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Highly Potent 2-Oxoester Inhibitors of Cytosolic Phospholipase A_2 (GIVA cPLA$_2$)

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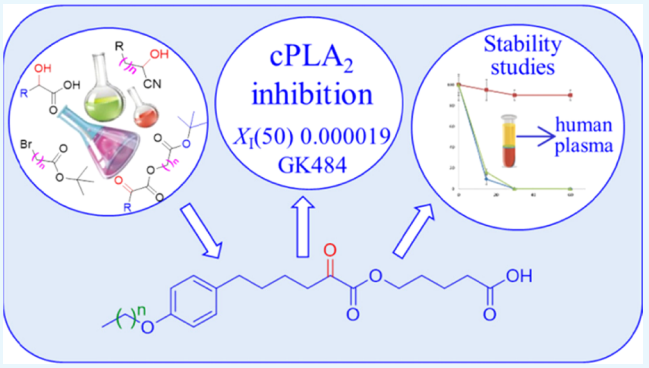
Supporting Information

ABSTRACT: Cytosolic phospholipase A_2 (GIVA cPLA$_2$) has attracted great interest as a medicinal target because it initiates the eicosanoid cascade and is involved in a number of inflammatory diseases. As a consequence, the development of potent synthetic inhibitors is of great importance. We have developed highly potent 2-oxoester inhibitors of GIVA cPLA$_2$ presenting $X_I(50)$ values between 0.000019 and 0.000066. We demonstrate that the 2-oxoester functionality is essential for in vitro inhibitory activity, making these inhibitors useful research reagents. However, their high reactivity results in rapid degradation of the inhibitors in human plasma, limiting their pharmaceutical utility without further modification.



INTRODUCTION

Phospholipases A_2 (PLA$_2$s) have attracted great interest as medicinal targets for more than 20 years because they are involved in a number of inflammatory diseases.[1] Among this superfamily of enzymes,[2] cytosolic PLA$_2$ (GIVA cPLA$_2$) stands out because it exhibits a marked preference for the hydrolysis of arachidonic acid at the sn-2 position of phospholipid substrates releasing arachidonic acid and initiating the eicosanoid cascade.[3] Both the physiological function and the role of cytosolic PLA$_2$ have been recently summarized by Leslie.[4] Due to the involvement of PLA$_2$s in various inflammatory diseases, many synthetic inhibitors have been developed in both academia and pharmaceutical companies.[1] Two recent review articles discuss the classes of PLA$_2$ inhibitors and highlight the in vitro activities and selectivity and the in vivo studies in animal models.[5,6]

Most recently, a randomized double-blind placebo-controlled dose-escalation first-in-man study to assess the safety and efficacy of a topical cytosolic PLA$_2$ inhibitor AVX001 (**1**, Figure 1) in patients with mild-to-moderate plaque psoriasis has demonstrated that treatment with AVX001 is well tolerated in doses up to 5%.[7] Pharmacological inhibition of GIVA cPLA$_2$ by inhibitor **2** (Figure 1) blocked *Streptococcus pneumoniae*-induced polymorphonuclear cells transepithelial migration in vitro, suggesting that this enzyme plays a crucial role in eliciting pulmonary inflammation during pneumococcal infection.[8] The daily administration of the indole-based inhibitor ASB14780 (**3**, Figure 1), which had been developed by Tomoo and colleagues,[9] markedly ameliorated liver injury and hepatic fibrosis following 6 weeks of treatment with CCl_4, indicating that a GIVA cPLA$_2$ inhibitor could be useful for the treatment of nonalcoholic fatty liver diseases, including fatty liver and hepatic fibrosis.[10] A few years ago, we presented new thiazolyl ketones as inhibitors of GIVA cPLA$_2$ and demonstrated the in vivo anti-inflammatory activity of inhibitor GK470 (**4**, Figure 1) in a collagen-induced arthritis model.[11] The anti-angiogenic effects of this inhibitor (now named as AVX235) in a patient-derived triple-negative basal-like breast cancer model was evaluated and significant tumor growth inhibition was observed after 8 days of treatment.[12] Most recent findings showed that blockage of GIVA cPLA$_2$ by either inhibitor **2** or pyrrophenone (**5**, Figure 1) sensitized aggressive breast cancer to doxorubicin by suppressing ERK and mTOR kinases.[13]

All of the above-described recent applications of synthetic GIVA cPLA$_2$ inhibitors highlight the importance of identifying new highly potent inhibitors to regulate the activity of GIVA cPLA$_2$. Last year, we reported the development of a novel class of GIVA cPLA$_2$ inhibitors, namely, 2-oxoesters.[14] 2-Oxoester GK452 (**6**, Figure 1), containing a biphenyl system and a free carboxyl group, led to highly potent and selective GIVA cPLA$_2$ in vitro inhibition ($X_I(50)$ 0.000078). The aim of the present work was to further understand the characteristics of 2-

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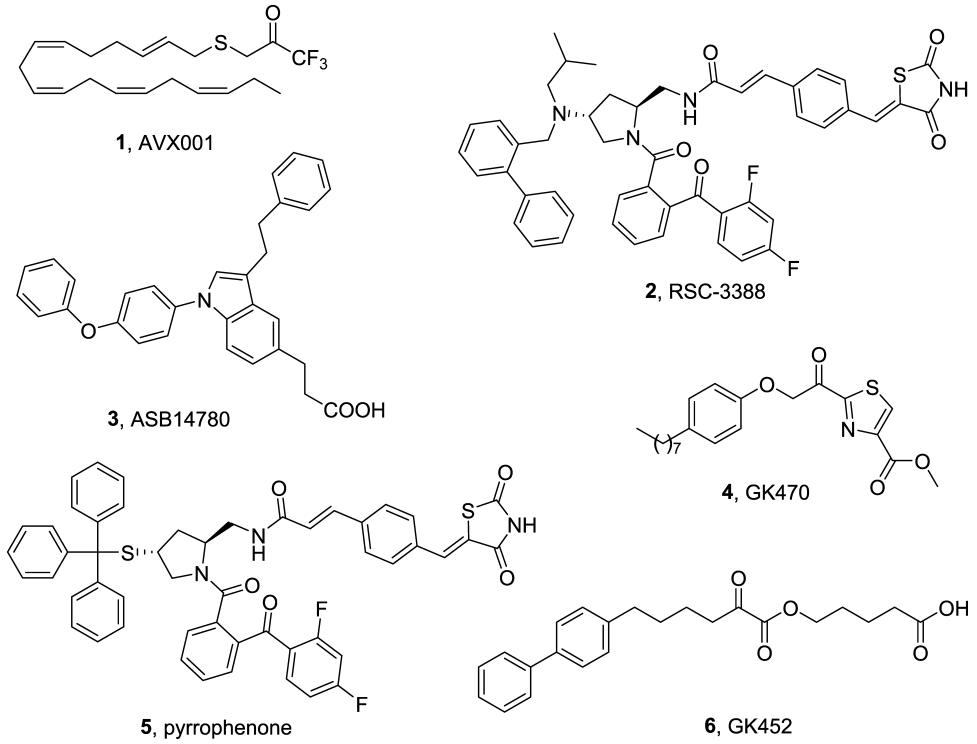
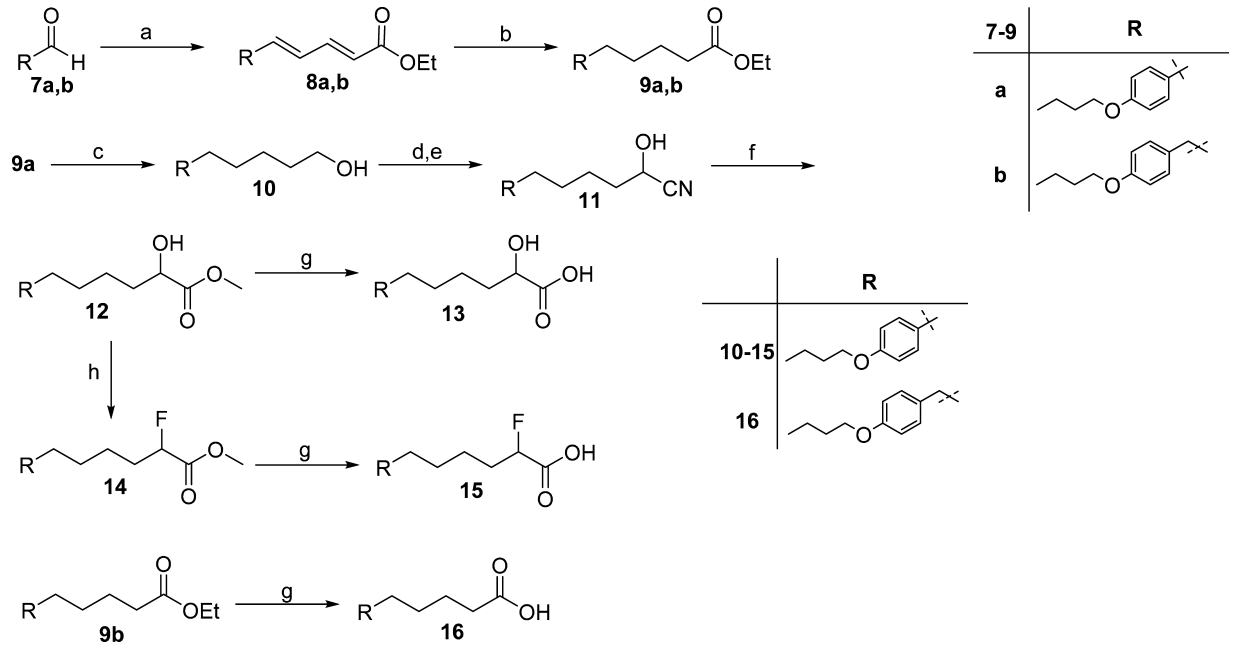


Figure 1. Structures of known GIVA cPLA$_2$ inhibitors.

Scheme 1



[a]Reagents and conditions: (a) $(C_2H_5O)_2P(O)CH_2CH{=}CHCO_2C_2H_5$, $LiOH{\cdot}H_2O$, tetrahydrofuran (THF), mol. sieves; (b) H_2, 10% Pd/C, EtOH; (c) DIBALH, Et_2O; (d) $C_6H_5I(O_2CCH_3)_2$, cat. (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl or (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (TEMPO), CH_2Cl_2; (e) (i) 4 N aq. $NaHSO_3$, CH_2Cl_2 (ii) 4 N aq. KCN; (f) 4 N HCl/MeOH; (g) 50% aq. NaOH, 1,4-dioxane; (h) diethylaminosulfur trifluoride (DAST), CH_2Cl_2.

oxoesters and explore the possibility of producing more potent GIVA cPLA$_2$ inhibitors.

RESULTS AND DISCUSSION

Synthesis of Inhibitors. To extend the structure−activity relationship studies on 2-oxoester inhibitors, we (a) shortened the length between the activated carbonyl group and the biphenyl functionality, (b) replaced the biphenyl system with a *para*-alkoxy-substituted phenyl group, and (c) explored the importance of the activated carbonyl group. To this end, a variety of 2-substituted carboxylic acids, required for the target compounds, were synthesized as described in Scheme 1.

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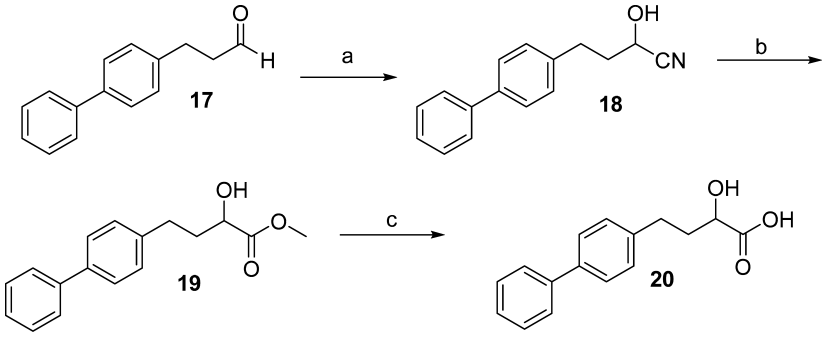
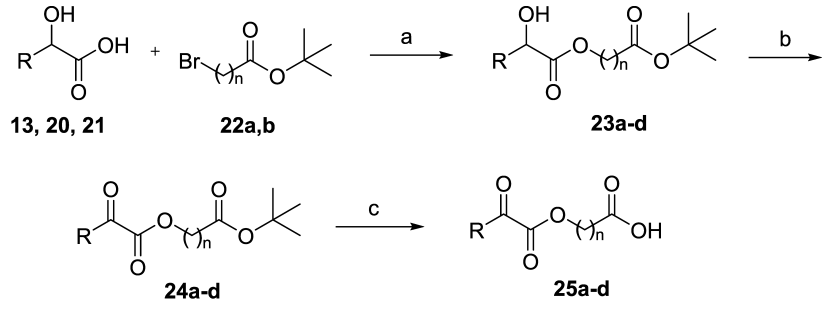
Scheme 2



[a]Reagents and conditions: (a) (i) 4 N aq. $NaHSO_3$, CH_2Cl_2, and (ii) 4 N aq. KCN; (b) 4 N HCl/MeOH; and (c) 50% aq. NaOH, 1,4-dioxane.

Scheme 3



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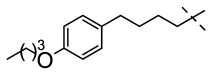
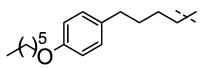
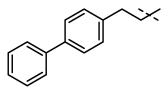
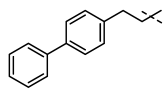
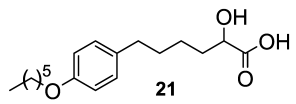
a		4
b		4
c		4
d		3

22	n
a	3
b	4



[a]Reagents and conditions: (a) 20% aq. Cs_2CO_3, *N*,*N*-dimethylformamide (DMF); (b) Dess–Martin periodinane, CH_2Cl_2; and (c) 50% trifluoroacetic acid (TFA)/CH_2Cl_2.

The synthesis of carboxylic acids **13**, **15**, and **16**, bearing a 4-butoxy-phenyl group, started with the Wadsworth–Horner–Emmons olefination reaction of aldehydes **7a**, **7b**[15] with triethyl phosphonocrotonate to give the corresponding unsaturated esters **8a,b**, which were hydrogenated to compounds **9a,b**. The reduction of **9a** led to alcohol **10**, which subsequently provided cyanohydrin **11**. Subsequent treatment with HCl in methanol gave 2-hydroxy methyl ester **12**, which was then saponified to 2-hydroxy acid **13** by treatment with aqueous NaOH. Treatment of **12** with the fluorinating agent diethylaminosulfur trifluoride (DAST)[16] resulted in 2-fluoro methyl ester **14**, consequently providing 2-fluoro carboxylic acid **15** after saponification. The carboxylic acid **16** was also obtained by alkaline hydrolysis of **9b**. The synthesis of 2-hydroxy acid **20** was accomplished by similar procedures starting from aldehyde **17**,[17] as depicted in Scheme 2.

The key step in the synthesis of 2-oxoesters was the reaction between cesium salt of 2-hydroxy carboxylic acids with appropriate *tert*-butyl bromoalkanoate **22a,b**[14] (Scheme 3). 2-Hydroxy esters **23a–d**, obtained from 2-hydroxy carboxylic acids **13**, **20**, and the previously synthesized **21**,[14] were then oxidized to the corresponding 2-oxoesters **24a–d** using the Dess–Martin periodinane reagent. Cleavage of *tert*-butyl protecting group led to the target compounds **25a–d** (Scheme 3).

Likewise, reaction of 2-fluoro carboxylic acid **15** and acid **16** with *tert*-butyl bromopentanoate (**22b**) resulted in derivatives **26a,b**, and subsequently, after the removal of *tert*-butyl group, to the desired products **27a,b** (Scheme 4). Finally, the synthesis of 2-oxoacid **29** was accomplished as shown in Scheme 5 by saponification of the corresponding 2-oxoester **28** under mild conditions.

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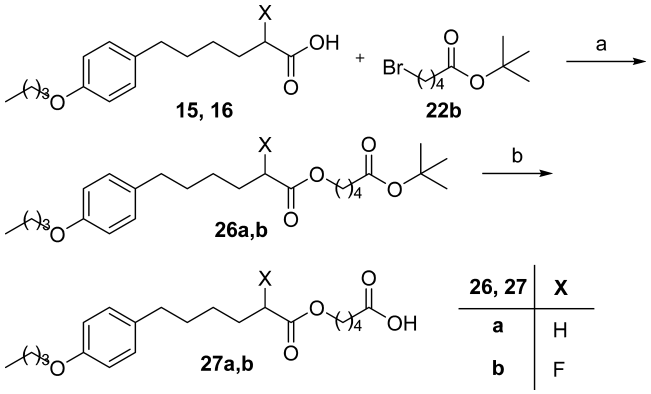
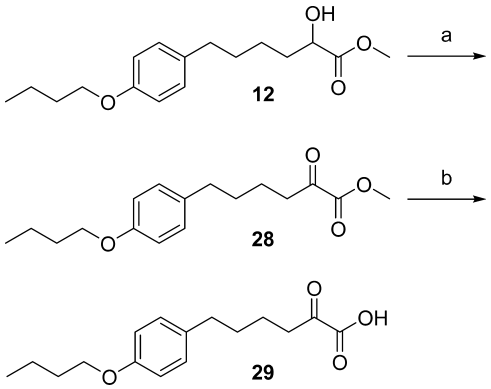
Scheme 4



[a]Reagents and conditions: (a) 20% aq. Cs_2CO_3, DMF and (b) 50% TFA/CH_2Cl_2.

Scheme 5



[a]Reagents and conditions: (a) Dess–Martin periodinane and (b) 20% aq. Cs_2CO_3, MeOH.

In Vitro Inhibition of GIVA cPLA$_2$, GVIA iPLA$_2$, and GV sPLA$_2$. All of the new compounds synthesized were tested for in vitro inhibition of human GIVA cPLA$_2$, calcium-independent phospholipase A$_2$ (GVIA iPLA$_2$), and secreted phospholipase A$_2$ (GV sPLA$_2$) using previously described mixed micelle-based assays.[18−20] The inhibition results are presented in Table 1, either as percent inhibition or as $X_I(50)$ values. At first, the percent of inhibition for each PLA$_2$ enzyme at a high mole fraction (0.091) of each inhibitor was determined. Then, the $X_I(50)$ values were measured for compounds that displayed more than 95% inhibition. The $X_I(50)$ is the mole fraction of the inhibitor in the total substrate interface required to inhibit the enzyme by 50%.

Because it is an important property of GIVA cPLA$_2$ inhibitors, the Clog *P* values for all the compounds tested, calculated by ChemDraw, are also included in Table 1. The Clog *P* value is a measure of hydrophobicity and represents the calculated partition coefficient in octanol/water on a logarithmic scale.

Inhibitor **6** (GK452) ($X_I(50)$ 0.000078)[14] is included in Table 1 for comparison purposes. When the distance between the oxoester functionality and the biphenyl system was decreased from four to two carbon atoms (compound **25c** (GK482) and compound **25d** (GK483)), the inhibitory potency against GIVA cPLA$_2$ considerably decreased. This decrease was 1 order of magnitude in the case of a four-carbon linker (compound **25c** $X_I(50)$ 0.00041), further decreasing (2 orders of magnitude) when the linker was further shortened (**25d** $X_I(50)$ 0.0012). These results are in accordance with our previous results[14] and confirm that the optimum distance between the free carboxyl group and the oxoester functionality corresponds to four carbon atoms. In addition, compound **25c** lost the selectivity because the inhibition of GVIA iPLA$_2$ was observed ($X_I(50)$ 0.0030). Thus, the four-carbon linker was maintained for all the other derivatives. The optimum length of the linker may be correlated with stereochemical reasons, rather than hydrophobicity.

Interestingly, when one ring of the biphenyl system was replaced by a *para*-hexyloxy group, an increase in the inhibitor potency against GIVA cPLA$_2$ was observed. Compound **25b** (GK484) is the most potent inhibitor of GIVA cPLA$_2$ ever reported with a $X_I(50)$ value of 0.000019, 4 times more potent than **6**. However, the replacement of the phenyl ring by the hexyloxy group resulted in an increase in the Clog *P* value (from 4.70 for **6** to 5.37 for **25b**). Thus, we shortened the hexyloxy chain to butyloxy one. Compound **25a** (GK504) ($X_I(50)$ 0.000066) is 3 times less potent than **25b**, but still slightly more potent than **6**. In addition, its Clog *P* value (4.31) is lower than 5, which is favorable because Lipinski's "rule of 5" predicts that poor absorption or permeation is more likely when CLog *P* is greater than 5.[21] Neither **25b** nor **25a** presented significant inhibition of GVIA iPLA$_2$ (86 and 77%, respectively, at a high mole fraction 0.091). In our experience, compounds that show inhibition of PLA$_2$ less than 90% at the highest concentration tested (which corresponds to about 50 μM inhibitor) always exhibit $X_I(50)$ values greater than 0.01.[11,22] The GIVA cPLA$_2$ inhibitor **25b** with a $X_I(50)$ 0.000019 is the most potent inhibitor of GIVA cPLA$_2$ ever reported and is at least 500-fold selective over GVIA iPLA$_2$.

To confirm the importance of the oxoester functionality, we removed the activated carbonyl group. Compound **27a** (GK505) abolished the inhibitory potency against GIVA cPLA$_2$. Because the only structural difference between **27a** and **25a** is the lack of the carbonyl group, a comparison of the inhibitory potencies of **25a** and **27a** makes it clear that the carbonyl group of the oxoester functionality is essential for GIVA cPLA$_2$ inhibition. To explore if an electron-withdrawing group at position 2 could contribute to the inhibitory potency, the fluoro derivative **27b** (GK506) was studied. However, again, no inhibition of GIVA cPLA$_2$ was observed. Finally, 2-oxoacid **29** (GK511) was evaluated in vitro and only a very weak inhibition of GIVA cPLA$_2$ was recorded (55% at a high mole fraction 0.091). Because this 2-oxoacid is a fragment of 2-oxoester **25a**, it is clear that the oxoester functionality is absolutely necessary for the inhibition.

Plasma Stability Studies. Determination of the stability of new chemical entities in plasma is important, as compounds (with the exception of pro-drugs), which rapidly degrade in plasma, generally show poor in vivo performance.[23] The stability of compounds **25a**, **25b**, and **27a** in human plasma was studied in a time-dependent manner. Our aim was to understand the difference between the two highly potent 2-oxoesters GIVA cPLA$_2$ inhibitors **25a** and **25b** in comparison to compound **27a** lacking the activated carbonyl group. A liquid chromatography-high resolution mass spectrometry (LC-HRMS) method was developed to measure the inhibitor levels in plasma. All of the HRMS spectra were obtained in electrospray ionization (ESI) negative ion mode. The molecular ion of inhibitor **25b** (GK484) was recorded at *m*/

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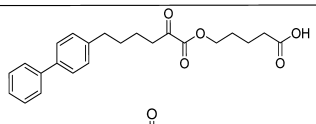
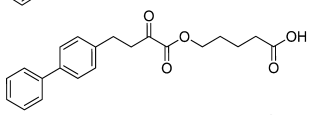
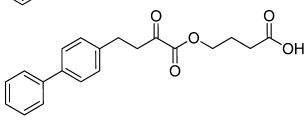
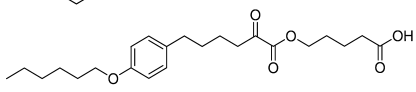
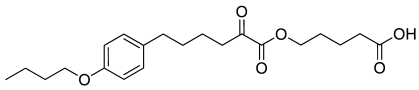
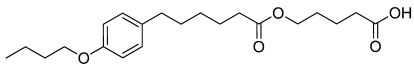
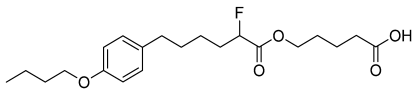
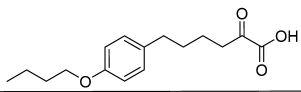
Table 1. In Vitro Inhibitory Potency and Selectivity of 2-Oxoesters

No (Code)	Structure	ClogP[a]	GIVA cPLA$_2$ % Inhibition[b]	GIVA cPLA$_2$ X_I(50)	GVIA iPLA$_2$ % Inhibition[b]	GVIA iPLA$_2$ X_I(50)	GV sPLA$_2$ % Inhibition[b]
6 (GK452)		4.70	>95%	0.000078 ± 0.00001	65±3		<25
25c (GK482)		3.79	>95%	0.00041± 0.00005	95±0.8	0.0030± 0.0004	39±4
25d (GK483)		3.87	>95%	0.0012± 0.0002	90±0.6		38±3
25b (GK484)		5.37	>95%	0.000019± 0.000002	86±2		<25
25a (GK504)		4.31	>95%	0.000066± 0.000003	77±2		29±2
27a (GK505)		5.39	42±1		68±5		<25
27b (GK506)		5.06	28±2		81±3		<25
29 (GK511)		3.27	55±3		28±3		28±6

[a]Calculated with ChemDraw. [b]% Inhibition at 0.091 mol fraction of each inhibitor.

z 405.2253 (Figure 2A), whereas in the MS/MS spectrum (Figure 2B), the most intense signal corresponds to the loss of $C_5H_8O_2$ (−100.0524), indicating the fragmentation of the ester bond. The HRMS spectra of inhibitors **25a** and **27a** are shown in the Supporting Information. In all cases of 2-oxoester inhibitors, the fragmentation of the ester bond was observed. Plasma samples incubated with each inhibitor were taken at 0, 15, 30, and 60 min and the molecular ion of each inhibitor was used to estimate the inhibitor level. The stability results are presented in Figure 2C as percent parent compound remaining at each time point. Inhibitors **25a** (GK504) and **25b** (GK484), containing the 2-oxo group, are not detectable in the plasma samples after 30 min, in contrast to **27a** (GK505), whose percentage remains close to 100% after 1 h. Apparently, the activated carbonyl group of the 2-oxoester functionality of inhibitors **25a** and **25b** increases the chemical reactivity of the ester bond toward hydrolysis in comparison to the less active simple ester bond of inhibitor **27a**. These results indicate that the activated carbonyl group, which is essential for the enzyme inhibition, makes the compounds susceptible to rapid degradation in human plasma. A compound like **27a**, lacking the activated carbonyl group, is rather stable in plasma, however lacking the inhibitory properties.

CONCLUSIONS

In conclusion, a number of new 2-oxoesters and analogues were synthesized. GK484 is the most potent inhibitor of GIVA cPLA$_2$ ever reported with a X_I(50) value of 0.000019. The study of the in vitro inhibitory activity highlighted the importance of the 2-oxoester group for the inhibitory potency against GIVA cPLA$_2$. The stability studies of 2-oxoesters in human plasma indicate that a fine-tuning between the reactivity of the carbonyl group and its stability will be needed in order for 2-oxoesters to be used in vivo as pharmacological agents.

EXPERIMENTAL SECTION

General. Merck Silica Gel 60254 aluminum plates and Silica Gel 60 (70−230 or 230−400 mesh) were used for thin-layer chromatography and chromatographic purification of products, respectively. For visualizing spots, UV light and/or phosphomolybdic acid was employed. Melting points were estimated by a Büchi 530 apparatus and uncorrected. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on Varian Mercury at 200, 50, and 188 MHz, respectively. $CDCl_3$ was used as the solvent. Chemical shifts are given in ppm and coupling constants (J) in Hz. Peak multiplicities are typified as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; qt, quintet; and m, multiplet. Dichloromethane was dried by standard procedures and stored over molecular sieves. No further purification of other solvents and chemicals was needed. The HRMS spectra were recorded on a Bruker Maxis Impact QTOF Spectrometer.

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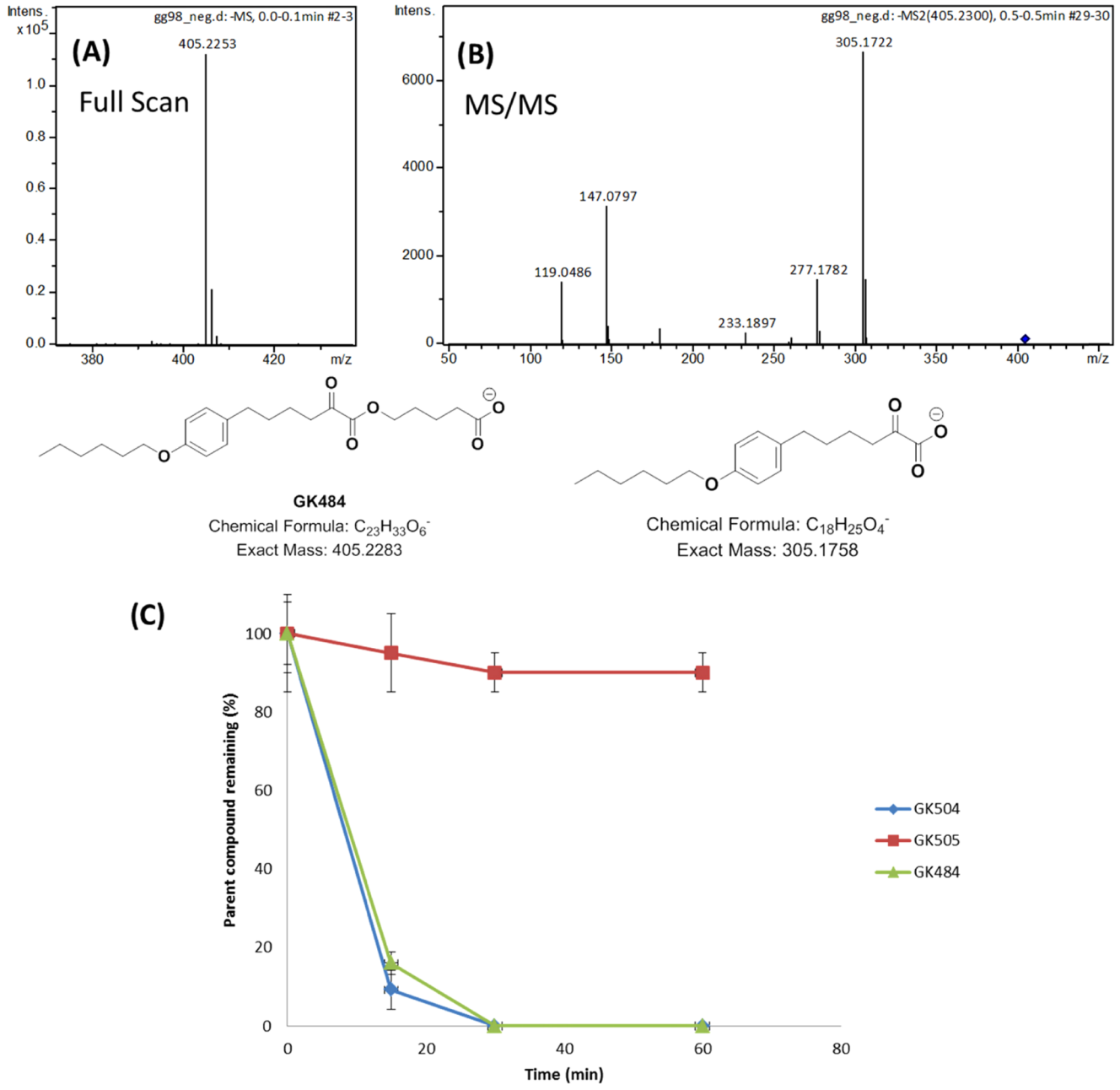

Figure 2. Precursor ion (A) and MS/MS HRMS spectrum (B) of inhibitor GK484. Time-dependent degradation of inhibitors GK484, GK504, and GK505 in human plasma (C).

Synthesis. Compound **7a** was commercially available, and compounds **7b**,[15] **17**,[17] and **21**[14] have been described elsewhere and their analytical data are in accordance with literature.

*General Procedure for Synthesis of Unsaturated Esters **8a,b**.* To a flame-dried, round-bottomed flask containing powdered molecular sieves (1 g), $LiOH{\cdot}H_2O$ (1.5 mmol, 36 mg) and a solution of aldehyde **7a,b** (1 mmol) in dry THF (10 mL) were added under Ar atmosphere. Triethyl 4-phosphonocrotonate (1.5 mmol, 375 mg) was added and the mixture was left under reflux overnight. The reaction mixture was filtered through celite and the solvent was evaporated under reduced pressure. The products were obtained after trituration with MeOH.

*Ethyl (2E,4E)-5-(4-Butoxyphenyl)penta-2,4-dienoate (**8a**).* Yield 60%; white solid; mp 57−60 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.49−7.18 (m, 3H, arom, ═CH), 6.89−6.50 (m, 4H, arom, 2 × ═CH), 5.87 (d, J = 15.2 Hz, 1H, ═CH), 4.17 (q, J = 7.1 Hz, 2H, CH_2OCO), 3.87 (t, J = 6.4 Hz, 2H, CH_2O), 1.82−1.60 (m, 2H, CH_2), 1.47−1.33 (m, 2H, CH_2), 1.25 (t, J = 7.1 Hz, 3H, CH_3CH_2OCO), 0.92 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 167.3, 160.2, 145.3, 140.4, 128.9, 128.7, 124.1, 120.1, 114.9, 67.9, 60.3, 31.5, 19.4, 14.6, 14.1; Anal. Calcd for $C_{17}H_{22}O_3$: C, 74.42; H, 8.08; found: C, 74.06; H, 8.53.

*Ethyl (2E,4E)-6-(4-Butoxyphenyl)hexa-2,4-dienoate (**8b**).* Yield 50%; white solid; mp 62−65 °C ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36−7.17 (m, 1H, ═CH), 7.03 (d, J = 8.4 Hz, 2H, arom) 6.81 (d, J = 8.4 Hz, 2H, arom), 6.15 (d, J = 8.0 Hz, 2H,

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2x=CH), 5.79 (d, J = 15.3 Hz, 1H, =CH), 4.16 (q, J = 7.1 Hz, 2H, CH_2OCO), 3.89 (t, J = 6.3 Hz, 2H, CH_2O), 3.59−3.30 (m, 2H, $PhCH_2$), 1.81−1.63 (m, 2H, CH_2), 1.45 (m, 2H, CH_2), 1.25 (t, J = 7.1 Hz, 3H, CH_3CH_2OCO), 0.96 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.3, 157.2, 143.9, 142.2, 129.9, 128.9, 128.4, 119.5, 66.9, 59.5, 113.9, 37.8, 30.8, 18.7, 13.7, 13.3; Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39; found: C, 74.64; H, 8.72.

General Procedure for Hydrogenation. To a solution of unsaturated esters **8a,b** (1 mmol) in EtOH (10 mL), 10% Pd/C was added and the mixture stirred overnight under H_2 atmosphere. The reaction mixture was filtered through celite and concentrated in vacuo to isolate the saturated product **9a,b**.

*Ethyl 5-(4-Butoxyphenyl)pentanoate (**9a**).* Yield 99%; colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.07 (d, J = 8.6 Hz, 2H, arom), 6.81 (d, J = 8.6 Hz, 2H, arom), 4.12 (q, J = 7.1 Hz, 2H, CH_2OCO), 3.92 (t, J = 6.4 Hz, 1H, CH_2O), 2.56 (t, J = 6.9 Hz, 2H, $PhCH_2$), 2.31 (t, J = 7.0 Hz, 2H, CH_2CO), 1.84−1.70 (m, 2H, CH_2), 1.69−1.56 (m, 4H, CH_2), 1.56−1.38 (m, 2H, CH_2), 1.24 (t, J = 7.1 Hz, 3H, CH_3CH_2O), 0.97 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.8, 157.5, 134.2, 129.4, 114.5, 67.8, 60.4, 34.9, 34.4, 31.7, 31.4, 24.8, 19.5, 14.5, 14.1; Anal. Calcd for $C_{17}H_{26}O_3$: C, 73.35; H, 9.41; found: C, 73.07; H, 9.69.

*Ethyl 6-(4-Butoxyphenyl)hexanoate (**9b**).* Yield 99%; colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.08−6.94 (d, J = 8.3 Hz, 2H, arom), 6.85−6.67 (d, J = 8.6 Hz, 2H, arom), 4.43−4.10 (m, 2H, CH_2OCO), 3.87 (t, J = 6.4 Hz, 2H, CH_2O), 2.64−2.39 (m, 2H, $PhCH_2$), 2.33−2.18 (m, 2H, CH_2CO), 1.86−1.30 (m, 10H, 5 × CH_2), 1.22 (t, J = 7.2 Hz, 3H, CH_3CH_2O), 0.94 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.5, 157.0, 134.1, 129.0, 114.3, 67.3, 59.9, 34.6, 34.0, 31.3, 29.6, 28.4, 24.6, 19.1, 14.0, 13.7; Anal. Calcd for $C_{18}H_{28}O_3$: C, 73.93; H, 9.65; found: C, 73.72; H, 9.82.

*5-(4-Butoxyphenyl)pentan-1-ol (**10**).* A solution of **9a** (1 mmol, 0.27 g) in anhydrous Et_2O (10 mL) under Ar was cooled at 0 °C. DIBALH (1.2 mmol, 1.2 mL) was added dropwise and the mixture was left at room temperature for 30 min. The reaction was quenched by adding ice at 0 °C and left to stir for 30 min. The mixture was filtered through celite and concentrated under reduced pressure. The residue was purified by column chromatography, using EtOAc/petroleum ether (bp 40−60 °C) 3:7 as eluent. Yield 90%; colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.07 (d, J = 8.5 Hz, 2H, arom), 6.81 (d, J = 8.6 Hz, 2H, arom), 3.93 (t, J = 6.5 Hz, 2H, CH_2O), 3.60 (t, J = 6.5 Hz, 2H, CH_2OH), 2.50 (t, J = 6.4 Hz, 2H, $PhCH_2$), 2.08 (brs, 1H, OH), 1.86−1.30 (m, 10H), 0.97 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 157.4, 134.7, 129.4, 114.5, 67.9, 63.0, 35.2, 32.8, 31.8, 31.6, 25.6, 19.5, 14.1; Anal. Calcd for $C_{15}H_{24}O_2$: C, 76.23; H, 10.24; found: C, 76.09; H, 10.32.

*6-(4-Butoxyphenyl)-2-hydroxyhexanenitrile (**11**).* To a stirring solution of alcohol **10** (1 mmol, 0.24 g) in dry CH_2Cl_2 (10 mL), iodobenzene diacetate (1.2 mmol, 0.39 g) and a catalytic amount of TEMPO (10%, 0.024 g) were added and the reaction mixture was left stirring at room temperature for 1 h. The reaction mixture was washed consecutively with 10% aqueous $Na_2S_2O_3$ (10 mL), 10% aqueous $NaHCO_3$ (10 mL), and brine (10 mL), dried over Na_2SO_4, and concentrated in vacuo. The aldehyde was then dissolved in CH_2Cl_2 (1.5 mL) and 4 N aqueous $NaHSO_3$ (2 mmol, 0.5 mL) and the mixture was left stirring for 30 min at room temperature. The organic solvent was removed under reduced pressure and H_2O (1.5 mL) was added. The mixture was cooled down to 0 °C and 4 N aqueous KCN (0.5 mL) was added dropwise within 2 h under vigorous stirring, followed by stirring overnight at room temperature. Then, H_2O (10 mL) was added and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography with EtOAc/petroleum ether (bp 40−60 °C) 3:7 as eluent. Yield 71%; colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.08 (d, J = 8.3 Hz, 2H, arom), 6.83 (d, J = 8.3 Hz, 2H, arom), 4.53−4.38 (m, 1H, CH), 3.95 (t, J = 6.4 Hz, 2H, CH_2O), 2.58 (t, J = 7.1 Hz, 2H, $PhCH_2$), 1.93−1.38 (m, 10H, 5 × CH_2), 0.98 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 157.5, 134.2, 129.5, 120.4, 114.7, 68.0, 61.4, 35.3, 35.0, 31.6, 31.3, 24.4, 19.5, 14.2; MS (ESI) m/z (%): 279 ($[M + NH_4]^+$, 100); Anal. Calcd for $C_{16}H_{23}NO_2$: C, 73.53; H, 8.87; N, 5.36; found: C, 73.32; H, 9.03; N, 5.18.

*4-([1,1′-Biphenyl]-4-yl)-2-hydroxybutanenitrile (**18**).* To a stirring solution of aldehyde **17** (1.0 mmol) in CH_2Cl_2 (1.5 mL), an aqueous solution of $NaHSO_3$ 6 M (0.25 mL, 1.5 mmol) was added and the mixture was left stirring for 30 min at room temperature. The organic solvent was evaporated under reduced pressure and H_2O (5 mL) was added. The mixture was cooled down to 0 °C and an aqueous solution of KCN 6 M (0.25 mL, 1.5 mmol) was added dropwise under vigorous stirring. The reaction was left stirring for 18 h at room temperature and then the mixture was extracted with CH_2Cl_2 (2 × 20 mL), washed with brine, and dried over Na_2SO_4. The organic solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography using EtOAc/petroleum ether (bp 40−60 °C) 2.5:7.5 as eluent. Yield 59%; white solid; mp 93−95 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.66−7.13 (m, 9H, arom), 4.16 (q, J = 7.2 Hz, 1H, CH), 3.76 (brs, 1H, OH), 2.90 (t, J = 7.4 Hz, 2H, $PhCH_2$), 2.22−2.13 (m, 2H, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 140.6, 139.3, 138.6, 128.8, 128.7, 127.3, 127.1, 126.9, 119.9, 60.2, 36.4, 30.2; MS (ESI) m/z (%): 255 ($[M + NH_4]^+$, 100); Anal. Calcd for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90; found: C, 80.64; H, 6.68; N, 5.73.

*General Procedure for Synthesis of 2-Hydroxy Esters **12** and **19**.* Cyanohydrins **11** and **18** (1 mmol) were dissolved in 4 N HCl/MeOH (10 mL) and the reaction mixture stirred for 24 h at room temperature. The organic solvent was evaporated under reduced pressure. The residue was dissolved in Et_2O (10 mL) and re-evaporated. Dilution and evaporation were repeated twice. The product was purified by flash column chromatography using EtOAc/petroleum ether (bp 40−60 °C) 2:8 as eluent.

*Methyl 6-(4-Butoxyphenyl)-2-hydroxyhexanoate (**12**).* Yield 65%; colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.07 (d, J = 8.6 Hz, 2H, arom), 6.81 (d, J = 8.6 Hz, 2H, arom), 4.25−4.10 (m, 1H, CH), 3.93 (t, J = 6.5 Hz, 2H, CH_2O), 3.77 (s, 3H, OCH_3), 2.83 (bs, 1H, OH), 2.63−2.48 (m, 2H, $PhCH_2$), 1.93−1.37 (m, 10H, CH_2), 0.97 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.0, 157.4, 134.5, 129.4, 114.5, 70.6, 67.9, 52.8, 35.3, 35.0, 34.5, 31.6, 24.6, 19.5, 14.1; MS (ESI) m/z (%): 312 ($[M + NH_4]^+$, 100); Anal. Calcd for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90; found: C, 69.18; H, 9.02.

*Methyl 4-([1,1′-Biphenyl]-4-yl)-2-hydroxybutanoate (**19**).* Yield 64%; colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.64−7.22 (m, 9H, arom), 4.25 (q, J = 4.0 Hz, 1H, CH), 3.77 (s, 3H, OCH_3), 2.84 (t, J = 8.2 Hz, 2H, $PhCH_2$), 2.30−1.84

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(m, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.6, 140.9, 140.1, 138.9, 129.0, 128.7, 127.1, 127.0, 126.9, 69.6, 52.5, 35.8, 30.6; MS (ESI) m/z (%): 288 ($[M + NH_4]^+$, 100); Anal. Calcd for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71; found: C, 75.32; H, 6.84.

*Methyl 6-(4-Butoxyphenyl)-2-fluorohexanoate (**14**).* A solution of diethylaminosulfur trifluoride (1.1 mmol, 0.15 mL) in CH_2Cl_2 (0.23 mL) was added to a flame-dried flask, under Ar atmosphere, and cooled down to −78 °C. A solution of ester **12** (1 mmol, 0.29 g) in anhydrous CH_2Cl_2 (0.3 mL) was added dropwise and the reaction stirred for 2 h at −78 °C and then overnight at room temperature. The reaction mixture was then concentrated under reduced pressure and the product was purified by column chromatography using EtOAc/petroleum ether (bp 40−60 °C) 2:8 as eluent. Yield 55%; yellowish oil; ^1H NMR (200 MHz, $CDCl_3$): δ 7.09 (d, J = 8.5 Hz, 2H, arom), 6.83 (d, J = 8.5 Hz, 2H, arom), 4.90 (dt, J = 49.0 Hz, J = 6.1 Hz, 1H, CHF), 3.94 (t, J = 6.4 Hz, 2H, CH_2O), 3.77 (s, 3H, OCH_3), 2.58 (t, J = 7.3 Hz, 2H, $PhCH_2$), 1.94−1.40 (m, 10H, CH_2), 0.99 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.3 (d, J = 23.9 Hz), 157.1, 133.7, 129.0, 114.1, 88.7 (d, J = 184.0 Hz), 67.4, 52.02, 34.5, 32.1 (d, J = 21.0 Hz), 31.2, 31.0, 23.8 (d, J = 3 Hz), 19.1, 13.7; ^{19}F NMR (188 MHz, $CDCl_3$): δ −150.03 (qt, J = 24.4 Hz). MS (ESI) m/z (%): 314 ($[M + NH_4]^+$, 100); Anal. Calcd for $C_{17}H_{25}FO_3$: C, 68.89; H, 8.50; found: C, 68.66; H, 8.71.

General Procedure for Saponification. To a stirring solution of methyl esters **9b**, **12**, **14**, and **19** (1 mmol) in 1,4-dioxane (10 mL), 2 N aqueous NaOH (2.0 mL) was added and the mixture stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the residue was dissolved in H_2O (10 mL) and extracted with Et_2O (3 × 10 mL). The aqueous layer was then acidified with 2 N HCl (4.0 mL) and extracted with EtOAc (3 × 10 mL) followed by drying over Na_2SO_4 and concentrating in vacuo.

*6-(4-Butoxyphenyl)-2-hydroxyhexanoic Acid (**13**).* Yield 95%; yellowish solid; mp 88−90 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 7.06 (d, J = 8.6 Hz, 2H, arom), 6.81 (d, J = 8.6 Hz, 2H, arom), 4.28−4.22 (m, 1H, CH), 3.93 (t, J = 6.5 Hz, 2H, CH_2O), 2.55 (t, J = 7.2 Hz, 2H, $PhCH_2$), 1.95−1.37 (m, 10H, 5 × CH_2), 0.97 (t, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 180.0, 157.4, 134.5, 129.4, 114.6, 70.4, 68.0, 35.0, 34.2, 31.6, 24.7, 19.5, 14.1; MS (ESI) m/z (%): 279 ($[M - H]^-$, 100); Anal. Calcd for $C_{16}H_{24}O_4$: C, 68.55; H, 8.63; found: C, 68.32; H, 8.75.

*6-(4-Butoxyphenyl)-2-fluorohexanoic Acid (**15**).* Yield 95%; yellowish solid; mp 65−67 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 9.90 (s, 1H, COOH), 7.08 (d, J = 8.5 Hz, 2H, arom), 6.83 (d, J = 8.6 Hz, 2H, arom), 4.95 (dt, J = 48.6 Hz, 6.4 Hz, 1H, CHF), 3.95 (t, J = 6.5 Hz, 2H, CH_2O), 2.58 (t, J = 7.2 Hz, 2H, $PhCH_2$), 2.06−1.36 (m, 10H, 5 × CH_2), 0.98 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.4 (d, J = 25 Hz), 157.1, 133.9, 129.1, 114.3, 88.2 (d, J = 185 Hz), 67.6, 34.6, 32.0 (d, J = 20.0 Hz), 31.3, 31.0, 23.9 (d, J = 2.5 Hz), 19.2, 13.8; ^{19}F NMR (188 MHz, $CDCl_3$): δ −150.4 (qt, J = 25.6 Hz). MS (ESI) m/z (%): 281 ($[M - H]^-$, 100); Anal. Calcd for $C_{16}H_{23}FO_3$: C, 68.06; H, 8.21; found: C, 67.93; H, 8.46.

*6-(4-Butoxyphenyl)hexanoic Acid (**16**).* Yield 95%; yellowish solid; mp 198−200 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 7.01 (d, J = 7.9 Hz, 2H, arom), 6.76 (d, J = 8.4 Hz, 2H, arom), 3.90 (m, 2H, CH_2O), 2.70−2.33 (m, 2H, $PhCH_2$), 2.15 (d, J = 6.6 Hz, 2H, CH_2CO), 1.85−1.10 (m, 10H, CH_2), 0.99 (t, J = 5.6 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 180.7, 156.9, 134.4, 128.9, 114.0, 67.5, 36.8, 34.7, 31.2, 29.1, 25.7, 19.0, 13.6; MS (ESI) m/z (%): 263 ($[M - H]^-$, 100); Anal. Calcd for $C_{16}H_{24}O_3$: C, 72.69; H, 9.15; found: C, 72.51; H, 9.29.

*4-([1,1′-Biphenyl]-4-yl)-2-hydroxybutanoic Acid (**20**).* Yield 90%; white solid;. mp 189−191 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 7.64−7.19 (m, 9H, arom), 4.18−4.10 (m, 1H, CH), 2.80 (t, J = 7.4 Hz, 2H, $PhCH_2$), 2.23−1.83 (m, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 177.8, 142.2, 141.7, 140.1, 138.7, 129.9, 129.7, 128.0, 127.7, 70.6, 37.2, 31.9; MS (ESI) m/z (%): 255 ($[M - H]^-$, 100); Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29; found: C, 74.78; H, 6.42

*General Procedure for Synthesis of **23a−d** and **26a,b**.* To a stirred solution of carboxylic acid (1 mmol) in tetrahydrofuran (THF) (6 mL) and 20% aqueous solution $CsCO_3$ (1.1 mmol, 1.8 mL) were added and left stirring for 10 min at 80 °C. The organic solvent was evaporated under reduced pressure and the residue was dissolved in *N*,*N*-dimethylformamide (DMF) (15 mL). Subsequently, the appropriate *tert*-butyl 5-bromoalkanoate, **22a** or **22b** (1.2 mmol), was added and the reaction mixture was left vigorously stirring under reflux for 72 h. The reaction mixture was concentrated in vacuo and then water (20 mL) was added and extracted with EtOAc (2 × 20 mL). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash column chromatography using EtOAc/petroleum ether (bp 40−60 °C) 3:7 or 2:8.

*5-(tert-Butoxy)-5-oxopentyl 6-(4-butoxyphenyl)-2-hydroxyhexanoate (**23a**).* Yield 35%; yellowish oil; ^1H NMR (200 MHz, $CDCl_3$): δ 7.06 (d, J = 8.5 Hz, 2H, arom), 6.79 (d, J = 8.6 Hz, 2H, arom), 4.19−4.11 (m, 3H, CH, CH_2OCO), 3.92 (t, J = 6.4 Hz, 2H, CH_2O), 2.81 (d, J = 5.8 Hz, 1H, OH), 2.60−2.48 (m, 2H, $PhCH_2$), 2.24 (t, J = 6.9 Hz, 2H, CH_2CO), 1.86−1.36 [m, 23H, 7 × CH_2, $C(CH_3)_3$], 0.95 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.6, 172.8, 157.4, 134.5, 129.4, 114.5, 80.6, 70.6, 67.9, 65.5, 35.1, 34.5, 31.7, 31.6, 28.3, 28.1, 24.7, 21.7, 19.5, 14.1; MS (ESI) m/z (%): 459 ([M + Na]$^+$, 100); Anal. Calcd for $C_{25}H_{40}O_6$: C, 68.78; H, 9.24; found: C, 68.51; H, 9.42.

*5-(tert-Butoxy)-5-oxopentyl 6-(4-(hexyloxy)phenyl)-2-hydroxyhexanoate (**23b**).* Yield 49%; colorless oil; ^1H NMR (200 MHz, $CDCl_3$): δ 7.05 (d, J = 8.7 Hz, 2H, arom), 6.79 (d, J = 8.7 Hz, 2H, arom), 4.23−4.04 (m, 3H, C*H*OH, CH_2O), 3.91 (t, J = 6.5 Hz, 2H, OCH_2), 2.82 (brs, 1H, OH), 2.54 (t, J = 7.0 Hz, 2H, $PhCH_2$), 2.41 (t, J = 7.0 Hz, 2H, CH_2CO), 1.89−1.10 [m, 27H, 9 × CH_2, $C(CH_3)_3$], 0.89 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.3, 172.5, 157.1, 134.2, 129.1, 114.2, 80.3, 70.3, 67.9, 65.2, 34.8, 34.2, 31.5, 31.4, 29.2, 28.0, 27.8, 25.7, 24.4, 22.6, 21.4, 14.0; MS (ESI) m/z (%): 487 ([M + Na]$^+$, 100); Anal. Calcd for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55; found: C, 69.62; H, 9.72.

*tert-Butyl 5-((4-([1,1′-Biphenyl]-4-yl)-2-hydroxybutanoyl)oxy)pentanoate (**23c**).* Yield 36%; colorless oil; ^1H NMR (200 MHz, $CDCl_3$): δ 7.62−7.22 (m, 9H, arom), 4.28−4.04 (m, 3H, OCH_2, CH), 2.82−2.78 (m, 3H, $PhCH_2$, OH), 2.33−1.94 (m, 4H, CH_2), 1.77−1.57 (m, 4H, 2 × CH_2), 1.44 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.1, 172.5, 140.9, 140.2, 138.9, 129.0, 128.7, 127.1, 127.0, 126.9, 80.3, 69.6, 65.3, 35.9, 34.8, 30.6, 28.0, 27.8, 21.4; MS (ESI) m/z (%): 430 ($[M + NH_4]^+$, 100); Anal. Calcd for $C_{25}H_{32}O_5$: C, 72.79; H, 7.82; found: C, 72.65; H, 7.98.

*4-(tert-Butoxy)-4-oxobutyl 4-([1,1′-biphenyl]-4-yl)-2-hydroxybutanoate (**23d**).* Yield 29%; colorless oil; ^1H NMR

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(200 MHz, $CDCl_3$): δ 7.64−7.22 (m, 9H, arom), 4.27−4.05 (m, 3H, OCH_2, CH), 2.83−2.78 (m, 3H, $PhCH_2$, OH), 2.30 (t, J = 7.2 Hz, 2H, CH_2CO), 2.09−1.86 (m, 4H, 2 × CH_2), 1.44 [m, 9H, $C(CH_3)_3$]; ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.0, 172.4, 141.0, 140.4, 138.7, 129.1, 128.6, 127.2, 127.1, 126.8, 80.4, 69.7, 65.2, 35.7, 34.6, 31.6, 28.2, 21.3; MS (ESI) m/z (%): 421 ([M + Na]$^+$, 100); Anal. Calcd for $C_{24}H_{30}O_5$: C, 72.34; H, 7.59; found: C, 72.16; H, 7.75.

*5-(tert-Butoxy)-5-oxopentyl 6-(4-butoxyphenyl)-2-fluorohexanoate (**26a**).* Yield 41%; yellowish oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.06 (d, J = 8.6 Hz, 2H, arom), 6.80 (d, J = 8.7 Hz, 2H, arom), 5.03−4.71 (dt, J = 48.0 Hz, J = 6.4 Hz, 1H, CHF), 4.20−4.06 (m, 2H, CH_2OCO), 3.92 (t, J = 6.5 Hz, 1H, CH_2O), 2.60−2.45 (m, 1H, $PhCH_2$), 2.24 (t, J = 6.6 Hz, 2H, CH_2CO), 2.01−1.23 [m, 23H, 7 × CH_2, $C(CH_3)_3$], 0.96 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.4, 169.9 (d, J = 23.9 Hz), 157.2, 133.8, 129.1, 114.19, 88.8 (d, J = 184.1 Hz), 80.2, 67.5, 64.9, 34.8, 34.6, 32.2 (d, J = 20.9 Hz), 31.3, 31.1, 28.0, 27.8, 23.9, 21.3, 19.2, 13.8; ^{19}F NMR (188 MHz, $CDCl_3$): δ −149.9 (qt, J = 24.4 Hz); MS (ESI) m/z (%): 456 ([M + NH_4]$^+$, 100); Anal. Calcd for $C_{25}H_{39}FO_5$: C, 68.47; H, 8.96; found: C, 68.31; H, 9.12.

*5-(tert-Butoxy)-5-oxopentyl 6-(4-butoxyphenyl)-hexanoate (**26b**).* Yield 52%; yellowish oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.03 (d, J = 8.5 Hz, 2H, arom), 6.78 (d, J = 8.6 Hz, 2H, arom), 4.05 (m, 2H, CH_2OCO), 3.89 (t, J = 6.4 Hz, 2H, CH_2O), 2.51 (t, J = 7.5 Hz, 2H, $PhCH_2$), 2.33−2.10 (m, 4H, 2 × CH_2CO), 1.81−1.16 [m, 23H, 7 × CH_2, $C(CH_3)_3$], 0.94 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.4, 172.3, 157.0, 134.0, 128.9, 114.0, 79.8, 67.3, 63.5, 34.7, 34.5, 33.9, 31.2, 31.1, 28.4, 27.8, 27.8, 24.6, 21.3, 19.0, 13.6; MS (ESI) m/z (%): 443 ([M + Na]$^+$, 100); Anal. Calcd for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59; found: C, 71.19; H, 9.71.

General Procedure for Oxidation of 2-Hydroxy Esters. To a stirring solution of 2-hydroxy esters **12** and **23a−d** (1 mmol) in dry CH_2Cl_2 (10 mL), Dess−Martin periodinane (1.1 mmol, 0.47 g) was added and the reaction mixture was stirred for 1.5 h at room temperature. The organic phase was washed with a mixture of $Na_2S_2O_3$ 10% and $NaHCO_3$ 10% (15 mL, 1:1, v/v) and then with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography using EtOAc−petroleum ether (bp 40−60 °C) 2:8 as eluent.

*5-(tert-Butoxy)-5-oxopentyl 6-(4-butoxyphenyl)-2-oxohexanoate (**24a**).* Yield 75%; yellowish oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.05 (d, J = 8.6 Hz, 2H, arom), 6.79 (d, J = 8.6 Hz, 2H, arom), 4.23 (t, J = 6.1 Hz, 2H, CH_2OCOCO), 3.91 (t, J = 6.4 Hz, 2H, CH_2O), 2.83 (t, J = 6.5 Hz, 2H, CH_2COCO), 2.55 (t, J = 6.6 Hz, 2H, $PhCH_2$), 2.25 (t, J = 6.8 Hz, 2H, CH_2COO), 1.82−1.23 [m, 21H, 6 × CH_2, $C(CH_3)_3$], 0.95 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, CDCl3): δ 194.6, 172.7, 161.4, 157.5, 134.0, 129.4, 114.5, 80.6, 67.8, 66.1, 39.4, 35.0, 34.9, 31.6, 31.1, 28.3, 28.0, 22.7, 21.6, 19.5, 14.1; MS (ESI) m/z (%): 452 ([M + NH_4]$^+$, 100); Anal. Calcd for $C_{25}H_{38}O_6$: C, 69.10; H, 8.81; found: C, 68.97; H, 8.99.

*5-(tert-Butoxy)-5-oxopentyl 6-(4-(hexyloxy)phenyl)-2-oxohexanoate (**24b**).* Yield 79%; colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.05 (d, J = 8.6 Hz, 2H, arom), 6.79 (d, J = 8.6 Hz, 2H, arom), 4.23 (t, J = 6.2 Hz, 2H, CH_2OCO), 3.91 (t, J = 6.5 Hz, 2H, OCH_2), 2.83 (t, J = 6.0 Hz, 2H, CH_2COCO), 2.55 (t, J = 6.2 Hz, 2H, $PhCH_2$), 2.25 (t, J = 7.1 Hz, 2H, CH_2COO), 1.84−1.53 (m, 10H, CH_2), 1.43 [s, 9H, $C(CH_3)_3$], 1.38−1.19 (m, 6H, CH_2), 0.89 (t, J = 6.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 194.3, 172.4, 161.1, 157.2, 133.7, 129.1, 114.3, 80.3, 67.9, 65.9, 39.1, 34.8, 34.6, 31.6, 30.8, 29.2, 28.0, 27.7, 25.7, 22.6, 22.4, 21.3, 14.0; MS (ESI) m/z (%): 480 ([M + NH_4]$^+$, 100); Anal. Calcd for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15; found: C, 69.91; H, 9.32.

*tert-Butyl 5-((4-([1,1′-Biphenyl]-4-yl)-2-oxobutanoyl)oxy)-pentanoate (**24c**).* Yield 70%; yellowish oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.67−7.22 (m, 9H, arom), 4.25 (t, J = 6.2 Hz, 2H, CH_2OCOCO), 3.22 (t, J = 6.9 Hz, 2H, CH_2COCO), 3.00 (t, J = 7.1 Hz, 2H, $PhCH_2$), 2.26 (t, J = 7.1 Hz, 2H, CH_2CO), 1.84−1.57 (m, 4H, 2 × CH_2), 1.44 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (50 MHz, $CDCl_3$): δ 193.3, 172.4, 160.8, 140.8, 139.3, 139.1, 128.8, 128.7, 127.2, 127.1, 126.9, 80.3, 66.0, 40.9, 34.8, 28.5, 28.0, 27.7, 21.3; MS (ESI) m/z (%): 428 ([M + NH_4]$^+$, 100); Anal. Calcd for $C_{25}H_{30}O_5$: C, 73.15; H, 7.37; found: C, 73.01; H, 7.59.

*4-(tert-Butoxy)-4-oxobutyl 4-([1,1′-biphenyl]-4-yl)-2-oxobutanoate (**24d**).* Yield 73%; colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.65−7.21 (m, 9H, arom), 4.28 (t, J = 6.4 Hz, 2H, CH_2OCOCO), 3.22 (t, J = 6.9 Hz, 2H, CH_2COCO), 2.99 (t, J = 7.2 Hz, 2H, $PhCH_2$), 2.33 (t, J = 7.2 Hz, 2H, CH_2CO), 2.09−1.91 (m, 2H, CH_2), 1.43 [s, 9H, 3 × CH_3]; ^{13}C NMR (50 MHz, $CDCl_3$): δ 194.5, 172.8, 159.8, 140.4, 139.4, 139.1, 128.9, 128.7, 127.4, 127.2, 126.8, 80.2, 65.1, 39.9, 34.6, 28.4, 28.2, 21.5; MS (ESI) m/z (%): 414 ([M + NH_4]$^+$, 100); Anal. Calcd for $C_{24}H_{28}O_5$: C, 72.71; H, 7.12; found: C, 72.50; H, 7.34.

*Methyl 6-(4-Butoxyphenyl)-2-oxohexanoate (**28**).* Yield 60%; colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.07 (d, J = 8.6 Hz, 2H, arom), 6.81 (d, J = 8.6 Hz, 2H, arom), 3.93 (t, J = 6.5 Hz, 2H, CH_2O), 3.85 (s, 3H, CH_3O), 2.86 (t, J = 6.9 Hz, 2H, CH_2CO), 2.57 (t, J = 6.9 Hz, 2H, $PhCH_2$), 1.82−1.40 (m, 8H, 4 × CH_2), 0.97 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 194.4, 161.7, 157.5, 134.0, 129.4, 114.5, 67.8, 53.2, 39.4, 34.9, 31.6, 31.1, 22.7, 19.5, 14.1; MS (ESI) m/z (%): 310 ([M + NH_4]$^+$, 100); Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.84; H, 8.27; found: C, 69.61; H, 8.38.

General Procedure for Cleavage of tert-Butyl Protecting Group. A solution of *tert*-butyl esters **24a−d**, **26a,b** (1 mmol) in CH_2Cl_2 (5 mL), and trifluoroacetic acid (TFA) (5 mL) was stirred for 1 h at room temperature. The organic solvent was evaporated under reduced pressure and then toluene (5 mL) was added and re-evaporated twice. The product was purified by precipitation with a mixture of EtOAc and petroleum ether (bp 40−60 °C) (5:95, v/v) or by column chromatography (CH_2Cl_2−MeOH, 95:5).

*5-((6-(4-Butoxyphenyl)-2-oxohexanoyl)oxy)pentanoic Acid (**25a**).* Yield 72%; white solid; mp 55−58 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.07 (d, J = 8.6 Hz, 2H, arom), 6.81 (d, J = 8.6 Hz, 2H, arom), 4.25 (t, J = 6.0 Hz, 2H, CH_2OCO), 3.93 (t, J = 6.5 Hz, 2H, CH_2O), 2.84 (t, J = 6.8 Hz, 2H, CH_2COCO), 2.57 (t, J = 6.8 Hz, 2H, $PhCH_2$), 2.41 (t, J = 6.8 Hz, 2H, CH_2COOH), 1.83−1.38 (m, 12H, 6 × CH_2), 0.96 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 194.2, 179.3, 161.1, 157.2, 133.7, 129.1, 114.3, 67.6, 65.7, 39.1, 34.6, 33.3, 31.3, 30.8, 27.6, 22.4, 20.9, 19.2, 13.8; HRMS (ESI) m/z: [M − H]$^-$ Calcd for $C_{21}H_{29}O_6^-$ 377.1970; found 377.1961; Anal. Calcd for $C_{21}H_{30}O_6$: C, 66.65; H, 7.99; found: C, 66.37; H, 8.16.

*5-((6-(4-(Hexyloxy)phenyl)-2-oxohexanoyl)oxy)pentanoic Acid (**25b**).* Yield 84%; white solid; mp 69−71 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.06 (d, J = 8.3 Hz, 2H, arom), 6.80 (d,

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J = 8.6 Hz, 2H, arom), 4.25 (t, J = 5.6 Hz, 2H, CH_2OCO), 3.91 (t, J = 6.5 Hz, 2H, OCH_2), 2.84 (t, J = 5.7 Hz, 2H, CH_2COCO), 2.55 (t, J = 6.7 Hz, 2H, $PhCH_2$), 2.40 (t, J = 6.6 Hz, 2H, CH_2COOH), 1.94−1.09 (m, 16H, CH_2), 0.89 (t, J = 5.6 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 194.3, 179.2, 161.1, 157.2, 133.7, 129.2, 114.3, 67.9, 65.7, 39.1, 34.6, 33.3, 31.6, 30.8, 29.2, 27.6, 25.7, 22.6, 22.4, 20.9, 14.0; HRMS (ESI) m/z: $[M - H]^-$ Calcd for $C_{23}H_{33}O_6^-$ 405.2283; found: 405.2253; Anal. Calcd for $C_{23}H_{34}O_6$: C, 67.96; H, 8.43; found: C, 67.79; H, 8.67.

*5-((4-([1,1′-Biphenyl]-4-yl)-2-oxobutanoyl)oxy)pentanoic Acid (**25c**).* Yield 79%; white solid; mp 114−116 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.65−7.14 (m, 9H, arom), 4.26 (t, J = 6.1 Hz, 2H, CH_2OCOCO), 3.22 (t, J = 6.5 Hz, 2H, CH_2CO), 3.00 (t, J = 7.2 Hz, 2H, $PhCH_2$), 2.40 (t, J = 6.9 Hz, 2H, CH_2COOH), 1.90−1.59 (m, 4H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 193.3, 179.2, 160.8, 140.8, 139.3, 139.1, 128.8, 128.7, 127.3, 127.1, 126.9, 65.8, 40.9, 33.3, 28.5, 27.6, 20.9; HRMS (ESI) m/z: $[M - H]^-$ Calcd for $C_{21}H_{21}O_5^-$ 353.1394; found: 353.1388; Anal. Calcd for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26; found: C, 70.95; H, 6.44.

*4-((4-([1,1′-Biphenyl]-4-yl)-2-oxobutanoyl)oxy)butanoic Acid (**25d**).* Yield 85%; white solid; mp 137−139 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.63−7.20 (m, 9H, arom), 4.30 (t, J = 6.1 Hz, 2H, CH_2OCOCO), 3.20 (t, J = 7.4 Hz, 2H, CH_2CO), 2.99 (t, J = 7.2 Hz, 2H, $PhCH_2$), 2.48 (t, J = 7.2 Hz, 2H, CH_2COOH), 2.14−1.96 (m, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 193.8, 179.7, 161.3, 141.2, 139.8, 139.6, 129.3, 129.2, 127.8, 127.6, 127.5, 65.2, 40.9, 29.7, 28.5, 23.4; HRMS (ESI) m/z: $[M - H]^-$ Calcd for $C_{20}H_{19}O_5^-$ 339.1238; found: 339.1234; Anal. Calcd for $C_{20}H_{20}O_5$: C, 70.58; H, 5.92; found: C, 70.41; H, 6.15.

*5-((6-(4-Butoxyphenyl)-2-fluorohexanoyl)oxy)pentanoic Acid (**27a**).* Yield 72%; low-melting-point yellow solid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.07 (d, J = 8.6 Hz, 2H, arom), 6.81 (d, J = 8.6 Hz, 2H, arom), 4.88 (dt, J = 48.0 Hz, J = 6.4 Hz, 1H, CHF), 4.20 (t, J = 5.6 Hz, 2H, CH_2OCO), 3.93 (t, J = 6.5 Hz, 2H, CH_2O), 2.56 (t, J = 7.3 Hz, 2H, $PhCH_2$), 2.40 (t, J = 6.6 Hz, 2H, CH_2COOH), 2.07−1.35 (m, 14H, 7 × CH_2), 0.97 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 179.3, 170.1 (d, J = 23.9 Hz), 157.2, 133.9, 129.1, 114.2, 88.8 (d, J = 184.1 Hz), 67.6, 64.8, 34.6, 33.3, 32.2 (d, J = 21.0 Hz), 31.3, 31.1, 27.7, 23.9 (d, J = 3 Hz), 20.9, 19.2, 13.8; ^{19}F NMR (188 MHz, $CDCl_3$) δ −149.9 (qt, J = 24.4 Hz); HRMS (ESI) m/z: $[M - H]^-$ Calcd for $C_{21}H_{30}FO_5^-$ 381.2083; found: 381.2080; Anal. Calcd for $C_{21}H_{31}FO_5$: C, 65.95; H, 8.17; found: C, 65.76; H, 8.31.

*5-((6-(4-Butoxyphenyl)hexanoyl)oxy)pentanoic Acid (**27b**).* Yield 99%; low-melting-point yellowish solid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 11.05 (s, 1H, COOH), 7.07 (d, J = 8.6 Hz, 2H, arom), 6.81 (d, J = 8.6 Hz, 2H, arom), 4.08 (t, J = 5.7 Hz, 2H, CH_2OCO), 3.93 (t, J = 6.5 Hz, 2H, CH_2O), 2.56 (t, J = 6.0 Hz, 2H, $PhCH_2$), 2.45−2.22 (m, 4H, 2 × CH_2), 1.85−1.20 (m, 14H, 7 × CH_2), 0.97 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 179.4, 173.9, 157.1, 134.2, 129.1, 114.1, 67.5, 63.6, 34.7, 34.1, 33.4, 31.3, 31.2, 28.6, 27.8, 24.7, 21.1, 19.2, 13.8; HRMS (ESI) m/z: $[M - H]^-$ Calcd for $C_{21}H_{31}O_5^-$ 363.2177; found: 363.2174; Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85; found: C, 69.01; H, 8.99.

*6-(4-Butoxyphenyl)-2-oxohexanoic Acid (**29**).* To a stirring solution of methyl ester **28** (1 mmol) in MeOH (10 mL), 20% aqueous solution of Cs_2CO_3 (2 mmol, 3.3 mL) was added and the mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the residue was dissolved in H_2O (10 mL) and extracted with Et_2O (3 × 10 mL). The aqueous layer was then acidified with 2 N HCl (4.0 mL) and extracted with EtOAc (3 × 10 mL), followed by drying over Na_2SO_4 and concentrating in vacuo. Yield 94%; white solid; mp 102−105 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.44 (s, 1H), 7.07 (d, J = 8.4 Hz, 2H, arom), 6.82 (d, J = 8.5 Hz, 2H, arom), 3.94 (t, J = 6.5 Hz, 2H, CH_2O), 2.94−2.61 (m, 1H, C*H*H), 2.57−2.21 (m, 3H, $PhCH_2$, CH*H*), 1.83−1.18 (m, 8H, 4 × CH_2), 0.97 (t, J = 7.3 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 195.4, 157.3, 133.6, 129.2, 114.3, 114.3, 67.6, 37.6, 34.5, 31.3, 30.8, 22.4, 19.2, 13.9; HRMS (ESI) m/z: $[M - H]^-$ Calcd for $C_{16}H_{21}O_4^-$ 277.1445; found: 277.1444; Anal. Calcd for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97; found: C, 68.89; H, 8.21.

In Vitro PLA_2 Activity Assays. Group-specific mixed micelle modified Dole assays were employed to determine the activity of human recombinant GIVA $cPLA_2$, GVIA $iPLA_2$, and GV $sPLA_2$.[18−20] To achieve optimum activity, the substrate was prepared using slightly different conditions for each enzyme: (i) GIVA $cPLA_2$ mixed micelle substrate consisted of 400 μM Triton X-100, 95.3 μM PAPC, 1.7 μM arachidonyl-1-^{14}C PAPC, and 3 μM PIP_2 in a buffer containing 100 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES) pH 7.5, 90 μM $CaCl_2$, 2 mM dithiothreitol (DTT), and 0.1 mg/mL bovine serum albumin; (ii) GVIA $iPLA_2$ mixed micelle substrate consisted of 400 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM arachidonyl-1-^{14}C PAPC in a buffer containing 100 mM HEPES pH 7.5, 2 mM adenosine 5′-triphosphate, and 4 mM DTT; and (iii) GV $sPLA_2$ mixed micelles substrate consisted of 400 μM Triton X-100, 98.3 μM PAPC, and 1.7 μM arachidonyl-1-^{14}C PAPC in a buffer containing 50 mM Tris−HCl pH 8.0, and 5 mM $CaCl_2$. Initially, the compounds were screened at 0.091 mol fraction (5 μL of 5 mM inhibitor in dimethyl sulfoxide) in substrate (495 μL). $X_I(50)$ values were determined for compounds exhibiting more than 95% inhibition. Inhibition curves were generated using GraphPad Prism 5.0 and the nonlinear regression by plotting percentage of inhibition vs log (mole fraction) to calculate the reported $X_I(50)$ and its associated error.

Plasma Stability Studies. The reactions were initiated by the addition of test compound to 200 μL of preheated (37 °C) human plasma to yield a final concentration of 1 mg/mL. Samples (50 μL) were taken at 0, 15, 30, and 60 min and acetonitrile (200 μL) was added. The samples were subjected to vortex mixing and then centrifugation for 5 min. The clear supernatants were analyzed by LC-HRMS/MS using an AB Sciex 4600 Triple TOF combined with a micro-LC Eksigent and an autosampler. Electrospray ionization (ESI)—negative mode—was used for the MS experiments. Halo C18 2.7 μm, 90 Å, 0.5 × 50 mm^2 from Eksigent was used as a column and the mobile phase consisted of a gradient (A: acetonitrile/0.01% formic acid—isopropanol 80/20 v/v; B: H_2O/0.01% formic acid). The data acquisition was carried out with MultiQuant from AB SCIEX (version 3.0). Each sample was studied in triplicate. The plot of the percentage of the remaining compound in comparison to the initial concentration vs time was designed.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01214.

HRMS spectra of inhibitors **25a** (GK504) and **27a** (GK505) (PDF)

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Notes

The authors declare no competing financial interest.

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