Design and Synthesis of a Novel and Potent Series of Inhibitors of Cytosolic Phospholipase A_2 Based on a 1,3-Disubstituted Propan-2-one Skeleton

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Using knowledge of the substrate specificity of $cPLA_2$ (phospholipases A_2), a novel series of inhibitors of this enzyme were designed based upon a three point model of inhibitor binding to the enzyme active site comprising a lipophilic anchor, an electrophilic serine "trap", and an acidic binding moiety. The resulting 1,3-diheteroatom-substituted propan-2-ones were evaluated as inhibitors of $cPLA_2$ in both aggregated bilayer and soluble substrate assays. Systematic variation of the lipophilic, electrophilic, and acidic groups revealed a well-defined structure−activity relationship against the enzyme. Optimization of each group led to compound **22** (AR-C70484XX), which contains a decyloxy lipophilic side chain, a 1,3-diaryloxypropan-2-one moiety as a unique serine trap, and a benzoic acid as the acidic binding group. AR-C70484XX was found to be among the most potent in vitro inhibitors of $cPLA_2$ described to date being more than 20-fold more active against the isolated enzyme ($IC_{50} = 0.03\ \mu M$) than the standard $cPLA_2$ inhibitor, arachidonyl trifluoromethyl ketone ($AACOCF_3$), and also greater than 10-fold more active than $AACOCF_3$ against the cellular production of arachidonic acid by HL60 cells ($IC_{50} = 2.8\ \mu M$).

Introduction

Phospholipases A_2 (PLA_2s) are a class of esterases that cleave the sn-2 ester bond of membrane phospholipids in both a regiospecific and a stereospecific manner.[1] Because the vast majority of cellular arachidonic acid is found esterified at this position of glycerophospholipids and free arachidonate is only produced by cells when appropriately activated, a PLA_2 is generally accepted as being the enzyme that is responsible for the cellular production of this important intermediate.[2] Arachidonic acid is further converted by cells into a number of proinflammatory metabolites, including prostaglandins and leukotrienes, molecules that have been the subject of many medicinal chemistry efforts,[3] either to block their biosynthesis or to antagonize the endpoint action of these mediators at specific receptors. In addition, the byproduct of arachidonic acid production by PLA_2 is a lysophospholipid, which on further enzymatic acetylation can lead to the formation of platelet-activating factor, yet another proinflammatory molecule of medicinal interest.[4] Thus, inhibition of the action of PLA_2 has the clear potential to block the cellular production of a wide range of proinflammatory lipid mediators and has therefore been the subject of intense study by the pharmaceutical industry.[5] It may therefore seem surprising that no inhibitor of PLA_2 has emerged as an antiinflammatory drug to date. However, there are good reasons for the lack of progress toward this aim.

First, for a long time, the only PLA_2s that had been described were all low molecular mass (∼14 kD) enzymes that have been secreted from cells and hence have subsequently been termed $sPLA_2$s. These $sPLA_2$s are characterized by 7 (or 8) disulfide bonds that endow the enzyme with extracellular stability. In addition, the enzymes demonstrate a lack of specificity for the fatty acid cleaved from the sn-2 position of phospholipids (although in contrast a reasonable specificity toward a phosphoethanolamine group in the sn-3 position) and require a high concentration ($>\mu M$) of Ca^{2+} for activation.[6] While none of these attributes would be expected of an intracellular PLA_2, it was nonetheless believed for some time that the cellular PLA_2 would be structurally and mechanistically similar; hence, much effort has been expended[7] on the search for inhibitors of the $sPLA_2$s as possible antiinflammatory agents. The combined Lilly and Shionogi groups have advanced an $sPLA_2$ inhibitor to clinical trials for the treatment of septic shock; however, it is now unclear whether the mechanism of action of this class of compound is through enzyme inhibition or antagonism of an $sPLA_2$ receptor.[8]

However, in the early 1990s, an intracellular PLA_2 was described,[9] the properties of which were much more consistent with those anticipated for the enzyme responsible for intracellular arachidonic acid production. This enzyme had a far higher molecular mass (approximately 85 kD) but contained no disulfide bridges. The enzyme showed a distinct preference for the hydrolysis of an arachidonoyl ester at the sn-2 position of phospholipids, both against purified substrates and in a natural phospholipid environment.[10] Furthermore, it was activated by much lower ($<\mu M$) levels of Ca^{2+} than $sPLA_2$, consistent with the levels required for the

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production of arachidonic acid (and its metabolites) within cells.[11] Notably, this intracellular PLA_2 was found within the cytosol and was named $cPLA_2$. Two further members of the $cPLA_2$ class, $cPLA_2\beta$ and $cPLA_2\gamma$, have been described recently.[12] Later, it was found[13] that when cells are activated $cPLA_2$ translocates largely to the nuclear membrane where the arachidonic acid can be produced in close proximity to the downstream enzymes 5-lipoxygenase (5-LO) and cyclooxygenase (CO). This provides compelling evidence that $cPLA_2$ production of arachidonic acid is concerted with its further biochemical transformation to proinflammatory eicosanoids[14] and lends support to the hypothesis that the inhibition of $cPLA_2$ would provide a valuable target in the search for a novel antiinflammatory agent.[15] This hypothesis became further strengthened with the publication from two separate laboratories that cPLA2 knockout mice have a much reduced output of inflammatory eicosanoids and associated resistance to downstream effects such as anaphylactic response, bronchial reactivity, and postischemic brain injury.[16]

A second reason for the apparent lack of progress in finding inhibitors of PLA_2s that might lead to clinical candidates is the complex nature of the in vitro screening of these enzymes. PLA_2s as their name suggests are lipases and as such have evolved to work optimally at a lipid−water interface. A large variety of screens have been described that involve the action of PLA_2 at an aggregated assembly of substrate such as that formed in bilayers, vesicles, and mixed micelles.[17] The great problem with all such screens is that inhibitors may work by interfering with the structure of the interface and inhibit the enzyme action not by binding to its catalytic site but merely by causing the enzyme to desorb from the lipid−water interface. This may be described as "nonspecific inhibition". Many different assay strategies have been employed in attempts to overcome this difficulty. For instance, Gelb and Jain have described[18] a "scooting assay" in which the enzyme is so strongly bound to (modified) substrate vesicles that nonspecific inhibition cannot occur. On the other hand, Lehr[19] has dispensed with isolated enzyme screening altogether, preferring to screen only in a cellular situation. We have previously described[20] a "dual-screening" strategy for $sPLA_2$, in which inhibitors are tested against the enzyme in the presence of a natural substrate bilayer and also against the enzyme working against a soluble substrate where the complexities associated with lipid aggregation are minimized. In this paper, we describe the use of both bilayer and soluble substrate screens for the discovery of novel and potent inhibitors of the cytosolic enzyme $cPLA_2$.

Inhibitor Design

At the outset of this work, we tested our entire collection of $sPLA_2$ inhibitors against $cPLA_2$, including the extremely potent $sPLA_2$ inhibitor AR-C67047, which we have previously reported.[21] None of the compounds showed significant inhibition of $cPLA_2$, as might be expected since data that later emerged showed the two classes of enzyme to have completely different catalytic mechanisms and quite distinct selectivities toward phospholipid substrate. We therefore turned our attention to the a priori design of inhibitors based on the natural substrate. A simple model of how one might expect substrate to bind to $cPLA_2$ is shown in Figure 1. Features that intrigued us were $cPLA_2$s selectivity for cleaving arachidonic acid from the sn-2 position of substrate, which suggested that there might be a specific binding site in the enzyme for the arachidonoyl group. In fact, Gelb has previously demonstrated[18b] that the double bonds of the arachidonate moiety, and particularly the first (Δ5,6) double bond, are highly beneficial for selectivity. In contrast, there was little evidence that the sn-2 ester was recognized at all. Turning to the phosphocholine group, it appeared that an anionic phosphate was required for recognition by the enzyme,[11] but there was no evidence that the choline, or any other quaternary ammonium group, was necessary suggesting that this part of the substrate plays little role in binding to enzyme. The model is completed by a proposed[22] catalytic diad with an activated serine OH group acting as the nucleophile[23] to react with the sn-2 ester group, thereby producing an intermediate oxyanion. This intermediate is likely to be stabilized by an "oxyanion hole" within the enzyme active site.

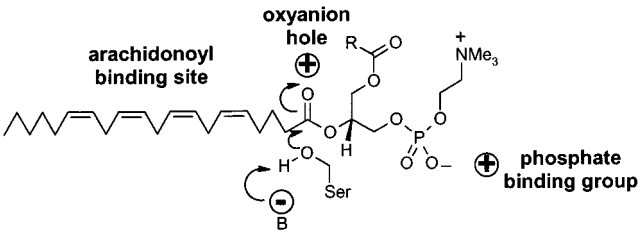


Figure 1. Simple model for substrate binding to cytosolic PLA_2.

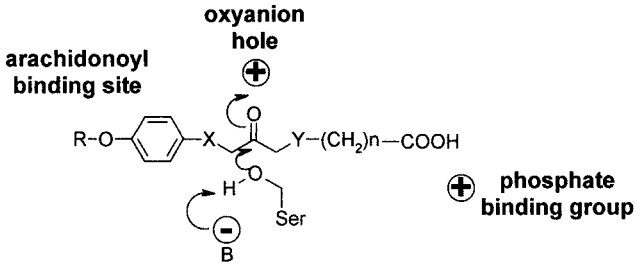


Figure 2. Simple model for inhibitor binding to cytosolic PLA_2.

This knowledge led to the design of novel inhibitors of $cPLA_2$ based on a model for inhibitor binding, which is shown in Figure 2. The model consists of three parts: first, a lipophilic substituent that incorporates aromatic rings to mimic (at least part of) the arachidonic acid-skipped tetraene unit; second, an electrophilic ketone as a serine "trap"; and third, an anionic group at a few atoms distance from the serine trap to mimic the phosphate group of the substrate. Our experience in the $sPLA_2$ area with the design of AR-C67047 showed that a carboxylic acid can be an excellent bioisostere for the monoanionic phosphodiester of substrate.[21] Therefore, in this model for a $cPLA_2$ inhibitor, we also replaced the phosphodiester with a carboxylic acid, conveniently positioned on an alkyl chain attached to the chosen serine trap. Subsequent publication[24] of the X-ray crystal structure of $cPLA_2$ showed this model to be largely correct; however, confirmation of the binding

mode of inhibitors will need to wait until the structure of $cPLA_2$ complexed to an inhibitor is reported.

The choice of electrophilic ketone to act as a serine trap deserves some discussion. Described in the literature, there are a number of different types of electrophilic ketones that may serve to interact with nucleophilic serine residues[25] (mainly within the context of serine protease inhibitors). For example, trifluoromethyl ketones are known to interact well with serine hydroxyl groups at the active site of serine proteases.[25] Indeed, while this work was in progress, the Merck-Frosst group described[26] arachidonyl trifluoromethyl ketone **1** (AA-$COCF_3$) as a potent inhibitor of $cPLA_2$.

1

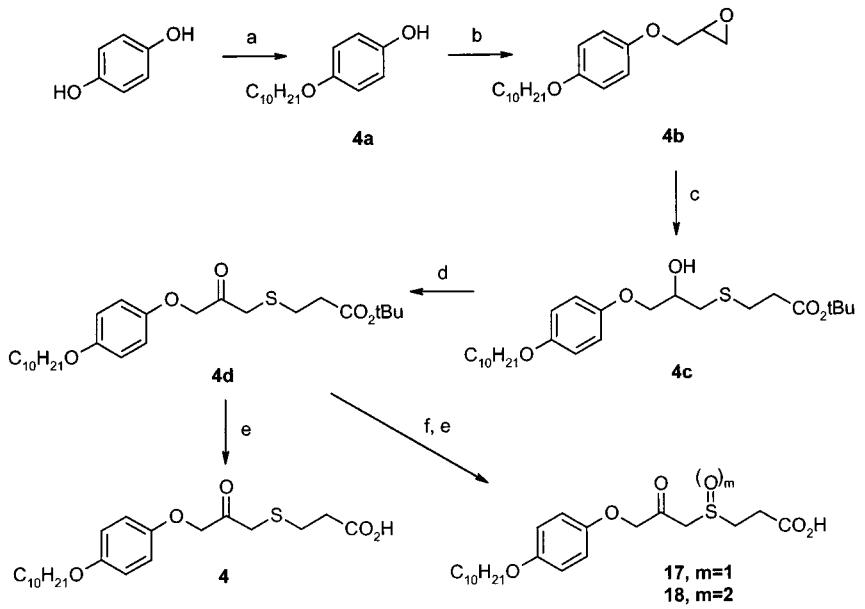
Later, it was shown[27] that this compound (**1**) functionally inhibits the products of $cPLA_2$ action in whole cell systems, although the mechanism of this cellular inhibition has been questioned.[28] However, while useful as a probe, this inhibitor suffers from some serious drawbacks as a starting point in the search for a clinical candidate. First, the skipped diene units of the arachidonyl side chain are metabolically labile, especially to oxidation. Second, because of the very strong electron-withdrawing effect of the trifluoromethyl group, trifluoromethyl ketones are exceptionally electrophilic and exist largely as hydrates. It is not possible to modify the electrophilicity of the trifluoromethyl ketone group in a controlled way, and because it is only the free ketone that can interact with the serine hydroxyl group, hydrated trifluoromethyl ketones may not appear to be as potent inhibitors of serine enzymes as might be expected. Additionally, the nature of the trifluoromethyl group makes it difficult to substitute on the CF_3 side of the ketone without changing the electron-withdrawing nature of the activating group substantially. Obviously, difluoromethyl analogues have reduced electrophilicity in comparison to the trifluoromethyl ketones and may be further substituted on the CF_2 group. For instance, some very elegant work describing substituted difluoromethyl ketone inhibitors of interleukin-1β converting enzyme has been reported.[29] However, while potent inhibitors of this type were found, these compounds remain highly electrophilic, and it is still difficult to control the electrophilicity of the pharmacophoric ketone group. Furthermore, it is likely that such highly electrophilic ketones will be rapidly metabolized. Indeed, the facile cellular reduction of compound **1** has been disclosed.[27a]

In our work, we felt it was essential to incorporate a ketone in which the electrophilicity of the carbonyl group could be altered over a wide range by structural modification. We chose to investigate the little-known 1,3-diheteroatom-substituted propan-2-one[30,31] group, where the flanking heteroatoms were either O or S atoms, as the interactive serine trap. On one side of the propan-2-one group, we introduced an aryloxy substituent to both activate the ketone group to nucleophilic attack and also to incorporate a planar all sp^2 moiety with high π-electron density to mimic the Δ5,6 double bond of arachidonic acid. We also appended a long alkyl chain on the distal side of the aromatic ring, to mimic the remainder of the lipid side chain of substrate. This alkyl chain was conveniently attached as an alkyloxy group. With the further incorporation of an alkyl-linked carboxylic acid group on the other side of the central ketone, we arrived at the prototype $cPLA_2$ inhibitor shown in Figure 2.

Chemistry

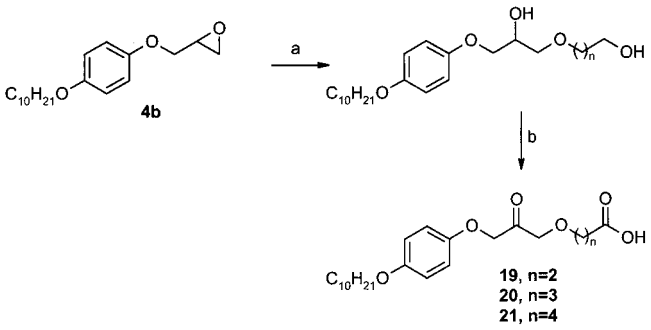
Scheme 1 outlines the chemical approach used to synthesize compounds **2**−**12** and **14**−**18**, as demonstrated by the preparation of compound **4**. 4-Hydroxyphenol was monoalkylated using decyl bromide under basic conditions to afford the 4-alkoxyphenol **4a** in moderate yield. This phenol was further reacted with epichlorohydrin to give the epoxy intermediate **4b** in

Scheme 1[a]



[a] (a) (1) NaH, DMF; (2) $C_{10}H_{21}Br$. (b) Epichlorohydrin, Cs_2CO_3, MeCN, reflux. (c) DABCO, $HS(CH_2)_2CO_2{}^tBu$, DMF, 100 °C. (d) Dess−Martin reagent, DCM. (e) TFA, DCM. (f) MCPBA, DCM.

Scheme 2[a]



[a] (a) (1) NaH, DMF; (2) $HO(CH_2)_nOH$. (b) PCC, DMF.

excellent yield. Ring opening of the **4b** with *tert*-butyl 3-mercaptopropanoate was best achieved in the presence of a catalytic amount of DABCO to give **4c**. Oxidation to give the intermediate ketone **4d** was carried out with mild oxidants such as the Dess−Martin reagent or the acetic anhydride−DMSO (dimethyl sulfoxide) system. In general, yields for this transformation were much lower when harsher oxidants, such as pyridinium dichromate (PDC) or PCC, were employed. Deprotection of the *tert*-butyl ester under standard conditions gave compound **4**. Alternatively, oxidation of **4d** with 1 or 2 equiv of MCPBA, followed by deprotection of the ester, afforded the sulfoxide **17** and sulfone **18**, respectively.

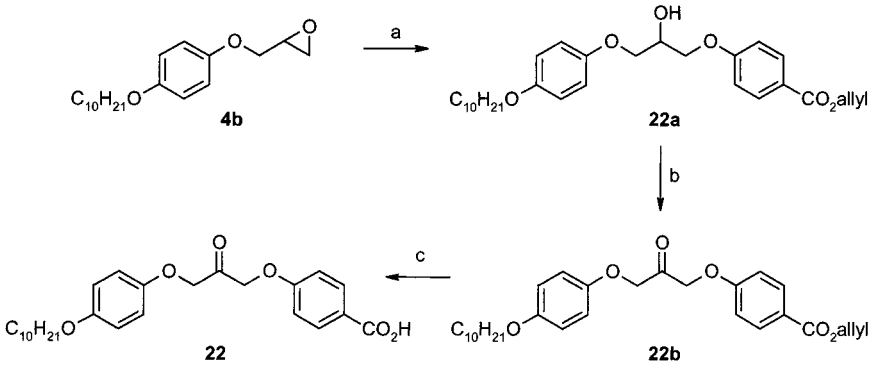
Oxygen-linked compounds **19**−**21** were prepared as shown in Scheme 2. Thus, the epoxy intermediate **4b** was reacted with the appropriate diol under strongly basic conditions. The resulting dihydroxy intermediates were not characterized but were oxidized directly to the keto-acid products using PCC, albeit in moderate overall yields.

Compounds **22**−**41**, **46**, and **49** were prepared by the route outlined for compound **22** in Scheme 3. In this series of compounds, the epoxide intermediate **4b** was ring-opened by a substituted phenol to afford the hydroxy intermediate **22a**. Subsequent oxidation of this alcohol with Dess−Martin reagent gave keto-ester **22b**, which upon deprotection gave the desired acid **22** in good yields.

Scheme 4 outlines the synthesis of the two des-oxy analogues **13** and **42**. The phenol **4a** was cleanly alkylated with 1,3-dibromopropane to afford an intermediate alkyl bromide in excellent yield. Reaction of this bromo compound with methyl 4-hydroxybenzoate gave the ester **42a**, deprotection of which gave the acid **42**. Similarly, reaction of the same bromo intermediate derived from **4a** with *tert*-butyl 3-mercaptopropanoate followed by acidic deprotection gave **13**.

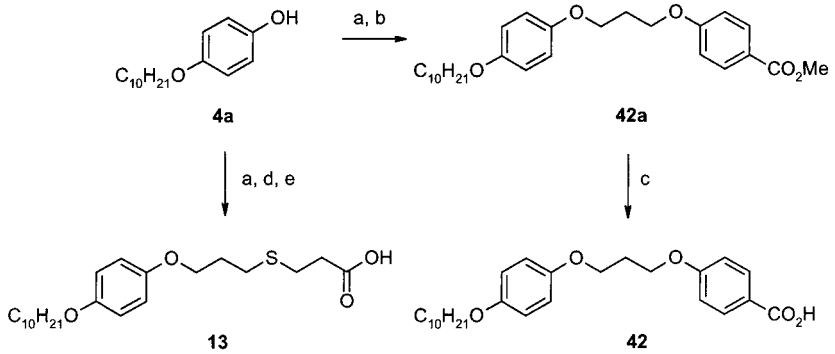
For the synthesis of analogues with only one heteroatom flanking the central ketone group, quite different chemistry was required. Scheme 5 outlines the synthesis of compound **44**, which contains a 2 carbon link to the benzoic acid moiety. 4-(5-Phenylpentylthio)phenol (prepared from 4-mercaptophenol in analogous fashion to **4a**) was alkylated very efficiently by 2-chloroacetonitrile to give **44a**. This compound was reduced with Dibal-H in toluene, and vinylmagnesium bromide was added cleanly to the resulting aldehyde (**44b**) to afford the allylic alcohol **44c**. This alcohol underwent the Heck reaction with methyl 4-iodobenzoate to give **44d** in moderate yield, which on deprotection gave **44**.

Scheme 3[a]



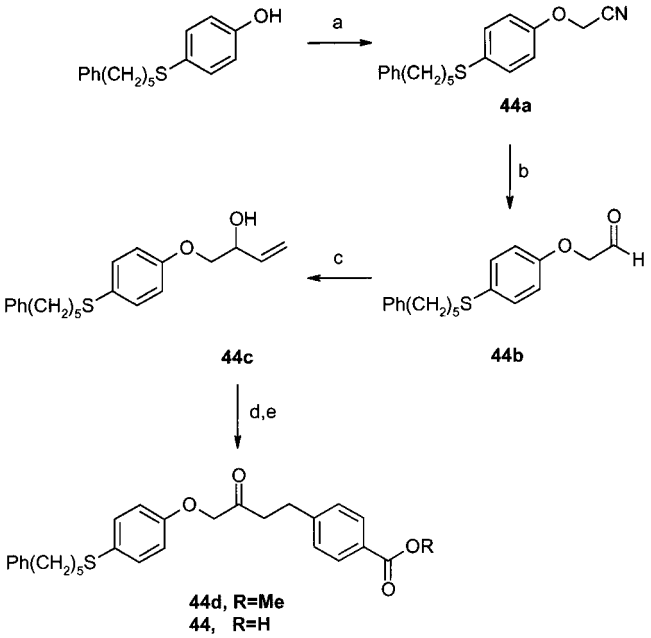
[a] (a) (1) $HOC_6H_4CO_2$allyl, DABCO, DMF, 100 °C. (b) Dess−Martin reagent, DCM. (c) $Pd(PPh_3)_4$AcOH, THF.

Scheme 4[a]



[a] (a) (1) Cs_2CO_3, DMF; (2) 1,3-dibromopropane. (b) 4-$HOC_6H_4CO_2Me$, Cs_2CO_3, MeCN, reflux. (c) LiOH, THF/H_2O. (d) $HS(CH_2)_2CO_2{}^tBu$, DABCO, DMF, 100 °C. (e) TFA, DCM.

Scheme 5[a]



[a] (a) Cs_2CO_3, DMF, $ClCH_2CN$. (b) Dibal-H, toluene, −40 °C. (c) VinylMgBr, THF, −10 °C. (d) $Pd(OAc)_2$, DMF, TEA, 4-$IC_6H_4CO_2Me$. (e) LiOH, THF/H_2O.

Compound **45**, which contains a 2 carbon link to the decyloxyphenyl substituent, was prepared by the route shown in Scheme 6. Thus, 4-(4-hydroxyphenyl)-2-oxobutane was alkylated with decyl bromide to afford **45a**. This methyl ketone was brominated using tetrabutylammonium tribromide to afford the corresponding bromomethyl ketone **45b** in good yield. Reaction of this compound with allyl 3-hydroxybenzoate proceeded efficiently with KF as the base to give **45c**, which was deprotected under standard conditions to give the target acid **45**.

Evaluation of Inhibitors

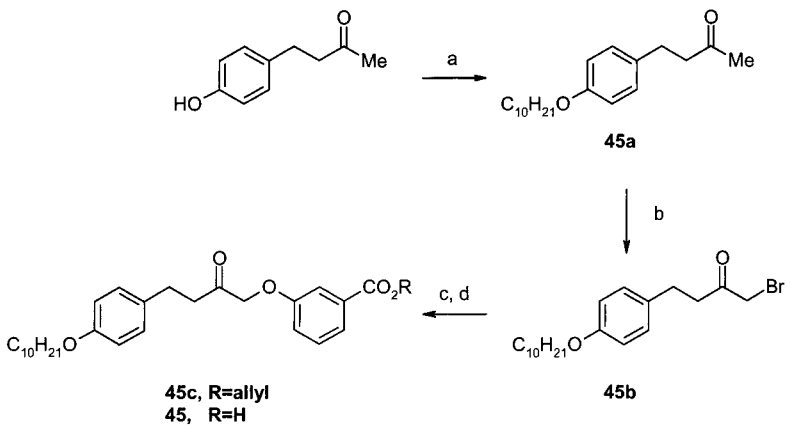
Initially, inhibitors were tested against the action of $cPLA_2$ in a bilayer-based assay in which the substrate is comprised of an aggregated form of phospholipid to which the enzyme binds reversibly. Subsequently, when it became available,[32] inhibitors were also tested in an assay where $cPLA_2$ is acting upon a simple, synthetic, and soluble substrate, wherein no aggregated assembly exists. Testing inhibitors in both assays (a dual screening strategy as described above) gave information on compounds that behaved differently in the two screens and that thus could be inhibiting in a nonspecific fashion. Finally, inhibitors were also tested in a cellular screen based on the stimulated production of arachidonic acid by HL60 cells.

Structure−Activity Relationships and Discussion

On the basis of our design principle as outlined above, we initially targeted propan-2-ones substituted on one side of the carbonyl group by a propanoic acid (linked by an S atom) to mimic the phosphate group and on the other side by aryloxy groups (to mimic part of the skipped tetraene of arachidonic acid). Thus, the 2-naphthoxy compound (**2**) and the 4-phenoxyphenoxy compound (**3**) were prepared but were shown to have no activity in the bilayer assay (see Table 1). Incorporation of a decyloxyphenoxy substituent, as in compound **4**, gave the first sub-micromolar inhibitor of $cPLA_2$ with an IC_{50} of 0.27 μM in the bilayer assay. This latter compound has a much higher lipophilicity than the former compounds, and at that point, it was not clear if the increased potency was due to the decyloxy group interacting with the enzyme or merely anchoring the inhibitor in the bilayer. However, the requirement for high lipophilicity is perhaps not surprising given the physical properties of the natural phospholipid substrate.

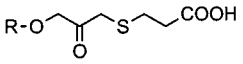
To investigate this lipophilic requirement, we synthesized analogues of compound **4** with the decyl chain truncated to 2 and 6 carbon atoms (compounds **5** and **6** in Table 1). These compounds had much reduced activity, with the least lipophilic (compound **5**) being totally inactive, again emphasizing the need for a lipophilic substituent at this position in the molecule. Surprisingly, increasing the lipophilicity by 2 orders of magnitude over compound **4** by extending the lipophilic alkyl group to 14 carbon atoms (as in compound **7**) gave no further increase in activity. Replacement of the decyloxy group of **4** with a 2-naphthylmethoxy group (common in inhibitors of 5-LO)[33] gave a compound (**8**) with somewhat reduced potency, but the residual activity did show that bulky aromatic groups can indeed be tolerated in this region of the inhibitor and that a linear alkyl group is not a necessity. Moreover, when the lipophi-

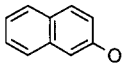
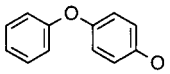
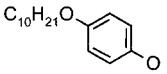
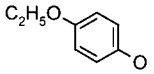
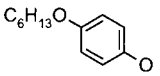
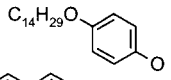
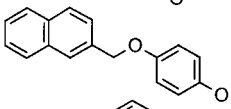
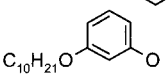
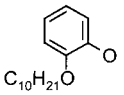
Scheme 6[a]



[a] (a) Cs_2CO_3, $C_{10}H_{21}Br$, DMF. (b) $BuN_4{\cdot}Br_3$, DCM/MeOH. (c) KF, 3-$HOC_6H_4CO_2$allyl, DMF, 100 °C. (d) $Pd(PPh_3)_4$, THF/AcOH.

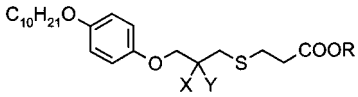
Table 1. Inhibition of Cytosolic PLA_2: Variation of the Lipophilic Aryl Substituent



compound	RO	Bilayer Assay[a] IC_{50} (μM)	cLogP[b]
2		>30[c]	2.6
3		>30[c]	3.7
4		0.27	6.3
5		>1000[c]	2.1
6		15	4.2
7		0.4	8.4
8		2.3	4.5
9		0.7	6.3
10		1.4	5.7

[a] Inhibition of $cPLA_2$ measured against an aggregated phospholipid substrate. IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] Lipophilicity as calculated by Daylight.[35] [c] No inhibition at this concentration.

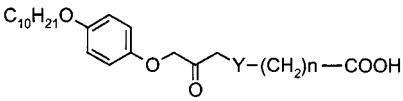
Table 2. Inhibition of Cytosolic PLA_2: Changes to the Ketone and Acid



compd	X, Y	R	bilayer assay IC_{50} (μM)[a]	cLog P[b]
4	=O	H	0.27	6.3
11	=O	Me	>10[c]	6.9
12	H, OH	H	3.6	5.9
13	H, H	H	1.9	6.9

[a] Inhibition of $cPLA_2$ measured against an aggregated phospholipid substrate. IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] Lipophilicity as calculated by Daylight.[35] [c] No inhibition at this concentration.

Table 3. Inhibition of Cytosolic PLA_2: Variation of the Acidic Chain



compd	Y	n	bilayer assay IC_{50} (μM)[a]	cLog P[b]
4	S	2	0.27	6.3
14	S	1	1.4	6.0
15	S	3	0.15	6.3
16	S	4	0.07	6.9
17	SO	2	1.0	5.4
18	SO_2	2	0.50	5.3
19	O	2	1.2	5.9
20	O	3	0.24	5.6
21	O	4	0.03	6.0

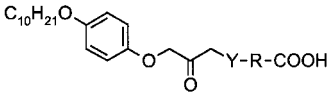
[a] Inhibition of $cPLA_2$ measured against an aggregated phospholipid substrate. IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] Lipophilicity as calculated by Daylight.[35]

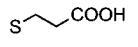
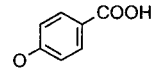
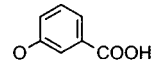
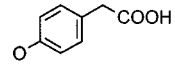
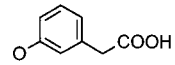
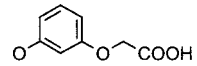
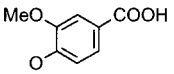
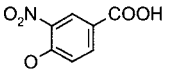
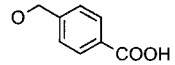
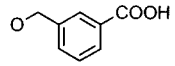
licity and activity of compound **8** are compared with that of compound **6**, it is clear that the sole reason for the reduced activity of **8** over **4** is its reduced lipophilicity. This could be taken as evidence that the role of the alkyl group may be only to partition the molecule into the bilayer. However, shape was also shown to be of some importance since the isomeric meta- and ortho-decyloxy compounds **9** and **10** (Table 1) are reduced in activity as compared to the prototype para-substituted compound **4**.

That an anionic group is a requirement for activity is suggested by the inactivity of the methyl ester **11** (Table 2). Although reduction of the ketone to the corresponding alcohol **12** or even to the methylene compound **13** gave weaker inhibition (3.6 and 1.9 μM, respectively) as compared to ketone **4**, these reduced analogues appear to have a surprising level of inhibitory activity. However, the similar degree of potency observed for both **12** and **13** strongly suggests that this level of activity (a few micromolars) might possibly be due to nonspecific inhibition in the aggregated bilayer assay. Indeed, a large number of lipophilic acids of varied structure produced apparent activity at this level in the bilayer assay and served to highlight the difficulties of screening solely with such aggregated assay systems (see following). At this stage, compound **4** was unique in our hands in that it inhibited $cPLA_2$ at submicromolar concentrations, and it gave us the confidence to explore this series further.

We next explored the nature of the chain linking the acid group to the electrophilic ketone (see Table 3). Shortening the carbon chain between the S atom and the acid to one methylene, as in compound **14**, gave a reduction in activity to 1.4 μM (possibly at the nonspecific level) whereas extending the chain to 3 or 4 carbon atoms gave compounds **15** and **16** with improved activity. At 70 nM, compound **16** was a particularly potent inhibitor of $cPLA_2$, and this low concentration of inhibitor dispelled any doubts about nonspecific inhibition by this compound, even in the bilayer assay. Attempts to increase the potency of compound **4** by making the ketone more electrophilic met with mixed success. For instance, oxidizing the linking sulfur atom to sulfoxide **17** or sulfone **18** gave weaker inhibitors, but this was probably due to the concomitant reduction in lipophilicity achieved by this transformation. Exchanging the linking sulfur atom for oxygen gave inhibitors **19**−**21** with broadly similar activity to the S series **4**, **15**, and **16**. In the oxygen series, the inhibitors have probably gained potency by having a more electrophilic ketone to interact with the serine hydroxyl, but they have also lost activity by having a shorter chain to the acidic group as well as being slightly less lipophilic. This balance of effects is likely to account for the similar overall

Table 4. Inhibition of Cytosolic PLA_2: Introduction of an Aromatic Group

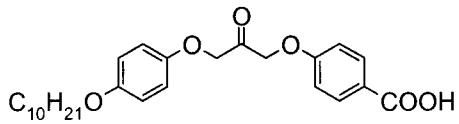


compound	Y−R−COOH	Bilayer Assay[a] IC_{50} (μM)	Soluble Assay[b] IC_{50} (μM)	cLogP[c]
4		0.27	2.2	6.3
22		0.008	0.03	7.8
23		0.050	NT[d]	7.8
24		0.017	0.15	7.2
25		0.060	NT[d]	7.2
26		0.060	0.20	7.2
27		0.074	0.27	7.4
28		0.020	0.12	7.5
29		0.040	FDR[e]	7.7
30		0.035	0.15	7.7

[a] Inhibition of $cPLA_2$ measured against an aggregated phospholipid substrate. IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] Inhibition of $cPLA_2$ measured against a soluble substrate. [c] Lipophilicity as calculated by Daylight.[35] [d] Not tested. [e] Flat dose response. Compound was poorly soluble.

inhibition observed. Nonetheless, compound **21** was the most potent inhibitor of this alkanoic acid series of $cPLA_2$ inhibitors having a measured IC_{50} of 30 nM in the bilayer assay.

Both the sulfur and the oxygen-linked series of inhibitors described in Table 3 have optimal activity with pentanoic acid substituents, and we were concerned that these compounds were extremely linear and flexible molecules. Therefore, we next turned our attention to restricting the conformation of the alkyl chain linking the ketone group to the acid function. After some investigation, compound **22** (Table 4), containing a para benzoic acid group linked by an oxygen atom to the propan-2-one serine trap, was discovered. This compound was extremely potent in the bilayer assay with an IC_{50} of 8 nM. At this time in our program, we also began testing our inhibitors against $cPLA_2$ in an assay that used a simplified and soluble substrate.[32] Compound **22** is also highly potent in this soluble substrate assay with an IC_{50} of 30 nM, notably inhibiting the action of the enzyme in a situation where there is no aggregated lipid present. Thus, compound **22** (designated AR-C70484XX) is a potent inhibitor of cPLA2 both in the presence and in the absence of a lipid−water interface.



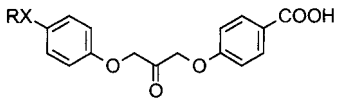
22, AR-C70484XX

The increased activity of compound **22** over compound **4** is probably due to both the conformational restriction of the acidic linking group and the increased electron-withdrawing effect of a second aryloxy group on the electrophilic ketone serine trap. In addition, incorporation of the benzoic acid moiety leads to an increase in lipophilicity. However, because the increased potency is seen in both the bilayer assay and the soluble substrate assay, this effect cannot be attributed solely to increased membrane anchoring (since no membrane exists in the latter assay).

The isomeric meta-substituted benzoic acid **23** is still very potent but less so in comparison with **22**, and the phenyl acetic acids **24** and **25** show a similar trend with the para-substituted compound (**24**) being the more active with an IC_{50} of 17 nM. Extending the acidic chain by a further oxygen atom, as in compound **26**, gave no change in potency as compared to the analogous meta-substituted compound **25**. To examine electronic effects further, we prepared compound **27** containing an extra methoxy group on the aryl ring and observed a drop in potency to 74 nM. This is possibly due to the electron-releasing effect of the ortho-methoxy group being relayed to the central carbonyl group via the aryl ring and the linking oxygen atom. Conversely, inclusion of an extra electron-withdrawing nitro group on the benzoic acid aryl ring, as in compound **28**, did give a potent inhibitor of $cPLA_2$ with an IC_{50} of 20 nM, although still 2- to 4-fold less active than the parent **22**. Steric effects accompanying the inclusion of these ortho substituents cannot be ruled out. Compounds **29** and **30**, in which a methylene has been inserted between the oxygen atom and the benzoic acid, are not as active as compound **22**, although in this case the reduced activity may be as a result of the poorer electron-withdrawing effect of benzyloxy as compared to aryloxy.

With compound **22** in hand, we reinvestigated the effect of the lipophilic substituent in this exceptionally potent benzoic acid series. From Table 5, it can be seen that systematically reducing the chain length of the decyloxy chain from 10 carbons to 8, 6, 4, and 2 (compounds **31**−**34**, respectively) produces a near linear reduction in potency. This effect is observed in both the bilayer and the soluble substrate assay, and because (as mentioned previously) there is no lipid interface present in the latter screen, the fall in activity cannot be due to membrane partitioning. A more likely explanation for this activity−lipophilicity correlation is that the decyloxy group is actually binding to a lipophilic pocket in the enzyme. Again, this would not be surprising given the lipophilic nature of the natural substrate. Similar to the result seen in Table 1, extending the chain beyond 10 carbon atoms to the dodecyloxy analogue (**35**) does not result in any further increase in potency in the

Table 5. Inhibition of Cytosolic PLA_2: Variation of the Lipophilic Aryl Substituent in the Benzoic Acid Series



compd	RX	bilayer assay IC_{50} (μM)[a]	soluble assay IC_{50} (μM)[b]	cLog P[c]
22	$C_{10}H_{21}O$	0.008	0.030	7.8
31	$C_8H_{17}O$	0.046	0.41	6.8
32	$C_6H_{13}O$	0.28	4.6	5.7
33	C_4H_9O	4.3	28	4.7
34	C_2H_5O	28	67	3.6
35	$C_{12}H_{25}O$	NT[d]	0.026	8.9
36	$Ph(CH_2)_5O$	0.048	0.46	6.6
37	$PhCH_2O$	2.2	16	4.9
38	4-$MeOPhCH_2O$	>3[e]	20	4.8
39	4-NO_2PhCH_2O	>1[e]	5.3	4.6

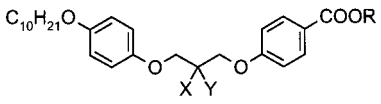
[a] Inhibition of $cPLA_2$ measured against an aggregated phospholipid substrate. IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] Inhibition of $cPLA_2$ measured against a soluble substrate. [c] Lipophilicity as calculated by Daylight.[35] [d] Not tested. [e] No inhibition at this concentration.

soluble substrate assay, suggesting that if the decyloxy chain binds to $cPLA_2$ then the lipophilic pocket is optimally filled with this substituent. This implies that the decyloxyphenoxymethyl carbonyl moiety of compound **22**, which was designed to mimic the arachidonyl group in substrate, is an excellent fit for the enzyme catalytic site.

Replacement of the decyloxy group of **22** with phenylpentyloxy gives a reasonably potent inhibitor of $cPLA_2$ (compound **36**, Table 5). However, comparison of this compound with the octyloxy-substituted analogue (**31**) shows that it is almost identical in terms of lipophilicity and potency. If the substituent at this position of the inhibitor is indeed binding within the enzyme, then a phenyl group at the end of the chain is clearly quite acceptable. Likewise, truncating the phenylpentyl group to a simple benzyl substituent produces a less potent inhibitor (**37**), but the similarity of the lipophilicity and activity of this compound with the butyloxy compound (**33**) further demonstrates that it is probably only the lipophilicity of each alkyl chain that contributes to activity. The corresponding para-methoxybenzyl compound **38** and the para-nitrobenzyl compound **39** show very similar potencies to the parent **37**.

Simple changes to the highly potent keto-acid **22**, such as esterification (compound **40**) or reduction (**41** and **42**), again largely destroy activity (see Table 6). While these effects were previously demonstrated for the earlier less potent inhibitor **4** (see Table 2), a comparison between the two series is quite illuminating. For instance, it is notable that the reduced hydroxy compounds of each series (**12** and **41**, respectively) give similar IC_{50} values in the bilayer assay, despite the parent ketones **4** and **22** having quite different potencies. This comparison lends support to the hypothesis that the level of activity seen with the alcohols in the bilayer assay is likely to be due to nonspecific inhibition. Furthermore, when alcohol **41** was tested in the soluble substrate assay, where no inhibition by interference with an aggregated substrate is possible per se, it was essentially inactive (see Table 6).

Table 6. Inhibition of Cytosolic PLA_2: Changes to the Ketone and Acid in the Benzoic Acid Series



compd	X, Y	R	bilayer assay IC_{50} (μM)[a]	soluble assay IC_{50} (μM)[b]	cLog P[c]
22	=O	H	0.008	0.030	7.8
40	=O	allyl	>10[d]	NT[e]	8.7
41	H, OH	H	1.3	53	7.7
42	H, H	H	>30[d]	26	8.8

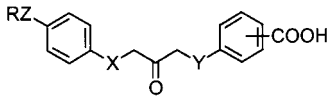
[a] Inhibition of $cPLA_2$ measured against an aggregated phospholipid substrate. IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] Inhibition of $cPLA_2$ measured against a soluble substrate. [c] Lipophilicity as calculated by Daylight.[35] [d] No inhibition at this concentration. [e] Not tested.

On the other hand, convincing evidence for the specific interaction of the active ketones of this series of inhibitors of $cPLA_2$ is demonstrated by simple changes to the activation of the electrophilic ketone moiety, as shown in Table 7. Compound **43** differs from **22** only by having a phenylpentylthio substituent on the aryl ring instead of the decyloxy group of the latter compound and shows similar high levels of potency against $cPLA_2$ in both aggregated and soluble substrate assays. Simple replacement of one of the linking oxygen atoms, which also serve to activate the ketone serine trap, by a methylene gives a compound (**44**) with very similar physical properties (compare cLogPs) but drastically reduced activity.

Similarly, in the meta-benzoic acid series, replacement of one of the activating oxygen atoms of compound **23** (Table 7) with methylene, as in compound **45**, leads to a substantial reduction in potency. Surprisingly, retaining the oxygen atom but inserting a methylene group between the oxygen and the decyloxyphenyl group, to give compound **46**, also reduces activity to a similar extent. While this insertion may reduce the electron withdrawing, and hence carbonyl-activating effect, of this group to a small degree, this is unlikely to be sufficient to account for the observed fall in potency. Indeed, an analogous insertion of a methylene group on the benzoic acid side, as in compound **30**, produced no fall in potency. Taken together, these results suggest that this series of inhibitors may be more tightly bound to enzyme around the alkoxy-substituted aryl ring than around the benzoic acid region. Because this alkoxy-substituted aryl ring was designed (see above) to mimic the Δ5,6 double bond of arachidonic acid, it may be hypothesized that insertion of an extra methylene group in this region of the inhibitor (as in compound **46**) dislocates the aryl ring such that it is no longer recognized by the enzyme as a bioisostere for the Δ5,6 double bond of the natural substrate.

In summary, we have described the design and synthesis of a completely novel series of inhibitors of $cPLA_2$ based on a 1,3-diheteroatom-substituted propan-2-one skeleton. These inhibitors show well-defined structure−activity relationships that point to the requirement of both lipophilic and electrophilic substituents for optimal potency. Most notably, the inhibitors show high levels of inhibitory potency in both aggregated bilayer-based and soluble substrate-based as-

Table 7. Inhibition of Cytosolic PLA_2: Effect of the Flanking Heteroatoms on Potency



compd	RZ	X	Y	acid	bilayer assay IC_{50} (μM)[a]	soluble assay IC_{50} (μM)[b]	cLog P[c]
43	$Ph(CH_2)_5S$	O	O	para	0.004	0.08	7.2
44	$Ph(CH_2)_5S$	O	CH_2	para	1.2	6.5	7.3
23	$C_{10}H_{21}O$	O	O	meta	0.050	NT[d]	7.8
45	$C_{10}H_{21}O$	CH_2	O	meta	0.690	2.0	8.0
46	$C_{10}H_{21}O$	CH_2O	O	meta	0.660	3.3	7.8
30	$C_{10}H_{21}O$	O	OCH_2	meta	0.035	0.15	7.7

[a] Inhibition of $cPLA_2$ measured against an aggregated phospholipid substrate. IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] Inhibition of $cPLA_2$ measured against a soluble substrate. [c] Lipophilicity as calculated by Daylight.[35] [d] Not tested.

Table 8. Inhibition of Cytosolic PLA_2: Comparison of Arachidonoyl Trifluoromethyl Ketone **1** and AR-C70484 **22**

compd	name	bilayer assay IC_{50} (μM)[a]	soluble assay IC_{50} (μM)[b]	HL60 cell assay IC_{50} (μM)[c]	cLog P[d]
1	$AACOCF_3$	NT[e]	0.800	29	7.9
22	AR-C70484XX	0.008	0.030	2.8	7.8

[a] Inhibition of $cPLA_2$ measured against an aggregated phospholipid substrate. IC_{50} values are the means of at least two independent determinations. Errors are within ±20%. [b] Inhibition of $cPLA_2$ measured against a soluble substrate. [c] Inhibition of ^3H-arachidonic acid production by stimulated HL60 cells. [d] Lipophilicity as calculated by Daylight.[35] [e] Not tested.

says and are among the most potent and druglike inhibitors of $cPLA_2$ described to date.[34] For comparison, compound **22** (AR-C70484XX) is more than 20-fold more potent than arachidonyl trifluoromethyl ketone (**1**) in the soluble substrate assay and also displays higher potency against arachidonic acid production in a cellular (HL60 cells) assay (see Table 8). Most importantly, compound **22**, by virtue of its design, is a "modular" molecule that, unlike arachidonyl trifluoromethyl ketone, lends itself to simple synthetic manipulation and therefore provides an excellent and potent starting point with which to further explore structure−activity relationships with $cPLA_2$. Studies on the reduction of both the high lipophilicity and the electrophilicity of this initial series of $cPLA_2$ inhibitors, while retaining potency against the enzyme, will be the subject of future papers.

Experimental Section

General. Proton magnetic resonance spectra were obtained on a Bruker AM360 (360 MHz) or Bruker AMX500 (500 MHz) instrument. Chemical shifts are reported as δ values (parts per million) downfield relative to Me_4Si as an internal standard. Mass spectra were obtained with Micromass Platform, Micromass LCT, and VG Sector spectrometers; ESI indicates electrospray ionization, APCI/NH_3 indicates atmospheric pressure chemical ionization in the presence of ammonia, FAB-(Xe) indicates fast atom bombardment ionization using xenon. The molecular ions were detected in either negative (−ve) or positive (+ve) mode. Combustion analyses were performed with a Carlo Erba EA1108 instrument. Melting points were determined on a Buchi 510 melting point apparatus with a silicone oil bath and are uncorrected. Chromatographies were carried out in flash mode using silica gel 60 (230−400 mesh) from E. Merck.

4-(Decyloxy)phenol (4a). Sodium hydride (10.92 g, 270 mmol, 60% dispersion in oil) was added batchwise over half an hour to a stirred solution of 1,4-hydroquinone (30 g, 270 mmol) in dry dimethylformamide (DMF, 700 mL) under nitrogen. After a further 20 min, a solution of decyl bromide (59.7 g, 270 mmol) in dry DMF (50 mL) was added over 5 min. The brown solution was stirred for 3 h, poured into water, and acidified with dilute hydrochloric acid. The reaction product was extracted with ethyl acetate, and the organic extract was washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by flash chromatography (hexane/ethyl acetate, 19:1 to 9:1) to give a white solid **4a** (24.23 g, 36%); mp 69−70 °C. ^1H nuclear magnetic resonance (NMR) ($CDCl_3$): δ 0.88 (t, 3H), 1.25−1.30 (m, 12H), 1.42 (m, 2H), 1.71−1.78 (m, 2H), 3.89 (t, 2H), 6.73−6.82 (m, 4H). Mass spectroscopy (MS) electron impact (EI): *m*/*z* 250 $(M)^+$.

2-[(4-(Decyloxy)phenoxy)methyl]oxirane (4b). A mixture of **4a** (23.0 g, 92 mmol), epichlorohydrin (42.6 g, 460 mmol), and cesium carbonate (30 g, 92 mmol) in acetonitrile (200 mL) was heated under reflux for 3 h. The mixture was cooled, poured into water, and extracted with ethyl acetate. The separated organic solution was washed with saturated brine solution and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (hexane/diethyl ether, 4:1) to give a white solid **4b** (25.56 g, 91%); mp 57−58 °C. ^1H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27−1.30 (m, 12H), 1.44 (m, 2H), 1.71−1.77 (m, 2H), 2.74−2.91 (m, 2H), 3.34 (m, 1H), 3.88 (t, 2H), 3.92−4.18 (m, 2H), 6.80−6.87 (m, 4H). MS (EI): *m*/*z* 306 $(M)^+$. Anal. ($C_{19}H_{30}O_3$) C, H.

1,1-Dimethylethyl 3-[(3-(4-(Decyloxy)phenoxy)-2-hydroxypropyl)thio]propanoate (4c). A solution of **4b** (2 g, 6.5 mmol) in acetonitrile (200 mL) was treated with 1,1-dimethylethyl 3-thiopropanoate (1.1 g, 9.8 mmol) and a catalytic amount of DABCO. The mixture was heated under reflux under nitrogen for 3 h, cooled, and then poured into water (400 mL). The precipitate was collected, dissolved in ethyl acetate, washed with brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the oily residue was purified by flash chromatography (hexane/ethyl acetate, 4:1) to give a colorless oil **4c** (1.76 g, 57%). ^1H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.24−1.27 (m, 14H), 1.45 (s, 9H), 1.71−1.77 (m, 2H), 2.52−2.56 (t, 2H), 2.71−2.90 (m, 4H), 3.88−3.91 (t, 2H), 3.97−3.99 (d, 2H), 4.07−4.15 (m, 1H), 6.83−6.86 (m, 4H). MS (EI): *m*/*z* 468 $(M)^+$.

1,1-Dimethylethyl 3-[(3-(4-(Decyloxy)phenoxy)-2-oxopropyl)thio]propanoate (4d). A solution of **4c** (1.79 g, 3.8 mmol) in dry DCM (5 mL) was treated with the Dess−Martin periodinane reagent (2.43 g, 5.7 mmol) and stirred under nitrogen at room temperature for 2 h. Ether (75 mL) and a solution of sodium thiosulfate (9.15 g, 37 mmol) in saturated sodium bicarbonate solution (75 mL) were added to the reaction mixture. After the mixture was stirred for 5 min, the organic layer was separated, washed with brine, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (hexane/ethyl acetate, 9:1) to give a colorless oil **4d** (1.14 g, 64%). ^1H

NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.24−1.28 (m, 14H), 1.45 (s, 9H), 1.71−1.77 (m, 2H), 2.53 (t, 2H), 2.77 (t, 2H), 3.42 (s, 2H), 3.89 (t, 2H), 4.71 (s, 2H), 6.84 (s, 4H). MS (EI): *m/z* 466 $(M)^+$.

3-[(3-(4-(Decyloxy)phenoxy)-2-oxopropyl)thio]propanoic Acid (4). A solution of **4d** (1.6 g, 3.3 mmol) in dry DCM (50 mL) was treated with trifluoroacetyl (TFA, 10 mL) and stirred at room temperature under nitrogen for 2 h. Toluene (30 mL) was added, and the solvents were evaporated under reduced pressure. The residue was recrystallized from ethyl acetate/hexane to give a white solid **4** (0.92 g, 80%); mp 95−96 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.71−1.77 (m, 2H), 2.68 (t, 2H), 2.81 (t, 2H), 3.44 (s, 2H), 3.89 (t, 2H), 4.70 (s, 2H), 6.84 (s, 4H). MS (EI): *m/z* 410 $(M)^+$. Anal. ($C_{22}H_{34}O_5S$) C, H, S.

The synthetic route described for the preparation of **4** was used to prepare compounds **2**, **3**, **5**−**12**, and **14**−**16**, starting with the appropriate protected acids and alkoxyphenols.

3-[(3-(2-Naphthyloxy)-2-oxopropyl)thio]propanoic Acid (2). mp 90−2 °C. ^{1}H NMR ($CDCl_3$): δ 2.68 (t, 2H), 2.82 (t, 2H), 3.48 (s, 2H), 4.88 (s, 2H), 7.01 (d, 1H), 7.23 (dd, 1H), 7.39 (t, 1H), 7.46 (t, 1H), 7.72 (d, 2H), 7.79 (d, 2H). MS (EI): *m/z* 304 (M^+). Anal. ($C_{16}H_{16}O_4S$) C, H, S.

3-[(3-(4-Phenoxyphenoxy)-2-oxopropyl)thio]propanoic Acid (3). mp 94−96 °C. ^{1}H NMR ($CDCl_3$): δ 2.69 (t, 2H), 2.82 (t, 2H), 3.45 (s, 2H), 4.75 (s, 2H), 6.88−7.06 (m, 6H), 7.26 (s, 1H), 7.31 (t, 2H). MS (EI): *m/z* 346 (M^+). Anal. ($C_{18}H_{18}O_5S$) C, H, S.

3-[(3-(4-Ethoxyphenoxy)-2-oxopropyl)thio]propanoic Acid (5). mp 78−79 °C. ^{1}H NMR ($CDCl_3$): δ 1.39 (t, 3H), 2.67 (t, 2H), 2.80 (t, 2H), 3.44 (s, 2H), 3.95−4.00 (q, 2H), 4.70 (s, 2H), 6.83 (s, 4H). MS (EI): *m/z* 370 (M + tetramethylsilane (TMS))$^+$. Anal. ($C_{14}H_{18}NO_5S$) C, H, S.

3-[(3-(4-Hexyloxyphenoxy)-2-oxopropyl)thio]propanoic Acid (6). mp 88−89 °C. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H), 1.3−1.5 (m, 6H), 1.73−1.77 (m, 2H), 2.67 (t, 2H), 2.81 (t, 2H), 3.44 (s, 2H), 3.89 (t, 2H), 4.70 (s, 2H), 6.83 (s, 4H). MS (EI): *m/z* 354 $(M)^+$. Anal. ($C_{18}H_{26}O_5S$) C, H, S.

3-[(3-(4-(Tetradecyloxy)phenoxy)-2-oxopropyl)thio]propanoic Acid (7). mp 91−93 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.2−1.4 (m, 20H), 1.45 (m, 2H), 1.72−1.76 (m, 2H), 2.67 (t, 2H), 2.81 (t, 2H), 3.44 (s, 2H), 3.89 (t, 2H), 4.70 (s, 2H), 6.83 (m, 4H). MS (EI): *m/z* 466 $(M)^+$. Anal. calcd for ($C_{26}H_{42}O_5S$) C, H, S, 6.87; found, 6.28.

3-[(3-(4-((2-Naphthyl)methoxy)phenoxy)-2-oxopropyl)-thio]propanoic Acid (8). mp 137−138 °C. ^{1}H NMR ($CDCl_3$): δ 2.66 (t, 2H), 2.79 (t, 2H), 3.43 (s, 2H), 4.69 (s, 2H), 5.18 (s, 2H), 6.85 (d, 2H), 6.95 (d, 2H), 7.47−7.53 (m, 3H), 7.82−7.87 (m, 4H). MS (EI): *m/z* 410 $(M)^+$. Anal. ($C_{23}H_{22}O_5S\cdot 0.5H_2O$) C, H, S.

3-[(3-(3-(Decyloxy)phenoxy)-2-oxopropyl)thio]propanoic Acid (9). mp 88 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.43 (m, 2H),1.76 (m, 2H), 2.65 (t, 2H), 2.81 (t, 2H), 3.44 (s, 2H), 3.92 (t, 2H), 4.74 (s, 2H), 6.46−6.56 (m, 3H), 7.18 (t, 1H). MS (EI): *m/z* 410 $(M)^+$. Anal. ($C_{22}H_{34}O_5S$) C, H, S.

3-[(3-(2-(Decyloxy)phenoxy)-2-oxopropyl)thio]propanoic Acid (10). mp 77 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.45 (m, 2H), 1.81 (m, 2H), 2.67 (t, 2H), 2.80 (t, 2H), 3.54 (s, 2H), 3.99 (t, 2H), 4.74 (s, 2H), 6.87−7.00 (m, 4H). MS (EI): *m/z* 410 $(M)^+$. Anal. ($C_{22}H_{34}O_5S$) C, H, S.

Methyl 3-[(3-(4-(Decyloxy)phenoxy)-2-oxopropyl)thio]-propanoate (11). mp 36−40 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27−1.30 (m, 12H), 1.44 (m, 2H), 1.77 (m, 2H), 2.63 (t, 2H), 2.81 (t, 2H), 3.43 (s, 2H), 3.70 (s, 3H), 3.90 (t, 2H), 4.71 (s, 2H), 6.84 (bs, 4H). MS (EI): *m/z* 424 $(M)^+$. Anal. ($C_{23}H_{36}O_5S\cdot 0.25H_2O$) C, H, S.

3-[(3-(4-(Decyloxy)phenoxy)-2-hydroxypropyl)thio]propanoic Acid (12). mp 102−104 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27−1.30 (m, 12H), 1.44 (m, 2H), 1.71−1.77 (m, 2H), 2.67 (t, 2H), 2.80−2.90 (m, 4H), 3.90 (t, 2H), 3.99 (d, 2H), 4.11 (m, 1H), 6.80−6.87 (m, 4H). MS (EI): *m/z* 412 $(M)^+$. Anal. ($C_{22}H_{36}O_5S$) C, H, S.

3-[(3-(4-(Decyloxy)phenoxy)-2-oxopropyl)thio]acetic Acid (14). mp 76−77 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.71−1.77 (m, 2H), 3.34 (s, 2H), 3.65 (s, 2H), 3.90 (t, 2H), 4.67 (s, 2H), 6.83 (m, 4H). MS (EI): *m/z* 396 $(M)^+$. Anal. calcd for ($C_{21}H_{32}O_5S$) C, H, S, 8.09; found, 7.38.

4-[(3-(4-(Decyloxy)phenoxy)-2-oxopropyl)thio]butanoic Acid (15). mp 90−91 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.71−1.77 (m, 2H), 1.89−1.96 (m, 2H), 2.48 (t, 2H), 2.59 (t, 2H), 3.39 (s, 2H), 3.89 (t, 2H), 4.71 (s, 2H), 6.84 (m, 4H). MS (EI): *m/z* 424 $(M)^+$. Anal. ($C_{23}H_{36}O_5S$) C, H, S.

5-[(3-(4-(Decyloxy)phenoxy)-2-oxopropyl)thio]pentanoic Acid (16). mp 88−89 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.43 (m, 2H), 1.64−1.77 (m, 6H), 2.37 (t, 2H), 2.54 (t, 2H), 3.38 (s, 2H), 3.89 (t, 2H), 4.72 (s, 2H), 6.84 (m, 4H). MS (ESI −ve): *m/z* 437 $(M - H)^-$. Anal. ($C_{24}H_{38}O_5S$) C, H, S.

3-[(3-(4-(Decyloxy)phenoxy)-2-oxopropyl)sulfinyl]propanoic Acid (17). A solution of **4d** (0.5 g, 1.1 mmol) in DCM (20 mL) was treated with *m*-chloroperbenzoic acid (0.27 g, 1.1 mmol). The reaction mixture was stirred at room temperature for 1 h and poured into a saturated aqueous sodium metabisulfite solution (20 mL). The organic layer was separated, washed with water and brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (hexane/ethyl acetate, 2:1) to give a solid (0.44 g). This solid was treated with a 4:1 mixture of TFA:DCM (25 mL) for 2 h at ambient temperature. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (hexane/ethyl acetate, 2:1 + 1% acetic acid) to give a white solid **17** (0.24 g, 62%); mp 116−117 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H), 1.25 (m, 12H), 1.38 (bs, 2H), 1.64−1.68 (m, 2H), 2.66 (t, 2H), 2.94−3.16 (m, 2H), 3.87 (t, 2H), 3.99−4.22 (dd, 2H), 4.83 (s, 2H), 6.82 (m, 4H), 12.55 (bs, 1H). MS (FAB-Xe): *m/z* 427 $(M + H)^+$. Anal. ($C_{22}H_{34}O_6S$) C, H, S.

3-[(3-(4-(Decyloxy)phenoxy)-2-oxopropyl)sulfonyl]propanoic Acid (18). A solution of **4d** (1.76 g, 3.8 mmol) in dry DCM (50 mL) was treated with *m*-chloroperbenzoic acid (2.04 g, 8.3 mmol) and stirred at room temperature for 2 h. Workup and deprotection were carried out as for **17** to give a white solid **18** (1.05 g, 63%); mp 138−140 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H), 1.25 (m, 12H), 1.38 (m, 2H), 1.63−1.68 (m, 2H), 2.71 (t, 2H), 3.48 (t, 2H), 3.88 (t, 2H), 4.62 (s, 2H), 4.89 (s, 2H), 6.83 (m, 4H), 12.64 (bs, 1H). MS (ESI −ve): *m/z* 441 $(M - H)^-$. Anal. ($C_{22}H_{34}O_7S\cdot 0.5H_2O$) C, H, S.

3-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]propanoic Acid (19). 1,3-Propanediol (1.1 mL, 15 mmol) was added to a stirred suspension of sodium hydride (0.36 g, 15 mmol) in anhydrous DMF (25 mL). After 15 min, **4b** (2 g, 6.5 mmol) was added, and the mixture was heated at 65 °C for 3.5 h. The reaction mixture was cooled and poured into dilute hydrochloric acid, and the product was extracted with ethyl acetate, which was washed with water and dried ($MgSO_4$). The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (hexane/ethyl acetate, 1:2) to give 3-[3-(4-(decyloxy)phenoxy)-2-hydroxypropoxy]propan-1-ol as a white solid (1.3 g). This solid (0.5 g, 1.3 mmol) and PDC (3.4 g, 9 mmol) in anhydrous DMF (25 mL) were stirred at room temperature for 18 h. The reaction mixture was poured into aqueous sodium metabisulfite solution and extracted with ethyl acetate, which was washed with brine and dried ($MgSO_4$). The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (hexane/ethyl acetate, 2:1 + 0.5% acetic acid) to give a white solid **19** (0.19 g, 37%); mp 69−70 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.43 (m, 2H), 1.73−1.77 (m, 2H), 2.72 (t, 2H), 3.82 (t, 2H), 3.89 (t, 2H), 4.42 (s, 2H), 6.82 (m, 4H). MS (EI): *m/z* 394 $(M)^+$. Anal. ($C_{22}H_{34}O_6\cdot 0.25H_2O$) C, H.

4-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]butanoic Acid (20). The procedure described for the preparation of **19** was applied to 1,4-butanediol to give **20** as a white solid; mp 70−72 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.73−1.77 (m, 2H), 1.97 (m, 2H), 2.51 (t, 2H), 3.58 (t,

2H), 3.89 (t, 2H), 4.36 (s, 2H), 4.67 (s, 2H), 6.83 (m, 4H). MS (ESI −ve): *m/z* 407 $(M - H)^-$. Anal. ($C_{23}H_{36}O_6$) C, H.

5-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]pentanoic Acid (21). The procedure described for the preparation of **19** was applied to 1,5-pentanediol to give **21** as a white solid; mp 69−71 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H), 1.25 (m, 12H), 1.40 (m, 2H), 1.54 (m, 2H), 1.64 (m, 2H), 2.24 (m, 2H), 2.50 (t, 2H), 3.44 (t, 2H), 3.87 (t, 2H), 4.27 (s, 2H), 4.79 (s, 2H), 6.82 (bs, 4H), 12.04 (bs, 1H). MS (ESI −ve): *m/z* 421 $(M - H)^-$. Anal. ($C_{24}H_{38}O_6$) C, H.

3-Propenyl 4-[3-(4-(Decyloxy)phenoxy)-2-hydroxypropoxy]benzoate (22a). 3-Propenyl 4-hydroxy benzoate (3.24 g, 18 mmol), prepared from 4-hydroxybenzoic acid, TMS-chloride, and 3-hydroxypropene, and **4b** (5.57 g, 18 mmol) in dry DMF (90 mL) were treated with a catalytic amount of DABCO (0.2 g) under nitrogen, and the mixture was heated under reflux for 3 h. The cooled solution was then poured into 2 N HCl (300 mL), extracted with ethyl acetate (3 × 75 mL), washed with brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (hexane/ethyl acetate, 4:1) to give a white powder **22a** (5.61 g, 64%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.26 (m, 12H), 1.42 (m, 2H), 1.76 (m, 2H), 2.59 (d, 1H), 3.90 (t, 2H), 4.11 (m, 2H), 4.19 (m, 2H), 4.39 (m, 1H), 4.80 (d, 2H), 5.43−5.27 (dd, 2H), 6.06 (m, 1H), 6.95 (m, 4H), 6.97 (d, 2H), 8.04 (d, 2H). MS (FAB-Xe): *m/z* 485 $(M + H)^+$.

3-Propenyl 4-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]benzoate (22b). Compound **22a** (4.85 g, 10 mmol) was oxidized by the procedure described for **4d** to afford a white solid **22b** (4.26 g, 88%); mp 68−69 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.43 (m, 2H), 1.76 (m, 2H), 3.91 (t, 2H), 4.79 (s, 2H), 4.84 (d, 2H), 4.99 (s, 2H), 5.29−5.39 (dd, 2H), 6.04 (m, 1H), 6.86 (m, 4H), 6.93 (d, 2H), 8.04 (d, 2H). MS (EI): *m/z* 482 $(M)^+$. Anal. ($C_{29}H_{38}O_6$) C, H.

4-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]benzoic Acid (22). A solution of **22b** (4.25 g, 8.8 mmol) in tetrahydrofuran (THF, 70 mL) was treated with $Pd(PPh_3)_4$ (1.15 g, 1 mmol), and nitrogen gas was bubbled through the solution for 20 min. Acetic acid (10 mL) was then added, and the reaction was stirred at room temperature under a nitrogen atmosphere for 4 h. A precipitate formed, which was collected and recrystallized from ethyl acetate to give a white solid **22** (3.02 g, 77%); mp 165−166 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.72−1.76 (m, 2H), 3.90 (t, 2H), 4.85 (s, 2H), 5.03 (s, 2H), 6.82 (d, 2H), 6.87 (d, 2H), 6.94 (d, 2H), 7.97 (d, 2H), 12.24 (bs, 1H). MS (ESI): *m/z* 442 $(M + H)^+$. Anal. ($C_{26}H_{34}O_6$, $0.25H_2O$) C, H.

The synthetic route described for the preparation of **22** was adapted to prepare compounds **23**−**41**, **43**, and **46** starting with the appropriate protected acids and alkoxyphenols, or in the case of **46**, alkoxybenzyl alcohol.

3-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]benzoic Acid (23). mp 133−134 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H), 1.25 (m, 12H), 1.38 (m, 2H), 1.66 (m, 2H), 3.88 (t, 2H), 4.95 (s, 2H), 5.10 (s, 2H), 6.85 (m, 4H), 7.21 (d, 2H), 7.41 (t, 1H), 7.44 (s, 1H), 7.55 (d, 1H), 13.09 (bs, 1H). MS (EI): *m/z* 442 $(M)^+$. Anal. ($C_{26}H_{34}O_6$) C, H.

4-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]phenylacetic Acid (24). mp 119−120 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.73−1.75 (m, 2H), 3.61 (s, 2H), 3.90 (t, 2H), 4.81 (s, 2H), 4.87 (s, 2H), 6.85 (m, 4H), 6.88 (d, 2H), 7.30 (d, 2H). MS (EI): *m/z* 456 $(M)^+$. Anal. ($C_{27}H_{36}O_6$) C, H.

3-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]phenylacetic Acid (25). mp 107−108 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.73−1.77 (m, 2H), 3.63 (s, 2H), 3.90 (t, 2H), 4.82 (s, 2H), 4.88 (s, 2H), 6.85 (m, 6H), 6.94 (d, 1H), 7.26 (d, 1H). MS (EI): *m/z* 456 $(M)^+$. Anal. ($C_{27}H_{36}O_6$) C, H.

[3-(3-(4-(Decyloxy)phenoxy)-2-oxopropoxy)phenoxy]acetic Acid (26). mp 111−112 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H), 1.25 (m, 12H), 1.39 (m, 2H), 1.63−1.69 (m, 2H), 3.88 (t, 2H), 4.65 (s, 2H), 4.92 (s, 2H), 4.99 (s, 2H), 6.50−6.54 (s and d, 3H), 6.82−6.88 (m, 4H), 7.18 (t, 1H), 13.05 (bs, 1H). MS (FAB-Xe): *m/z* 473 $(M + H)^+$. Anal. ($C_{27}H_{36}O_7$) C, H.

4-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]-3-methoxybenzoic Acid (27). mp 157−158 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.74−1.78 (m, 2H), 3.90 (t, 2H), 3.95 (s, 3H), 4.82 (s, 2H), 5.06 (s, 2H), 6.79 (d, 1H), 6.85 (m, 4H), 7.63−7.72 (m, 3H). MS (EI): *m/z* 472 $(M)^+$. Anal. ($C_{27}H_{36}O_7$) C, H.

4-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]-3-nitrobenzoic Acid (28). mp 165−167 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H), 1.25 (m, 12H), 1.39 (m, 2H), 1.67 (m, 2H), 3.88 (t, 2H), 4.95 (s, 2H), 5.35 (s, 2H), 6.84−6.90 (m, 4H), 7.39 (d, 1H), 8.12 (d, 1H), 8.37 (s, 1H), 13.40 (bs, 1H). MS (EI): *m/z* 487 $(M)^+$. Anal. ($C_{26}H_{33}NO_8 \cdot 0.25H_2O$) C, H, N.

4-[(3-(4-(Decyloxy)phenoxy)-2-oxopropoxy)methyl]benzoic Acid (29). mp 125−126 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H), 1.25 (m, 12H), 1.38 (m, 2H), 1.63−1.68 (m, 2H), 3.87 (t, 2H), 4.42 (s, 2H), 4.63 (s, 2H), 4.83 (s, 2H), 6.83 (m, 4H), 7.48 (d, 2H), 7.94 (d, 2H), 12.96 (bs, 1H). MS (ESI −ve): *m/z* 455 $(M - 1)^-$. Anal. ($C_{27}H_{36}O_6 \cdot 0.5H_2O$) C, H.

3-[(3-(4-(Decyloxy)phenoxy)-2-oxopropoxy)methyl]benzoic Acid (30). mp 95−97 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.30 (m, 12H), 1.43 (m, 2H), 1.72−1.76 (m, 2H), 3.89 (t, 2H), 4.43 (s, 2H), 4.68 (s, 2H), 4.71 (s, 2H), 6.82 (m, 4H), 7.50 (t, 1H), 7.63 (d, 1H), 8.01 (m, 2H). MS (EI): *m/z* 456 $(M)^+$. Anal. ($C_{27}H_{36}O_6 \cdot 0.5H_2O$) C, H.

4-[3-(4-(Octyloxy)phenoxy)-2-oxopropoxy]benzoic Acid (31). mp 162−163 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H), 1.2−1.45 (m, 10H), 1.63−1.68 (m, 2H), 3.88 (t, 2H), 4.93 (s, 2H), 5.12 (s, 2H), 6.83−6.89 (m, 4H), 7.01 (m, 2H), 7.87 (m, 2H), 12.67 (bs, 1H). MS (ESI −ve): *m/z* 413 $(M - H)^-$. Anal. ($C_{24}H_{30}O_6$) C, H.

4-[3-(4-(Hexyloxy)phenoxy)-2-oxopropoxy]benzoic Acid (32). mp 162−163 °C. ^{1}H NMR ($CDCl_3$): δ 0.90 (t, 3H), 1.3−1.4 (m, 4H), 1.73−1.77 (m, 2H), 3.90 (t, 2H), 4.81 (s, 2H), 4.98 (s, 2H), 6.85 (m, 4H), 6.91 (d, 2H), 8.00 (d, 2H). MS (EI): *m/z* 386 $(M)^+$. Anal. ($C_{22}H_{26}O_6$) C, H.

4-[3-(4-(Butoxy)phenoxy)-2-oxopropoxy]benzoic Acid (33). mp 156−157 °C. ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 3H), 1.36−1.47 (m, 2H), 1.62−1.70 (m, 2H), 3.89 (t, 2H), 4.94 (s, 2H), 5.13 (s, 2H), 6.83−6.90 (m, 4H), 7.02 (d, 2H), 7.88 (d, 2H), 12.69 (bs, 1H). MS (ESI +ve): *m/z* 359 $(M + H)^+$. Anal. ($C_{20}H_{22}O_6$) C, H.

4-[3-(4-(Ethoxy)phenoxy)-2-oxopropoxy]benzoic Acid (34). mp 172−173 °C. ^{1}H NMR (DMSO-d_6): δ 1.29 (t, 3H), 3.92−3.98 (q, 2H), 4.94 (s, 2H), 5.13 (s, 2H), 6.83−6.90 (m, 4H), 7.02 (d, 2H), 7.87 (d, 2H), 12.68 (bs, 1H). MS (ESI −ve): *m/z* 329 $(M - H)^-$. Anal. ($C_{18}H_{18}O_6$) C, H.

4-[3-(4-(Dodecyloxy)phenoxy)-2-oxopropoxy]benzoic Acid (35). mp 169−170 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.2−1.3 (m, 16H), 1.43 (m, 2H), 1.71 (m, 2H), 3.88 (t, 2H), 4.89 (s, 2H), 5.07 (s, 2H), 6.82−6.86 (m, 4H), 6.96 (d, 2H), 7.91 (d, 2H). MS (FAB-Xe): *m/z* 471 $(M + H)^+$. Anal. ($C_{28}H_{38}O_6$) C, H.

4-[3-(4-(5-Phenylpentyloxy)phenoxy)-2-oxopropoxy]benzoic Acid (36). mp 163−164 °C. ^{1}H NMR ($CDCl_3$): δ 1.45−1.55 (m, 2H), 1.67−1.82 (m, 4H), 2.65 (t, 2H), 3.91 (t, 2H), 4.95 (s, 2H), 5.01 (s, 2H), 6.86 (m, 4H), 6.94 (d, 2H), 7.18 (m, 3H), 7.28 (m, 2H), 8.05−8.08 (m, 2H). MS (EI): *m/z* 448 $(M)^+$. Anal. ($C_{27}H_{28}O_6$, H_2O) C, H.

4-[3-(4-(Phenylmethoxy)phenoxy)-2-oxopropoxy]benzoic Acid (37). mp 173−175 °C. ^{1}H NMR (DMSO-d_6): δ 3.77 (s, 2H), 3.89 (s, 2H), 4.01 (s, 2H), 6.32−6.49 (dd, 4H), 6.52 (d, 2H), 6.86−7.06 (m, 5H), 7.64 (d, 2H). MS (ESI −ve): *m/z* 391 $(M - H)^-$. Anal. ($C_{23}H_{20}O_6$, $0.25H_2O$) C, H.

4-[3-(4-((4-Methoxyphenyl)methoxy)phenoxy)-2-oxopropoxy]benzoic Acid (38). mp 196−198 °C. ^{1}H NMR ($CDCl_3$): 3.75 (s, 3H), 4.94 (bs, 4H), 5.14 (s, 2H), 6.91 (m, 6H), 7.00 (d, 2H), 7.35 (d, 2H), 7.87 (d, 2H). MS (FAB-Xe): *m/z* 422 $(M)^+$. Anal. ($C_{24}H_{22}O_7$) C, H.

4-[3-(4-((4-Nitrophenyl)methoxy)phenoxy)-2-oxopropoxy]benzoic Acid (39). mp 207−209 °C. ^{1}H NMR ($CDCl_3$): 4.87 (s, 2H), 5.03 (s, 2H), 5.18 (s, 2H), 6.92 (m, 6H), 7.67 (d, 2H), 7.97 (d, 2H), 8.23 (d, 2H). MS (TOF ES −ve): *m/z* 436.1027 $(M - H)^-$. ($C_{23}H_{19}NO_8$) requires 436.1033.

3-Propenyl 4-[3-(4-(Decyloxy)phenoxy)-2-oxopropoxy]-benzoate (40). mp 68−69 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.43 (m, 2H), 1.76 (m, 2H), 3.91 (t, 2H), 4.79 (s, 2H), 4.84 (d, 2H), 4.99 (s, 2H), 5.29−5.39 (dd, 2H), 6.04 (m, 1H), 6.86 (m, 4H), 6.93 (d, 2H), 8.04 (d, 2H). MS (EI): *m*/*z* 482 $(M)^+$. Anal. ($C_{29}H_{38}O_6$) C, H.

4-[3-(4-(Decyloxy)phenoxy)-2-hydroxypropoxy]benzoic Acid (41). mp 136−137 °C. ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H), 1.25 (m, 12H), 1.38 (m, 2H), 1.66 (m, 2H), 3.87 (t, 2H), 3.97 (m, 2H), 4.07−4.15 (m, 3H), 5.41 (s, 1H), 6.82−6.90 (m, 4H), 7.04 (d, 2H), 7.88 (d, 2H), 12.64 (bs, 1H). MS (ESI −ve): *m*/*z* 443 $(M - H)^-$. Anal. ($C_{26}H_{36}O_6 \cdot 0.25H_2O$) C, H.

4-[3-(4-(5-Phenylpentylthio)phenoxy)-2-oxopropoxy]-benzoic Acid (43). mp 131−132 °C. ^{1}H NMR (DMSO-d_6): δ 1.34−1.41 (m, 2H), 1.49−1.59 (m, 4H), 2.53 (t, 2H), 2.85 (t, 2H), 5.03 (s, 2H), 5.14 (s, 2H), 6.93 (d, 2H), 7.15−7.17 (s and d, 3H), 7.24−7.31 (2d, 4H), 7.90 (d, 2H), 12.72 (bs, 1H). MS (EI): *m*/*z* 464 $(M)^+$. Anal. ($C_{27}H_{28}O_5S$) C, H, S.

3-[3-((4-(Decyloxy)phenyl)methyl)-2-oxopropoxy]benzoic Acid (46). mp 104−106 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.2−1.3 (m, 16H), 1.45 (m, 2H), 1.76 (m, 2H), 3.95 (t, 2H), 4.30 (s, 2H), 4.57 (s, 2H), 4.85 (s, 2H), 6.89 (d, 2H), 7.14 (d, 1H), 7.27 (d, 2H), 7.41 (t, 1H), 7.57 (s, 1H), 7.76 (d, 1H). MS (ESI −ve): *m*/*z* 455 $(M - H)^-$. Anal. ($C_{27}H_{36}O_6$) C, H.

Methyl 4-[3-(4-(Decyloxy)phenoxy)propoxy]benzoate (42a). A solution of 1,3-dibromopropane (10 g, 50 mmol), **4a** (1.26 g, 5 mmol), and Cs_2CO_3 (1.8 g, 5.5 mmol) in acetonitrile was heated under reflux for 6 h. The cold mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was taken up in ethyl acetate (250 mL), washed with water (3 × 75 mL) and brine (100 mL), dried ($MgSO_4$), and evaporated to dryness. The solid residue was triturated with ice-cold acetonitrile to afford 3-(4-(decyloxy)phenoxy)propyl bromide as a white solid (1.23 g, 66%). MS (EI): 370/372 $(M)^+$. This solid (0.56 g, 1.5 mmol), methyl 4-hydroxybenzoate (0.25 g, 1.6 mmol), and Cs_2CO_3 (0.6 g, 1.8 mmol) in acetonitrile (25 mL) were heated under reflux for 6 h. The cold mixture was poured into water (250 mL) and extracted with DCM (250 mL). The extract was washed with water and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 10:1) to give a white solid **42a** (0.5 g, 75%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.26−1.30 (m, 12H), 1.43 (m, 2H), 1.70−1.78 (m, 2H), 2.22−2.28 (m, 2H), 3.88 (s, 3H), 3.90 (t, 2H), 4.11 (t, 2H), 4.21 (t, 2H), 6.82 (bs, 4H), 6.91 (d, 2H), 7.97 (d, 2H). MS (EI): *m*/*z* 422 $(M)^+$.

4-[3-(4-(Decyloxy)phenoxy)propoxy]benzoic Acid (42). $LiOH \cdot H_2O$ (0.2 g, 4.8 mmol) and **42a** (0.44 g, 1 mmol) in THF/H_2O (3:1, 20 mL) were stirred at room temperature for 18 h. The mixture was poured into 2 N HCl (100 mL) and extracted with ethyl acetate (2 × 100 mL). The extract was washed with water and brine, dried ($MgSO_4$), and evaporated under reduced pressure to give a white solid that was crystallized from ethyl acetate **42** (0.42 g, 98%); mp 149−150 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.26−1.30 (m, 12H), 1.42 (m, 2H), 1.69−1.76 (m, 2H), 2.21−2.28 (m, 2H), 3.91 (t, 2H), 4.10 (t, 2H), 4.21 (t, 2H), 6.78−6.84 (m, 4H), 6.92 (d, 2H), 7.95 (d, 2H). MS (EI): *m*/*z* 428 $(M)^+$. Anal. ($C_{26}H_{36}O_5 \cdot 0.25H_2O$) C, H.

3-[(3-(4-(Decyloxy)phenoxy)propyl)thio]propanoic Acid (13). The synthetic sequence used to prepare **42a** and **42** was applied to give **13**; mp 92−93 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.41 (m, 2H), 1.75 (m, 2H), 2.03 (m, 2H), 2.66 (t, 2H), 2.73 (t, 2H), 2.82 (t, 2H), 3.89 (t, 2H), 4.00 (t, 2H), 6.82 (m, 4H). MS (EI): *m*/*z* 396 $(M)^+$. Anal. ($C_{22}H_{36}O_4S$) C, H, S.

[4-(5-Phenylpentylthio)phenoxy]acetonitrile (44a). 4-((5-Phenylpentyl)thio)phenol (2.73 g, 10 mmol), bromoacetonitrile (1.44 g, 12 mmol), and cesium carbonate (4.8 g, 14 mmol) in acetonitrile (50 mL) were heated under reflux for 4 h. The cold mixture was poured into 2 N HCl (200 mL) and extracted with DCM (3 × 100 mL). The organic extract was washed with water and brine, dried ($MgSO_4$), and evaporated under reduced pressure to give a solid. This solid was passed down a silica gel column (hexane/DCM, 1:1) to give a colorless oil **44a** (2.39 g, 77%). ^{1}H NMR ($CDCl_3$): δ 1.40−1.48 (m, 2H), 1.56−1.66 (m, 4H), 2.59 (t, 2H), 2.84 (t, 2H), 4.73 (s, 2H), 6.90 (d, 2H), 7.15−7.19 (m, 3H), 7.27−7.32 (m, 2H), 7.35 (d, 2H). MS (EI): *m*/*z* 311 $(M)^+$.

[4-(5-Phenylpentylthio)phenoxy]acetaldehyde (44b). Dibal-H (1 M in toluene, 9.5 mL, 9.5 mmol) was added to a solution of **44a** (2 g, 6.5 mmol) in toluene (10 mL) at −40 °C, under a nitrogen atmosphere. The solution was allowed to warm to room temperature and stirred at this temperature for 1 h. Saturated ammonium chloride solution (20 mL) and ether (20 mL) were added followed by a solution of concentrated H_2SO_4 (8 mL) in water (32 mL). The mixture was stirred vigorously for 18 h and then extracted with ethyl acetate (250 mL). The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated to dryness to give a yellow oil **44b** (1.66 g, 82%). ^{1}H NMR ($CDCl_3$): δ 1.43−1.47 (m, 2H), 1.59−1.65 (m, 4H), 2.58 (t, 2H), 2.81 (t, 2H), 4.54 (s, 2H), 6.82 (d, 2H), 7.13−7.19 (m, 3H), 7.24−7.39 (m, 4H), 9.90 (s, 1H). MS (EI): *m*/*z* 314 $(M)^+$.

4-[4-(5-Phenylpentylthio)phenoxy]-3-hydroxybut-1-ene (44c). Vinylmagnesium bromide (1 M in THF, 7.5 mL, 7.5 mmol) was added to the crude aldehyde **44b** (∼1.7 g, 5.5 mmol) in THF (35 mL) at −10 °C, under a nitrogen atmosphere. It was stirred at −10 °C for 1 h and then allowed to warm to room temperature. A saturated solution of ammonium chloride (100 mL) was added, and the product was extracted into ethyl acetate (3 × 50 mL). The solvent was evaporated to dryness, and the residue was passed down a silica gel column (hexane/ethyl acetate, 4:1) to afford a clear oil **44c** (0.62 g, 33%). ^{1}H NMR ($CDCl_3$): δ 1.43−1.48 (m, 2H), 1.57−1.65 (m, 4H), 2.35 (d, 1H), 2.59 (t, 2H), 2.81 (t, 2H), 3.85−4.02 (m, 2H), 4.55 (m, 1H), 5.27−5.47 (m, 2H), 5.89−5.99 (m, 1H), 6.85 (d, 2H), 7.14−7.29 (m, 5H), 7.31 (2d, 2H).

4-[4-(4-(5-Phenylpentylthio)phenoxy)-3-oxobutyl]benzoic Acid (44). 4-Iodobenzoic acid (0.45 g, 1.8 mmol), **44c** (0.60 g, 1.75 mmol), and a catalytic amount of palladium(II) acetate (0.06 g) in acetonitrile (30 mL) were treated with triethylamine (10 mL) and heated under reflux for 18 h. The cold reaction mixture was filtered through Celite, poured into 2 N HCl (100 mL), and extracted with ethyl acetate (3 × 50 mL). The extract was washed with saturated sodium bicarbonate solution (2 × 50 mL), water, and brine, dried ($MgSO_4$), and evaporated to dryness. The residue was passed down a silica gel column (DCM + 3% MeOH) to give a white solid, which crystallized from ethyl acetate **44** (0.19 g, 23%); mp 141−142 °C. ^{1}H NMR ($CDCl_3$): δ 1.43−1.48 (m, 2H), 1.57−1.65 (m, 4H), 2.58 (t, 2H), 2.82 (t, 2H), 2.97−3.03 (m, 4H), 4.51 (s, 2H), 6.76 (d, 2H), 7.14−7.31 (m and d, 9H), 8.01 (d, 2H). MS (ESI −ve): *m*/*z* 461 $(M - H)^-$. Anal. ($C_{28}H_{30}O_4S$) C, H, S.

4-(4-Decyloxyphenyl)-2-oxobutane (45a). 4-(4-Hydroxyphenyl)-2-oxobutane (5.0 g, 30 mmol), decyl bromide (6.8 g, 30.5 mmol), and Cs_2CO_3 (10 g, 30.5 mmol) in DMF (50 mL) were stirred at room temperature for 18 h. The mixture was poured into 2 N HCl (500 mL) and extracted with ethyl acetate (2 × 250 mL). The extract was washed with water and then brine, dried ($MgSO_4$), and evaporated under reduced pressure to give a white solid **45a** (8.68 g, 94%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.24−1.30 (m, 12H), 1.43 (m, 2H), 1.71−1.78 (m, 2H), 2.13 (s, 3H), 2.69−2.74 (m, 2H), 2.80−2.84 (m, 2H), 3.92 (t, 2H), 6.82 (d, 2H), 7.05 (d, 2H).

4-(4-Decyloxyphenyl)-2-oxobutyl Bromide (45b). A solution of **45a** (3.05 g, 10 mmol) in DCM (100 mL) and methanol (50 mL) was treated with tetrabutylammonium tribromide (5.3 g, 11 mmol) and stirred at room temperature for 18 h. The solvent was removed under reduced pressure, and the residue was passed down a silica gel column (hexane/ethyl acetate, 20:1) to afford the product **45b** as a colorless oil (2.03 g, 53%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.72−1.78 (m, 2H), 2.87−2.96 (m, 4H), 3.84 (s, 2H), 3.92 (t, 2H), 6.81 (d, 2H), 7.08 (d, 2H). MS (EI): *m*/*z* 382/384 $(M)^+$.

3-Propenyl 3-[4-(4-Decyloxyphenyl)-2-oxobutoxy]benzoate (45c). 3-Propenyl 3-hydroxybenzoate (0.32 g, 1.8 mmol) and **45b** (0.60 g, 1.5 mmol) in DMF (10 mL) were treated with KF (0.175 g, 3 mmol) and stirred at room temperature for 2 h

and then at 100 °C for 3 h. The mixture was partitioned between 2 N HCl (100 mL) and ethyl acetate (250 mL). The organic phase was washed with ammonium chloride water and brine, dried ($MgSO_4$), and evaporated to dryness. The residue was passed down a silica gel column (hexane/ethyl acetate, 7:1) to afford a colorless oil **45c** (0.59 g, 80%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.27 (m, 12H), 1.44 (m, 2H), 1.72−1.78 (m, 2H), 2.90 (bs, 4H), 3.92 (t, 2H), 4.56 (s, 2H), 4.82 (d, 2H), 5.28−5.43 (dd, 2H), 5.98−6.07 (m, 1H), 6.81 (d, 2H), 7.04−7.11 (m, 3H), 7.35 (t, 1H), 7.53 (bs, 1H), 7.71 (d, 1H).

3-[4-(4-Decyloxyphenyl)-2-oxobutoxy]benzoic Acid (45). Deprotection of **45c** as described for **22** gave a white solid **45**; mp 109−110 °C. ^{1}H NMR (DMSO-d_6): δ 0.94 (t, 3H), 1.3−1.47 (m, 12H), 1.56 (m, 2H), 1.76 (m, 2H), 2.8−2.9 (m, 4H), 3.98 (t, 2H), 4.97 (s, 2H), 6.90 (d, 2H), 7.16−7.20 (m, 3H), 7.46 (t, 2H), 7.61 (d, 1H). MS (EI): *m*/*z* 440 $(M)^+$. Anal. ($C_{27}H_{36}O_5$) C, H.

Assay of cPLA$_2$ Using a Bilayer Substrate. (a) Preparation of Substrate. The substrate was prepared by mixing 1-stearoyl-2-[^{14}C]arachidonylphosphatidylcholine (36.8 μL of a 0.18 MBq/mL solution in ethanol, specific activity 2.02 GBq/mmol), 1-stearoyl-2-arachidonylphosphatidylcholine (PC) (19.1 μL of a 10 mg/mL solution in ethanol/$CHCl_3$ (4:1)), phosphatidylinositol 4,5-bisphosphate (PIP_2) (18.1 μL of a 0.5 mg/mL solution in methanol/$CHCl_3$/H_2O/1 M aqueous HCl (20:9:1:0.01)), and diacylglycerol (DAG) (36.8 μL of a 1 mg/mL solution in methanol) in a polypropylene test tube (50 mL), and the mixture was dried thoroughly under a stream of nitrogen. Tris HCl, pH 7.4, buffer (17 mL of 50 mM solution) was added, and the mixture was vortex mixed for 60 s and then sonicated at room temperature for 2 min. The tube was placed in dry ice until frozen (approximately 20 min). The frozen substrate was sonicated at room temperature until thawed (approximately 15 min) and then finally vortex mixed for 60 s.

(b) Assay of cPLA$_2$ Activity. $CaCl_2$ (50 μL of a 100 μM aqueous solution) and KCl (50 μL of a 1 M aqueous solution) were added to Tris HCl, pH 7.4, buffer (135 μL of a 50 mM solution) in each tube of a 96 well plate, followed by substrate (250 μL) prepared as above. A solution of inhibitor in DMSO (5 μL) was then added. cPLA$_2$ enzyme was diluted appropriately in assay buffer to give approximately 10% conversion when 10 μL was used. The final assay composition was 7.4 μM 1-stearoyl-2-arachidonylphosphatidylcholine (containing 30 000 dpm ^{14}C substrate), 0.24 μM PIP_2, 1.7 μM DAG, 10 μM $CaCl_2$, and 100 mM KCl in assay buffer. The final assay volume was 500 μL. The reaction was started with enzyme and performed at 37 °C for 10 min and then stopped with a 25 mM aqueous solution of ethylenediaminetetraacetic acid containing 2.5% Triton X-100 (500 μL). The released arachidonate was extracted by adding sufficient anhydrous sodium sulfate to saturate each tube, followed by acetic acid in hexane (5 mL, 0.4% (v/v)). The organic layer was transferred to a scintillation vial and evaporated under a stream of air, and the released radioactivity was determined by liquid scintillation counting. Inhibition of enzyme activity was determined by comparing to control reaction with no inhibitor added, after correcting for radioactivity released in the absence of enzyme.

Assay of cPLA$_2$ Using a Soluble Substrate. A stock solution of substrate was prepared by adding 7-(2-Oxo-6-heptenoxy)-2H-1-benzopyran-2-one in DMSO (20 mM) to a *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES), pH 7.2, buffer (50 mM), containing KCl (315 mM) and EGTA (3.15 mM), to give a final substrate concentration of 31 μM. A solution of inhibitor in DMSO (5 μL) was added to each well of a microtiter plate followed by 100 μL of the substrate solution. The reaction was started by the addition of purified cPLA$_2$ enzyme diluted in the same buffer as the substrate (100 μL). The fluorescence was measured immediately in a Molecular Devices fMax fluorescence plate reader at 355 nm excitation, 460 nm emission filter settings. The plates were incubated at room temperature in the dark for 2 h, and the fluorescence was measured again in the fMax plate reader. Inhibition of enzyme activity was determined by comparing the increase in fluorescence with control reaction with no inhibitor added, after correcting for background hydrolysis in the absence of enzyme.

Ionophore-Induced Release of Arachidonic Acid from DMSO-Differentiated HL60 Cells. The ability of compounds to inhibit cPLA$_2$ activity in intact cells was evaluated using HL60s differentiated with DMSO to resemble neutrophils. HL60 cells were differentiated by resuspending at 10^6 cells/mL in 1.3% DMSO in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 iu/mL penicillin, 100 iu/mL streptomycin, and 2.5 μg/mL amphotericin B. Cultures were used for compound evaluation on days 4 and 5 at which point they reached their maximum ability to mobilize incorporated arachidonic acid in response to ionophore. Cells were incubated with ^{3}H-arachidonic acid for a long enough time period for labeling of choline, ethanolamine, serine, and inositol-containing phospholipids to approach equilibrium. ^{3}H-arachidonic acid (7 GBq/mmol) was added to differentiating cells on day 3 or 4 giving 5500 Bq/mL of culture. Cells were spun down and washed twice with 20% of the starting volume of Tyrodes buffer (137 mM NaCl, 5.7 mM glucose, 10 mM HEPES buffer, 27 mM KCl, 0.4 mM NaH_2PO_4, 1.8 mM $CaCl_2$, and 1.0 mM $MgCl_2$, pH 7.4) and finally resuspended in Tyrodes buffer. The assay was performed in 96 well polypropylene plates. Compounds were initially dissolved in DMSO and then diluted in Tyrodes buffer to give a constant 3.75% DMSO. A solution of inhibitor (40 μL) was preincubated with cells (90 μL, 4×10^5) for 10 min at 37 °C before initiating release of arachidonic acid by the addition of calcium ionophore A23187 (20 μL of a 15 μM solution in Tyrodes buffer containing bovine serum albumin (BSA) (7.5 mg/mL)). Addition of BSA alone to control wells was used to estimate background release. After 3 min, ice-cold methanol (150 μL) was added to stop the reaction, and the mixture was left at 0 °C for 20 min. A portion (200 μL) was removed from each well for filtration through a 96 well filter plate, and the filtrate (100 μL) was dried onto a Luma-Plate for counting. Recovery of released arachidonic acid was 80% by this process. Background release in the absence of ionophore (less than 30% of the release in the presence of ionophore) was subtracted before calculating the percentage inhibition.

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