UC San Diego
UC San Diego Previously Published Works

Title
Synthesis of Polyfluoro Ketones for Selective Inhibition of Human Phospholipase A2 Enzymes

Permalink
https://escholarship.org/uc/item/1bt0c292

Journal
Journal of Medicinal Chemistry, 51(24)

ISSN
0022-2623

Authors
Baskakis, Constantinos
Magrioti, Victoria
Cotton, Naomi
et al.

Publication Date
2008-12-25

DOI
10.1021/jm800649q

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

NIH Public Access
Author Manuscript
J Med Chem. Author manuscript; available in PMC 2009 December 25.

Published in final edited form as:
J Med Chem. 2008 December 25; 51(24): 8027–8037. doi:10.1021/jm800649q.

Synthesis of Polyfluoro Ketones for Selective Inhibition of Human Phospholipase A_2 Enzymes

Constantinos Baskakis[†], **Victoria Magrioti**[†], **Naomi Cotton**[‡], **Daren Stephens**[‡], **Violetta Constantinou-Kokotou**[§], **Edward A. Dennis**[*,‡], and **George Kokotos**[*,†]
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; Chemical Laboratories, Agricultural University of Athens, Athens 11855, Greece

Abstract

The development of selective inhibitors for individual PLA_2 enzymes is necessary in order to target PLA_2-specific signaling pathways; but it is challenging due to the observed promiscuity of known PLA_2 inhibitors. In the current work, we present the development and application of a variety of synthetic routes to produce pentafluoro, tetrafluoro and trifluoro derivatives of activated carbonyl groups in order to screen for selective inhibitors and characterize the chemical properties that can lead to selective inhibition. Our results demonstrate that the pentafluoroethyl ketone functionality favors selective inhibition of the GVIA $iPLA_2$, a very important enzyme for which specific, potent reversible inhibitors are needed. We find that 1,1,1,2,2-pentafluoro-7-phenyl-heptan-3-one (FKGK11) is a selective inhibitor of GVIA $iPLA_2$ ($X_I(50) = 0.0073$). Furthermore, we conclude that the introduction of an additional fluorine atom at the α′ position of a trifluoromethyl ketone constitutes an important strategy for the development of new potent GVIA $iPLA_2$ inhibitors.

Keywords

Calcium-independent phospholipase A_2; inhibitors; pentafluoroethyl ketones; polyfluoro ketones; phospholipase A_2

Introduction

Phospholipase A_2 (PLA_2) enzymes catalyze the hydrolysis of the *sn*-2 ester bond of glycerophospholipids producing free fatty acids and lysophospholipids.[1,2] Both products are precursor signaling molecules that are involved in a plethora of biological functions. The PLA_2 superfamily currently consists of fifteen groups and many subgroups of which a number of enzymes differ in primary sequence, structure and catalytic mechanism.[1] Among the various PLA_2 enzymes, Group IVA $cPLA_2$ (GIVA $cPLA_2$) is considered the rate-limiting provider of arachidonic acid and lysophospholipids that can be converted into prostaglandins, leukotrienes and PAF, respectively.[1–3] Another major intracellular PLA_2, the calcium-independent PLA_2 (GVIA $iPLA_2$) appears to be the primary phospholipase for basal metabolic functions

*To whom correspondence should be addressed. For G.K.: (phone) (30210) 7274462; (fax) (30210) 7274761; (e-mail) gkokotos@chem.uoa.gr. For E.A.D.: (phone) 858-534-3055; (fax) 858-534-7390; (e-mail) edennis@ucsd.edu.
†University of Athens
‡University of California, San Diego
§Agricultural University of Athens

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

within the cell.[1,2,4,5] Both intracellular enzymes share the same catalytic mechanism of utilizing a serine residue as the nucleophile. The PLA_2 superfamily also includes a type of small, secreted phospholipase ($sPLA_2$) that is characterized by a catalytic His/Asp dyad as well as a catalytic Ca^{2+}.[1,2,6] A well-studied example of this class is the human Group V secreted phospholipase A_2 (GV $sPLA_2$).[7] In many cases the activity of $sPLA_2$ has been shown to be dependent on or linked to the activity of GIVA $cPLA_2$.[8–10]

Various classes of synthetic compounds have been studied as inhibitors of human GIVA $cPLA_2$, GVIA $iPLA_2$ and GV $sPLA_2$; and the results are summarized in recent review articles.[11,12] One of the most potent inhibitors of GIVA $cPLA_2$ is pyrrophenone (**1**, Figure 1).[13] Other recently reported inhibitors include 2-propanone derivatives combined with the indole ring (e.g. **2**, Figure 1)[14–16] and a series of indole derivatives[17–19] presented by Wyeth (for example compounds **3a** and **3b**, Figure 1) of which Efipladib (**3b**) is currently in phase I clinical trials.[19] Our laboratories have reported on the development of 2-oxoamide inhibitors of GIVA $cPLA_2$ (e.g. **4a–d**, Figure 1). [20–26]

Historically, the first potent inhibitor of GIVA $cPLA_2$ was a trifluoromethyl ketone analogue of arachidonic acid ($AACOCF_3$) in which the carboxyl group was replaced by $COCF_3$ (**5**, Figure 2).[27] This analogue was shown to be a slow- and tight-binding inhibitor of GIVA $cPLA_2$ and its mechanism of inhibition has been characterized via ^{19}F NMR and ^{13}C NMR.[28] Trifluoromethyl ketone analogues of H-linolenic and linoleic acid as well as the analogue of palmitic acid (**6**, Figure 2) also inhibit GIVA $cPLA_2$.[29,30] Furthermore, a variety of trifluoromethyl ketones have been analyzed with phospholipid vesicle-, detergent-phospholipid mixed micelle-, and natural membrane-based assays.[31]

$AACOCF_3$ has been used as a tool to study the role of GIVA $cPLA_2$ inhibition in various animal models. Using this inhibitor, it was demonstrated that GIVA $cPLA_2$ plays an important role in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis.[32] $AACOCF_3$ was also used to study possible contributions of central nervous PLA_2 enzymes to the development of allodynia after facial carrageenan injection in mice.[33] Intrathecal administration of $AACOCF_3$ prevented thermal hyperalgesia induced by intraplantar carrageenan as well as formalin-induced flinching in a dose-dependent manner.[34] Intrathecal injection of $AACOCF_3$, at antihyperalgesic doses, decreased the release of prostaglandin PGE-2 into spinal dialysate-evoked *N*-methyl-D-aspartate (NMDA).[35] Similarly, treatment of prion-infected cell lines indicated a pivotal role for PLA_2 enzymes in prion diseases.[35] Even so, the various *in vivo* activities of $AACOCF_3$ should be viewed with some caution, since this inhibitor is not selective for GIVA $cPLA_2$ and has been reported to cause cell lysis.[36] Additional trifluoromethyl ketone derivatives are also observed to inhibit GIVA $cPLA_2$.[37–40] For example, BMS-229724[41] (**7**, Figure 2) was reported to be a tight-binding inhibitor of GIVA $cPLA_2$ possessing anti-inflammatory activity in skin inflammation models.[41]

Trifluoromethyl ketone analogs of arachidonic and palmitic acids also inhibit GVIA $iPLA_2$.[42] Both compounds inhibited macrophage GVIA $iPLA_2$ in a concentration-dependent manner and, in contrast to GIVA $cPLA_2$, GIVA $iPLA_2$ showed a preference for the saturated fatty chain.[42] Inhibition studies of a variety of trifluoromethyl ketones as inhibitors of GVIA $iPLA_2$ in mixed-micelle assays found that one trifluoromethyl ketone (**8**, Figure 2) is a potent inhibitor of GVIA $iPLA_2$ presenting a $X_I(50)$ value of 0.0043, which is ten-fold more potent than the corresponding value against GIVA $cPLA_2$.[31]

Continuing our efforts to synthesize selective inhibitors for the various PLA_2 enzyme types, we designed a variety of polyfluoro ketone-based derivatives. In this work, we present routes for the synthesis of polyfluoro ketones and demonstrate their inhibition of the three major

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

human PLA_2 enzymes: GIVA $cPLA_2$, GVIA $iPLA_2$ and GV $sPLA_2$, but with vastly different specificities. Of particular note is the development of specific GVIA $iPLA_2$ inhibitors.

Design and Synthesis of Polyfluoro Ketones

We designed a variety of polyfluoro ketones and examples of such activated carbonyl functionalities are depicted in Figure 3. The rationale behind our design of polyfluoro ketones was based on: (a) Increase of the carbonyl reactivity by introduction of additional fluorine atoms at the β- or α′-positions. The inductive effect of additional fluorine atoms may increase carbonyl reactivity against nucleophiles, such as the active-site serine hydroxyl group in GIVA $cPLA_2$ and GVIA $iPLA_2$; and (b) Increase of the inhibitor binding affinity to the target enzymes. Additional fluorine atoms at the β- or α′-position may contribute to the development of additional interactions, further stabilizing the enzyme-inhibitor complex. Recently, it has become clear that fluorine can enhance binding efficacy and selectivity in pharmaceuticals due to a variety of multipolar C-F···H-N, C-F···C=O, and C-F···H-C_α interactions between a fluorinated ligand and protein binding-site.[43,44] Since the natural substrates of PLA_2 enzymes are long chain phospholipids, we chose to attach the polyfluoro ketone functionality to a long aliphatic chain as well as to short or medium chains carrying a non-substituted or para-alkoxy (or aryloxy) substituted ring.

Among the existing methods, the synthesis of trifluoromethyl ketones through conversion of carboxylic acids into chlorides followed by subsequent treatment with trifluoroacetic anhydride and pyridine[45] has found wide application. We observe that simple carboxylic acids, amino acids and peptides,[46] and even lipophilic glyceride analogues, as we have demonstrated for the synthesis of potent gastric lipases inhibitors,[47] are able to produce trifluoromethyl ketones in satisfactory yields. For the synthesis of pentafluoroethyl ketones, carboxylic acids **9a–c** were converted to chlorides by treatment with oxalyl chloride and then to the target compounds **10a–c** using pentafluoropropionic anhydride and pyridine (Figure 4). For comparison purposes, we prepared pentafluoroethyl ketone **11** corresponding to palmitic acid as well as trifluoromethyl ketones **12a,b** corresponding to pentafluoro derivatives **10b,c**.

The synthesis of various trifluoromethyl and pentafluoroethyl ketones is depicted in Figure 5. The hydroxymethyl group of compounds **13a,b** was oxidized to an aldehyde by the NaClO/TEMPO method.[48] Wittig olefination of aldehydes **14a,b** and Wadworth-Horner-Emmons reaction led to elongation of the chain by two or four carbon atoms, respectively. After hydrogenation and saponification, carboxylic acids **17a,b** and **18a,b** were converted to fluoroketones **19a,b**, **20a,b** and **21** as described above. The trifluoromethyl ketone **23** was prepared from he t known carboxylic acid **22** (Figure 6).

Tetrafluoro derivative **26** was synthesized as shown in Figure 7. The replacement of the hydroxyl group of methyl 2-hydroxy-hexadecanoate (**24**) with fluorine was carried out by treatment with diethylaminosulfur trifluoride (DAST), a well-known fluorinating agent.[49] Treatment of methyl ester **25** by (trifluoromethyl)trimethylsilane in the presence of a catalytic amount of cesium fluoride, followed by hydrolysis of silyl ether intermediate,[50] led directly to tetrafluoro derivative **26**. It should be noted that a 2-fluorocarboxylic acid cannot transform into a trifluoromethyl ketone by conversion to chloride and treatment with anhydride and pyridine, probably because the intermediate ketene required for such a transformation[45] cannot be formed.

To synthesize pentafluoro derivative **30**, we explored two different routes (Figures 8 and 9). Reaction of diethyl oxalate with Grignard reagent[51] **27** led to 2-oxoester **28** (Figure 8). DAST is an efficient reagent for the conversion of 2-oxoesters to 2,2-difluoroesters;[52,53] therefore, 2-oxoester **28** was fluorinated by treatment with DAST and ethyl ester **29** was converted to

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

trifluoromethyl ketone **30** as described above. Alternatively, compound **30** was prepared starting from aldehyde **31** (Figure 9). Formation of cyanohydrin **32** was followed by methanolysis and finally oxidation to produce 2-oxoester **34**. By similar procedures to those described above, the pentafluoro derivative **30** was prepared.

Electrophilic ketones, like fluoroketones, may exist in equilibrium with their corresponding hydrates (gem diols) depending on the environment. Based on the ^{1}H NMR data, the trifluoromethyl ketones and the pentafluoroethyl ketones synthesized in this work were found to exist solely in their ketone forms in chloroform solution. However, tetrafluoro derivative **26** appears to be a mixture of ketone-hydrate form in a ratio 1:2, whereas pentafluoro derivative **30** is completely hydrated (see NMR data in experimental section).^{19}F NMR spectroscopic data confirm the existence of the hydrated form in the cases of compounds **26** and **30**.

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

All synthesized inhibitors were tested for inhibition of human GIVA cPLA$_2$, GVIA iPLA$_2$ and GV sPLA$_2$ using previously described mixed micelle-based assays.[20,21,24,25] The resulting degrees of inhibition are presented in Table 1 as either percent inhibition or $X_I(50)$ values. Initially, the percent of inhibition for each PLA$_2$ enzyme at 0.091 mole fraction of each inhibitor was determined; and, $X_I(50)$ values were estimated for compounds that displayed greater than 90% inhibition. The $X_I(50)$ is the mole fraction of the inhibitor in the total substrate interface required to inhibit the enzyme by 50%.

In accordance with the literature, the long-chain saturated palmitoyl trifluoromethyl ketone **6** inhibits both intracellular enzymes GIVA cPLA$_2$ and GVIA iPLA$_2$ at a similar level. In this work, we show that compound **6** is also a weak inhibitor of GV sPLA$_2$ (79% inhibition at 0.091 mole fraction). However, compound **8** is considered to be a selective inhibitor of GVIA iPLA$_2$ with an observed $X_I(50)$ 0.0096, while high mole fraction of the inhibitor causes only 38% inhibition of GIVA cPLA$_2$ and does not affect GV sPLA$_2$.

The introduction of a pentafluoroethyl ketone functionality led to adverse effects depending on the nature of the chain. 1,1,1,2,2-Pentafluoro-7-phenyl-heptan-3-one (**10a**, FKGK11) presents slightly higher inhibitory activity on GVIA iPLA$_2$ ($X_I(50)$ 0.0073) than the corresponding trifluoromethyl derivative **8**. The dose-response curve for the inhibition of GVIA iPLA$_2$ by pentafluoroethyl ketone **10a** is shown in Figure 10. In addition, it demonstrates selective inhibition for GVIA iPLA$_2$ since high mole fractions (0.091) do not affect GVIA cPLA$_2$ and caused slight inhibition (28%) of GV sPLA$_2$. Interestingly, the long-chain saturated pentafluoroethyl ketone **11** abolished the inhibitory potency and selectivity, demonstrating only 50% inhibition of GVIA iPLA$_2$ and 43% inhibition of GV sPLA$_2$ at 0.091 mole fraction.

In pentafluoroethyl derivatives, increasing the chain length (from four to five or six carbon atoms) between the activated carbonyl group and the aromatic ring resulted in decreased selectivity for GVIA iPLA$_2$. Derivatives **10b** and **10c** (five and six carbon atoms, respectively) inhibit GVIA iPLA$_2$ at a similar level as inhibitor **10a** ($X_I(50)$ 0.0065). However, both **10b** and **10c** are weak inhibitors of GIVA cPLA$_2$ (56% and 65%, respectively) and GV sPLA$_2$ (46% and 75%, respectively). For the trifluoromethyl ketone derivatives **12a** and **12b**, the inhibitory activity increased as the chain length increased between the carbonyl group and the aromatic ring. Both **12a** and **12b** are more potent inhibitors of GVIA iPLA$_2$ ($X_I(50)$ 0.0025 and $X_I(50)$ 0.0018, respectively) than compound **8;** however, these compounds also weakly inhibit GIVA cPLA$_2$ (62% and 68%, respectively) and GV sPLA$_2$ (48% and 53%, respectively) at 0.091 mole fraction. These results demonstrate that an increase of carbon atoms between the activated carbonyl group and the aromatic ring leads to a loss in selectivity.

Trifluoromethyl ketones **19a**, **19b**, **20a** and **20b** containing a medium (hexyloxy) or a long (decyloxy) chain substituent at the *para* position of the aromatic ring inhibit both GIVA $cPLA_2$ and GVIA $iPLA_2$. The dose-response curves for the inhibition of GVIA $iPLA_2$ and GIVA $cPLA_2$ by 1,1,1-trifluoro-6-(4-hexyloxy-phenyl)-hexan-2-one (**20a**, FKGK2) are shown in Figure 11. Comparison of **19a** with **20a** and **19b** with **20b** shows that the increase of the chain length between the carbonyl group and the aromatic ring from two to four carbon atoms results in increased inhibitory potency for both GIVA $cPLA_2$ and GVIA $iPLA_2$. All of these compounds (**19a**, **19b**, **20a** and **20b**) also inhibit GV $sPLA_2$. Thus, trifluoromethyl ketones containing an alkoxy group at the *para* position of the aromatic group can be considered to be pan inhibitors of the all three enzymes: GIVA $cPLA_2$, GVIA $iPLA_2$ and GV $sPLA_2$. In particular, compound **20a** is an inhibitor suitable for applications involving the inhibition of both intracellular and extracellular PLA_2 enzymes. The replacement of the hexyloxy by a benzyloxy group led to derivative **23**, which weakly inhibited all the three PLA_2 enzymes. Comparison of inhibitors **8**, **20a**, **20b** and **23** demonstrates that the introduction of an alkoxy or a benzyloxy group in the aromatic ring destroys the selectivity for GVIA $iPLA_2$.

Comparison of pentafluoroethyl ketone **21** with the corresponding trifluoromethyl ketone **19a** reinforces our observation that pentafluoroethyl ketone functionality favors the inhibition of GVIA $iPLA_2$ ($X_I(50)$ 0.0075). However, the presence of a hexyloxy substituent leads to loss of selectivity for GVIA $iPLA_2$, since compound **21** weakly inhibits GIVA $cPLA_2$ (73%) and GV $sPLA_2$ (86%) at 0.091 inhibitor mole fraction.

Comparison of compound **26** with **6** shows that the introduction of an additional fluorine atom at the α′ position in a long chain saturated derivative results in a derivative with slightly better activity for GIVA $cPLA_2$ ($X_I(50)$ 0.0167) than the parent trifluoromethyl ketone **6** ($X_I(50)$ 0.0223). More importantly, tetrafluoro derivative **26** is approximately twenty-fold more potent inhibitor of GVIA $iPLA_2$ ($X_I(50)$ 0.0011) than the trifluoro derivative **6** ($X_I(50)$ 0.0195). To our knowledge, compound **26** is the most potent inhibitor of GVIA $iPLA_2$ reported, indicating that introduction of an additional fluorine atom at the α′ position constitutes an important strategy for the development of new potent GVIA $iPLA_2$ inhibitors. However, the tetrafluoro derivative **26** also inhibits GIVA and GVA PLA_2. Interestingly, the introduction of two fluorine atoms at the α′ position in an aromatic ring containing derivative destroyed the inhibitory potency and the selectivity for GVIA $iPLA_2$. For example, at 0.091 mole fraction, derivative **30** is a weak inhibitor of GVIA $iPLA_2$ (49%), GV $sPLA_2$ (59%), and presents no significant inhibition of GIVA $cPLA_2$ (27%).

Our data indicates the importance of screening selective inhibitors against multiple enzyme classes within the PLA_2 superfamily. As mentioned above, our work shows that the known inhibitor palmitoyl trifluoromethyl ketone **6**, reported to strictly inhibit intracellular GVIA $iPLA_2$ and GIVA $cPLA_2$, also weakly inhibits GV $sPLA_2$. Similarly, some of our synthesized trifluoromethyl, pentafluoroethyl and tetrafluoro derivatives (for example, compounds **20a**, **21**, **26**) were found to inhibit GV $sPLA_2$. Futhermore, Gelb et al. demonstrated that difluoro ketones similar to **36** (Figure 12) inhibit cobra venom PLA_2.[54] Therefore activated ketones, such as polyfluoro ketones, are likely to inhibit serine enzymes, GIVA $cPLA_2$ and GVIA $iPLA_2$, as well as histidine enzymes like secreted PLA_2.

Bromoenol lactone (BEL) **37** (Figure 12) is considered to be a selective and irreversible GVIA $iPLA_2$ and has been widely applied to study potential biological roles for GVIA $iPLA_2$.[55,56] However, Turk et al. have recently reported that BEL inactivates GVIA $iPLA_2$ by generating a diffusible bromomethyl keto acid that alkylates cysteine thiols, rather than creating an acyl-enzyme intermediate with the active-site serine.[57] Therefore, it is likely that BEL affects multiple enzymes and should be used with appropriate caution when studying potential roles

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

of GVIA $iPLA_2$.[57] These observations lead us to design selective inhibitors of GVIA $iPLA_2$ such as the pentafluoroethyl ketone **10a**.

In conclusion, we developed and applied a variety of synthetic routes to produce various pentafluoro, tetrafluoro and trifluoro derivatives containing activated carbonyl groups. We studied their *in-vitro* activity on the three major human PLA_2 enzyme classes and demonstrated that the pentafluoroethyl ketone functionality favors GVIA $iPLA_2$ inhibition. Furthermore, 1,1,1,2,2-pentafluoro-7-phenyl-heptan-3-one (**10a**) was shown to be a selective inhibitor of GVIA $iPLA_2$. Additionally, introduction of an additional fluorine atom at the α′ position of a trifluoromethyl ketone constitutes an important strategy for the development of new potent GVIA $iPLA_2$ inhibitors. The tetrafluoro derivative of palmitic acid **26** is observed to be the most potent inhibitor of GVIA $iPLA_2$ to date; however, it also inhibits GIVA $cPLA_2$ and GV $sPLA_2$. Polyfluoro ketones displaying an array of selectivities for the major PLA_2 enzyme classes will prove to be valuable tools for the *in-vivo* characterization of the roles of PLA_2 enzymes. Furthermore, we found that these compounds do not show cytotoxcity toward cells in culture and we are currently utilizing these polyfluoro ketone derivatives for the comparison of intracellular versus extracellular PLA_2 enzyme roles in animal models of neurological disorders such as multiple sclerosis, spinal cord injury and peripheral nerve injury.[58]

Experimental Section

Synthesis of fluoroketone inhibitors

Melting points were determined on a Buchi 530 apparatus and are uncorrected. Nuclear magnetic resonance spectra were obtained on a Varian Mercury spectrometer (^{1}H NMR recorded at 200 MHz, ^{13}C NMR recorded at 50 MHz, ^{19}F NMR recorded at 188 MHz) and are referenced in ppm relative to TMS for ^{1}NMR and ^{13}C NMR, and relative to TFA as an internal standard for ^{19}F NMR. Thin layer chromatography (TLC) plates (silica gel 60 F_{254}) and silica gel 60 (230–400 mesh) for flash column chromatography were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid, in EtOH stain. Tetrahydrofuran (THF), toluene, and Et_2O were dried by standard procedures and stored over molecular sieves or Na. All other solvents and chemicals were reagent grade and used without further purification. All the products gave satisfactory elemental analysis results.

General Procedure for the Synthesis of Pentafluoroethyl Ketones

Oxalyl chloride (0.38 g, 3 mmol) and N,N-dimethylformamide (40 μL) were added to a solution of carboxylic acid (1 mmol) in dry dichloromethane (40 mL). After 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 pentafluoropropionic 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) 1/9].

1,1,1,2,2-Pentafluoro-7-phenyl-heptan-3-one (10a)

Yield 53%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.31-7.17 (5H, m, Ph), 2.80 (2H, t, J=6.2 Hz, CH_2), 2.66 (2H, t, J=6.6 Hz, CH_2), 1.73-1.67 (4H, m, 2×CH_2); ^{13}C NMR: δ 194.2 (t, $J_{C\text{-}C\text{-}F}$=26 Hz, CO), 141.6 (Ph), 128.4 (Ph), 128.3 (Ph), 125.9 (Ph), 117.8 (qt, $J_{C\text{-}F3}$=287 Hz, $J_{C\text{-}CF2}$=34 Hz, CF_3), 106.8 (tq, $J_{C\text{-}F2}$=267 Hz, $J_{C\text{-}CF3}$=38 Hz, CF_2), 37.1 (CH_2), 35.5 (CH_2),

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

30.3 (CH_2), 21.9 (CH_2); ^{19}F NMR: δ -4.1 (CF_3), -45.5 (CF_2); MS (ESI) m/z (%): 279 (M^-, 100). Anal. ($C_{13}H_{13}F_5O$) C, H.

1,1,1,2,2-Pentafluoro-8-phenyl-octan-3-one (10b)

Yield 75%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.35-7.21 (5H, m, Ph), 2.79 (2H, t, J=6.8 Hz, CH_2), 2.68 (2H, t, J=7.4 Hz, CH_2), 1.80-1.68 (4H, m, 2×CH_2), 1.48-1.40 (2H, m, CH_2); ^{13}C NMR: δ 194.3 (t, $J_{C\text{-}C\text{-}F}$=26 Hz, CO), 142.2 (Ph), 128.3 (Ph), 128.2 (Ph), 125.7 (Ph), 117.8 (qt, $J_{C\text{-}F3}$=285 Hz, $J_{C\text{-}CF2}$=34 Hz, CF_3), 106.9 (tq, $J_{C\text{-}F2}$=265 Hz, $J_{C\text{-}CF3}$=37 Hz, CF_2), 37.2 (CH_2), 35.6 (CH_2), 31.0 (CH_2), 28.2 (CH_2), 22.1 (CH_2); ^{19}F NMR: δ -4.2 (CF_3), -45.6 (CF_2); MS (ESI) m/z (%): 293 (M^-, 100). Anal. ($C_{14}H_{15}F_5O$) C, H.

1,1,1,2,2-Pentafluoro-9-phenyl-nonan-3-one (10c)

Yield 60%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.31-7.18 (5H, m, Ph), 2.76 (2H, t, J=6.8 Hz, CH_2), 2.64 (2H, t, J=8.0 Hz, CH_2), 1.72-1.58 (4H, m, 2×CH_2), 1.44-1.34 (4H, m, 2×CH_2); ^{13}C NMR: δ 194.4 (t, $J_{C\text{-}C\text{-}F}$=26 Hz, CO), 142.5 (Ph), 128.4(Ph), 128.3 (Ph), 125.7 (Ph), 117.8 (qt, $J_{C\text{-}F3}$=285 Hz, $J_{C\text{-}CF2}$=34 Hz, CF_3), 106.9 (tq, $J_{C\text{-}F2}$=265 Hz, $J_{C\text{-}CF3}$ = 37 Hz, CF_2), 37.3 (CH_2), 35.8 (CH_2), 31.1 (CH_2), 28.8 (CH_2), 28.5 (CH_2), 22.2 (CH_2); ^{19}F NMR: δ -4.2 (CF_3), -45.6 (CF_2); MS (ESI) m/z (%): 307 (M^-, 100). Anal. ($C_{15}H_{17}F_5O$) C, H.

1,1,1,2,2-Pentafluoro-octadecan-3-one (11)

Yield 24%; colorless oil; ^{1}H NMR ($CDCl_3$): δ 2.75 (2H, t, J=7.4 Hz, CH_2), 1.67 (2H, t, J=7.0 Hz, CH_2), 1.38-1.20 (24H, m, 12×CH_2), 0.88 (3H, t, J=7.0 Hz, CH_3); ^{13}C NMR: δ 194.5 (t, $J_{C\text{-}CF2}$=26 Hz, CO), 117.8 ppm (qt, $J_{C\text{-}F3}$=285 Hz, $J_{C\text{-}CF2}$=34 Hz, CF_3), 106.9 ppm (tq, $J_{C\text{-}F2}$=265 Hz, $J_{C\text{-}CF3}$ = 38 Hz, CF_2), 37.4 (CH_2), 31.9 (CH_2), 30.3 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 28.7 (CH_2), 22.7 (CH_2), 22.3 (CH_2), 14.1 (CH_3); ^{19}F NMR: δ -4.2 (CF_3), -45.6 (CF_2); MS (ESI) m/z (%): 357 (M^-, 93). Anal. ($C_{18}H_{31}F_5O$) C, H.

1,1,1,2,2-Pentafluoro-5-(4-hexyloxy-phenyl)-pentan-3-one (21)

Yield 76%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.10 (2H, d, J=8.6 Hz, Ph), 6.85 (2H, d, J=8.6 Hz, Ph), 3.94 (2H, t, J=6.6 Hz, CH_2O), 3.02 (2H, t, J=7.0 Hz, CH_2), 2.96 (2H, t, J=7.0 Hz, CH_2), 1.86-1.73 (2H, m, CH_2), 1.60-1.25 (6H, m, 3×CH_2), 0.93 (3H, t, J=6.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 193.5 (t, $J_{C\text{-}C\text{-}F}$=26 Hz, CO), 157.9 (Ph), 130.9 (Ph), 129.2 (Ph), 117.8 (qt, $J_{C\text{-}F3}$ =286 Hz, $J_{C\text{-}CF2}$=34 Hz, CF_3), 114.7 (Ph), 106.8 (tq, $J_{C\text{-}F2}$=265 Hz, $J_{C\text{-}CF3}$=38 Hz, CF_2), 68.0 (CH_2O), 39.4 (CH_2), 31.6 (CH_2), 29.3 (CH_2), 27.5 (CH_2), 25.7 (CH_2), 22.6 (CH_2), 13.9 (CH_3). ^{19}F NMR: δ -4.2 (CF_3), -45.6 (CF_2). MS (ESI) m/z (%): 351 (M^-, 100). Anal. ($C_{17}H_{21}F_5O_2$) C, H.

Synthesis of Trifluoromethyl Ketones

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

1,1,1-Trifluoro-7-phenylheptan-2-one (12a).[59]

Yield 45%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.34-7.19 (5H, m, Ph), 2.76-2.62 (4H, m, 2×CH_2), 1.77-1.66 (4H, m, 2×CH_2), 1.46-1.39 (2H, m, CH_2); ^{13}C NMR: δ 191.8 (q, $J_{C\text{-}C\text{-}F}$=35 Hz, *C*OCF$_3$), 142.2 (Ph), 128.3 (Ph), 128.2 (Ph), 125.7 (Ph), 115.5 (q, $J_{C\text{-}F}$=290 Hz, CF_3), 36.2 (CH_2), 35.7 (CH_2), 30.9 (CH_2), 28.2 (CH_2), 22.2 (CH_2); ^{19}F NMR: δ -1.5 (CF_3); MS (ESI) m/z (%): 243 (M^-, 100).

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

1,1,1-Trifluoro-8-phenyloctan-2-one (12b).[59]

Yield 42%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.28-7.17 (5H, m, Ph), 2.72-2.60 (4H, m, 2×CH_2), 1.70-1.61 (4H, m, 2×CH_2), 1.42-1.24 (4H, m, 2×CH_2); ^{13}C NMR: δ 191.4 (q, $J_{C\text{-}C\text{-}F}$=35 Hz, *C*OCF_3), 142.5 (Ph), 128.3 (Ph), 128.2 (Ph), 125.7 (Ph), 115.6 (q, $J_{C\text{-}F}$=291 Hz, CF_3), 36.3 (CH_2), 35.8 (CH_2), 31.1 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 22.3 (CH_2); ^{19}F NMR: δ -1.5 (CF_3); MS (ESI) m/z (%): 257 (M^-, 100).

1,1,1-Trifluoro-4-(4-hexyloxy-phenyl)-butan-2-one (19a)

Yield 53%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.10 (2H, d, *J*=8.6 Hz, Ph), 6.84 (2H, d, *J*=8.6 Hz, Ph), 3.93 (2H, t, *J*=6.2 Hz, CH_2O), 3.10-2.92 (4H, m, 2×CH_2), 1.82-1.62 (2H, m, CH_2), 1.55-1.22 (6H, m, 3×CH_2), 0.91 (3H, t, *J*=6.6 Hz, CH_3); ^{13}C NMR: δ 190.7 (q, $J_{C\text{-}C\text{-}F}$ = 35 Hz, *C*OCF_3), 157.9 (Ph), 131.0 (Ph), 129.2 (Ph), 115.5 (q, $J_{C\text{-}F}$=292 Hz, CF_3), 114.7 (Ph), 68.0 (OCH_2), 38.3 (CH_2), 31.6 (CH_2), 29.2 (CH_2), 27.5 (CH_2), 25.7 (CH_2), 22.6 (CH_2), 13.9 (CH_3); ^{19}F NMR: δ -1.5 (CF_3); MS (ESI) m/z (%): 301 (M^-, 100). Anal. ($C_{16}H_{21}F_3O_2$) C, H.

4-(4-Decyloxy-phenyl)-1,1,1-trifluoro-butan-2-one (19b)

Yield 46%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.12 (2H, d, *J*=8.6 Hz, Ph), 6.85 (2H, d, *J*=8.6 Hz, Ph), 3.95 (2H, t, *J*=6.6 Hz, CH_2O), 3.05-2.85 (4H, m, 2×CH_2), 1.81-1.62 (2H, m, CH_2), 1.56-1.22 (14H, m, 7×CH_2), 0.92 (3H, t, *J*=6.8 Hz, CH_3); ^{13}C NMR: δ 190.5 (q, $J_{C\text{-}C\text{-}F}$=35 Hz, *C*OCF_3), 157.9 (Ph), 131.0 (Ph), 129.2 (Ph), 115.5 (q, $J_{C\text{-}F}$=292 Hz, CF_3), 114.6 (Ph), 68.0 (CH_2O), 38.3 (CH_2), 31.8 (CH_2), 29.6 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 27.4 (CH_2), 26.0 (CH_2), 22.7 (CH_2), 14.0 (CH_3); ^{19}F NMR: δ -1.5 (CF_3); MS (FAB) m/z (%): 358 (M^+, 85). Anal. ($C_{20}H_{29}F_3O_2$) C, H.

1,1,1-Trifluoro-6-(4-hexyloxy-phenyl)-hexan-2-one (20a)

Yield 45%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.09 (2H, d, *J*=8.0 Hz, Ph), 6.85 (2H, d, *J*=8.0 Hz, Ph), 3.95 (2H, t, *J*=6.6 Hz, CH_2), 2.74 (2H, t, *J*=6.6 Hz, CH_2), 2.60 (2H, t, *J*=6.2 Hz, CH_2), 1.82-1.62 (6H, m, 3×CH_2), 1.46-1.25 (6H, m, 3×CH_2), 0.94 (3H, t, *J*=6.8 Hz, CH_3); ^{13}C NMR: δ 191.4 (q, $J_{C\text{-}C\text{-}F}$=34 Hz, *C*OCF_3), 157.9 (Ph), 133.4 (Ph), 129.1 (Ph), 115.4 (q, $J_{C\text{-}F}$=290 Hz, CF_3), 114.4 (Ph), 67.9 (CH_2O), 36.1 (CH_2), 34.5 (CH_2), 31.6 (CH_2), 30.6 (CH_2), 29.3 (CH_2), 25.7 (CH_2), 22.6 (CH_2), 21.8 (CH_2), 13.9 (CH_3); ^{19}F NMR: δ -1.6 (CF_3); MS (FAB) m/z (%): 330 (M^+, 23). Anal. ($C_{18}H_{25}F_3O_2$) C, H.

6-(4-Decyloxy-phenyl)-1,1,1-trifluoro-hexan-2-one (20b)

Yield 46%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.08 (2H, d, *J*=8.6 Hz, Ph), 6.84 (2H, d, *J*=8.6 Hz, Ph), 3.94 (2H, t, *J*=6.6 Hz, CH_2O), 2.73 (2H, t, *J*=6.6 Hz, CH_2), 2.59 (2H, t, *J*=7.0 Hz, CH_2), 1.82-1.62 (6H, m, 3×CH_2), 1.45-1.22 (14H, m, 7×CH_2), 0.90 (3H, t, *J*=6.8 Hz, CH_3); ^{13}C NMR: δ 191.6 (q, $J_{C\text{-}C\text{-}F}$=35 Hz, *C*OCF_3), 157.7 (Ph), 133.7 (Ph), 129.4 (Ph), 115.8 (q, $J_{C\text{-}F}$ = 292 Hz, CF_3), 114.6 (Ph), 68.2 (CH_2O), 36.4 (CH_2), 34.8 (CH_2), 32.1 (CH_2), 30.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 26.6 (CH_2), 26.3 (CH_2), 22.9 (CH_2), 22.1 (CH_2), 14.32 (CH_3); ^{19}F NMR: δ -1.5 (CF_3); MS (FAB) m/z (%): 386 (M^+, 100). Anal. ($C_{22}H_{33}F_3O_2$) C, H.

4-(4-Benzyloxy-phenyl)-1,1,1-trifluoro-butan-2-one (23)

Yield 43%; yellowish solid; mp 71–72 °C; ^{1}H NMR ($CDCl_3$): δ 7.46-7.35 (5H, m, Ph), 7.15 (2H, d, *J*=8.4 Hz, Ph), 6.95 (2H, d, *J*=8.4 Hz, Ph), 5.07 (2H, s, $PhCH_2$), 3.12-2.85 (4H, m, 2×CH_2); ^{13}C NMR: δ 190.7 (q, $J_{C\text{-}C\text{-}F}$ = 35 Hz, *C*OCF_3), 157.4 (Ph), 136.9 (Ph), 131.5 (Ph), 129.2 (Ph), 128.5 (Ph), 127.9 (Ph), 127.4 (Ph), 115.4 (q, $J_{C\text{-}F}$=290 Hz, CF_3), 114.9 (Ph), 70.0 (CH_2O), 38.3 (CH_2), 27.4 (CH_2); ^{19}F NMR: δ -1.4 (CF_3); MS (ESI) m/z (%): 307 (M^-, 100). Anal. ($C_{17}H_{15}F_3O_2$) C, H.

NIH-PA Author Manuscript

Intermediate compounds **14a,b** and **22** were prepared by known methods and their spectroscopic data were in accordance with those in the literature.[60,61]

Horner-Wadsworth-Emmons Olefination

A suspension of aldehyde **14a** or **14b** (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.

Ethyl (2*E*,4*E*)-5-(4-Hexyloxy-phenyl)-penta-2,4-dienoate (16a)

Yield 71%; white solid; mp 68–69 °C; ^{1}H NMR ($CDCl_3$): δ 7.48-7.20 (3H, m, CH, Ph), 6.90-6.75 (3H, m, CH, Ph), 6.71 (1H, d, *J*=15.4 Hz, CH), 5.94 (1H, d, *J*=15.4 Hz, CHCOO), 4.23 (2H, q, *J*=7.4 Hz, O$CH_2$$CH_3$), 3.97 (2H, t, *J*=6.2 Hz, CH_2O), 1.85-1.62 (2H, m, $CH_2$$CH_2O$), 1.45-1.02 (9H, m, 3×$CH_2$, CH_3), 0.92 (3H, t, *J*=6.8 Hz, CH_3); ^{13}C NMR: δ 167.2 (COO), 160.0 (Ph), 145.0 (CH), 140.2 (CH), 131.9 (Ph), 128.6 (Ph), 123.9 (CH), 119.9 (CH), 114.7 (Ph), 68.0 (CH_2O), 60.2 (O$CH_2$$CH_3$), 31.6 ($CH_2$), 29.1 ($CH_2$), 25.7 ($CH_2$), 22.6 ($CH_2$), 14.3 ($CH_3$), 14.0 ($CH_3$). Anal. ($C_{19}H_{26}O_3$) C, H.

Ethyl (2*E*,4*E*)-5-(4-Decyloxy-phenyl)-penta-2,4-dienoate (16b)

Yield 65%; white solid; mp 80–81 °C; ^{1}H NMR ($CDCl_3$): δ 7.45-7.38 (3H, m, CH, Ph), 6.88-6.80 (3H, m, CH, Ph), 6.78 (1H, d, *J*=12 Hz, CH), 5.94 (1H, d, *J*=15.4 Hz, CHCOO), 4.23 (2H, q, *J*=7.4 Hz, O$CH_2$$CH_3$), 3.97 (2H, t, *J*=6.6 Hz, CH_2O), 1.81-1.75 (2H, m, $CH_2$$CH_2O$), 1.50-1.14 (17H, m, 7×$CH_2$, CH_3), 0.89 (3H, t, *J*=6.8 Hz, CH_3); ^{13}C NMR: d167.3 (COO), 160.0 (Ph), 145.1 (CH), 140.2 (CH), 131.9 (Ph), 128.6 (Ph), 124.0 (CH), 119.9 (CH), 114.8 (Ph), 68.1 (CH_2O), 60.2 (CH_2), 31.9 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 26.0 (CH_2), 22.7 (CH_2), 14.3 (CH_3), 14.1 (CH_3). Anal. ($C_{23}H_{34}O_3$) C, H.

Wittig Olefination

A solution of aldehyde **14a** or **14b** (1 mmol) and methyl (triphenylphosphanylidene)acetate (0.334 g, 1 mmol) in dry dichloromethane (3 mL) was refluxed under argon for 24 h. 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.

Methyl (*E*)-3-(4-Hexyloxy-phenyl)-acrylate (15a)

Yield 93%; white solid; mp 84–85 °C; ^{1}H NMR ($CDCl_3$): δ 7.63 (1H, d, *J*=15.8 Hz, C*H*=CHCO), 7.43 (2H, d, *J*=8.8 Hz, Ph), 6.87 (2H, d, *J*=8.8 Hz, Ph), 6.28 (1H, d, *J*=15.8 Hz, CHCOO), 3.95 (2H, t, *J*=6.4 Hz, CH_2O), 3.77 (3H, s, OCH_3), 1.76 (2H, m, $CH_2$$CH_2O$), 1.46-1.21 (6H, m, 3×$CH_2$), 0.89 (3H, t, *J*=6.8 Hz, CH_3); ^{13}C NMR: δ 167.7 (COO), 161.0 (Ph), 144.6 (CH), 129.6 (Ph), 126.8 (Ph), 115.0 (CH), 114.7 (Ph), 68.1 (CH_2O), 51.5 (OCH_3), 31.5 (CH_2), 29.0 (CH_2), 25.6 (CH_2), 22.5 (CH_2), 13.9 (CH_3). Anal. ($C_{16}H_{22}O_3$) C, H.

Methyl (*E*)-3-(4-Decyloxy-phenyl)-acrylate (15b)

Yield 92%; white solid; mp 75–76 °C; ^{1}H NMR ($CDCl_3$): δ 7.63 (1H, d, *J*=15.8 Hz, C*H*=CHCOO), 7.37 (2H, d, *J*=8.8 Hz, Ph), 6.85 (2H, d, *J*=8.8 Hz, Ph), 6.23 (1H, d, *J*=15.8 Hz, CHCOO), 3.87 (2H, t, *J*=6.6 Hz, CH_2O), 3.71 (3H, s, OCH_3), 1.78-1.62 (2H, m, $CH_2$$CH_2O$), 1.40-1.22 (14H, m, 7×$CH_2$), 0.84 (3H, t, *J*=7 Hz, CH_3); ^{13}C NMR: δ 167.4 (COO), 160.8 (Ph), 144.3 (CH), 129.4 (Ph), 126.6 (Ph), 114.8 (CH), 114.5 (Ph), 67.8 (CH_2O), 51.2 (OCH_3), 31.7

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

(CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 25.8 (CH_2), 22.5 (CH_2), 13.9 (CH_3). Anal. ($C_{20}H_{30}O_3$) C, H.

Hydrogenation and Saponification of Unsaturated Esters

A mixture of the unsaturated ester (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 vacum to afford the saturated acid as a white solid.

3-(4-Hexyloxy-phenyl)-propanoic acid (17a)

Yield 90%; white solid; mp 70–72 °C; ^1H NMR ($CDCl_3$): δ 7.14 (2H, d, J=8.2 Hz, Ph), 6.86 (2H, d, J=8.2 Hz, Ph), 3.96 (2H, t, J=6.6 Hz, CH_2O), 2.93 (2H, t, J=7.6 Hz, CH_2), 2.67 (2H, t, J=7.6 Hz, CH_2), 1.76-1.60 (2H, m, CH_2), 1.41-1.30 (6H, m, 3×CH_2), 0.92 (3H, t, J=6.7 Hz, CH_3); ^{13}C NMR: δ 179.0 (COO), 157.6 (Ph), 132.0 (Ph), 129.1 (Ph), 114.5(Ph), 67.9 (CH_2O), 35.9 (CH_2), 31.5 (CH_2), 29.7 (CH_2), 29.2 (CH_2), 25.7 (CH_2), 22.5 (CH_2), 14.0 (CH_3). Anal. ($C_{15}H_{22}O_3$) C, H.

3-(4-Decyloxy-phenyl)-propanoic acid (17b)

Yield 96%; white solid; mp 74–76 °C; ^1H NMR ($CDCl_3$): δ 7.14 (2H, d, J=8.2 Hz, Ph), 6.86 (2H, d, J=8.2 Hz, Ph), 3.95 (2H, t, J=6.5 Hz, CH_2O), 2.93 (2H, t, J=7.7 Hz, C$H_2$$CH_2$COO), 2.67 (2H, t, J=7.7 Hz, CH_2COO), 1.85-1.68 (2H, m, C$H_2$$CH_2O$), 1.50-1.21 (14H, br s, 7×CH_2), 0.92 (3H, t, J=6.2 Hz, CH_3); ^{13}C NMR: δ 179.3 (COO), 157.6 (Ph), 132.0 (Ph), 129.1 (Ph), 114.5 (Ph), 67.9 (CH_2O), 35.9 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 26.0 (CH_2), 22.6 (CH_2), 14.1 (CH_3). Anal. ($C_{19}H_{30}O_3$) C, H.

5-(4-Hexyloxy-phenyl)-pentanoic acid (18a)

Yield 96%; white solid; mp 90–91 °C; ^1H NMR ($CDCl_3$): δ 7.03 (2H, d, J=8.4 Hz, Ph), 6.77 (2H, d, J=8.4 Hz, Ph), 3.88 (2H, t, J=6.2 Hz, CH_2O), 2.52 (2H, t, J=6.8 Hz, CH_2), 2.32 (2H, t, J=6.7 Hz, CH_2COO), 1.80-1.60 (6H, m, 3×CH_2), 1.60-1.21 (6H, m, 3×CH_2), 0.89 (3H, t, J=6.7 Hz, CH_3); ^{13}C NMR: δ 180.1 (COO), 157.3 (Ph), 133.9 (Ph), 129.2 (Ph), 114.4 (Ph), 68.0 (CH_2O), 34.6 (CH_2), 33.9 (CH_2), 31.6 (CH_2), 31.0 (CH_2), 29.3 (CH_2), 25.7 (CH_2), 24.2 (CH_2), 22.6 (CH_2), 14.0 (CH_3). Anal. ($C_{17}H_{26}O_3$) C, H.

5-(4-Decyloxy-phenyl)-pentanoic acid (18b)

Yield 94%; white solid; mp 101–102 °C; ^1H NMR ($CDCl_3$): δ 7.08 (2H, d, J=8.4 Hz, Ph), 6.82 (2H, d, J=8.4 Hz, Ph), 3.93 (2H, t, J=6.2 Hz, CH_2O), 2.57 (2H, t, J=6.8 Hz, PhCH_2), 2.37 (2H, t, J=7 Hz, CH_2COOH), 1.80-1.60 (6H, m, 3×CH_2), 1.51-1.22 (14H, m, 7×CH_2), 0.89 (3H, t, J=6.6 Hz, CH_3); ^{13}C NMR: δ 179.5 (COO), 157.2 (Ph), 133.8 (Ph), 129.1 (Ph), 114.3 (Ph), 67.9 (CH_2O), 34.5 (CH_2), 33.8 (CH_2), 31.8 (CH_2), 30.9 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 26.0 (CH_2), 24.2 (CH_2), 22.6 (CH_2), 14.0 (CH_3). Anal. ($C_{21}H_{34}O_3$) C, H.

Methyl 2-Fluoro-hexadecanoate (25)

Compound **24** (1 mmol) was added to a solution of bis(2-methoxyethyl)amino-sulfur-trifluoride, Deoxofluor (0.2 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 quenched with saturated aqueous $NaHCO_3$ (2.5 mL). The organic phase was then washed with brine and

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residual oil was purified by flash column chromatography on silica gel eluting with EtOAc-petroleum ether (bp 40–60 °C) 3/7. Yield 64%; yellowish oil; ^{1}H-NMR ($CDCl_3$): δ 4.86 (1H, dt, J_{H-F}=49.2 Hz, J_{H-H}=6.6 Hz, CH), 3.74 (3H, s, OCH_3), 2.00-1.72 (2H, m, CH_2), 1.45-1.10 (24H, br, 12×CH_2), 0.83 (3H, t, J=6.2 Hz, CH_3); ^{13}C NMR: δ 170.4 (d, J_{C-C-F}=24 Hz, COO), 88.9 (d, J_{C-F}=183 Hz, CF), 52.0 (OCH_3), 32.3 (d, J_{C-C-F}=21, CH_2), 31.9 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 24.6 (CH_2), 24.4 (CH_2), 24.3 (CH_2), 22.7 (CH_2), 14.0 (CH_3); ^{19}F NMR: δ -120.8 (m, CF). Anal. ($C_{17}H_{33}FO_2$) C, H.

1,1,1,3-Tetrafluoro-heptadecan-2-one (in equilibrium with 1,1,1,3-Tetrafluoro-heptadecane-2,2-diol) (26)

A solution of compound **25** (173 mg, 0.6 mmol) and trifluoromethyltrimethylsilane (170 μL, 1.15 mmol) in ethylene glycol dimethyl ether (0.55 mL) at 0 °C was treated with cesium fluoride (3 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 (Na_2SO_4). The solvent was evaporated under reduced pressure and the residual oil was purified by flash column chromatography on silica gel eluting with EtOAc-petroleum ether (bp 40–60 °C) 3/7. Yield 58%; white solid; mp 34–35 °C; ^{1}H-NMR ($CDCl_3$): δ 5.23 (1/3H, dt, J_{H-F}=48.2 Hz, J_{H-H}=6.2 Hz, CH), 4.65 (2/3H, dt, J_{H-F}=49.4 Hz, J_{H-H}=6.6 Hz, CH), 3.74 (2/3H, s, OH), 3.49 (2/3H, s, OH), 2.08-1.27 (26H, m, 13×CH_2), 0.89 (3H, t, J=7 Hz, CH_3); ^{13}C NMR: δ 122.6 (q, J_{C-F3}=286 Hz, CF_3), 115.4 (q, J_{C-F3}=290 Hz, CF_3) 92.9 [C $(OH)_2$], 92.4 (d, J_{C-F}=186 Hz, CF), 32.1 (CH_2), 31.6 (d, J_{C-C-F}=20 Hz, CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 28.5 (CH_2), 28.1 (CH_2), 22.7 (CH_2), 22.3 (CH_2), 14.3 (CH_3); ^{19}F NMR: δ 1.6 (CF_3), -5.3 (CF_3), -121.7 (CF); MS (ESI) m/z (%): 343 (M^-, 100).

Ethyl 2,2-Difluoro-5-phenyl-pentanoate (29)

To a stirring mixture of magnesium (350 mg, 14.6 mmol) and iodine in dry THF (10 ml), (3-bromo-propyl)-benzene (2.87 g, 14.4 mmol) was added dropwise under N_2 atmosphere. Once the Grignard reagent was formed, the resulting mixture was added dropwise to a cooled (−78 °C) solution of diethyl oxalate (1.6 mL, 11.8 mmol) in dry ether (17.3 mL). The reaction mixture was stirred at −78 °C for 45 min and then was quenched with 1N HCl. The aqueous layer was extracted with ether (3 × 25 mL) and the combined organic layers were washed with brine, dried (Na_2SO_4) and the solvent was evaporated in vacuo. After flash column chromatography, a mixture of methyl 2-oxo-5-phenyl-pentanoate **(28)** with diethyl oxalate was obtained and treated with DAST (1 eq) at room temperature. After stirring for 4 h at 45 °C, the reaction mixture was quenched with ice water. The reaction mixture was diluted with dichlolomethane and the organic phase was then washed with brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residual oil was purified by flash column chromatography on silica gel eluting with EtOAc-petroleum ether (bp 40–60 °C) 1/9. Yield 69%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.38-7.12 (5H, m, Ph), 4.30 (2H, q, J=6.8 Hz, OCH_2), 2.68 (2H, t, J=7.4 Hz, $PhCH_2$), 2.21-1.93 (2H, m, CH_2CF_2), 1.90-1.75 (2H, m, CH_2), 1.34 (3H, t, J=6.8 Hz, CH_3); ^{13}C NMR: δ 164.2 (t, J_{C-C-F}=24 Hz, COO), 140.9 (Ph), 128.4 (Ph), 128.3 (Ph), 126.1 (Ph), 116.2 (t, J_{C-F}=248 Hz, CF_2), 62.7 (OCH_2), 34.9 (CH_2), 33.8 (t, J_{C-C-F}=23 Hz, CH_2CF_2), 23.0 (t, $J_{C-C-C-F}$=4 Hz, $CH_2CH_2CF_2$), 13.8 (CH_3); ^{19}F NMR: δ - 28.0 (t, J=17 Hz, CF_2). Anal. ($C_{13}H_{16}F_2O_2$) C, H.

1,1,1,3,3-Pentafluoro-6-phenyl-hexane-2,2-diol (30)

It was prepared following the method used for the synthesis of compound **26**. Yield 35%; yellowish oil; ^{1}H NMR ($CDCl_3$): δ 7.41-7.18 (5H, m, Ph), 3.93 (2H, br, 2×OH), 2.69 (2H, t,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

J=7.6 Hz, $PhCH_2$), 2.22-1.88 (4H, m, 2×CH_2); ^{13}C NMR: δ 141.3 (Ph), 128.4 (Ph), 126.3 (Ph), 126.1 (Ph), 121.5 (q, *J*=286 Hz, CF_3), 120.7 (t, *J*=249 Hz, CF_2), 92.3 [$C(OH)_2$], 35.2 (CH_2), 30.8 (t, $J_{C\text{-}C\text{-}F2}$=23 Hz, CH_2CF_2), 22.5 (t, $J_{C\text{-}C\text{-}C\text{-}F2}$=2.4 Hz, $CH_2CH_2CF_2$); ^{19}F NMR: δ –3.2 (CF_3), –36.4 (CF_2); Ms (ESI) m/z (%): 283 (M^-, 65), 213 (100). Anal. ($C_{12}H_{13}F_5O_2$) C, H.

2-Hydroxy-5-phenyl-pentanenitrile (32).[62]

A solution of 4-phenylbutanal **31** (0.56 g, 3.78 mmol) and $NaHSO_3$ (0.59 g in 1 ml H_2O) in dichloromethane was stirred for 30 min at room temperature. After the formation of the white salt, the organic solvent was evaporated and water (3.8 mL) was added. The mixture cooled to 0 °C and an aqueous solution of KCN (0.368 g, 567 mmol in 1 mL H_2O) was added dropwise. The reaction mixture was stirred for another 18 h at room temperature and then CH_2Cl_2 (10 mL) and water (10 mL) were added. The organic phase was washed with brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residual oil was purified by flash column chromatography on silica gel eluting with EtOAc-petroleum ether (bp 40–60 °C) 2/8 to give 0.653 g (99%) of the title compound as a clear oil; ^1H-NMR ($CDCl_3$): δ 7.32-7.15 (5H, m, Ph), 4.40 (1H, t, *J*=8.8 Hz, CH), 2.63 (2H, t, *J*=6.6 Hz, CH_2), 1.90-1.70 (4H, m, 2×CH_2); ^{13}C NMR: 141.5 (Ph), 128.7 (Ph), 128.6 (Ph), 126.3 (Ph), 120.4 (CN), 61.2 (CH), 35.3 (CH_2), 34.7 (CH_2), 26.4 (CH_2). Anal. ($C_{11}H_{13}NO$) C, H.

Methyl 2-Hydroxy-5-phenyl-pentanoate (33).[63]

Compound **32** (0.63 g, 3.59 mmol) was treated with HCl (0.6 mL. 6N) in MeOH for 18 h at room temperature. The organic solvent was evaporated and an aqueous solution of K_2CO_3 was added to neutralize the pH of the mixture. After extraction with EtOAc (3 × 15 mL), the combined organic phases were washed with brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residual oil was purified by flash column chromatography on silica gel eluting with EtOAc-petroleum ether (bp 40–60 °C) 3/7 to give 0.56 g (79%) of the title compound as a clear oil; ^1H-NMR ($CDCl_3$): δ 7.30-7.12 (5H, m, Ph), 4.22 (1H, t, *J*=4.0 Hz, CH), 3.74 (3H, s, OCH_3), 3.18 (1H, s, OH), 2.66 (2H, t, *J*=6.6 Hz, CH_2), 1.85-1.62 (4H, m, 2×CH_2); ^{13}C NMR: 175.4 (COO), 141.6 (Ph), 128.1 (Ph), 128.0 (Ph), 125.5 (Ph), 70.1 (CHOH), 52.1 (OCH_3), 35.2 (CH_2), 33.6 (CH_2), 26.3 (CH_2). Anal. ($C_{12}H_{16}O_3$) C, H.

Methyl 2-Oxo-5-phenyl-pentanoate (34)

Compound **33** (0.20 g, 0.96mmol) was dissolved in CH_2Cl_2 (20 mL) and treated with Dess-Martin periodinane (0. 43 g) under stirring for 40 min. The organic phase was washed with brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residual oil was purified by flash column chromatography on silica gel eluting with EtOAc-petroleum ether (bp 40–60 °C) 3/7 to give 0.195 g (99%) of the title compound as an yellowish oil; ^1H-NMR ($CDCl_3$): δ 7.31-7.15 (5H, m, Ph), 3.85 (3H, s, OCH_3), 2.86 (2H, t, *J*=6.6 Hz, CH_2), 2.63 (t, *J*=6.6 Hz, 2H, CH_2), 1.71-1.62 (2H, m, CH_2); ^{13}C NMR: 194.0 (CO), 161.2 (COO), 141.8 (Ph), 128.3 (Ph), 128.0 (Ph), 125.8 (Ph), 52.8 (OCH_3), 35.5 (CH_2), 30.5 (CH_2), 22.5 (CH_2). Anal. ($C_{12}H_{14}O_3$) C, H.

Methyl 2,2-Difluoro-5-phenyl-pentanoate (35)

A solution of compound **34** (0.404 g, 1.67 mmol) in CH_2Cl_2 (3.3 mL) was treated dropwise with DAST (0.489 mL, 3.6 mmol) at room temperature. After heating at 55 °C for 5 h, it was poured into H_2O, cautiously neutralized by the addition of solid K_2CO_3, and extracted with $CHCl_3$ (2 × 15 mL). The organic solvent was dried over Na_2SO_4, filtered and evaporated, and the crude product purified by flash column chromatography on silica gel eluting with EtOAc-petroleum ether (bp 40–60 °C) 1/9 to give 0.202 g (50%) of the title compound as an yellowish oil; ^1H NMR ($CDCl_3$): δ 7.32-7.12 (5H, m, Ph), 4.10 (3H, s, OCH_3), 2.69 (2H, t, *J*=7.4 Hz,

PhCH$_2$), 2.21-1.90 (2H, m, CH$_2$CF$_2$), 1.80-1.72 (2H, m, CH$_2$); ^{13}C NMR: δ 164.2 (t, $J_{C\text{-}C\text{-}F}$=33 Hz, COO), 140.9 (Ph), 128.4 (Ph), 128.3 (Ph), 126.1 (Ph), 116.2 (t, $J_{C\text{-}F}$=248 Hz, CF$_2$), 52.7 (OCH$_3$), 34.9 (CH$_2$), 33.8 (t, $J_{C\text{-}C\text{-}F}$=23 Hz, *C*H$_2$CF$_2$), 23.0 (t, $J_{C\text{-}C\text{-}C\text{-}F}$=2.4 Hz, *C*H$_2CH_2CF_2$); ^{19}F NMR: δ -28.0 (2F, t, *J*=17 Hz, CF$_2$). MS (ESI) m/z (%): 229 (M$^+$+1, 100). Anal. ($C_{12}H_{14}F_2O_2$) C, H.

In-vitro PLA$_2$ Assays

Phospholipase A$_2$ activity was determined using the previously described modified Dole assay[20] with buffer and substrate conditions optimized for each enzyme as described previously[20,21,24,25]: (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 buffer containing 100 mM HEPES pH 7.5, 90 μM CaCl$_2$, 2 mM DTT and 0.1 mg/ml BSA; (ii) GVI iPLA$_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 99 μM DPPC, and 1.5 μM ^{14}C-labeled DPPC in buffer containing 200 mM HEPES pH 7.0, 1 mM ATP, 2 mM DTT and 0.1 mg/ml BSA; and (iii) GV sPLA$_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 99 μM DPPC, and 1.5 μM ^{14}C-labeled DPPC in buffer containing 50 mM Tris pH 8.0 and 5 mM CaCl$_2$.

In-vitro PLA$_2$ Inhibition Studies

Initial screening of compounds at 0.091 mole fraction inhibitor in mixed-micelles was carried out. We considered compounds displaying 25% or less inhibition to have no inhibitory affect (designated N.D.). We report average percent inhibition (and standard error, n=3) for compounds displaying more than 25% and less than 90% enzyme inhibition. If percent inhibition was greater than 90%, we determined its $X_I(50)$ by plotting percent inhibition vs. inhibitor molar fraction (7 points; typically 0.005 to 0.091 mole fraction). Inhibition curves were modeled in Graphpad Prism using either a linear (x, y intercept = 0) or non-linear regression (one-site binding model - hyperbola, BMAX = 100) to calculate the reported $X_I(50)$ and associated error values.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH GM 20,501 and GM64611 (E.A.D.). The project is co-funded by the European Social Fund and National Resources – (EPEAEK II) (G.K.)

Abbreviations

AACOCF$_3$
arachidonyl trifluoromethyl ketone

ATP
adenosine triphosphate

BEL
bromoenol lactone, DAST, diethylaminosulfur trifluoride

Deoxofluor
bis(2-methoxyethyl)amino-sulfur-trifluoride

DIBALH
diisobutylaluminium hydride

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

DPPC
1,2-dipalmitoylphosphotidylcholine

DTT
dithiothreitol

EAE
experimental autoimmune encephalomyelitis

EtOAc
ethyl acetate

GIVA cPLA$_2$
Group VIA cytosolic phospholipase A$_2$

GV sPLA$_2$
Group V secreted phospholipase A$_2$

GVIA iPLA$_2$
Group VIA calcium-independent phospholipase A$_2$

NMDA
N-methyl-D-aspartate

PAF
platelet activating factor

PAPC
1-palmitoyl, 2-arachidonal phosphatidylcholine

PIP$_2$
phosphatidyl inositol (4,5)-bisphosphate

TBAF
tetra-n-butylammonium fluoride

TEMPO
2,2,6,6-tetramethylpiperidine-1-yloxy free radical

TFA
trifluoroacetic acid

THF
tetrahydrofuran

TLC
thin-layer chromatography

TMS
tetramethylsilane

References

1. Schaloske RH, Dennis EA. The phospholipase A(2) superfamily and its group numbering system. Biochim Biophys Acta 2006;1761:1246–1259. [PubMed: 16973413]
2. Kudo I, Murakami M. Phospholipase A(2) enzymes. Prostag Oth Lipid M 2002:68–69. 3–58.
3. Leslie CC. Regulation of the specific release of arachidonic acid by cytosolic phospholipase A2. Prostaglandins Leukot Essent Fatty Acids 2004;70:373–376. [PubMed: 15041029]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

4. Winstead MV, Balsinde J, Dennis EA. Calcium-independent phospholipase A(2): structure and function. Biochim Biophys Acta 2000;1488:28–39. [PubMed: 11080674]
5. Balsinde J, Balboa MA. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A(2) in activated cells. Cellular Signalling 2005;17:1052–1062. [PubMed: 15993747]
6. Berg OG, Gelb MH, Tsai M-D, Jain MK. Interfacial enzymology: The secreted phospholipase A_2-paradigm. Chem Rev 2001;101:2613–2654. [PubMed: 11749391]
7. Balestrieri B, Arm JP. Group V $sPLA_2$: Classical and novel functions. Biochim Biophys Acta 2006;1761:1280–1288. [PubMed: 16945583]
8. Mounier CM, Ghomashchi F, Lindsay MR, James S, Singer AG, Parton RG, Gelb MH. Arachidonic acid release from mammalian cells transfected with human groups IIA and X secreted phospholipase A_2 occurs predominantly during the secretory process and with the involvement of cytosolic phospholipase $A_{2-\alpha}$. J Biol Chem 2004;279:25024–25038. [PubMed: 15007070]
9. Satake Y, Diaz BL, Balestrieri B, Lam BK, Kanaoka Y, Grusby MJ, Arm JP. Role of group V phospholipase A2 in zymosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption. J Biol Chem 2004;279:16488–16494. [PubMed: 14761945]
10. Shirai Y, Balsinde J, Dennis EA. Localization and functional interrelationships among cytosolic Group IV, secreted Group V, and Ca^{2+}-independent Group VI phospholipase A2s in P388D1 macrophages using GFP/RFP constructs. Biochim Biophys Acta 2005;1735:119–129. [PubMed: 15967714]
11. Magrioti V, Kokotos G. Synthetic inhibitors of Group IVA and Group VIA phospholipase A_2. Anti-Inflammatory Anti-Allergy Agents Med Chem 2006;5:189–203.
12. Reid RC. Inhibitors of secretory phospholipase A_2 Group IIA. Curr Med Chem 2005;12:3011–3026. [PubMed: 16378502]
13. Seno K, Okuno T, Nishi K, Murakami Y, Yamada K, Nakamoto S, Ono T. Pyrrolidine inhibitors of human cytosolic phospholipase A_2. Part 2: synthesis of potent and crystallized 4-triphenylmethylthio derivative 'Pyrrophenone'. Bioorg Med Chem Lett 2001;11:587–590. [PubMed: 11229777]
14. Ludwig J, Bovens S, Brauch C, Elfringhoff AS, Lehr M. Design and synthesis of 1-indol-1-yl-propan-2-ones as inhibitors of human cytosolic phospholipase A(2)alpha. J Med Chem 2006;49:2611–2620. [PubMed: 16610804]
15. Hess M, Elfringhoff AS, Lehr M. 1-(5-Carboxy- and 5-carbamoylindol-1-yl)propan-2-ones as inhibitors of human cytosolic phospholipase $A_2\alpha$: Bioisosteric replacement of the carboxylic acid and carboxamide moiety. Bioorg Med Chem 2007;15:2883–2891. [PubMed: 17320401]
16. Fritsche A, Elfringhoff AS, Fabian J, Lehr M. 1-(2-Carboxyindol-5-yloxy)propan-2-ones as inhibitors of human cytosolic phospholipase $A_2\alpha$: Synthesis, biological activity, metabolic stability, and solubility. Bioorg Med Chem 2008;16:3489–3500. [PubMed: 18321717]
17. Lee KL, Foley MA, Chen LR, Behnke ML, Lovering FE, Kirincich SJ, Wang WH, Shim J, Tam S, Shen MWH, Khor SP, Xu X, Goodwin DG, Ramarao MK, Nickerson-Nutter C, Donahue F, Ku MS, Clark JD, Mckew JC. Discovery of ecopladib, an indole inhibitor of cytosolic phospholipase A(2) alpha. J Med Chem 2007;50:1380–1400. [PubMed: 17305324]
18. Lee KL, Behnke ML, Foley MA, Chen L, Wang W, Vargas R, Nunez J, Tam S, Mollova N, Xu X, Shen MWH, Ramarao MK, Goodwin DG, Nickerson-Nutter CL, Abraham WM, Williams C, Clark JD, McKew JC. Benzenesulfonamide indole inhibitors of cytosolic phospholipase $A_2\alpha$: Optimization of in vitro potency and rat pharmacokinetics for oral efficacy. Bioorg Med Chem 2008;16:1345–1358. [PubMed: 17998165]
19. McKew JC, Lee KL, Shen MWH, Thakker P, Foley MA, Behnke ML, Hu B, Sum FW, Tam S, Hu Y, Chen L, Kirincich SJ, Michalak R, Thomason J, Ipek M, Wu K, Wooder L, Ramarao MK, Murphy EA, Goodwin DG, Albert L, Xu X, Donahue F, Ku MS, Keith J, Nickerson-Nutter CL, Abraham WM, Williams C, Hegen M, Clark JD. Indole cytosolic phospholipase A_2 α inhibitors: Discovery and in vitro and in vivo characterization of 4-{3-[5-Chloro-2-(2-{[(3,4-dichlorobenzyl)sulfonyl] amino}ethyl)-1-(diphenylmethyl)-1*H*-indol-3-yl]propyl}benzoic acid, efipladib. J Med Chem 2008;51:3388–3413. [PubMed: 18498150]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

20. Kokotos G, Kotsovolou S, Six DA, Constantinou-Kokotou V, Beltzner CC, Dennis EA. Novel 2-oxoamide inhibitors of human Group IVA phospholipase A_2. J Med Chem 2002;45:2891–2893. [PubMed: 12086476]
21. Kokotos G, Six DA, Loukas V, Smith T, Constantinou-Kokotou V, Hadjipavlou-Litina D, Kotsovolou S, Chiou A, Beltzner CC, Dennis EA. Inhibition of Group IVA cytosolic phospholipase A_2 by novel 2-oxoamides in vitro, in cells and in vivo. J Med Chem 2004;47:3615–3628. [PubMed: 15214789]
22. Constantinou-Kokotou V, Peristeraki A, Kokotos CG, Six DA, Dennis EA. Synthesis and activity of 2-oxoamides containing long chain beta-amino acids. J Pept Sci 2005;11:431–435. [PubMed: 15635664]
23. Yaksh TL, Kokotos G, Svensson CI, Stephens D, Kokotos CG, Fitzsimmons B, Hadjipavlou-Litina D, Hua XY, Dennis EA. Systemic and intrathecal effects of a novel series of phospholipase A(2) inhibitors on hyperalgesia and spinal prostaglandin E-2 release. J Pharmacol Exp Ther 2006;316:466–475. [PubMed: 16203828]
24. Stephens D, Barbayianni E, Constantinou-Kokotou V, Peristeraki A, Six DA, Cooper J, Harkewicz R, Deems RA, Dennis EA, Kokotos G. Differential inhibition of Group IVA and Group VIA phospholipases A(2) by 2-oxoamides. J Med Chem 2006;49:2821–2828. [PubMed: 16640343]
25. Six DA, Barbayianni E, Loukas V, Constantinou-Kokotou V, Hadjipavlou-Litina D, Stephens D, Wong AC, Magrioti V, Moutevelis-Minakakis P, Baker S, Dennis EA, Kokotos G. Structure-activity relationship of 2-oxoamide inhibition of group IVA cytosolic phospholipase A_2 and group V secreted phopholipase A_2. J Med Chem 2007;50:4222–4235. [PubMed: 17672443]
26. Moutevelis-Minakakis P, Neokosmidi A, Filippakou M, Stephens D, Dennis EA, Kokotos G. Synthesis of lipophilic 2-oxoamides based on H-aminobutyric and δ-aminovaleric analogues and their activity against phospholipase A_2. J Pept Sci 2007;13:634–641. [PubMed: 17631670]
27. Street IP, Lin HK, Laliberte F, Ghomashchi F, Wang Z, Perrier H, Tremblay NM, Huang Z, Weech PK, Gelb MH. Slow- and tight-binding inhibitors of the 85-kDa human phospholipase A_2. Biochemistry 1993;32:5935–5940. [PubMed: 8018213]
28. Trimble LA, Street IP, Perrier H, Tremblay NM, Weech PK, Bernstein MA. NMR structural studies of the tight complex between a trifluoromethyl ketone inhibitor and the 85-kDa human phospholipase A_2. Biochemistry 1993;32:12560–12565. [PubMed: 8251473]
29. Amandi-Burgermeister E, Tibes U, Kaiser BM, Friebe WG, Scheuer WV. Suppression of cytokine synthesis, integrin expression and chronic inflammation by inhibitors of cytosolic phospholipase A_2. Eur J Pharmacol 1997;326:237–250. [PubMed: 9196277]
30. Conde-Frieboes K, Reynolds LJ, Lio YC, Hale MR, Wasserman HH, Dennis EA. Activated ketones as inhibitors of intracellular Ca^{2+}-dependent and Ca^{2+}-independent phospholipase A_2. J Am Chem Soc 1996;118:5519–5525.
31. Ghomashchi F, Loo R, Balsinde J, Bartoli F, Apitz-Castro R, Clark JD, Dennis EA, Gelb MH. Trifluoromethyl ketones and methyl fluorophosphonates as inhibitors of group IV and VI phospholipases A_2: structure-function studies with vesicle, micelle, and membrane assays. Biochim Biophys Acta 1999;1420:45–56. [PubMed: 10446289]
32. Kalyvas A, David S. Cytosolic phospholipase A_2 plays a key role in the pathogenesis of multiple sclerosis-like disease. Neuron 2004;41:323–335. [PubMed: 14766173]
33. Yeo JF, Ong WY, Ling SF, Farooqui AA. Intracerebroventricular injection of phospholipases A2 inhibitors modulates allodynia after facial carrageenan injection in mice. Pain 2004;112:148–155. [PubMed: 15494195]
34. Svensson CI, Lucas KK, Hua XY, Powell HC, Dennis EA, Yaksh TL. Spinal phospholipase A2 in inflammatory hyperalgesia: Role of the small, secretory phospholipase A_2. Neuroscience 2005;133:543–553. [PubMed: 15885922]
35. Bate C, Reid S, Williams A. Phospholipase A2 inhibitors or platelet-activating factor antagonists prevent prion replication. J Biol Chem 2004;279:36405–36411. [PubMed: 15210691]
36. Risse D, Elfringhoff AS, Lehr M. Determination of the cell lytic properties of amphiphilic inhibitors of the cytosolic phospholipase A2 against human platelets by measuring the liberation of serotonin with high-performance liquid chromatography and fluorescence detection. J Chromatogr B 2002;769:185–190.
37. Kazuyoshi, Y., Kenjiro, O., Mayumi, K., Sei K. Patent JP09268153A, 1997.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

38. Banville, J., Marinier, A., Gai, Y., Plamondon, S., Roy, S., Balasubramanian, N. Patent US 6,414,179 B1, 2002.
39. Banville, J., Plamondon, S., Gai, Y., Balasubramanian, N. Patent US 6,492,550 B2, 2001.
40. Banville, J., Remillard, R., Balasubramanian, N., Bouthillier, G., Martel, A. Patent US 6,924,391 B2, 2005.
41. Burke JR, Davern LB, Stanley PL, Gregor KR, Banville J, Remillard R, Russell JW, Brassil PJ, Witmer MR, Johnson G, Tredup JA, Tramposch KM. BMS-229724 is a tight-binding inhibitor of cytosolic phospholipase A2 that acts at the lipid/water interface and possesses anti-inflammatory activity in skin inflammation models. J Pharmacol Exp Ther 2001;298:376–385. [PubMed: 11408565]
42. Ackermann EJ, Conde-Frieboes K, Dennis EA. Inhibition of macrophage Ca^{2+}-independent phospholipase A_2 by bromoenol lactone and trifluoromethyl ketones. J Biol Chem 1995;270:445–450. [PubMed: 7814408]
43. Müller K, Faeh C, Diederich F. Fluorine in pharmaceuticals: Looking beyond intuition. Science 2007;317:1881–1886. [PubMed: 17901324]
44. Bohm HJ, Banner D, Bendels S, Kansy M, Kuhn B, Muller K, Obst-Sander U, Stahl M. Fluorine in medicinal chemistry. ChemBioChem 2004;5:637–643. [PubMed: 15122635]
45. Boivin J, El Kaim L, Zard SZ. A new and efficient synthesis of trifluoromethyl ketones from carboxylic acids. Part I Tetrahedron 1995;51:2573–2584.
46. Gelb MH, Svaren JP, Abeles RH. Fluoro ketone inhibitors of hydrolytic enzymes. Biochemistry 1985;24:1813–1821. [PubMed: 2990541]
47. Kokotos G, Kotsovolou S, Verger R. Novel trifluoromethyl ketones as potent gastric lipase inhibitors. ChemBioChem 2003;4:90–95. [PubMed: 12512081]
48. Leanna MR, Sowin TJ, Morton HE. Synthesis of α-amino and α-alkoxy aldehydes via oxoammonium oxidation. Tetrahedron Lett 1992;33:5029–5032.
49. Middleton WJ. New fluorinating reagents. Dialkylaminosulfur fluorides. J Org Chem 1975;5:574–578.
50. Singh RP, Cao G, Kirchmeier RL, Shreeve JM. Cesium fluoride catalysed trifluoromethylation of esters, aldehydes, and ketones with (trifluoromethyl)trimethylsilane. J Org Chem 1999;64:2873–2876. [PubMed: 11674359]
51. Creary X. Reaction of organometallic reagents with ethyl trifluoroacetate and diethyl oxalate. Formation of trifluoromethyl ketones and α-keto esters via stable tetrahedral adducts. J Org Chem 1987;52:5026–5030.
52. Erni B, Khorana HG. Fatty acids containing photoactivable carbene precursors. Synthesis and photochemical properties of 3,3-bis(1,1-difluorohexyl)diazirine and 3-(1,1-difluorooctyl)-3*H*-diazirine. J Am Chem Soc 1980;102:3888–3896.
53. Middleton WJ, Bingham EM. α,α-Difluoroarylacetic acids: preparation from (diethylamino)sulphur trifluoride and α-oxoarylacetates. J Org Chem 1980;45:2883–2887.
54. Yuan W, Berman RJ, Gelb MH. Synthesis and evaluation of phospholipid analogues as inhibitors of cobra venom phospholipase A_2. J Am Chem Soc 1987;109:8071–8081.
55. Balsinde J, Dennis EA. Distinct roles in signal transduction for each of the phospholipase A_2 enzymes present in P388D1 macrophages. J Biol Chem 1996;271:6758–6765. [PubMed: 8636097]
56. Balsinde J, Dennis EA. Function and inhibition of intracellular calcium-independent phospholipase A_2. J Biol Chem 1997;272:16069–16072. [PubMed: 9195897]
57. Song H, Ramanadham S, Bao S, Hsu FF, Turk JA. Bromoenol lactone suicide substrate inactivates group VIA phospholipase A_2 by generating a diffiusible bromomethyl keto acid that alkylates cysteine thiols. Biochemistry 2006;45:1061–1073. [PubMed: 16411783]
58. López-Vales R, Navarro X, Shimizu T, Baskakis C, Kokotos G, Constantinou-Kokotou V, Stephens D, Dennis EA, David S. Intracellular phospholipase A_2 group IVA and group VIA play important roles in Wallerian degeneration and axon regeneration after peripheral nerve injury. Brain. 2008in press
59. Leung D, Du W, Hardouin C, Cheng H, Hwang I, Cravatt BF, Boger DL. Discovery of an exceptionally potent and selective class of fatty acid amide hydrolase inhibitors enlisting proteome-

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

wide selectivity screening: concurrent optimization of enzyme inhibitor potency and selectivity. Bioorg Med Chem Lett 2005;15:1423–1428. [PubMed: 15713400]

60. Pez D, Leal I, Zuccotto F, Boussard C, Brun R, Croft SL, Yardley V, Ruiz Perez LM, Gonzalez Pacanowska D, Gilbert IH. 2,4-Diaminopyrimidines as inhibitors of leishmanial and trypanosomal dihydrofolate reductase. Bioorg Med Chem 2003;11:4693–4711. [PubMed: 14556785]
61. Lwein AH, Szewczyk J, Wilson JW, Ivy Carroll F. Galanthamine analogs: 6*H*-benzofuro[3a,3,2,-*e*,*f*][1]benzazepine and 6*H*-benzofuro[3a,3,2,-*e*,*f*][3]benzazepine. Tetrahedron 2005;61:7144–7152.
62. Roda G, Riva S, Danieli B. Almond oxynitrilase-catalyzed transformation of aldehydes is strongly influenced by napthyl and alkoxy substituents. Tetrahedron: Asymmetry 1999;10:3939–3949.
63. Rho HS, Ko BS. Regioselective deoxygenation of the cyclic thionocarbonates of 2,3-dihydroxy esters with magnesium in methanol. Synth Commun 1999;29:2875–2880.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

1, Pyrrophenone

2

3a, X = O, R = 3-Cl, 4-Cl

3b, X = CH_2, R = 3-Cl, 4-Cl, Efipladib

4a, R^1 = H, R^2 = H, n = 1

4b, R^1 =$(CH_2)_2CH_3$, R^2 = H, n = 1

4c, R^1 =$(CH_2)_2CH_3$, R^2 = H, n = 2

4d, R^1 = H, R^2 = Et, n = 1 (AX048)

Figure 1.

Some known inhibitors of GIVA $cPLA_2$.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

5, $AACOCF_3$

6

7

8

Figure 2.
Trifluoromethyl ketone inhibitors of GIVA $cPLA_2$ and GVIA $iPLA_2$.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

O F F α F β F F F

O α´ α F F F F

O α´ α F F F F F

Figure 3.
Polyfluoro ketone functionalities.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

9-10	n
a	4
b	5
c	6

Figure 4.

Reagents and conditions: (a) i) $(COCl_2)_2$, CH_2Cl_2; ii) $(CF_3CF_2CO)_2O$, pyridine, CH_2Cl_2.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

13-20	n
a	5
b	9

Figure 5.

Reagents and conditions: (a) NaOCl, TEMPO, NaBr, $NaHCO_3$, toluene/EtOAc, H_2O; (b) $Ph_3P{=}CHCOOCH_3$, CH_2Cl_2; (c) $C_2H_5OOCH{=}CHCH_2P({=}O)(OC_2H_5)$, LiOH, THF; (d) i) H_2, 10% Pd, ii) NaOH, CH_3OH; (e) i) $(COCl_2)_2$, CH_2Cl_2, ii) $(CF_3CO)_2O$, pyridine, CH_2Cl_2.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Figure 6.
Reagents and conditions: (a) i) $(COCl_2)_2$, CH_2Cl_2, ii) $(CF_3CO)_2O$, pyridine, CH_2Cl_2.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Figure 7.
Reagents and conditions: (a) Deoxofluor, dry CH_2Cl_2; (b) i) $(CH_3)_3SiCF_3$, CsF, $CH_3OCH_2CH_2OCH_3$, ii) conc. HCl.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

MgBr
a
OEt
b
OEt
c
27
28
29
HO OH
CF_3
F F
F F
30

Figure 8.

Reagents and conditions: (a) dry Et_2O, diethyl oxalate; (b) Et_2NSF_3; (c) i) $(CH_3)_3SiCF_3$, CsF, $CH_3OCH_2CH_2OCH_3$, ii) conc. HCl.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Figure 9.

Reagents and conditions: (a) $NaHSO_3$, KCN, CH_2Cl_2; (b) HCl, MeOH; (c) Dess-Martin periodinate, CH_2Cl_2; (d) Et_2NSF_3, CH_2Cl_2; (e) i) $(CH_3)_3SiCF_3$, CsF, $CH_3OCH_2CH_2OCH_3$, ii) conc. HCl.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

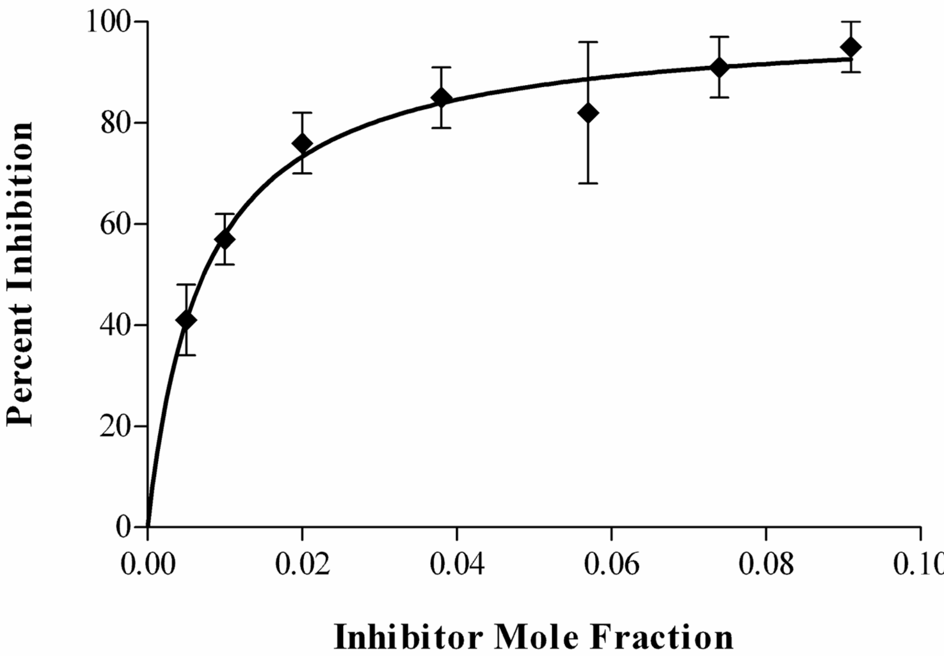

Figure 10.

Inhibition curve for pentafluoro ketone **10a** in a mixed-micelle assay with human GVIA $iPLA_2$. Non-linear regression (hyperbolic) estimated a $X_I(50)$ value of 0.0073 ± 0.0007. Compound **10a** inhibited GIVA $cPLA_2$ less than 25% and GV $sPLA_2$ approximately 28% at 0.091 mole fraction.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

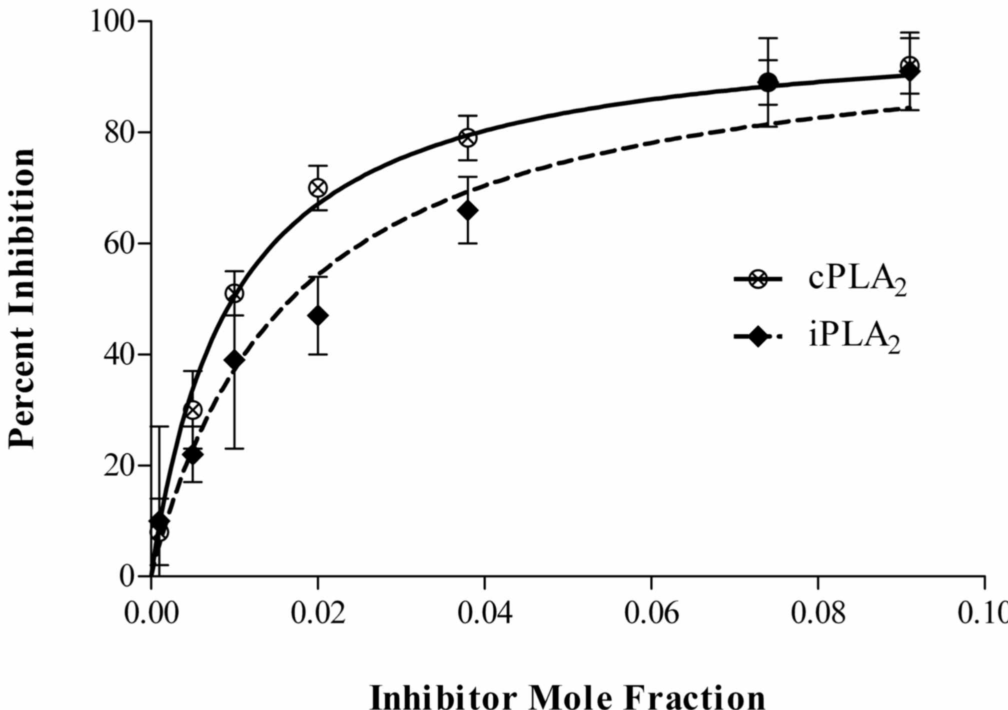

Figure 11.

Inhibition curves for trifluoromethyl ketone **20a** in a mixed-micelle assay with human GIVA $cPLA_2$ and GVIA $iPLA_2$. Non-linear regressions (hyperbolic) estimated $X_I(50)$ values of 0.0169 ± 0.0021 and 0.0098 ± 0.0006 for GIVA $cPLA_2$ and GVIA $iPLA_2$, respectively. Compound **20a** inhibited GV $sPLA_2$ approximately 86% at 0.091 mole fraction.

Figure 12.
Structures of difluoro ketone inhibitor 36 of cobra venom PLA_2 and BEL inhibitor **37**.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Inhibition of PLA_2 by fluoroketones

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

No	Structure	GIVA cPLA$_2$		GVIA iPLA$_2$		GV sPLA$_2$	
		% Inhibition	$X_I(50)$	% Inhibition	$X_I(50)$	% Inhibition	$X_I(50)$
6		96 ± 2	0.0223 ± 0.0023	92 ± 3	0.0195 ± 0.0053	79 ± 9	
11		N.D.		50 ± 13		43 ± 8	
8		38 ± 2		96 ± 3	0.0096 ±0.0008	N.D.	
10a		N.D.		98 ± 16	0.0073 ± 0.0007	28 ± 1	
12a		62 ± 5		96 ± 6	0.0025 ± 0.0003	48 ± 6	
10b		56 ± 4		98 ± 5	0.0065 ± 0.001	46 ± 8	
12b		68 ± 6		99 ± 10	0.0018 ± 0.0005	53 ± 14	
10c		65 ± 12		98 ± 4	0.0065 ± 0.0008	75 ± 10	
19a		91 ± 2	0.0199 ± 0.0025	85 ± 4	0.0328 ±0.0035	82 ± 8	
20a		92 ± 3	0.0098 ± 0.0006	91 ± 4	0.0169 ±0.0021	86 ± 2	
19b		96 ± 2	0.0156 ± 0.0019	94 ± 8	0.0208 ±0.0032	80 ± 6	
20b		95 ± 2	0.0116 ± 94 ± 8	94 ± 8	0.0166 ±0.0022	84 ± 7	

No	Structure	GIVA $cPLA_2$		GVIA $iPLA_2$		GV $sPLA_2$	
		% Inhibition	$X_I(50)$	% Inhibition	$X_I(50)$	% Inhibition	$X_I(50)$
23		88 ± 1		71 ± 14		49 ± 12	
21		73 ± 4		95 ± 5	0.0075 ±0.0011	86 ± 4	
30		27 ± 3		49 ± 12		59 ± 12	
26		94 ± 2	0.0167 ± 0.0018	93 ± 4	0.0011 ± 0.0002	86 ± 10	0.0236 ± 0.004

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript