Inhibition of Secreted Phospholipase A_2. 4-Glycerol Derivatives of 4,5-Dihydro-3-(4-tetradecyloxybenzyl)-1,2,4-4*H*-oxadiazol-5-one with Broad Activities

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Secreted phospholipases A_2 (sPLA$_2$s) have been reported to play an important role in various inflammatory conditions and thus represent an attractive therapeutic target. Previous SAR studies from our laboratory have revealed certain important features of our recently discovered specific hGIIA sPLA$_2$ inhibitors, and we report here the synthesis and biological activities of glycerol-containing derivatives of our lead compound **III** (Figure 1). Efficient and selective synthesis methods have been developed to make glycerol trisubstituted by different groups on desired positions. In terms of biological activities, the best compounds (**A3**, **A6**, and **A15**) are more active than **III** (Figure 1), as potent as Me-Indoxam, an sPLA$_2$s inhibitor of reference, against hGIIA, hGV, and hGX sPLA$_2$s and at least 10 times less active toward the GIB enzymes in two *in vitro* assay systems. By synthesis of enantiopure (*S*)-**A6**, we demonstrated that no important improvement of the inhibitory potency could be achieved by this approach. Furthermore, the results show that the global lipophilicity is likely responsible for the anti-PLA$_2$ activity and two oxadiazolone moieties seem too big to be accommodated by the active site of the hGIIA enzyme.

Introduction

Phospholipases A_2 (PLA$_2$, phosphatidyl 2-acylhydrolase, EC 3.1.1.4) are involved in various physiopathological processes by promoting the release of free fatty acids and lyso-phospholipids from membrane glycero-phospholipids. The implication of certain PLA$_2$s in inflammation has been demonstrated by their ability to regulate in the inflammatory reaction the amount of arachidonic acid (AA), precursor for the formation of prostaglandins, leukotrienes, and thromboxanes, three of the most powerful lipid inflammatory mediators. Meanwhile, the lyso-phospholipids released in the enzymatic reaction result in certain settings in the formation of platelet-activating factor (PAF), another potent mediator of inflammation.

To date, 12 groups of PLA$_2$s (group I or GI to group XII or GXII) are known and classified mainly into two categories: intracellular PLA$_2$s, including cytosolic PLA$_2$s (cPLA$_2$s) and Ca^{2+}-independent PLA$_2$s (iPLA$_2$s), and secreted PLA$_2$s (sPLA$_2$s) of which a great number have been cloned and expressed in the past decade and the characterization remains to be an active area.[1,2] Until recently, the increased serum PLA$_2$ activity has been attributed to hGIIA enzyme in different human inflammatory diseases,[3] such as septic shock,[4] rhumatoid arthritis,[5,6] Crohn's disease,[7] acute pancreatitis,[8] or partly to hGIIA enzyme in psoriasis[9] and adult respiratory distress syndrome.[10] However, a large body of evidence indicates that the group V and group X sPLA$_2$s also contribute to the production of various inflammatory mediators in mammalian cells, as well as in tissues pretreated with pro-inflammatory agents or isolated from patients suffering from various inflammatory diseases.[11−20] It has been shown recently that exogenously added group V or group X sPLA$_2$s to mammalian cells leads to arachidonic acid (AA) release,[20−22] which is also observed in mammalian cells transfected with groups IIA and X sPLA$_2$s.[23] Data from GV PLA$_2$-null mice have provided clear evidence of a role for GV PLA$_2$ in regulating eicosanoid generation in response to an acute innate stimulus of the immune system *in vivo*,[24] suggesting a potential function of this enzyme in innate immunity. Moreover, GV PLA$_2$ is detected in human atherosclerotic aortic lesions and aortic root sections from apolipoprotein E-deficient mice.[25] Similarly, GX PLA$_2$ is detected in foam cell lesions in the arterial intima of high fat-fed apolipoprotein E-deficient mice.[26] Therefore, modification of plasma lipoproteins by GV and GX PLA$_2$s may be more relevant than that by GIIA PLA$_2$ to the pathogenesis of atherosclerosis. Recent studies have indicated the presence of various types of cross-talk between sPLA$_2$ and cPLA$_2\alpha$ resulting in effective arachidonic acid (AA) release. By working on cPLA$_2\alpha$-deficient mice and by using specific sPLA$_2$ and cPLA$_2\alpha$ inhibitors, GX PLA$_2$ has been found to induce AA release and eicosanoid production without activation of cPLA$_2\alpha$.[27]

The various distribution of each group of mammalian sPLA$_2$s in healthy or inflamed tissues[14−17,28] suggests that these enzymes play different roles in physiological and pathophysiological conditions. It is thus important to develop selective inhibitors of each sPLA$_2$ isozyme and inhibitors with broad spectrum activities to all inflammatory-related sPLA$_2$s to study the physiopathological functions of each sPLA$_2$ under different biological conditions. Many GIIA sPLA$_2$ inhibitors have been reported, and to date the most potent ones are those described by Lilly and Shionogi research laboratories.[29−32] Previous work from our laboratory (Figure 1) has shown that molecules as simple as the amidine **I**[33] and the piperazine derivative **II**[34] are fairly potent inhibitors of GIIA sPLA$_2$. They also inhibit the enzymatic activity of GIB PLA$_2$ which is known to play a

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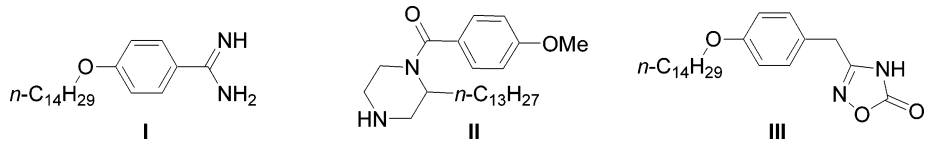


Figure 1. Previous $sPLA_2$s inhibitors discovered in our laboratory.

critical role in digestion, indicating that the inhibition is not enzyme-specific. An extensive structure−activity relationship study has led to the identification of the lead compound **III** (Figure 1), which is more specific for hGIIA than GIB $sPLA_2$. One of the key elements of this specificity appears to be the presence of the oxadiazolone cycle.[35]

We have also reported that when the chain length in **III** (Figure 1) was shortened, some new interactions should be created by introducing new structural elements to maintain the activity and the specificity. The design has been assisted by molecular modeling.[36] On the other hand, when the C14 chain in **III** (Figure 1) was split up to two equal fragments and assembled in the same molecule by disubstitution, the activity decreased significantly unless a long chain such as C14 was present as one of both the substituents.[37] These results prompted us to examine the importance of the hydrophobic part of **III** (Figure 1) in a different series. Given that the glycerol backbone is a natural structural element in the native substrates of PLA_2s, we designed and synthesized a series of glycerol derivatives of **III** (Figure 1) through efficient and selective alkylations of the glycerol backbone. In these pseudosubstrate analogues, the hydrophilic head of the substrates was replaced by an oxadiazolone ring and different alkyl chains were attached to the two other positions of glycerol. Their biological activities were evaluated in two different *in vitro* assay systems using group IB, IIA, V, and X $sPLA_2$s. The best inhibitor was found to be highly active at sub-micromolar concentrations against hGIIA, hGV, and hGX $sPLA_2$s, and about 10 times less potent toward the GIB enzymes. Me-Indoxam, a $sPLA_2$ inhibitor of reference, displays comparable activities in the same assay conditions.

Chemistry. In this work, we needed *O*-dihomo or *O*-diheteroalkyl-(4-cyanophenyl)glycerols and *O*-dihomo or *O*-diheteroalkyl-(4-cyanomethylphenyl)glycerols as key intermediates. Wheeler and Willson[38] have reported that phenol can be condensed with 3-chloro-1,2-propanediol to provide 1-*O*-phenylglycerol in a 64% yield. The same procedure was thus applied to our syntheses (Scheme 1), and the compounds **1a**−**d** were obtained with comparable or better yields: 60% for **1a**, 65% for **1b**,[39] quantitative for **1c**, and 60% for **1d**. Dihomoalkylation of **1a**−**c** using different alkyl bromides led to compounds **2a**−**c** and **3a**−**c** (Scheme 1).

Direct dialkylation of **1d** under the same conditions as for **1a**−**c** failed, since a simultaneous alkylation of the alpha CH_2 of the nitrile function occurred in high proportion (90%). Consequently, a new synthetic pathway was designed. The alcohols **2e**, **3e** were prepared either by reduction of the esters **2b**, **3b** or acid cleavage of the acetal function of **2c**, **3c** in the presence of pyridinium *p*-toluenesulfonate (PPTS). Bromination of **2e**, **3e**, followed by a substitution reaction using sodium cyanide gave the corresponding nitriles, **2d**, **3d** in 60% yield. Dissymmetrical substitution strategy leading to **6a**−**12a** is also outlined in Scheme 1. The primary hydroxyl group of **1a** was selectively blocked with a trityl group (**4a**), and the remaining secondary hydroxyl group was alkylated after removal of the proton by NaH, using the corresponding bromide to yield **5a**. Acid-catalyzed hydrolysis of the trityl group was then carried out, leading to the free alcohol function from which the ethers **6a**−**12a** were formed with the suitable alkyl bromides as above.

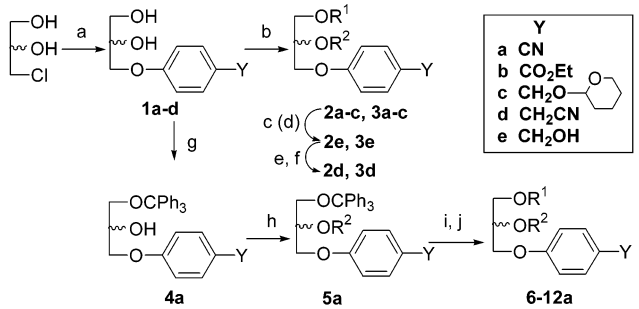
As shown in Scheme 2, the nitrile group of the intermediates **2a, 3a, 5a**−**12a**, **2d**, and **3d** was converted into the amidoxime function with NH_2OH, formed *in situ* from its HCl salt and K_2CO_3. Treatment of these amidoximes **13**−**24** with phenyl chloroformate yielded the corresponding carbonates which, after heating to reflux in toluene, provided the final oxadiazolone derivatives **A1**−**A12**. The trityl group in **A6** was removed by acid hydrolysis to afford **A13**.

Following Wheeler and Wilson's method and starting from the commercially available 2,3-dibromo-1-propanol and 1,3-dibromo-2-propanol (Scheme 3), compounds **25a**, **25b** and the precursors of **28a**, **28**−**30b** were obtained in good yields and next substituted under the conditions as described above to afford **26a**, **26b**, **28a**, **28**−**30b**. The esters **26b**, **28**−**30b** were subsequently converted into the nitrile homologues **26e**, **28**−**30e**. The diamidoximes **27a**, **27e**, **31a**, **31e**, **32e** and the monoamidoximes **33**−**34** resulted from hydroxylamination of the corresponding dinitrile compounds in the same manner as for **13**−**24**. Their transformation into the carbonate intermediates and then the ring closure were performed using the same procedure as above to yield the target molecules, **B1**−**B2**, **C1**−**C3**, and **A14**−**A15**

Results and Discussion

We have recently reported a series of hGIIA $sPLA_2$ specific inhibitors, and the specificity appears to be related to the presence of an oxadiazolone ring as the hydrophilic head, which in its anionic form should play a role of Ca^{2+} ligand at the active site of the enzyme.[35,37] On the other hand, the potency of an inhibitor is largely dependent on the nature and the disposition of the hydrophobic group(s).[36,37] While the optimal linear chain

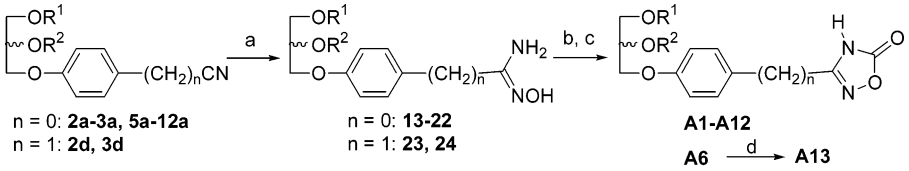
Scheme 1[a]



Compd	R^1	R^2	Compd	R^1	R^2
2a-e	*n*-C_7H_{15}	*n*-C_7H_{15}	**7a**	*n*-C_5H_{11}	*n*-$C_{14}H_{29}$
3a	$C_6H_5(CH_2)_4$	$C_6H_5(CH_2)_4$	**8a**	*n*-$C_{10}H_{21}$	*n*-$C_{14}H_{29}$
3b-e	*n*-C_9H_{19}	*n*-C_9H_{19}	**9a**	$C_6H_5(CH_2)_4$	*n*-$C_{14}H_{29}$
4a	$(C_6H_5)_3C$	H	**10a**	$C_6H_5CH_2$	*n*-$C_{14}H_{29}$
5a	$(C_6H_5)_3C$	*n*-$C_{14}H_{29}$	**11a**	$(C_6H_5)_2CH$	*n*-$C_{14}H_{29}$
6a	C_2H_5	*n*-$C_{14}H_{29}$	**12a**	*m*-$MeOC_6H_4CH_2$	*n*-$C_{14}H_{29}$

[a] Reagents and conditions: (a) *p*-HOPhY, EtONa, EtOH, rt; (b) (i) NaH, DMF, rt, (ii) R^1Br (=R^2Br); (c) **2b** or **3b**, $LiAlH_4$, dry Et_2O, rt; (d) **2c** or **3c**, PPTS, EtOH, 50 °C; (e) 15% HBr in acetic acid, 0 °C; (f) NaCN, DMF, rt; (g) Ph_3CCl, Py, cat. DMAP, CH_2Cl_2, rt; (h) (i) NaH, DMF, rt, (ii) R^2Br; (i) 1 N HCl, THF, rt; (j) NaH, DMF, rt, (ii) R^1Br.

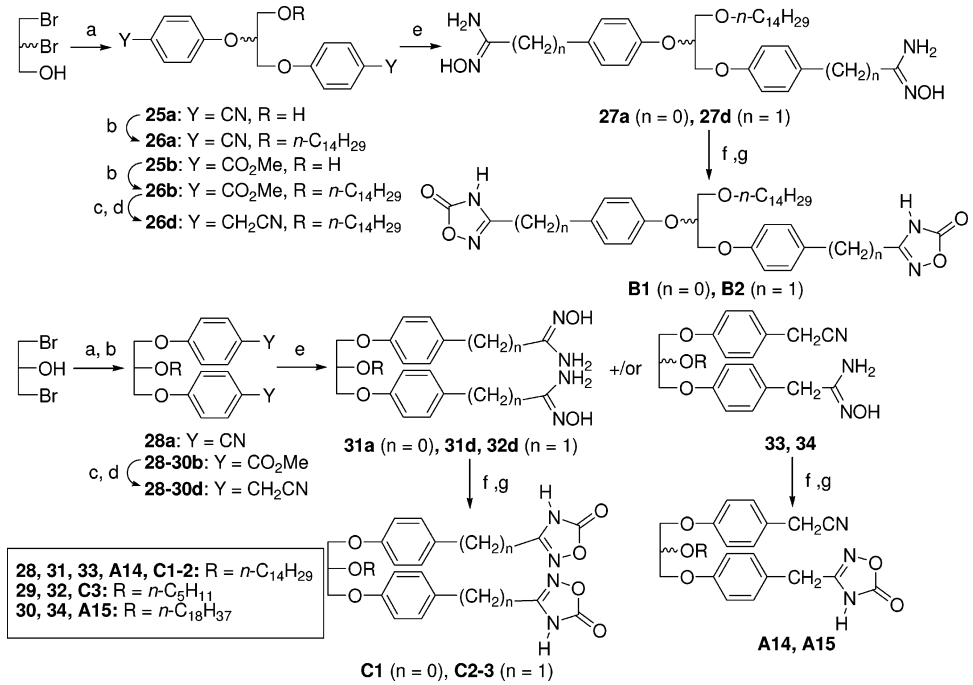
Scheme 2[a]



Compd	n	R^1	R^2	Compd	n	R^1	R^2
13, A1	0	C_2H_5	*n*-$C_{14}H_{29}$	**20, A8**	0	*m*-$MeOC_6H_4CH_2$	*n*-$C_{14}H_{29}$
14, A2	0	*n*-C_5H_{11}	*n*-$C_{14}H_{29}$	**21, A9**	0	$C_6H_5(CH_2)_4$	$C_6H_5(CH_2)_4$
15, A3	0	*n*-$C_{10}H_{21}$	*n*-$C_{14}H_{29}$	**22, A10**	0	*n*-C_7H_{15}	*n*-C_7H_{15}
16, A4	0	$C_6H_5CH_2$	*n*-$C_{14}H_{29}$	**23, A11**	1	*n*-C_7H_{15}	*n*-C_7H_{15}
17, A5	0	$C_6H_5(CH_2)_4$	*n*-$C_{14}H_{29}$	**24, A12**	1	*n*-C_9H_{19}	*n*-C_9H_{19}
18, A6	0	$(C_6H_5)_3C$	*n*-$C_{14}H_{29}$	**A13**	0	H	*n*-$C_{14}H_{29}$
19, A7	0	$(C_6H_5)_2CH$	*n*-$C_{14}H_{29}$				

[a] Reagents and conditions: (a) NH_2OH HCl, K_2CO_3, absol EtOH, reflux; (b) PhOCOCl, Et_3N, CH_2Cl_2, 0 °C; (c) toluene, reflux; (d) 2 N HCl, THF, rt.

Scheme 3[a]



[a] Reagents and conditions: (a) *p*-HOPhY, EtONa, EtOH, rt; (b) (i) NaH, DMF, rt, (ii) RBr; (c) $LiAlH_4$, THF, rt; (d) (i) 15% HBr in acetic acid, 0 °C; (ii) NaCN, DMF, rt; (e) NH_2OH HCl, K_2CO_3, absol EtOH, reflux; (f) PhOCOCl, Et_3N, CH_2Cl_2, 0 °C; (g) toluene, reflux.

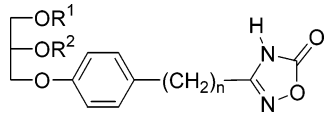
length is composed of 14 carbons (C14),[37] a much shorter chain (C5) is needed when it is terminated by an aromatic group, such as an indole ring.[36] According to our docking study, the indole ring can generate new $\pi-\pi$ or cation$-\pi$ interactions with certain residues of hGIIA $sPLA_2$, which is likely to compensate for the loss of Van der Waals forces between the inhibitor and the enzyme induced by the cutoff of a part of the C14 chain.[36] To better understand the mechanism of action and more deeply evaluate the importance of the hydrophobic part for the activity and selectivity, we describe here the synthesis and structure−activity relationship (SAR) study of a series of glycerol derivatives of **III** (Figure 1).

On the basis of our earlier results,[37] a C14 chain was maintained at the 2-position of glycerol in 10 compounds of the **A** series (**A1**−**A8**, **A13**−**A14**) and oxadiazolone attached to the 3-position through a phenyl or benzyl as linkers. The OH function at the 1-position was either free or substituted with various groups. In regard to the other members of the **A** series, either the two same alkyl groups (**A9**−**A12**) or C18 at the 2-position (**A15**) were used.

All the glycerol derivatives **A1**−**A15** were first submitted to an *in vitro* fluorimetric assay (see Experimental Section) to determine their inhibitory potency and selectivity against human group IIA PLA_2 ($hPLA_2$) versus porcine group IB ($pPLA_2$). The reference compound used in this assay condition is one of the specific and active site targeted hGIIA PLA_2 inhibitors of Lilly Laboratories, LY311727, as previously reported.[37]

The results in Table 1 show that the presence of a free alcohol function at the 1-position of glycerol is not favorable to the biological activity. **A13** displays a weak activity, even though it presents a lipophilicity close to that of **III** (Figure 1),[35] our home reference compound (log $P = 7$). This implies that, as in the natural substrates, a certain length of alkyl chain is necessary at this position to establish supplemental Van der Waals

Table 1. Inhibition of the Enzymatic Activity of Porcine Pancreatic PLA_2 (pGIB) and Human Group IIA PLA_2 (hGIIA) by Glycerol Derivatives of **III** (Figure 1), Determined by Using the Fluorimetric Assay



compd	n	R^1	R^2	IC_{50} (μM) pGIB	IC_{50} (μM) hGIIA	log P^a
A1	0	C_2H_5	*n*-$C_{14}H_{29}$	>40	29.8 ± 0.3	7.34
A2	0	*n*-C_5H_{11}	*n*-$C_{14}H_{29}$	>100	8.3 ± 0.2	8.72
A3	0	*n*-$C_{10}H_{21}$	*n*-$C_{14}H_{29}$	>100	0.28 ± 0.02	11.5
A4	0	$C_6H_5CH_2$	*n*-$C_{14}H_{29}$	>25	14.8 ± 0.2	8.48
A5	0	$C_6H_5(CH_2)_4$	*n*-$C_{14}H_{29}$	2.2 ± 0.2	0.29 ± 0.03	10.0
A6	0	$(C_6H_5)_3C$	*n*-$C_{14}H_{29}$	>50	0.9 ± 0.1	11.8
(*S*)-**A6**	0	$(C_6H_5)_3C$	*n*-$C_{14}H_{29}$	>50	0.33 ± 0.12	11.8
A7	0	$(C_6H_5)_2CH$	*n*-$C_{14}H_{29}$	5.6 ± 0.1	0.28 ± 0.02	10.1
A8	0	*m*-$MeOC_6H_4CH_2$	*n*-$C_{14}H_{29}$	39 ± 1	2.2 ± 0.1	8.56
A9	0	$C_6H_5(CH_2)_4$	$C_6H_5(CH_2)_4$	>100	76.7 ± 0.3	6.51
A10	0	*n*-C_7H_{15}	*n*-C_7H_{15}	>100	50.9 ± 0.1	6.30
A11	1	*n*-C_7H_{15}	*n*-C_7H_{15}	>100	11.6 ± 0.3	7.12
A12	1	*n*-C_9H_{19}	*n*-C_9H_{19}	>100	2.7 ± 0.1	9.18
A13	0	H	*n*-$C_{14}H_{29}$	>100	56.0 ± 0.4	6.59
A14	1	*p*-$NCCH_2C_6H_4$	*n*-$C_{14}H_{29}$	9.6 ± 0.1	1.1 ± 0.1	8.94
A15	1	*p*-$NCCH_2C_6H_4$	*n*-$C_{18}H_{37}$	36.6 ± 0.4	0.15 ± 0.03	11.0
III (Figure 1)[35,37]				>100	4.0 ± 0.9	7.08
LY311727[37]				8.0	0.47	

[a] Calculated using the Rekker's hydrophobic fragmental constants.

interactions with the hydrophobic side chains of certain $sPLA_2$s residues. Indeed, alkylating this OH leads to an increasing activity and selectivity as a function of the chain length. Attaching an ethyl group to this position provides compound **A1**, twice as active as the nonsubstituted **A13** (Table 1). Further increase was observed when a pentyl group was used (**A2**) and the activity is particularly enhanced with decyl (**A3**), 100 times more active than **A1**. The difference can be explained by the increase of the global molecular lipophilicity of 4.1 units of log *P* and by the creation of new hydrophobic interactions between this alkyl chain and the corresponding residues of hGIIA PLA_2. It is important to note that the selectivity of these compounds toward hGIIA versus GIB enzyme also increases as a function of the chain length. This increase in selectivity suggests that the presence of decyl in **A3** seems unable to create sufficient interactions with the porcine GIB PLA_2 enzyme (see **A5** and **A7** below), as it does in the case of hGIIA PLA_2, since it still remains inactive at 100 μM, the highest concentration tested against this enzyme.

The substitution at the 1-position by arylalkyl groups in the compounds **A4**−**A7** was performed in order to study possible steric, electronic, and/or hydrophobic effects induced by these groups. As shown in Table 1, a benzyl group at the 1-position (**A4**) leads to an activity equivalent to that of the isolipophilic **A2**. However, separating the phenyl from the oxygen by three more methylenes gives the compound **A5** which is about 50 times more active than **A4**. Unfortunately, the selectivity decreases significantly in parallel. Replacing the benzyl moiety in **A4** by a diphenylmethyl (**A7**) leads to a scenario similar to that of **A5.** Taken together, these results and those described above for the linear chains suggest that the hydrophobic pocket of the hGIIA enzyme, well-known to naturally accommodate the lipophilic part of substrates, is big enough in depth and in volume and well distanced from the active site to tolerate both kinds of molecules. However, it should not be the case for the GIB enzyme, since the decrease in selectivity is only observed when arylalkyl groups are used, implying that some new interactions are likely created in this case and consequently increase the affinity of the inhibitors to the GIB enzyme. Interestingly, when a trityl group was used, the selectivity was restored almost completely (**A6**). The steric hindrance induced by the third phenyl compared to **A7** seems responsible for this recovery of selectivity, and the potency depends on both the steric hindrance and the global lipophilicity.

To complete this approach, we undertook to incorporate substituted phenyl groups onto the glycerol backbone. Introducing *m*-methoxybenzyl to the 3-position of glycerol (**A8**) increased the anti-PLA_2 activity by 7 fold, as compared to the isolipophilic benzyl derivative (**A4**), indicating that the presence of a methoxy group at this position is favorable to the binding of the inhibitor to the hGIIA enzyme. In compound **A14**, two structural modifications were performed: (i) insertion of a methylene between the oxadiazolone and the phenyl and (ii) direct connection of the phenyl group to the 3-position of glycerol with a *p*-cyanomethyl substitution. These modifications had little influence on activity but decreased selectivity compared to **A8**. Interestingly, an elongation by four methylenes of the alkyl chain at the 2-position of glycerol (**A15**) improved the activity by a factor of 7, in agreement with our earlier observations,[37] as well as the selectivity. The latter is a specific feature for the glycerol derivative **A15.**

It has been frequently reported that one enantiomer could be much more potent than the corresponding racemates. In order to check out if it can happen to this series of compounds, the (*S*)-isomer of **A6** was synthesized and tested under the same conditions. Unfortunately, no important improvement of the inhibitory potency was achieved, as it was found only a little more active toward hGIIA PLA_2 than the racemate with an IC_{50} of 0.33 ± 0.12 μM against 0.9 ± 0.1 μM for the latter in our fluorimetric assay (Table 1). Given this fact and the structure similarity of all final compounds in the **A** series, no synthesis of other enantiopure compounds was performed.

Another type of modification in the **A** series was to use two identical chains for both the substituents R^1 and R^2. The compound **A10** in which two heptyls are connected to the 1- and 2-positions of glycerol showed much less activity, compared with **III** (Figure 1), as already observed in our previous work in which two C7 chains are connected to the different positions

Table 2. Inhibition of the Enzymatic Activity of Human Group IB (hGIB), Group IIA (hGIIA), Group V (hGV) and Group X (hGX) PLA_2s by Selected Glycerol Derivatives of **III** (Figure 1), Determined by Using the Radiometric Assay

	IC_{50} (μM)				
compd	hGIB	hGIIA	hGV	hGX	log P^a
A3	10	1	0.55	1	11.5
A4	>100	>100	>100	14	8.48
A5	15	26	22	12	10.0
A6	5.75	2	0.66	1.25	11.8
A7	18	5	2	5.3	10.1
A14	7.6	7.6	1.73	5	8.94
A15	8.7	1.15	1	0.9	11.0
Me-IDX	3.3	0.18	0.27	4.57	ND

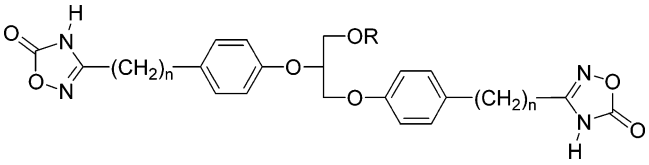
[a] Calculated using the Rekker's hydrophobic fragmental constants

of the phenyl group.[37] When both the heptyl groups were replaced by 4-phenylbutyl (**A9**), the potency is even worse, implying that the presence of the phenyl groups is likely not to induce supplemental interactions with hGIIA enzyme. The difference between **A11** and **A10** can be explained by the insertion of a methylene between the phenyl and oxadiazolone moieties. The 5-fold gain in potency probably results from a more optimal length spacer between the Ca^{2+} ligand and the hydrophobic pockets of the enzyme, a higher lipophilicity, and flexibility due to the disruption of the conjugation between both the two rings. No longer linker was introduced, since we found earlier in a different series of **III** (Figure 1) derivatives that two methylenes did not make the inhibitor more active than one methylene.[37] The fact that **A12** is a stronger inhibitor than **A11** is probably due to the increase of two units of log *P* caused by the presence of longer chains.

In order to check the influence of relative positions of the three moieties on the glycerol backbone to the activity and selectivity, the position isomer of **A11,** 1,3-*O*-di-*n*-heptyl-2-*O*-[4-(4,5-dihydro-5-oxo-1,2,4-4*H*-oxadiazol-3-yl)methylphenyl]-glycerol, was prepared and tested (data not shown). No difference in terms of activity and selectivity was observed between **A11** and this position isomer, suggesting that this factor is not important.

A radiometric assay (see Experimental Section) was then used in this work, not only to confirm the results obtained with the fluorimetric assay, but also to analyze the effects of our inhibitors on other groups of $sPLA_2$s, such as hGV and hGX enzymes, since they have been reported to be both emerging inflammatory-type enzymes[11−20] and seem to be more relevant than hGIIA PLA_2 to certain pathogeneses.[24−27] Moreover, to make the data more relevant, hGIB PLA_2 instead of porcine GIB PLA_2 was employed in this assay system. A preliminary screening (data not shown) using a single concentration of 10 μM led to the selection of the glycerol derivatives listed in Table 2 for their IC_{50} determination in this new assay. Me-Indoxam (Me-IDX), a well-known reference compound,[40] was tested under the same conditions for comparison. Some differences, especially in term of the IC_{50} values of the inhibitors toward hGIIA PLA_2 and the hGIIA versus GIB selectivity, have been observed between the results obtained from the two assay systems. It is likely due to the difference of the GIB enzymes used on one hand and the assay conditions on the other hand. In fact, the GIB PLA_2 used in the fluorimetric assay is of porcine pancreatic source, while that used in the second assay is human recombinant enzyme. It is not really surprising to observe a difference of IC_{50} values, bearing in mind this detail, neither in consequence of the hGIIA versus GIB selectivity. Meanwhile, taking into account the high lipophilicity of the tested compounds, some nonspecific interactions could happen between

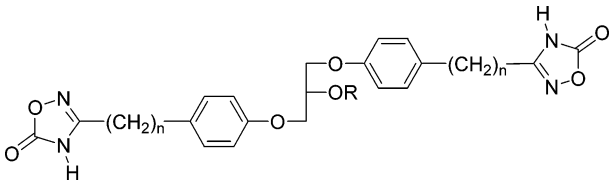
Table 3. Influence of Two Oxadiazolone-Containing Moieties at 2- and 3-Positions of the Glycerol Backbone on the Activity and Specificity, Determined by Using the Fluorimetric Assay



			IC_{50} (μM)		
compd	n	R	pGIB	hGIIA	log P^a
B1	0	*n*-$C_{14}H_{29}$	63.6 ± 0.4	21.0 ± 0.5	7.05
B2	1	*n*-$C_{14}H_{29}$	57.8 ± 0.2	3.1 ± 0.1	7.64

[a] Calculated using the Rekker's hydrophobic fragmental constants.

Table 4. Influence of Two Oxadiazolone-Containing Moieties at 1- and 3-Positions of the Glycerol Backbone on the Activity and Specificity, Determined by Using the Fluorimetric Assay



			IC_{50} (μM)		
compd	n	R	pGIB	hGIIA	log P^a
C1	0	*n*-$C_{14}H_{29}$	>100	47.7 ± 0.2	7.05
C2	1	*n*-$C_{14}H_{29}$	39.9 ± 0.1	2.1 ± 0.1	7.64
C3	1	*n*-C_5H_{11}	>100	30.5 ± 0.5	2.97

[a] Calculated using the Rekker's hydrophobic fragmental constants.

the inhibitors and the membrane substrate used in the radiometric assay, leading to an underestimation of the inhibitory potency, as often observed in similar works. It is worthwhile to emphasize that the best compounds (**A3**, **A6**, and **A10**) were found to be the same in both the cases. In addition, they possess a good inhibitory activity, not only against hGIIA PLA_2 but also against hGV and hGX enzymes. Although they are a little less active than Me-Indoxam to inhibit hGIIA and hGV PLA_2s, our inhibitors appear quite potent against hGX enzyme and possess the same profile as Me-Indoxam for the different groups of $sPLA_2$s. Taking into account all of these results, certain compounds of this series are interesting enough to be further investigated and may represent useful tools to study physiological and pathological roles of various secreted PLA_2s.

Among the synthetic hGIIA PLA_2 inhibitors reported, Lilly laboratories have shown that the inhibitory activity of a molecule can be greatly enhanced when a second calcium ligand is introduced and well positioned in their indole derivatives.[29−31] On the basis of our previous results,[36−37] we argued that the oxadiazolone ($pK_a = 5.5$) should be present in anionic form at neutral pH, which, by chelating the calcium ion at the catalytic center of the enzyme, could play a critical role for the activity and the selectivity of the **III** (Figure 1) derivatives. To improve the potency of our inhibitors, two oxadiazolone heterocycles were connected to either the 2,3- (**B1** and **B2)** or 1,3-positions (**C1-C3**) of glycerol. The length of the unique alkyl chain, as well as the linker between the phenyl and the oxadiazolone, was also varied to study their influences. The results are listed in Table 3 and Table 4 due to the difference of the general structures.

Compared with the compound **A1** of equivalent lipophilicity in the previous series, **B1** (Table 3) shows the same activity but a worse selectivity, suggesting that doubling the oxadi-

azolone heterocycle leads to no improvement in the activity. However, introducing a methylene group as in the **A** series has a beneficial effect since compound **B2** is seven times more active and selective than **B1**.

The same scenario was observed for the compounds with two oxadiazolone rings connected to 1,3-positions of the glycerol backbone (Table 4). It means that the position of the second oxadiazolone ring has little influence on the activity and the selectivity. By contrast, a sufficient chain length is required to maintain the biological properties, since compound **C3**, more hydrophilic than **C2**, is obviously less active.

Taken together, the inhibition data of the compounds **B** and **C** seem to indicate that the active site of hGIIA enzyme could not accommodate two calcium ligands as big as two oxadiazolones.

Conclusion

In this work, we have developed an efficient and selective method to anchor different alkyl and/or arylalkyl groups on the glycerol backbone. In consequence, a certain number of glycerol derivatives of **III** (Figure 1) encompassing one or two oxadiazolone rings were synthesized, and their *in vitro* inhibitory potencies toward different secreted PLA_2s, including GIB, hGIIA, hGV, and hGX were evaluated in two distinct *in vitro* assay conditions. Several of them (**A3**, **A6**, and **A15**) are active with sub-micromolar IC_{50} values and selective against all inflammatory-type $sPLA_2$s tested (GIIA, GV, GX) versus GIB enzyme. By synthesis of enantiopure (*S*)-**A6**, we demonstrated that no important improvement of the inhibitory potency could be achieved by this approach. Apparently, the global lipophilicity plays a determinant role in their anti-PLA_2 activity, while the presence of aromatic group(s) induces a negative influence on selectivity. On the other hand, our results with compounds **B** and **C** suggest that the active site of the GIIA enzyme would not be large enough to accommodate two oxadiazolone moieties. These SAR data, associated with our earlier results, prompted us to design novel compounds for which the synthesis and inhibitory profiles are now under investigation.

Experimental Section

Chemistry. All materials were obtained from commercial suppliers and used without further purification or purified as described. Thin layer chromatography was performed on TLC plastic sheets of silica gel 60F254 (layer thickness 0.2 mm) from Merck. Column chromatography purification was carried out on silica gel 60 (70−230 mesh ASTM, Merck). All melting points were determined on a digital melting point apparatus (Electrothermal) and are uncorrected. The structures of all compounds were confirmed by IR and NMR spectra. IR spectra were obtained in paraffin oil with an ATI Mattson Genesis Series FTIR spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 200 spectrometer using hexamethyldisiloxane (HMDS) as an internal standard. Chemical shifts are given in ppm, coupling constant in Hertz, and peak multiplicities are assigned as follows: s for singlet, d for doublet, t for triplet, br s for broad singlet, and m for multiplet. Rotary powers were determined with a Jasco P-1010 polarimeter. Elemental analyses were obtained from the "Service régional de microanalyse" (Université Pierre et Marie Curie, Paris, France) and were within ±0.4% of theoretical values.

Biological Materials. Porcine pancreatic $sPLA_2$ and fatty acid-free bovine serum albumin (BSA) (fraction V) were purchased from Sigma (Paris, France), and hGIIA $sPLA_2$ was synthesized as previously reported.[41] HGIB, hGV, and hGX $sPLA_2$s were expressed and purified as described earlier.[23,42] The fluorescent substrate for PLA_2 assay, 1-hexadecanoyl-2-(10-pyrenyldecanoyl)-*sn*-glycero-3-phosphoglycerol (*β*-pyC-10-PG), was obtained from Molecular Probes (Leiden, Netherlands). Me-Indoxam is a kind gift from Dr. M. H. Gelb (University of Washington, Seattle, WA).

(±)-1-*O*-(4-Cyanophenyl)glycerol (1a). To absolute ethanol (100 mL) was added sodium (1.15 g, 50.0 mmol) piece by piece, and the mixture was heated to reflux till complete disappearance of the sodium. 4-Cyanophenol (5.0 g, 42 mmol) was then added gradually through the condenser, followed by freshly distilled 3-chloro-1,2-propanediol (5.10 g, 46.2 mmol) dropwise. The mixture was heated to reflux for further 1 h and the solvent evaporated. The residue was dissolved in water and extracted with ether. The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford an oil which was purified on silica gel column chromatography ($MeOH/CH_2Cl_2$, 5:95, v/v) to give **1a** (4.9 g, 60% yield) as a white solid: mp 97−99 °C (litt. 95−98 °C);[43] ^1H NMR ($CDC1_3$) δ 7.52 (d, 2H, J = 8.6, H_{ar}), 6.93 (d, 2H, J = 8.6, H_{ar}), 3.99 (br s, 2H, OH), 3.72−3.40 (m, 5H, CH_2O, CH).

(*S*)-**1a** was obtained according to the same procedure from (*S*)-3-chloro-1,2-propanediol: $[\alpha]^{20}_D$ = −4.13 c 5.11 (CH_3COCH_3).

(±)-1-*O*-[4-(Tetrahydropyran-2-yloxymethyl)phenyl]glycerol (1c). Several drops of concentrated HCl (36%) were added dropwise to a mixture of 3,4-dihydro-2*H*-pyran (12.9 g, 0.153 mol) and 4-hydroxybenzyl alcohol (20.0 g, 0.160 mol) in anhydrous THF (150 mL) at 0 °C. The reaction mixture was stirred at room temperature overnight and then reduced to dryness. The residue was taken up in ether, washed with H_2O to neutral pH, and dried over $MgSO_4$. After filtration, the solvent was evaporated and the crude product chromatographed (Et_2O/petroleum ether, 1:4, v/v) to provide 4-(tetrahydropyran-2-yloxymethyl)phenol[44] as a colorless oil (18.5 g, 58% yield). It was then used to prepare the compound **1c** (quantitative yield) as a colorless oil, following the same procedure as for **1a**, after purification with silica gel column chromatography using $MeOH/CH_2Cl_2$ (2:98, v/v) as eluent: ^1H NMR ($CDCl_3$) δ 7.18 (d, 2H, J = 8.7, H_{ar}), 6.78 (d, 2H, J = 8.6, H_{ar}), 4.63 (d, 1H, J = 11.6, O*CH*$_2$C$_6$H$_4$), 4.60 (t, 1H, J = 3.3, OCHO), 4.35 (d, 1H, J = 11.6, O*CH*$_2$C$_6$H$_4$), 3.99−3.41 (m, 9H, CH_2OH, CHOH, *CH*$_2$OC$_6$H$_4$, CH_2*CH*$_2$O), 1.75−1.47 (m, 6H, CH_2-CH_2CH_2).

(±)-3-*O*-(4-Cyanophenyl)-1,2-*O*-di-*n*-heptylglycerol (2a). A solution of **1a** (2.00 g, 10.4 mmol) in dry DMF (25 mL) was added dropwise to a suspension of NaH (60% dispersion in mineral oil, 1.24 g, 31.0 mmol) in dry DMF (150 mL). The mixture was stirred at room temperature for 1 h, and 1-bromoheptane (5.56 g, 26.0 mmol) was then added dropwise. After being stirred at room temperature for 12h, the reaction mixture was diluted with water (100 mL) and extracted with ether. The combined organic layers were dried ($MgSO_4$) and filtered, and the solvent was evaporated under reduced pressure to give an oil which was purified by silica gel column chromatography (Et_2O/petroleum ether, 1:4, v/v) to afford **2a** (2.2 g, 55% yield) as a colorless oil: ^1H NMR ($CDCl_3$) δ 7.50 (d, 2H, J = 8.7, H_{ar}), 6.69 (d, 2H, J = 8.7, H_{ar}), 4.13−3.95 (m, 2H, *CH*$_2$OC$_6$H$_4$), 3.73−3.69 (m, 1H, CH), 3.57−3.49 (m, 4H, CH_2OC_7, CHO*CH*$_2$C$_6$), 3.39 (t, 2H, J = 6.5, OCH_2C_6), 1.50−1.47 (m, 4H, $OCH_2CH_2C_5$), 1.20−1.09 (m, 16H, CH_2), 0.80−0.77 (m, 6H, CH_3).

(±)-3-*O*-(4-Cyanophenyl)-1-*O*-triphenylmethylglycerol (4a). To a solution of **1a** (10.0 g, 52.0 mmol) in CH_2Cl_2 (300 mL) were added triethylamine (20.0 mL, 142 mmol), trityl chloride (16.5 g, 59.0 mmol), and 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 0.2 g). The mixture was stirred at room temperature for 48 h and then washed with water to neutral pH. The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by silica gel chromatography (pure CH_2Cl_2) to afford **4a** (13.5 g, 60% yield) as a viscous oil: ^1H NMR ($CDCl_3$) δ 7.48 (d, 2H, J = 8.6, H_{ar}), 7.42−7.28 (m, 6H, H_{ar}), 7.24−7.10 (m, 9H, H_{ar}), 6.80 (d, 2H, J = 8.6, H_{ar}), 4.04−3.96 (m, 3H, CH_2-OPh, CH), 3.27 (d, 2H, J = 4.9, CH_2O), 2.48 (s, 1H, OH).

(*S*)-**4a** was obtained according to the same procedure from (*S*)-**1a**: $[\alpha]^{20}_D$ = −5.82 c 4.35 ($CHCl_3$).

(±)-3-*O*-(4-Cyanophenyl)-2-*O*-tetradecyl-1-*O*-triphenylmethylglycerol (5a). Compound **5a** was prepared according to the same procedure as for **2a** from **4a** in 70% yield as a viscous oil after

purification by rapid silica gel column chromatography (Et_2O/petroleum ether, 5:95, v/v): ^1H NMR ($CDCl_3$) δ 7.47 (d, 2H, J = 8.9, H_{ar}), 7.43−7.34 (m, 6H, H_{ar}), 7.24−7.16 (m, 9H, H_{ar}), 6.80 (d, 2H, J = 8.9, H_{ar}), 4.10−3.97 (m, 2H, CH_2OPh), 3.68−3.63 (m, 1H, CH), 3.49−3.46 (m, 2H, OCH_2C_{13}), 3.20 (d, 2H, J = 5.2, CH_2OCPh_3), 1.50−1.42 (m, 2H, OCH_2CH_2), 1.18−1.04 (m, 22H, CH_2), 0.77 (t, 3H, J = 5.5, CH_3).

(*S*)-**5a** was obtained according to the same procedure from (*S*)-**4a**: $[\alpha]^{20}_D$ = −5.29 c 4.61 ($CHCl_3$).

General Procedure to Prepare 6a−12a. Compound **5a** (10 mmol) was heated to reflux in THF (100 mL) in the presence of concentrated HCl (2 mL) for 1 h. The solvent was removed and the residue taken up into ether, washed with H_2O to neutral pH, and dried over $MgSO_4$. The crude product was purified by silica gel column chromatography to provide 1-*O*-(4-cyanophenyl)-2-*O*-tetradecylglycerol as a white solid in 90% yield: mp 67−69 °C; ^1H NMR ($CDCl_3$) δ 7.50 (d, 2H, J = 8.9, H_{ar}), 6.85 (d, 2H, J = 8.9, H_{ar}), 4.05 (d, 2H, J = 4.6, CH_2OPh), 3.90−3.40 (m, 5H, CH_2-OH, $CHOC_{14}$, OCH_2C_{13}), 2.10 (s, 1H, OH), 1.60−1.40 (m, 2H, $OCH_2CH_2C_{12}$), 1.18 (m, 22H, CH_2), 0.80 (t, 3H, J = 6.1, CH_3).

The above alcohol was used to prepare **6a−12a** according to the same procedure as described for **2a** using the corresponding alkyl bromides. The crude products were purified by silica gel column chromatography using ether/petroleum ether (2:8, v/v) as eluent to give pure **6a−12a** as a colorless oil.

(±)-3-*O*-(4-Cyanophenyl)-1-*O*-ethyl-2-*O*-tetradecylglycerol (6a). 70% yield: ^1H NMR ($CDCl_3$) δ 7.51 (d, 2H, J = 8.8, H_{ar}), 6.80 (d, 2H, J = 8.8, H_{ar}), 4,14−3.96 (m, 2H, $CH_2OC_6H_4$), 3.74−3.60 (m, 1H, CH), 3.58−3.41 (m, 6H, OCH_2), 1.44−1.53 (m, 2H, $OCH_2CH_2C_{12}$), 1.19 (m, 22H, CH_2), 1.13 (t, 3H, J = 7.0, OCH_2CH_3), 0.81 (t, 3H, J = 6.5, CH_3).

(±)-3-*O*-(4-Hydroxymethylphenyl)-1,2-*O*-di-*n*-heptylglycerol (2e). Method A: A solution of **2b** (2.00 g, 4.58 mmol) in dry ether (20 mL) was added dropwise to a suspension of $LiAlH_4$ (0.35 g, 9.2 mmol) in dry ether (50 mL), and the reaction was stirred at room temperature for further 1 h. The excess of $LiAlH_4$ was neutralized by slow addition of NaOH (20%, 20 mL) to the mixture, and the precipitate was then filtered. The aqueous layer was extracted three times with ether, and the combined organic layers were washed with water. After being dried over $MgSO_4$, the solution was concentrated to dryness to provide pure **2e** as a white solid in quantitative yield.

Method B: Pyridinium *p*-toluenesulfonate (PPTS, 0.52 g, 2.1 mmol) was added to a solution of **2c** (2.00 g, 4.18 mmol) in ethanol (50 mL), and the mixture was stirred at 60 °C for 12 h. After dilution with water, it was then extracted three times with ether. The organic layers were washed with water, dried ($MgSO_4$), and concentrated to provide pure **2e** as a white solid in quantitative yield: mp 66−68 °C; ^1H NMR ($CDCl_3$) δ 7.20 (d, 2H, J = 8.7, H_{ar}), 6.80 (d, 2H, J = 8.7, H_{ar}), 4.50 (s, 2H, CH_2OH), 4.10−3.90 (m, 2H, $CH_2OC_6H_4$), 3.70 (m, 1H, CH), 3.60−3.45 (m, 4H, $CHOCH_2C_6$, CH_2OC_7), 3.40 (t, 2H, J = 6.6, OCH_2C_6), 1.70 (br s, 1 H, OH), 1.60−1.40 (m, 4H, $OCH_2CH_2C_5$), 1.40−1.10 (m, 16H, CH_2), 0.80 (t, 6H, J = 5.7, CH_3).

(±)-3-*O*-(4-Cyanomethylphenyl)-1,2-*O*-di-*n*-heptylglycerol (2d). The compound **2e** (4.00 g, 10.0 mmol) in glacial acetic acid (60 mL) was added to 33% HBr in CH_3CO_2H (50 mL) at 0 °C, and the solution was stirred at room temperature for 30 min. It was then poured onto ice−water (500 g), and the precipitate was recovered by filtration. The solid was dissolved in Et_2O (200 mL) and washed with H_2O to neutral pH. The organic layer was dried over $MgSO_4$ and concentrated to dryness to provide the corresponding bromide in quantitative yield. It was used without further purification.

The bromide derivative (2.00 g, 4.37 mmol) and NaCN (0.25 g, 5.6 mmol) in dry DMF (25 mL) were stirred at room temperature for 12 h. The mixture was diluted with water (100 mL) and extracted several times with ether. The organic layers were dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give an oil which was purified by silica gel column chromatography (ether/petroleum ether, 1:9, v/v) to afford 1.2 g (70% yield) of **2d** as a colorless oil: ^1H NMR ($CDCl_3$) δ 7.15 (d, 2H, J = 8.7, H_{ar}), 6.80 (d, 2H, J = 8.7, H_{ar}), 4.00 (m, 2H, $CH_2OC_6H_4$), 3.70 (m, 1H, CH), 3.60 (s, 2H, CH_2CN), 3.50 (m, 4H, $CHOCH_2C_6$, CH_2OC_7), 3.40 (t, 2H, J = 6.6, OCH_2C_6), 1.50 (m, 4H, $OCH_2CH_2C_5$), 1.20 (m, 16H, CH_2), 0.80 (t, 6H, J = 5.5, CH_3).

General Procedure to Synthesize the Amidoximes 13−24. Each of the nitriles **2a**, **3a**, **5a−12a, 2d**, and **3d**, hydroxylamine hydrochloride (5 equiv), and K_2CO_3 (5.5 equiv) were heated to reflux in absolute ethanol for 18 h. The salts were filtered, and the filtrate was evaporated under reduced pressure and then purified by column chromatography to give the corresponding amidoximes.

(±)-1-*O*-Ethyl-3-*O*-[4-(*N*-hydroxylamidino)phenyl]-2-*O*-tetradecylglycerol (13). Compound **13** was obtained as a viscous oil from **6a** in 90% yield after chromatography using $MeOH/CH_2Cl_2$ (1:99, v/v) as eluent: ^1H NMR ($CDCl_3$) δ 7.50 (d, 2H, J = 8.5, H_{ar}), 6.80 (d, 2H, J = 8.5, H_{ar}), 4.79 (br s, 2H, NH_2), 4.09−3.92 (m, 2H, $CH_2OC_6H_4$), 3.71−3.66 (m, 1H, CH), 3.58−3.39 (m, 6H, OCH_2C_{13}, CH_2OEt, OCH_2Me), 1.52 (m, 2H, $CH_2CH_2C_{12}$), 1.19 (m, 22H, CH_2), 1.13 (t, 3H, J = 7.0, OCH_2CH_3), 0.81 (t, 3H, J = 6.2, $CH_2CH_2CH_3$).

General Procedure to Synthesize Oxadiazolones A1−A13. Phenyl chloroformate (1.1 equiv) was added to a solution of amidoxime (1.0 equiv) in $CHCl_3$ in the presence of triethylamine (1.5 equiv) at 0 °C. Upon completion, the solution was stirred for 1 h, washed with water to neutral pH, and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the carbonate, dissolved in toluene, was heated to reflux for 18 h. After evaporation of the solvent, the residue was chromatographed to yield pure oxadiazolone derivative.

(±)-3-*O*-[4-(4,5-Dihydro-5-oxo-1,2,4−4*H*-oxadiazol-3-yl)phenyl]-2-*O*-tetradecyl-1-*O*-triphenylmethylglycerol (A6). In 55% yield as a white solid using $MeOH/CH_2Cl_2$ (2:98, v/v) for the chromatography: mp 109−111 °C; ^1H NMR ($CDC1_3$) δ 7.60 (d, 2H, J = 8.8, H_{ar}), 7.40−7.36 (m, 6H, H_{ar}), 7.36−7.22 (m, 9H, H_{ar}), 6.90 (d, 2H, J = 8.9, H_{ar}), 4.15−4.00 (m, 2H, $CH_2OC_6H_4$), 3.72−3.67 (m, 1H, CH), 3.48 (t, 2H, J = 6.5, OCH_2C_{13}), 3.20 (d, 2H, J = 5.1, CH_2OCPh_3), 1.49−1.46 (m, 2H, $OCH_2CH_2C_{12}$), 1.28−1,18 (m, 22H, CH_2), 0.80 (t, 3H, J = 6.4, CH_3). Anal. ($C_{44}H_{54}N_2O_5$): C, H, N.

(*S*)-**A6** was obtained according to the same procedure from (*S*)-**5a**: $[\alpha]^{20}_D$ = −6.20 c 3.00 ($CHCl_3$).

Fluorimetric Assay. PLA_2 activity is evaluated by the method of Radvanyi et al.[45] using the fluorescent phospholipid analogue β-pyC-10-PG as the substrate. This assay is specific for secretory PLA_2, cytosolic PLA_2 being inactive on substrate with a pyrene group at the *sn*-2 position.[46] In a total volume of 1 mL, the standard medium contains 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EGTA, 2 μM substrate, 0.1% fatty acid free BSA solution in water, and 1 ng of hGIIA PLA_2 or 60 ng of $pPLA_2$. The fluorescence (λ_{ex} = 342 nm and λ_{em} = 388 nm) of the enzymatic reaction medium (blank) is recorded for 1 min with a spectrofluorimeter LS50 (Perkin-Elmer). The reaction is then initiated by addition of $CaCl_2$ (10 mM, final concentration). The increase in fluorescence is continuously recorded for 2 min, and PLA_2 activity is calculated as described by Radvanyi et al.[45] The inhibitor in ethanol (or DMSO) was added to the reaction medium after introduction of BSA. The activity is expressed in micromoles of fluorescent β-pyC-10-PG hydrolyzed per min and per mg of PLA_2. This allows the determination of the IC_{50} values (concentration of inhibitors producing 50% inhibition) of each compound.

Radiometric Assay.[42] Preparation of autoclaved and [^{3}H]oleate-labeled *E. coli* membranes and $sPLA_2$ assays were performed essentially as described previously.[42] Briefly, $sPLA_2$ assays were performed at 25 °C in a total volume of 100 μL consisting of 140 mM NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM $CaCl_2$, 0.1% bovine serum albumin, and 100 000 dpm of [^{3}H]oleate-labeled *E. coli* membranes. Incubation times and sample volumes were adjusted to ensure hydrolysis rates within the linear range of enzymatic assays. Typically, 3−10 μL of solution containing different $sPLA_2$s were incubated for 30−60 min at 20 °C to measure $sPLA_2$ activity. Reaction mixtures were stopped by adding 300 μL of 0.1 M EDTA,

pH 8.0, and 1% fatty acid-free bovine serum albumin. After centrifugation at 10 000*g* for 3 min, 300 μL of supernatant containing hydrolyzed phospholipids was counted. Control incubations in the absence of added $sPLA_2$ were carried out in parallel and used to calculate specific hydrolysis.

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Supporting Information Available: Routine experimental procedures and spectroscopic data, as well as elemental analysis data, of the test compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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