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Review

Marine natural products targeting phospholipases A_2

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ABSTRACT

Phospholipases A_2 (PLA_2s) form a family of enzymes catalyzing the hydrolysis of membrane phospholipids into arachidonic acid, which is the major precursor of pro-inflammatory eicosanoids. As a result, PLA_2s have been considered as potential targets in anti-inflammatory drug discovery.

Marine natural products are a rich source of bioactive compounds, including PLA_2 inhibitors. Here, we review the properties of marine PLA_2 inhibitors identified since the first discovery of PLA_2 inhibitory activity in the marine natural product manoalide in the mid 1980s.

Contents

1. Introduction

Inflammation is the response of vascular tissues to harmful stimuli such as injury, pathogens, or irritants. While inflammation normally functions as a defense mechanism in higher animals, deregulated inflammation is implicated in a large number of diseases such as autoimmune diseases, allergies, asthma, rheumatoid arthritis, inflammatory bowel diseases, pelvic inflammatory diseases, glomerulonephritis, atherosclerosis, myocardial ischemia, and cancer [1–3]. The process of inflammation is controlled by a group of substances called chemical mediators [1]. Endogenous chemical mediators consist of vasoactive amines, cytokines, bradikinin, fibrin, complement components, eicosanoids, platelet activating factor (PAF), nitric oxide (NO), and neuropeptides [1]. Eicosanoids, in particular, play a critical role in virtually every step of inflammation. Eicosanoids, which comprise prostaglandins, prostacyclins, thromboxanes, and leukotrienes, are a family of oxygenated fatty acid metabolized by cyclooxygenases (COX) and lipoxygenases (LOX) from arachidonic acid [1]. Despite the extensive efforts invested in developing drugs that suppress the conversion of arachidonic acid into pro-inflammatory eicosanoids, the latter approach has been unsuccessful. Undesired side-effects resulting from the lack of specificity of COX and LOX are

Abbreviations: 5-HPTETE, 5-hydroperoxyeicosatetraenoic acid; COX, cyclooxygenase; $cPLA_2$, cytosolic PLA_2; IP_3, inositol 1,4,5-triphosphate; $iPLA_2$, calcium independent PLA_2; LOX, lipoxygenase; (Lp)PLA_2, lipoprotein-associated PLA_2; LT, leukotriene; NO, nitric oxide; PAF, platelet-activating factor; PG, prostaglandin; PLA_2, phospholipase A_2; PLC, phospholipase C; ROS, reactive oxygen species; $sPLA_2$, secretory PLA_2; TX, thromboxane.

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responsible for the failure of the concept [2]. As an alternative, the quest for inhibitors of phospholipases A_2 (PLA_2s), the enzymes that catalyze the hydrolysis of membrane phospholipids into arachidonic acid, has opened up a new research avenue in anti-inflammatory drug discovery [2,4–6]. As a matter of fact, PLA_2s isolated from snake venom have been shown to induce all the inflammatory symptoms of snakebite such as acute pain, oedema, hypotension, hemorrhage, and neuromuscular junction blockage. Furthermore, rheumatoid arthritis, asthma, psoriasis, myocardial ischemia, and pancreatis have all been shown to be associated with elevated levels of serum PLA_2. Lysophospholipids produced by PLA_2s have also been shown to induce gastric ulceration in rats, and to induce an inflammation similar to acute cholecystitis in the gall bladder mucosa [3]. Here, we review the properties of marine PLA_2 inhibitors identified since the first discovery of PLA_2 inhibitory activity in the marine natural product manoalide (**1**), by research groups lead by Edward Dennis [4] and by Robert Jacobs [5] at the universities of San Diego and Santa Barbara, respectively, in the mid 1980s.

2. The PLA_2-mediated inflammation signaling cascade

PLA_2s are lipolytic enzymes found in almost all types of cells. They specifically hydrolyze the 2-acyl ester bond of 1,2-diacyl-*sn*-3-glycerophospholipids such as arachidonic acid. Fifteen different PLA_2s have been characterized to date. They are grouped into four families: secreted PLA_2s ($sPLA_2$s), cytosolic PLA_2s ($cPLA_2$s), lipoprotein associated PLA_2s ((Lp)PLA_2), and calcium-independent PLA_2s ($iPLA_2$s) [2,5,6]. The calcium-dependent $sPLA_2$s are commonly found in snake, scorpion, and bee venom. They are of low molecular weight (13–15 kDa) and characteristically contain a histidine residue in their catalytic site [2,6]. The mode of action of $sPLA_2$s involves a nucleophilic attack onto the phospholipid's *sn*-2 bond. While the role of $sPLA_2$s in inflammation remains poorly understood, it has been suggested that $sPLA_2$s induce an increase in $cPLA_2$-dependent eicosanoid release, and that they synergize with other pro-inflammatory mediators [2,6]. $cPLA_2$s are 85 kDa enzymes containing a serine and an aspartic acid residue in the active site. Noteworthy, $cPLA_2$s are the only PLA_2s with specificity for arachidonic acid at the phospholipase *sn*-2 position. $cPLA_2$s are calcium-dependent enzymes activated by extra-cellular stimulations from pathogens, tissue injury, or physical or chemical stresses. The cytolic concentrations of calcium required for PLA_2 activation result from the cleavage of phospholipids into inositol 1,4,5-triphosphate (IP_3) by phospholipase C (PLC), followed by the binding of IP_3 to calcium channels in the endoplasmic reticulum [7]. Because of their central role in mediating the generation of eicosanoids and of PAFs, and hence in mediating inflammation, $cPLA_2$s have been recognized as very attractive targets in drug discovery, despite some rare side-effects including the formation of intestinal ulcers, and several pharmaceutical companies, such as Pfizer have started to develop promising $cPLA_2$-specific drug candidates [7–9]. Unlike $cPLA_2$s, (Lp)PLA_2s, or platelet aggregation factor acetylhydrolases (PAF-AHs), have anti-inflammatory properties, as they are able to degrade the pro-inflammatory signaling molecules PAFs by cleaving their acetyl group at the *sn*-2 position. However, (Lp)PLA_2s have become an important target in PLA_2 inhibitory drug discovery, as they are known to lead to coronary heart diseases [6]. $iPLA_2$s have complex and still poorly understood implications in signaling pathways. $iPLA_2$s play a role in bone formation, apoptosis, insulin secretion, sperm development, and axon regeneration [2,6]. The present review focuses only on inhibitors of $sPLA_2$s and $cPLA_2$s. The latter two are present in most types of cells, and both of them are known to be implicated in inflammation through eicosanoid biosynthesis [6,9–11].

As illustrated in Fig. 1, PLA_2s initiate the pro-inflammatory signaling cascade by catalyzing the hydrolysis of the *sn*-2 acyl ester bond of membrane phospholipids, leading to the release of the ω-6 fatty acid arachidonic acid and of lysophospholipids [7]. Next, arachidonic acid is oxygenated into prostaglandin (PG) PGH_2 by COX, or into 5-hydroperoxyeicosatetraenoic acid (5-HPTETE) by LOX. The conversion of arachidonic acid to PGH_2 by COX occurs in two steps. First, two molecules of O_2 are added as two peroxide linkages, and a 5-membered carbon ring is formed near the middle of the fatty acid chain, leading to an unstable intermediate prostaglandin G (PGG_2). One of the peroxide linkages then sheds a single oxygen atom to form the PGH_2 [1]. PGH_2 is the unstable precursor of PGD_2, PGE_2, PGI_2, and thomboxane A_2 (TXA_2) [1]. PGE_2 and PGI_2 enhance edema formation and leukocyte infiltration by promoting blood flow in the inflamed region, and they stimulate the pain-inducing activity of bradykinin and autacoids. PGE_2 induces pain, heat, and fever. TXA_2 triggers platelet aggregation [1]. LOX converts arachidonic acid into lipid hydroxyperoxides that exert relevant functions as mediators of inflammation: 5-hydroperoxyeicosatetraenoic acid (5-HPTETE) is spontaneously reduces to 5-hydroxyeicosatetraenoic acid (5-HETE), which is further converted by 5-lipoxygenase to leukotriene A_4. LTA_4 may be converted to LTB_4. LTB_4 is a potent chemoattractant for polymorphonuclear leukocytes. It activates neutrophil functional responses, leading to the generation of free oxygen free radicals and to the release of lysosomal enzymes. LTB_4 also causes the adhesion and chemotaxis of leukocytes, it stimulates aggregation, enzyme release, generation of superoxide in neutrophils, and it makes blood vessels more permeable [10]. Eosinophils, mast cells, and alveolar macrophages use LTC_4 synthase to conjugate glutathione with LTA_4 to make LTC_4, which is transported outside the cell where a glutamic acid moiety is removed to make LTD_4. LTD_4 is then cleaved by dipeptidases to make LTE_4. LTC_4, LTD_4, and LTE_4 play an important role in atherosclerosis, in asthma, in allergenic rhinitis, and in inflammatory gastrointestinal diseases. Eicosanoids also activate the production of pro-inflammatory reactive oxygen species (ROS), nitric oxide (NO), and cytokines [3,9,10,13,14]. The lysophospholipids produced during the conversion of membrane phospholipids to arachidonic acid are a precursor for PAF. In addition, lysophospholipids induce the activation and extravasion of pro-inflammatory leukocytes and activate the secretion of pro-inflammatory histamine by mast cells [7].

3. Marine PLA_2 inhibitors

PLA_2 activity has been reported in several marine organisms, including hard and soft corals, jellyfish, starfish, sea anemones, and soft corals, and marine snails [11,12]. Hence, from an ecological perspective, it is not surprising that marine organisms have developed potent PLA_2 inhibitors, which may be used as chemical defences in their natural environment. Marine PLA_2 inhibitors reported to date are primarily terpenoids isolated from sponges, nudibranchs, and algae. Their chemical and biological properties are described below and summarized in Table 1. The chemical structures of the compounds are shown in Fig. 2.

3.1. PLA_2 inhibiting sesquiterpenes

One of the most investigated marine PLA_2 inhibitors is the merosesquiterpene bolinaquinone (**1**) isolated from the sponge *Dysidea* sp. Bolinaquinone (**1**) has been shown to inhibit the enzymatic activity of $sPLA_2$ with an IC_{50} value of 100 nM [13]. While the inhibition of $sPLA_2$ by bolinaquinone (**1**) is very potent, it is not selective against this enzyme. Bolinaquinone **1** is known to also affects $cPLA_2$ [13–19]. Bolinaquinone (**1**) is known to reduce

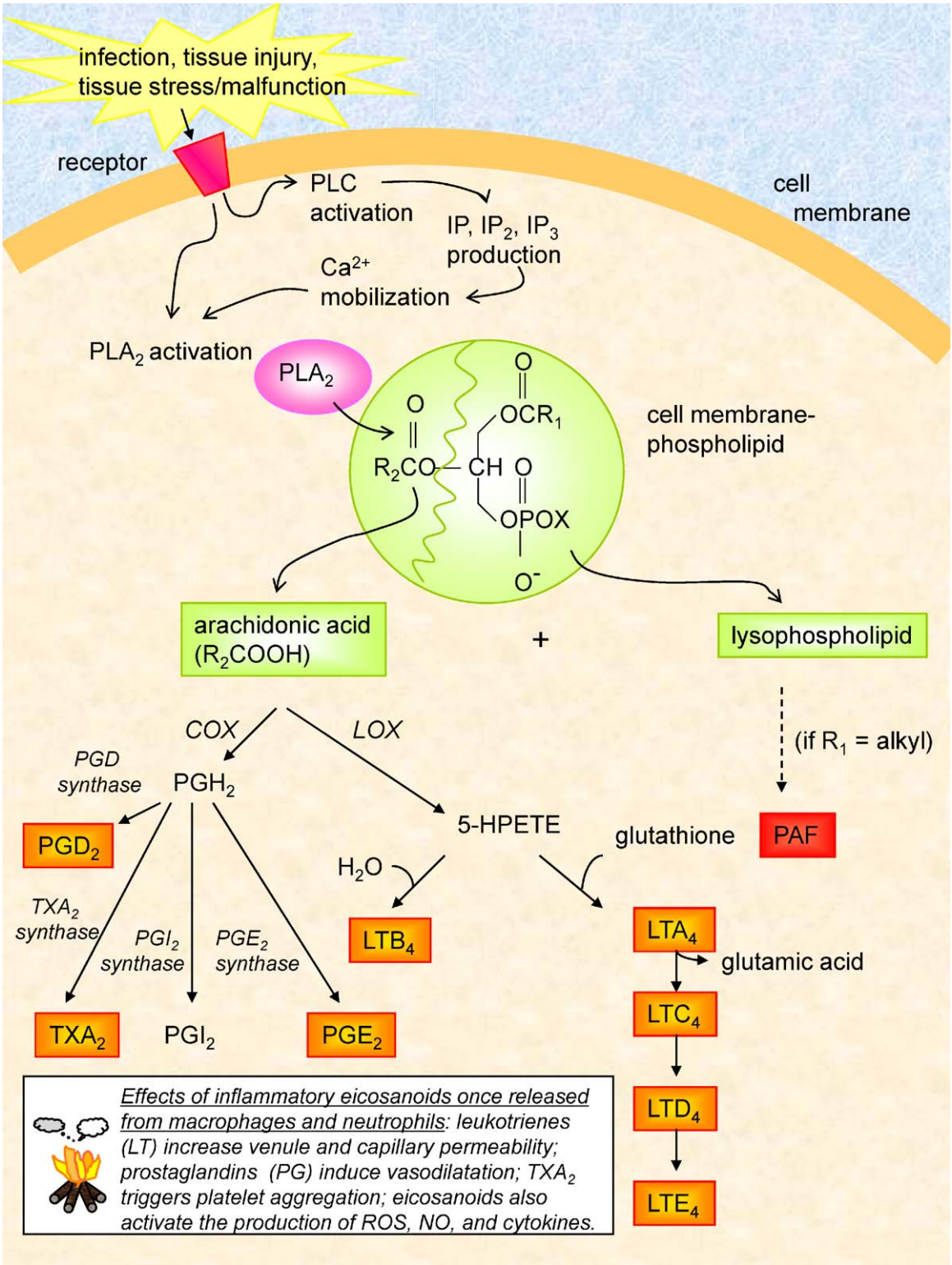

Fig. 1. The PLA_2-mediated inflammation signaling cascade. $cPLA_2$s are calcium-dependent enzymes activated by extra-cellular stimulations from pathogens, tissue injury, or physical or chemical stresses. The cytolic concentrations of calcium required for PLA_2 activation result from the cleavage of phospholipids into IP_3 by PLC, followed by the binding of IP_3 to calcium channels in the endoplasmic reticulum. PLA_2s hydrolyze the *sn*-2 acyl ester bond of membrane phospholipids, which leads to the release of arachidonic acid and lysophospholipids. Arachidonic acid is oxygenated into PGH_2 by COX, or into 5-HPTETE by LOX. PGH_2 is the unstable precursor of PGD_2, PGE_2, PGI_2, and thomboxane A_2 (TXA_2). PGE_2 and PGI_2 enhance edema formation, pain induction, and fever development. TXA_2 triggers platelet aggregation. LOX converts arachidonic acid into 5-HPTETE, which is spontaneously reduces to 5-HETE, and then to leukotriene A_4. LTA_4 may be converted to LTB_4, a potent chemoattractant for polymorphonuclear leukocytes. Eosinophils, mast cells, and alveolar macrophages conjugate glutathione with LTA_4 to make LTC_4, which is transported outside the cell where a glutamic acid moiety is removed to make LTD_4. LTD_4 is then cleaved by dipeptidases to make LTE_4. LTC_4, LTD_4, and LTE_4 play an important role in atherosclerosis, asthma, allergenic rhinitis, and inflammatory gastrointestinal diseases. The lysophospholipids produced during the conversion of membrane phospholipids to arachidonic acid are a precursor for PAF.

Table 1
Bioactivity of marine PLA_2 inhibitors.

Compound	Source organism	Target PLA_2	IC_{50} (μM)	References
Sesquiterpenes				
Bolinaquinone **1**	*Dysidea* sp. (S)	Non-specific	0.1	[13]
Ilimaquinone **2**	*Hippiospongia metachromia* (S)	Bee venom $sPLA_2$	<270	[19,20]
Avarol **3**	*D. avara* (S)	$sPLA_2$	2	[17–19]
Avarone **4**	*D. avara* (S)	$sPLA_2$	2	[17–19]
Dysidine **5**	*D. avara* (S)	$sPLA_2$	2	[16–19]
Dysidiotronic acid **6**	*D. avara* (S)	$sPLA_2$	2.6	[18,19]
Cavernolide **7**	*Fasciospongia cavernosa* (sponge)	$sPLA_2$	8.8	[16,18,20,21]
Rhipocephalin **8**	*Rhipocephalus phoenix* (GA)	Bee venom $sPLA_2$	>4.0	[21]
Caulerpyne **9**	*Caulerpa prolifera* (GA)	Bee venom $sPLA_2$	>4.0	[21]
Diterpenes				
Gracilin A **10**	*Aplysilla* sp. (S)	Bee venom $sPLA_2$	5	[20]
Aplyroseol 1 **11**	*Aplysilla* sp. (S)	Bee venom $sPLA_2$	5	[20]
12-Acetoxytetrahydroaplysulphurin1 **12**	*Aplysilla* sp. (S)	Bee venom $sPLA_2$	5	[20]
Dendrillolide A **13**	*Dendrilla* sp. (S)	Bee venom $sPLA_2$	5	[20]
Norrisolide **14**	*Dendrilla* sp. (S)	Bee venom $sPLA_2$	5	[20]
Epitaondiol **15**	*Stypopodium flabelliforme* (BA)	Human $sPLA_2$	3.8	[19,23]
Sesterterpenes				
Manoalide **20**	*Luffariella variabilis* (S)	Human $sPLA_2$	1.7	[4,5,27,29,30]
		Snake venom $sPLA_2$	<0.1	
		$cPLA_2$	10	
Secomanoalide **21**	*L. variabilis* (S)	Snake venom $sPLA_2$	<0.1	[20,29,30]
Luffariellolide **22**	*L. variabilis* (S)	Bee venom $sPLA_2$	0.2	[20,29,30]
Luffariellin A–B **23–24**	*L. variabilis* (S)	Bee venom $sPLA_2$	0.06	[20,29,30]
Luffolide **25**	*L. variabilis* (S)	Bee venom $sPLA_2$	0.04	[20,29,30]
Luffariellin C–D **26–27**	*Chromodoris* sp. (N)	Snake venom $sPLA_2$	0.2	[29,30]
Deoxymanoalide **28**	*Chromodoris* sp. (N)	Snake venom $sPLA_2$	0.2	[29,30]
Deoxysecomanoalide **29**	*Chromodoris* sp. (N)	Snake venom $sPLA_2$	0.5	[29,30]
Cacospongiolide B **30**	*Fasciospongia cavernosa* (S)	Human and bee venom $sPLA_2$	0.3	[29,30]
Cyclolinteinone **31**	*Cacospongia linteiformis* (S)	Bee venom $sPLA_2$	25	[29,30]
Variabilin **32**	Various sponges	Human $sPLA_2$ and $cPLA_2$	6.9	[29,30]
Halistanol sulphate 1 **33**	*Halichondria* sp. (S)	Bee venom $sPLA_2$	50	[18,20,29,30]
Petrosaspongiolide M **34**	*Petrosaspongia nigra* (S)	Human and bee venom $sPLA_2$	0.6	[18,29,30]
Scalaradial **35**	*Cacospongia mollior* (S)	Bee venom $sPLA_2$ and $cPLA_2$	0.6	[18,20,29,30]
Aplyolide **36**	*Aplysinopsis elegans* (S)	Human $sPLA_2$	10.5	[18]
Palinurin **37**	*Ircinia echinata* (S)	Bee venom $sPLA_2$	50	[18,29,30]
Palauolol **38**	*Fascaplysinopsis* sp. (S)	Bee venom $sPLA_2$	0.8	[18,29,30]
Palauolide **39**	*Fascaplysinopsis* sp. (S)	Bee venom $sPLA_2$	0.8	[18,29,30]
Cladocorans A–B **40–41**	*Cladocora cespitosa* (C)	$sPLA_2$	<2.0	[37]
Bromohydroquinones				
Cymopol **42**	*Cymopolia barbata* (GA)	Bee venom $sPLA_2$	>4.7	[21]
Cyclocymopol **43**	*Cymopolia barbata* (GA)	Bee venom $sPLA_2$	>3.7	[21]
Alkaloids				
Spongidine A–D **44–47**	*Spongia* sp. (S)	Human $sPLA_2$	10	[38]
Bromophenols				
Vidalol A–B **48–49**	*Vidilia obtusaloba* (RA)	Bee venom $sPLA_2$	5	[20]
Methoxylated fatty acid				
MMHDA **50**	*Ishige okamurae* (BA)	Bacterial PLA_2	2	[39]

BA, brown alga; C, coral, F, fungus; GA, green alga; N, nudibranch; N.A., not available; RA, red alga; S, sponge.

LTB_4 production in neutrophils and NO and PGE_2 production in macrophages [13–19]. Another, closely related sesquiterpenoid quinone, ilimaquinone (**2**) isolated from the sponge *Hippiospongia metachromia* [20], has also been shown to inhibit PLA_2 (IC_{75} = 270 μM against bee venom $sPLA_2$) [19]. The anti-psoriasis sesquiterpene hydroquinone avarol (**3**) and the sesquiterpene quinones avarone (**4**) and dysidine (**5**) isolated from the sponge *Dysidea avara* inhibit $sPLA_2$ activity and PGE_2 release in keratinocytes and in monocytes (IC_{50} = 2 μM). Furthermore, avarol has been shown to reduce eicosanoid release and ROS generation in stimulated leukocytes [17–19]. Dysidiotronic acid (**6**) isolated from *Dysidea* sp. also inhibits $sPLA_2$ (IC_{50} = 2.6 μM) [18,19]. The sesquiterpene lactone cavernolide (**7**) isolated from the sponge *Fasciospongia cavernosa* inhibits $sPLA_2$ activation (IC_{50} = 8.8 μM), as well as iNOS and COX-2 gene expression [18,20,21]. Amongst sesquiterpenes isolated from algae, rhipocephalin (**8**) extracted from the green alga *Rhipocephalus phoenix* has been shown to inhibit bee venom $sPLA_2$ (IC_{100} = 4.1 μM), and caulerpyne (**9**) produced by the green alga *Caulerpa prolifera* inhibits bee venom $sPLA_2$ activity with an IC_{92} value of 4.2 μM [21].

3.2. PLA_2 inhibiting diterpenes

The diterpenes gracilin A (**10**), aplyroseol 1 (**11**), and 12-acetoxytetrahydroaplysulphurin 1 (**12**) isolated from *Aplysilla* sp. sponges [22], and dendrillolide A (**13**) and norrisolide (**14**) isolated from the sponge *Dendrilla* sp. inhibit bee venom $sPLA_2$ with IC_{50} values around 5 μM [20]. They all contain a masked 1,4-dialdehyde function, which has been suggested to play a key role in their bioactivity [20]. The meroditerpne epitaondiol (**15**) isolated from the brown alga *Stypopodium flabelliforme* inhibits TXB_2 production by potently inhibiting human $sPLA_2$ (IC_{50} = 3.8 μM) [19,23]. The tetra- and bicyclic diterpenes phomactins A–C (**16–18**) isolated from the marine fungus *Phoma* sp. are potent PAF antagonists. While the precise mode of action of **16–18** remains poorly understood, it is likely that these three compounds may act as PAF antagonists by inhibiting $cPLA_2$s or by activating (Lp)PLA_2 [9,23,24]. The arabidose-containing diterpene fuscoside B (**19**) isolated from the gorgonian *Eunicea fusca* has not been reported as a PLA_2 inhibitor, but it has been shown to inhibit the conversion of

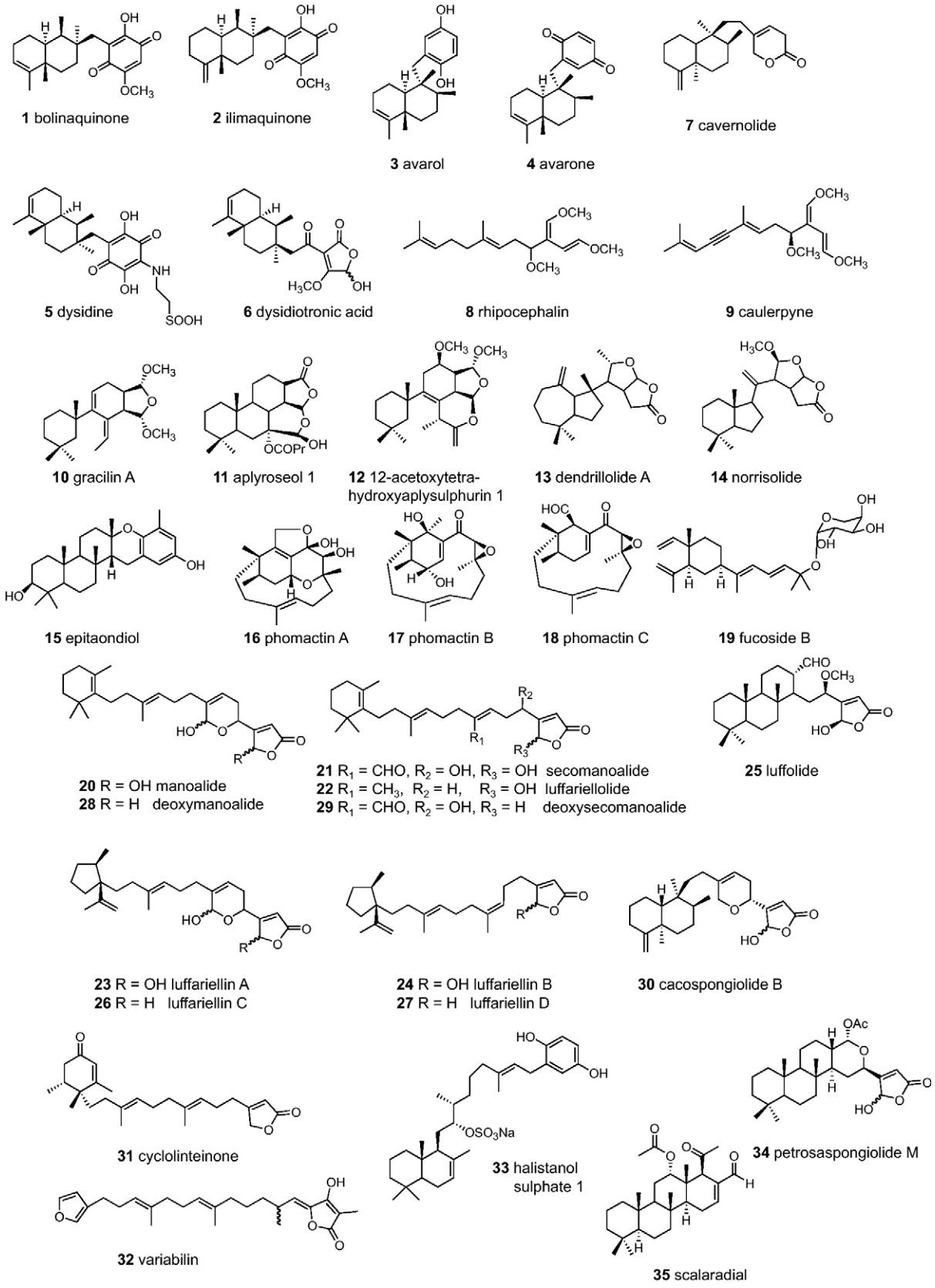

Fig. 2. Molecular structure of marine PLA_2 inhibitors.

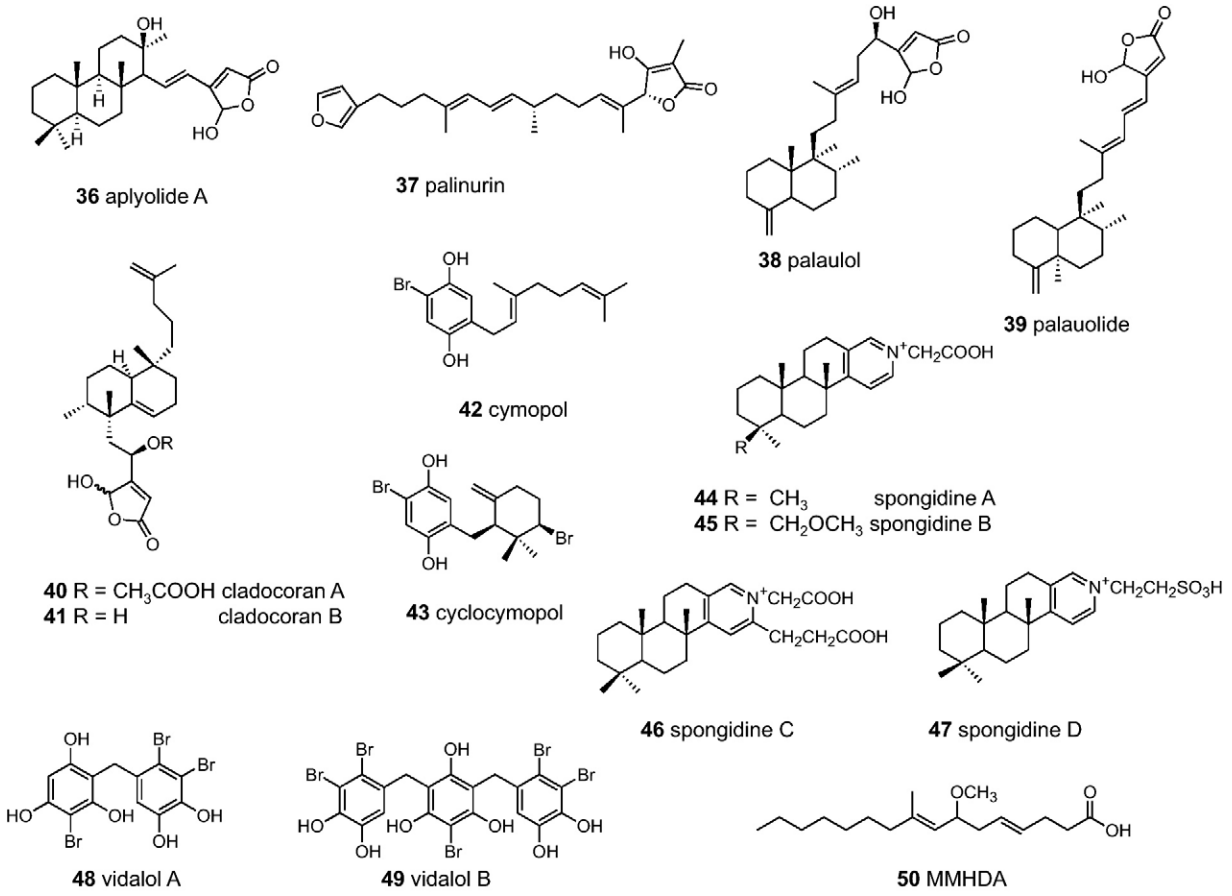

Fig. 2. (*Continued*).

arachidonic acid to LTB_4 by inhibiting 5-LO (IC_{50} = 18 μM) [18,25].

3.3. PLA_2 inhibiting sesterterpenes

Sesterterpenes have an outstanding potential as anti-inflammatory compounds. The sesterterpene manoalide (**20**), which was isolated for the first time in the early 1980s from the sponge *Luffariella variabilis* by Scheuer et al. [26], became the first marine natural product reported as PLA_2 inhibitor, and it remains, to date, the most investigated marine PLA_2 antagonist. The PLA_2 inhibiting properties of manoalide (**20**) were discovered simultaneously by research groups lead by Edward Dennis [4] and by Robert Jacobs [5] at the universities of San Diego and Santa Barbara, respectively, in the mid 1980s. Both groups confirmed that PLA_2 inhibition was responsible for the previously observed potent anti-inflammatory properties of manoalide (**20**) [8,14,15,18,27,28]. Like bolinaquinone (**1**), manoalide (**20**) is a non-specific inhibitor of PLA_2s [27]. Manoalide (**20**) inhibits human $sPLA_2$ (IC_{50} = 1.7 μM); snake venom $sPLA_2$ (IC_{50} = 0.03 μM); and $cPLA_2$ (IC_{50} = 10 μM) [8,14,15,18,27,28]. Manoalide (**20**) has been shown to inhibit $cPLA_2$ (IC_{50} = 10 μM) and phospholipase C [27]. Mechanistic studies revealed that the PLA_2 inhibitory activity of manoalide (**20**) results from the irreversible binding of two of the compound's masked aldehyde groups (the α-hydroxydihydropyran ring and the γ-hydroxybutenolide ring) to lysine residues at the active site of PLA_2 [15,28–30]. Manoalide (**20**) was licensed to Allergan Pharmaceuticals and reached Phase II clinical trials as a topical antipsoriatic, its development was however, discontinued due to formulation problems [14,28]. In addition to manoalide (**20**), several analogues of the molecule have been isolated from sponges belonging to the genus *Luffariella*, as well from other sponges. The major manoalide analogues include secomanoalide (**21**), which has the same potency as manoalide (**20**), luffariellolide (**22**) (IC_{50} = 230 nM against bee venom $sPLA_2$), luffariellins A (**23**) and B (**24**) (IC_{50} = 60 nM against bee venom $sPLA_2$), and luffolide (**25**) (IC_{50} = 40 nM against bee venom $sPLA_2$) [20]. Manoalide analogues have also been isolated from nudibranchs of the *Chromodoris* genus, which prey primarily on *Luffariella* sp. sponges [29]. Noteworthy, the nudibranch derived compounds, which include luffariellins C (**26**) and D (**27**), and deoxymanoalide (**28**) (IC_{50} = 0.2 μM against snake venom PLA_2) and deoxysecomanoalide (**29**) (IC_{50} = 0.5 μM against snake venom PLA_2), are all reduced (deoxy) counterparts of spongean manoalide analogues, and their PLA_2 inhibitory activity is a ten-fold weaker than the ones observed in the sponges [29,30]. Other PLA_2 inhibiting sesterterpenes isolated from various marine sponges include cacospongiolide B (**30**) (IC_{50} = 300 nM against human and bee venom $sPLA_2$), cyclolinteinone (**31**) (IC_{50} = 25 μM against bee venom $sPLA_2$), variabilin (**32**) (IC_{50} = 6.9 μM against human $sPLA_2$ and $cPLA_2$), halistanol sulphate 1 (**33**) (IC_{50} = 16 μg/mL against bee venom $sPLA_2$), petrosaspongiolide M (**34**) (IC_{50} = 1.6 μM against human $sPLA_2$; 0.6 μM against bee venom PLA_2), scalaradial (**35**) (IC_{50} = 1.6 nM against bee $sPLA_2$ and $cPLA_2$), aplyolide (**36**) (IC_{50} = 10.5 μM against human $sPLA_2$), palinurin (**37**) (IC_{50} = 50 μM against bee venom $sPLA_2$), palauolol (**38**) (IC_{50} = 0.8 μg/mL against bee venom $sPLA_2$), and palauolide (**39**) (IC_{50} = 0.8 μg/mL against bee venom $sPLA_2$) [12,14,18,20,31–34]. Molecular modelling studies have revealed that petrosaspongiolide M (**34**) inhibits PLA_2 *via* a non-covalent recognition between petrosaspongiolide M (**34**) and the enzyme, followed by a nucleophilic attack by the PLA_2 *N*-terminus onto the masked aldehyde at C-25 of the pharmacophoric γ-hydroxybutenolide ring of petrosapongiolide M (**34**) [30,32,35,36].

Petrosaspongiolide M (**34**) also inhibits the expression of iNOS and COX-2, and, as a result, the production of NO and PGE_2, respectively, and NF-κB activation [30,32–35]. Studies performed by Monti et al. have revealed that, although scalaradial (**35**) does bind covalently to bee venom PLA_2, the key step in the PLA_2 inhibitory activity of scalaradial (**35**) is, as observed with petrosaspongiolide M (**34**), its nonvalent binding to the enzyme's active site [33]. The furanoses-terterpene palinurin (**37**) isolated from the sponge *Ircinia echinata* has been shown to inhibit TXB_2 (IC_{50} = 5 μM), and the furan ring is thought to be the pharmacophore of the molecule [36]. The sesterterpenes cladocoran A (**40**) and B (**41**) isolated from the coral *Cladocora cespitosa* inhibit $sPLA_2$ (IC_{50} = 0.78 μM and 1.95 μM, respectively) [37]. Cladocoran A (**40**) and B (**41**) caught the attention of Miyako et al. because of their possession of a γ-hydroxybutenolide moiety as in manoalide (**20**) and cacospongiolide B (**30**). Interestingly, studies on diastereoisomers of cladocoran A (**40**) and B (**41**) revealed that the presence of a γ-hydroxybutenolide moiety itself is not sufficient for PLA_2 inhibitory activity, and that the size and shape of the molecule also play critical roles towards the compounds' potency [37].

3.4. Non-terpenoid marine PLA_2 inhibitors

The bromohydroquinones cymopol (**42**) and cyclocymopol (**43**) isolated from the green alga *Cymopolia barbata* inhibit bee venom $sPLA_2$ activity with IC_{98} values of 4.7 and 3.4 μM, respectively [21]. The pyridinium alkaloids spongidines A–D (**44**–**47**) isolated the sponge *Spongia* sp. inhibit human $sPLA_2$ (IC_{50} = 10 μM) [38], and the bromophenols vidalol A (**48**) and B (**49**) isolated from the red alga *Vidilia obtusaloba* inhibit bee venom $sPLA_2$ (IC_{50} = 1.6 μg/mL) despite lacking a γ-hydroxybutenolide or masked 1,4-dialdehyde group [20]. Finally, one of the most recently discovered marine PLA_2 inhibitors, namely the methoxylated fatty acid 7-methoxy-9-methylhexadeca-4,8-dienoic acid (MMHDA) (**50**) isolated from the brown alga *Ishige okamurae* has been shown to inhibit bacterial PLA_2 (IC_{50} = 2 μg/mL) [39].

4. Future perspectives and concluding remarks

Given the critical role of inflammation in diseases, identifying and developing novel anti-inflammatory drug candidates is of great importance in drug discovery. PLA_2s play a very important role in inflammation, and they are hence regarded as an interesting target for anti-inflammatory drugs. Fifty marine natural products and counting have been identified as potent PLA_2 inhibitors. Although the quest for novel marine PLA_2 inhibitors faded a little during the 1990s, the last three years have been associated with a fresh spark of enthusiasm into this field of research. Additionally, significant progress has been made recently in the classification and characterization of the different families of phospholipases, and in the understanding of the biochemistry and biology of PLA_2s. We can therefore expect a high number of novel, highly promising PLA_2 inhibitors to be developed over the next few years, from marine sources, as well as from terrestrial organisms or synthetically produced. Researchers working in this field of research are still facing some major challenges, as they need to find compounds that express high levels of specificity to the PLA_2s that they are inhibiting. The development of a thorough understanding of the chemical and biological properties of the various types of PLA_2s, and of their specificity to various diseases, is also a critical point that needs to be addressed, as is the precise understanding of the mechanism of action of PLA_2-targeting drug candidates. Only recently have the specific biological roles of the different classes of PLA_2s, and of the different isoforms within these classes, started to become understood, and even though a relatively large number of marine natural products have been tested for their PLA_2 inhibitory effects, most of them have only been screened against a single class of PLA_2s. For the tested compounds to become potential drug candidates, or to become useful research tools in fundamental biology, it is absolutely critical to screen their bioactivity against each one of the four PLA_2 classes, and against various PLA_2 isoforms, and to establish the specificity of the compounds for their target PLA_2. Specific PLA_2 inhibitors are indeed more likely to be bioactive at lower concentrations than non-specific inhibitors, and they are less prone to induce undesired side-effects [8]. Amongst the marine natural products included in the present review article, bolinaquinone (**1**) and manoalide (**20**) and its analogues have been shown to potently inhibit PLA_2s, but in a non-specific manner. Manoalide (**20**) has been valued as a potential drug candidate, and it has been taken forward to clinical trials, but it had to be dropped due to formulation problems. To our knowledge, the sesterterpenes palauolol (**38**) and palauolide (**39**) have only been evaluated for their potential to inhibit bee venom $sPLA_2$. Yet, their IC_{50} values were rather promising, and if the compounds' bioactivity could be shown to be paralleled with a good level of specificity, then **38** and **39** could potentially be considered as promising drug candidates, based on their PLA_2 inhibiting properties. Finally, when considering PLA_2-inhibiting compounds to be taken forward into more advanced studies, it is important to make sure that the compounds in question do not completely abolish the PLA_2 activity. Instead, they should only bring PLA_2 activity down to the basal level, as some vital cellular housekeeping depends on basal levels of PLA_2 activity.

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