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Commentary

Marine natural products as targeted modulators of the transcription factor NF-κB

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ABSTRACT

The inducible transcription factor nuclear factor-kappaB (NF-κB) plays an important role in the regulation of immune, inflammatory and carcinogenic responses. While normal NF-κB activation is necessary for cell survival and immunity, deregulated NF-κB expression is a characteristic phenomenon in cancer development, as well as in several inflammatory diseases. Hence, NF-κB has become a major target in drug discovery, and several natural and synthetic compounds have been investigated for their potential to inhibit NF-κB. Here, we discuss the applications of marine natural products, in particular, as novel, potent NF-κB inhibitors. With the oceans covering two-thirds of the Earth's surface, and with the uniqueness of the environmental properties of marine habitats, it is easily understandable that organisms thriving in the oceans constitute a rich source of chemically unique and biomedically powerful secondary metabolites. Since the early 1960s, significant effort has been placed on the pharmacological evaluation of marine secondary metabolites. Noteworthy achievements of this field of biomedically guided marine exploration, a scientific endeavour often referred to as the search for "*Drugs from the Sea*", include the discovery of numerous potent anti-cancer, anti-inflammatory, antimicrobial, and analgesic compounds. The chemical characteristics and molecular targets of marine NF-κB inhibitors discovered to date are presented and discussed in the context of marine chemical ecology.

1. Secondary metabolites as a source of natural drugs

Many of the medicines prescribed today are natural products obtained from terrestrial plants and microorganisms. The use of plant-derived secondary metabolites as drugs has come to us as a legacy of folk medicine based on herbal remedies [1,2]. Some well-known examples are the anti-malarial drug quinine obtained from fever-tree (*Cinchona officinalis*) bark, the analgesics codeine and morphine isolated from opium-poppy (*Papaver somniferum*) latex, and the anti-cancer substance silymarin isolated from the milk thistle (*Silybum marianum*) [2,3]. Natural products isolated from marine organisms have also been shown to have a great potential

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Abbreviations: IκB, inhibitor of NF-κB; IKK, kinase of IκB; MIC, minimal inhibitory concentration; MNP, marine natural product; NF-κB, nuclear factor-κB; PLA_2, phospholipase A_2; ROS, reactive oxygen species; TNF-α, tumour necrosis factor α; TNFR1, TNF-α receptor 1; TRADD, TNFR1-associated death domain; TRAF2, TNF-receptor-associated factor 2; VEGF, vascular endothelial growth factor.

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in drug discovery [4]. With the ocean covering 70% of the Earth's surface, and with the uniqueness of the environmental conditions present in the oceans, it is easily understandable why the ocean can be considered as a very promising source of natural drugs – or synthetic derivatives thereof – for the future [4].

With the continuous emergence of new diseases and the development of drug resistance in harmful bacteria, viri, and cancer cells, there is a continuous need for the development of new drugs with novel mechanisms of action. In the present review, we discuss the potential of marine natural products to inhibit the activation of the transcription factor nuclear factor κB (NF-κB), which has been shown to play a key role in cancer development and inflammation [5–7]. Since the research for marine NF-κB inhibitors is still at a relatively early stage, examples of terrestrial natural products are used to illustrate our current understanding of the molecular targets, of the mechanisms of action, and of the chemical properties of the pharmacophores of natural products inhibiting the transcription factor NF-κB.

2. The transcription factor NF-κB: its activation pathway and its implications to diseases

Transcription is an important regulatory event in the pathway leading to gene expression. Transcription factors regulate transcription by binding to specific sequences present within the promoter, enhancer, or other regulatory regions of DNA. There are several well-characterized signalling pathways and transcription factors that are known to be involved in the development of cancer and of other diseases, including rheumatoid arthritis, asthma, inflammatory bowel disease, atherosclerosis, cystic fibrosis, diabetes, AIDS, and Alzheimer's disease [8,9]. Of these, the transcription factor NF-κB plays an exceptionally important role because of the rapidity of its activation and because of the extremely large number of genes regulated by NF-κB that are implicated in diseases, and in carcinogenesis and inflammation in particular [7–9]. NF-κB activation is now considered as the key element to the long-recognized link between inflammation and cancer [10].

NF-κB is an inducible transcription factor found in virtually all types of cells. It is a dimer of proteins belonging to the Rel family, which includes RelA (p65), RelB, c-Rel, p50 (NF-κB1), and p52 [11]. All five Rel family proteins contain, at their N-terminus, a well-conserved Rel homology domain (RHD) consisting of approximately 300 amino acids which is responsible for the dimerization of NF-κB, for the interactions of NF-κB with its cytoplasmic inhibitory protein, IκB, and for the binding of NF-κB to DNA [11]. At the C-terminus of the RHD, Rel proteins contain a nuclear localization signal (NLS) which allows the activated NF-κB protein to translocate into the nucleus [11]. p65, RelB, and c-Rel, which are biosynthesized as active proteins, also contain a terminal transactivation domain (TAD) at their C-terminal end which is required for the activation of transcription [11]. p50 and p52, on the other hand, are first synthesized as large precursors (p105 and p100, respectively) which lack a terminal transactivation domain. Hence, both p50 and p52 are transcriptionally inactive [11]. The most common form of NF-κB is the p50/p65 heterodimer [11]. NF-κB is normally found in the cytoplasm, in an inactive form as it is bound to IκB. NF-κB activation is triggered by stimulation from extra-cellular ionizing radiation, from oxidative stress, from pro-inflammatory cytokines such as TNF-α or IL-1β, or from various toxins, which culminate in the activation of the IκB kinase complex IKK [6,11]. IKK phosphorylates IκB. The phosphorylation is followed by Lys^{48}-linked (Ub^{K48}) polyubiquitination of IκB, which marks the protein for degradation by the 26S proteasome [12]. Once freed from IκB, NF-κB translocates into the nucleus, where it activates its target genes [6,11]. To date, there are still many unknowns about the detailed pathway of NF-κB activation upstream of the phosphorylation of IκB by IKK. The most commonly reported pathway involves the binding of the cytokine TNF-α to its cytoplasmic membrane receptor TNFR1, followed by the recruitment of several signalling proteins to the cytoplasmic domains of the receptor [13]. The first protein recruited to TNFR1 is the TNFR1-associated death domain protein (TRADD), which serves as a platform to recruit the TNF-receptor-associated factor 2 (TRAF2) and the receptor-interacting protein (RIP). TRAF2 is an ubiquitin ligase whose major ubiquitination targets are RIP and TRAF2 itself [13]. Ub^{K63}-polyubiquitinated RIP binds to and activates a protein kinase complex composed of the MAP3K TGFβ (transforming growth factor β)-activated kinase 1 (TAK1) and of TAK1's two adapter proteins TAB1 and TAB2 [14]. Both RIP and TAK1 activate the MAP3K MEKK3 [15]. Activated TAK1, MEKK3, and other MAP3Ks phosphorylate, and thereby activate IKK [13,15]. Over-expression of TNFR1, TRADD, or TRAF2 alone has been shown to be sufficient to activate NF-κB, although the mechanism of action remains unknown [16]. One possible explanation is that, by virtue of being present in the cell in large concentration, these over-expressed proteins aggregate into complexes that can activate downstream events in the absence of ligands [17].

The present review focuses mainly on the effects of natural products on molecular targets along the "classical", or "canonical" NF-κB activation pathway described above. The canonical pathway remains, today, the most studied NF-κB activation pathway. Other NF-κB activation pathways, which are not discussed here, include the NIK (NF-κB inducing kinase)-dependent "alternative" pathway [6,11], the "p105 pathway" [18], and IKK-independent NF-κB activation pathways such as the casein kinase 2 (CK2) pathway that are triggered by short wavelength UV radiation, γ-radiation, or various chemotherapeutic agents [19–21].

3. Molecular targets for NF-κB inhibitors

Several different strategies can be envisioned for inhibiting NF-κB activation or function. One possibility is to interfere with the binding of NF-κB to DNA [22]. However, given the large interaction surface mediating the binding of NF-κB to DNA, it is rather unlikely to find small natural products that specifically block NF-κB-DNA binding [22]. Nevertheless, sesquiterpene lactones, which have been hypothesised to interfere directly with NF-κB-DNA binding, are the most widely published class of natural products cited as NF-κB

inhibitors [22,23]. The bioactivity of the sesquiterpene lactones can possibly be explained by a Michael-type conjugate addition of the nucleophilic cysteine sulfhydryl groups Cys^{38} and Cys^{120} in the p65 monomer of NF-κB to one or more α,β-unsaturated carbonyl groups of the sesquiterpene lactones [23,24]. Within α,β-unsaturated carbonyl groups, the electrons of the C=C bond are delocalized. Consequently, the C=C bond of the resonance structures are electrophilic and can bind to nucleophiles such as the cysteine sulfhydryl groups of p65 through conjugate addition reactions [25]. Cys^{38} is located within the DNA-binding domain of p65, and the Michael addition of Cys^{38} in p65 to the α,β-unsaturated carbonyl groups of the sesquiterpene lactones interferes with the activation of NF-κB primarily by preventing the binding of NF-κB to DNA [23].

Unlike Michael acceptors, a few compounds, including gallic acid (**1**), are able to specifically inhibit the binding of p50 to DNA, by making strong hydrogen bonds with amino acids, serine Ser^{66} in particular, that play an important role in the binding of p50 to DNA. These hydrogen bonds lead to steric hindrance in the DNA-binding region of p50 and affects the three-dimensional conformation required for DNA binding [26].

Another strategy of interfering with the process of NF-κB activation is to target the proteasomal degradation of IκB [27]. The inhibition of the proteasome has recently started to be viewed as a highly promising novel approach to cancer therapy [27], and some proteasome inhibitors, including the synthetic peptide boronate Velcade® (also known as bortezomib or PS-341) (**2**) [27] and the γ-lactam PS-519 (**3**) [28], have entered clinical trials as anti-cancer molecules. Velcade® (**2**) (Millennium Pharmaceuticals, Inc., and Johnson & Johnson Pharmaceutical Research & Development) is the first anti-cancer drug on the market to target the proteasome, and, thereby, also the first anti-cancer drug on the market to target NF-κB activation. Velcade® (**2**) has been shown to be a very successful drug against multiple myeloma, which is one of the most common types of haematological malignancies. Unfortunately, in many cases, proteasome- or other protease-inhibition is not specific because the proteasome is involved in the degradation of a wide range of polyubiquinated proteins [29]. In particular, the proteasome plays an important role in the regulation of the cell cycle, as mitosis is regulated by cyclins and cyclin-dependent kinases, which are the most important substrate of the proteasomal degradation machinery [30].

A slightly higher degree of specificity can be achieved with inhibitors of the ubiquitin ligases and ubiquitin-conjugating enzymes responsible for the phosphorylation-dependent polyubiquitination of IκB [31,32] and for the activation of RIP, TAK1, and of other MAP3Ks implicated in IKK activation [13,33,34]. Ubiquitination involves the sequential action of three different types of enzymes, namely the ubiquitin activation enzymes E1, the ubiquitin conjugating enzymes E2, and the ubiquitin protein ligase enzymes E3 [14]. The enzymes implicated in ubiquitination function as dynamic switches that can influence signalling outputs in dramatically different ways, based on the location of the ubiquitin chains on the target molecule and based on the three-dimensional structure and chemical composition of the ubiquitin chains [34,35]. The rare ubiquitination inhibitors identified to date include the E1 inhibitors panepophenanthrin (**4**) and himeic acid A (**5**) isolated from terrestrial fungi [36,37], and various phenylarsenoxides capable of inhibiting E3 enzymes [38].

The most effective and selective approach for the inhibition of NF-κB activation is provided by inhibitors of the IKK activity [22,29]. As a matter of fact, there is little or no evidence that IKK might phosphorylate proteins involved in other pathways than the NF-κB activation cascade [6,29]. Although the mechanism of action of IKK targeting NF-κB inhibitors remains largely unknown, it has been speculated that the inhibition of IKK results from a formation of hydrogen bonds between the IKK inhibitors and IKK [29]. Natural products specifically targeting the kinase activity of IKK include the two benzoquinones herbimycin A (**6**) and geldanamycin (**7**) isolated from marine and terrestrial bacteria [39].

NF-κB activation can also be slowed down by antioxidants such as vitamins C (ascorbic acid) and E (α-tocopherol), coenzyme Q10, and a variety of polyphenolics [22,29,40–42], or by selenium compounds required for the enzymatic activity of cellular antioxidants such as glutathione and thioredoxin. Antioxidants reduce the level of reactive oxygen species (ROS) which could otherwise activate NF-κB [22,29,40].

Finally, with the continuously growing understanding of the NF-κB activation pathway, new molecular targets to inhibit NF-κB, such as the TNF-α receptor TNFR1 and its associated proteins TRADD and TRAF2, are being discovered at a rapid pace (see Fig. 1 for chemical structures **1**–**7**).

4. Secondary metabolites modulating the NF-κB activation pathway: chemical characteristics, molecular targets, structure–activity relationships

A large number of natural products from various chemical classes, including sesquiterpene lactones, kaurene diterpenes, triterpenes, phenolics, and γ-lactams, have been tested for NF-κB inhibitory properties since the first report of a plant-derived NF-κB inhibitor, sodium salicylate, by Kopp and Ghosh in 1994 [43]. A selection of terrestrial natural products reported as potent NF-κB inhibitors, with minimal inhibitory concentration (MIC) values ranging from 1 to 320 μM, are listed in Table 1 (see Fig. 2 for chemical structures **8**–**22**).

As mentioned in Section 3, sesquiterpene lactones, which have been hypothesised to interfere directly with NF-κB-DNA binding, are the most widely published class of natural products cited as NF-κB inhibitors [22,23]. Not all, but the majority of sesquiterpene lactones possess at least one α,β-unsaturated carbonyl group that can act as Michael acceptors for the nucleophilic cysteine sulfhydryl groups Cys^{38} and Cys^{120} of p65 [23,24]. These include α-methylene-γ-lactones, α,β-unsaturated esters, and α,β-unsaturated cyclopentenones. Michael-type addition of α,β-unsaturated carbonyl groups to NF-κB appears, in the first instance, to be unspecific, as other proteins involved in a wide range of cellular functions may be modulated in the same way [22]. As a matter of fact, compounds that act as Michael acceptors are often rejected from pharmaceutical trials due to their interferences with vital proteins [23,44]. Furthermore, *in vivo*, the bioactive potential of Michael acceptors is severely attenuated, since Michael acceptors can be scavenged by

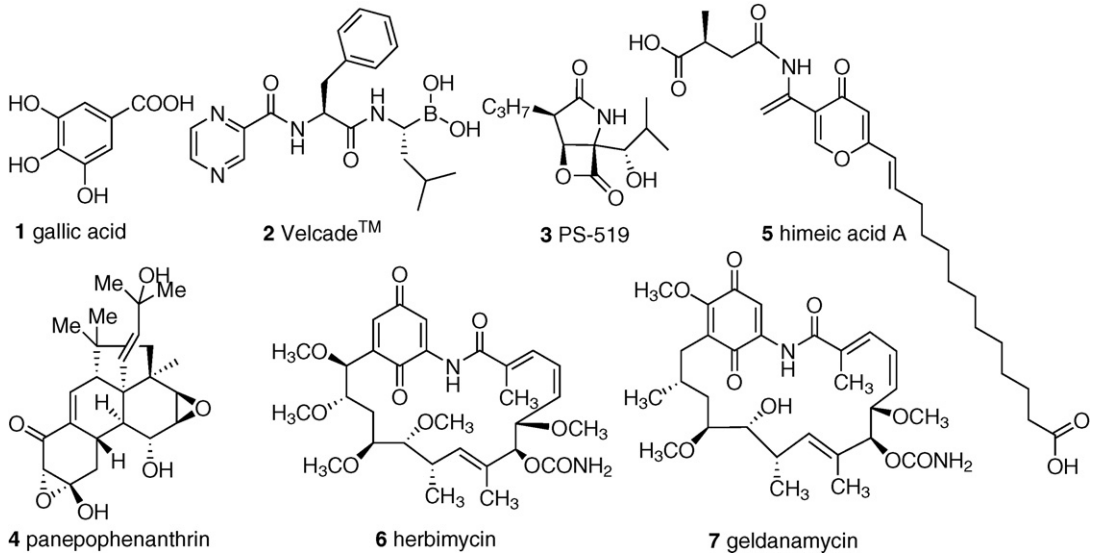

Fig. 1 – Chemical structures 1–7.

glutathione, a cytoplasmic triterpenes with a cysteine residue [25]. Hence, Michael acceptors are unlikely to be good drug candidates. Nevertheless, the numerous NF-κB-related structure–activity relationship (SAR) studies performed on natural Michael acceptors have doubtlessly contributed to a better knowledge of the chemistry of the pharmacophores of NF-κB inhibitors. The NF-κB inhibitory potential of sesquiterpene lactones has been shown to depend on the number of α,β-unsaturated carbonyl functional groups [45]. In sesquiterpene lactones containing only one α,β-unsaturated carbonyl function, the IC_{100} value tends to be an order of magnitude higher than in sesquiterpene lactones with two or more α,β-unsaturated carbonyl groups [23,45]. Sesquiterpene lactones with two α,β-unsaturated carbonyl groups, such as helenalin (**10**), can use their bifunctionality to alkylate both the Cys^{38} thiol located in the DNA-binding domain of p65 and the Cys^{120} thiol located in a proximal loop [23]. This creates a cross-link between Cys^{38} and Cys^{120} in the p65 molecule and prevents the binding of the phenol ring of Tyr^{36} present in the gap between Cys^{38} and Cys^{120} to thymine residues in the DNA backbone [23]. Beside the number and the electron affinity of α,β-unsaturated carbonyl functions, the most important

Table 1 – A selection of terrestrial natural products inhibiting NF-κB

Chemical compound	Source organism	Molecular targets	MIC (μM)	References
Isoprenoids: di- and tri-terpenoids				
Oleandrin (**8**)	*Nerium oleander*	TRAF2, IKK	9	[43]
Kamebakaurin (**9**)	*Isodon japonicus*	IKK	27	[22,40,44]
Isoprenoids: sesquiterpene lactones				
Helenalin (**10**)	*Arnicae* sp.	DNA-binding	10	[22,45]
Parthenolide (**11**)	Feverfew (*Tanacetum parthenium*)	IKK	30	[22,46]
Phenolics				
Curcumin (**12**)	*Curcuma longa*	IKK	10	[22,28,47]
Resveratrol (**13**)	Red grapes (*Vitis* sp.)	Unspecific (nuclear translocation of NF-κB; ROS)	5	[22,28,48]
Epigallocatechin-3-gallate (EGCG) (**14**)	Green tea (*Thea sinensis*)	26S proteasome	20	[22]
Emodin (**15**)	Rhubarb (*Rheum palmatum*)	IκB degradation, DNA-binding	185	[22,49–51]
Silymarin (family of silybin A (**16**) analogues)	Milk thistle (*Silybum marianum*)	Phosphorylation and degradation of IκB	25	[3]
Flavokavain A (**17**)	Kava-kava (*Piper methysticum*)	IKK	320	[52]
Miscellaneous				
Panepophenanthrin (**4**) (phenanthrene)	*Panus rudis*	Ubiquitination	250	[35]
Himeic acid A (**5**) (pyrone derivative)	*Aspergillus* sp.	Ubiquitination	50	[36]
Herbimycin A (**6**) (benzoquinone)	Bacteria	IKK	1	[38]
Geldanamycin (**7**) (benzoquinone)	Bacteria	IKK	6	[38]
Omuralide (**18**) (lactone-γ-lactam)	Actinomycete	26S proteasome	1	[26,53,54]
Gliotoxin (**19**) (piperazine metabolite)	Fungus	26S proteasome	2	[22,26,55]

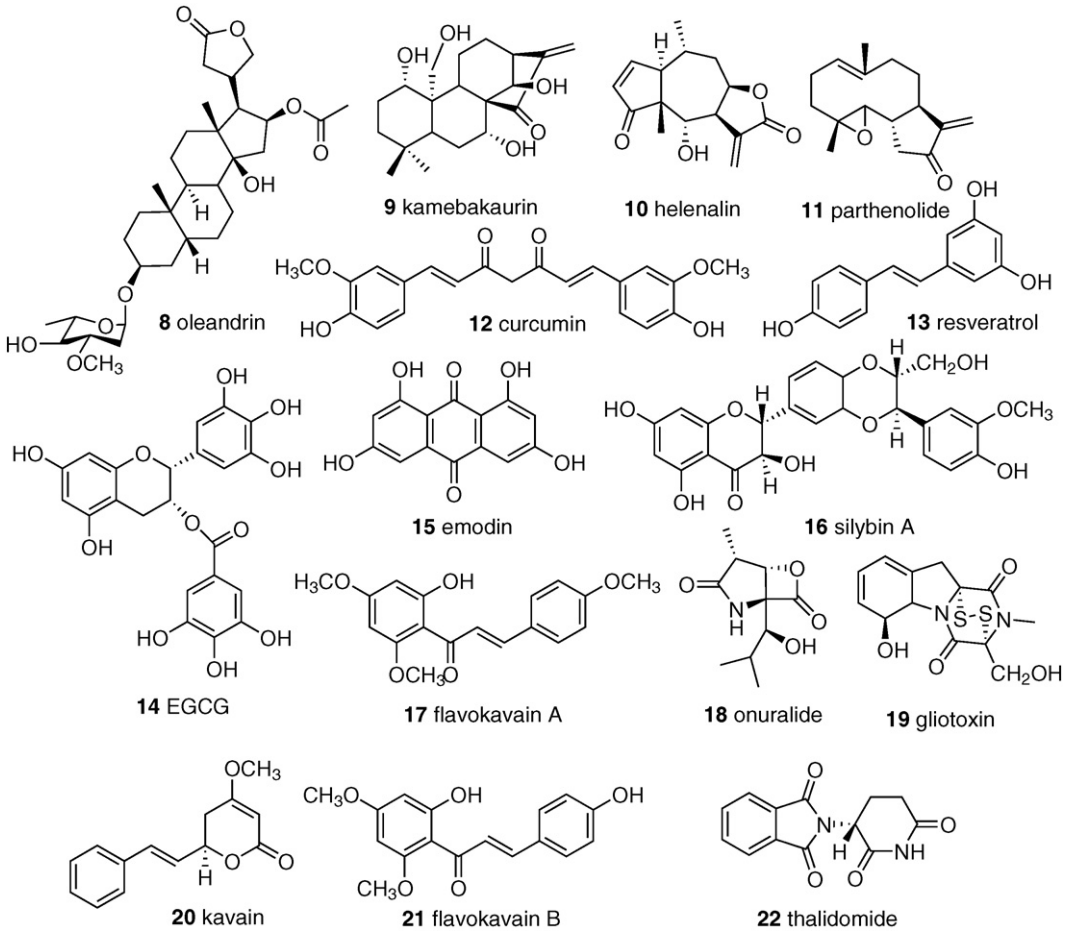

Fig. 2 – Chemical structures 8–22.

parameter for NF-κB inhibition is the occurrence of an α-methylene-γ-lactone moiety [23,45]. The number of α,β-unsaturated acyl residues and their position with respect to the α-methylene-γ-lactone moiety also play an important role in the inhibition of NF-κB [45]. This observation may be explained by the fact that α,β-unsaturated acyl residues can facilitate the reaction of sesquiterpene lactones with NF-κB by forming hydrogen bonds with NF-κB, thereby stabilizing the covalent bonds between NF-κB and the sesquiterpene lactone [45]. Finally, and in particular in sesquiterpene lactones with more rigid skeletons, the presence of α,β-unsaturated carbonyl functions integrated in a ring system plays an important role in the bioactivity of the molecule [23,45].

Another SAR study performed in the context of the investigation of the NF-κB inhibitory potential of terrestrial natural products focused on the major lactones and chalcones from the ethnobotanical plant kava-kava (*Piper methysticum*) [46]. This study revealed that the kava-derived lactone kavain (**20**) and the two kava-derived chalcones flavokavain A (**17**) and flavokavain B (**21**) are capable of inhibiting TNF-α induced NF-κB activation by preventing IκB degradation. The kava chalcones tend to have a stronger NF-κB inhibitory potential than their lactone counterparts. The reduction of the cinnamyl chain of kavalactones does not have any effect on the bioactivity of the tested compounds. The functional groups of ring B of the kavalactones and ring A or the α,β-unsaturated carbonyl group of the central chain of kavachalcones are likely to be the pharmacophore of the NF-κB inhibiting kava derivatives. Ketal or *methoxy* groups on ring A of kavalactones or on ring B of kavachalcones appear to hinder the latter's bioactivity.

A third major SAR study performed on terrestrial natural products in the context of NF-κB inhibition was based on analogues of the phenolic compound curcumin (**12**) isolated from the perennial herb *Curcuma longa* [47]. Zambre *et al.* showed that the enolizable β-diketone system is the major pharmacophore of curcumin (**12**) [47].

Several terrestrial natural products and synthetic compounds with NF-κB inhibitory properties have entered clinical trial, and some of them, including arsenic trioxide (Fowler's solution), Velcade® (bortezomib) (**2**), and Thalomid® ((R)-thalidomide) (**22**), have become available on the market and have been used in the clinic for the treatment of various types of cancer [48,49]. Although there has been some concern regarding the lack of specificity of currently known natural NF-κB inhibitors [50] and regarding possible side effects of NF-κB-inhibition therapies [50], the clinical and experimental results obtained so far in terms of using NF-κB inhibitors in the clinic have been highly promising. Significant enthusiasm for the

use of NF-κB inhibitors as a new anti-cancer treatment has been generated, both at the chemopreventive level and at the therapeutic level [5,6,51]. The proteasome inhibitor Velcade® (**22**), for instance, has been very successful in the U.S.A, as well as in Europe and Japan, as a drug to treat multiple myeloma, which is the second most common type of haematological malignancy [49].

5. Marine chemical ecology and evolution of the immune system in marine invertebrates: potential explanations for the presence of NF-κB inhibitors in marine organisms

Finding a novel and potent bioactive natural product by random screening is, in itself, a great achievement in drug discovery. But the additional understand the evolutionary or ecological reason, if there is any, behind the production of the bioactive compound is to be viewed as a yet greater achievement. Information on the ecological aspect of the production of natural products can, indeed, be used to rapidly discover additional sources of the same or very similar compounds, to biotechnologically increase the production of the metabolite of interest, and to increase the sustainability of the isolation of the bioactive compound. For several decades, marine organisms have provided marine natural products chemists with a rich source of unusual metabolites [4]. The function of these compounds in their natural habitat is linked to various aspects of species survival, such as predator deterrence, prevention of fouling, inhibition of overgrowth, and protection from ultraviolet radiation [52]. The richest sources of anti-cancer marine natural products have been soft-bodied and mainly sessile organisms, such as sponges, cnidarians, sea slugs, and tunicates, that lack physical defence against their predators, and that hence rely on chemical defence mechanisms using cytotoxic secondary metabolites [4]. It may not be directly obvious why, from an evolutionary or ecological point of view, marine organisms would produce secondary metabolites that possess NF-κB inhibitory properties. However, a closer investigation of the biology of marine organisms reveals interesting reasons for the discovery of potent NF-κB inhibitors amongst marine natural products.

From the evolutionary point of view, one interesting potential explanation for the finding of NF-κB inhibitors in marine organisms is the fact that marine invertebrates and fish, no matter how distantly related to us they appear to be, possess, in many cases, NF-κB or closely related analogues, and are hence likely to have developed some metabolites that can counterbalance the effect of NF-κB [53–58]. As a matter of fact, the canonical NF-κB activation pathway has a very ancient evolutionary origin reaching all the way back to the "living fossil" and most ancient arthropod, namely the horseshoe crab (*Carcinoscorpius rotundicauda*) [56,59]. Oysters (*Crassostera gigas*) have been shown to possess IKK-like proteins that share structural and functional properties with their mammalian homologues [60]. In the interesting case of ascidians, the NF-κB proteins As-rel1 and As-rel2 have been shown to be involved in the regulation of the formation and degradation of the notochord [61]. Ascidians are unique in the sense that they start their larval life as vertebrates, bearing, like all other chordates, a notochord at the centre of the larval tail, and then gradually losing their notochord to become invertebrate adults [61]. Interestingly, it has been shown by Povelones *et al.* that an NF-κB analogue, apNF-κB, is constitutively present in the cells of the sea slug *Aplysia* sp. In the case of crush injury, the loss of apNF-κB leads to a rapid injury signal [57]. According to Merlo *et al.*, the regulation of NF-κB plays a crucial role in long-term memory in crab (*Chasmagnathus* sp.) [58].

From an ecological point of view, it is particularly noteworthy that several bacteria and viri have been reported to modulate NF-κB activity in host cells in order to increase their chances to survive as parasites within the host [62,63]. Pancer *et al.* have shown that sea urchins use a NF-κB analogue, spNF-κB to protect themselves against apoptosis-inducing compounds released by the diatoms on which they graze, and to respond to bacterial infection and other pathogens [53,64].

Harsh environmental conditions have also led to the evolution of various chemical defence mechanisms in marine organisms, and it is probable to find strong antioxidants that could act as attenuators of redox signals involved in the NF-κB activation pathways [22]. Strong antioxidants isolated from marine organisms include the carotenoid astaxanthin (**23**) produced by various algae and microorganisms, the terpenoid bromohydroquinone cymopol (**24**) isolated from the green alga *Cymopolia barbata*, the brominated diphenyl methane

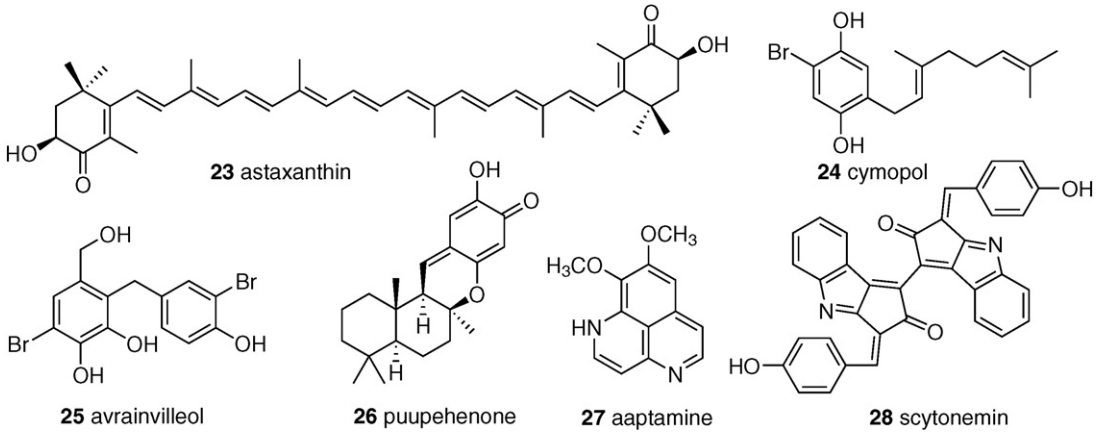

Fig. 3 – Chemical structures *23–28*.

Table 2 – Marine natural products in clinical trials or on the market as anti-cancer or anti-inflammatory drugs

On the market			
Ara-C (**29**)	Nucleoside	*Cryptotethya crypta* (**S**)	Cytosar[1]
Phase III			
E7389 (**30**)	Macrolide	Inspired by halichondrin (*Halichondria okadai* (**S**))	Eisai Inc.[1]
Yondelis™ (ET-743) (**31**)[a]	Isoquinolone	*Ecteinascidia turbinate* (**T**)	Pharmamar[2], J&J[1,b]
Neovastat™ (AE-941) (**32**)	Aminosteroid	(Various elasmobranchs)	Aeterna[3]
Phase II			
Bryostatin 1 (**33**)	Macrolide	*Bugula neritina* (**Br**)	GPC Biotech[4]
IPL-576092 (**34**)	Steroid	Inspired by contignasterol (*Petrosia contignata* (**S**))	Inflazyme[3], Sanofi-Aventis[5]
Soblidotin (TZT1027) (**35**)	Depsipeptide	Inspired by dolastatin 10 (*Dolabella auricularia* (**T**))	Teikoku Pharm[1]
Aplidin (**36**)	Depsipeptide	*Aplidium albicans* (**T**)	Pharmamar[2]
Kahalalide F (**37**)	Depsipeptide	*Elysia rufescens* (**M**)[c]	Pharmamar[2]
Squalamine (**38**)	Aminosteroid	(Various elasmobranchs)	Genaera[1]
Phase I			
Spisulosine (ES285) (**39**)	Lipid	*Spisula polynyma* (**M**)	Pharmamar[2]
Salinosporamide A (NPI0052) (**40**) taltobulin	β-Lactone-γ-lactam	*Salinospora tropica* (**Ba**)	Nereus pharmaceuticals[1]
(HTI 286) (**41**)	Tripeptide	Inspired by hemiasterlin (various sponges)	Wyeth[1]
LY355703 (**42**)	Dioxandiaza-cyclohexa-decenetetrone	Inspired by cryptophycin (*Nostoc* sp. (**Cy**))	Eli Lilly Research Laboratories[1]
Zalypsis	Alkaloid	Inspired by jorumycin (**43**) (*Jorunna funebris* (**M**))	Pharmamar[2]
NVP-LAQ824 (**44**)	Indolic cinnamyl hydroxonate	Inspired by psammaplin A (various sponges)	Novartis oncology[3]
KRN-7000 (**45**)	Glycosphingo-lipid	Inspired by agelasphin (*Agelas mauritianus* (**S**))	Kirin brewery[6]

S, sponge; **T**, tunicate; **Br**, bryozoan; **M**, mollusc; **Ba**, bacterium. Superscript numbers: 1, U.S.A.; 2, Spain; 3, Canada; 4, Germany; 5, France; 6, Japan. [a]Granted orphan drug status by the EC and the U.S. FDA for soft tissue sarcomas and ovarian cancer. [b]Johnson & Johnson. [c]From *Bryopsis* sp. (green alga) diet. Compiled from Rawat *et al.* (2006) [66], Simmons *et al.* (2005) [67], Jimeno *et al.* (2004) [68], Nagle *et al.* (2004) [1], Newman and Cragg (2004)[69], and Haefner (2003) [70].

avrainvilleol (**25**) isolated from the tropical green alga *Avrainvillia* sp., the sesquiterpene quinone puupehenone (**26**) isolated from certain deep-water sponges, the alkaloid aaptamine (**27**) isolated from the sponge *Aaptos aaptos*, and the UV sunscreen pigment scytonemin (**28**) isolated from various cyanobacteria [42,65] (see Fig. 3 for chemical structures **23–28**).

6. Chemical and biological properties of marine natural products modulating the NF-κB activation pathway

Since the early 1960s, significant effort has been placed on the pharmacological evaluation of marine secondary metabolites. Noteworthy achievements of this field of biomedically guided marine exploration, a scientific endeavour often referred to as the search for ''*Drugs from the Sea*'', include the discovery of numerous potent antimicrobial, anti-cancer, and anti-inflammatory compounds [4]. Marine natural products that are currently in clinical trials or on the market as anti-cancer or anti-inflammatory drugs are listed in Table 2, and their cellular or molecular targets are shown in Fig. 4 (see Fig. 5 for chemical structures **29–45**).

Despite the abundance of marine natural products reported to have pharmaceutical activity, relatively few marine compounds have been reported to date as NF-κB inhibitors. The marine natural products reported at present as NF-κB inhibitors can be divided into four categories: (a) compounds targeting the IKK-dependent degradation of IκB, (b) compounds specifically interfering with the proteolytic activity of the 26S proteasome, (c) compounds interfering with the binding of NF-κB to its DNA binding site, and (d) compounds with unknown mechanisms of action. Marine natural products that inhibit IκB degradation encompass the sunscreen pigment scytonemin (**28**) isolated from various cyanobacteria [1], the sesterterpene lactone cacospongionolide B (**46**) isolated from the sponge *Fasciospongia cavernosa* [71], and the sesterterpene lactone petrosaspongiolide M (**47**) isolated from the sponge *Petrosaspongia nigra* [71]. The lactone-γ-lactam salinosporamide A (**40**) isolated from the bacterium *Salinispora tropica* [72], the oxazole alkaloid mycalolide A (**48**) isolated from a *Mycale* sp. sponge [73], and the sterol acetate agosterol C (**49**) isolated from the sponge *Acanthodendrilla* sp. [74] have been reported as 26S proteasome inhibitors. The sesterterpene lactone cyclolinteinone (**50**) isolated from the sponge *Cacospongia linteiformis* has been shown to prevent the disappearance of IκB from the cytoplasm, but the exact mechanism of action remains unknown [75]. The alkaloid hymenialdisine (**51**) isolated from the sponges *Acanthella aurantiaca* [76]. The red pigment cycloprodigiosin hydrochloride (**52**) isolated from the bacterium *Pseudoalteromonas denitrificans* [77], the macrocyclic trichothecene verracurin A (**53**) isolated from the marine fungus *Myrothecium roridum* [78], the quinone

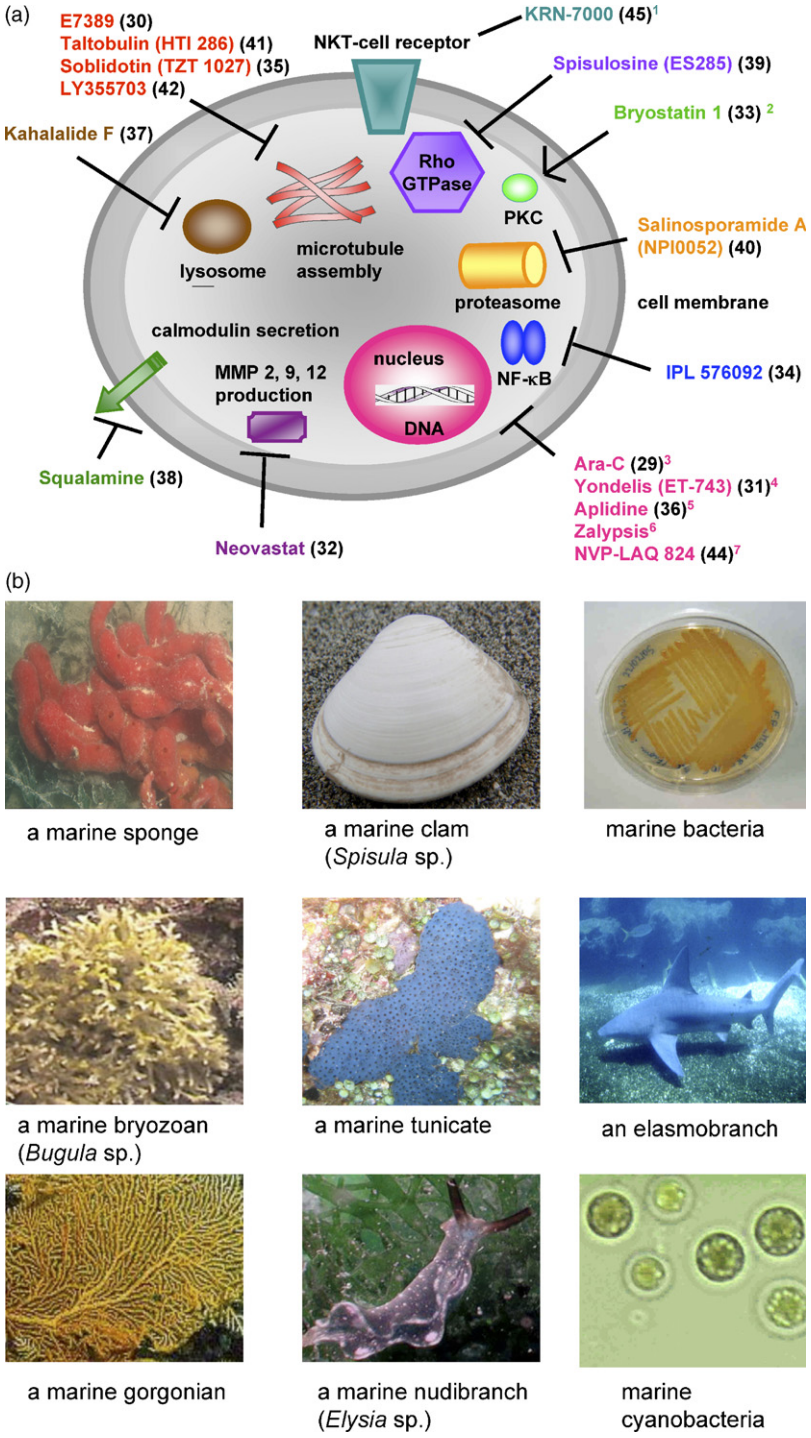

Fig. 4 – Marine natural products in clinical trials as anti-cancer or anti-inflammatory drugs. The chemical structure numbers are shown in brackets. Superscript numbers: 1, activation of the nuclear killer (NK) T-cell receptor; 2, activation of protein kinase C (PKC); 3, inhibition of DNA synthesis; 4, inhibition of ornithin decarboxylase; 5, alkylation of DNA; 6, inhibition of DNA binding; 7, inhibition of histone deacetylase.

ilimaquinone (**54**) isolated from the sponge *Hippospongia metachromia* [79], the thiazoline-containing lipid curacin A (**55**) isolated from the cyanobacterium *Lyngbya majuscula* [80], the polyacetylenic alcohol petrocortyne A (**56**) [81], and the steroid IPL 576092 (**34**) [82] have been shown to be potent inhibitors of NF-κB, but their mechanisms of action remain unknown. Most plausibly, cycloprodigiosin hydrochloride interferes with interactions between p65 and some transcriptional co-activators such as the camp response element-binding protein (CBP) or p300 [77]. The molecular targets and the MIC values of the NF-κB inhibiting marine natural products listed above are shown in Fig. 6.

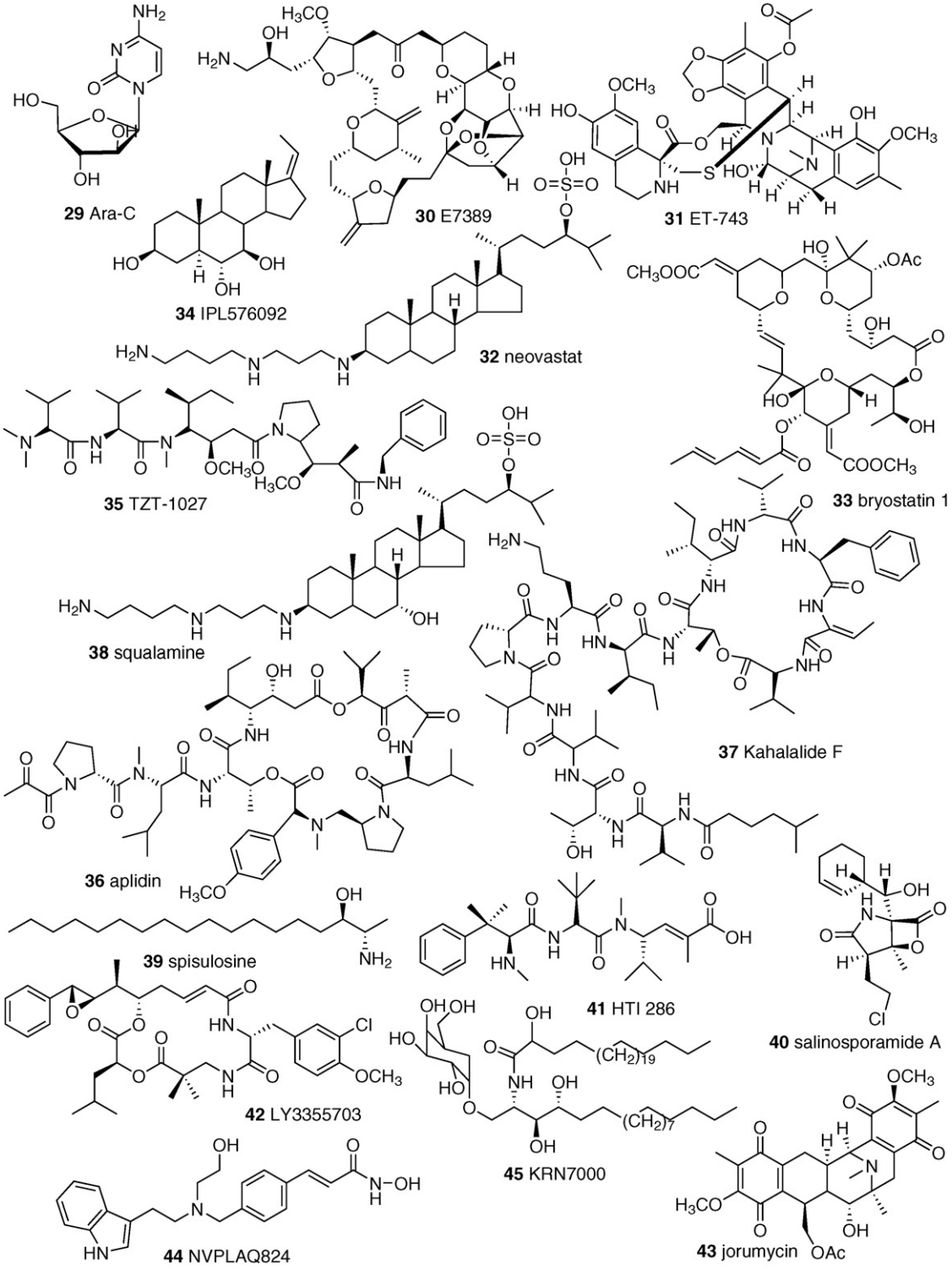

Fig. 5 – Chemical structures 29–45.

Recently, scientists have identified a series of marine natural products that interfere with the small GTPase Ras, which is known to play an important role in IKK-dependent NF-κB activation [83] (see Fig. 7 for chemical structures **46–56**). Rhopaloic acid A (**57**) and five other closely related terpenoids isolated from the marine sponge *Hippospongia* sp. strongly inhibit the Ras-converting enzyme RCE, a Ras protease that recognizes and activates both farnesylated and geranylgeranylated Ras [1]. Kinases downstream of Ras along the Ras-PI3K-Akt/Protein kinase B (PKB) signalling pathway, including phosphoinositide 3-kinase (PI3K) and Akt, have also been recently reported as NF-κB activators

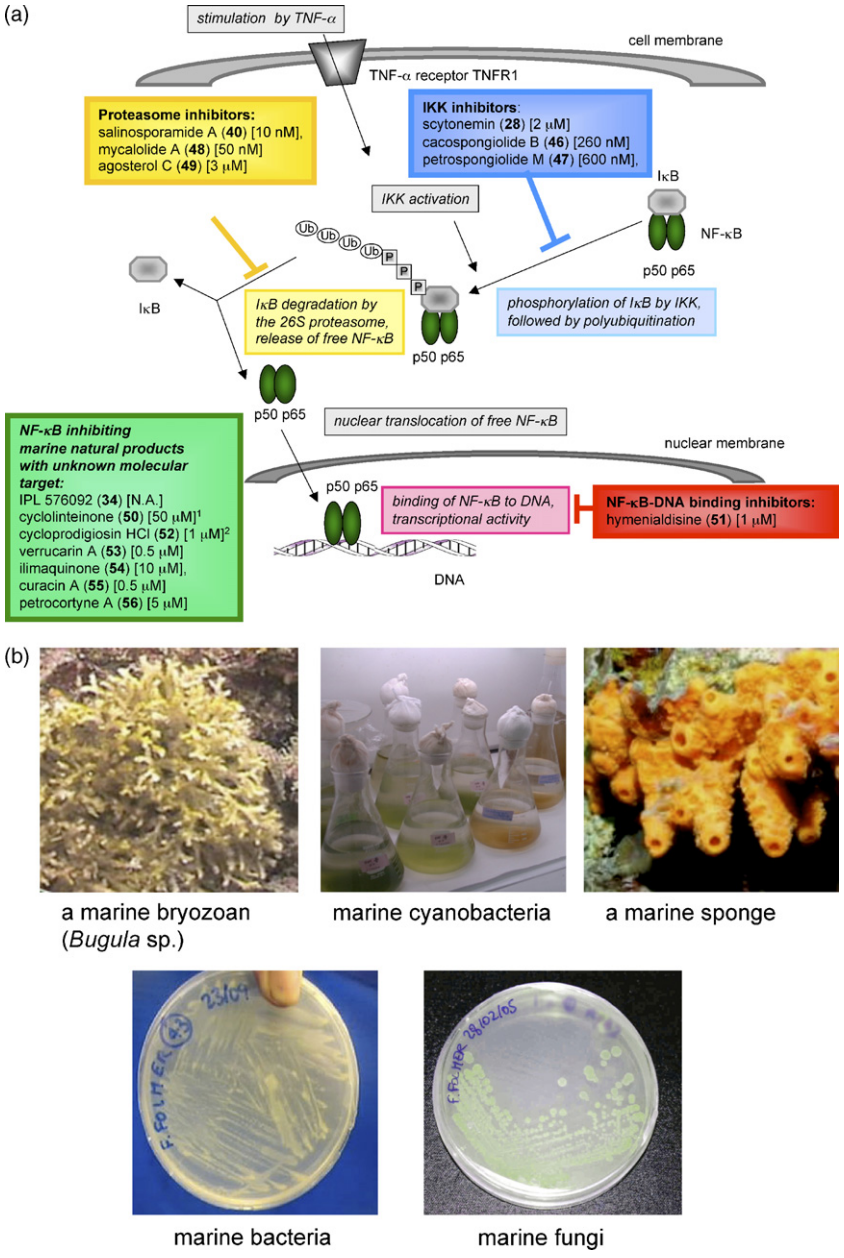

Fig. 6 – Molecular targets of marine natural products known to inhibit NF-κB activation. The chemical structure numbers are shown in brackets, and the IC_{50} values are given in square brackets. Superscript numbers: 1, Cyclolinteinone (50) has been shown to prevent the degradation of IκB, but the exact mechanism of action remains unknown; 2, most plausibly, cycloprodigiosin HCl (52) interferes with interactions between p65 and some transcriptional co-activators. (N.A.: not available).

[69,84,85]. Halenaquinone (**58**), a polyketide isolated from the sponge *Xestospongia exigua*, has been shown to inhibit PI3K [1], and the sesterterpene scalaradial (**59**) isolated from the sponge *Cacospongia mollior* has been shown to interfere with the phosphorylation of Akt [86].

Marine natural products have also been intensively investigated for their potential to inhibit phospholipase A_2 (PLA_2), and the effects of marine natural products on PLA_2 has been the subject of several reviews [87–89]. PLA_2 is an enzyme that cleaves the ester linkage at the β-position of phospholipids to release arachidonic acid, which is metabolized into pro-inflammatory prostaglandins [89]. Thommesen *et al.* have highlighted a strong correlation between the inhibition of PLA_2 and the inhibition of TNF-α-induced NF-κB, suggesting that the pro-inflammatory function of PLA_2 results from an activation of NF-κB, in addition to the release of arachidonic acid [90]. Furthermore, Terracciano *et al.* have shown that the same pharmacophore (a hemiacetal) is responsible for the PLA_2-inhibitory activity and the IKK-inhibitory activity of several marine natural products [89]. Marine natural products with potent PLA_2-inhibitory potentials include the sesterterpenoid manoalide (**60**) isolated from the sponge *Luffariella*

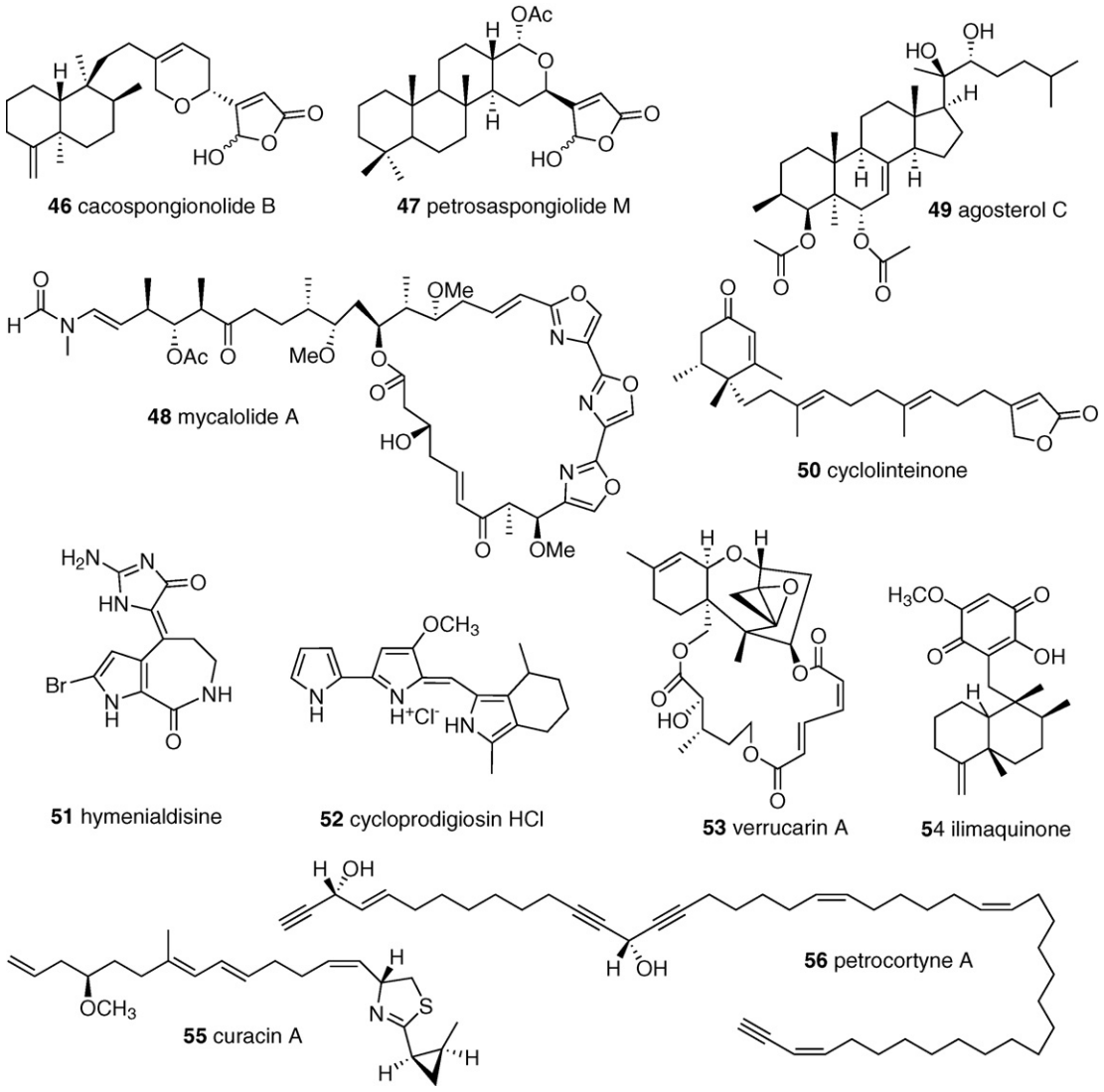

Fig. 7 – Chemical structures 46–56.

variabilis, the sesterterpene lactone cacospongionolide B (**46**) isolated from the sponge *Fasciospongia cavernosa*, and the sesterterpene lactone petrosaspongiolide M (**47**) isolated from the sponge *Petrosaspongia niger* [87,89]. The mechanism of action of all the marine PLA_2 inhibitors listed above is through the formation of a Schiff base with lysine residues of PLA_2 [87,89].

Finally, some research efforts have been spent on a quest for marine activators of the inositol phosphatase SHIP-1 (SH2-containing inositol 5′-phosphatase 1) which, for several years, used to be considered as a cellular inhibitor of NF-κB activity [91]. Amongst marine natural products shown to activate SHIP-1 is the aromatic sesquiterpene pelorol (**61**) isolated from the sponge *Dactylospongia elegans* [91]. However, there is growing evidence that SHIP-1 does not always act as an inhibitor of NF-κB, but that it can also induce NF-κB, depending on the cell type and on the nature of the extra-cellular signals [92] (see Fig. 8 for chemical structures **57**–**61**).

It can be concluded that marine, as well as terrestrial natural products are very promising drug candidates in the context of NF-κB-dependent cancer development and inflammation. As a matter of fact, some marine natural products with demonstrated NF-κB inhibitory potential are currently in clinical trials as anti-cancer or anti-inflammatory drugs. Noteworthy, a number of marine natural products that potently inhibit NF-κB do not bear α,β-unsaturated carbonyl functions which are widely recognized as the most common NF-κB inhibitory pharmacophore, but which are often associated with adverse side effects. Instead, marine NF-κB inhibitors such as salinosporamide A (NPI0052) (**40**), verracurin A (**53**), and curacin A (**55**) rely on more reactive functional groups such as γ-lactams and epoxide rings that have no, or at least only mild side effects. Unfortunately, marine drug discovery research has been dogged since its early days by the difficulty to supply sufficient material for drug development [93]. The development of a new drug requires amounts of pure compounds that exceed by large amounts the quantities that are generally feasible to collect from a marine environment without affecting the natural population [93]. Developing drugs from marine organisms that

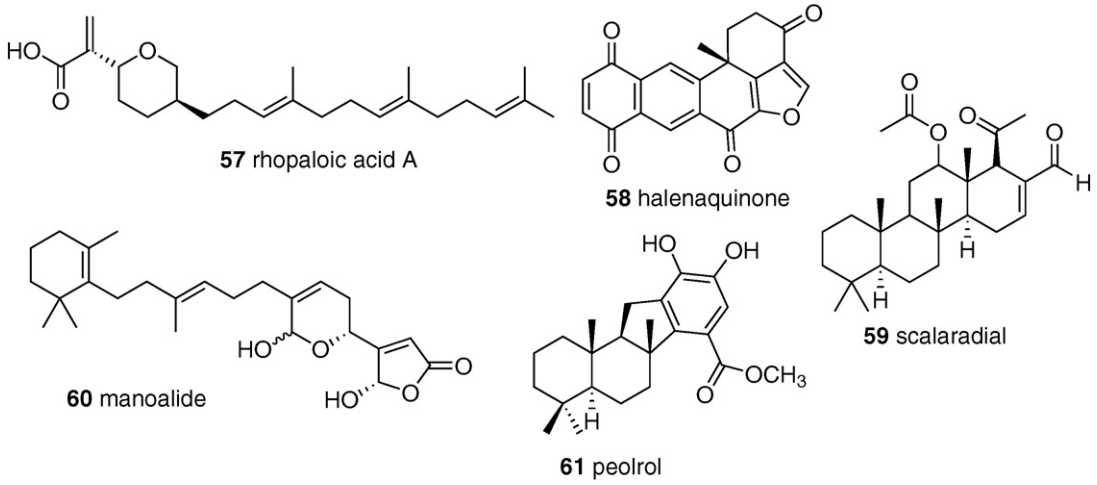

Fig. 8 – Chemical structures 57–61.

are found below the intertidal zone is extremely challenging, since it is extremely difficult to localize and identify the source of the compounds of interest if the source is located at depths that are difficult to access, and at which visibility is poor. Even modern mariculture techniques and *in vitro* tissue culture methods have not yet provided successful solutions to the supply issue associated with marine drug discovery [93]. Nevertheless, if the compound of interest was originally isolated from a bacterium, fungus, or microalga, the source latter can, in some cases, be cultured at a large scale by fermentation [93]. Additionally, advances in biotechnology, particularly in the ability to transfer genetic material from one bacterium to another, has opened up the exiting possibility of transferring segments of DNA that are responsible for the biosynthesis of secondary metabolites from slow-growing or unculturable bacteria into easily cultured bacteria such as *Escherichia coli* [94,95]. The use of universal gene cloning and expression has even made it possible to clone and express genes encoding for natural products both from prokaryotes and from eukaryotes [95]. Finally, the rapidly developing field of metabonomics may soon provide extremely useful tools for marine drug discovery. In particular, it will provide crucial information on how to solve the bioprospecting problem associated with marine drug discovery [96].

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