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Potent and Selective Fluoroketone Inhibitors of Group VIA Calcium-Independent Phospholipase A₂

George Kokotos^{*,†}, Yuan-Hao Hsu[‡], John E. Burke[‡], Constantinos Baskakis[†], Christoforos G. Kokotos[†], Victoria Magrioti[†], and Edward A. Dennis^{*,‡}

Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece; Department of Chemistry and Biochemistry and Department of Pharmacology, School of Medicine, MC 0601, University of California, San Diego, La Jolla, California 92093-0601

Abstract

Group VIA calcium-independent phospholipase A₂ (GVIA iPLA₂) has recently emerged as a novel pharmaceutical target. We have now explored the structure-activity relationship between fluoroketones and GVIA iPLA₂ 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 iPLA₂ (X₁(50) 0.0002) ever reported. Being 195 and >455 times more potent for GVIA iPLA₂ than for GIVA cPLA₂ and GV sPLA₂, respectively, makes it a valuable tool to explore the role of GVIA iPLA₂ in cells and *in vivo* models. 1,1,1,2,2,3,3-Heptafluoro-8-(naphthalene-2-yl) octan-4-one inhibited GVIA iPLA₂ with a X₁(50) value of 0.001, while inhibiting the other intracellular GIVA cPLA₂ and GV sPLA₂ at least 90-times less potently. Hexa- and octa-fluoro ketones were also found to be potent inhibitors of GVIA iPLA₂; however they are not selective.

Keywords

heptafluoropropyl ketones; inhibitors; polyfluoro ketones; phospholipase A₂

Introduction

The phospholipase A₂ (PLA₂) 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 PLA₂ superfamily currently contains 15 separate, identifiable groups and various subgroups.^{2,3} The three predominant types of PLA₂ found in human tissues are the cytosolic (such as the GIVA cPLA₂), the secreted (such as the GIIA and GV sPLA₂), and the calcium-independent (such as the GVIA iPLA₂) enzymes. GIVA cPLA₂ is generally considered a pro-inflammatory enzyme which is the rate-limiting provider of arachidonic acid and lysophospholipids.⁴ In many cases, the activity of secreted PLA₂ has been shown to be dependent on or linked to the activity of GIVA cPLA₂.^{5–7} The calcium-independent Group VIA iPLA₂ (GVIA iPLA₂), typically referred to in the literature as iPLA₂, 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

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

[†]University of Athens

[‡]University of California, San Diego

iPLA₂ 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 iPLA₂ also plays important roles in numerous cell types, although they may differ from cell to cell. Recent review articles discuss the role of GVIA iPLA₂ in signaling and pathological conditions (for example, cancer and ischemia).^{16–20}

The GVIA iPLA₂ 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 cPLA₂ and GVIA iPLA₂ 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 PLA₂,^{23,24} while methyl arachidonyl 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 iPLA₂ inhibitor and has been widely applied to study potential biological roles for GVIA iPLA₂.^{11,26} Turk et al. have recently studied the inactivation mechanism of GVIA iPLA₂ 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 iPLA₂. Our laboratories have previously reported on the development of 2-oxoamide inhibitors targeting GIVA cPLA₂.^{28–32} We have demonstrated that 2-oxoamides containing a free carboxyl group are selective inhibitors of GIVA cPLA₂ and most recently we have determined the location of such an inhibitor bound in the active site of GIVA cPLA₂ using a combination of deuterium exchange mass spectrometry and molecular dynamics.³³ 2-Oxoamides based on amino acid esters show cross-reactivity for both GIVA cPLA₂ and GVIA iPLA₂,^{30,32} while most recently we identified a 2-oxoamide based on a pseudodipeptide which preferentially inhibits GVIA iPLA₂.³⁴

The development of selective inhibitors for the three main human PLA₂ enzymes is an important goal, and we have synthesized and assayed a variety of polyfluoroketones for their activity on GIVA cPLA₂, GVIA iPLA₂ and GV sPLA₂. 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 iPLA₂.³⁶ Trifluoromethyl ketone **3** (FKGK2, Figure 1) can be considered to be a pan inhibitor of all three enzymes: GIVA cPLA₂, GVIA iPLA₂ and GV sPLA₂. The tetrafluoro derivative **4** was found to be the most potent GVIA iPLA₂ inhibitor, although it is not selective.³⁶ The selective GVIA iPLA₂ 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 iPLA₂ is a novel target for the development of new therapies for multiple sclerosis. The recently emerged important pharmaceutical significance of GVIA iPLA₂ and the lack of potent and selective GVIA iPLA₂ inhibitors, prompted us to extend our studies towards 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 phospholipases A₂s.

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 iPLA₂ inhibitor **2** (pentafluoroethyl ketone) and the tetrafluoro derivative **4**, which is a potent GVIA iPLA₂ 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 analogs of the lead GVIA iPLA₂ 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 (**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 (C₂F₅CO)₂O and pyridine, led to the formylated derivative **12b**. Under these conditions we were unable to prepare the non-formylated derivative.

The synthesis of tetrafluoro derivatives **18a,b** was accomplished by procedures developed earlier³⁶ (Scheme 4). On the basis of ¹H and ¹⁹F 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 iPLA₂, GIVA cPLA₂ and GV sPLA₂

All synthesized inhibitors were tested for inhibition of human GVIA iPLA₂ based on a modification of the previously described mixed micelle-based assay.³⁰ The mixed micelle assay employed herein used 1-palmitoyl-2-arachidonyl-phosphatidylcholine (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-dipalmitoyl-phosphatidylcholine (DPPC) as substrate. This change was made in order to use the same substrate for iPLA₂ as for cPLA₂, so as to better compare the specificities of both i and cPLA₂ toward the same substrate. This also improved the consistency of the standard error in the assay. Using 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 cPLA₂, and GV sPLA₂, the previously reported mixed micelle-based assays were used.^{28,29,31} The resulting values of GVIA iPLA₂ inhibition are presented in Figure 2 as either percent inhibition or $X_I(50)$ values. Initially, the percent of inhibition for each PLA₂ enzyme at 0.091 mole fraction of each inhibitor was determined, and $X_I(50)$ values were determined for all compounds toward GVIA iPLA₂ and for the other two enzymes for all inhibitors that displayed greater than 90% inhibition. However, for two additional iPLA₂ inhibitor examples, we also determined their $X_I(50)$ toward cPLA₂ 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 iPLA₂ 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 iPLA₂, although not potent. They were also weak inhibitors of the other two PLA₂s.

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 iPLA₂, since a high mole fraction of the inhibitor (0.091) does not inhibit either GIVA cPLA₂ or GV sPLA₂ at all. The furan-based inhibitor **12b** was not a potent inhibitor.

Pentafluoroethyl derivative **21** based on the oleyl chain inhibited GVIA iPLA₂ better than the corresponding palmitoyl derivative.³⁶ Heptafluoropropyl derivative **22** was a weaker inhibitor of GVIA iPLA₂ than derivative **21**. Both **21** and **22** were able to inhibit weakly the other two enzymes. This data indicates that for the inhibition of GVIA iPLA₂ 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 iPLA₂ with a $X_I(50)$ 0.0066. This derivative does not inhibit at all the other intracellular enzyme GIVA cPLA₂ and inhibits weakly GV sPLA₂ (77%) at 0.091 mole fraction.

Both the *para*-hexyloxy substituted pentafluoroethyl and heptafluoropropyl derivatives **12c** and **12d** inhibited GVIA iPLA₂ with $X_I(50)$ 0.0084 and 0.0136, respectively. Comparing **12c** and **12d** with **2** and **6a**, it seems that the *para*-hexyloxy substitution had a negative effect on the GVIA iPLA₂ 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 iPLA₂ ($X_I(50)$ 0.0029 and 0.0024, respectively). However, all the derivatives bearing a substituent at the *para* position failed to be selective for any PLA₂ 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)³⁵ proved to be a very potent inhibitor of GVIA iPLA₂ ($X_I(50)$ 0.0002), exhibiting 7 times higher inhibition than inhibitor **2**. Compound **12g** (FKGK18) also inhibited GIVA cPLA₂, 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 iPLA₂ than on GIVA cPLA₂. It was also a very weak inhibitor of GV sPLA₂ (37% at 0.091 mole fraction) which implies that it is >455 times selective for iPLA₂. Both pentafluoroethyl and heptafluoropropyl derivatives **12h** and **12i** were potent inhibitors of GVIA iPLA₂ ($X_I(50)$ 0.0006 and 0.0010, respectively). However, both **12h** and **12i** inhibited weakly GIVA cPLA₂ and GV sPLA₂. The dose response curves for the inhibition of GVIA iPLA₂ by **12g** and **12i** are presented in Figure 3.

Both the tetrafluoro derivatives **18a** and **18b** were potent inhibitors of GVIA iPLA₂ ($X_I(50)$ 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 iPLA₂ ($X_I(50)$ 0.0005 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 octa-fluoro derivatives proved selective.

The chemical mapping of the GVIA iPLA₂ active site through structure-activity studies, carried out in the present and the previous work,³⁶ allow 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 fluorine may enhance binding efficacy and selectivity in pharmaceuticals due to a variety of multipolar C-F^{δ+}H-N, C-F^{δ+}C=O, and C-F^{δ+}H-C_α interactions between a fluorinated ligand and protein binding-sites.^{42,43} The “fluorophilic” binding site of GVIA iPLA₂ 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 iPLA₂ 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 α' position of a pentafluoroethyl or a heptafluoropropyl ketone (compounds **24** and **25**) significantly increased the inhibitory potency for GVIA iPLA₂. We therefore determined the X_I(50) for cPLA₂ which was 0.038 ± 0.002. Inhibitor **25** is 76-times and >180-times more potent for GVIA iPLA₂ than for GIVA cPLA₂ and GV sPLA₂, respectively. Inhibitor **12a** is also of interest because it inhibits GVIA iPLA₂ (X_I(50) 0.0036) without affecting at all GIVA cPLA₂ and GV sPLA₂. The presence of a naphthyl group proved to be of paramount importance. Trifluoromethyl ketone **12g** is the most potent inhibitor of GVIA iPLA₂ (X_I(50) 0.0002) ever reported. Being 195 and >455 times more potent for GVIA iPLA₂ than for GIVA cPLA₂ and sPLA₂, 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 iPLA₂. Some of them present interesting selectivity over the intracellular GIVA cPLA₂ and the secreted GV sPLA₂. Applying these inhibitors as tools for studies in animal models, the role of GVIA iPLA₂ in various inflammatory diseases may be explored. Since it has become clear that GVIA iPLA₂ 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 (¹H NMR recorded at 200 MHz, ¹³C NMR recorded at 50 MHz, ¹⁹F NMR recorded at 188 MHz) and are referenced in ppm relative to TMS for ¹H NMR and ¹³C NMR and relative to TFA as an internal standard for ¹⁹F NMR. Thin layer chromatography (TLC) plates (silica gel 60 F₂₅₄) 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 Et₂O 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. Intermediate **11a** was prepared by known methods,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 μ L) were added to a solution of carboxylic acid (1 mmol) in dry dichloromethane (40 mL). After 3 h stirring 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 stirring 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 stirring 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. ^1H 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, $2 \times \text{CH}_2$). ^{13}C NMR: δ 194.0 (t, $J_{\text{C-CF}_2}$ = 26 Hz, CO), 141.6 (Ph), 130.0–103.5 (m, $2 \times \text{CF}_2$, 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. ^1H 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, $2 \times \text{CH}_2$), 1.42–1.35 (2H, m, CH_2). ^{13}C NMR: δ 194.4 (t, $J_{\text{C-CF}_2}$ = 26 Hz, CO), 142.4 (Ph), 130.2–103.5 (m, $2 \times \text{CF}_2$, 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. ($\text{C}_{15}\text{H}_{15}\text{F}_7\text{O}$) C, H.

1,1,1,2,2,3,3-Heptafluoro-8-(4-hexyloxyphenyl)octan-4-one (12d)

Yield 62%; yellowish oil. ^1H 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, $6 \times \text{CH}_2$), 0.88 (3H, t, J = 6.2 Hz, CH_3). ^{13}C NMR: δ 194.2 (t, $J_{\text{C-CF}_2}$ = 26.0 Hz, CO), 157.6 (Ph), 132.0 (Ph), 130.2–103.5 (m, $2 \times \text{CF}_2$, 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. ($\text{C}_{20}\text{H}_{25}\text{F}_7\text{O}_2$) C, H.

1,1,1,2,2,3,3-Heptafluoro-8-(naphthalen-2-yl)octan-4-one (12i)

Yield 45%; yellowish oil. ^1H NMR (CDCl_3): δ 7.90–7.20 (7H, m, Ph), 2.85–2.70 (4H, m, $2 \times \text{CH}_2$), 1.85–1.70 (4H, m, $2 \times \text{CH}_2$). ^{13}C NMR: δ 194.2 (t, $J_{\text{C-CF}_2}$ = 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 , $2 \times \text{CF}_2$), 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. ($\text{C}_{18}\text{H}_{15}\text{F}_7\text{O}$) 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), DMAP (122 mg, 1 mmol), *N,O*-dimethyl hydroxyamine hydrochloride (98 mg, 1 mmol), NMM (0.11 mL, 1 mmol) and WSCl^+HCl (192 mg, 1 mmol) were added consecutively at room temperature. The reaction mixture was left stirring for 18 h. It was then washed with an aqueous solution of HCl

1N (3 × 10 mL), brine (1 × 10 mL), an aqueous solution of NaHCO₃ 5% (3 × 10 mL) and brine (1 × 10 mL). The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure. The amide 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. ¹H NMR (CDCl₃): 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, CH₃O), 3.25 (3H, s, CH₃). ¹³C NMR: δ 167.0 (CO), 143.2 (CH), 139.6 (CH), 136.2 (Ph), 128.7 (Ph), 126.9 (Ph), 126.8 (CH), 119.0 (CH), 61.7 (CH₃O), 32.3 (CH₃). 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 Et₂O (5 mL) at –78 °C was added pentafluoriodoethane (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 H₂O and acidified with a 10% solution of KHSO₄. The layers were separated and the aqueous layer was extracted with Et₂O (3 × 15 mL). The combined organic layers were washed with a 5% solution of NaHCO₃ (40 mL) and dried over MgSO₄. 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. ¹H NMR (CDCl₃): 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). ¹³C 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, CF₂, CF₃). ¹⁹F NMR: δ –4.3 (CF₃), –46.0 (CF₂). MS (ESI) *m/z* (%): 276 (M⁺, 100). Anal. (C₁₃H₉F₅O) 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. ¹H NMR (CDCl₃): δ 7.40–7.20 (2H, m, Ph), 7.00–6.83 (3H, m, Ph), 4.02 (2H, t, *J* = 7 Hz, OCH₂), 3.02 (2H, t, *J* = 6.6 Hz, CH₂CO), 2.30–2.10 (2H, m, CH₂). ¹³C NMR: δ 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, CF₃), 114.4 (CH), 106.8 (tq, *J*_{C–F₂} = 265 Hz, *J*_{C–CF₃} = 38 Hz, CF₂), 68.0 (CH₂O), 34.1 (CH₂), 22.3 (CH₂). ¹⁹F NMR: δ –4.1 (CF₃), –45.6 (CF₂). MS (ESI) *m/z* (%): 281 [(M–H)[–], 100]. Anal. (C₁₂H₁₁F₅O₂) C, H.

5-(6,6,7,7,7-Pentafluoro-5-oxoheptyl)furan-2-carboxaldehyde (12b)

Yield 34%; yellowish oil. ¹H NMR (CDCl₃): δ 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 × CH₂), 1.76–1.69 (4H, m, 2 × CH₂). ¹³C NMR: δ 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, CF₃), 109.2 (arom), 106.8 (tq, *J*_{C–F₂} = 265 Hz, *J*_{C–CF₃} = 38 Hz, CF₂), 36.9 (CH₂), 28.1 (CH₂), 26.4 (CH₂), 21.7 (CH₂). ¹⁹F NMR: δ –4.0 (CF₃), –45.5 (CF₂). MS (ESI) *m/z* (%): 299 [(M+H)⁺, 100]. Anal. (C₁₂H₁₁F₅O₃) C, H.

1,1,1,2,2-Pentafluoro-7-(4-hexyloxyphenyl)heptan-3-one (12c)

Yield 61%; yellowish oil. ¹H NMR (CDCl₃): δ 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, OCH₂), 2.75 (2H, t, *J* = 6.6 Hz, CH₂), 2.57 (2H, t, *J* = 6.2

Hz, CH₂), 1.77-1.62 (6H, m, 3 × CH₂), 1.44-1.27 (6H, m, 3 × CH₂), 0.90 (3H, t, *J* = 6.8 Hz, CH₃). ¹³C NMR: δ 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, CF₃), 114.4 (Ph), 106.8 (tq, *J*_{C-F2} = 265 Hz, *J*_{C-CF3} = 38 Hz, CF₂), 68.0 (CH₂O), 37.2 (CH₂), 34.5 (CH₂), 31.8 (CH₂), 31.6 (CH₂), 29.3 (CH₂), 27.5 (CH₂), 25.7 (CH₂), 22.6 (CH₂), 14.0 (CH₃). ¹⁹F NMR: δ -4.1 (CF₃), -45.6 (CF₂). MS (ESI) *m/z* (%): 379 [(M-H)⁻, 100]. Anal. (C₁₉H₂₅F₅O₂) C, H.

1,1,1,2,2-Pentafluoro-6-(4-octylphenoxy)hexan-3-one (12f)

Yield 70%; yellowish oil. ¹H NMR (CDCl₃): δ 7.10 (2H, d, *J* = 8 Hz, Ph), 6.81 (2H, d, *J* = 8 Hz, Ph), 3.99 (2H, t, *J* = 6.6 Hz, CH₂), 3.00 (2H, t, *J* = 6.6 Hz, CH₂), 2.57 (2H, t, *J* = 6.2 Hz, CH₂), 2.41-2.14 (2H, m, CH₂), 1.64-1.58 (2H, m, CH₂), 1.38-1.21 (10H, m, 5 × CH₂), 0.91 (3H, t, *J* = 6.8 Hz, CH₃). ¹³C NMR: δ 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, CF₃), 114.4 (Ph), 106.8 (tq, *J*_{C-F2} = 267 Hz, *J*_{C-CF3} = 38 Hz, CF₂), 65.8 (CH₂O), 35.4 (CH₂), 34.5 (CH₂), 31.5 (CH₂), 30.6 (CH₂), 29.3 (CH₂), 25.7 (CH₂), 22.6 (CH₂), 21.9 (CH₂), 14.2 (CH₃). ¹⁹F NMR: δ -4.2 (CF₃), -45.6 (CF₂). MS (ESI) *m/z* (%): 393 [(M-H)⁻, 100]. Anal. (C₂₀H₂₇F₅O₂) C, H.

1,1,1,2,2-Pentafluoro-7-(naphthalen-2-yl)heptan-3-one (12h)

Yield 38%; yellowish oil. ¹H NMR (CDCl₃): δ 7.88-7.28 (7H, m, Ph), 2.83-2.78 (4H, m, 2 × CH₂), 1.80-1.74 (4H, m, 2 × CH₂). ¹³C NMR: δ 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, CF₃), 107.2 (tq, *J*_{C-F2} = 265 Hz, *J*_{C-CF3} = 38 Hz, CF₂), 37.4 (CH₂), 35.9 (CH₂), 30.5 (CH₂), 22.2 (CH₂). ¹⁹F NMR: δ -4.1 (CF₃), -45.5 (CF₂). MS (ESI) *m/z* (%): 329 [(M-H)⁻, 100]. Anal. (C₁₇H₁₅F₅O) 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 (CDCl₃): δ 7.10 (2H, d, *J* = 8 Hz, Ph), 6.80 (2H, d, *J* = 8 Hz, Ph), 3.99 (2H, t, *J* = 6.6 Hz, OCH₂), 2.95 (2H, t, *J* = 6.6 Hz, CH₂), 2.54 (2H, t, *J* = 6.2 Hz, CH₂), 2.20-2.10 (2H, m, CH₂), 1.61-1.51 (2H, m, CH₂), 1.28-1.21 (10H, m, 5 × CH₂), 0.88 (3H, t, *J* = 6.8 Hz, CH₃). ¹³C NMR: δ 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, CF₃), 114.2 (Ph), 65.8 (CH₂O), 35.0 (CH₂), 33.1 (CH₂), 31.9 (CH₂), 31.7 (CH₂), 31.6 (CH₂), 31.5 (CH₂), 29.5 (CH₂), 22.6 (CH₂), 22.4 (CH₂), 14.0 (CH₃). ¹⁹F NMR: δ -1.5 (s, CF₃). MS (ESI) *m/z* (%): 343 [(M-H)⁻, 100]. Anal. (C₁₉H₂₇F₃O₂) C, H.

1,1,1-Trifluoro-6-(naphthalen-2-yl)hexan-2-one (12g)

Yield 39%; yellowish oil. ¹H NMR (CDCl₃): δ 7.81-7.29 (7H, m, Ph), 2.81-2.73 (4H, m, 2 × CH₂), 1.79-1.73 (4H, m, 2 × CH₂). ¹³C NMR: δ 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, CF₃), 36.2 (CH₂), 35.6 (CH₂), 30.3 (CH₂), 22.0 (CH₂). ¹⁹F NMR: δ -1.5 (s, CF₃). MS (ESI) *m/z* (%): 279 [(M-H)⁻, 100]. Anal. (C₁₆H₁₅F₃O) 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, 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 sodium hydroxide 1N (1 mL, 1 mmol). The mixture was stirred at room temperature for 12 h, acidified with 1N HCl and extracted with EtOAc (3 × 10 mL). The solvent was removed in vacuo to afford the saturated acid. Yield 66%; white solid; mp 40–41 °C. ¹H NMR (CDCl₃): δ 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, CH₂), 2.36 (2H, t, *J* = 6.0 Hz, CH₂), 1.73–1.63 (4H, m, 2 × CH₂). ¹³C NMR: δ 178.4 (CO), 155.5 (arom), 140.9 (arom), 110.0 (arom), 104.9 (arom), 33.8 (CH₂), 27.6 (CH₂), 27.4 (CH₂), 24.1 (CH₂).

(2E,4E)-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 (CDCl₃): δ 7.58–7.22 (6H, m, Ph, CH), 7.05–6.90 (2H, m, CH), 6.00 (1H, d, *J* = 15 Hz, CH). ¹³C NMR: δ 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-Octyl-phenoxy)butyric acid ethyl ester (15)

A mixture of *p*-octyl-phenol (206 mg, 1 mmol), K₂CO₃ (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 solvent 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 (CDCl₃): δ 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, OCH₂CH₃), 3.92 (2H, t, *J* = 6.6 Hz, OCH₂), 2.60–2.45 (4H, m, 2 × CH₂), 2.18–2.05 (2H, m, CH₂CH₂COO), 1.65–1.42 (2H, m, CH₂), 1.38–1.21 (13H, br, 5 × CH₂, CH₃), 0.90 (3H, t, *J* = 6.8 Hz, CH₃). ¹³C NMR: δ 173.4 (CO), 157.1 (Ph), 135.3 (Ph), 129.4 (Ph), 114.4 (Ph), 66.9 (CH₂O), 60.5 (OCH₂CH₃), 35.3 (CH₂), 32.1 (CH₂), 32.0 (CH₂), 31.5 (CH₂), 31.0 (CH₂), 29.7 (CH₂), 29.5 (CH₂), 24.9 (CH₂), 22.9 (CH₂), 14.5 (CH₃), 14.3 (CH₃). Anal. (C₂₀H₃₂O₃) 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 stirring for 2 h at –78 °C and another 3 h at room temperature, the reaction mixture was quenched with saturated aqueous NaHCO₃ (2.5 mL). The organic phase was then washed with brine and dried (Na₂SO₄). 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. ¹H NMR (CDCl₃): δ 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, CH₃O), 2.67 (2H, t, *J* = 6.6 Hz, PhCH₂), 2.04–1.78 (4H, m, 2 × CH₂). ¹³C NMR: δ 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 (CH₃), 35.1 (CH₂), 31.8 (d, $J_{C-CF} = 20.8$ Hz, CH₂CHF), 25.9 (CH₂). ¹⁹F NMR: δ -114.1 (CF). MS (ESI) m/z (%): 212 [(M+H)⁺, 100]. Anal. (C₁₂H₁₅FO₂) C, H.

Methyl 6-Phenyl-2-fluorohexanoate (17b)

Yield 52%; colourless oil. ¹H NMR (CDCl₃): δ 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, CH₃O), 2.64 (2H, t, $J = 6.6$ Hz, PhCH₂), 2.10-1.46 (6H, m, 3 $\times$ CH₂). ¹³C NMR: δ 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 (CH₃), 35.4 (CH₂), 32.0 (d, $J_{C-CF} = 20.8$ Hz, CH₂CHF), 30.7 (CH₂), 23.8 (d, $J_{C-C-CF} = 3.0$ Hz CH₂). ¹⁹F NMR: δ -114.1 (CF). Anal. (C₁₃H₁₇FO₂) C, H.

Synthesis of 1,1,1,3-Tetrafluoro Ketones

Method A

A solution of compound **17a** or **17b** (1 mmol) and trifluoromethyltrimethylsilane (283 μ L, 1.92 mmol) in ethylene glycol dimethyl ether (0.92 mL) at 0 °C was treated with cesium fluoride (4 mg). After stirring for 30 min at 0 °C and another 18 h at 25 °C the reaction mixture was treated with concentrated HCl (1 mL). After stirring 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 (Na₂SO₄). 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 trifluoromethyl-trimethylsilane (1 mL, 6.9 mmol) in toluene (9 mL) at μ 78 °C was treated with TBAF 1.0 M (45 μ L) in THF. After stirring for 2 h at 25 °C the intermediate silyl ether was formed and then it was treated by TBAF 1.0 M (1.2 mmol) in THF and glacial (3 drops) acetic acid. The reaction mixture stirred for 30 min at 25 °C and diluted with EtOAc (10 mL). The organic phase was washed first with saturated solution of K₂CO₃ and then with brine and dried (Na₂SO₄). 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. ¹H NMR (CDCl₃): δ 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 (3/4H, s, OH), 3.49 (3/4H, s, OH), 2.68 (2H, t, $J = 6.2$ Hz, CH₂), 1.90-1.10 (4H, m, 2 $\times$ CH₂). ¹³C NMR: 141.6 (Ph), 128.4 (Ph), 126.1 (Ph), 125.9 (Ph), 122.6 (q, $J_{C-F3} = 286$ Hz, CF₃), 92.4 (d, $J_{C-F} = 175$ Hz, CF), 92.2 [m, C(OH)₂], 35.4 (CH₂), 31.8 (d, $J_{C-F} = 20$ Hz, CH₂), 27.6 (d, $J_{C-F} = 20$ Hz, CH₂). ¹⁹F NMR: δ 1.6 (CF₃), -5.3 (CF₃), -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. ¹H NMR (CDCl₃): δ 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, CH₂), 1.84-1.80 (2H, m, CH₂), 1.74-1.42 (4H, m, 2 $\times$ CH₂). ¹³C NMR: 142.1 (Ph), 128.3 (Ph), 125.8 (Ph), 125.3 (Ph), 122.6 (q, $J_{C-F3} = 286$ Hz, CF₃), 92.3 (d, $J_{C-F} = 175$ Hz, CF), 92.2 [m, C(OH)₂], 35.6 (CH₂), 31.0 (CH₂), 27.8 (d, $J_{C-F} = 20$ Hz, CH₂), 24.6 (d,

$J_{C-C-F} = 2.6$ Hz, CH₂). ¹⁹F NMR: δ 1.6 (CF₃), -5.3 (CF₃), -120.8 (CHF). MS (ESI) m/z (%): 261 [(M-H)⁻, 100].

In Vitro PLA₂ Assays

Phospholipase A₂ activity was determined using the previously described modified Dole assay²⁸ 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 follow: (i) GIVA cPLA₂ substrate mixed-micelles were composed of 400 μ M Triton X-100, 97 μ M PAPC, 1.8 μ M ¹⁴C-labeled PAPC, and 3 μ M PIP₂ in buffer containing 100 mM HEPES pH 7.5, 90 μ M CaCl₂, 2 mM DTT and 0.1 mg/ml BSA; (ii) GVI iPLA₂ substrate mixed-micelles were composed of 400 μ M Triton X-100, 98.3 μ M PAPC, and 1.7 μ M ¹⁴C-labeled PAPC in buffer containing 100 mM HEPES pH 7.5, 2 mM ATP and 4 mM DTT; and (iii) GV sPLA₂ substrate mixed-micelles were composed of 400 μ M Triton X-100, 99 μ M DPPC, and 1.5 μ M ¹⁴C-labeled DPPC in buffer containing 50 mM Tris pH 8.0 and 5 mM CaCl₂.

In Vitro PLA₂ Inhibition Studies

Initial screening of compounds at 0.091 mole 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 7 concentrations between 0.00091 and 0.091 mole fraction). Inhibition curves were modeled in Graphpad Prism 5.0 using non-linear 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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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-piperazine ethane sulfonic acid
NMM	N-methylmorpholine

PAPC	1-palmitoyl-2-arachidonylphosphatidylcholine
PIP ₂	phosphatidyl inositol (4,5)-bisphosphate
TBAF	tetra-n-butylammonium fluoride
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
Tris	tris(hydroxymethyl)aminomethane
TMS	tetramethylsilane
WSCl•HCl	<i>N</i> -(3-dimethylaminopropyl)- <i>N'</i> -ethyl carbodiimide hydrochloride

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1 BEL **2 FKGK11** **3 FKGK2** **4**

Figure 1.
Some known inhibitors of GVIA iPLA₂.

Figure 2. GVIA iPLA₂ % inhibition at 0.091 mole fraction of inhibitor in mixed micelles (top) and X_I(50) values (bottom). Standard error (n = 3) for average % inhibition and X_I(50) values for all synthesized compounds is indicated.

Figure 3.

Dose-response curves for GVIA iPLA₂ inhibition by inhibitors **12g** and **12i**. Inhibition of the activity of human GVIA iPLA₂ was tested on mixed-micelles containing 100 μ M PAPC and 400 μ M Triton X-100. Inhibition curves were generated using Graphpad Prism with a non-linear regression targeted at symmetrical sigmoidal curves based on plots of % inhibition versus log(inhibitor concentration). The reported $X_1(50)$ values were calculated from the resultant plots.

Figure 4.
Model for the binding mode of fluoroketone inhibitors in the active-site crevice of GVIA iPLA₂.

Scheme 1.

Reagents and conditions: (a) (i) $(\text{COCl})_2$, CH_2Cl_2 , (ii) $(\text{C}_3\text{F}_7\text{CO})_2\text{O}$, pyridine, CH_2Cl_2 .

Scheme 2.

Reagents and conditions: (a) $\text{C}_2\text{H}_5\text{OOCCH}=\text{CHCH}_2\text{P}(=\text{O})(\text{OC}_2\text{H}_5)_2$, LiOH, THF; (b) (i) NaOH, 1,4-dioxane; (c) DMAP, NMM, WSCl^+HCl , $\text{CH}_3\text{ONHCH}_3^+\text{HCl}$, CH_2Cl_2 ; (d) $\text{CF}_3\text{CF}_2\text{I}$, $\text{CH}_3\text{Li}^+\text{LiBr}$, Et_2O .

Scheme 3.

Reagents and conditions: (a) (i) (COCl)₂, CH₂Cl₂, (ii) (CF₃CO)₂O or (C₂F₅CO)₂O or (C₃F₇CO)₂O, pyridine, CH₂Cl₂; (b) C₂H₅OOCCH=CHCH₂P(=O)(OC₂H₅)₂, LiOH, THF; (c) H₂, 10% Pd/C; (d) NaOH, CH₃OH; (e) Br(CH₂)₃COOEt, K₂CO₃, acetone.

Scheme 4.

Reagents and conditions: (a) DAST, CH_2Cl_2 or $(\text{CH}_3\text{OCH}_2\text{CH}_2)_2\text{NSF}_3$, CH_2Cl_2 ; (b) (i) $(\text{CH}_3)_3\text{SiCF}_3$, CsF, $\text{CH}_3\text{OCH}_2\text{CH}_2\text{OCH}_3$ or $(\text{CH}_3)_3\text{SiCF}_3$, TBAF, toluene, (ii) conc. HCl or TBAF, CH_3COOH , THF.

Table 1

b by fluoroketones.^a

Structure	GVIA iPLA ₂		GIVA cPLA ₂		GV sPLA ₂
	% Inhibition	X ₁ (50)	% Inhibition	X ₁ (50)	% Inhibition
99.4 ± 0.1	0.0014 ± 0.0001	N.D.		28 ± 1
99.4 ± 0.0	0.0022 ± 0.0001	32.6 ± 4.0		61.8 ± 6.7
98.4 ± 0.3	0.0030 ± 0.0002	N.D.		N. D.

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Structure	GVIA iPLA ₂		GIVA cPLA ₂		GV sPLA ₂
	% Inhibition	X ₁ (50)	% Inhibition	X ₁ (50)	% Inhibition
94.3 ± 1.5	0.0390 ± 0.0024	44.8 ± 3.7		67.3 ± 7.7
97.0 ± 1.3	0.0258 ± 0.0016	82.0 ± 1.2		65.8 ± 1.8
96.0 ± 0.8	0.0313 ± 0.0025	59.0 ± 3.6		N. D.
99.1 ± 0.5	0.0036 ± 0.0001	N. D.		N. D.

Structure	GVIA iPLA ₂		GIVA cPLA ₂		GV sPLA ₂	
	% Inhibition	X _I (50)	% Inhibition	X _I (50)	% Inhibition	% Inhibition
80.6 ± 2.5	0.0574 ± 0.0030	57.0 ± 1.9		33.9 ± 18.4
96.4 ± 0.6	0.0066 ± 0.0005	N. D.		76.9 ± 5.3
98.3 ± 0.2	0.0084 ± 0.0006	76.1 ± 1.8		71.7 ± 3.6
95.8 ± 1.3	0.0136 ± 0.0006	43.7 ± 3.2		76.9 ± 2.2
99.4 ± 0.1	0.0029 ± 0.0001	91.5 ± 0.9	0.022 ± 0.001	61.4 ± 5.3
99.4 ± 0.1	0.0024 ± 0.0001	88.8 ± 0.7		63.0 ± 6.2	

Structure	GVIA iPLA ₂		GIVA cPLA ₂		GV sPLA ₂
	% Inhibition	X _I (50)	% Inhibition	X _I (50)	% Inhibition
99.9 ± 0.1	0.0002 ± 0.0000	80.8 ± 1.5		36.8 ± 7.9
99.8 ± 0.0	0.0006 ± 0.0000	77.1 ± 1.8		58.4 ± 5.7
99.7 ± 0.2	0.0010 ± 0.0001	55.8 ± 2.1		46.3 ± 10.0
97.6 ± 0.2	0.0025 ± 0.0001	52.1 ± 2.2		N. D.
98.8 ± 0.2	0.0013 ± 0.0000	66.0 ± 3.7		N. D.