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Original article

# Inhibition of secretory phospholipase $A_2$. 1-Design, synthesis and structure–activity relationship studies starting from 4-tetradecyloxybenzamidine to obtain specific inhibitors of group II $sPLA_2$s

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**Abstract**

Starting from 4-tetradecyloxybenzamidine (**PMS815**), a non-specific inhibitor of GI and GII $PLA_2$s, we report in this work the discovery of the specificity through design, synthesis and structure–activity relationships studies of different kinds of **PMS815** derivatives. The leading compound, 4,5-dihydro-3-(4-tetradecyloxybenzyl)-1,2,4-4*H*-oxadiazol-5-one (**9b**, **PMS1062**) exhibits a micromolar $IC_{50}$ towards three group II $PLA_2$s, while inactive towards four group I and one group III enzymes in two in vitro enzymatic assay conditions. It is also able to block the $PLA_2$-II activities induced by LPS and IL-6 in $HepG_2$ cell line and no cytotoxicity is observed when **PMS1062** is tested up to a concentration of 100 μM in two different cell lines (A549 and LLC-$PK_1$).


*Keywords:* $sPLA_2$ inhibitors; Oxadiazolone; Structure–activity relationships; Cytotoxicity

## 1. Introduction

Phospholipases $A_2$ ($PLA_2$s) are ubiquitous enzymes that catalyze the hydrolysis of the ester bond at the sn-2 position of glycerophospholipids. The fatty-acid released, such as arachidonic acid (AA), may be enzymatically metabolized into potent pro-inflammatory mediators known as eicosanoids (prostaglandins, leucotrienes and thromboxanes), while the lyso-phospholipid, other product of the $PLA_2$ catalyzed reaction in the case of lyso-platelet-activating-factor (lyso-PAF), may be converted by the PAF-acetyltransferase into PAF, another well-known pro-inflammatory mediator.

The $PLA_2$ family encompasses two classes of enzymes, either intracellular (cytosolic $PLA_2$s and $Ca^{2+}$-independent $PLA_2$s) or secretory ($sPLA_2$s). According to the similarity of their polypeptide sequence, $sPLA_2$s have been classified initially into three groups [1]: group I ($PLA_2$-I) including $PLA_2$s from mammalian pancreatic secretions and *Elapidae* venoms; group II ($PLA_2$-II) comprising $PLA_2$s from *Crotalidae* and *Viperidae* venoms as well as the mammalian non-pancreatic secretory $PLA_2$ and group III ($PLA_2$-III) from bee and lizard venoms. Thanks to the success of genomics, a great number of mammalian $sPLA_2$s have been recently identified and cloned [2–5]. Among the 10 human $sPLA_2$s known up to date [2], it remains true that the most studied are pancreatic or group IB $PLA_2$ ($PLA_2$-IB) and non-pancreatic or group IIA $PLA_2$ ($PLA_2$-IIA), especially in regards to their association with various inflammatory diseases [6].

$PLA_2$-IB is a digestive enzyme synthesized by pancreatic acinar cells as a proenzyme, which is secreted through the

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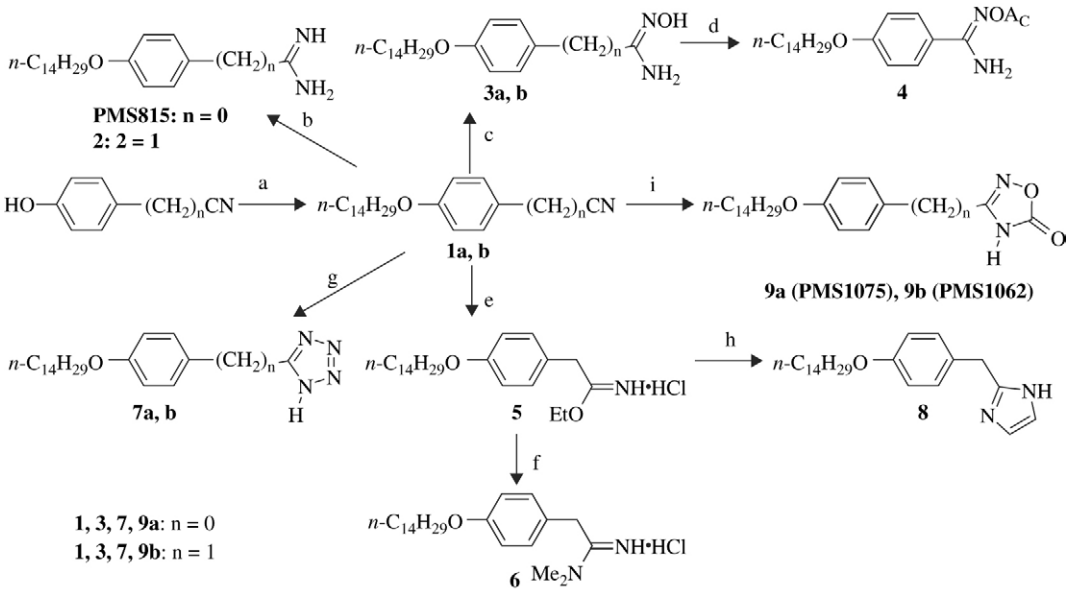



[a]Reagents: (a) -1) NaOH, abs EtOH, -2) $CH_3(CH_2)_{13}Br$, DMF; (b) -1) $NH_4Cl$, $AlMe_3$, toluene, 5 °C, -2) **1a,** or **b**, toluene, 110 °C; (c) $NH_2OH$•HCl, $K_2CO_3$, abs EtOH, reflux; (d) $Ac_2O$, $Et_3N$, $CH_2Cl_2$; (e) abs EtOH, HCl gas; (f) $Me_2NH$, MeOH, 0 °C; (g) $NaN_3$, $NH_4Cl$, DMF, 100 °C; (h) -1) $NH_2CH_2CH(OEt)_2$, abs EtOH, -2) 2 N HCl, reflux; (i) -1) 2-ethylhexyl chloroformate, pyridine, 0 °C, -2) xylene, reflux.

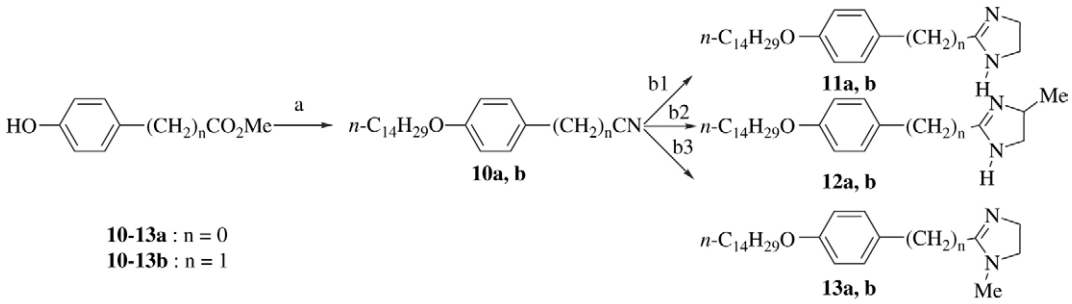
Scheme 1.

pancreatic duct to the lumen of the small intestine and cleaved by trypsin into enzymatically active form [7]. In acute pancreatitis, characterized by destruction of pancreatic tissue, $PLA_2$-IB is released into the circulation, but its role in pancreatic or any other tissue damages is still hypothetical [6]. $PLA_2$-IIA activity is shown to be highly elevated in many inflammatory tissues such as rheumatoid arthritic joints [8], psoriatic skin [9], and serum of patients suffering from pancreatitis [10] or gram negative shock [11]. Furthermore, its concentration increases in blood plasma in generalized inflammatory response resulting from infections, chronic inflammatory diseases, acute pancreatitis, trauma and surgical operations [6]. Even if $PLA_2$-IIA shows no preference for any fatty-acid at the sn-2 position, in particular arachidonic acid [12], its expression seems related to the potentiation and perpetuation of inflammatory reactions [13]. In addition, this expression is inducible in a wide variety of cells and tissues, such as macrophages [14], as well as hepatoma-derived cells in vitro [15] and hepatocytes in vivo [16], when they are stimulated by LDL, LPS, cytokines such as interleukin-6 (IL-6), tumor necrosis factor (TNF), interleukin-1 (IL-1) etc.

The crystal structures of s$PLA_2$-IB and -IIA, free or complexed with a substrate analogue, have shown that several conserved features at the active site are defined by the presence of a hydrophobic channel lined on one side by the N-terminal helix [17]. This hydrophobic channel binds a single phospholipid molecule and then hydrolyzes it following interfacial recognition between the enzyme and the aggregated substrate. This structural similarity shows how much it is difficult to obtain inhibitors specific of one particular enzyme.

Due to the involvement of lipid mediators in diverse pathological processes, the suppression of their production has long been considered as therapeutical strategies. In a previous paper, we have demonstrated that compounds as simple as 4-alkoxybenzamidines could inhibit secretory $PLA_2$ in a competitive manner [18]. Particularly, **PMS815** (Scheme 1, $n = 0$) showed interesting $IC_{50}$ of 3 and 5 μM, respectively, for group I bovine pancreatic $PLA_2$ and group II $PLA_2$ from rabbit platelet lysate.

In order to clarify the mode of action of such kind of compounds and find specific inhibitors of group II $PLA_2$, several structural modifications shown in Scheme 1 were investigated. Different substitutions were performed on the $sp^2$ and the $sp^3$ nitrogens of the amidine and their influence on the inhibitory potency studied. Our SAR study was also enlarged by using various heterocycles as imidazoline, oxazolidine, imidazole, tetrazole and oxadiazolone. This led us to the discovery of some specific inhibitors of group II $PLA_2$ versus group I and III enzymes. The leading compound, 4,5-dihydro-3-(4-tetradecyloxybenzyl)-1,2,4-4*H*-oxadiazol-5-one (**9b**, **PMS1062**) exhibits a micromolar $IC_{50}$ towards a number of group II $PLA_2$s, while inactive at 100 μM, the highest concentration used, towards different group I and group III enzymes in two in vitro enzymatic assay conditions, one using an aggregated and the other using a monomeric substrate. It was also shown to be able to block the $PLA_2$-II activities induced by LPS and IL-6 in $HepG_2$ cell line. Moreover, no cytotoxicity was observed when **PMS1062** was tested up to a concentration of 100 μM in two different cell lines (A549 and $LLC\text{-}PK_1$). Furthermore, in a carrageenan-induced oedema model, although completely inactive when administrated per os, likely due to its high lipophilicity, **PMS1062** was a little less active than indomethacin, when i.p. administrated. These results suggest that **PMS1062** can act likely as a $PLA_2$-II




[a] Reagents: (a) -1) NaOH, abs EtOH, -2) $CH_3(CH_2)_{13}Br$, DMF; (b) $AlMe_3$, -1) EDA, -2) 1,2-diaminopropane, -3) *N*-MeEDA, toluene, reflux.

Scheme 2.

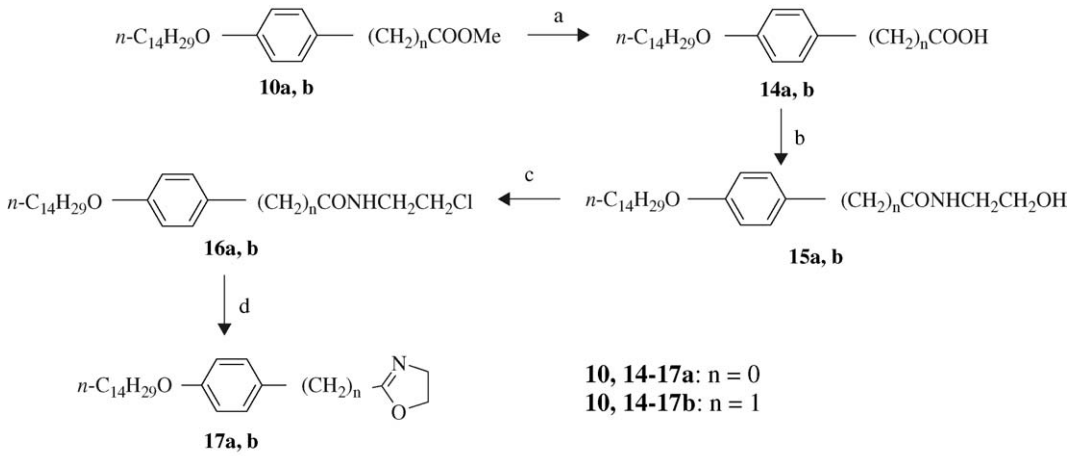
inhibitor on the acute inflammation model in which the involvement of $PLA_2$-II has been established, whereas, its high lipophilic character could prevent it from being well biodistributed per os. This will be further addressed in our following manuscripts.

## 2. Chemistry

The majority of the compounds reported in this work were prepared as depicted in Scheme 1. **1b** was synthesized using a similar protocol as described previously to prepare **1a** [18], while NaOH was used instead of NaH to obtain the corresponding phenolate, and then treated with $NH_2Al(CH_3)Cl$, freshly prepared from trimethylaluminum and ammonium chloride according to the method developed to obtain **PMS815** [19], to afford **2**. The nitriles **1a**, **b** were converted into the amidoximes **3a**, **b** and acetylamidoxime **4** following a method adapted from Diana et al. [20], while the dimethylamidine **6** needed the treatment of the intermediate iminoether hydrochloride **5** with dimethylamine according to McFarland et al. [21]. The tetrazole ring analogues **7a**, **b** were prepared from **1a**, **b** by the method of Harfenist et al. [22], with $NaN_3$ and ammonium chloride while the imidazole derivative **8** was synthesized from **5** using the method of Robertson et al. [23] with aminoacetaldehyde diethylacetal. The oxadiazolone moiety of **9a (PMS 1075)** and **9b (PMS1062)** was obtained by reaction of the amidoxime **3a**, **b** with 2-ethylhexyl chloroformate and reflux of the intermediate in xylene, according to the method of Kohara et al. [24]. Imidazoline derivatives **11a**, **b**, **12a**, **b** and **13a**, **b**, were synthesized following the method of Rondu et al. [25] starting from the corresponding esters **10a**, **b** and using trimethylaluminum and substituted (or not) ethylene diamine (EDA) (Scheme 2). These last esters were also used in the preparation of the oxazolines **17a**, **b** (Scheme 3): the acids **14a**, **b**, resulting from the hydrolysis of the esters **10a**, **b**, were condensed with ethanolamine by action of *N,N'*-dicyclohexylcarbodiimide (DCC) to give **15a**, **b**, of which the alcohol function was converted into chloride by thionyl chloride to give **16a**, **b**. Intramolecular cyclization of **16a**, **b** occurred in alkaline conditions to afford **17a**, **b**.

## 3. Results and discussion

We have previously published that compounds as simple as 4-alkoxybenzamidines could be potent $sPLA_2$ inhibitors




[a] Reagents: (a) 20% NaOH, EtOH; (b) DCC, $Et_3N$, DMF, EtOAc; (c) $SOCl_2$, $CHCl_3$; (d) NaOH pellets, abs EtOH.

Scheme 3.

Table 1a
Inhibition of the enzymatic activity of porcine pancreatic $PLA_2$ (GIB) and $PLA_2$ from rabbit platelet lysates (GIIA) by amidine analogues with the $IC_{50}$ values determined using spectrofluorimetric assay

| Compound | Z | $IC_{50}$ (μM)[a] | |
|---|---|---|---|
| | | GIB | GIIA |
| **1a (PMS815)** | | 2.8 ± 0.5 | 2.5 ± 0.5 |
| **3a** | | > 100 | > 100 |
| **4** | | > 100 | > 100 |
| **11a** | | 1.8 ± 0.1 | 1.9 ± 0.2 |
| **12a** | | 2.0 ± 0.2 | 2.1 ± 0.3 |
| **13a** | | 2.4 ± 0.1 | 2.3 ± 0.5 |
| **17a** | | > 100 | > 100 |
| **7a** | | > 100 | 46 1 |
| **PMS 1075** | | > 100 | 11 ± 1 |
| LY311727 [28] | | 8.0 ± 0.1 | 0.47 ± 0.04 |

[a] Mean S.D.

and the leader of the series possessed an alkyl chain of 14 carbons (compound **1a** or **PMS815**, Scheme 1) [19]. In this work, the amidine function of **PMS815** was substituted or replaced by different heterocycles for the purpose to improve the inhibitory activity, as well as to reach a specific inhibition of $sPLA_2$-IIA versus $sPLA_2$-IB.

### 3.1. Non-cyclic analogues of *PMS815* (Table 1a)

When the hydrogen on the so-called $sp^2$ nitrogen of **PMS815** was replaced by a hydroxy or an acetoxy group, **3a** and **4** thus obtained lost the ability to inhibit both $PLA_2$-I and -II activities. This could be explained by either the decrease of 1–2 units of log *P* due to the introduction of the OH function (compound **3a**), or suppression of the interactions between this hydrogen and certain residues of the enzyme (compound **4**).

As shown in Table 1b, insertion of a methylene group between the amidine function and the phenyl moiety (compound **2**) did not induce a significant improvement of the inhibitory activity on both group I and II enzymes. The *N*-hydroxylation of **2** led to compound **3b**, which lost its capacity to inhibit the $sPLA_2$s activities, confirming the results obtained with its homologue **3a**. However, when two methyls were used to substitute the so-called $sp^3$ nitrogen of **2**, the compound **6**, obtained through the intermediate **5**, showed an enhanced inhibitory effect on both group I and II $PLA_2$s (two or three times). These results indicate that the presence of a more basic nitrogen at this position due to the dimethylation, to which a protonation can happen at neutral pH used in our in vitro enzymatic assay, seems to be favorable for the anti-$sPLA_2$ activity.

Table 1b
Inhibition of the enzymatic activity of porcine pancreatic $PLA_2$ (GIB) and $PLA_2$ from rabbit platelet lysates (GIIA) by amidine homologue derivatives with the $IC_{50}$ values determined using spectrofluorimetric assay

| Compound | n | Z | $IC_{50}$ (μM)[a] | |
|---|---|---|---|---|
| | | | Group IB | Group IIA |
| **PMS815** | 0 | | 2.8 ± 0.5 | 2.5 ± 0.5 |
| **2** | 1 | | 1.9 ± 0.3 | 1.7 ± 0.5 |
| **3b** | 1 | | > 100 | > 100 |
| **6** | 1 | | 0.9 ± 0.4 | 1.3 ± 0.2 |
| **11b** | 1 | | 1.3 ± 0.5 | 1.5 ± 0.3 |
| **12b** | 1 | | 1.4 ± 0.4 | 1.4 ± 0.7 |
| **13b** | 1 | | 1.9 ± 0.3 | 1.7 ± 0.5 |
| **8** | 1 | | > 100 | > 100 |
| **17b** | 1 | | > 100 | > 100 |
| **7b** | 1 | | 70 ± 2 | 34 ± 1 |
| **9b (PMS1062)** | 1 | | > 100 | 4.0 ± 0.9 |
| LY311727 [28] | | | 8.0 ± 0.1 | 0.47 ± 0.04 |

[a] Mean S.D.

### 3.2. Cyclic analogues of *PMS815* (Table 1a,b)

Given that the above structural modifications only improved faintly the anti-$PLA_2$ activity, but not at all the selectivity, we have diversified our SAR study by using different heterocycles in the place of the amidine function. Based on the fact that the *N*,*N*-dimethylamidine analogues did not significantly affect the activity, the amidine motif was firstly integrated in an imidazoline cycle (compounds **11a**, **b**). This modification induced no amelioration of the selectivity and the inhibition potency remained almost the same as that of the linear analogues. None of both properties was influenced by the C or *N*-methylation of the imidazoline ring (compounds **12a**, **b** and **13a**, **b**). These results are in agreement with those related to **6**, since the imidazoline, substituted or not, remains basic and thus protonable in the enzymatic assay conditions. This protonation is likely important for the formation of a hydrogen bond between the His48 of the enzyme and the inhibitors, as proposed previously [19].

Along with this argument, the imidazole derivative (compound **8**), although containing the amidine motif and possessing almost the same lipophilicity, was shown to be inactive against both enzymes in the spectrofluorimetric assay, as its $pK_a$ is very close to 7. The fact that the oxazoline derivatives (compounds **17a**, **b**) were neither active, implies that the integration of the amidine motif would be necessary to the establishment of important interactions between the inhibitors and certain residues of the enzymes.

Tetrazole derivatives **7a**, **b** were the first compounds which showed a little selectivity towards $PLA_2$-IIA from rabbit platelet lysates versus $PLA_2$-IB, although their activity was much weaker than the amidine and the imidazoline counterparts. When submitted to the in vitro spectrofluorimetric enzymatic assay, **7a** (Table 1a) was completely inactive at 100 μM, the highest concentration tested, against porcine pancreatic $PLA_2$ (GIB), while possessed an $IC_{50}$ of 46.2 μM against $PLA_2$ from rabbit platelet lysates (GIIA). Similarly, **7b** was twofold more potent against GIIA $PLA_2$ than GIB $PLA_2$ (Table 1b).

### 3.3. Replacement of the tetrazole by an oxadiazolone ring: towards $PLA_2$-II specificity

The first results obtained with the tetrazole ring, bioisostere of carboxylic acid function, encouraged us to investigate

Table 2
Inhibition by **PMS1062** of the enzymatic activity of different sPLA$_2$s in the in vitro fluorimetric (FA) and spectrophotometric (SA) assays

| | Group I | | | | Group II | | |
|---|---|---|---|---|---|---|---|
| | hGIB | pGIB | bGIB | NNA | hGIIA | RPL | CB |
| FA-IC$_{50}$ (μM)[a] | > 100 | > 100 | > 100 | > 100 | 3.4 ± 0.4 | 4.0 ± 0.9 | ND |
| SA-IC$_{50}$ (μM)[a] | > 100 | > 100 | > 100 | > 100 | 0.40 ± 0.06 | ND | 0.10 ± 0.01 |

Group I: human pancreatic (hGIB), porcine pancreatic (pGIB), bovine pancreatic (bGIB) and *N. naja atra* of *Elapidae* venom (NNA); group II: human non-pancreatic (hGIIA), rabbit platelet lysates (RPL) and *C. durissus terrifucus* venom (CB).
[a] Mean S.D.

other heterocycles possessing an acid character. Oxadiazolone was chosen because it was described to be an indispensable component in a great number of bioactive molecules, such as anti-5-lipoxygenase and anti-cyclooxygenase agents [26], antagonists of angiotensine II receptor [24] and an anti-thrombotic prodrug [27].

Indeed, **9a** (**PMS1075**), in which the oxadiazolone was linked directly to the phenyl group, shows a much better selectivity and activity than **7a** corresponding to the tetrazole analogue (Table 1a). No activity was detected at 100 μM of this inhibitor when PLA$_2$-I was used in the fluorimetric assay conditions, however, the IC$_{50}$ of **PMS1075** was four-times smaller than that of **7a**, when PLA$_2$-II was used (Table 1a). More interestingly, when the homologue **9b** (**PMS1062**) was submitted to the same assay conditions, we found that the activity and the selectivity were more advantageously enhanced (Table 1b). Apparently, this gain of both the properties is related to the flexibility increase between the oxadiazolone and the phenyl rings, since the conjugation between these rings in the case of **9a** is disrupted by the insertion of a methylene group in the case of **9b**.

To confirm the selectivity of **9b** against GIIA PLA$_2$ versus other groups of sPLA$_2$s, the study was enlarged to various group I and II PLA$_2$s, as well as group III PLA$_2$ of different species. For all the other PLA$_2$s-I tested, including human pancreatic, bovine pancreatic PLA$_2$s and that from the venom of *Naja naja atra* of *Elapidae*, **PMS1062** at 100 μM had not any detectable effect on their enzymatic activity in our fluorimetric conditions as observed in the case of porcine pancreatic PLA$_2$. The same observation was noted when the assay was carried out with a group III PLA$_2$ from the bee venom of *Apis mellifera*. By contrast, a similar IC$_{50}$ of micromolar magnitude (Table 2) was found in this fluorimetric assay for all the other group II PLA$_2$s used, including human secretory non-pancreatic one and that from the venom of *Crotalus durissus terrificus* (basic sub-unit of the crotoxine, CB).

To verify the reliability of our fluorimetric assay, one of the specific hGIIA PLA$_2$ inhibitors of Lilly Company, LY311727 [28], was tested in the same conditions as for our compounds. It exhibited effectively a more potent inhibitory activity towards hGIIA PLA$_2$ with an IC$_{50}$ of 0.47 μM than towards porcine pancreatic PLA$_2$ with an IC$_{50}$ of 8 μM (Table 1a,b). Our inhibition data of this compound against hGIIA PLA$_2$ is well consistent with its apparent $K_B$ of 0.27 μM against the same enzyme obtained in a tissue-based assay [29], although its specificity is observed less in our fluorimetric assay conditions than reported previously using the same assay (no effect on porcine pancreatic PLA$_2$ at 10 μM). This could be due to the difference of the two assay systems and in particular to the side-specific effect of the inhibitor on the substrate vesicles in the case of our fluorimetric assay (Table 2).

### *3.4. Spectrophotometric competition assay using a monomeric 1,2-di-n-hexanoyl-1,2-dithio-sn-glycero-3-phosphoglycerol, lithium salt (thio-PG) substrate and specific inhibition of group II PLA$_2$s by **PMS1062***

One compound could appear as an inhibitor by interfering simply with the organized substrate, which has been shown to be preferred by interfacial activating PLA$_2$s, and thus preventing it from enzymatic action or leading to a sub-estimation of a real inhibition. On the other hand, an inhibition could occur in an irreversible manner, if one molecule reacted with one of the important residues at the active site of an enzyme. In the latter case, the molecule would be in general excluded from a therapeutical application. In consequence, these features needed to be addressed through elucidating the mechanism of action of **PMS1062**.

Spectrophotometric competition assay was therefore developed and the substrate in this assay was maintained under the critical micellar concentration (CMC). The substrate, 1,2-di-*n*-hexanoyl-1,2-dithio-sn-glycero-3-phosphoglycerol, lithium salt (thio-PG), was prepared in our laboratory and the CMC was determined to be about 1 mM (data to be published elsewhere). It has been observed in this assay conditions that CB and human recombinant (group II) PLA$_2$s could be inhibited by **PMS1062** with a sub-micromolar IC$_{50}$ (Table 2), whereas neither *N. naja atra*, bovine, porcine and human pancreatic PLA$_2$s (group I), nor bee venom PLA$_2$ (group III) were sensible to **PMS1062**, up to a concentration of 100 μM (Table 2). These results provided solid evidence that **PMS1062** is a specific and active site-targeted inhibitor of group II PLA$_2$s. The difference between the IC$_{50}$ values obtained by the fluorimetric and the spectrophotometric assays could be explained by the different assay conditions, and probably also by a sub-estimation in the fluorimetric conditions.

The reversibility of this inhibition was demonstrated by a dilution experience. When a preformed human group II PLA$_2$-**PMS1062** complex was diluted, a total recovery of the PLA$_2$ activity was observed.

The above interesting activities of **PMS1062** in our in vitro enzymatic assays encouraged us to study its pharmacological properties using different cell lines and a carrageenan-

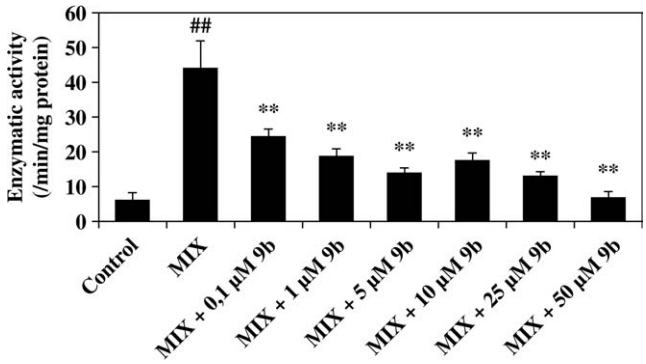



Fig. 1. Effects of **PMS1062** on $sPLA_2$ activity in $HebG_2$ cells, stimulated with LPS and IL6 (MIX). ##: $p < 0.01$ compared to control; ** $P < 0.01$ compared to MIX.

induced oedema model. As reported [16], stimulation with LPS + Il-6 (MIX) induced an increase of $sPLA_2$ activity in $HepG_2$ cells, by sevenfold in our experimental conditions compared to the control. However, co-treatment with MIX plus **PMS1062** (50–0.1 μM, $n = 6$) resulted in a decrease of this activity by 84–45% with an $IC_{50}$ of 0.5 μM (Fig. 1). We observed that 50 μM of **PMS1062** could completely suppress the LPS and IL-6 induced $sPLA_2$ activity in $HepG_2$ cells.

Cytotoxicity of **PMS1062** was assessed using MTT and neutral red assays. As can be seen in Table 3, cell viability is not altered even at 100 μM, the highest dose used in both A549 and LLC-$PK_1$ cell lines, indicating that **PMS1062** is poorly cytotoxic. Moreover, the effect of **PMS1062** on the release of two marker enzymes, γ-GT and NAG, which is directly correlated to tubular cell damages, was assessed using LLC-$PK_1$ cells. Since **PMS1062** has no effect on this release at all (data not shown), it seems to be non-nephrotoxic and not cytotoxic for LLC-$PK_1$ cells.

Anti-inflammatory property of **PMS1062** has also been evaluated on acute response where mammalian $PLA_2$-II is involved, such as in carrageenan-induced oedema formation. When i.p. administrated, **PMS1062** was able to reduce the oedema formation in the rear rat paw by 30% at a dose of 30 mg $kg^{-1}$ (results not shown), while indomethacin, a well-known anti-inflammatory agent, by 40% at a dose of 10 mg $kg^{-1}$ in the same experimental conditions. However, when administrated per os, this inhibition disappeared completely. These results suggest that **PMS1062** can act likely as a $PLA_2$-II inhibitor on the acute inflammation model in which the involvement of $PLA_2$-II has been established, whereas, its high lipophilic character could prevent it from being well biodistributed per os. This will be further addressed in our following manuscripts.

Table 3
Effects of **PMS1062** on cell viability expressed by $IC_{50}$ (μM), determined ($n = 6$) at the end of 24 or 48 h incubation by MTT and Neutral Red methods using A549 and LLC-$PK_1$ cell lines (see Section 5)

| | MTT | | Neutral Red | |
|---|---|---|---|---|
| | 24 h | 48 h | 24 h | 48 h |
| A549 | > 100 | > 100 | > 100 | > 100 |
| LLC-$PK_1$ | > 100 | > 100 | > 100 | > 100 |

## 4. Conclusion

Starting from a non-specific inhibitor of group I and II $PLA_2$s, **PMS815**, we describe in this work how a rational SAR study led us towards the specific inhibition of $sPLA_2$-II. Using three groups of $sPLA_2$s of different species and two in vitro enzymatic assay conditions, we have demonstrated that the leading compound, **PMS1062** is an active site-targeted and specific inhibitor of group II $PLA_2$s and in addition, this inhibition seems to be reversible. Furthermore, biological studies using cellular or animal models provide evidences that this kind of compounds could be of therapeutical interests in the treatment of inflammation by targeting specifically group II $sPLA_2$s. More detailed SAR studies will be described in the following manuscripts and we are actually to enlarge our specificity determination to recently reported human group III, V and X $sPLA_2$s.

## 5. Experimental section

### 5.1. Chemistry

All chemicals were of reagent quality and were used without further purification. The purity of each compound was checked by thin-layer chromatography on TLC plastic sheets (silica gel 60F 254, layer thickness 0.2 mm) from Merck. Column chromatography purification was carried out on silica gel 60 (particle size 0.063–0.200 mm) from Merck without any special treatment. All melting points were determined in a digital melting point apparatus (Electrothermal) and are uncorrected. The structures of all compounds were confirmed by IR and $^1H$ NMR spectra. IR spectra were obtained with an ATI Mattson GENESIS SERIES FTIR infrared spectrometer, and $^1H$ NMR spectra were recorded in $CDCl_3$ or DMSO-$d_6$ on a Bruker AC 200 spectrometer using hexamethyldisiloxane (HMDS) as an internal standard. Chemical shifts are reported in parts per million ($\delta$) and coupling constants expressed in Hz. Elemental analyses were carried out for C, H and N by the Service Régional de Microanalyse de l'Université Paris 6 and are within ± 0.4% of theoretical values.

#### 5.1.1. 4-Tetradecyloxyphenylacetonitrile (*1b*)

To an ice-bath cooled solution of 4-hydroxybenzyl cyanide (6.00 g, 45.1 mmol) in absolute EtOH (30 ml) was added dropwise a solution of NaOH (1.90 g, 47.5 mmol) in EtOH (20 ml). After stirred for 15 min, the solution was evaporated to dryness and the residue, taken up in *N*,*N*-dimethylformamide (DMF, 30 ml), was added dropwise to a solution of 1-bromotetradecane (13 g, 47.5 mmol) in DMF (200 ml) at 0 °C. After stirred at room temperature for 4 h, the mixture was diluted with water, extracted with EtOAc. The organic phases were dried over $MgSO_4$, filtered and evaporated. Purification on a silica gel column (ether/spirit) yielded **1b** (7.1 g, 48%) as a white solid: m.p. 68 °C; Rf 0.81 (MeOH/$CH_2Cl_2$,

5:95, v/v); IR (KBr, $cm^{-1}$) 2245 (CN), 1597 (C=C); $^1H$ NMR ($CDCl_3$) 7.15, 6.89 (2d, 4H, $J$ = 8.70, $H_{ar}$), 3.86 (t, 2H, $J$ = 6.52, $CH_2O$), 3.59 (s, 2H, $CH_2CN$), 1.70 (m, 2H, C**H$_2$**$CH_2O$), 1.36–1.19 (m, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.37, $CH_3$).

*5.1.2. 4-Tetradecyloxyphenylacetamidine hydrochloride (**2**)*

To a suspension of ammonium chloride (4.82 g, 90.0 mmol) in dry benzene (90 ml) at 5 °C was added dropwise a solution of trimethylaluminum in toluene (2.0 M, 45 ml, 90 mmol). The mixture was stirred at room temperature for 2 h and then added to a stirred solution of **1b** (15.0 g, 41.4 mmol) in dry toluene (150 ml). After heated to reflux for 6 h at 110 °C under argon atmosphere, the solution was poured on to a stirred suspension of silica gel (120 g) in chloroform (600 ml) and stirred for a further 5 min at room temperature. The silica gel was filtered and washed with chloroform to eliminate the unreacted nitrile **1b**. The amidine hydrochloride **2** was then recovered by methanol as colorless crystals after evaporation of the solvent (6.32 g, 40%): m.p. 88 °C; Rf 0.23 (MeOH/$CHCl_3$/$NH_4OH$, 20:80:02, v/v/v); IR (KBr, $cm^{-1}$) 3196 (N–H), 1688 (C=N), 1613 (C=C); $^1H$ NMR (DMSO-$d_6$) 8.95 (br s, 4H, NH), 7.32, 6.84 (2 d, 4H, $J$ = 8.31, $H_{ar}$), 3.87 (t, 2H, $J$ = 6.00, $CH_2O$), 3.57 (s, 2H, $CH_2CN$), 1.61 (qt, 2H, $J$ = 6.38, C**H$_2$**$CH_2O$), 1.18 (s, 22H, $CH_2$), 0.79 (t, 3H, $J$ = 6.37, $CH_3$). Anal. ($C_{22}H_{39}ClN_2O$) C, H, N.

*5.1.3. 4-Tetradecyloxyphenylacetamidoxime (**3b**)*

A mixture of **1b** (5.00 g, 15.2 mmol), $K_2CO_3$ (11.0 g, 79.7 mmol) and hydroxylamine hydrochloride (5.3 g, 76 mmol) in absolute EtOH (60 ml) was heated to reflux for 18 h. The suspension was filtered and the solid washed with hot absolute EtOH. The filtrate was concentrated in vacuo and the residue purified on a silica gel column using $CH_2Cl_2$ as eluent to give **3b** as white crystals (4.0 g, 74%): m.p. 102 °C; Rf 0.18 (MeOH/$CH_2Cl_2$, 5:95, v/v); IR (KBr, $cm^{-1}$) 3456 (N–H), 3150 (O–H), 1656 (C=N), 1610 (C=C); $^1H$ NMR ($CDCl_3$) 9.41 (br s, 1H, $D_2O$ exchange, OH), 7.13, 6.77 (2d, 4H, $J$ = 8.62, $H_{ar}$), 4.41 (s, 2H, $NH_2$), 3.85 (t, 2H, $J$ = 6.52, $CH_2O$), 3.55 (s, 2H, $CH_2CN$), 1.68 (qt, 2H, $J$ = 6.70, C**H$_2$**$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.81(s, 3H, $J$ = 6.56, $CH_3$). Anal. ($C_{22}H_{38}N_2O_2$) C, H, N.

*5.1.4. 4-Tetradecyloxybenzylamidoxime (**3a**)*

4-Tetradecyloxybenzylamidoxime (**3a**) was prepared in 76% yield following the same procedure as for **3b** but starting from 4-tetradecyloxybenzonitrile **1a**: m.p. 110 °C; Rf 0.17 (MeOH/$CH_2Cl_2$, 5:95, v/v); IR (KBr, $cm^{-1}$) 3449, 3348 (N–H), 3240 (O–H), 1653 (C=N), 1612 (C=C); $^1H$ NMR ($CDCl_3$) 9.44 (br s, 1H, $D_2O$ exchange, OH), 7.57, 6.89 (2 d, 4H, $J$ = 8.68, $H_{ar}$), 5.71 (s, 2H, $NH_2$), 3.94 (t, 2H, $J$ = 6.32, $CH_2O$), 1.69 (m, 2H, C**H$_2$**$CH_2O$), 1.23 (s, 22H, $CH_2$), 0.83 (t, 3H, $J$ = 6.56, $CH_3$). Anal. ($C_{21}H_{36}N_2O_2$) C, H, N.

*5.1.5. O-Acetyl-4-tetradecyloxyphenylacetamidoxime (**4**)*

A mixture of **3b** (0.90 g, 2.48 mmol), acetic anhydride (0.30 g, 2.94 mmol) and $Et_3N$ (0.30 g, 2.97 mmol) in $CH_2Cl_2$ (100 ml) was stirred at room temperature for 2 h. Salts were eliminated by washing with $H_2O$ and the organic layer was dried ($MgSO_4$), filtered and evaporated to dryness. Crystallization in ether/$CH_2Cl_2$ (2:1, v/v) yielded **4** as white crystals (0.43 g, 43%): m.p. 140 °C; Rf 0.12 (MeOH/$CH_2Cl_2$, 5:95, v/v); IR (KBr, $cm^{-1}$) 3426, 3320 (N–H), 1737 (C=O), 1625 (C=N); $^1H$ NMR ($CDCl_3$) 7.12, 6.79 (2 d, 4H, $J$ = 8.49, $H_{ar}$), 4.57 (s, 2H, $NH_2$), 3.86 (t, 2H, $J$ = 6.50, $CH_2O$), 3.45 (s, 2H, $CH_2CN$), 2.10 (s, 3H, $CH_3CO$), 1.67 (qt, 2H, $J$ = 6.90, C**H$_2$**$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.82 (t, 3H, $J$ = 6.56, $CH_3$). Anal. ($C_{24}H_{40}N_2O_3$) C, H, N.

*5.1.6. 4-Tetradecyloxyphenylethoxyacetimine hydrochloride (**5**)*

A solution of **1b** (2.4 g, 7.3 mmol) in absolute EtOH (60 ml) was chilled in an ice-bath and saturated with dry HCl gas. The solution was then standed at –18 °C to give **5** as white crystals (2.1 g, 70%). The title compound was used immediately in the next step upon formation due to its instability.

*5.1.7. N,N-Dimethyl-4-tetradecyloxybenzylamidine hydrochloride (**6**)*

To an ice-chilled and stirred solution of $Me_2NH$ (0.20 g, 4.4 mmol) in MeOH (25 ml), the iminoether hydrochloride **5** (1.5 g, 3.6 mmol) was added portionwise. After stirred overnight at room temperature, the solution was evaporated to dryness and the residue prepurified by crystallization in MeOH/$CH_2Cl_2$/ether (1:1:1, v/v/v). A silica gel column chromatography using 5% MeOH in $CH_2Cl_2$ as eluent yielded **6** as white crystals (1.0 g, 75%): m.p. 149 °C; Rf 0.42 (MeOH/$CH_2Cl_2$, 20:80, v/v); IR (KBr, $cm^{-1}$) 3160 (N–H), 1611 (C=N); $^1H$ NMR ($CDCl_3$) 10.08 (s, 1H, NH), 9.60 (s, 1H, NH), 7.14, 6.78 (2d, 4H, $J$ = 8.60, $H_{ar}$), 4.00 (s, 2H, $CH_2Ph$), 3.85 (t, 2H, $J$ = 6.46, $CH_2O$), 3.28 (s, 3H, $CH_3N$), 3.00 (s, 3H, $CH_3N$), 1.70 (qt, 2H, $J$ = 6.70, C**H$_2$**$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.82 (t, 3H, $J$ = 6.64, $CH_3$). Anal. ($C_{24}H_{42}N_2O$) C, H, N.

*5.1.8. 2-(4-Tetradecyloxyphenyl)-1H-tetrazole (**7a**)*

A mixture of **1a** (3.00 g, 9.52 mmol), $NaN_3$ (0.80 g, 12.5 mmol) and $NH_4Cl$ (0.67 g, 12.5 mmol) in DMF (50 ml) was stirred at 100 °C for 12 h. The solvent was evaporated under reduced pressure and the residue partitioned in an aqueous NaOH solution (20%) and ether. The aqueous phase was poured onto 1 N HCl and the precipitate purified by silica gel chromatography using 2% MeOH in $CHCl_3$ as eluent to yield **7a** as a white solid (1.00 g, 29%): m.p. 134 °C; Rf 0.29 (MeOH/$CHCl_3$, 1:9, v/v); IR (KBr, $cm^{-1}$) 3379 (N–H), 1614 (C=N); $^1H$ NMR (DMSO-$d_6$) 7.96, 7.13 (2d, 4H, $J$ = 8.60, $H_{ar}$), 4.00 (t, 2H, $J$ = 6.40, $CH_2O$), 1.73 (qt, 2H, $J$ = 7.40, C**H$_2$**$CH_2O$), 1.23 (m, 22H, $CH_2$), 0.84 (t, 3H, $J$ = 6.00, $CH_3$). Anal. ($C_{21}H_{34}N_4O$·HCl) C, H, N.

*5.1.9. 2-(4-Tetradecyloxybenzyl)-1H-tetrazole (**7b**)*

2-(4-Tetradecyloxybenzyl)-1H-tetrazole (**7b**) was obtained in 30% yield following the same procedure as for **7a** but start-

ing from **1b** and treatment of the hydrochloride salt by sodium hydrogen carbonate: m.p. 68 °C; Rf 0.47 (MeOH/$CHCl_3$, 1:9, v/v); IR (KBr, $cm^{-1}$) 3434 (N–H), 1613 (C=N); $^1H$ NMR ($CDCl_3$) 9.50 (br s, 1H, $D_2O$ exchange, NH), 7.16, 6.86 (2d, 4H, $J$ = 8.53, $H_{ar}$), 4.18 (s, 2H, $CH_2Ph$), 3.89 (t, 2H, $J$ = 6.38, $CH_2O$), 1.66 (qt, 2H, $J$ = 6.26, C**H**$_2$$CH_2O$), 1.22 (m, 22H, $CH_2$), 0.83 (t, 3H, $J$ = 5.90, $CH_3$). Anal. ($C_{22}H_{36}N_4O$) C, H, N.

*5.1.10. 2-(4-Tetradecyloxybenzyl)-1H-imidazole (**8**)*

A mixture of **5** (2.00 g, 4.86 mmol) and aminoacetaldehyde diethyl acetal (0.64 g, 4.47 mmol) in absolute EtOH (70 ml) was stirred at room temperature for 72 h. After evaporation of the solvent, the residue was taken up in 2 N HCl (30 ml) and heated at 90 °C for 1 h. The solution was then neutralized with an aqueous NaOH solution (20%) and the precipitate, dissolved in $CH_2Cl_2$, was washed with water. The organic layer was dried over $MgSO_4$ and concentrated to dryness. The residue was crystallized in $CH_2Cl_2$/ether (1:5, v/v) to yield **8** as white crystals (0.70 g, 40%): m.p. 131 °C; Rf 0.38 (MeOH/$CHCl_3$, 10:90, v/v); IR (KBr, $cm^{-1}$) 3445 (N–H), 1611 (C=N), 1584 (C=C); $^1H$ NMR ($CDCl_3$) 9.50 (br s, 1H, NH), 7.00, 6.77 (2 d, 4H, $J$ = 8.53, $H_{ar}$), 6.86 (s, 2H, CHN), 3.95 (s, 2H, $CH_2Ph$), 3.84 (t, 2H, $J$ = 6.54, $CH_2O$), 1.69 (qt, 2H, $J$ = 6.52, C**H**$_2$$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 5.94, $CH_3$). Anal. ($C_{24}H_{38}N_2O$•1/2$H_2O$) C, H, N.

*5.1.11. 4,5-Dihydro-3-(4-tetradecyloxyphenyl)-4H-1,2,4-oxadiazol-5-one **9a** (**PMS 1075**)*

A mixture of **3a** (0.50 g, 1.38 mmol) and pyridine (0.14 g, 1.88 mmol) in DMF (10 ml) was stirred 5 min at 0 °C. 2-Ethylhexyl chloroformate (0.27 g, 1.40 mmol) was added dropwise and the solution was stirred for 45 min at 0 °C. Then the mixture was diluted with water (40 ml) and extracted with EtOAc (2 × 40 ml). The combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was heated to reflux for 2 h in toluene (50 ml) and concentrated. Crystallization at room temperature with 30% EtOAc in chloroforme provided **9a** as colorless crystals (0.22 g, 43%): m.p. 151 °C; Rf 0.30 (MeOH/$CH_2Cl_2$, 5:95, v/v); IR (KBr, $cm^{-1}$) 3301 (N–H), 1813 (C=O), 1615 (C=N); $^1H$ NMR ($CDCl_3$) 9.70 (br s, 1H, NH), 7.72, 7.00 (2d, 4H, $J$ = 8.83, $H_{ar}$) 4.00 (t, 2H, $J$ = 6.40, $CH_2O$), 1.71 (m, 2H, C**H**$_2$$CH_2O$), 1.23 (m, 22H, $CH_2$), 0.82 (t, 3H, $J$ = 6.62, $CH_3$). Anal. ($C_{22}H_{34}N_2O_3$) C, H, N.

*5.1.12. 4,5-Dihydro-3-(4-tetradecyloxybenzyl)-4H-1,2,4-oxadiazol-5-one (**9b, PMS1062**)*

4,5-Dihydro-3-(4-tetradecyloxybenzyl)-4*H*-1,2,4-oxadiazol-5-one (**9b**, **PMS1062**) was obtained in 61% yield following the same procedure as for **9a**, but starting from **2b**, as beige crystals: m.p. 123–124 °C; IR (KBr, $cm^{-1}$) 3116 (NH), 1839 (C=O), 1728 (C=N); $^1H$ NMR ($CDCl_3$) 7.10, 6.79 (2d, 2H, $J$ = 8.68, $H_{ar}$), 3.85 (t, 2H, $J$ = 6.53, $CH_2O$), 3.72 (s, 2H, $CH_2Ph$), 1.70 (dt, 2H, $J$ = 6.87, C**H**$_2$$CH_2O$), 1.20 (m, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.44, $CH_3$). Anal. ($C_{23}H_{36}N_2O_3$) C, H, N.

*5.1.13. Methyl 4-tetradecyloxybenzoate (**10a**)*

Methyl 4-tetradecyloxybenzoate (**10a**) was obtained in 93% yield using the same protocol as for **1b**, but starting from methyl 4-hydroxybenzoate and with a reaction time of 7 h, as white crystals (MeOH, 4 °C): m.p. 59.0–60.5 °C; Rf 0.52 (ether/spirit, 10:90, v/v); IR (KBr, $cm^{-1}$) 1723 (C=O), 1609 (C=C); $^1H$ NMR ($CDCl_3$) 7.88, 6.80 (2d, 4H, $J$ = 8.84, $H_{ar}$), 3.90 (t, 2H, $J$ = 6.51, $CH_2O$), 3.78 (s, 3H, $CH_3O$), 1.70 (dt, 2H, $J$ = 7.09, C**H**$_2$$CH_2O$), 1.18 (s, 22H, $CH_2$), 0.79 (t, 3H, $J$ = 6.05, $CH_3$).

*5.1.14. Methyl 4-tetradecyloxyphenylacetate (**10b**)*

Methyl 4-tetradecyloxyphenylacetate (**10b**) was obtained in 93% yield using the same protocol as for **1b**, but starting from methyl 4-hydroxyphenylacetate, as white crystals (MeOH): m.p. 37 °C; Rf 0.64 (ether/spirit, 10:90, v/v); IR (KBr, $cm^{-1}$) 1720 (C=O), 1614 (C=C); $^1H$ NMR ($CDCl_3$) 7.11, 6.78 (2d, 4H, $J$ = 8.42, $H_{ar}$), 3.86 (t, 2H, $J$ = 6.49, $CH_2O$), 3.61 (s, 3H, $OCH_3$), 3.49 (s, 2H, $CH_2Ph$), 1.69 (m, 2H, C**H**$_2$$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.29, $CH_3$).

*5.1.15. 4,5-Dihydro-2-(4-tetradecyloxybenzyl)-1H-imidazole (**11b**)*

In a cooled (0 °C) three-necked round-bottom flask equipped with a condenser and a dropping funnel under argon atmosphere were introduced successively, using a syringe, dry toluene (100 ml), trimethylaluminum ($AlMe_3$, 2.0 M in toluene, 40 ml, 80 mmol), ethylenediamine (EDA, 1.80 g, 30 mmol) and finally a solution of **10b** (5.00 g, 13.8 mmol) in dry toluene (30 ml). The mixture was heated to reflux for 4 h and the excess of $AlMe_3$ was hydrolyzed with an aqueous NaOH solution (20%, 100 ml). The salts were filtered and the filtrate concentrated to dryness. The residue was then dissolved in $CHCl_3$, washed with water, dried over $MgSO_4$ and filtered. After removal of the solvent, the product was chromatographied on a silica gel column using firstly 2%, then 10% MeOH in $CH_2Cl_2$ as eluents to yield **11b** as white crystals (1.50 g, 30%): m.p. 103 °C; Rf 0.24 (MeOH/$CHCl_3$/$NH_4OH$, 20:80:2, v/v/v); IR (KBr, $cm^{-1}$) 3173 (N–H), 1610 (C=N); $^1H$ NMR ($CDCl_3$) 7.10, 6.78 (2d, 4H, $J$ = 8.77, $H_{ar}$), 3.85 (t, 2H, $J$ = 6.49, $CH_2O$), 3.65 (s, 1H, NH), 3.47 (s, 4H, $CH_2N$), 3.46 (s, 2H, $CH_2CN$), 1.72 (m, 2H, C**H**$_2$$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.14, $CH_3$). Anal. ($C_{24}H_{40}N_2O$•1/2$H_2O$) C, H, N.

*5.1.16. 4,5-Dihydro-2-(4-tetradecyloxyphenyl)-1H-imidazole (**11a**)*

4,5-Dihydro-2-(4-tetradecyloxyphenyl)-1*H*-imidazole (**11a**) was obtained in 41% yield following the same procedure as for **11b** but starting from **10a**: m.p. 144 °C; Rf 0.40 (MeOH/$CHCl_3$/$NH_4OH$, 20:80:2, v/v/v); IR (KBr, $cm^{-1}$) 3334 (N–H), 1610 (C=N), 1561 (C=C); $^1H$ NMR of **11a** hydrochloride (DMSO-$d_6$) 10.39 (s, 2H, $D_2O$ exchange, NH), 7.92, 7.12 (2d, 4H, $J$ = 8.82, $H_{ar}$), 4.00 (t, 2H, $J$ = 6.62, $CH_2O$), 3.90 (s,

4H, $CH_2N$), 1.65 (m, 2H, C**H$_2$**$CH_2O$), 1.17 (s, 22H, $CH_2$), 0.79 (t, 3H, $J$ = 6.45, $CH_3$). Anal. ($C_{23}H_{37}N_2O$•3/4$H_2O$) C, H, N.

*5.1.17. 4,5-Dihydro-4-methyl-2-(4-tetradecyloxybenzyl)-1H-imidazole (**12b**)*

4,5-Dihydro-4-methyl-2-(4-tetradecyloxybenzyl)-1*H*-imidazole (**12b**) was obtained in 32% yield following the same procedure as for **11b** but using 1,2-diaminopropane: m.p. 59 °C; Rf 0.32 (MeOH/$CHCl_3$/$NH_4OH$, 20:80:2, v/v/v); IR (KBr, $cm^{-1}$) 3125 (N–H), 1605 (C=N); $^1$H NMR ($CDCl_3$) 7.10, 6.77 (2d, 4H, $J$ = 8.60, $H_{ar}$), 3.86 (m, 3H, $CH_2O$ and CHN), 3.63 (t, 1H, $J$ = 10.00, $CH_2N$), 3.46 (s, 2H, $CH_2Ph$), 3.00 (m, 2H, $D_2O$ exchange, $CH_2N$ and NH), 1.70 (qt, 2H, $J$ = 6.74, C**H$_2$**$CH_2O$), 1.18 (m, 22H, $CH_2$), 0.82 (t, 3H, $J$ = 6.60, $CH_3$). Anal. ($C_{25}H_{42}N_2O$•$H_2O$) C, H, N.

*5.1.18. 4,5-Dihydro-4-methyl-2-(4-tetradecyloxyphenyl)-1H-imidazole (**12a**)*

4,5-Dihydro-4-methyl-2-(4-tetradecyloxyphenyl)-1*H*-imidazole (**12a**) was obtained in 35% yield following the same procedure as for **12b** but starting from **10a** and 1,2-diaminopropane: m.p. 71 °C; Rf 0.33 (MeOH/$CHCl_3$/$NH_4OH$, 20:80:2, v/v/v); IR (KBr, $cm^{-1}$) 3203 (N–H), 1601 (C=N); $^1$H NMR ($CDCl_3$) 7.63, 6.82 (2d, 4H, $J$ = 8.72, $H_{ar}$), 3.93 (m, 5H, $CH_2O$, CHN and $CH_2N$), 3.27 (br s, 1H, NH), 1.71 (qt, 2H, $J$ = 7.62, C**H$_2$**$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.66, $CH_3$). Anal. ($C_{24}H_{40}N_2O$•1/2$H_2O$) C, H, N.

*5.1.19. 4,5-Dihydro-1-methyl-2-(4-tetradecyloxybenzyl)-1H-imidazole (**13b**)*

4,5-Dihydro-1-methyl-2-(4-tetradecyloxybenzyl)-1*H*-imidazole (**13b**) was obtained in 36% yield following the same procedure as for **11b** but using *N*-methylethylenediamine: m.p. 75 °C; Rf 0.44 (MeOH/$CHCl_3$/$NH_4OH$, 20:80:2, v/v/v); IR (KBr, $cm^{-1}$) 1619 (C=N); $^1$H NMR ($CDCl_3$) 7.10, 6.76 (2d, 4H, $J$ = 8.54, $H_{ar}$), 3.85 (t, 2H, $J$ = 6.50, $CH_2O$), 3.62 (t, 2H, $J$ = 9.57, $CH_2N$), 3.47 (s, 2H, $CH_2CN$), 3.20 (t, 2H, $J$ = 9.57, $CH_2N$), 2.62 (s, 3H, $CH_3N$), 1.67 (qt, 2H, $J$ = 6.56, C**H$_2$**$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.00, $CH_3$). Anal. ($C_{25}H_{42}N_2O$•5/4$H_2O$) C, H, N.

*5.1.20. 4,5-Dihydro-1-methyl-2-(4-tetradecyloxyphenyl)-1H-imidazole (**13a**)*

4,5-Dihydro-1-methyl-2-(4-tetradecyloxyphenyl)-1*H*-imidazole (**13a**) was obtained in 39% yield following the same procedure as for **13b** but starting from **10a**: m.p. 47 °C; Rf 0.37 (MeOH/$CHCl_3$/$NH_4OH$, 20:80:2, v/v/v); IR (KBr, $cm^{-1}$) 1614 (C=N); $^1$H NMR ($CDCl_3$) 7.42, 6.83 (2d, 4H, $J$ = 8.55, $H_{ar}$), 3.90 (t, 2H, $J$ = 6.50, $CH_2O$), 3.78 (t, 2H, $J$ = 9.53, $CH_2N$), 3.37 (t, 2H, $J$ = 9.53, $CH_2N$), 2.74 (s, 3H, $CH_3N$), 1.73 (qt, 2H, $J$ = 6.64, C**H$_2$**$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.00, $CH_3$). Anal. ($C_{24}H_{40}N_2O$•3/5 $H_2O$) C, H, N.

*5.1.21. 4-Tetradecyloxyphenylacetic acid (**14b**)*

To a solution of **10b** (3.0 g, 8.0 mmol) in methanol (25 ml) was added 20% NaOH solution (10 ml) and the mixture was heated to reflux for 4 h. The solution was then acidified and the solvent removed under reduced pressure. The residue was taken up in $CH_2Cl_2$ and washed with $H_2O$, dried over $Na_2SO_4$. The title compound was obtained upon evaporation of the solvent, followed by a recrystallization in ethanol as pale yellow crystals (1.8 g, 65%): m.p. 70 °C; Rf 0.15 (MeOH/$CH_2Cl_2$, 2:98, v/v); IR (KBr, $cm^{-1}$) 3033 (OH), 1738 (C=O), 1584 (C=C); $^1$H NMR ($CDCl_3$) 8.70 (br s, 1H, OH), 7.12, 6.78 (2d, 4H, $J$ = 8.15, $H_{ar}$), 3.86 (t, 2H, $J$ = 6.54, $CH_2O$), 3.50 (s, 2H, $CH_2Ph$), 1.70 (m, 2H, C**H$_2$**$CH_2O$), 1.19 (m, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.08, $CH_3$). Anal. ($C_{22}H_{36}O_3$) C, H, N.

*5.1.22. 4-Tetradecyloxybenzoic acid (**14a**)*

4-Tetradecyloxybenzoic acid (**14a**) was obtained quantitatively following the same procedure as for **14b** but starting from **10a** and without purification by recrystallization: m.p. 98.2–99.6 °C, Rf 0.43 (MeOH/$CH_2Cl_2$, 5:95, v/v); IR (KBr, $cm^{-1}$) 3173 (OH), 1649 (C=O); $^1$H NMR ($CDCl_3$) 7.98, 6.86 (2d, 4H, $J$ = 8.90, $H_{ar}$), 3.96 (t, 2H, $J$ = 6.52, $CH_2O$), 1.74 (dt, 2H, $J$ = 6.87, C**H$_2$**$CH_2O$), 1.20 (s, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.40, $CH_3$). Anal. ($C_{21}H_{34}O_3$) C, H, N.

*5.1.23. N-(2-Hydroxyethyl)-4-tetradecyloxyphenylacetamide (**15b**)*

Ethanolamine (38.5 μl, 0.54 mmol) and **14b** (200 mg, 0.57 mmol) were stirred in DMF (10 ml) and EtOAc (10 ml) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC, 119 mg, 0.58 mmol) and $Et_3N$ (121 μl, 0.86 mmol) overnight. The reaction mixture was diluted with EtOAc (40 ml) and washed with 2 N HCl (15 ml). The aqueous phase was extracted once again with EtOAc (40 ml) and the combined organic phases were washed with $H_2O$, 10% $NaHCO_3$, $H_2O$ and brine. After dried over $MgSO_4$, the solution was evaporated to dryness and the residue was purified by silica gel column chromatography (MeOH/$CH_2Cl_2$) to afford **15b** as a white solid (187 mg, 83%): m.p. 105.2–107.2 °C; Rf 0.5 (MeOH/$CHCl_3$, 1:9, v/v); IR (KBr, $cm^{-1}$) 3268 (OH), 3094 (NH), 1634 (C=O); $^1$H NMR ($CDCl_3$) 7.09, 6.80 (2d, 4H, $J$ = 6.22, $H_{ar}$), 5.90 (br s, 1H, NH), 3.87 (t, 2H, $J$ = 6.28, $CH_2OPh$), 3.59 (t, 2H, $J$ = 4.74, C**H$_2$**OH), 3.46 (s, 2H, $CH_2Ph$), 3.31 (t, 2H, $J$ = 4.74, $CH_2N$), 1.75 (m, 2H, C**H$_2$**$CH_2OPh$), 1.19 (m, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 5.83, $CH_3$).

*5.1.24. N-(2-Hydroxyethyl)-4-tetradecyloxybenzamide (**15a**)*

*N*-(2-Hydroxyethyl)-4-tetradecyloxybenzamide (**15a**) was obtained in 89% yield using the same procedure as for **15b** from **14a**: m.p. 91.3–92.7 °C; Rf 0.5 (MeOH/$CHCl_3$, 1:9, v/v); IR (KBr, $cm^{-1}$) 3303 (OH, NH), 1636 (C=O); $^1$H NMR ($CDCl_3$) 7.66, 6.82 (2d, 4H, $J$ = 6.84, $H_{ar}$), 6.63 (t, 1H, $J$ = 5.00, NH), 3.90 (t, 2H, $J$ = 7.36, $CH_2O$), 3.74 (t, 2H, $J$ = 4.64, C**H$_2$**OH), 3.52 (m, 2H, $CH_2N$), 3.00 (br s, 1H, $D_2O$ exchange, OH), 1.75 (qt, 2H, $J$ = 7.20, C**H$_2$**$CH_2O$), 1.19 (s, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.00, $CH_3$).

*5.1.25. N-(2-Chloroethyl)-4-tetradecyloxybenzamide (**16a**)*

To a solution of **15a** (6.00 g, 15.9 mmol) in $CHCl_3$ (40 ml) at 0 °C was added dropwise thionyl chloride (2.0 ml, 27.4 mmol) in $CHCl_3$ (40 ml) and the mixture was stirred at room temperature overnight. It was then washed with 1 M $Na_2CO_3$, dried over $MgSO_4$, filtered and concentrated. The residue was purified on a silica gel column using $CHCl_3$ as eluent to afford **16a** as a yellow solid (3.8 g, 60%): m.p. 108.4–109.9 °C; Rf 0.42 ($CHCl_3$); IR (KBr, $cm^{-1}$) 3305 (N–H), 1632 (C=O), 1608 (C=C); $^1$H NMR($CDCl_3$) 7.65, 6.87 (2d, 4H, $J$ = 8.66, $H_{ar}$), 6.51 (s, 1H, NH), 3.92 (t, 2H, $J$ = 6.46, $CH_2O$), 3.7 (m, 4H, $Cl(CH_2)_2N$), 1.72 (qt, 2H, $J$ = 7.00, $C\mathbf{H_2}CH_2O$), 1.19 (m, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 5.92, $CH_3$).

*5.1.26. N-(2-Chloroethyl)-4-tetradecycloxyphenylacetamide (**16b**)*

*N*-(2-Chloroethyl)-4-tetradecycloxyphenylacetamide (**16b**) was obtained in 30% yield using the same procedure as for **16a** from **15b**: m.p. 112.4–113.3 °C; Rf 0.42 ($CHCl_3$); IR (KBr, $cm^{-1}$) 3275 (N–H), 1641 (C=O); $^1$H NMR($CDCl_3$) 7.16, 6.84 (2d, 4H, $J$ = 6.98, $H_{ar}$), 4.55 (s, 1H, NH), 3.90 (t, 2H, $J$ = 6.46, $CH_2O$), 3.56 (m, 2H, $ClCH_2$), 3.47 (m, 2H, $CH_2N$), 3.32 (s, 2H, $CH_2Ph$), 1.72 (qt, 2H, $J$ = 7.00, $C\mathbf{H_2}CH_2O$), 1.19 (m, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 5.92, $CH_3$).

*5.1.27. 4,5-Dihydro-2-(4-tetradecyloxyphenyl)-1,3-oxazole (**17a**)*

Sodium hydroxide pellets (0.52 g, 13.0 mmol) were dissolved in absolute ethanol (20 ml) and a solution of **16a** (5.00 g, 12.6 mmol) in the same solvent (30 ml) was added dropwise. The mixture was stirred at 50 °C overnight. The salts were filtered and the filtrate was evaporated in vacuo. The residue was taken up in chloroform and washed with water. The organic phase was then dried over $MgSO_4$, filtered and concentrated to yield **17a** as bright crystals (3.63 g, 80%): m.p. 56 °C; Rf 0.44 (MeOH/$CHCl_3$, 5:95, v/v); IR (KBr, $cm^{-1}$) 1649 (C=N), 1611 (C=C); $^1$H NMR ($CDCl_3$) 7.79, 6.85 (2d, 4H, $J$ = 8.86, $H_{ar}$), 4.33 (t, 2H, $J$ = 7.76, $C\mathbf{H_2}CH_2N$), 3.96 (t, 2H, $J$ = 7.76, $CH_2N$), 3.91 (t, 2H, $J$ = 6.46, $CH_2OPh$), 1.68 (qt, 2H, $J$ = 5.46, $C\mathbf{H_2}CH_2O$), 1.19 (m, 22H, $CH_2$), 0.81 (t, 3H, $J$ = 6.40, $CH_3$). Anal. ($C_{23}H_{37}NO_2$) C, H, N.

*5.1.28. 4,5-Dihydro-2-(4-tetradecyloxybenzyl)-1,3-oxazole (**17b**)*

4,5-Dihydro-2-(4-tetradecyloxybenzyl)-1,3-oxazole (**17b**) was obtained in 25% yield following the same procedure as for **17a** but starting from **16b**: m.p. 48 °C; Rf 0.37 (MeOH/$CHCl_3$, 5:95, v/v); IR (KBr, $cm^{-1}$) 1671 (C=N), 1586 (C=C); $^1$H NMR ($CDCl_3$) 7.12, 6.76 (2 d, 4H, $J$ = 8.57, $H_{ar}$), 4.13 (t, 2H, $J$ = 9.62, $C\mathbf{H_2}CH_2N$), 3.84 (t, 2H, $J$ = 6.50, $CH_2OPh$), 3.73 (t, 2H, $J$ = 9.62, $CH_2N$), 3.46 (s, 2H, $CH_2Ph$), 1.65 (qt, 2H, $J$ = 6.72, $C\mathbf{H_2}CH_2O$), 1.18 (s, 22H, $CH_2$), 0.80 (t, 3H, $J$ = 6.40, $CH_3$). Anal. ($C_{24}H_{39}NO_2\bullet 6/5H_2O$) C, H, N.

*5.2. Biology*

Bovine and porcine pancreatic (GIB) $PLA_2$s and fatty-acid free bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Human recombinant GIB $PLA_2$ was a kind gift from the late Dr. H. Verheij and human recombinant GIIA $PLA_2$, basic sub-unit of the GIIA $sPLA_2$ from *C. durissus terrificus* venom (CB), GIB $sPLA_2$ from *N. naja atra* venom (NNA) and bee venom $sPLA_2$ (GIII) were gifts from Dr. C. Bon (Institut Pasteur, Paris, France). Rabbit platelet lysates containing a GII $sPLA_2$ were prepared as published previously [12]. The fluorescent substrate for $PLA_2$ assay, 1-hexadecanoyl-2-(10-pyrenedecanoyl)-*sn*-glycero-3-phosphoglycerol, ammonium salt (β-py-$C_{10}$-PG) was from Molecular Probes (Eugene, OR). LY311727 was a kind gift from Lilly Company (Lilly Research Laboratories, Eli Lilly and Company, IN).

*5.2.1. Spectrofluorimetric $PLA_2$ assay*

$PLA_2$ activity was evaluated by the method of Radvanyi et al. [30] using the fluorescent phospholipid analogue, β-py-$C_{10}$-PG as the substrate. This assay is specific for secretory $PLA_2$, cytosolic $PLA_2$ being inactive on substrates with a pyrene group at the sn-2 position. Bovine pancreatic $PLA_2$ or $PLA_2$ from rabbit platelet lysate were used to test the effect of the various inhibitors. In a total volume of 1 ml, the standard reaction medium contained 50 mM Tris–HCl (pH 7.5), 100 mM NaCl, 1 mM ethyleneglycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 2 μM substrate, fatty-acid free BSA solution in water (15 μM or 0.1%) and 5 nM of pancreatic $PLA_2$ or 10 μl of platelet lysate (0.4 μg of proteins). The fluorescence of the enzymatic reaction medium (blank) was recorded for 1 min with a spectrofluorimeter SFM 25 (Kontron Instruments) equipped with a Xenon lamp. The reaction was then initiated by addition of $CaCl_2$ (10 mM, final concentration). The increase in fluorescence was continuously recorded for 2 min and $PLA_2$ activity was calculated as described by Radvanyi et al. [30]. When used, the solution of inhibitor in ethanol was added to the reaction medium after introduction of BSA. The activity was expressed in micromoles of fluorescent β-py-$C_{10}$-PG hydrolyzed per min and per mg of bovine pancreatic $PLA_2$, or in nmol of fluorescent β-py-$C_{10}$-PG hydrolyzed per min per $10^9$ cells, in the case of rabbit platelet lysate. The standard error of the mean of three independent experiments was less than 10%. This allowed the determination of the $IC_{50}$ values (concentration of inhibitor producing 50% inhibition) of each compound.

*5.2.2. Spectrophotometric UV assay*

Bovine pancreatic $PLA_2$ (group I), human recombinant $PLA_2$ (group II), or *C. durissus terrificus* venom enzyme (group II) were used to test the potency of various inhibitors. Hydrolysis of thiophospholipid-PG was followed by allowing the released lysothiophospholipid to react with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and monitoring the absorbance at 412 nm using a spectrophotometer UV Unikon

810 Kontron equipped with a recorder. The buffer used contains 50 mM Tris–HCl (pH 7.5), 0.5 M NaCl, 1 mM EGTA. Fresh stock of DTNB (10 mM) was prepared in ethanol. Thiophospholipid-PG was dissolved in distilled water at the appropriate concentrations to cover submicellar test conditions. The final concentration of calcium chloride was of 5 mM. Briefly, 500 μl of the buffer solution was placed in a polystyrene cuvette, followed by addition of 10 mM DTNB (5 μl), 10 mM substrate (10 μl), 5 μl of ethanol (or appropriate concentrations of the inhibitors), 100 to 300 ng of one of the enzymes (in 5 μl of the same buffer) and finally 0.5 M calcium solution (5 μl). TNB anion absorbance was recorded at 412 nm every 30 s for 20 min to calculate residual activity.

*5.2.3. Cell cultures*

LLC-$PK_1$ cells (American Type Culture Collection, CRL-1392, ICN Biochemicals, passage 200–220) and human epithelial cell line A549 (European Collection of Cell Culture, Sigma-Aldrich) were, respectively, grown in DMEM or in RPMI 1640 supplemented with 10% fetal calf serum (FCS, Biowhittaker), 1% penicillin (5000 IU $ml^{-1}$), 5000 mg $ml^{-1}$ streptomycin and 1% glutamine (complete DMEM or RPMI) under a 5% $CO_2$–95% $O_2$ atmosphere at 37 °C. Cells were seeded in 96-well plates at a density of $2.0 \times 10^5$ cells per ml DMEM or RPMI (100 μl per well) for viability studies or in 6-well dishes (2 ml per well) for the measurement of the γ-glutamyl-transferase (γ-GT) release and the $sPLA_2$ activity in the culture medium.

*5.2.4. Cell viability assessment*

A549 cells were incubated with **PMS1062**, dissolved in DMSO and further diluted in culture medium without FCS (0.1–100 μM), and antibiotics in DMEM or in RPMI for 24 or 48 h. Cell viability was assessed by colorimetric assays, using neutral red or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue, MTT), as previously described [31]. The plates were read at 535 nm on a spectrophotometer (ELX 808, BIO-TEK Instruments) and the results were expressed as percentage of the control. The $IC_{50}$ value was determined from the cytotoxicity dose-response curve.

*5.2.5. γ-GT and N-acetylglucosaminidase (NAG) activity assays*

After 24 or 48 h incubation with **PMS1062**, the release of the marker enzymes for apical membrane γ-GT and NAG was determined in the culture medium (Boehringer kit). Enzyme activities were expressed as milliunits per volume of the culture medium supernatant.

*5.2.6. $sPLA_2$ activity assay in $HepG_2$ cells*

$HepG_2$ cells (a kind gift of Dr. E. Ninio, Inserm U525) were grown in complete DMEM. Cells were seeded into 6-well dishes at a density of $10^5$ cells per ml (2 ml per well) in phenol red free RPMI 1640 medium (Sigma-Aldrich). Cells were incubated for 24 h with a mixture of 10 μg $ml^{-1}$ lipopolysaccharide (LPS) and 10 ng $ml^{-1}$ Il-6 (so-called MIX) in the absence or in the presence of **PMS1062** (0.1–50 μM, so-called MIX + **PMS1062**). $sPLA_2$ activity in the supernatant was assessed as previously described. Protein concentration was determined by the BCA method (Sigma-Aldrich), using albumin bovine as a standard.

*5.2.7. Statistical analysis*

Results were given as the mean of triplicate values of enzymatic activities ± standard deviation (S.D.). Statistical significance was assessed by the Mann–Whitney non-parametric free sample test with $P \leq 0.05$.

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## References

[1] E.A. Dennis, J. Biol. Chem. 261 (1994) 13057–13060.
[2] E. Valentin, G. Lambeau, Biochim. Biophys. Acta 1488 (2000) 59–70.
[3] I. Kudo, M. Murakami, Prostaglandins Other Lipid Mediat. 68–69 (2002) 3–58.
[4] J. Ishizaki, N. Suzuki, K. Higashino, Y. Yokota, T. Ono, K. Kawamoto, N. Fujii, K. Hanasaki, J. Biol. Chem. 274 (1999) 24973–24979.
[5] M.H. Gelb, E. Valentin, F. Ghomashchi, M. Lazdunski, G. Lambeau, J. Biol. Chem. 275 (2000) 39823–39826.
[6] T.J. Nevalainen, M.M. Haapamäki, J.M. Grönroos, Biochim. Biophys. Acta 1488 (2000) 83–90.
[7] J. Belleville, J. Clement, Bull. Soc. Chim. Biol. (Paris) 50 (1968) 1419–1424.
[8] J.S. Bomalaski, M.A. Clark, Arthritis Rheum. 36 (1993) 190–198.
[9] S. Forster, E. Ilderton, J.F.B. Norris, R. Summerly, H.J. Yardley, Br. J. Dermatol. 112 (1985) 135–147.
[10] L.J. Formela, S.W. Galloway, A.N. Kingsnorth, Br. J. Surg. 82 (1995) 6–13.
[11] P. Vadas, K. Scott, G. Smith, I. Rajkovic, B.D. Schouten, R. Singh, W. Pruzanski, Life Sci. 50 (1992) 807–811.
[12] C. Mounier, H. Faili, B.B. Vargaftig, C. Bon, M. Hatmi, Eur. J. Biochem. 216 (1993) 169–175.
[13] P. Vadas, J. Browning, J. Edelson, W. Pruzanski, J. Lip. Med. 8 (1993) 1–30.
[14] W. Pruzanski, P. Vadas, Immunol. Today 12 (1991) 143–146.
[15] M. Triggiani, F. Granata, A. Oriente, V. De Marino, M. Gentile, C. Calabrese, C. Palumbo, G. Marone, J. Immunol. 164 (2000) 4908–4915.
[16] R.M. Crowl, T.J. Stoller, R.R. Conroy, C.R. Stoner, J. Biol. Chem. 266 (1991) 2647–2651.
[17] J.P. Wery, R.W. Schevitz, D.K. Clawson, J.L. Bobbitt, E.R. Dow, G. Gamboa, T. Goodson Jr., R.B. Hermann, R.M. Kramer, D.B. McClure, E.D. Mihelich, J.E. Putman, J.D. Sharp, D.H. Stark, C. Teater, M.W. Warrick, N.D. Jones, Nature 352 (1991) 79–82.
[18] D.L. Scott, S.P. White, J.L. Browning, J.J. Rosa, M.H. Gelb, P.B. Sigler, Science 254 (1991) 1007–1010.
[19] M. Aitdafoun, C. Mounier, F. Heymans, C. Binisti, C. Bon, J.-J. Godfroid, Biochem. Pharmacol. 51 (1996) 737–742.
[20] G.D. Diana, D.L. Volkots, T.J. Nitz, T.R. Bailey, M.A. Long, N. Vescio, S. Aldous, D.C. Pevear, F.J. Dutko, J. Med. Chem. 37 (1994) 2421–2422.

[21] W.J. McFarland, L. Harold, J. Howes Jr., J. Med. Chem. 15 (1972) 365–368.

[22] M. Harfenist, D.J. Heuser, C.T. Joyner, J.F. Batchelor, H.L. White, J. Med. Chem. 39 (1996) 1857–1863.

[23] D.W. Robertson, J.H. Krushinski, E.E. Beedle, J.D. Leander, D.T. Wong, R.C. Rathbun, J. Med. Chem. 29 (1986) 1577–1586.

[24] Y. Kohara, K. Kubo, E. Imamiya, T. Wada, Y. Inada, T. Naka, J. Med. Chem. 39 (1996) 5228–5235.

[25] F. Rondu, G. Le Bihan, X. Wang, A. Lamouri, E. Touboul, G. Dive, T. Bellahsene, B. Pfeiffer, P.B. Renard, B.D. Guardiola-Lemaitre, D. Manechez, L. Penicaud, A. Ktorza, J.-J. Godfroid, J. Med. Chem. 40 (1998) 3793–3803.

[26] P.C. Unangst, G.P. Shrum, D.T. Connor, R.D. Dyer, D.J. Schrier, J. Med. Chem. 35 (1992) 3691–3698.

[27] K. Rehse, S. Bade, Arch. Pharm. (Weinheim) 329 (1996) 535–540.

[28] R.W. Schevitz, N.J. Bach, D.G. Carlson, N.Y. Chirgadze, D.K. Clawson, R.D. Dillard, S.E. Draheim, L.W. Hartley, N.D. Jones, E.D. Mihelich, J.L. Olkowski, D.W. Snyder, C.D. Sommers, J.P. Wery, Nat. Struct. Biol. 2 (1995) 458–465.

[29] C.Z. Dong, A. Romieu, C.M. Mounier, F. Heymans, B.P. Roques, J.-J. Godfroid, Biochem. J. 365 (2002) 505–511.

[30] F. Radvanyi, L. Jordan, F. Russo-Marie, C. Bon, Analytic. Biochem. 117 (1989) 103–109.

[31] F. Massicot, A. Lamouri, C. Martin, C. Pham-Huy, F. Heymans, J.M. Warnet, J.-J. Godfroid, J.R. Claude, J. Lipid Mediat. Cell Signal. 15 (1997) 203–214.