necrosis factor (TNF) receptor, the TNF-related apoptosis-inducing ligand (TRAIL) receptors (TRAIL-R), and Fas (16,17). The binding of the receptors to adaptor proteins activates the cysteine protease caspase 8. In turn, caspase 8, which is an initiator caspase, activates the effecter caspase 3 (16,17). Caspase 8 also truncates, and thereby activates, the pro-apoptotic Bcl-2 (B-cell lymphoma gene 2) protein Bid (Bcl-2 homology domain (BH)3 interacting domain death agonist), which, along with the intrinsic apoptosis activators like radiation, toxins, and hypoxia, activate the pro-apoptotic Bcl-2 proteins Bax (Bcl-2 associated X protein) and Bak (Bcl-2 antagonist/killer). Signalling through p53 following DNA damage (7,18), or through the mitogen-activated protein kinases (MAPK) JNK (c-Jun NH2-terminal kinase) and p38 (7,17) also activates the intrinsic apoptotic pathway. The interaction of Bak and Bad with the anti-apoptotic mitochondria-bound Bcl-2 proteins Bcl-2 and Bcl-X leads to the permeabilization of the outer mitochondrial membrane, and eventually of both the outer and inner mitochondrial membranes. The permeabilization of the outer mitochondrial membrane induces the leakage of pro-apoptotic molecules including cytochrome c and Smac (second mitochondria-derived activator of caspase) from the mitochondrial inter-membrane space into the cytosol. The release of cytochrome c activates the initiator caspase 9 which, in turn, activates the effecter caspase 3, while Smac inhibits various cytosolic inhibitor of apoptosis proteins (IAP) (7,19,20). The permeabilization of both mitochondrial membranes leads to a dissipation of the mitochondrial trans-membrane potential, which is required for numerous mitochondrial functions, including ion transport and energy conservation. In an advanced stage of mitochondrial failure, the cells will switch to the oncosis pathway (16-18,21,22). Another intrinsic apoptosis pathway has been associated with the

induction of stress of the endoplasmic reticulum. The endoplasmic reticulum is responsible for proper calcium homeostasis and for the post-translational modification required for proteins to obtain their mature conformation. If cytotoxic conditions lead to a severe disruption of the calcium homeostasis, the endoplasmic reticulum signals for the cells to undergo apoptosis, primarily through the activation of the murine caspase 12 or to the human caspase 4, which lead to the activation of the effecter caspase 3 (17). The effector caspase 3 downstream of the three major apoptosis pathways is responsible for the activation of the endonuclease which leads to chromatin condensation, for the cleavage of certain substrates, including actin, lamins, poly-ADP ribose polymerase (PARP) and protein kinase C (PKC)-d, for the reorganization of the cytoskeleton, for the ruffling of the cellular membrane, for the packaging of the cellular content into apoptosomes, and for the externalization of phosphatidylserine to the outer plasma membrane which signals the apoptosomes for a rapid engulfed and destruction by the neighbouring cells, without induction of an inflammatory response (1,16-18,20,23). In addition to its pro-apoptotic effects through the intrinsic pathway as described above, JNK activates the transcription factor c-Jun, which increases transcription of Fas ligand, and which upregulates various pro-apoptotic Bcl-2 proteins (17). Survival signals through anti-apoptotic growth factors, through the activation of PKC, of p21ras, of the PI3K (phosphoinositide 3-kinase)/Akt (protein kinase B) pathway, or of the NF-kB (nuclear transcription factor-kB) activation block pro-apoptotic signalling pathways, mainly through the activation of anti-apoptotic members of the Bcl-2 familiy IAPs.

Autophagy, or programmed cell death type II, is triggered primarily by fasting (5), by endoplasmic reticulum stress resulting from an accumulation of misfolded proteins (24), and by various other genotoxic stress factors (13). The major cellular inducers of autophagy, in response to starvation, to endoplasmic reticulum stress, or to low cellular energy levels, are the eukaryotic initiation factor 2α (eIF2α) and the 5′-AMP-activated protein kinase (AMPK). Other cellular inducers of autophagy include the tumor suppressor protein p53, the endoplasmic reticulum membrane-associated protein Ire-1, and calcium (Criollo et al., 2007; Maiuri et al., 2007a; Meijer and Codogno, 2006; Rubinsztein et al., 2007). The downstream execution of autophagy is regulated by proteins belonging to the Atg family which mediate the maturation and the expansion of autophagosomes. Lysosomal transmembrane proteins assist with the fusion of the autophagosomes with lysosomes, and various lysosomal cysteine proteases ensure the degradation of autophagosomal contents (5). The major cellular inhibitor of autophagy is the TOR (target of rapamycin) kinase. TOR represses autophagy in response to growth factor and insulin-like signals during nutrient abundance (5,25,26).

Oncosis, or programmed cell death type III, is a predominantly uncontrolled, passive form of cell death. Oncosis is induced by severe physical or chemical insults onto the cells [16]. Oncotic cells undergo a physical enlargement of the endoplasmic reticulum and of the mitochondria. The consequent rupture of the organelles' membranes disturbs the intracellular ion homeostasis and ultimately causes the swelling and the osmotic lysis of the cells. The lysed cells release a range of signalling molecules, including heat shock proteins and various proteases, that ultimately induce inflammation and may stimulate cell-mediated immunity (15,16,27). Liver cells have been shown to possess a shared intrinsic apoptosis/oncosis pathway. Depending on the severity of the mitochondrial membrane permeability, liver cells can undergo a cytochrome c mediated apoptotic cell death or an oncotic cell death. Unlike apoptosis, oncosis does not require

energy. For this reason, if cellular ATP levels have been depleted as a result of a severe loss in mitochondrial function, oncosis may be the only way to complete the process of cell death (15-17,21).

## Tools available to screen for modulators of the various types of cell death

Several techniques are now available to investigate the pro-apoptotic effects of test compounds (16). The cell morphology characteristic for apoptotic cells can be observed by microscopy. Various dyes, including 4',6-diamidino-2-phenylindole (DAPI) and Annexin V, can assist with the microscopy analysis. The dyes can be used to flow cytometry studies. DAPI is a fluorescent dye that binds to DNA and can visualize apoptotic body formation as a consequence of chromosomal fragmentation (28). Annexin V is used to detect cells that express phosphatidylserine on their cell surface, as it is the case in apoptosis and in autophagy (3,17). TUNEL (Terminal deoxynucleotidyl transferase mediated dUTP Nick End Labeling) is a fluorescence-based technique used to detect DNA fragmentation (3). Finally, Western blot analysis can be used to monitor the activation of caspases and the cleavage of substrates such as PARP by the latter (3). Several of the techniques listed above can also be used to search for inducers of autophagy or oncosis. As in apoptosis, cells undergoing autophagy exteriorize phosphatidylserine, but oncotic cells don't. The activation of caspases is characteristic for apoptosis, but does not occur during oncosis, and only happens at a very late stage, if at all, during autophagy (3). The dye propidium iodide, which cannot permeate cell membranes, is a marker for cells with permeabilized or lysed membranes. The formation of vesicular bodies during autophagy can be monitored using the lysosomotropic agent acridine orange (29,30). The accumulation of autophagosomes can also be detected biochemically or microscopically, as pro-autophagic proteins belonging to the Atg family conjugate with phosphatidylethanolamine. The resulting complexes, which stably associate with the autophagosomal membrane, can be fluorescently tagged for microscopy studies (5,30,31). The major features of oncosis which can be used to monitor the induction of the latter by test compounds include the dramatic swelling of the cells and the induction of inflammatory responses (3,15,21).

## The orchestration of the various types of programmed cell death by marine natural products

Many marine organisms, and sessile, soft-bodied invertebrates lacking physical defence mechanisms, have developed potent toxins as chemical defences against their predators. The toxins produced by marine organisms display unique chemical and biological features of scientific interest, and the hypothesis established by the pioneers in the field of marine natural products research in the 1960's that at least some of these very potent marine toxins could have promising cytotoxic effects on cancer cells has long since been verified (32-34). Of the numerous cytotoxic marine natural products identified to date, several have been described as activators of apoptosis, autophagy, or oncosis (33,35-38). From an ecological or evolutionary perspective, this doesn't come too much as a surprise, as caspase-mediated apoptosis is an evolutionary highly conserved process found already in place in basal metazoan phyla such as sponges (39) and cnideria (40) and even in pre-metazoan phytoplankton (41). Apoptosis plays critical roles in the metamorphosis of sea urchins (42) and of ascidians (40) and in various host/parasite interactions (43). Autophagy protects mussels (44) and sea cucumbers (45) against various environmental stresses, including starvation,

reactive oxygen species, copper accumulation, and UV radiation and sea cucumber from "body melting" under UV radiation. Both apoptosis and autophagy have been shown to have severe ecological implications in coral bleaching in coral bleaching (46).

Here, we describe the effects of cytotoxic marine natural products on the three different pathways of programmed cell death. The association of the different marine natural products with their target cell death pathways is summarized in figure 1.

## The activation of apoptosis by marine natural products

The sesquiterpenoid euplotin-C (fig. 1 and 2, 1) isolated from the marine ciliate *Euplotes crassus* has been reported as an activator the pro-apoptotic Bcl-2 protein Bax through the activation of p53, leading to the loss of mitochondrial membrane potential and to the activation of the caspase cascade (47).

The diatom *Phaeodactylum tricornutum* has yielded two pro-apoptotic galactolipids, but their mechanism of action remains unknown (48).

Several marine natural products isolated from dinoflagellate have been reported to induce apoptosis. These include the polyether amine azaspiracid-1 (fig. 2, 2) from *Protoperidinium crassipes*, which is known to activate the caspase cascade and to induce chromatin condensation (49,50), the red tide toxin brevetoxin B (fig. 1 and 2, 3) isolated from various genera of dinoflagellates, including *Gymnodinium breve* and *Karenia brevis*, which, in addition to other toxic effects, activates the intrinsic apoptotic pathway (20,50), the amino acid domoic acid (fig. 1 and 2, 4) released by *Pseudo-nitzschia* sp. during algal blooms, which has been reported to lead to both apoptotic cell shrinkage and oncotic cell swelling (51), and the diarrhetic shellfish poison okadaic acid (fig. 1 and 2, 5) produced by *Prorocentrum* sp. and from *Dinophysis* sp. Okadaic acid triggers the extrinsic apoptosis pathway through Fas (50,52,53). The macrolide pectenotoxin 2 (fig. 1 and 2, 6) isolated from the dinoflagellate *Dinophysis* sp. activates pro-apoptotic members of the Bcl-2 family (54,55). The carotenoid peridinin (fig. 1 and 2, 7) isolated from the dinoflagellate *Heterocapsa triquetra* has been shown to activate both the extrinsic and intrinsic apoptotic pathways (56).

Research on the natural products produced by marine bacteria has boomed over the last decade. Intensive screening of extracts from cultured marine bacteria has let to a 10% hit rate in cytotoxicity assays. Amongst the cytotoxic extracts, a distribution of 3 pro-apoptotic extracts to 7 oncosis-inducing extracts has been observed (57). The bis-indole alkaloid staurosporine (fig. 1 and 2, 8) isolated from marine *Streptomyces* sp. has been shown to induce apoptosis in neuronal cells in TUNEL studies, DNA laddering studies, and by microscopy. Staurosporine activates the intrinsic apoptosis pathway and induces a loss in mitochondrial membrane potential (58). The red pigment cycloprodigiosin hydrochloride (Fig. 2, 9) from *Pseudoalteromonas denitrificans* induces apoptosis by potently inhibiting the anti-apoptotic transcription factor NF-kB (59-61). Iron chelators such as desferrioxamine B (fig. 1 and 2, 10) isolated from *Vibrio* sp. have been shown to increase the expression of the pro-apoptotic Bcl-2 protein Bax, to decrease the expression of the anti-apoptotic protein Bcl-2, and to activate p53 and $p21^{WAF1/CIP1}$ which are both known to induce apoptosis (62,63). The b-carboline alkaloid norharman (fig. 1 and 2, 11) from *Pseudoalteromonas piscida* has been shown to induce apoptosis in various studies, including TUNEL and microscopy. Norharman leads to

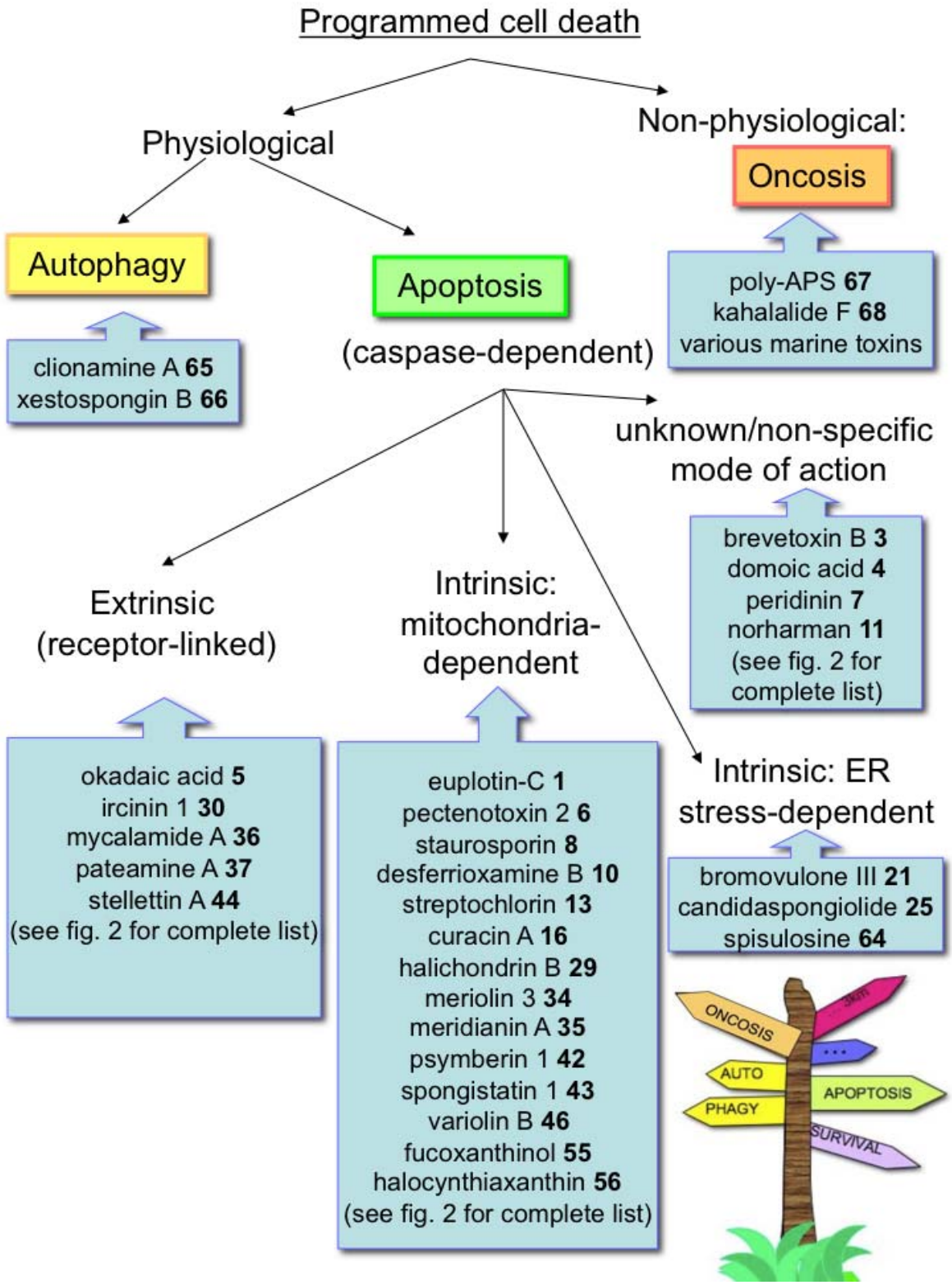


**Figure 1.** The programmed cell death pathways (*from* [3] (Lockshin RA and Zakeri Z. Apoptosis, autophagy, and more. International Journal of Cellular Biology 2004;36:2405-19), with modifications), with examples of marine natural products targeting the various types of cell death.

the condensation of chromatin and to the degradation of DNA, but the detailed mechanism of action remains unknown (64). The b-lactam salinosporamide A (fig. 2, 12) isolated from *Salinospora tropicana* downregulates several anti-apoptotic Bcl-2 members and inhibits the anti-apoptotic transcription factor NF-kB (65). The oxazole streptochlorin (fig. 1 and 2, 13) from various *Streptomyces* species activates the pro-apoptotic Bcl-2 protein Bax and inhibits the anti-apoptototic protein Bcl-2. Streptochlorin has been shown to activate the caspase cascade [66]. The pigment violacein (fig. 2, 14) isolated from *Chromobacterium violaceum* induces apoptosis by inhibiting the anti-apoptotic transcription factor NF-kB and the anti-apoptotic protein kinase B (Akt) (67).

Pro-apoptotic marine natural products isolated from cyanobacteria include the light-harvesting biliptortein C-phycocyanins (fig. 2, 15) from *Spirulina platensis*. C-phycocyanins activates the pro-apoptotic protein Bax and inhibits the anti-apoptotic protein Bcl-s. As a result, C-phycocyanins induces the typical apoptosis cell morphology as observed by microscopy and induces the cleavage of the apoptosis substrate molecule PARP (68). The lipid curacin A (fig. 1 and 2, 16) obtained from the cyanobacterium *Lyngbya majuscula* potently induces intrinsic apoptosis (19). The lipopeptide somocystinamide A (fig. 2, 17) isolated from the same species of cyanobacterium activates the extrinsic apoptotic pathway (69).

Leptosin C (fig. 2, 18) and other closely related epipolythiodiketopiperazines isolated from *Leptoshaeria* sp. activate apoptosis by inhibiting the anti-apoptotic protein kinase B (Akt) (38,50,70).

The sesquiterpenoid laurinterol (fig. 2, 19) isolated from the red alga *Laurencia okamurai* induces apoptosis through the activation of p53 and p21 (71).

The diterpene eupalmerin acetate (fig. 2, 20) isolated from the gorgonian octocoral *Eunicea succinea* induces the phosphorylation of JNK and activates the pro-apoptotic Bcl-2 member Bax (72).

The cyclopentenone prostanoids bromovulone III (fig. 1 and 2, 21) and clavulon II (fig. 2, 22) isolated from the soft coral *Clavularia viridis* activate the caspase cascade and induce a loss in mitochondrial membrane potential. Bromovulone III, but not clavulon II has been reported to primarily induce the activation of caspase 12, suggesting that it is mainly involved in endoplasmic reticulum stress-related apoptosis (38,73).

Numerous sponge-derived natural products have been identified as apoptosis inducers. Amongst them are the brominated alkaloid aeroplysinin-1 (fig. 2, 23) brominated isolated from *Aplisina aerophoba* whose detailed mechanism of action remains to be investigated (37,74,75), the nucleoside analogue ara-C (cytarabine) (fig. 2, 24) isolated from *Cryptotethia crypta*, which modulates the effects of the protein kinase PKC on JNK, on the MAPK p38, and on the transcription factor NF-kB (76), the polyketide candidaspongiolide (fig. 1 and 2, 25) from *Candidaspongia* sp., which is primarily implicated in endoplasmic reticulum stress-related apoptosis (1), the polyacetylene dideoxypetrosynol A (fig. 2, 26) from *Petrosia* sp., which activates the mitochondria-related intrinsic apoptosis pathway (37,77), and the polyketide discodermolide (fig. 2, 27) isolated from the deep-sea sponge *Discodermia dissoluta* (78). Despite the several apoptotic features induced by discodermolide, it has been suggested that its cytotoxicity is due to effects on microtubule function, rather than to its pro-apototic properties (36,79), although it is not excluded that these two processes may be interlinked (80). As a matter of fact, interference with the microtubule assembly lead to cell cycle arrest at the G2-M phase and to the consequent

activation of the pro-apoptotic Bcl-2 protein Bad (19). Further sponge-derived apoptosis inducers include the isomalabaricane triterpene geoditin A (fig. 2, 28) isolated from *Geodia japonica*, which triggers the dissipation of the mitochondrial membrane potential (38,81), the large polyether macrolide halichondrin B (fig. 1 and 2, 29) isolated from various sponges and its synthetic analogue E7389 currently in clinical trials (EISAI), which activate the mitochondria-related intrinsic apoptotic pathway (19,37,82), the sesterterpene ircinin 1 (fig. 1 and 2, 30) isolated from various sponges, which induced extrinsic apoptosis through Fas (38,83), and the depsipeptide jasplakinolide (fig. 2, 31) isolated from various sponges, which stabilizes actin filaments and thereby preventing the overexpression of the anti-apoptotic Bcl-2 protein Bcl-XL (36,84-86). The isomalabaricane triterpene jaspolide B (fig. 2, 32) isolated from *Jaspis* sp. has been shown by flow cytometry to induce apoptosis, but it is also known to trigger the dissassembly of tubulin (64). The pro-apoptotic macrolide latrunculin A (fig. 2, 33) isolated from the sponges *Negombatta magnifica* and *Latrunculia* sp. is primarily implicated in the inhibition of actin polymerization (87,88). A series of potent pro-apoptotic azaindoles called meriolins, which include meriolin 3 (fig. 1 and 2, 34), have been synthesized as hybrids between spongean guanidine alkaloids belonging to the variolin family and analogues of the brominated indole meridianin A (fig. 1 and 2, 35) isolated from the ascidian *Aplidium meridianum* (89). Both meriolin 3 and meridianin A potently inhibit cyclin-dependent kinases by occupying the ATP binding site of the enzyme's catalytic subunit. As a result, meriolin 3 and meridianin A activate the Bax and Bak-dependent mitochondrial apoptosis pathway (90). Two alkaloids isolated from *Mycale* sp. sponges, mycalamide A (fig. 1 and 2, 36) and pateamine A (fig. 1 and 2, 37), have been shown to enhance Fas-mediated apoptosis through their interaction with p21*ras* signalling pathways (16,36). Theopederin-type polyketides such as onnamide A (fig. 2, 38) isolated from the same genus activate the pro-apoptotic transforming growth factor (TGF)-b pathway which leads to the activation of the MAPK p38 and of JNK (38,91). The macrolide peloruside A (fig. 2, 39) isolated from *Mycale hentscheli* induces apoptosis through the microtubule stabilization followed by mitotic cell cycle arrest (37,92). The alkaloid psammaplin A (fig. 2, 40) produced by a variety of marine sponges induces the expression of $p21^{WAF1}$ (28,38,93). Psammaplin A has been recognized as a very potent anti-cancer compound, but because of its physiological instability, the compound is undruggable. A synthetic analogue of psammaplin A, NVP-LAQ824 (Novartis Pharmaceutical), which possesses the same pro-apoptotic properties, has entered clinical trials for solid tumour and leukaemia (94). The dimeric bromotyrosine alkaloid psammaplysene A (fig. 2, 41) from *Psammaplysilla* sp. induces apoptosis by increasing the expression and relocalization of the transcription factor Forkhead box O (FOXO)1 into the nucleus in PTEN-deficient cancer cells. FOXO1, which is located downstream of Akt in the PI3K/Akt signalling pathway, is normally sequestered in the cytoplasm. The nuclear translocation of FOXO1 leads to an increase in TRAIL and FasL expression (95). A pederin-type polyketide related to onnamide A, psymberin1 (fig. 1 and 2, 42), has been isolated from the sponge *Psammocinia* sp. and has been shown to activate the caspase cascade. Psymberin1 is potentially produced by endosymbiotic bacteria belonging to the genus *Pseudomonas*, rather than by the sponge (96). The macrolide spongistatin 1 (fig. 1 and 2, 43) isolated from *Spongia* sp. activates the intrinsic apoptotic signalling pathway. 44 has indeed been shown to induce the release of cytochrome c and of Smac, and to consequently induce the degradation of XIAP and the activation of caspase 9 (97). The

isomalabaricane triterpene stellettin A (fig. 1 and 2, 44) isolated from *Rhabdastrella globostellata* has been shown the Fas-dependent extrinsic apoptotic pathway (38,98). The macrolide swinholide 1 (fig. 2, 45) isolated from *Theonella swinhoei* induces the same pro-apoptotic mechanisms as latrunculin A (38,88), while the guanidine alkaloid variolin B (fig. 1 and 2, 46) isolated from the Antarctic sponge *Kirkpatrickia variolosa* possesses the same pro-pro-apoptotic mode of action as the ascidian-derived meridianins and the synthetic meriolins (38,89,99).

Like latrunculin A and swinholide 1, the macrolide aplyronine A (fig. 1 and 2, 47) isolated from the sea slug *Aplysia kurodai* induces apoptosis through the depolarization of actin filaments (2,100), while the pentapeptides dolastatin 10 (fig. 2, 49) isolated from *Dolabella auricularia*, its synthetic derivative auristatin-PE (soblidotin, TZT-1027) (fig. 2, 48), and its natural analogue dolastain 15 (fig. 2, 50) interfere with tubulin polymerization (19). Compounds 48-50 have been shown to activate both the Fas-dependent extrinsic and the mitochondria-dependent intrinsic apoptotic pathways (100-103). It has now been confirmed that dolastatins 10 and 15 actually produced by the sea slug's cyanobacterial diet, *Symploca* sp. (19). Dolastatin 10 has failed phase II clinical trial (19), but to the best of our knowledge it's synthetic analogue auristatin-PE is still undergoing advanced clinical trials for treating solid tumors.

An impressive proportion of the marine natural products that have reached clinical trials as antitumor agents are derived from ascidians and have potent pro-apoptotic properties (94,104). These include the cyclic depsipeptides aplidine (dehydrodidemnin B, Aplidin® (Pharmamar)) (fig. 2, 51) and didemnin B (fig. 2, 52) isolated from *Aplidium albicans* and from *Trididemnum solidum*, respectively, and the isoquinoline alkaloid ecteinascidin 743 (trabectedin, Yondelis® (PharmaMar and OrthoBiotech)) (fig. 2, 53) isolated from *Ecteinascidia turbinata*. Compounds (fig. 2, 52-54) upregulate JNK and MAPK p38, resulting in the downstream release of cytochrome C, the activation of Smac and of caspases 3 and 9, the cleavage of PARP, and the formation of apoptotic bodies. Additionally, aplidine and didemnin B have been shown to activate the TRAIL-dependent pro-apoptotic signalling cascade (36,105-108). As for the natural product 49 isolated from sea slugs, it has, however, been hypothesized that compounds aplidine and didemnin B are actually produced by cyanobacteria, rather than by the ascidians themselves (104). Cardiotoxicity has caused didemnin B to be dropped from clinical trials, while the closely related, yet non-cardiotoxic and more potent analogue aplidine (PharmaMar) is in late phase II clinical trials for solid and haemological malignant neoplasias and in phase I trials for acute paediatric leukaemia, and ecteinascidin 743 (PharmaMar) has received marketing authorization from the European Commission for the treatment of advanced or metastatic soft tissue carcinoma and for ovarian cancer (109). Other ascidian-derived pro-apoptotic natural products include the alkaloid ascididemnin (fig. 2, 54) isolated from *Amphimedon* sp., which upregulates JNK (37,110), the carotenoids fucoxanthinol (fig. 1 and 2, 55) and halocynthiaxanthin (fig. 1 and 2, 56) from *Halocynthia roretzi* which modulate the balance betwen pro- and anti-apoptotic Bcl-2 proteins (38,111), the brominated indole meridianin A meridianin A isolated from *Aplidium meridianum,* which, as described above, inhibits cyclin-dependent kinases and thereby activates the intrinsic apoptosis pathway (89,90), the macrolide iejimalide B (fig. 2, 57) isolated from *Eudistoma rigida,* which inhibits survivin and $p21^{WAF1/CIP1}$ (112), the imidazole-type alkaloid polycarpine (fig. 2, 58) from *Polycarpa aurata,* which activates the phosphorylation of p53 through JNK

(113), the alkaloid ritterazine B (fig. 2, 59) from *Ritterella toikoka*, which promotes apoptosis by potently inducing cell cycle arrest at the G2/M checkpoint (114), and the fatty acid turbinamide (fig. 2, 60) isolated from *Sydnium turbinatum*, whose detailed mode of action remains unknown (36,115).

The enolic sulphated sterol arenicolsterol (fig. 2, 61) isolated from the annelid *Arenicola cristata* has been shown by Annexin V-based microscopy to induce apoptosis, but the precise mechanism of action remains to be investigated (23). On the other hand, a second worm-derived marine natural product, the bis-steroid cephalostatin 1 (fig. 2, 62) isolated from *Cephalodiscus gilchristi*, is probably one of the most intensively investigated pro-apoptotic marine natural products (17). Cephalostatin 1, which is closely related to ritterazine B (114) selective triggers the release of Smac (17,38,116). Cephalostatin 1 also activates caspase 9, but without the upstream mitochondrial release of cytochrome c or of apoptosomes. As shown by Lopez-Anton *et al*. (117), the non-classical activation of caspase 9 mediated by cephalostatin 1 is triggered by the activation of caspase 4 through the endoplasmic reticulum stress-related apoptotic signalling cascade (17).

The macrolide bryostatin 1 (fig. 2, 63) isolated from the bryozoan Bugula neritina is a potent inhibitor of PKC. Bryostatin 1 64 has been shown to prevent the anti-apoptotic Bcl-2 protein Bcl-XL from from blocking the mitochondrial release of cytochrome C (36,118).

Finally, the sphingolipid derivative spisulosine (fig. 1 and 2, 64) isolated from the clam *Mactromeris polynima* specifically induces the endoplasmic reticulum stress-dependent apoptotic signalling cascade, leading to the activation of the initiator caspase 12 (119).

The cellular targets of the pro-apototic marine natural products listed above are shown in figure 2.

### Marine inducers of autophagy

The aminosteroid clionamine A (fig. 1, 65) isolated from the sponge *Cliona celata* has been shown by microscopy using the Atg family autophagy marker LC3 fused to green fluorescent protein to induce autophagy (31). The alkaloid xestospongin B (fig. 1, 66) isolated from the sponge *Xestospongia exigua* has been shown to induce autophagy through its inositol triphosphate receptor antagonistic properties (5,120).

### Marine inducers of oncosis

Several marine natural products are known to be harshly cytotoxic, and to induce acute cell death that lacks the typical characteristics of apoptosis or of autophagy (32,35,36,38,51,121,122). Marine natural products reported to specifically induce oncosis in human cells include a series of pore-forming toxins such as the polymeric 3-alkylpyridinium salts (poly-APS) (fig. 1, 67) isolated from the sponge *Reniera* sp. (123) and various jellyfish-, sponge-, sea anemone-, worm-, and bacteria-derived secondary metabolites (123-130). The depsipeptide kahalalide F (fig. 1, 68) isolated from the sea slug *Elysia rufescens* leads to several oncosis-specific hallmarks, including loss of mitochondrial potential, cytoplasmic swelling and vacuolisation, without affecting any caspase-dependent signalling pathway (37,131). The neurotoxin domoic acid which was described earlier in the present review as a pro-apoptotic compound, can simultaneously induce apoptosis (characterized by shrunk cells) and oncosis (characterized by swollen cells). After 24 hours of treatment, oncosis dominates over apoptosis (51,132).

## CONCLUDING REMARKS

As shown in the present review, numerous marine natural products are potent modulators of programmed cell death. The majority of the compounds described in the review specifically induce apoptosis, while there are very few reports

on marine natural products specifically targeting cell death types II and III. This observation could possibly be explained by a bias towards apoptosis in the interest of pharmaceutical research and in the availability of tools to screen for activators of the various types of cell death. With the recent spark in interest for modulators of autophagy as cancer therapeutics or as chemical tools to study the genetics of autophagy (10,25,31,133), one can expect a proportional increase in discovery of novel marine natural products targeting autophagy within the next decades. Some biotechnological interest has also been focusing on programmed cell death type III-inducing marine natural products such as the polymeric 3-alkylpyridinium salts (poly-APS) isolated from the sponge *Reniera* sp. (123) which have been used for cellular transfection. It can be concluded that marine natural products have been and will continue to be a very promising source of molecules orchestrating the various types of programmed cell death.

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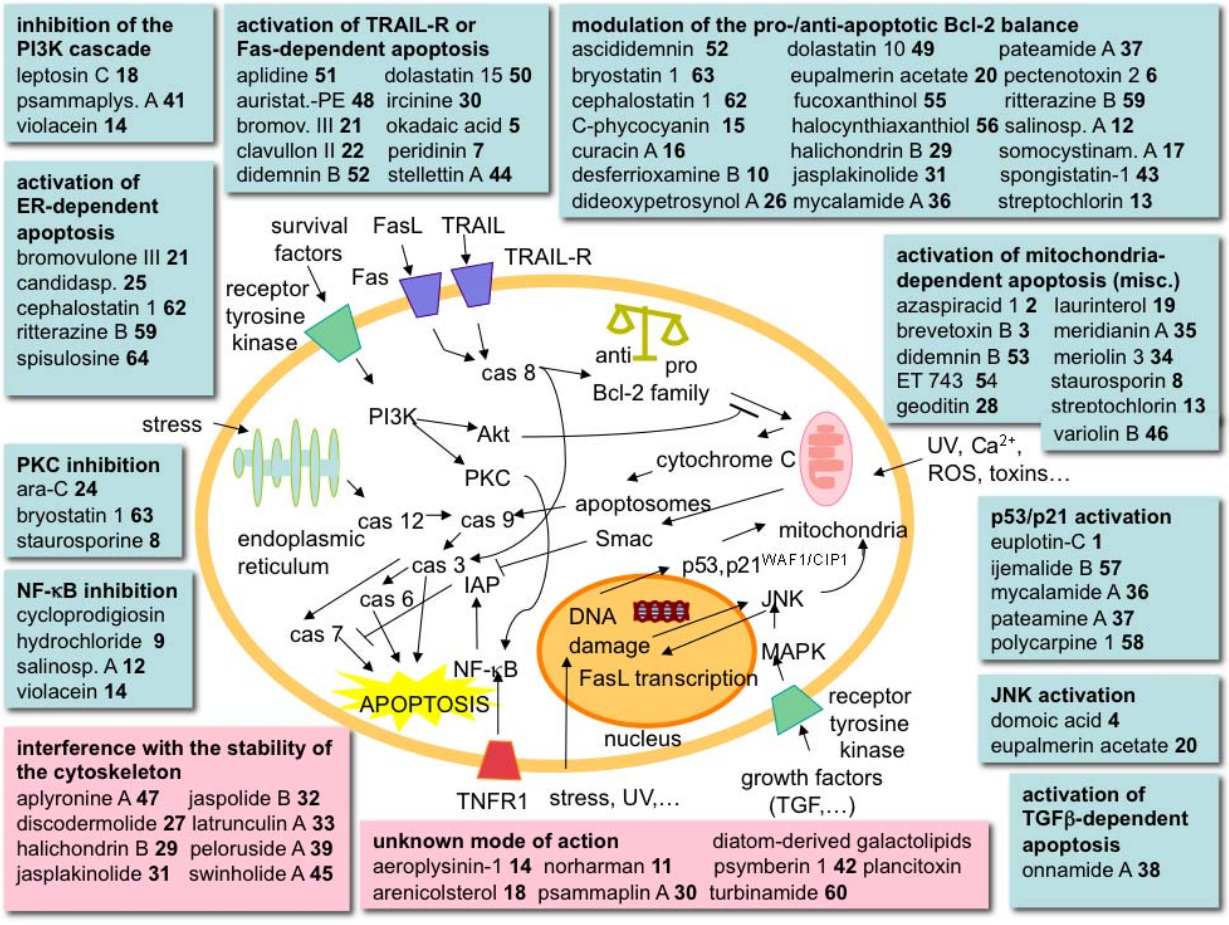


**Figure 2.** The cellular targets of pro-apoptotic marine natural products.

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