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# Potent and Selective Fluoroketone Inhibitors of Group VIA Calcium-Independent Phospholipase $A_2$

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Group VIA calcium-independent phospholipase $A_2$ (GVIA iPLA$_2$) has recently emerged as a novel pharmaceutical target. We have now explored the structure−activity relationship between fluoroketones and GVIA iPLA$_2$ inhibition. The presence of a naphthyl group proved to be of paramount importance. 1,1,1-Trifluoro-6-(naphthalen-2-yl)hexan-2-one (FKGK18) is the most potent inhibitor of GVIA iPLA$_2$ ($X_I(50) = 0.0002$) ever reported. Being 195 and $>455$ times more potent for GVIA iPLA$_2$ than for GIVA cPLA$_2$ and GV sPLA$_2$, respectively, makes it a valuable tool to explore the role of GVIA iPLA$_2$ in cells and in vivo models. 1,1,1,2,2,3,3-Heptafluoro-8-(naphthalene-2-yl)octan-4-one inhibited GVIA iPLA$_2$ with a $X_I(50)$ value of 0.001 while inhibiting the other intracellular GIVA cPLA$_2$ and GV sPLA$_2$ at least 90 times less potently. Hexa- and octafluoro ketones were also found to be potent inhibitors of GVIA iPLA$_2$; however, they are not selective.

## Introduction

The phospholipase $A_2$ (PLA$_2$) superfamily consists of many different groups of enzymes that catalyze the hydrolysis of the ester bond at the sn-2 position of various phospholipids.[1] The products of the hydrolysis are a free fatty acid and a lysophospholipid, both of which may generate second messengers that play important physiological roles. The PLA$_2$ superfamily currently contains 15 separate, identifiable groups and various subgroups.[2,3] The three predominant types of PLA$_2$ found in human tissues are the cytosolic (such as the GIVA cPLA$_2$[a]), the secreted (such as the GIIA and GV sPLA$_2$), and the calcium-independent (such as the GVIA iPLA$_2$) enzymes. GIVA cPLA$_2$ is generally considered a proinflammatory enzyme that is the rate-limiting provider of arachidonic acid and lysophospholipids.[4] In many cases, the activity of secreted PLA$_2$ has been shown to be dependent on or linked to the activity of GIVA cPLA$_2$.[5−7] The calcium-independent group VIA iPLA$_2$ (GVIA iPLA$_2$), typically referred to in the literature as iPLA$_2$, is actually a group of cytosolic enzymes ranging from 85 to 88 kDa and expressed as several distinct splice variants of the same gene.[8] GVIA iPLA$_2$ has long been proposed as a homeostatic enzyme involved in basal metabolism within the cell.[9−15] However, a number of studies suggest that GVIA iPLA$_2$ also plays important roles in numerous cell types, although they may differ from cell to cell. Recent review articles discuss the role of GVIA iPLA$_2$ in signaling and pathological conditions (for example, cancer and ischemia).[16−20]

The GVIA iPLA$_2$ enzyme contains a consensus lipase motif, Gly-Thr-Ser*-Thr-Gly, with the catalytic serine confirmed by site-directed mutagenesis.[8,21] Both of the intracellular enzymes GIVA cPLA$_2$ and GVIA iPLA$_2$ share the same catalytic mechanism utilizing a serine residue as the nucleophile. The various inhibitor classes of both enzymes are summarized in a recent review article.[22] Arachidonyl trifluoromethyl ketone has been shown to function as a tight binding, reversible inhibitor of both GIVA and GVIA PLA$_2$,[23,24] while methylarachidonyl fluorophosphonate functions as an irreversible inhibitor of both enzymes.[25] Bromoenol lactone (BEL) (**1**, Figure 1) has previously been considered to be a selective and irreversible GVIA iPLA$_2$ inhibitor and has been widely applied to study potential biological roles for GVIA iPLA$_2$.[11,26] Turk et al. have recently studied the inactivation mechanism of GVIA iPLA$_2$ by **1** (BEL),[27] and they concluded that it is likely that this inhibitor affects multiple enzymes and should be used with appropriate caution when studying potential roles of GVIA iPLA$_2$. Our laboratories have previously reported on the development of 2-oxoamide inhibitors targeting GIVA cPLA$_2$.[28−32] We have demonstrated that 2-oxoamides containing a free carboxyl group are selective inhibitors of GIVA cPLA$_2$, and most recently we have determined the location of such an inhibitor bound in the active site of GIVA cPLA$_2$ using a combination of deuterium exchange

*To whom correspondence should be addressed. For G.K.: (phone) (30210) 7274462; (fax) (30210) 7274761; (e-mail) gkokotos@chem.uoa.gr. For E.A.D.: (phone) 858-534-3055; (fax) 858-534-7390; (e-mail) edennis@ucsd.edu.

[a] Abbreviations: ATP, adenosine triphosphate; BEL, bromoenol lactone, BSA, bovine serum albumin; DAST, diethylaminosulfur trifluoride; DMAP, 4-dimethylaminopyridine; DPPC, 1,2-dipalmitoylphosphatidylcholine; DTT, dithiothreitol; EtOAc, ethyl acetate; GIVA cPLA$_2$, group IVA cytosolic phospholipase $A_2$; GV sPLA$_2$, group V secreted phospholipase $A_2$; GVIA iPLA$_2$, group VIA calcium-independent phospholipase $A_2$; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NMM, *N*-methylmorpholine; PAPC, 1-palmitoyl-2-arachidonylphosphatidylcholine; PIP$_2$, phosphatidylinositol (4,5)-bisphosphate; TBAF, tetra-*n*-butylammonium fluoride; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; Tris, tris(hydroxymethyl)aminomethane; TMS, tetramethylsilane; WSCI·HCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride.

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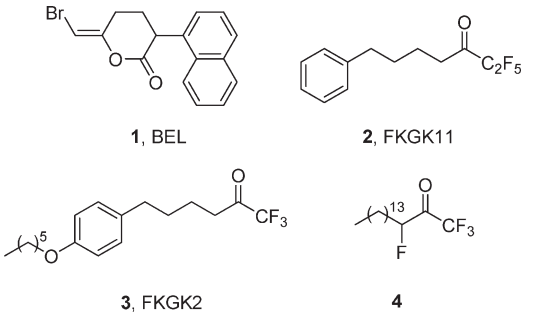



**Figure 1.** Some known inhibitors of GVIA iPLA$_2$.

mass spectrometry and molecular dynamics.[33] 2-Oxoamides based on amino acid esters show cross-reactivity for both GIVA cPLA$_2$ and GVIA iPLA$_2$,[30,32] while most recently we identified a 2-oxoamide based on a pseudodipeptide that preferentially inhibits GVIA iPLA$_2$.[34]

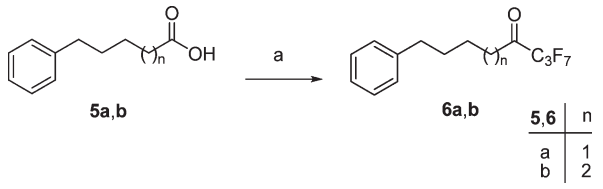
The development of selective inhibitors for the three main human PLA$_2$ enzymes is an important goal, and we have synthesized and assayed a variety of polyfluoroketones for their activity on GIVA cPLA$_2$, GVIA iPLA$_2$, and GV sPLA$_2$. We previously found that 1,1,1,2,2-pentafluoro-7-phenylheptan-3-one (FKGK11)[35] (**2**, Figure 1) is a selective inhibitor of GVIA iPLA$_2$.[36] Trifluoromethyl ketone **3** (FKGK2, Figure 1) can be considered to be a pan inhibitor of all three enzymes: GIVA cPLA$_2$, GVIA iPLA$_2$, and GV sPLA$_2$. The tetrafluoro derivative **4** was found to be the most potent GVIA iPLA$_2$ inhibitor, although it is not selective.[36] The selective GVIA iPLA$_2$ inhibitor **2** was successfully used to study the role of this enzyme in neurological disorders such as peripheral nerve injury and multiple sclerosis.[37,38] We successfully demonstrated that inhibitor **2** causes a beneficial therapeutic effect in experimental autoimmune encephalomyelitis,[38] the animal model of multiple sclerosis. This indicates that GVIA iPLA$_2$ is a novel target for the development of new therapies for multiple sclerosis. The recently emerged important pharmaceutical significance of GVIA iPLA$_2$ and the lack of potent and selective GVIA iPLA$_2$ inhibitors prompted us to extend our studies toward the discovery of such inhibitors. In this work, we report the synthesis of a variety of new fluoroketones and the study of their selectivity on the three main human phospholipase A$_2$ compounds.

## Design and Synthesis of Polyfluoroketones

The rationale behind our design of polyfluoroketones was based on the hypothesis that the introduction of more than three fluorine atoms adjacent to a carbonyl group may increase either the carbonyl reactivity or the inhibitor binding affinity to the target enzyme.[36] This hypothesis was confirmed, and in fact, such a design led to the selective GVIA iPLA$_2$ inhibitor **2** (pentafluoroethyl ketone) and the tetrafluoro derivative **4**, which is a potent GVIA iPLA$_2$ inhibitor, although it is not a selective inhibitor. In the present work, our aim was to extend the structure−activity relationship studies on the potency and the selectivity of heptafluoropropyl ketones and analogues of the lead GVIA iPLA$_2$ inhibitors **2**, **3**, and **4**.

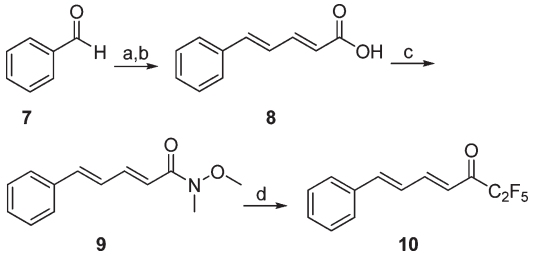
For the synthesis of heptafluoropropyl ketones, carboxylic acids **5a**,**b** were converted to chlorides by treatment with oxalyl chloride and then to the target compounds **6a**,**b** using heptafluorobutanoic anhydride and pyridine (Scheme 1). Wadsworth−Horner−Emmons reaction of benzaldehyde

**Scheme 1**[a]




[a] Reagents and conditions: (a) (i) $(COCl)_2$, $CH_2Cl_2$; (ii) $(C_3F_7CO)_2O$, pyridine, $CH_2Cl_2$.

**Scheme 2**[a]




[a] Reagents and conditions: (a) $C_2H_5OOCCH{=}CHCH_2P(=O)(OC_2H_5)_2$, LiOH, THF; (b) NaOH, 1,4-dioxane; (c) DMAP, NMM, WSCI·HCl, $CH_3ONHCH_3$·HCl, $CH_2Cl_2$; (d) $CF_3CF_2I$, $CH_3Li$·LiBr, $Et_2O$.

(**7**) with triethyl phosphonocrotonate,[39] followed by saponification, led to unsaturated acid **8** (Scheme 2). We previously showed that α,β-unsaturated acids may be converted into pentafluoroethyl ketones by treatment of the corresponding Weinreb amide with (pentafluoroethyl)lithium.[40] Following this procedure, the Weinreb amide **9** was converted to the unsaturated pentafluoroethyl ketone **10**.

Various trifluoromethyl, pentafluoroethyl, and heptafluoropropyl ketones **12a**−**i** were synthesized as depicted in Scheme 3. Reaction of furfural (**13**) with triethyl phosphonocrotonate, followed by hydrogenation and saponification, produced acid **11b**. However, treatment of this acid with oxalyl chloride, followed by $(C_2F_5CO)_2O$ and pyridine, led to the formylated derivative **12b**. Under these conditions we were unable to prepare the nonformylated derivative.

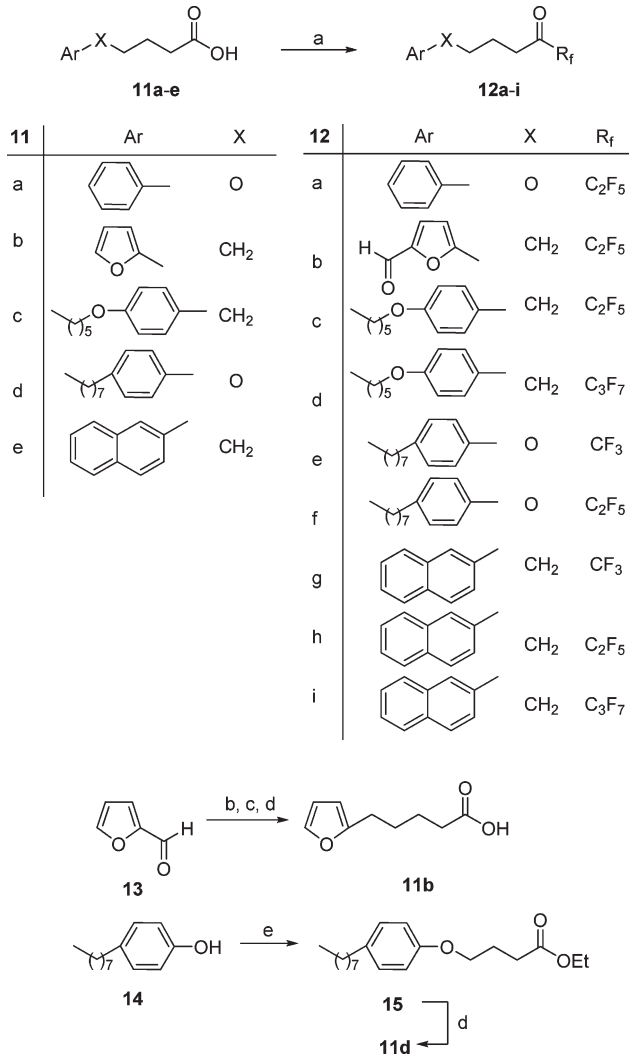
The synthesis of tetrafluoro derivatives **18a**,**b** was accomplished by procedures developed earlier[36] (Scheme 4). On the basis of $^1$H and $^{19}$F NMR data, tetrafluoro derivatives **18a**,**b** appear to be a mixture of ketone−hydrate form.

Fluoroketones **19**−**25** (for structures, see Table 1), which were used in the in vitro assays, were prepared as described previously.[40]

## In Vitro Inhibition of GVIA iPLA$_2$, GIVA cPLA$_2$, and GV sPLA$_2$

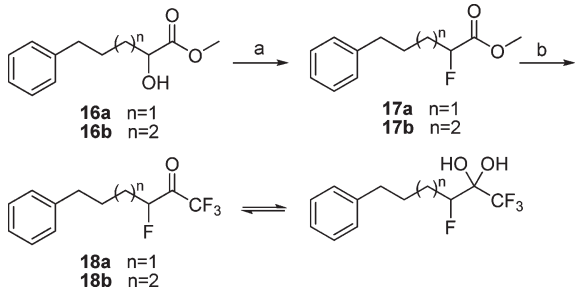
All synthesized inhibitors were tested for inhibition of human GVIA iPLA$_2$ based on a modification of the previously described mixed micelle-based assay.[30] The mixed micelle assay employed herein used 1-palmitoyl-2-arachidonylphosphatidylcholine (PAPC) as substrate, and the specific conditions employed herein were somewhat different from those employed in the previous mixed micelle assay which employed 1,2-dipalmitoylphosphatidylcholine (DPPC) as substrate. This change was made in order to use the same substrate for iPLA$_2$ as for cPLA$_2$, to better compare the specificities of both iPLA$_2$ and cPLA$_2$ toward the same substrate. This also improved the consistency of the standard

**Scheme 3**[a]




[a] Reagents and conditions: (a) (i) $(COCl)_2$, $CH_2Cl_2$; (ii) $(CF_3CO)_2O$ or $(C_2F_5CO)_2O$ or $(C_3F_7CO)_2O$, pyridine, $CH_2Cl_2$; (b) $C_2H_5OOCCH{=}CHCH_2P(=O)(OC_2H_5)_2$, LiOH, THF; (c) $H_2$, 10% Pd/C; (d) NaOH, $CH_3OH$; (e) $Br(CH_2)_3COOEt$, $K_2CO_3$, acetone.

**Scheme 4**[a]




[a] Reagents and conditions: (a) DAST, $CH_2Cl_2$ or $(CH_3OCH_2CH_2)_2NSF_3$, $CH_2Cl_2$; (b) (i) $(CH_3)_3SiCF_3$, CsF, $CH_3OCH_2CH_2OCH_3$ or $(CH_3)_3SiCF_3$, TBAF, toluene; (ii) conc HCl or TBAF, $CH_3COOH$, THF.

error in the assay. By use of this more refined assay, a $X_I(50) = 0.0014$ was determined for the lead inhibitor **2**, lower than that determined previously (0.0073).[36] To test the selectivity of the synthesized inhibitors toward GIVA $cPLA_2$ and GV $sPLA_2$, the previously reported mixed micelle-based assays were used.[28,29,31] The resulting values of GVIA $iPLA_2$ inhibition are presented in Figure 2 as either percent inhibition or $X_I(50)$ values. Initially, the percent of inhibition for each $PLA_2$ enzyme at 0.091 mol fraction of each inhibitor was determined, and $X_I(50)$ values were determined for all compounds toward GVIA $iPLA_2$ and for the other two enzymes for all inhibitors that displayed greater than 90% inhibition. However, for two additional $iPLA_2$ inhibitor examples, we also determined their $X_I(50)$ toward $cPLA_2$ in order to calculate their relative specificities. The $X_I(50)$ is the mole fraction of the inhibitor in the total substrate interface required to inhibit the enzyme by 50%. The inhibition results for all three enzymes are summarized in Table 1.

The replacement of the pentafluoroethyl group of inhibitor **2** ($X_I(50) = 0.0014$) by the heptafluoropropyl group led to inhibitor **6a** ($X_I(50) = 0.0022$), which resulted in a slightly decreased potency of the GVIA $iPLA_2$ inhibition. Extension of the carbon chain by one carbon atom produced inhibitor **6b**, which also resulted in a slightly decreased potency ($X_I(50) = 0.0030$). Compounds **19** and **20**, which carry a hydroxyl group instead of the carbonyl group of inhibitors **2** and **6a**, were surprisingly found to be inhibitors of GVIA $iPLA_2$, although they were not potent. They were also weak inhibitors of the other two $PLA_2$ compounds.

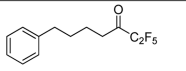
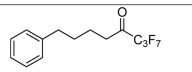
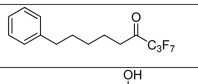
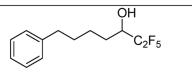
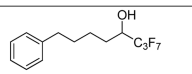
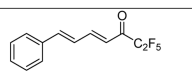
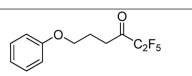
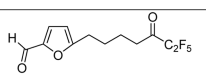
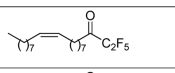
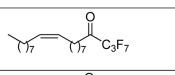
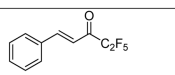
We observed that the insertion of two unsaturated bonds, while keeping the distance between the phenyl and the activated carbonyl group constant, significantly reduced the inhibitory activity of **10** by 22 times. When the carbon atom next to the phenyl group of **2** was replaced by oxygen, compound **12a** was 2.5 times less potent than inhibitor **2**. At the same time, we found that inhibitor **12a** is selective for GVIA $iPLA_2$, since a high mole fraction of the inhibitor (0.091) does not inhibit either GIVA $cPLA_2$ or GV $sPLA_2$ at all. The furan-based inhibitor **12b** was not a potent inhibitor.

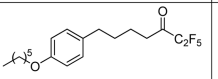
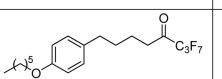
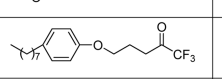
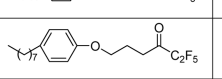
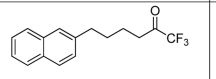
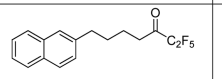
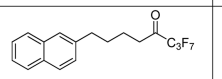
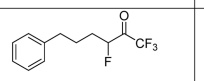
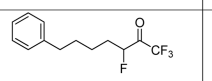
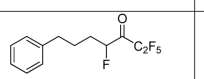
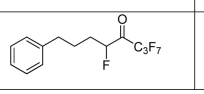
Pentafluoroethyl derivative **21** based on the oleyl chain inhibited GVIA $iPLA_2$ better than the corresponding palmitoyl derivative.[36] Heptafluoropropyl derivative **22** was a weaker inhibitor of GVIA $iPLA_2$ than derivative **21**. Both **21** and **22** were able to inhibit weakly the other two enzymes. These data indicate that for the inhibition of GVIA $iPLA_2$ a chain bearing an aromatic ring rather than a long aliphatic saturated or unsaturated chain has to be attached to the activated carbonyl. Reducing the distance between the phenyl and the carbonyl group and inserting a trans double bond led to compound **23**, which inhibits GVIA $iPLA_2$ with $X_I(50) = 0.0066$. This derivative does not inhibit at all the other intracellular enzyme GIVA $cPLA_2$ and inhibits weakly GV $sPLA_2$ (77%) at 0.091 mol fraction.

Both the *p*-hexyloxy substituted pentafluoroethyl and heptafluoropropyl derivatives **12c** and **12d** inhibited GVIA $iPLA_2$ with $X_I(50)$ of 0.0084 and 0.0136, respectively. Comparing **12c** and **12d** with **2** and **6a**, it seems that the *p*-hexyloxy substitution had a negative effect on the GVIA $iPLA_2$ inhibition. Interestingly, when the oxygen atom was moved between the aromatic group and the carbonyl next to the phenyl group, both the trifluoromethyl and the pentafluoroethyl derivatives **12e** and **12f** strongly inhibited GVIA $iPLA_2$ ($X_I(50)$ of 0.0029 and 0.0024, respectively). However, all the derivatives bearing a substituent at the para position failed to be selective for any $PLA_2$ enzyme.

The replacement of the phenyl group of inhibitor **2** by a naphthyl group led to excellent results. 1,1,1-Trifluoro-6-(naphthalen-2-yl)hexan-2-one (FKGK18, **12g**)[35] proved to

**Table 1.** Inhibition of $PLA_2$ by Fluoroketones[a]

| No | Structure | GVIA iPLA$_2$ | | GIVA cPLA$_2$ | | GV sPLA$_2$ |
|---|---|---|---|---|---|---|
| | | % Inhibition | $X_I(50)$ | % Inhibition | $X_I(50)$ | % Inhibition |
| **2** |  | 99.4 ± 0.1 | 0.0014 ± 0.0001 | N.D. | | 28 ± 1 |
| **6a** |  | 99.4 ± 0.0 | 0.0022 ± 0.0001 | 32.6 ± 4.0 | | 61.8 ± 6.7 |
| **6b** |  | 98.4 ± 0.3 | 0.0030 ± 0.0002 | N.D. | | N. D. |
| **19** |  | 94.3 ± 1.5 | 0.0390 ± 0.0024 | 44.8 ± 3.7 | | 67.3 ± 7.7 |
| **20** |  | 97.0 ± 1.3 | 0.0258 ± 0.0016 | 82.0 ± 1.2 | | 65.8 ± 1.8 |
| **10** |  | 96.0 ± 0.8 | 0.0313 ± 0.0025 | 59.0 ± 3.6 | | N. D. |
| **12a** |  | 99.1 ± 0.5 | 0.0036 ± 0.0001 | N. D. | | N. D. |
| **12b** |  | 84.4 ± 2.1 | 0.0262 ± 0.0006 | N. D. | | 41.9 ± 4.4 |
| **21** |  | 95.5 ± 0.4 | 0.0192 ± 0.0007 | 41.4 ± 3.4 | | 67.0 ± 6.4 |
| **22** |  | 80.6 ± 2.5 | 0.0574 ± 0.0030 | 57.0 ± 1.9 | | 33.9 ± 18.4 |
| **23** |  | 96.4 ± 0.6 | 0.0066 ± 0.0005 | N. D. | | 76.9 ± 5.3 |

| No | Structure | GVIA iPLA$_2$ | | GIVA cPLA$_2$ | | GV sPLA$_2$ |
|---|---|---|---|---|---|---|
| | | % Inhibition | $X_I(50)$ | % Inhibition | $X_I(50)$ | % Inhibition |
| **12c** |  | 98.3 ± 0.2 | 0.0084 ± 0.0006 | 76.1 ± 1.8 | | 71.7 ± 3.6 |
| **12d** |  | 95.8 ± 1.3 | 0.0136 ± 0.0006 | 43.7 ± 3.2 | | 76.9 ± 2.2 |
| **12e** |  | 99.4 ± 0.1 | 0.0029 ± 0.0001 | 91.5 ± 0.9 | 0.022 ± 0.001 | 61.4 ± 5.3 |
| **12f** |  | 99.4 ± 0.1 | 0.0024 ± 0.0001 | 88.8 ± 0.7 | | 63.0 ± 6.2 |
| **12g** |  | 99.9 ± 0.1 | 0.0002 ± 0.0000 | 80.8 ± 1.5 | | 36.8 ± 7.9 |
| **12h** |  | 99.8 ± 0.0 | 0.0006 ± 0.0000 | 77.1 ± 1.8 | | 58.4 ± 5.7 |
| **12i** |  | 99.7 ± 0.2 | 0.0010 ± 0.0001 | 55.8 ± 2.1 | | 46.3 ± 10.0 |
| **18a** |  | 97.6 ± 0.2 | 0.0025 ± 0.0001 | 52.1 ± 2.2 | | N. D. |
| **18b** |  | 98.8 ± 0.2 | 0.0013 ± 0.0000 | 66.0 ± 3.7 | | N. D. |
| **24** |  | 100 ± 0.1 | 0.0005 ± 0.0000 | 68.4 ± 1.5 | | 39.1 ± 12.6 |
| **25** |  | 100.0 ± 0.1 | 0.0005 ± 0.0000 | 79.9 ± 1.0 | | 36.1 ± 8.2 |

[a] Average percent inhibition and standard error ($n = 3$) are reported for each compound at 0.091 mol fraction. $X_I(50)$ values were determined for inhibitors with greater than 90% inhibition. N.D. signifies compounds with less than 25% inhibition (or no detectable inhibition).

be a very potent inhibitor of GVIA iPLA$_2$ ($X_I(50) = 0.0002$), exhibiting 7 times higher inhibition than inhibitor **2**. Compound **12g** also inhibited GIVA cPLA$_2$ but at a significant lower level, so we determined its $X_I(50)$ which was 0.039 ± 0.001. It is 195 times more potent on GVIA iPLA$_2$ than on GIVA cPLA$_2$. It was also a very weak inhibitor of GV sPLA$_2$ (37% at 0.091 mol fraction), which implies that it is >455 times selective for iPLA$_2$. Both pentafluoroethyl and heptafluoropropyl derivatives **12h** and **12i** were potent inhibitors of GVIA iPLA$_2$ ($X_I(50)$ of 0.0006 and 0.0010, respectively). However, both **12h** and **12i** inhibited weakly GIVA cPLA$_2$ and GV sPLA$_2$. The dose response curves for the inhibition of GVIA iPLA$_2$ by **12g** and **12i** are presented in Figure 3.

Both the tetrafluoro derivatives **18a** and **18b** were potent inhibitors of GVIA iPLA$_2$ ($X_I(50)$ of 0.0025 and 0.0013, respectively). 1,1,1,2,2,4-Hexafluoro-7-phenylheptan-3-one **24** (FKGK21)[35] and 1,1,1,2,2,3,3,5-octafluoro-7-phenyloctan-4-one **25** (FKGK22)[35] were even more potent inhibitors of GVIA iPLA$_2$ ($X_I(50) = 0.0005$ for both). Comparing **24** and **25** with **2** and **6a**, we observe that the insertion of an additional fluorine atom at the α′ position of either the pentafluoroethyl or the heptafluoropropyl ketone results in improved inhibitory potency. However, none of the tetra-, hexa-, and octafluoro derivatives proved selective.

The chemical mapping of the GVIA iPLA$_2$ active site through structure−activity studies, carried out in the present and the previous work,[36] allowed us to understand some of its features, although no crystal structure has been reported. The enzyme−inhibitor complex is likely to be stabilized by an "oxyanion hole" and other features shown in Figure 4. Although long saturated or unsaturated chains may be bound by the enzyme, the presence of an aromatic ring facilitates inhibitor−enzyme binding. The aromatic system, preferably an extended one such as the naphthyl moiety, may be accommodated in an enzyme binding pocket able to create aromatic−aromatic interactions. The aromatic system should have a distance from the carbonyl group corresponding to a four-carbon chain. We propose that the fluorine atoms, apart from their role in increasing the carbonyl reactivity, may contribute to additional interactions of the inhibitor with a "fluorophilic" region of the enzyme. The perfluoroalkyl chain may interact with a "fluorophilic" binding site through a variety of bonds involving fluorine. Recently, it has become clear that

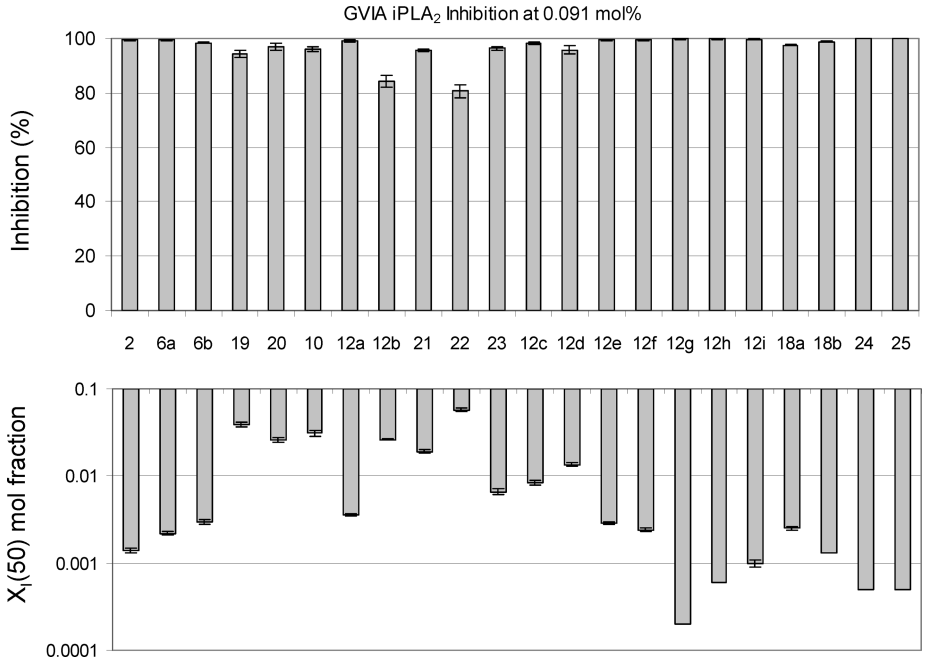



**Figure 2.** GVIA iPLA$_2$ % inhibition at 0.091 mol fraction of inhibitor in mixed micelles (top) and $X_I(50)$ values (bottom). Standard error ($n = 3$) for average % inhibition and for $X_I(50)$ values for all synthesized compounds is indicated.

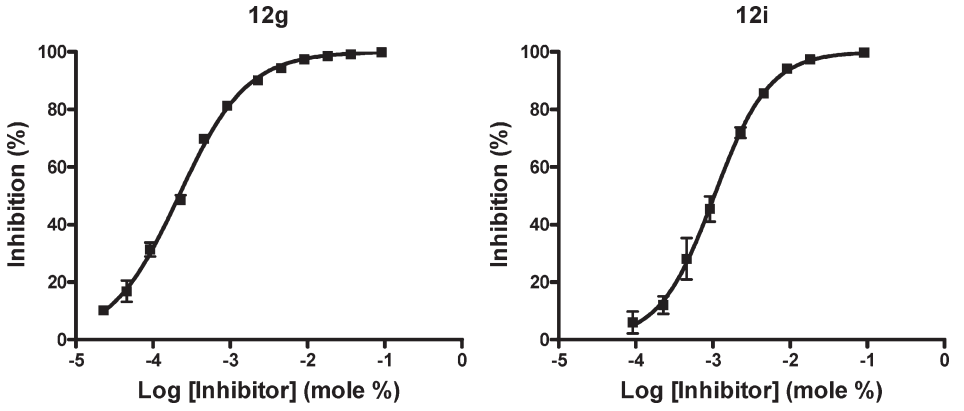



**Figure 3.** Dose−response curves for GVIA iPLA$_2$ inhibition by inhibitors **12g** and **12i**. Inhibition of the activity of human GVIA iPLA$_2$ was tested on mixed micelles containing 100 $\mu$M PAPC and 400 $\mu$M Triton X-100. Inhibition curves were generated using Graphpad Prism with a nonlinear regression targeted at symmetrical sigmoidal curves based on plots of % inhibition versus log(inhibitor concentration). The reported $X_I(50)$ values were calculated from the resultant plots.

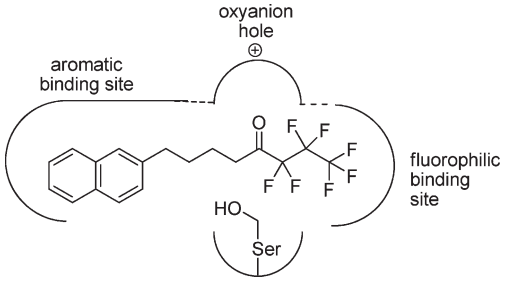



**Figure 4.** Model for the binding mode of fluoroketone inhibitors in the active-site crevice of GVIA iPLA$_2$.

fluorine may enhance binding efficacy and selectivity in pharmaceuticals because of a variety of multipolar C−F···H−N, C−F···C=O, and C−F···H−C$_\alpha$ interactions between a fluorinated ligand and protein binding sites.[41,42] The "fluorophilic" binding site of GIVA iPLA$_2$ should be large enough to accommodate even a heptafluoropropyl group.

In the present study, we identified five fluoroketones (**12g**, **12h**, **12i**, **24**, and **25**) that are more potent inhibitors of GVIA iPLA$_2$ than the lead inhibitor **2**, which has been successfully used in animal models of neurological disorders.[37,38] The introduction of one fluorine atom at the $\alpha'$ position of a pentafluoroethyl or a heptafluoropropyl ketone (compounds **24** and **25**) significantly increased the inhibitory potency for GVIA iPLA$_2$. We therefore determined the $X_I(50)$ for cPLA$_2$ which was 0.038 ± 0.002. Inhibitor **25** is 76 times and >180 times more potent for GVIA iPLA$_2$ than for GIVA cPLA$_2$ and GV sPLA$_2$, respectively. Inhibitor **12a** is also of interest because it inhibits GVIA iPLA$_2$ ($X_I(50) = 0.0036$) without affecting at all GIVA cPLA$_2$ and GV sPLA$_2$. The presence of a naphthyl group proved to be of paramount importance. Trifluoromethyl ketone **12g** is the most potent inhibitor of GVIA iPLA$_2$ ($X_I(50) = 0.0002$) ever reported. Being 195

and > 455 times more potent for GVIA iPLA$_2$ than for GIVA cPLA$_2$ and sPLA$_2$, respectively, makes it a valuable tool for ex vivo and in vivo studies.

In conclusion, we developed new, very potent inhibitors of the calcium-independent GVIA iPLA$_2$. Some of them present interesting selectivity over the intracellular GIVA cPLA$_2$ and the secreted GV sPLA$_2$. By application of these inhibitors as tools for studies in animal models, the role of GVIA iPLA$_2$ in various inflammatory diseases may be explored. Since it has become clear that GVIA iPLA$_2$ is a novel target for the development of novel therapies, fluoroketone inhibitors may become leads for the development of novel medicines, in particular for complex neurological disorders such as multiple sclerosis.

## Experimental Section

**Synthesis of Fluoroketone Inhibitors.** Melting points were determined on a Buchi 530 apparatus and are uncorrected. Nuclear magnetic resonance spectra were obtained on a Varian Mercury spectrometer ($^1$H NMR recorded at 200 MHz, $^{13}$C NMR recorded at 50 MHz, $^{19}$F NMR recorded at 188 MHz) and are referenced in ppm relative to TMS for $^1$H NMR and $^{13}$C NMR and relative to TFA as an internal standard for $^{19}$F NMR. Thin layer chromatography (TLC) plates (silica gel 60 $F_{254}$) and silica gel 60 (230−400 mesh) for flash column chromatography were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid, in EtOH stain. Tetrahydrofuran, toluene, and $Et_2O$ were dried by standard procedures and stored over molecular sieves or Na. All other solvents and chemicals were reagent grade and used without further purification. All tested compounds possessed ≥95% purity as determined by combustion analysis. Intermediates **11a** and **11e** were prepared by known methods,[43,44] and its spectroscopic data were in accordance with those in the literature.

**General Procedure for the Synthesis of Heptafluoropropyl Ketones.** Oxalyl chloride (0.38 g, 3 mmol) and *N*,*N*-dimethylformamide (40 μL) were added to a solution of carboxylic acid (1 mmol) in dry dichloromethane (40 mL). After the mixture was stirred for 3 h at room temperature, the solvent and excess reagent were evaporated under reduced pressure and the residue was dissolved in dry dichloromethane (10 mL). Pyridine (0.64 mL, 8 mmol) and heptafluorobutanoic anhydride (1.5 mL, 6 mmol) were added dropwise to this solution at 0 °C consecutively. After being stirred at 0 °C for 30 min and at room temperature for 1.5 h, the reaction mixture was cooled again at 0 °C and water (2 mL) was added dropwise. After being stirred for 30 min at 0 °C and another 30 min at room temperature, the reaction mixture was diluted with dichloromethane (10 mL). The organic phase was then washed with brine and dried ($Na_2SO_4$). The solvent was evaporated under reduced pressure, and the residual oil was purified by flash column chromatography [EtOAc−petroleum ether (bp 40−60 °C), 5/95].

**1,1,1,2,2,3,3-Heptafluoro-8-phenyloctan-4-one (6a).**[40] Yield 59%; yellowish oil. $^1$H NMR ($CDCl_3$): δ 7.32−7.15 (5H, m, Ph), 2.77 (2H, t, $J$ = 6.2 Hz, $CH_2$), 2.65 (2H, t, $J$ = 6.6 Hz, $CH_2$), 1.71−1.59 (4H, m, 2 × $CH_2$). $^{13}$C NMR: δ 194.0 (t, $J_{C-CF2}$ = 26 Hz, CO), 141.6 (Ph), 130.0−103.5 (m, 2 × $CF_2$, $CF_3$), 128.4 (Ph), 128.3 (Ph), 125.9 (Ph), 37.7 ($CH_2$), 35.4 ($CH_2$), 30.3 ($CH_2$), 21.9 ($CH_2$). $^{19}$F NMR: δ −9.4 ($CF_3$), −49.9 ($CF_2$), −55.4 ($CF_2$). MS (ESI) $m/z$ (%): 329 [(M − H)$^-$, 100].

**1,1,1,2,2,3,3-Heptafluoro-9-phenylnonan-4-one (6b).** Yield 76%; yellowish oil. $^1$H NMR ($CDCl_3$): δ 7.38−7.15 (5H, m, Ph), 2.74 (2H, t, $J$ = 6.2 Hz, $CH_2$), 2.63 (2H, t, $J$ = 6.6 Hz, $CH_2$), 1.78−1.60 (4H, m, 2 × $CH_2$), 1.42−1.35 (2H, m, $CH_2$). $^{13}$C NMR: δ 194.4 (t, $J_{C-CF2}$ = 26 Hz, CO), 142.4 (Ph), 130.2−103.5 (m, 2 × $CF_2$, $CF_3$), 128.6 (Ph), 128.5 (Ph), 126.4 (Ph), 38.1 ($CH_2$), 35.9 ($CH_2$), 31.3 ($CH_2$), 29.9 ($CH_2$), 22.5 ($CH_2$). $^{19}$F NMR: δ −9.4 ($CF_3$), −49.9 ($CF_2$), −55.4 ($CF_2$). MS (ESI) $m/z$ (%): 343 [(M − H)$^-$, 100]. Anal. ($C_{15}H_{15}F_7O$) C, H.

**1,1,1,2,2,3,3-Heptafluoro-8-(4-hexyloxyphenyl)octan-4-one (12d).** Yield 62%; yellowish oil. $^1$H NMR ($CDCl_3$): δ 7.05 (2H, d, $J$ = 8.2 Hz, Ph), 6.87 (2H, d, $J$ = 8.2 Hz, Ph), 3.91 (2H, t, $J$ = 6.6 Hz, $OCH_2$), 2.74 (2H, t, $J$ = 7.7 Hz, $CH_2$), 2.56 (2H, t, $J$ = 7.7 Hz, $CH_2$), 1.78−1.22 (12H, m, 6 × $CH_2$), 0.88 (3H, t, $J$ = 6.2 Hz, $CH_3$). $^{13}$C NMR: δ 194.2 (t, $J_{C-C-F}$ = 26.0 Hz, CO), 157.6 (Ph), 132.0 (Ph), 130.2−103.5 (m, 2 × $CF_2$, $CF_3$), 129.1 (Ph), 114.5 (Ph), 68.0 ($CH_2O$), 38.0 ($CH_2$), 34.8 ($CH_2$), 31.8 ($CH_2$), 30.8 ($CH_2$), 29.5 ($CH_2$), 25.9 ($CH_2$), 22.8 ($CH_2$), 22.1 ($CH_2$), 14.3 ($CH_3$). $^{19}$F NMR: δ −9.4 ($CF_3$), −49.9 ($CF_2$), −55.4 ($CF_2$). Anal. ($C_{20}H_{25}F_7O_2$) C, H.

**1,1,1,2,2,3,3-Heptafluoro-8-(naphthalen-2-yl)octan-4-one (12i).** Yield 45%; yellowish oil. $^1$H NMR ($CDCl_3$): δ 7.90−7.20 (7H, m, Ph), 2.85−2.70 (4H, m, 2 × $CH_2$), 1.85−1.70 (4H, m, 2 × $CH_2$). $^{13}$C NMR: δ 194.2 (t, $J_{C-CF2}$ = 26 Hz, CO), 139.3 (Ph), 133.8 (Ph), 132.3 (Ph), 128.4 (Ph), 127.9 (Ph), 127.7 (Ph), 127.4 (Ph), 126.7 (Ph), 126.2 (Ph), 125.9 (Ph), 125.0−102.0 (m, $CF_3$, 2 × $CF_2$), 37.7 ($CH_2$), 35.6 ($CH_2$), 30.2 ($CH_2$), 22.0 ($CH_2$). $^{19}$F NMR: δ −8.8 ($CF_3$), −50.0 ($CF_2$), −55.5 ($CF_2$). MS (ESI) $m/z$ (%): 379 [(M − H)$^-$, 100]. Anal. ($C_{18}H_{15}F_7O$) C, H.

**(2*E*,4*E*)-*N*-Methoxy-*N*-methyl-5-phenylpenta-2,4-dienamide (9).**[45] To a stirred solution of carboxylic acid (175 mg, 1 mmol) in $CH_2Cl_2$ (7 mL) were added DMAP (122 mg, 1 mmol), *N*,*O*-dimethylhydroxyamine hydrochloride (98 mg, 1 mmol), NMM (0.11 mL, 1 mmol), and WSCI·HCl (192 mg, 1 mmol) consecutively at room temperature. The reaction mixture was left stirring for 18 h. It was then washed with an aqueous solution of 1 N HCl (3 × 10 mL), brine (1 × 10 mL), an aqueous solution of 5% $NaHCO_3$ (3 × 10 mL), and brine (1 × 10 mL). The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure. The amide was purified by flash chromatography, eluting with the appropriate mixture of EtOAc−petroleum ether (40−60 °C), 1/9, to afford the desired product. Yield 67%; white solid. $^1$H NMR ($CDCl_3$): δ 7.55−7.20 (6H, m, Ph, CH), 6.90−6.80 (2H, m, 2 × CH), 6.57 (1H, d, $J$ = 15 Hz, CH), 3.70 (3H, s, $CH_3O$), 3.25 (3H, s, $CH_3$). $^{13}$C NMR: δ 167.0 (CO), 143.2 (CH), 139.6 (CH), 136.2 (Ph), 129.8 (Ph), 128.7 (Ph), 126.9 (Ph), 126.8 (CH), 119.0 (CH), 61.7 ($CH_3O$), 32.3 ($CH_3$). MS (ESI) $m/z$ (%): 218 (M$^+$, 100).

**(4*E*,6*E*)-1,1,1,2,2-Pentafluoro-7-phenylhepta-4,6-dien-3-one (10).** To a stirring solution of the Weinreb amide **9** (78 mg, 0.36 mmol) in $Et_2O$ (5 mL) at −78 °C was added pentafluoroiodoethane (0.7 mL, 1.80 mmol) followed by dropwise addition of a MeLi·LiBr solution 1.6 M in ether (1.2 mL, 1.80 mmol). The reaction mixture was stirred at −78 °C for 3 h. Once the reaction was finished, the reaction mixture was poured into $H_2O$ and acidified with a 10% solution of $KHSO_4$. The layers were separated, and the aqueous layer was extracted with $Et_2O$ (3 × 15 mL). The combined organic layers were washed with a 5% solution of $NaHCO_3$ (40 mL) and dried over $MgSO_4$. The organic solvent was evaporated in vacuo and the residue was purified by column chromatography, eluting with EtOAc−petroleum ether (40−60 °C), 2/98. Yield 90%; yellow oil. $^1$H NMR ($CDCl_3$): δ 7.74 (1H, dd, $J_1$ = 15.0 Hz, $J_2$ = 10.6 Hz, CH), 7.56−7.44 (2H, m, Ph), 7.42−7.32 (3H, m, Ph), 7.25−6.88 (2H, m, CH), 6.65 (1H, d, $J$ = 15.4 Hz, CH). $^{13}$C NMR: δ 182.1 (t, $J_{C-C-F}$ = 25.4 Hz, CO), 149.8 (CH), 146.7 (CH), 135.3 (Ph), 130.4 (Ph), 129.0 (Ph), 127.9 (Ph), 125.9 (CH), 119.9 (CH), 130.0−107.0 (m, $CF_2$, $CF_3$). $^{19}$F NMR: δ −4.3 ($CF_3$), −46.0 ($CF_2$). MS (ESI) $m/z$ (%): 276 (M$^-$, 100). Anal. ($C_{13}H_9F_5O$) C, H.

**Synthesis of Pentafluoroethyl Ketones.** The synthesis of pentafluoroethyl ketones was carried out following the procedure described above for heptafluoropropyl ketones except that pentafluoropropionic anhydride was used instead of heptafluorobutanoic anhydride. The products were purified by flash column chromatography [EtOAc−petroleum ether (bp 40−60 °C), 1/9].

**1,1,1,2,2-Pentafluoro-6-phenoxyhexan-3-one (12a).** Yield 60%; yellowish oil. $^1$H NMR ($CDCl_3$): δ 7.40−7.20 (2H, m, Ph), 7.00−6.83 (3H, m, Ph), 4.02 (2H, t, $J$ = 7 Hz, $OCH_2$), 3.02

(2H, t, $J$ = 6.6 Hz, $CH_2CO$), 2.30−2.10 (2H, m, $CH_2$). $^{13}C$ NMR: $\delta$ 194.0 (t, $J_{C-C-F}$ = 26.4 Hz, CO), 158.5 (Ph), 133.4 (Ph), 129.5 (Ph), 121.0 (Ph), 125.0−110.0 (m, $CF_3$), 114.4 (Ph), 106.8 (tq, $J_{C-F2}$ = 265 Hz, $J_{C-CF3}$ = 38 Hz, $CF_2$), 68.0 ($CH_2O$), 34.1 ($CH_2$), 22.3 ($CH_2$). $^{19}F$ NMR: $\delta$ −4.1 ($CF_3$), −45.6 ($CF_2$). MS (ESI) $m/z$ (%): 281 [$(M - H)^-$, 100]. Anal. ($C_{12}H_{11}F_5O_2$) C, H.

**5-(6,6,7,7,7-Pentafluoro-5-oxoheptyl)furan-2-carboxaldeyde (12b).** Yield 34%; yellowish oil. $^1H$ NMR ($CDCl_3$): $\delta$ 9.49 (1H, s, CHO), 7.16 (1H, d, $J$ = 3.8 Hz, arom), 6.26 (1H, d, $J$ = 3.6 Hz, arom), 2.78−2.74 (4H, m, 2 × $CH_2$), 1.76−169 (4H, m, 2 × $CH_2$). $^{13}C$ NMR: $\delta$ 193.9 (t, $J_{C-C-F}$ = 26.4 Hz, CO), 177.0 (CHO), 162.7 (arom), 151.9 (arom), 123.7 (arom), 117.6 (qt, $J_{C-F3}$ = 286 Hz, $J_{C-CF2}$ = 34 Hz, $CF_3$), 109.2 (arom), 106.8 (tq, $J_{C-F2}$ = 265 Hz, $J_{C-CF3}$ = 38 Hz, $CF_2$), 36.9 ($CH_2$), 28.1 ($CH_2$), 26.4 ($CH_2$), 21.7 ($CH_2$). $^{19}F$ NMR: $\delta$ −4.0 ($CF_3$), −45.5 ($CF_2$). MS (ESI) $m/z$ (%): 299 [$(M + H)^+$, 100]. Anal. ($C_{12}H_{11}F_5O_3$) C, H.

**1,1,1,2,2-Pentafluoro-7-(4-hexyloxyphenyl)heptan-3-one (12c).** Yield 61%; yellowish oil. $^1H$ NMR ($CDCl_3$): $\delta$ 7.06 (2H, d, $J$ = 8.4 Hz, Ph), 6.82 (2H, d, $J$ = 8.4 Hz, Ph), 3.93 (2H, t, $J$ = 6.6 Hz, $OCH_2$), 2.75 (2H, t, $J$ = 6.6 Hz, $CH_2$), 2.57 (2H, t, $J$ = 6.2 Hz, $CH_2$), 1.77−1.62 (6H, m, 3 × $CH_2$), 1.44−1.27 (6H, m, 3 × $CH_2$), 0.90 (3H, t, $J$ = 6.8 Hz, $CH_3$). $^{13}C$ NMR: $\delta$ 194.2 (t, $J_{C-C-F}$ = 26.4 Hz, CO), 157.5 (Ph), 133.4 (Ph), 129.2 (Ph), 117.6 (qt, $J_{C-F3}$ = 286 Hz, $J_{C-CF2}$ = 34 Hz, $CF_3$), 114.4 (Ph), 106.8 (tq, $J_{C-F2}$ = 265 Hz, $J_{C-CF3}$ = 38 Hz, $CF_2$), 68.0 ($CH_2O$), 37.2 ($CH_2$), 34.5 ($CH_2$), 31.8 ($CH_2$), 31.6 ($CH_2$), 29.3 ($CH_2$), 27.5 ($CH_2$), 25.7 ($CH_2$), 22.6 ($CH_2$), 14.0 ($CH_3$). $^{19}F$ NMR: $\delta$ −4.1 ($CF_3$), −45.6 ($CF_2$). MS (ESI) $m/z$ (%): 379 [$(M - H)^-$, 100]. Anal. ($C_{19}H_{25}F_5O_2$) C, H.

**1,1,1,2,2-Pentafluoro-6-(4-octylphenoxy)hexan-3-one (12f).** Yield 70%; yellowish oil. $^1H$ NMR ($CDCl_3$): $\delta$ 7.10 (2H, d, $J$ = 8 Hz, Ph), 6.81 (2H, d, $J$ = 8 Hz, Ph), 3.99 (2H, t, $J$ = 6.6 Hz, $CH_2$), 3.00 (2H, t, $J$ = 6.6 Hz, $CH_2$), 2.57 (2H, t, $J$ = 6.2 Hz, $CH_2$), 2.41−2.14 (2H, m, $CH_2$), 1.64−1.58 (2H, m, $CH_2$), 1.38−1.21 (10H, m, 5 × $CH_2$), 0.91 (3H, t, $J$ = 6.8 Hz, $CH_3$). $^{13}C$ NMR: $\delta$ 194.0 (t, $J_{C-CF2}$ = 26 Hz, CO), 156.6 (Ph), 135.5 (Ph), 129.1 (Ph), 117.8 (qt, $J_{C-F3}$ = 287 Hz, $J_{C-CF2}$ = 34 Hz, $CF_3$), 114.4 (Ph), 106.8 (tq, $J_{C-F2}$ = 267 Hz, $J_{C-CF3}$ = 38 Hz, $CF_2$), 65.8 ($CH_2O$), 35.4 ($CH_2$), 34.5 ($CH_2$), 31.5 ($CH_2$), 30.6 ($CH_2$), 29.3 ($CH_2$), 25.7 ($CH_2$), 22.6 ($CH_2$), 21.9 ($CH_2$), 14.2 ($CH_3$). $^{19}F$ NMR: $\delta$ −4.2 ($CF_3$), −45.6 ($CF_2$). MS (ESI) $m/z$ (%): 393 [$(M - H)^-$, 100]. Anal. ($C_{20}H_{27}F_5O_2$) C, H.

**1,1,1,2,2-Pentafluoro-7-(naphthalen-2-yl)heptan-3-one (12h).** Yield 38%; yellowish oil. $^1H$ NMR ($CDCl_3$): $\delta$ 7.88−7.28 (7H, m, Ph), 2.83−2.78 (4H, m, 2 × $CH_2$), 1.80−1.74 (4H, m, 2 × $CH_2$). $^{13}C$ NMR: $\delta$ 194.4 (t, $J_{C-CF2}$ = 26 Hz, CO), 139.4 (Ph), 133.9 (Ph), 132.4 (Ph), 128.4 (Ph), 127.9 (Ph), 127.6 (Ph), 127.4 (Ph), 126.7 (Ph), 126.2 (Ph), 125.9 (Ph), 118.1 (qt, $J_{C-F3}$ = 287 Hz, $J_{C-CF2}$ = 35 Hz, $CF_3$), 107.2 (tq, $J_{C-F2}$ = 265 Hz, $J_{C-CF3}$ = 38 Hz, $CF_2$), 37.4 ($CH_2$), 35.9 ($CH_2$), 30.5 ($CH_2$), 22.2 ($CH_2$). $^{19}F$ NMR: $\delta$ −4.1 ($CF_3$), −45.5 ($CF_2$). MS (ESI) $m/z$ (%): 329 [$(M - H)^-$, 100]. Anal. ($C_{17}H_{15}F_5O$) C, H.

**Synthesis of Trifluoromethyl Ketones.** The synthesis of trifluoromethyl ketones was carried out following the procedure described above for heptafluoropropyl ketones except that trifluoroacetic anhydride was used instead of heptafluorobutanoic anhydride. The products were purified by flash column chromatography [EtOAc−petroleum ether (bp 40−60 °C), 3/7].

**1,1,1-Trifluoro-5-(4-octylphenoxy)pentan-2-one (12e).** Yield 32%; yellowish oil. $^1H$ NMR ($CDCl_3$): $\delta$ 7.10 (2H, d, $J$ = 8 Hz, Ph), 6.80 (2H, d, $J$ = 8 Hz, Ph), 3.99 (2H, t, $J$ = 6.6 Hz, $OCH_2$), 2.95 (2H, t, $J$ = 6.6 Hz, $CH_2$), 2.54 (2H, t, $J$ = 6.2 Hz, $CH_2$), 2.20−2.10 (2H, m, $CH_2$), 1.61−1.51 (2H, m, $CH_2$), 1.28−1.21 (10H, m, 5 × $CH_2$), 0.88 (3H, t, $J$ = 6.8 Hz, $CH_3$). $^{13}C$ NMR: $\delta$ 193.9 (t, $J_{C-CF2}$ = 26 Hz, CO), 156.5 (Ph), 135.5 (Ph), 129.3 (Ph), 115.8 (q, $J_{C-F}$ = 292 Hz, $CF_3$), 114.2 (Ph), 65.8 ($CH_2O$), 35.0 ($CH_2$), 33.1 ($CH_2$), 31.9 ($CH_2$), 31.7 ($CH_2$), 31.6 ($CH_2$), 31.5 ($CH_2$), 29.5 ($CH_2$), 22.6 ($CH_2$), 22.4 ($CH_2$), 14.0 ($CH_3$). $^{19}F$ NMR: $\delta$ −1.5 (s, $CF_3$). MS (ESI) $m/z$ (%): 343 [$(M - H)^-$, 100]. Anal. ($C_{19}H_{27}F_3O_2$) C, H.

**1,1,1-Trifluoro-6-(naphthalen-2-yl)hexan-2-one (12g).** Yield 39%; yellowish oil. $^1H$ NMR ($CDCl_3$): $\delta$ 7.81−7.29 (7H, m, Ph), 2.81−2.73 (4H, m, 2 × $CH_2$), 1.79−1.73 (4H, m, 2 × $CH_2$). $^{13}C$ NMR: $\delta$ 194.4 (t, $J_{C-C-F}$ = 26 Hz, CO), 139.4 (Ph), 133.9 (Ph), 132.3 (Ph), 128.4 (Ph), 127.9 (Ph), 127.7 (Ph) 127.4 (Ph), 126.7 (Ph), 126.2 (Ph), 125.9 (Ph), 115.8 (q, $J_{C-F}$ = 292 Hz, $CF_3$), 36.2 ($CH_2$), 35.6 ($CH_2$), 30.3 ($CH_2$), 22.0 ($CH_2$). $^{19}F$ NMR: $\delta$ −1.5 (s, $CF_3$). MS (ESI) $m/z$ (%): 279 [$(M - H)^-$, 100]. Anal. ($C_{16}H_{15}F_3O$) C, H.

**5-(Furan-2-yl)pentanoic Acid (11b).**[46] A suspension of aldehyde **13** (0.096 g, 1 mmol), triethyl 4-phosphonocrotonate (0.37 g, 1.5 mmol), lithium hydroxide (0.036 g, 1.5 mmol), and molecular sieves (beads, 4−8 mesh, 1.5 g/mmol aldehyde) in dry tetrahydrofuran (10 mL) was refluxed under argon for 24 h. The reaction mixture was then cooled to room temperature and filtered through a thin pad of Celite and the solvent evaporated under reduced pressure. The residual oil was purified by chromatography on silica gel, eluting with ether−petroleum ether (bp 40−60 °C), 1/9. A mixture of the unsaturated ester (135 mg, 0.7 mmol) in dry 1,4-dioxane (7 mL) and 10% palladium on activated carbon (0.07 g) was hydrogenated for 12 h under atmospheric conditions. After filtration through a pad of Celite, the solvent was removed in vacuo to give the saturated compound. The solution of the saturated ester in methanol (1.4 mL) was treated with 1 N sodium hydroxide (1 mL, 1 mmol). The mixture was stirred at room temperature for 12 h, acidified with 1 N HCl, and extracted with EtOAc (3 × 10 mL). The solvent was removed in vacum to afford the saturated acid. Yield 66%; white solid; mp 40−41 °C. $^1H$ NMR ($CDCl_3$): $\delta$ 10.00 (1H, br, COOH), 7.29−7.27 (1H, m, arom), 6.27−6.25 (1H, m, arom), 6.00−5.97 (1H, m, arom), 2.64 (2H, t, $J$ = 6.4 Hz, $CH_2$), 2.36 (2H, t, $J$ = 6.0 Hz, $CH_2$), 1.73−1.63 (4H, m, 2 × $CH_2$). $^{13}C$ NMR: $\delta$ 178.4 (CO), 155.5 (arom), 140.9 (arom), 110.0 (arom), 104.9 (arom), 33.8 ($CH_2$), 27.6 ($CH_2$), 27.4 ($CH_2$), 24.1 ($CH_2$).

**(2*E*,4*E*)-5-Phenylpenta-2,4-dienoic Acid (8).**[47] Benzaldehyde was treated with triethyl 4-phosphonocrotonate, and the resulting ester was saponified as described above. Yield 76%; white solid; mp 165−166 °C. $^1H$ NMR ($CDCl_3$): $\delta$ 7.58−7.22 (6H, m, Ph, CH), 7.05−6.90 (2H, m, CH), 6.00 (1H, d, $J$ = 15 Hz, CH). $^{13}C$ NMR: $\delta$ 169.3 (CO), 145.5 (CH), 140.6 (CH), 136.4 (Ph), 128.9 (Ph), 128.4 (Ph), 127.1 (Ph), 126.2 (CH), 121.1 (CH).

**4-(4-Octylphenoxy)butyric Acid Ethyl Ester (15).** A mixture of *p*-octylphenol (206 mg, 1 mmol), $K_2CO_3$ (415 mg, 3 mmol), and ethyl 4-bromobutyrate (215 mg, 1.1 mmol) in acetone (7.6 mL) was refluxed overnight. The reaction mixture was then cooled to room temperature and the soslvent evaporated under reduced pressure. The residual oil was purified by flash column chromatography on silica gel, eluting with EtOAc−petroleum ether (bp 40−60 °C), 1/9. Yield 71%; colorless oil. $^1H$ NMR ($CDCl_3$): $\delta$ 7.07 (2H, d, $J$ = 8.8 Hz, Ph), 6.81 (2H, d, $J$ = 8.8 Hz, Ph), 4.17 (2H, q, $J$ = 7 Hz, $OCH_2CH_3$), 3.92 (2H, t, $J$ = 6.6 Hz, $OCH_2$), 2.60−2.45 (4H, m, 2 × $CH_2$), 2.18−2.05 (2H, m, $CH_2CH_2COO$), 1.65−1.42 (2H, m, $CH_2$), 1.38−1.21 (13H, br, 5 × $CH_2$, $CH_3$), 0.90 (3H, t, $J$ = 6.8 Hz, $CH_3$). $^{13}C$ NMR: $\delta$ 173.4 (CO), 157.1 (Ph), 135.3 (Ph), 129.4 (Ph), 114.4 (Ph), 66.9 ($CH_2O$), 60.5 ($OCH_2CH_3$), 35.3 ($CH_2$), 32.1 ($CH_2$), 32.0 ($CH_2$), 31.5 ($CH_2$), 31.0 ($CH_2$), 29.7 ($CH_2$), 29.5 ($CH_2$), 24.9 ($CH_2$), 22.9 ($CH_2$), 14.5 ($CH_3$), 14.3 ($CH_3$). Anal. ($C_{20}H_{32}O_3$) C, H.

**α-Fluorination of α-Hydroxy Methyl Esters.** Compound **16a** or **16b** (1 mmol) was added to a solution of DAST (0.14 mL, 1 mmol) in dry dichloromethane (0.2 mL) at −78 °C. After being stirred for 2 h at −78 °C and another 3 h at room temperature, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (2.5 mL). The organic phase was then washed with brine and dried ($Na_2SO_4$). The solvent was evaporated under reduced pressure, and the residual oil was purified by flash column chromatography on silica gel, eluting with EtOAc−petroleum ether (bp 40−60 °C), 3/7.

**Methyl 5-Phenyl-2-fluoropentanoate (17a).** Yield 60%; yellowish oil. $^1$H NMR ($CDCl_3$): $\delta$ 7.35−7.02 (5H, m, Ph), 4.98 (1H, dt, $J_{H-F}$ = 48.2 Hz, $J_{H-H}$ = 6.2 Hz, CHF), 3.78 (3H, s, $CH_3O$), 2.67 (2H, t, $J$ = 6.6 Hz, $PhCH_2$), 2.04−1.78 (4H, m, 2 × $CH_2$). $^{13}$C NMR: $\delta$ 170.2 (d, $J_{C-CF}$ = 23.5 Hz, CO), 141.3 (Ph), 128.4 (Ph), 128.3 (Ph), 125.9 (Ph), 88.8 (d, $J_{C-F}$ = 183.3 Hz, CHF), 52.1 ($CH_3$), 35.1 ($CH_2$), 31.8 (d, $J_{C-CF}$ = 20.8 Hz, $CH_2$CHF), 25.9 ($CH_2$). $^{19}$F NMR: $\delta$ −114.1 (CF). MS (ESI) $m/z$ (%): 212 [$(M + H)^+$,100]. Anal. ($C_{12}H_{15}FO_2$) C, H.

**Methyl 6-Phenyl-2-fluorohexanoate (17b).** Yield 52%; colorless oil. $^1$H NMR ($CDCl_3$): $\delta$ 7.35−7.16 (5H, m, Ph), 4.86 (1H, dt, $J_{H-F}$ = 48.2 Hz, $J_{H-H}$ = 6.2 Hz, CHF), 3.78 (3H, s, $CH_3O$), 2.64 (2H, t, $J$ = 6.6 Hz, $PhCH_2$), 2.10−1.46 (6H, m, 3 × $CH_2$). $^{13}$C NMR: $\delta$ 170.2 (d, $J_{C-CF}$ = 23.5 Hz, CO), 141.9 (Ph), 128.2 (Ph), 128.1 (Ph), 125.6 (Ph), 88.8 (d, $J_{C-F}$ = 183.3 Hz, CHF), 52.0 ($CH_3$), 35.4 ($CH_2$), 32.0 (d, $J_{C-CF}$ = 20.8 Hz, $CH_2$CHF), 30.7 ($CH_2$), 23.8 (d, $J_{C-C-CF}$ = 3.0 Hz $CH_2$). $^{19}$F NMR: $\delta$ −114.1 (CF). Anal. ($C_{13}H_{17}FO_2$) C, H.

**Synthesis of 1,1,1,3-Tetrafluoro Ketones. Method A.** A solution of compound **17a** or **17b** (1 mmol) and trifluoromethyltrimethylsilane (283 $\mu$L, 1.92 mmol) in ethylene glycol dimethyl ether (0.92 mL) at 0 °C was treated with cesium fluoride (4 mg). After being stirred for 30 min at 0 °C and another 18 h at 25 °C, the reaction mixture was treated with concentrated HCl (1 mL). After being stirred for another 18 h at 25 °C, the reaction mixture was diluted with EtOAc (10 mL). The organic phase was then washed with brine and dried ($Na_2SO_4$). The solvent was evaporated under reduced pressure, and the residual oil was purified by flash column chromatography on silica gel, eluting with EtOAc−petroleum ether (bp 40−60 °C), 3/7.

**Method B.** A solution of compound **17a** or **17b** (1 mmol) and trifluoromethyltrimethylsilane (1 mL, 6.9 mmol) in toluene (9 mL) at −78 °C was treated with 1.0 M TBAF (45 $\mu$L) in THF. After the mixture was stirred for 2 h at 25 °C the intermediate silyl ether was formed and then it was treated with 1.0 M TBAF (1.2 mmol) in THF and with glacial acetic acid (3 drops). The reaction mixture was stirred for 30 min at 25 °C and diluted with EtOAc (10 mL). The organic phase was washed first with saturated solution of $K_2CO_3$ and then with brine and dried ($Na_2SO_4$). The solvent was evaporated under reduced pressure, and the residual oil was purified by flash column chromatography on silica gel, eluting with EtOAc−petroleum ether (bp 40−60 °C), 3/7.

**1,1,1,3-Tetrafluoro-6-phenylhexan-2-one (in Equilibrium with 1,1,1,3-Tetrafluoro-6-phenyl-2,2-*gem*-hexanodiol) (18a).** Yield 47% (method A), 93% (method B); yellowish oil. $^1$H NMR ($CDCl_3$): $\delta$ 7.34−7.15 (5H, m, Ph), 5.23 (1/4H, dm, $J_{H-F}$ = 48.2 Hz, CH), 4.65 (3/4H, dm, $J_{H-F}$ = 48.2 Hz, CH), 3.74 ($^3/_4$H, s, OH), 3.49 ($^3/_4$H, s, OH), 2.68 (2H, t, $J$ = 6.2 Hz, $CH_2$), 1.90−1.10 (4H, m, 2 × $CH_2$). $^{13}$C NMR: $\delta$ 141.6 (Ph), 128.4 (Ph), 126.1 (Ph), 125.9 (Ph), 122.6 (q, $J_{C-F3}$ = 286 Hz, $CF_3$), 92.4 (d, $J_{C-F}$ = 175 Hz, CF), 92.2 [m, $C(OH)_2$], 35.4 ($CH_2$), 31.8 (d, $J_{C-F}$ = 20 Hz, $CH_2$), 27.6 (d, $J_{C-F}$ = 20 Hz, $CH_2$). $^{19}$F NMR: $\delta$ 1.6 ($CF_3$), −5.3 ($CF_3$), −120.9 (CHF). MS (ESI) $m/z$ (%): 247 [$(M - H)^-$, 100].

**1,1,1,3-Tetrafluoro-7-phenylheptan-2-one (in Equilibrium with 1,1,1,3-Tetrafluoro-7-phenyl-2,2-*gem*-heptanodiol) (18b).** Yield 45% (method A), 94% (method B); yellowish oil. $^1$H NMR ($CDCl_3$): $\delta$ 7.32−7.15 (5H, m, Ph), 5.20 (1/6H, dm, $J_{H-F}$ = 48.2 Hz, CH), 4.63 (5/6H, dm, $J_{H-F}$ = 48.2 Hz, CH), 2.64 (2H, t, $J$ = 7.4 Hz, $CH_2$), 1.84−1.80 (2H, m, $CH_2$), 1.74−1.42 (4H, m, 2 × $CH_2$). $^{13}$C NMR: $\delta$ 142.1 (Ph), 128.3 (Ph), 125.8 (Ph), 125.3 (Ph), 122.6 (q, $J_{C-F3}$ = 286 Hz, $CF_3$), 92.3 (d, $J_{C-F}$ = 175 Hz, CF), 92.2 [m, $C(OH)_2$], 35.6 ($CH_2$), 31.0 ($CH_2$), 27.8 (d, $J_{C-F}$ = 20 Hz, $CH_2$), 24.6 (d, $J_{C-C-F}$ = 2.6 Hz, $CH_2$). $^{19}$F NMR: $\delta$ 1.6 ($CF_3$), −5.3 ($CF_3$), −120.8 (CHF). MS (ESI) $m/z$ (%): 261 [$(M - H)^-$, 100].

**In Vitro $PLA_2$ Assays.** Phospholipase $A_2$ activity was determined using the previously described modified Dole assay[28] with buffer and substrate conditions optimized for each enzyme as described previously.[29,31,34] The specific assay conditions employed for the studies reported in this manuscript for each enzyme are as follows: (i) GIVA $cPLA_2$ substrate mixed-micelles were composed of 400 $\mu$M Triton X-100, 97 $\mu$M PAPC, 1.8 $\mu$M $^{14}$C-labeled PAPC, and 3 $\mu$M $PIP_2$ in buffer containing 100 mM HEPES, pH 7.5, 90 $\mu$M $CaCl_2$, 2 mM DTT, and 0.1 mg/mL BSA; (ii) GVI $iPLA_2$ substrate mixed-micelles were composed of 400 $\mu$M Triton X-100, 98.3 $\mu$M PAPC, and 1.7 $\mu$M $^{14}$C-labeled PAPC in buffer containing 100 mM HEPES, pH 7.5, 2 mM ATP, and 4 mM DTT; (iii) GV $sPLA_2$ substrate mixed-micelles were composed of 400 $\mu$M Triton X-100, 99 $\mu$M DPPC, and 1.5 $\mu$M $^{14}$C-labeled DPPC in buffer containing 50 mM Tris, pH 8.0, and 5 mM $CaCl_2$.

**In Vitro $PLA_2$ Inhibition Studies.** Initial screening of compounds at 0.091 mol fraction inhibitor in mixed micelles was carried out. Compounds displaying 25% or less inhibition of the assays were considered to have no inhibitory affect (designated N.D.). We report average percent inhibition (and standard error, $n$ = 3) for compounds displaying less than 90% enzyme inhibition. If the percent inhibition was greater than 90%, we determined its $X_I(50)$ by plotting percent inhibition vs inhibitor mole fraction (typically seven concentrations between 0.000 91 and 0.091 mol fraction). Inhibition curves were modeled in Graphpad Prism 5.0 using nonlinear regression targeted at symmetrical sigmoidal curves based on plots of % inhibition versus log(inhibitor concentration) to calculate the reported $X_I(50)$ and associated error values.

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