New potent and selective polyfluoroalkyl ketone inhibitors of GVIA calcium-independent phospholipase A₂

Victoria Magrioti¹, Aikaterini Nikolaou a, Annetta Smyrniotou b, Ishita Shah c,d, Violetta Constantinou-Kokotoub, Edward A. Dennis c,d, George Kokotosa,a

¹Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece
²Chemical Laboratories, Agricultural University of Athens, Athens 11855, Greece
³Department of Chemistry and Biochemistry, School of Medicine, MC 0601, University of California, San Diego, La Jolla, CA 92093-0601, USA
⁴Department of Pharmacology, School of Medicine, MC 0601, University of California, San Diego, La Jolla, CA 92093-0601, USA

ABSTRACT

Group VIA calcium-independent phospholipase A₂ (GVIA iPLA₂) has recently emerged as an important pharmaceutical target. Selective and potent GVIA iPLA₂ 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 iPLA₂ inhibitors. 1,1,1,2,2-Pentafluoro-7-(4-methoxyphenyl)heptan-3-one (GK187) is the most potent and selective GVIA iPLA₂ inhibitor ever reported with a Kᵢ(50) value of 0.0001, and with no significant inhibition against GIVA cPLA₂ or GV sPLA₂. We also compare the inhibition of two difluoromethyl ketones on GVIA iPLA₂, GIVA cPLA₂ and GV sPLA₂.

Keywords:
Phospholipase A₂
Inhibitor
GVIA iPLA₂
Polyfluoroalkyl ketone
Pentafluoroethyl ketones

A R T I C L E   I N F O
Article history:
Received 17 May 2013
Accepted 6 July 2013
Available online 16 July 2013

1. Introduction

Phospholipases A₂ 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 PLA₂ superfamily currently consists of 16 different groups and various subgroups.² Three of the most important types of PLA₂s that can be found in human tissues are the secreted (such as GIIA and GV sPLA₂), the cytosolic GIVA cPLA₂ and the calcium-independent GVIA iPLA₂.

GVIA cPLA₂ is considered to be a proinflammatory enzyme that is the rate-limiting provider of arachidonic acid and lysophospholipids.³ GIVA cPLA₂ is regulated by intracellular calcium, and calcium binding to the C2 domain of GIVA cPLA₂ can activate the enzyme, resulting in the localization of the enzyme to the phospholipid membrane.⁴,⁵ Furthermore, the activity of sPLA₂s has been suggested to be dependent on or linked to the activity of cPLA₂.⁶–⁸

GVIA iPLA₂ is a phospholipase A₂ that can be characterized by its calcium-independent activity. It was purified and characterized from macrophages in 1994⁹ 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 cPLA₂ and GVIA iPLA₂ share the same catalytic mechanism utilizing a serine residue as the nucleophile, while the active site serine of GVIA iPLA₂ lies within a lipase consensus sequence (Gly-X-Ser519-X-Gly) on top of the catalytic domain.² GVIA iPLA₂ is known to be a homeostatic enzyme involved in basal metabolism within the cell.¹³–¹⁹ Several studies also suggest that GVIA iPLA₂ plays significant 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 (e.g., diabetes, Barth syndrome, ischemia and cancer).²⁰–²⁷

Various GVIA iPLA₂ inhibitor classes have been discussed in recent review articles.²²–²³ The first reported GVIA iPLA₂ inhibitors were the trifluoromethyl ketones,³¹ tricarbonyls³² and methyl fluorophosphonates³³ 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 iPLA₂ and cPLA₂ activity towards PC in vitro.³⁴ Bromoeno lactone (BEL, Fig. 1) was considered to be a selective, irreversible GVIA iPLA₂ inhibitor and was used to study potential

© 2013 Elsevier Ltd. All rights reserved.
biological functions of GVIA iPLA2. The inactivation mechanism of GVIA iPLA2 by BEL has been studied by Turk and co-workers. 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 GVsPLA2. 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. 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. 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. 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 pataxin. 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). 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 trifluoromethyl 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 unsaturated 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 pentafluoropropyl 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) C3H7OOCCCH2CH2P(=O(OH)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 trifluromethyl and pentafluoroethyl ketones 12 and 13, a Wittig olefination reaction between aldehyde 8 and methyl (triphenylphosphinylidene)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 GIVA 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. The inhibition results are presented in Table 1, either as percent inhibition or as X(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(50) values were measured for compounds that displayed greater than 95% inhibition. The X(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 trifluromethyl 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 5d 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 both present X(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 dioxyalane 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 fluoroalketones (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, 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 than 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(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 CFC13 (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 prepared similarly as described above.
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 (Na2SO4). 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, CDCl3); δ 8.10–7.30 (m, 7H, arom), 3.13 (t, 2H, CH2J = 5.8 Hz), 2.77 (t, 2H, CH2J = 5.8 Hz), 1.86–1.79 (m, 4H, CH2); 13C NMR (50 MHz, CDCl3); δ 191.39 (q, CO, J = 35 Hz), 137.62, 133.87, 131.66, 128.82, 126.78, 125.83, 125.49, 123.55, 115.51 (q, CF3, J = 290 Hz), 36.20, 32.67, 29.71, 22.36; 19F NMR (188 MHz, CDCl3); δ -79.7 (CF3); MS (ESI) m/z (%): 275.2 ([M–H]+, 100); Anal. Calc’d for C16H12F3O: 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, CDCl3); δ 7.17–6.93 (m, 4H, arom), 2.74 (t, 2H, CH2J = 6.6 Hz), 2.63 (t, 2H, CH2J = 7.2 Hz), 1.80–1.56 (m, 4H, CH2CH2); 13C NMR (50 MHz, CDCl3); 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, CF3, J = 291 Hz), 115.1 (d, J = 21 Hz), 36.1, 34.6, 30.5, 21.8; 19F NMR (188 MHz, CDCl3); δ -79.8 (CF3), -118.0 (F); MS (ESI) m/z (%): 247.2 ([M–H]+, 85); Anal. Calc’d for C16H12F3O: 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, CDCl3); δ 7.53 (d, 2H, arom, J = 8.0 Hz), 7.27 (d, 2H, arom, J = 8.0 Hz), 2.71 (t, 4H, CH2J = 7.0 Hz), 1.78–1.60 (m, 4H, CH2); 13C NMR (50 MHz, CDCl3); δ 191.4 (t, CO, J = 35 Hz), 145.9, 132.6, 128.2, 125.6, 124.5 (q, CF3, J = 270 Hz), 115.8 (q, CF3, J = 290 Hz), 36.2, 35.5, 30.3, 22.1; 19F NMR (188 MHz, CDCl3); δ -62.8 (CF3), -79.8 (CF3); MS (ESI) m/z (%): 297.1 ([M–H]+, 100); Anal. Calc’d for C16H12F3O: 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).

Yield 40%; Yellow oil; 1H NMR (200 MHz, CDCl3); δ 7.09 (d, 2H, arom, J = 8.6 Hz), 6.83 (d, 2H, arom, J = 8.6 Hz), 3.79 (s, 3H, OCH3), 2.74 (t, 2H, CH2J = 6.6 Hz), 2.68 (t, 2H, CH2J = 6.8 Hz), 1.80–1.60 (m, 4H, CH2); 13C NMR (50 MHz, CDCl3); δ 191.4 (q, CO, J = 34 Hz), 157.8, 133.6, 129.9, 115.5 (q, CF3, J = 290 Hz), 113.7, 55.2, 36.2, 34.5, 30.6, 21.9; 19F NMR (188 MHz, CDCl3); δ -79.8 (CF3); MS (ESI) m/z (%): 259.2 ([M–H]+, 100); Anal. Calc’d for C16H12F3O: 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%; Yellow oil; 1H NMR (200 MHz, CDCl3); δ 7.70–7.20 (m, 9H, arom), 2.80–2.60 (m, 4H, CH2), 1.30–1.60 (m, 4H, CH2); 13C NMR (50 MHz, CDCl3); 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, CF3, J = 290 Hz), 36.2, 35.1, 30.4, 22.0; 19F NMR (188 MHz, CDCl3); δ -79.7 (CF3); MS (ESI) m/z (%): 305.2 ([M–H]+, 100).
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 trifluorooacetic 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; \(^{1}H\) NMR (200 MHz, CDCl\(_3\)): \(\delta\) 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, 8H, CH\(_2\)); \(^{13}C\) NMR (50 MHz, CDCl\(_3\)): \(\delta\) 194.2 (t, CO, J = 27 Hz), 137.6, 133.9, 131.7, 128.8, 126.8, 125.8, 125.5, 123.5, 117.8 (qt, CF\(_3\), J\(_{CF3,J}= 285\) Hz, J\(_{CF3,J}= 34\) Hz), 109.5 (tq, CF\(_3\), J\(_{CF3,J}\)= 265 Hz, J\(_{CF3,J}\)= 38 Hz), 37.2, 32.7, 29.7, 22.3; \(^{19}F\) NMR (188 MHz, CDCl\(_3\)): \(\delta\) −82.3 (CF\(_3\)), −123.7 (CF\(_2\)); MS (ESI) m/z (%): 379.2 ([M–H]+, 100); Anal. Calc for C\(_{17}H\(_23\)F\(_6\)O: 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; \(^{1}H\) NMR (200 MHz, CDCl\(_3\)): \(\delta\) 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, 6H, CH\(_2\)); \(^{13}C\) NMR (50 MHz, CDCl\(_3\)): \(\delta\) 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\(_3\)): \(\delta\) −82.3 (CF\(_3\)), −118.0 (F), −123.8 (CF\(_2\)); MS (ESI) m/z (%): 329.2 ([M–H]+, 100); Anal. Calc for C\(_{17}H\(_{23}\)F\(_5\)O: 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)phenylheptan-3-one (6c)

Yield 65%; Yellow oil; \(^{1}H\) NMR (200 MHz, CDCl\(_3\)): \(\delta\) 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\(_3\)): \(\delta\) 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\(_{CF3,J}= 285\) Hz, J\(_{CF3,J}= 34\) Hz), 109.5 (tq, CF\(_3\), J\(_{CF3,J}\)= 265 Hz, J\(_{CF3,J}\)= 38 Hz), 37.1, 35.4, 30.0, 21.8; \(^{19}F\) NMR (188 MHz, CDCl\(_3\)): \(\delta\) −82.3 (CF\(_3\)), −82.4 (CF\(_3\)), −123.8 (CF\(_2\)); MS (ESI) m/z (%): 347.1 ([M–H]+, 95); Anal. Calc for C\(_{17}H\(_{23}\)F\(_5\)O: 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-trifluoromethyl)phenylheptan-3-one (6d)

Yield 31%; Colorless oil; \(^{1}H\) NMR (200 MHz, CDCl\(_3\)): \(\delta\) 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\(_3\)): \(\delta\) 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\(_3\)): \(\delta\) −82.3 (CF\(_3\)), −123.8 (CF\(_2\)); MS (ESI) m/z (%): 309.2 ([M–H]+, 72); Anal. Calc for C\(_{17}H\(_{23}\)F\(_5\)O: 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; \(^{1}H\) NMR (200 MHz, CDCl\(_3\)): \(\delta\) 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, 8H, CH\(_2\)); \(^{13}C\) NMR (50 MHz, CDCl\(_3\)): \(\delta\) 194.2 (t, CO, J = 27 Hz), 141.0, 140.7, 138.9, 128.8, 128.7, 127.1, 126.9, 126.4, 125.7, 117.8 (qt, CF\(_3\), J\(_{CF3,J}= 285\) Hz, J\(_{CF3,J}= 34\) Hz), 106.9 (tq, CF\(_3\), J\(_{CF3,J}\)= 265 Hz, J\(_{CF3,J}\)= 38 Hz), 37.1, 35.1, 30.3, 21.9; \(^{19}F\) NMR (188 MHz, CDCl\(_3\)): \(\delta\) −82.3 (CF\(_3\)), −123.7 (CF\(_2\)); MS (ESI) m/z (%): 355.2 ([M–H]+, 100); Anal. Calc for C\(_{24}H\(_{25}\)F\(_7\)O: 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; \(^{1}H\) NMR (200 MHz, CDCl\(_3\)): \(\delta\) 7.89 (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, OCH3), 2.95–2.60 (m, 4H, CH2), 1.90–1.60 (m, 4H, CH2); 13C NMR (50 MHz, CDCl3): δ 194.2 (t, CO, J = 27 Hz), 157.2, 136.7, 133.0, 129.0, 128.9, 127.6, 126.8, 123.3, 118.8, 117.8 (qt, CF3, J = 285 Hz, J = 34 Hz), 106.9 (qt, CF3, J = 285 Hz, J = 34 Hz), 105.6, 55.2, 37.2, 35.4, 30.3, 21.9; 19F NMR (188 MHz, CDCl3): δ −82.3 (CF3), −123.8 (CF2); MS (ESI) m/z (%): 393.9 [M−H]−. 100; Anal. Calcld for C17H13F2O3: C, 62.20; H, 3.99. Found: C, 62.29; H, 3.95.

4.2.3. 1,1,1,2,3,3-Heptfluoro-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 heptafluorobutanolic 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; 1H NMR (200 MHz, CDCl3): δ 8.06–7.73 (m, 7H, arom), 3.14 (t, 2H, CH2, J = 7.2 Hz), 2.82 (t, 2H, CH2, J = 7.0 Hz), 1.86–1.80 (m, 4H, CH2); 13C NMR (50 MHz, CDCl3): δ 193.9 (t, CO, J = 26 Hz), 176.3, 133.9, 130.0–102.5 (m, CF2, CF3), 128.8, 126.8, 126.0, 125.8, 125.5, 123.5, 37.8, 32.7, 29.6, 22.4; 19F NMR (188 MHz, CDCl3): δ −81.05 (CF2)−121.56 (CF2)−127.08 (CF2); MS (ESI) m/z (%): 379.1 [M−H]−, 100; Anal. Calcld for C18H13F2O: 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 Et2O (1 mL), a solution of bromide 14a or 14b (1 mmol) in dry Et2O (9 mL) was added dropwise under N2 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 (Na2SO4), 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; 1H NMR (200 MHz, CDCl3): δ 7.40–7.10 (m, 5H, CH), 5.66 (t, 1H, CHF2, J = 54.0 Hz), 2.77–2.55 (m, 4H, CH2), 1.99 (quintet, 2H, CH2, J = 8.0 Hz); 13C NMR (50 MHz, CDCl3): δ 199.6 (t, CO, J = 26.0 Hz), 140.9, 128.5, 128.4, 126.1, 109.8 (d, CHF2, J = 250 Hz), 35.2, 34.7, 23.8; 19F NMR (188 MHz, CDCl3): δ −127.4 (d, CHF2, J = 54.5 Hz); MS (ESI) m/z (%): 197.1 [M−H]−, 100; Anal. Calcld for C11H11F2O: C, 66.66; H, 6.10. Found: C, 66.78; H, 6.06.

4.2.5. 1,1-Difluoro-6-phenylhexan-2-one (16b)

Yield 45%; Colorless oil; 1H NMR (200 MHz, CDCl3): δ 7.37–7.07 (m, 5H, CH), 5.66 (t, 1H, CHF2, J = 54.0 Hz), 2.78–2.52 (m, 4H, CH2), 1.81–1.58 (m, 4H, CH2); 13C NMR (50 MHz, CDCl3): δ 199.7 (t, CO, J = 26 Hz), 140.2, 128.3, 125.8, 109.8 (d, CHF2, J = 251 Hz), 35.8, 35.5, 30.6, 21.9; 19F NMR (188 MHz, CDCl3): δ −127.4 (d, CHF2, J = 54.5 Hz); MS (ESI) m/z (%): 211.2 [M−H]−, 100; Anal. Calcld for C12H12F2O: C, 67.91; H, 6.65. Found: C, 67.82; H, 6.69.

4.3. In vitro PLA2 assays.

The activity of cPLA2, iPLA2 and sPLA2 were determined using modified Dole Assay.40,41 The buffer and substrate conditions were optimized for each enzyme assay as follows: (i) GVA cPLA2 substrate mixed-micelles were composed of 400 μM Triton X-100, 97 μM PAPC, 1.8 μM 14C-labeled PAPC, and 3 μM PIP2 in 100 mM HEPES buffer, pH 7.5, with 90 μM CaCl2, 2 mM DTT, and 0.1 mg/ml BSA; (ii) GVA iPLA2 substrate mixed-micelles were composed of 400 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM 14C-labeled PAPC in buffer containing 100 mM HEPES, pH 7.5, 2 mM ATP, and 4 mM DTT; (iii) GV sPLA2 substrate mixed-micelles were composed of 400 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM 14C-labeled PAPC in buffer containing 50 mMTris, pH 8.0, and 5 mM CaCl2.
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

This work was supported by the European Social Fund and National Resources Herakleitos II (A.S.) and by NIH Grant GM20501 (E.A.D.).

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.

References and notes

23. Lei, X.; Barbour, S. E.; Ramanadham, S. Biochimie 2010, 92, 627.
34. Hsu, Y.-H.; Dumlao, D. S.; Cao, J.; Dennis, E. A. Plos One 2013, 8, e59267.