New potent and selective polyfluoroalkyl ketone inhibitors of GVIA calcium-independent phospholipase A$_2$

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ABSTRACT

Group VIA calcium-independent phospholipase A$_2$ (GVIA iPLA$_2$) has recently emerged as an important pharmaceutical target. Selective and potent GVIA iPLA$_2$ inhibitors can be used to study its role in various neurological disorders. In the current work, we explore the significance of the introduction of a substituent in previously reported potent GVIA iPLA$_2$ inhibitors. 1,1,1,2,2-Pentafluoro-7-(4-methoxyphenyl)heptan-3-one (GK187) is the most potent and selective GVIA iPLA$_2$ inhibitor ever reported with a $X_I(50)$ value of 0.0001, and with no significant inhibition against GIVA cPLA$_2$ or GV sPLA$_2$. We also compare the inhibition of two difluoromethyl ketones on GVIA iPLA$_2$, GIVA cPLA$_2$, and GV sPLA$_2$.

1. Introduction

Phospholipases A$_2$ are the enzymes that hydrolyse the ester bond of phospholipids at the sn-2 position releasing free fatty acids and lysophospholipids.[1] Both of the products of this hydrolysis may generate second messengers that play significant pharmacological roles, and especially when the released free fatty acid is arachidonic acid. The PLA$_2$ superfamily currently consists of 16 different groups and various subgroups.[2] Three of the most important types of PLA$_2$s that can be found in human tissues are the secreted (such as GIIA and GV sPLA$_2$), the cytosolic GIVA cPLA$_2$ and the calcium-independent GVIA iPLA$_2$.

GIVA cPLA$_2$ is considered to be a proinflammatory enzyme that is the rate-limiting provider of arachidonic acid and lysophospholipids.[3] GIVA cPLA$_2$ is regulated by intracellular calcium, and calcium binding to the C2 domain of GIVA PLA$_2$ can activate the enzyme, resulting in the localization of the enzyme to the phospholipid membrane.[4,5] Furthermore, the activity of sPLA$_2$s has been suggested to be dependent on or linked to the activity of cPLA$_2$.[6–8]

GVIA iPLA$_2$ is a phospholipase A$_2$ that can be characterized by its calcium-independent activity. It was purified and characterized from macrophages in 1994[9] and it functions through a catalytic serine at the active site in a patatin-like α/β-hydrolase domain. It is a 752-amino acid protein with a molecular mass of 85 kDa that contains eight ankyrin repeats and a catalytic domain.[10–12] Both intracellular enzymes GIVA cPLA$_2$ and GVIA iPLA$_2$ share the same catalytic mechanism utilizing a serine residue as the nucleophile, while the active site serine of GVIA iPLA$_2$ lies within a lipase consensus sequence (Gly-X-Ser519-X-Gly) on top of the catalytic domain.[2] GVIA iPLA$_2$ is known to be a homeostatic enzyme involved in basal metabolism within the cell.[13–19] Several studies also suggest that GVIA iPLA$_2$ plays significant 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 (e.g., diabetes, Barth syndrome, ischemia and cancer).[20–27]

Various GVIA iPLA$_2$ inhibitor classes have been discussed in recent review articles.[2,28–30] The first reported GVIA iPLA$_2$ inhibitors were the trifluoromethyl ketones,[31] tricarbonyls[32] and methyl fluorophosphonates[33] of fatty acids, such as arachidonic acid. They were not very potent, nor selective inhibitors, while the methyl fluorophosphonates were also irreversible. Most recently, cardiolipin was found to inhibit iPLA$_2$ and cPLA$_2$ activity towards PC in vitro.[34]

Bromoenol lactone (BEL, Fig. 1) was considered to be a selective, irreversible GVIA iPLA$_2$ inhibitor and was used to study potential
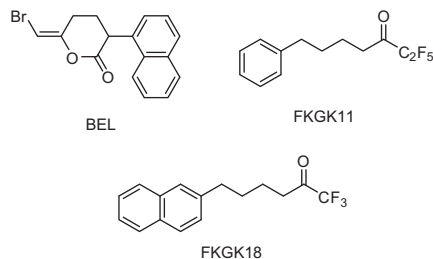
Figure 1. Some known iPLA₂ inhibitors.

biological functions of GVIA iPLA₂.[13,31] The inactivation mechanism of GVIA iPLA₂ by BEL has been studied by Turk and co-workers.[35] It is likely that this inhibitor affects multiple enzymes and should be used with appropriate caution when studying potential roles of GVIA iPLA₂.

The development of selective inhibitors for the three main human PLA₂ enzymes is of paramount importance. Our groups have previously synthesized and assayed a series of polyfluoroalkyl ketones for their activity on GIVA cPLA₂, GVIA iPLA₂, and GVs-PLA₂.[36,37] When compared to a trifluoromethyl ketone, it was found that the corresponding pentafluoroethyl ketone favored selective GVIA iPLA₂ inhibition. FKGK11 (Fig. 1) was found to be a selective GVIA iPLA₂ inhibitor, while FKGK18 (Fig. 1) was identified as the most potent GVIA PLA₂ inhibitor yet reported.[37] Selective PLA₂ inhibitors may contribute to the clarification of the role of each PLA₂ class in various disorders. Using the selective GVIA iPLA₂ inhibitor FKGK11, a selective GIVA cPLA₂ inhibitor, and a pan-PLA₂ inhibitor, the role of the various classes of PLA₂ in an animal model of multiple sclerosis, EAE, was studied.[38] According to the results of that study, GIVA cPLA₂ plays a role in the onset of the disease, while GVIA iPLA₂ plays a key role in both the onset and the progression of the disease. Therefore, it appears that GVIA iPLA₂ is a target enzyme for the development of novel therapies for multiple sclerosis.[38] Furthermore, in a very recent article, the inhibition mechanism of GVIA iPLA₂ by a fluoroketone ligand was examined using a combination of deuterium exchange mass spectrometry (DXMS) and molecular dynamics (MD), while models for iPLA₂ were built by homology with the known structure of patatin.[39] The discovery of the precise binding mode of fluoroketone ligands to iPLA₂ should greatly improve our ability to design new inhibitors with higher potency and selectivity.

Based on previous results, we have explored further this family of GVIA iPLA₂ inhibitors and herein we describe our most recent results.

2. Results and discussion

2.1. Design of inhibitors

The design of the novel polyfluoroalkyl ketones was based on the optimization of the activity and selectivity of iPLA₂ inhibitors that we have presented in previous work, such as FKGK11 and FKGK18 (Fig. 1).[36,37] Having established that the best linker between a polyfluoroalkyl ketone and an aromatic ring is a chain of four methylene groups, we introduced in the aromatic ring different substituents and studied the effect of these substituents on the affinity towards GVIA iPLA₂, as well as the selectivity towards GVIA iPLA₂ when compared to GIVA cPLA₂ and GV sPLA₂ activity. Several substituents were introduced in para position, such as a fluorine atom, a methoxy group, a phenyl group, and a trifluoromethyl group. Also, the isomer of the most potent iPLA₂ inhibitor FKGK18 was prepared, where the naphthyl group was attached to the linker at the 1-position.

Furthermore, compounds **12** and **13** were synthesized as the structurally restricted analogues of the inhibitor FKGK11 to determine the effect that the second phenyl group would have on the activity of the inhibitor.

Finally, two difluoromethyl ketones were prepared that resembled the structure of inhibitor FKGK11 in order to identify the effect that different number of fluorine atoms would have on the activity of polyfluoroalkyl ketones.

The inhibition studies showed high activity and selectivity for compounds **5d** and **6d** that had a methoxy group at *para* position. Thus, we prepared another series of substituted polyfluoroalkyl ketones bearing one or two methoxy groups in different positions of the phenyl or in the naphthalene group to see the effect on inhibition and selectivity.

2.2. Synthesis of inhibitors

For the synthesis of trifluomethyl and pentafluoroethyl ketones **5a–j** and **6a–j**, a Wadsworth–Horner–Emmons olefination reaction of the corresponding commercially available substituted aromatic aldehydes **1a–j** with triethyl phosphonocrotonate yielded the usaturated esters **2a–j** (Scheme 1). Hydrogenation with 10% Pd/C gave esters **3a–j**, followed by saponification to afford acids **4a–j**.

After treating compounds **4a–j** with oxalyl chloride, the corresponding chlorides were treated with trifluoroacetic or pentafluoropropionic anhydride and pyridine to yield trifluoromethyl ketones **5a–j** and pentafluoropropyl ketones **6a–j**. In the case of heptafluorobutyl ketone **7a**, the corresponding chloride was treated with heptafluorobutyric anhydride and pyridine.
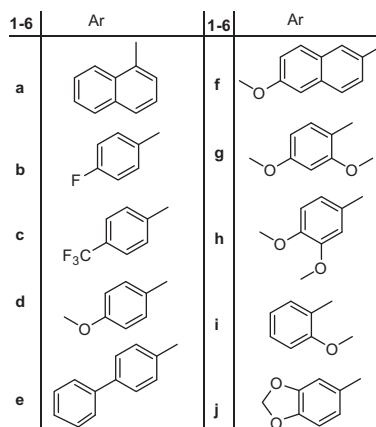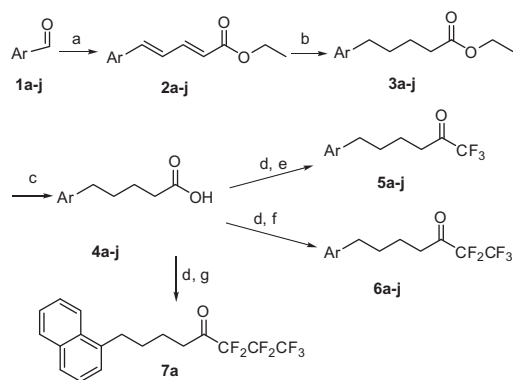
Scheme 1. Reagents and conditions: (a) C₂H₅OOCCH=CHCH₂P(=O)(OC₂H₅)₂, LiOH, THF, reflux; (b) H₂, 10% Pd/C, EtOH; (c) NaOH 1 N, EtOH; (d) (COCl)₂, DMF, CH₂Cl₂; (e) pyridine, (CF₃CO)₂O, CH₂Cl₂, 0 °C to rt; (f) pyridine, (CF₃CF₂CO)₂O, CH₂Cl₂, 0 °C to rt; (g) pyridine, (CF₃CF₂CF₂CO)₂O, CH₂Cl₂, 0 °C to rt.

For the synthesis of trifluomethyl and pentafluoroethyl ketones **12** and **13**, a Wittig olefination reaction between aldehyde **8** and methyl (triphenylphosphanylidene)acetate yielded usaturated ester **9** (Scheme 2). Catalytic hydrogenation, followed by saponification gave compound **11**. Ketones **12** and **13** were prepared similarly as described above.

The difluoromethyl ketones were prepared from bromides **14a** and **14b**, after being treated with magnesium, and the corresponding Grignard reagents were slowly added to ethyl difluoroacetate at −78 °C to yield ketones **16a** and **16b** (Scheme 3).

## 2.3. In vitro inhibition of GIIA sPLA₂, GIVA cPLA₂ and GVIA iPLA₂

All synthesized inhibitors were tested for inhibition of human GIVA cPLA₂, GVIA iPLA₂ and GV sPLA₂ using previously described mixed micelle-based assays.[40–42] The inhibition results are presented in Table 1, either as percent inhibition or as $X_I(50)$ values. At first, the percent of inhibition for each PLA₂ enzyme at 0.091 mole fraction of each inhibitor was determined; and, then the $X_I(50)$ values were measured for compounds that displayed greater than 95% inhibition. The $X_I(50)$ is the mole fraction of the inhibitor in the total substrate interface required to inhibit the enzyme by 50%.

The isomer of the most potent iPLA₂ inhibitor FKGK18, compound **5a** seems to be a 9-times weaker inhibitor towards GVIA iPLA₂, while there is no significant selectivity towards GIVA cPLA₂. Interestingly enough, the pentafluoro and heptafluoro ketone analogues **6a** and **7a** are even weaker iPLA₂ inhibitors. The methoxy group in position 6 of the naphthalene group also seems to lower the inhibitory potency of FKGK18 in compounds **5f** and **6f**.

Compounds **5b–e** and **6b–e** were prepared as substituted analogues of FKGK11. Most of these compounds presented excellent iPLA₂ inhibition, with the exception of compounds **5e** and **6e**, which were 13-fold and ninefold weaker towards iPLA₂ than FKGK11. It was interesting though that trifluoromethyl ketone **5e**, 6-(biphenyl-4-yl)-1,1,1-trifluorohexan-2-one (GK174), seemed to be a more potent inhibitor towards GIVA cPLA₂ than for GVIA iPLA₂. Compounds **5b** (GK176) and **5c** (GK178) proved to be as potent as FKGK18, and compound **5c** showed even better selectivity when compared to GIVA cPLA₂ and GV sPLA₂. The most potent inhibitors proved to be compounds **5d** (1,1,1-trifluoro-6-(4-methoxyphenyl)hexan-2-one, GK177) and **6d** (1,1,1,2,2-pentafluoro-7-(4-methoxyphenyl)heptan-3-one, GK187) bearing a methoxy group at the *para* position of the phenyl substituent. They
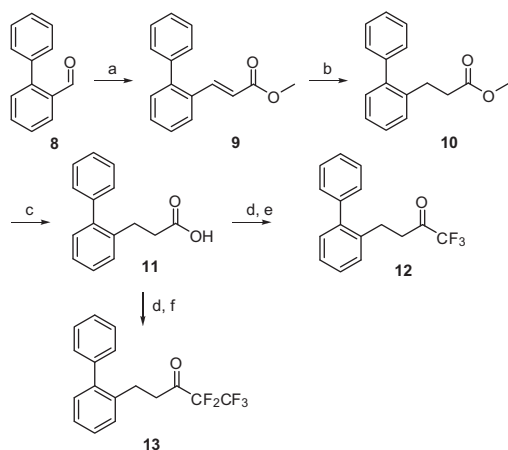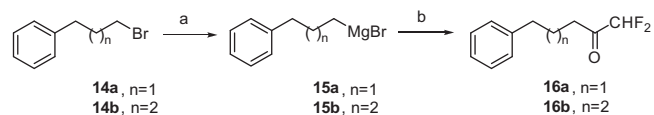
Scheme 3. Reagents and conditions: (a) Mg, dry Et₂O; (b) CHF₂COOEt, dry Et₂O, −78 °C.

both present $X_I(50)$ values of 0.0001 and they are much more selective to GVIA iPLA₂ when compared to GIVA cPLA₂ and GV sPLA₂.

Taking into consideration these results, we prepared and tested in vitro a series of other polyfluoroalkyl ketones bearing one or two methoxy groups in different positions of the phenyl group, in order to find even more potent and selective GVIA iPLA₂ inhibitors. However, compounds **5g–j** and **6g–j** lost the potency that inhibitors **5d** and **6d** presented. The most potent of this group were compounds **5j** and **6j**, bearing a dioxolane ring on the phenyl group.

The structurally restricted analogues of inhibitor FKGK11 **12** and **13** did not give optimum potency; instead they were weak, yet selective, iPLA₂ inhibitors.

Finally, the two difluoromethyl ketones **16a** and **16b** that are analogues of FKGK11 presented good selectivity, but low activity, towards GVIA iPLA₂ when compared with GIVA cPLA₂ and GV sPLA₂.

3. Conclusion

In the present study, we identified six fluoroketones (**5b**, **5c**, **5d**, **6b**, **6c**, and **6d**) that are very potent inhibitors of GVIA iPLA₂. All of them are more potent than the previous lead inhibitor FKGK11, which has been successfully used in animal models of neurological disorders,[38] but compounds **5d** and **6d** are also more potent than the most potent iPLA₂ inhibitor FKGK18 in the literature. Especially, compound **6d** (GK187) is the most potent, but also the most selective iPLA₂ inhibitor presented, since it shows less that 25% inhibition against GIVA cPLA₂ and 32.8% against GV sPLA₂ at 0.091 mol fraction.

In conclusion, a series of potent GVIA iPLA₂ inhibitors was developed. The introduction of a methoxy group at the *para* position of the phenyl group of the lead compound FKGK11 resulted in the most potent GVIA iPLA₂ inhibitor ever reported ($X_I(50) = 0.0001$). By the use of these inhibitors in studies in animal models, the role of GVIA iPLA₂ in inflammatory conditions or neurological diseases may be further explored. Since GVIA iPLA₂ has emerged as a novel target for drug discovery, the identification of potent and selective iPLA₂ inhibitors is of paramount importance.

4. Experimental section

4.1. General

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 were recorded in chloroform (CDCl₃), using CHCl₃ residual peak as the ¹H internal reference (7.27 ppm); and the central peak of CDCl₃ at 77.0 ppm for ¹³C NMR. All ¹⁹F NMR chemical shifts were referenced to CFCl₃ (0.0 ppm). 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

Scheme 2. Reagents and conditions: (a) CH₃OOCCH=PPh₃, dry THF; (b) H₂, 10% Pd/C, MeOH; (c) NaOH 1 N, MeOH; (d) (COCl)₂, DMF, CH₂Cl₂; (e) pyridine, (CF₃CO)₂O, CH₂Cl₂, 0 °C to rt; (f) pyridine, (CF₃CF₂CO)₂O, CH₂Cl₂, 0 °C to rt.

Table 1
Inhibition of PLA_2 by fluoroketones[a]

Number	GVIA $iPLA_2$		GIVA $cPLA_2$	GV $sPLA_2$
	% Inhibition at 0.091	$X_I(50)$	% Inhibition at 0.091	% Inhibition at 0.091
FKGK11[37]	99.4	0.0014 ± 0.0001	N.D.	28.0
FKGK18[37]	99.9	0.0002 ± 0.0000	80.8	63.0
5a	98.7	0.0018 ± 0.0002	77.4	31.9
6a	96.4	0.0034 ± 0.0002	64.0	29.4
7a	76.7		60.4	57.8
5b	98.5	0.0002 ± 0.0000	41.4	N.D.
6b	98.2	0.0003 ± 0.0001	N.D.	29.3
5c	99.9	0.0002 ± 0.0000	83.9	30.4
6c	98.9	0.0003 ± 0.0001	62.5	36.9
5d	99.8	0.0001 ± 0.0000	54.3	N.D.
6d	99.8	0.0001 ± 0.0000	N.D.	32.8
5e	97.9	0.0189 ± 0.0045	96.5 $X_I(50)$: 0.0074 ± 0.0003	40.5
6e	94.6	0.0134 ± 0.0017	72.6	38.3
5f	92.6		85.5	41.7
6f	92.5		54.7	63.1
5g	89.6		64.3	N.D.
6g	95.6	0.0553 ± 0.0056	35.6	38.7
5h	62.5		28.8	N.D.
6h	78.3		N.D.	N.D.
5i	82.8		58.9	N.D.
6i	95.5	0.0263 ± 0.0047	30.5	39.1
5j	98.7	0.0025 ± 0.0002	41.2	N.D.
6j	99.5	0.0019 ± 0.0003	38.3	29.4
12	75.0		N.D.	
13	90.4		N.D.	
16a	86.5		N.D.	N.D.
16b	87.4		N.D.	N.D.

[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 95% inhibition. N.D. signifies compounds with less than 25% inhibition (or no detectable inhibition).

reagent grade and used without further purification. All the products gave satisfactory elemental analysis results.

4.2. Chemistry

4.2.1. Synthesis of trifluoromethyl ketones

Oxalyl chloride (1.5 mL, 3 mmol) and N,N-dimethylformamide (40 μL) were added to a solution of carboxylic acid **4a–j** or **11** (1 mmol) in dry dichloromethane (40 mL). After 2 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 trifluoroacetic anhydride (0.85 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 to 1/9].

4.2.1.1. 1,1,1-Trifluoro-6-(naphthalen-1-yl)hexan-2-one (5a).
Yield 26%; Yellow oil; ^1H NMR (200 MHz, $CDCl_3$): δ 8.10–7.30 (m, 7H, arom), 3.13 (t, 2H, CH_2, J = 5.8 Hz), 2.77 (t, 2H, CH_2, J = 5.8 Hz), 1.86–1.79 (m, 4H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 191.39 (q, CO, J = 35 Hz), 137.62, 133.87, 131.66, 128.82, 126.78, 125.98, 125.83, 125.49, 123.55, 115.51 (q, CF_3, J = 290 Hz), 36.20, 32.67, 29.71, 22.36; ^{19}F NMR (188 MHz, $CDCl_3$): δ −79.7 (CF_3); MS (ESI) m/z (%): 279.2 ([M−H]⁻, 100); Anal. Calcd for $C_{16}H_{15}F_3O$: C, 68.56; H, 5.39. Found: C, 68.47; H, 5.42.

4.2.1.2. 1,1,1-Trifluoro-6-(4-fluorophenyl)hexan-2-one (5b).
Yield 38%; Colorless oil; ^1H NMR (200 MHz, $CDCl_3$): δ 7.17–6.93 (m, 4H, arom), 2.74 (t, 2H, CH_2, J = 6.6 Hz), 2.63 (t, 2H, CH_2, J = 7.2 Hz), 1.80–1.56 (m, 4H, CH_2CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 191.3 (q, CO, J = 45 Hz), 161.3 (d, C-F, J = 242 Hz), 137.2, 129.6 (d, J = 8 Hz), 115.5 (q, CF_3, J = 291 Hz), 115.1 (d, J = 21 Hz), 36.1, 34.6, 30.5, 21.8; ^{19}F NMR (188 MHz, $CDCl_3$): δ −79.8 (CF_3), −118.0 (F); MS (ESI) m/z (%): 247.2 ([M−H]⁻, 85); Anal. Calcd for $C_{12}H_{12}F_4O$: C, 58.07; H, 4.87. Found: C, 58.16; H, 4.85.

4.2.1.3. 1,1,1-Trifluoro-6-(4-(trifluoromethyl)phenyl)hexan-2-one (5c).
Yield 16%; Yellow oil; ^1H NMR (200 MHz, $CDCl_3$): δ 7.53 (d, 2H, arom, J = 8.0 Hz), 7.27 (d, 2H, arom, J = 8.0 Hz), 2.71 (t, 4H, CH_2, J = 7.0 Hz), 1.78–1.60 (m, 4H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 191.4 (t, CO, J = 35 Hz), 145.9, 132.6, 128.2, 125.6, 124.5 (q, CF_3, J = 270 Hz), δ 115.8 (q, CF_3, J = 290 Hz), 36.2, 35.5, 30.3, 22.1; ^{19}F NMR (188 MHz, $CDCl_3$): δ −62.8 (CF_3), −79.8 (CF_3); MS (ESI) m/z (%): 297.1 ([M−H]⁻, 100); Anal. Calcd for $C_{13}H_{12}F_6O$: C, 52.36; H, 4.06. Found: C, 52.48; H, 4.01.

4.2.1.4. 1,1,1-Trifluoro-6-(4-methoxyphenyl)hexan-2-one (5d)[43,44].
Yield 40%; Yellow oil; ^1H NMR (200 MHz, $CDCl_3$): δ 7.09 (d, 2H, arom, J = 8.6 Hz), 6.83 (d, 2H, arom, J = 8.6 Hz), 3.79 (s, 3H, OCH_3), 2.74 (t, 2H, CH_2, J = 6.6 Hz), 2.68 (t, 2H, CH_2, J = 6.8 Hz), 1.80–1.60 (m, 4H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 191.4 (q, CO, J = 34 Hz), 157.8, 133.6, 129.9, 115.5 (q, CF_3, J = 290 Hz), 113.7, 55.2, 36.2, 34.5, 30.6, 21.9; ^{19}F NMR (188 MHz, $CDCl_3$): δ −79.8 (CF_3). MS (ESI) m/z (%): 259.2 ([M−H]⁻, 100); Anal. Calcd for $C_{13}H_{15}F_3O_2$: C, 60.00; H, 5.81. Found: C, 60.11; H, 5.76.

4.2.1.5. 6-(Biphenyl-4-yl)-1,1,1-trifluorohexan-2-one (5e).
Yield 39%; Yellowish oil; ^1H NMR (200 MHz, $CDCl_3$): δ 7.70–7.20 (m, 9H, arom), 2.80–2.60 (m, 4H, CH_2), 1.90–1.60 (m, 4H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 191.4 (q, CO, J = 35 Hz), 140.9, 140.7, 138.9, 128.9, 128.8, 128.7, 127.2, 127.1, 127.0, 126.9, 115.5 (q, CF_3, J = 290 Hz), 36.2, 35.1, 30.4, 22.0; ^{19}F NMR (188 MHz, $CDCl_3$): δ −79.7 (CF_3); MS (ESI) m/z (%): 305.2 ([M−H]⁻,

100); Anal. Calcd for $C_{18}H_{17}F_3O$: C, 70.58; H, 5.59. Found: C, 70.69; H, 5.54.

4.2.1.6. 1,1,1-Trifluoro-6-(6-methoxynaphthalen-2-yl)hexan-2-one (5f).
Yield 53%; Yellow solid; mp 51–53 °C; ^1H NMR (200 MHz, CDCl$_3$): δ 7.70 (d, 2H, arom, J = 8.2 Hz), 7.55 (s, 1H, arom), 7.30 (d, 1H, arom, J = 8.0 Hz), 7.16 (d, 1H, arom, J = 8.2 Hz), 7.14 (s, 1H, arom), 3.92 (s, 3H, OCH$_3$), 2.90–2.55 (m, 4H, CH$_2$), 1.90–1.50 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 191.4 (q, CO, J = 35 Hz), 157.2, 136.7, 133.0, 129.0, 128.8, 127.6, 126.8, 126.2, 118.7, 115.5 (q, CF$_3$, J = 290 Hz), 105.5, 55.2, 36.1, 35.3, 30.3, 21.9; ^{19}F NMR (188 MHz, CDCl$_3$): δ −79.7 (CF$_3$); MS (ESI) m/z (%): 309.3 ([M−H]$^−$, 100); Anal. Calcd for $C_{17}H_{17}F_3O_2$: C, 65.80; H, 5.52. Found: C, 65.88; H, 5.48.

4.2.1.7. 6-(2,4-Dimethoxyphenyl)-1,1,1-trifluorohexan-2-one (5g).
Yield 16%; Yellow oil; ^1H NMR (200 MHz, CDCl$_3$): δ 7.02 (d, 1H, arom, J = 7.2 Hz), 6.45 (s, 1H, arom), 6.42 (d, 1H, arom, J = 7.2 Hz), 3.80 (s, 6H, OCH$_3$), 2.75 (t, 2H, CH$_2$, J = 6.6 Hz), 2.58 (t, 2H, CH$_2$, J = 6.8 Hz), 1.80–1.50 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 191.6 (q, CO, J = 35 Hz), 159.2, 158.2, 129.9, 122.4, 115.5 (q, CF$_3$, J = 291 Hz), 103.7, 98.4, 55.3, 55.2, 36.2, 29.1, 29.0, 21.9; ^{19}F NMR (188 MHz, CDCl$_3$): δ −79.7 (CF$_3$); MS (ESI) m/z (%): 289.3 ([M−H]$^−$, 100); Anal. Calcd for $C_{14}H_{17}F_3O_3$: C, 57.93; H, 5.90. Found: C, 57.87; H, 5.92.

4.2.1.8. 6-(3,4-Dimethoxyphenyl)-1,1,1-trifluorohexan-2-one (5h).
Yield 26%; Yellow oil; ^1H NMR (200 MHz, CDCl$_3$): δ 6.90–6.50 (m, 3H, arom), 3.83 (s, 3H, OCH$_3$), 3.81 (s, 3H, OCH$_3$), 2.70 (t, 2H, CH$_2$, J = 5.8 Hz), 2.55 (t, 2H, CH$_2$, J = 7.0 Hz), 1.85–1.40 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 191.3 (q, CO, J = 35 Hz), 148.7, 147.1, 134.1, 120.0, 115.4 (q, CF$_3$, J = 291 Hz), 111.4, 111.0, 55.7, 55.6, 36.1, 35.0, 30.5, 21.8; ^{19}F NMR (188 MHz, CDCl$_3$): δ −79.8 (CF$_3$); MS (ESI) m/z (%): 289.1 ([M−H]$^−$, 100); Anal. Calcd for $C_{14}H_{17}F_3O_3$: C, 57.93; H, 5.90. Found: C, 57.82; H, 5.94.

4.2.1.9. 1,1,1-Trifluoro-6-(2-methoxyphenyl)hexan-2-one (5i).
Yield 29%; Yellowish oil; ^1H NMR (200 MHz, CDCl$_3$): δ 7.30–7.05 (m, 2H, arom), 6.95–6.75 (m, 2H, arom), 3.82 (s, 3H, OCH$_3$), 2.82–2.54 (m, 4H, CH$_2$), 1.83–1.50 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 191.6 (q, CO, J = 35 Hz), 157.3, 130.0, 129.8, 127.1, 120.3, 115.5 (q, CF$_3$, J = 291 Hz), 110.1, 55.1, 36.1, 29.6, 28.8, 22.0; ^{19}F NMR (188 MHz, CDCl$_3$): δ −79.9 (CF$_3$); MS (ESI) m/z (%): 259.2 ([M−H]$^−$, 100); Anal. Calcd for $C_{13}H_{15}F_3O_2$: C, 60.00; H, 5.81. Found: C, 59.87; H, 5.87.

4.2.1.10. 6-(Benzo[d][1,3]dioxol-5-yl)-1,1,1-trifluorohexan-2-one (5j).
Yield 70%; Yellow oil; ^1H NMR (200 MHz, CDCl$_3$): δ 6.82–6.42 (m, 3H, arom), 5.92 (s, 2H, OCH$_2$O), 2.73 (t, 2H, CH$_2$, J = 6.6 Hz), 2.57 (t, 2H, CH$_2$, J = 6.6 Hz), 1.90–1.50 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 191.4 (q, CO, J = 35 Hz), 147.6, 145.7, 135.4, 121.0, 115.5 (q, CF$_3$, J = 291 Hz), 108.7, 108.1, 100.7, 36.1, 35.1, 30.6, 21.8; ^{19}F NMR (188 MHz, CDCl$_3$): δ −79.8 (CF$_3$); MS (ESI) m/z (%): 273.4 ([M+H]$^+$, 100); Anal. Calcd for $C_{14}H_{15}F_3O_2$: C, 61.76; H, 5.55. Found: C, 61.87; H, 5.49.

4.2.1.11. 4-(Biphenyl-2-yl)-1,1,1-trifluorobutan-2-one (12).
Yield 67%; Yellow oil; ^1H NMR (200 MHz, CDCl$_3$): δ 7.60–7.10 (m, 9H, arom), 3.04 (t, 2H, CH$_2$, J = 7.0 Hz), 2.83 (t, 2H, CH$_2$, J = 7.0 Hz); ^{13}C NMR (50 MHz, CDCl$_3$): δ 190.5 (q, CO, J = 35 Hz), 142.0, 141.1, 136.6, 130.4, 128.4, 127.7, 127.2, 126.7, 115.4 (q, CF$_3$, J = 291 Hz), 37.3, 26.0; ^{19}F NMR (188 MHz, CDCl$_3$): δ −79.7 (CF$_3$). MS (ESI) m/z (%): 277.2 ([M−H]$^−$, 100); Anal. Calcd for $C_{16}H_{13}F_3O$: C, 69.06; H, 4.71. Found: C, 69.15; H, 4.68.

4.2.2. Synthesis of pentafluoroethyl ketones
The synthesis of pentafluoroethyl ketones was carried out following the procedure described above for trifluoromethyl ketones, except that pentafluoropropionic anhydride was used instead of trifluoroacetic anhydride. The products were purified by flash column chromatography [EtOAc–petroleum ether (bp 40–60 °C) 5/95 to 1/9].

4.2.2.1. 1,1,1,2,2-Pentafluoro-7-(naphthalen-1-yl)heptan-3-one (6a).
Yield 65%; Yellow oil; ^1H NMR (200 MHz, CDCl$_3$): δ 8.06–7.32 (m, 7H, arom), 3.13 (t, 2H, CH$_2$, J = 7.2 Hz), 2.81 (t, 2H, CH$_2$, J = 7.0 Hz), 1.86–1.80 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.2 (t, CO, J = 27 Hz), 137.6, 133.9, 131.7, 128.8, 126.8, 126.0, 125.8, 125.5, 123.5, 117.8 (qt, CF$_3$, J_1 = 285 Hz, J_2 = 34 Hz), 109.5 (tq, CF$_2$, J_1 = 265 Hz, J_2 = 38 Hz), 37.2, 32.7, 29.7, 22.3; ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.3 (CF$_3$), −123.7 (CF$_2$); MS (ESI) m/z (%): 329.2 ([M−H]$^−$, 100); Anal. Calcd for $C_{17}H_{15}F_5O$: C, 61.82; H, 4.58. Found: C, 61.87; H, 4.55.

4.2.2.2. 1,1,1,2,2-Pentafluoro-7-(4-fluorophenyl)heptan-3-one (6b).
Yield 53%; Yellow oil; ^1H NMR (200 MHz, CDCl$_3$): δ 7.20–7.00 (m, 2H, arom), 6.98–6.80 (m, 2H, arom), 2.78 (t, 2H, CH$_2$, J = 6.8 Hz), 2.62 (t, 2H, CH$_2$, J = 7.0 Hz), 1.82–1.60 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.2 (t, CO, J = 24 Hz), 161.3 (d, C–F, J = 242 Hz), 137.2, 129.6 (d, J = 8 Hz), 115.1 (d, J = 21 Hz), 122.0–100.0 (m, CF$_2$, CF$_3$), 37.1, 34.7, 30.5, 21.8; ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.3 (CF$_3$), −118.0 (F), −123.8 (CF$_2$); MS (ESI) m/z (%): 297.1 ([M−H]$^−$, 100); Anal. Calcd for $C_{13}H_{12}F_6O$: C, 52.36; H, 4.06. Found: C, 52.42; H, 4.03.

4.2.2.3. 1,1,1,2,2-Pentafluoro-7-(4-(trifluoromethyl)phenyl)heptan-3-one (6c).
Yield 65%; Yellow oil; ^1H NMR (200 MHz, CDCl$_3$): δ 7.57 (d, 2H, arom, J = 5.2 Hz), 7.31 (d, 2H, arom, J = 5.2 Hz), 2.81 (t, 2H, CH$_2$, J = 4.4 Hz), 2.74 (t, 2H, CH$_2$, J = 4.4 Hz), 1.80–1.66 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.1 (t, CO, J = 27 Hz), 145.7, 132.4, 129.3, 128.6, 128.0, 125.3 (q, C–CF$_3$, J = 4 Hz), 121.6, 117.8 (qt, CF$_3$, J_1 = 285 Hz, J_2 = 34 Hz), 109.5 (tq, CF$_2$, J_1 = 265 Hz, J_2 = 38 Hz), 37.1, 35.4, 30.0, 21.8; ^{19}F NMR (188 MHz, CDCl$_3$): δ −62.8 (CF$_3$), −82.4 (CF$_3$), −123.8 (CF$_2$); MS (ESI) m/z (%): 347.1 ([M−H]$^−$, 95); Anal. Calcd for $C_{14}H_{12}F_8O$: C, 48.29; H, 3.47. Found: C, 48.38; H, 3.43.

4.2.2.4. 1,1,1,2,2-Pentafluoro-7-(4-methoxyphenyl)heptan-3-one (6d).
Yield 31%; Colorless oil; ^1H NMR (200 MHz, CDCl$_3$): δ 7.12 (d, 2H, arom, J = 5.6 Hz), 6.87 (d, 2H, arom, J = 5.8 Hz), 3.83 (s, 3H, OCH$_3$), 2.79 (t, 2H, CH$_2$, J = 6.6 Hz), 2.62 (t, 2H, CH$_2$, J = 6.8 Hz), 1.79–1.61 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.3 (t, CO, J = 27 Hz), 157.8, 133.7, 129.2, 113.8, 122.0–100.0 (m, CF$_2$, CF$_3$), 55.2, 37.2, 34.6, 30.6; ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.3 (CF$_3$), −123.8 (CF$_2$); MS (ESI) m/z (%): 309.2 ([M−H]$^−$, 72); Anal. Calcd for $C_{14}H_{15}F_5O_2$: C, 54.20; H, 4.87. Found: C, 54.32; H, 4.84.

4.2.2.5. 7-(Biphenyl-4-yl)-1,1,1,2,2-pentafluoroheptan-3-one (6e).
Yield 63%; Yellow low mp solid; mp 32–34 °C; ^1H NMR (200 MHz, CDCl$_3$): δ 7.80–7.20 (m, 9H, arom), 2.83 (t, 2H, CH$_2$, J = 6.8 Hz), 2.73 (t, 2H, CH$_2$, J = 7.0 Hz), 1.95–1.60 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.2 (t, CO, J = 27 Hz), 141.0, 140.7, 138.9, 128.8, 128.7, 127.1, 127.0, 126.9, 126.4, 125.7, 117.8 (qt, CF$_3$, J_1 = 285 Hz, J_2 = 34 Hz), 106.9 (tq, CF$_2$, J_1 = 265 Hz, J_2 = 38 Hz), 37.1, 35.1, 30.3, 21.9; ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.3 (CF$_3$), −123.7 (CF$_2$); MS (ESI) m/z (%): 355.2 ([M−H]$^−$, 100); Anal. Calcd for $C_{19}H_{17}F_5O$: C, 64.04; H, 4.81. Found: C, 64.16; H, 4.78.

4.2.2.6. 1,1,1,2,2-Pentafluoro-7-(6-methoxynaphthalen-2-yl)heptan-3-one (6f).
Yield 60%; Yellow solid; mp 42–44 °C; ^1H NMR (200 MHz, CDCl$_3$): δ 7.69 (d, 2H, arom, J = 8.8 Hz), 7.54

(s, 1H, arom), 7.28 (d, 1H, arom, *J* = 9.4 Hz), 7.15 (d, 1H, arom, *J* = 8.2 Hz), 7.13 (s, 1H, arom), 3.91 (s, 3H, OCH$_3$), 2.95–2.60 (m, 4H, CH$_2$), 1.90–1.60 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.2 (t, CO, *J* = 27 Hz), 157.2, 136.7, 133.0, 129.0, 128.9, 127.6, 126.8, 126.3, 118.8, 117.8 (qt, CF$_3$, J_1 = 285 Hz, J_2 = 34 Hz), 106.9 (tq, CF$_2$, J_1 = 265 Hz, J_2 = 38 Hz), 105.6, 55.2, 37.2, 35.4, 30.3, 21.9; ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.3 (CF$_3$), −123.8 (CF$_2$); MS (ESI) *m/z* (%): 359.3 ([M−H]$^-$, 100); Anal. Calcd for C$_{18}$H$_{17}$F$_5$O$_2$: C, 60.00; H, 4.76. Found: C, 60.17; H, 4.71.

4.2.2.7. 7-(2,4-Dimethoxyphenyl)-1,1,1,2,2-pentafluoroheptan-3-one (6g).
Yield 52%; Yellow oil; ^{1}H NMR (200 MHz, CDCl$_3$): δ 6.99 (d, 1H, arom, *J* = 7.8 Hz), 6.42 (s, 1H, arom), 6.40 (d, 1H, arom, *J* = 7.8 Hz), 3.77 (s, 6H, OCH$_3$), 2.76 (t, 2H, CH$_2$, *J* = 6.4 Hz), 2.55 (t, 2H, CH$_2$, *J* = 7.0 Hz), 1.78–1.50 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.4 (t, CO, *J* = 26 Hz), 159.2, 158.2, 129.9, 122.4, 117.8 (qt, CF$_3$, J_1 = 285 Hz, J_2 = 34 Hz), 106.9 (tq, CF$_2$, J_1 = 266 Hz, J_2 = 38 Hz), 103.7, 98.4, 55.3, 55.1, 37.2, 29.0, 22.6, 21.9; ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.3 (CF$_3$), −123.8 (CF$_2$); MS (ESI) *m/z* (%): 339.3 ([M−H]$^-$, 100); Anal. Calcd for C$_{15}$H$_{17}$F$_5$O$_3$: C, 52.94; H, 5.04. Found: C, 52.87; H, 5.07.

4.2.2.8. 7-(3,4-Dimethoxyphenyl)-1,1,1,2,2-pentafluoroheptan-3-one (6h).
Yield 48%; Yellowish oil; ^{1}H NMR (200 MHz, CDCl$_3$): δ 6.90–6.60 (m, 3H, arom), 3.88 (s, 3H, OCH$_3$), 3.86 (s, 3H, OCH$_3$), 2.78 (t, 2H, CH$_2$, *J* = 5.4 Hz), 2.59 (t, 2H, CH$_2$, *J* = 6.4 Hz), 1.90–1.42 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.2 (t, CO, *J* = 26 Hz), 148.8, 147.2, 134.2, 120.1, 117.8 (qt, CF$_3$, J_1 = 285 Hz, J_2 = 34 Hz), 111.5, 111.1, 106.8 (tq, CF$_2$, J_1 = 265 Hz, J_2 = 38 Hz), 55.8, 55.7, 37.1, 35.0, 30.5, 21.8; ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.4 (CF$_3$), −123.8 (CF$_2$); MS (ESI) *m/z* (%): 339.3 ([M−H]$^-$, 100); Anal. Calcd for C$_{15}$H$_{17}$F$_5$O$_3$: C, 52.94; H, 5.04. Found: C, 52.87; H, 5.09.

4.2.2.9. 1,1,1,2,2-Pentafluoro-7-(2-methoxyphenyl)heptan-3-one (6i).
Yield 32%; Yellowish oil; ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.35–7.05 (m, 2H, arom), 6.95–6.75 (m, 2H, arom), 3.81 (s, 3H, OCH$_3$), 2.78 (t, 2H, CH$_2$, *J* = 6.6 Hz), 2.63 (t, 2H, CH$_2$, *J* = 6.6 Hz), 1.90–1.50 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.4 (t, CO, *J* = 26 Hz), 157.3, 130.0, 129.8, 127.2, 120.4, 117.8 (qt, CF$_3$, J_1 = 285 Hz, J_2 = 34 Hz), 110.2, 109.5 (tq, CF$_2$, J_1 = 265 Hz, J_2 = 38 Hz), 55.1, 37.2, 29.6, 28.8, 21.9; ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.4 (CF$_3$), −123.8 (CF$_2$); MS (ESI) *m/z* (%): 309.1 ([M−H]$^-$, 85); Anal. Calcd for C$_{14}$H$_{15}$F$_5$O$_2$: C, 54.20; H, 4.87. Found: C, 54.29; H, 4.84.

4.2.2.10. 7-(Benzo[d][1,3]dioxol-5-yl)-1,1,1,2,2-pentafluoroheptan-3-one (6j).
Yield 67%; Yellow oil; ^{1}H NMR (200 MHz, CDCl$_3$): δ 6.90–6.50 (m, 3H, arom), 5.92 (s, 2H, OCH$_2$O), 2.78 (t, 2H, CH$_2$, *J* = 5.8 Hz), 2.57 (t, 2H, CH$_2$, *J* = 7.0 Hz), 1.85–1.50 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 194.2 (t, CO, *J* = 26 Hz), 147.6, 145.7, 135.4, 121.0, 117.8 (qt, CF$_3$, J_1 = 285 Hz, J_2 = 34 Hz), 108.7, 106.9 (tq, CF$_2$, J_1 = 265 Hz, J_2 = 38 Hz), 100.8, 37.1, 35.2, 30.6, 21.7; ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.4 (CF$_3$), −123.8 (CF$_2$); MS (ESI) *m/z* (%): 323.2 ([M+H]$^+$, 100); Anal. Calcd for C$_{15}$H$_{15}$F$_5$O$_2$: C, 55.90; H, 4.69. Found: C, 55.98; H, 4.65.

4.2.2.11. 5-(Biphenyl-2-yl)-1,1,1,2,2-pentafluoropentan-3-one (13).
Yield 48%; Yellowish oil; ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.70–7.10 (m, 9H, arom), 3.03 (t, 2H, CH$_2$, *J* = 7.0 Hz), 2.85 (t, 2H, CH$_2$, *J* = 7.0 Hz); ^{13}C NMR (50 MHz, CDCl$_3$): δ 193.4 (t, CO, *J* = 26 Hz), 142.0, 141.1, 136.6, 130.4, 129.1, 128.9, 128.4, 126.7, 117.7 (qt, CF$_3$, J_1 = 285 Hz, J_2 = 34 Hz), 106.8 (tq, CF$_2$, J_1 = 265 Hz, J_2 = 38 Hz), 38.4 (CH$_2$), 26.0 (CH$_2$); ^{19}F NMR (188 MHz, CDCl$_3$): δ −82.4 (CF$_3$), −123.9 (CF$_2$); MS (ESI) *m/z* (%): 327.2 ([M−H]$^-$,

100); Anal. Calcd for C$_{17}$H$_{13}$F$_5$O: C, 62.20; H, 3.99. Found: C, 62.29; H, 3.95.

4.2.3. 1,1,1,2,2,3,3-Heptafluoro-8-(naphthalen-1-yl)octan-4-one (7a)
The synthesis of heptafluoropropyl ketone **7a** was carried out following the procedure described above for trifluoromethyl ketones, except that heptafluorobutanoic anhydride was used instead of trifluoroacetic anhydride. The product was purified by flash column chromatography [EtOAc–petroleum ether (bp 40–60 °C) 5/95]. Yield 54%; Yellow low mp solid; mp 31–32 °C; ^{1}H NMR (200 MHz, CDCl$_3$): δ 8.06–7.33 (m, 7H, arom), 3.14 (t, 2H, CH$_2$, *J* = 7.2 Hz), 2.82 (t, 2H, CH$_2$, *J* = 7.0 Hz), 1.86–1.80 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 193.9 (t, CO, *J* = 26 Hz), 137.6, 133.9, 130.0–102.5 (m, CF$_2$, CF$_3$), 128.8, 126.8, 126.0, 125.8, 125.5, 123.5, 37.8, 32.7, 29.6, 22.4; ^{19}F NMR (188 MHz, CDCl$_3$): δ −81.05 (CF$_3$), −121.56 (CF$_2$), −127.08 (CF$_2$); MS (ESI) *m/z* (%): 379.1 ([M−H]$^-$, 100); Anal. Calcd for C$_{18}$H$_{15}$F$_7$O: C, 56.85; H, 3.98. Found: C, 56.72; H, 4.03.

4.2.4. Synthesis of difluoromethyl ketones
To a stirring mixture of magnesium (24 mg, 1 mmol) and iodine in dry Et$_2$O (1 mL), a solution of bromide **14a** or **14b** (1 mmol) in dry Et$_2$O (9 mL) was added dropwise under N$_2$ atmosphere. Once the Grignard reagent was formed, it was added dropwise to a cooled (−78 °C) solution of ethyl difluoroacetate (62 mg, 0.5 mmol) in dry ether (0.5 mL). The reaction mixture was stirred at −78 °C for 45 min and then was quenched with 1 N HCl. The aqueous layer was extracted with ether (3 × 25 mL) and the combined organic layers were washed with brine, dried (Na$_2$SO$_4$) and the solvent was evaporated in vacuo. The product was purified by flash column chromatography [EtOAc–petroleum ether (bp 40–60 °C) 5/95].

4.2.4.1. 1,1-Difluoro-5-phenylpentan-2-one (16a).
Yield 56%; Colorless oil; ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.40–7.10 (m, 5H, CH), 5.66 (t, 1H, CHF$_2$, *J* = 54.0 Hz), 2.77–2.55 (m, 4H, CH$_2$), 1.99 (quintet, 2H, CH$_2$, *J* = 8.0 Hz); ^{13}C NMR (50 MHz, CDCl$_3$): δ 199.6 (t, CO, *J* = 26.0 Hz), 140.9, 128.5, 128.4, 126.1, 109.8 (d, CHF$_2$, *J* = 250 Hz), 35.2, 34.7, 23.8; ^{19}F NMR (188 MHz, CDCl$_3$): δ −127.4 (d, CHF$_2$, *J* = 54.5 Hz); MS (ESI) *m/z* (%): 197.1 ([M−H]$^-$, 100); Anal. Calcd for C$_{11}$H$_{12}$F$_2$O: C, 66.66; H, 6.10. Found: C, 66.78; H, 6.06.

4.2.5. 1,1-Difluoro-6-phenylhexan-2-one (16b)[45]
Yield 45%; Colorless oil; ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.37–7.07 (m, 5H, CH), 5.66 (t, 1H, CHF$_2$, *J* = 54 Hz), 2.78–2.52 (m, 4H, CH$_2$), 1.81–1.58 (m, 4H, CH$_2$); ^{13}C NMR (50 MHz, CDCl$_3$): δ 199.7 (t, CO, *J* = 26 Hz), 140.2, 128.3, 125.8, 109.8 (d, CHF$_2$, *J* = 251 Hz), 35.8, 35.5, 30.6, 21.9; ^{19}F NMR (188 MHz, CDCl$_3$): δ −127.4 (d, CHF$_2$, *J* = 54.5 Hz); MS (ESI) *m/z* (%): 211.2 ([M−H]$^-$, 100); Anal. Calcd for C$_{12}$H$_{14}$F$_2$O: C, 67.91; H, 6.65. Found: C, 67.82; H, 6.69.

4.3. In vitro PLA$_2$ assays.
The activity of cPLA$_2$, iPLA$_2$ and sPLA$_2$ were determined using modified Dole Assay.[40–42] The buffer and substrate conditions were optimized for each enzyme assay as follows: (i) GIVA cPLA$_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 97 μM PAPC, 1.8 μM ^{14}C-labeled PAPC, and 3 μM PIP$_2$ in 100 mM HEPES buffer, pH 7.5, with 90 μM CaCl$_2$, 2 mM DTT, and 0.1 mg/ml BSA; (ii) GVIA iPLA$_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM ^{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 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM ^{14}C-labeled PAPC in buffer containing 50 mM Tris, pH 8.0, and 5 mM CaCl$_2$.

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 for compounds displaying less than 95% enzyme inhibition. If the percent inhibition was greater than 95%, we determined its $X_I(50)$ by plotting percent inhibition versus 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.

Acknowledgments

Supplementary data

Supplementary data (the synthesis and characterization data of all the intermediates) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.07.010.

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